El Colegio de la Frontera Sur

Análisis taxonómico y biogeográfico de la acarofauna (Acari: Hydrachnidia) en sistemas cársticos de la Península de Yucatán (México)

Tesis

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Hacemos constar que hemos revisado y aprobado la tesis titulada: Análisis taxonómico y biogeográfico de la acarofauna (Acari: Hydrachnidia) en sistemas cársticos de la Península de Yucatán (México).

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Dedicatoria

Tal vez la felicidad sea esto, no sentir que debes estar en otro lado, haciendo otra cosa, siendo alguien más.
Isaac Asimov

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* La presente tesis no debe de ser considerada como una publicación en el sentido del Código Internacional de Nomenclatura Zoológica (ICZN); y lo nombres científicos mencionados en ésta, no deben ser citados en ninguna forma.

*This thesis is not to be regarded as a publication in the sense of the International Code of Zoological Nomenclature (ICZN); and scientific names mentioned in it must not be cited in any form.

RESUMEN

Los ácaros acuáticos constituyen uno de los grupos más diversos en los cuerpos de agua dulce, cerca de 7500 especies han sido descritas en el mundo; no obstante, se estima que esta cifra solo representa el 30% del total de especies esperadas. Estos organismos establecen fuertes interacciones bióticas con su comunidad, como parásitos, depredadores o presas. Además, están adaptados para explotar microambientes con regímenes físicos y químicos específicos, así como atributos bióticos particulares en los cuerpos de agua; por lo anterior, se consideran uno de los grupos más sensibles y excepcionales bioindicadores de las condiciones de un hábitat. A pesar de lo anterior, han sido descartados en los estudios de calidad del agua. Además, constituyen una variable importante en la dinámica ecológica del ambiente donde se encuentran. Esta falta de interés en el papel que desempeñan en los ecosistemas acuáticos se debe principalmente a la falta de tradición taxonómica en el país para este grupo, pero también a las dificultades de identificación debido a su complejo ciclo de vida, alta diversidad morfológica y dimorfismo sexual. En México se tienen registradas 258 especies, 35 de ellas descritas en la Península de Yucatán, sin embargo, desde la década de los 80´s, con algunas excepciones, no se ha realizado alguna contribución al conocimiento taxonómico de este grupo. Esto constituye un vacío importante, sobre todo en los ecosistemas predominantes en esta región: cenotes y lagunas cársticas. El objetivo de este estudio fue analizar la acarofauna en los sistemas cársticos en algunos sistemas representativos de la Península de Yucatán. Se analizaron, asimismo sus patrones de distribución utilizando datos morfológicos y moleculares, basados en el análisis de un gen estandarizado para el reconocimiento de la biodiversidad: la primera mitad del ADN que codifica para la citocromo c oxidasa I (COI o COX), conocido coloquialmente como códigos de barras de la vida. Para la parte morfológica se realizó un análisis detallado del género *Arrenurus* el cual es de los recolectados con mayor frecuencia. En total fueron muestreados 24 sitios. En primera instancia, los ácaros acuáticos fueron identificados morfológicamente a nivel de género con literatura y claves taxonómicas especializadas de Cook 1974 y 1980. Para el COI, se obtuvieron en total 607 secuencias y se identificaron 18 géneros: *Arrenurus, Atractides, Centrolimnesia, Eylais, Geayia, Hydrodroma, Hydryphantes, Hygrobates, Koenikea, Krendowskia, Limnesia, Limnochares, Mamersellides, Mideopsis, Neumania, Piona, Torrenticola* y

Unionicola, correspondientes a 77 grupos genéticos o especies putativas. El índice de similitud utilizando los datos moleculares indica que existen ensambles de ácaros acuáticos característicos en cada uno de los sitios, así como una distribución restringida de la mayoría de las especies.

Finalmente, se proporciona una lista actualizada de las especies del género *Arrenurus* en México y se brindan tres nuevos registros de este género para la Península de Yucatán, además de la descripción de cuatro nuevas especies de los subgéneros *Megaluracarus y Dadayella* incrementando a 264 las especies registradas para el país y a 42 para el género *Arrenurus*. Adicionalmente se brinda la descripción de una nueva especie del género *Litarachna* para la bahía de Corozal (Belice) misma que forma parte de la más extensa, conocida como Bahía de Chetumal.

Palabras clave: Hydrachnidia; *Arrenurus*; morfología; COI, patrones de distribución.

CAPITULO I
INTRODUCCION

Introducción

Los ácaros acuáticos representan el grupo de arácnidos más numeroso, diverso y ecológicamente importante en todos los ambientes de agua dulce (Proctor et al. 2015; Di Sabatino et al. 2008), incluyendo aguas intersticiales, ambientes lóticos, lénticos y charcas temporales (Di Sabatino et al. 2003). Proctor et al. (2015) reportaron que en lagos eutrofizados pueden alcanzar hasta 2000 individuos por metro cuadrado, agrupados en 75 especies y 25 géneros, mientras que en ambientes lóticos una muestra equivalente puede contener hasta 5000 individuos de hasta 50 especies y 30 géneros. Lo anterior contextualiza la idea respecto a su importancia en el ecosistema que habitan, debido a las múltiples interacciones bióticas que establecen en su comunidad. Por ejemplo, como parásitos durante su etapa larval se ha demostrado su influencia en la supervivencia y longevidad de sus hospederos (insectos) (Lanciani 1979). Como depredadores durante la etapa de adulto y deutoninfa han sido calificados como voraces, siendo capaces de moldear y afectar poblaciones de microcrustáceos como cladóceros y ostrácodos, así como larvas de quironómidos entre otros (Paterson 1970; Gliwicz y Biesiadka 1975; Cassano et al. 2002; Proctor y Pritchard 1989).

Desde el punto de vista del biomonitoreo, se ha demostrado la viabilidad de los ácaros acuáticos como bioindicadores de los ecosistemas que habitan, ya que existe una clara y predecible relación entre la calidad de un cuerpo de agua y la composición de su acarofauna (Goldschmidt 2016), lo que los convierte en una importante herramienta de biomonitoreo.

En México se han registrado 258 especies hasta el momento (Cook 1954; Cook 1956; Cook 1974; Cook 1980; Viets 1987; Otero-Colina 1988; Cramer y Cook 1992a; Cramer y Cook 1992b; Cramer y Cook 1996; Cramer 2000; Marín-Hernández y Cramer-Hemkes 2009). Sin embargo, este conocimiento es heterogéneo, ya que existen regiones del país mejor estudiadas que otras; particularmente la Península de Yucatán: Quintana Roo, Campeche y Yucatán están catalogados con un conocimiento limitado en cuanto a géneros registrados, en contraste con otros estados como Veracruz, Oaxaca y Guerrero (Goldschmidt et al. 2015). Un ejemplo de esto es el estado de Quintana Roo, en el cual no se continuaron las investigaciones comenzadas en la época de los 80´s por Cook (1980) y Otero-Colina (1987, 1988) con la excepción

de la descripción de una nueva especie del género *Arrenurus* (Ramírez-Sánchez y Rivas 2013), tendencia que se ve reflejada en el resto del país.

En general, en cuanto a la diversidad taxonómica de los ácaros acuáticos en México, se considera que es una de las mejores conocidas (Goldschmidt 2002; Goldschmidt et al. 2015), Cook (1980) enunció que la acarofauna neotropical (incluyendo la del sureste mexicano) se encontraba entre las más ampliamente exploradas, no obstante, también expresó que se encontraban bastante lejos de conocer las faunas locales. Por su parte, Ramírez-Sánchez y Rivas (2013) afirmaron que, respecto a la fauna de ácaros acuáticos, México había sido extensivamente investigado; sin embargo, reconocieron que casi en cada viaje de colecta se encontraban especies nuevas, particularmente en regiones con cuerpos lénticos permanentes. En este mismo sentido, Smith y colaboradores (2001) estimaron que para Norteamérica se esperan un total de 1500 especies por lo que la mitad de ellas aún no estaba descrita. Mientras que Goldschmidt (2002) a través de diferentes modelaciones estimó que para el neotrópico se esperan entre 2100 y 5500 especies. En suma, es posible inferir que el conocimiento taxonómico en el país y especialmente en la Península de Yucatán es aún bastante limitado y que faltan numerosas especies por descubrir.

Aunado al limitado conocimiento taxonómico, el estudio del grupo resulta en sí mismo un reto, debido al complejo ciclo de vida de los ácaros, compuesto de seis estados: huevo, larva, deutoninfa, protoninfa, tritoninfa y adulto. Además, el dimorfismo sexual presente en algunas familias, ha resultado en que sea difícil la identificación de estadios tempranos como larvas o deutoninfas, o que haya descripciones en las que solo se conoce al macho, por ejemplo, *Arrenurus (Truncaturus) tucumanensis* Cook, 1980 (descrito en Tucumán, Argentina) o bien, están basadas en la hembra como es el caso de *Dadayella zempoala* Cook, 1980 (descrita en Zempoala, México). La adición de información genética a los estudios taxonómicos se ha usado de manera exitosa para relacionar a distintos estadios de una misma especie, así como entre machos y hembras que presentan dimorfismo sexual marcado (Alarcón-Elbal et al. 2020). En el mismo orden de ideas, las descripciones que contienen distintas fuentes de información (morfológica, molecular, ecológica, etc.) permiten alcanzar un mayor grado de certidumbre en la delimitación o reconocimiento de una especie (Fisher et al. 2015; Pešić y Smit 2018; Pešić y Smit 2020). La generación e inclusión de este tipo de información ha permitido, a través de las bases públicas de datos, el acceso directo a las evidencias generadas, lo que coadyuva a la colaboración, intercambio y

comparación de datos, resultando en la resolución de problemas taxonómicos (Więcek et al. 2020).

Por lo tanto, resulta fundamental emprender líneas de investigación que permitan una rápida evaluación inicial de la posible diversidad y distribución de ácaros acuáticos en la Península de Yucatán, así como la descripción de especies que amalgamen distintas fuentes de información.

Marco teórico

Los estudios sobre ácaros acuáticos en México comenzaron formalmente con la descripción de dos especies colectadas en Guanajuato, una del género *Limnesia* y otra del género *Eylais,* por el naturalista Alfred Dugés en 1834 y 1873 respectivamente. Posteriormente, Marshall (1936) contribuyó con la primera compilación de ácaros acuáticos para Yucatán en la que se describieron tres nuevas especies y seis registros para la región. Particularmente, durante las décadas de los 70´s y 80´s los listados taxonómicos y descripciones de especies experimentaron un crecimiento sustantivo, debido a las contribuciones de Vidrine (1985), Otero-Colina (1987) pero principalmente de Cook (1974, 1980), quien registró 177 especies, de las cuales 139 eran nuevas para el país. Este autor trabajó especialmente en el sureste mexicano, con excepción del estado de Quintana Roo, en donde solo existen algunos registros que no suman más de diez especies (Cook 1980; Otero-Colina 1988).

Paralelamente, la Dra. Anita Hoffmann impulsó de forma extraordinaria los estudios sobre ácaros en México. Destacando en este campo una de sus estudiantes la Dra. Cristina Cramer adscrita al Laboratorio de Acarología de la Facultad de Ciencias quien durante los 80’s inició el proyecto “Taxonomía, ecología y distribución de los ácaros acuáticos de México” del cual se derivaron 45 descripciones de especies (Cramer 2000; Cramer y Cook 1992a, 1992b, 1996; Marín-Hernández y Cramer-Hemkes 2009).

Desafortunadamente, durante las dos primeras décadas del siglo XXI, las publicaciones y estudios de ácaros acuáticos en México han disminuido notablemente, destacando únicamente el registro de cuatro nuevas especies del subgénero *Megaluracarus* (Ramírez-Sánchez y Rivas 2013).

Durante la última década, la integración de caracteres moleculares y la exploración morfológica con equipos como el microscopio electrónico de barrido han permitido obtener un espectro más amplio de respuestas en la solución de preguntas

taxonómicas específicas Ejemplo de lo anterior son los estudios conducidos por Alarcón Elbal et al. (2020) en el que se combinaron datos morfológicos (microscopía confocal) y moleculares (secuencias de la subunidad I del gen COI) para asociar una larva no descrita y parasitando una pupa de mosquito, con los correspondientes adultos y deutoninfas. Sin estas herramientas, lo anterior hubiese resultado más complicado, debido a la necesidad de rastrear las larvas directamente de una hembra grávida, es así que este enfoque es prometedor para identificar larvas. Adicionalmente con los datos generados fue posible elucidar interacciones huésped-parásito entre dos especies. En el mismo sentido, destaca la descripción de *Torrenticola trimaculata* por Fisher (2015) en Norteamérica, en el que se realiza un detallado estudio de la morfología de dicha especie utilizando el microscopio óptico y el electrónico de barrido, lo cual fue robustecido con secuencias del gen COI. Finalmente, otro estudio relevante, en relación con la inclusión de la información genética, es el realizado por Więcek et al. (2019) en el que se explora la diversidad de especies de *Hydrodroma* Koch, 1837 en Norteamérica y Europa. Los resultados presentados confirmaron el estatus de las especies presentes en Europa *Hydrodroma despiciens* Müller, 1776, *H. pilosa* Besseling, 1940 e *H. torrenticola* Walter, 1908; no obstante, se descartó que las especies en Norteamérica sean las mismas que en Europa. Más aún, los autores corroboraron que se trataban de especies nuevas, y destaca en este estudio el empleo de secuencias genéticas generadas en el laboratorio de código de barras de Ecosur, unidad Chetumal (Montes-Ortiz y Elías-Gutiérrez 2018), demostrando que las especies distribuidas en los estados de Quintana Roo y Campeche no corresponden a ninguna de las descritas en Europa y forman un grupo separado de las presentes en Canadá.

No obstante, en México, hasta 2021 no existía algún estudio taxonómico que integrara a los marcadores moleculares. Sin embargo, sí hay un par de trabajos faunísticos publicados recientemente donde se reportan los primeros códigos de barras para ácaros acuáticos en el país (Elías-Gutiérrez et al. 2018; Montes-Ortiz y Elías-Gutiérrez 2018). Actualmente, existen 835 secuencias en la base de datos de BOLD SYSTEMS registradas en la zona sur de México y 71,464 en todo el mundo. Sin embargo, de las anteriores sólo el 0.5% posee una identificación hasta nivel de especie, las restantes se encuentran delegadas en niveles taxonómicos superiores, incluso únicamente reconocidos como parte del orden Trombidiformes. De tal manera se puede señalar que aún falta bastante trabajo para lograr descripciones morfológicas detalladas y

asignar a todas las secuencias de ácaros existentes una identidad taxonómica (Young et al. 2012; Telfer et al. 2015).

Respecto a los patrones de distribución de los ácaros acuáticos, Goldschmidt (2007) destacó que el estado del conocimiento de la acarofauna en América es aún bastante pobre para poder obtener una clasificación zoogeográfica. A pesar de lo anterior, en las aproximaciones de patrones globales de los ácaros acuáticos, la Península de Yucatán ha sido considerada con una alta afinidad a la región neotropical, zona que ocupa el segundo lugar de endemismos, así como una región de alta diversidad (Di Sabatino et al. 2007). Considerando su historia geológica y características hidrogeoquímicas, la Península de Yucatán podría tratarse de un área única en cuanto a la diversidad de este grupo.

Justificación

Dado que los ácaros acuáticos son uno de los componentes característicos y notables en términos de abundancia y riqueza entre los invertebrados acuáticos (Di Sabatino et al. 2000), así como el grupo más diversificado de los diferentes linajes de ácaros que han invadido secundariamente tanto ambientes lóticos como lénticos, no es de sorprender que se estime la existencia de cerca de 10,000 especies a nivel mundial (Di Sabatino et al. 2008). En particular para el Neotrópico, Goldschmidt (2002) evaluó que podrían esperarse hasta 5440 especies, como ya se ha mencionado.

En 2015, mientras se llevaba a cabo el Congreso Latinoamericano de macroinvertebrados de agua dulce en Querétaro, Goldschmidt y colaboradores, realizaron una colecta de ácaros acuáticos en una sola área con distintos microhábitats. Sin un esfuerzo de muestreo considerable lograron recolectar 112 especímenes de diez géneros y siete familias, cifras que posicionaron a esta entidad en una posición privilegiada en cuanto a la diversidad de este grupo (Goldschmidt et al. 2015). Otro claro ejemplo de esto es el estudio llevado a cabo en el Cenote Azul (Quintana Roo), en donde se tenían registradas solo cuatro especies de ácaros acuáticos, cifra que incrementó a 15 especies putativas representadas por 15 clusters genéticos o Barcode Index Number (BIN) utilizando los códigos de barras de la vida (Montes-Ortiz y Elías Gutiérrez 2018). También en Quintana Roo, en la Laguna de Bacalar se registraron 26 especies putativas identificadas solo a nivel de familia (Elías-Gutiérrez et al. 2018). Estos datos ponen en evidencia la potencial diversidad aún no descrita de estos sitios y en particular en la Península de Yucatán.

Por lo tanto, la tarea para catalogar la biodiversidad de ácaros acuáticos en México es enorme, ya que los procesos acelerados de destrucción, fragmentación y alteración de los ambientes dulceacuícolas conlleva a extinciones de especies que aún no han sido descritas (Rosso de Ferradás y Fernández 2005).

En ese mismo contexto, destaca la importancia de los ácaros acuáticos como bioindicadores de la calidad del agua, debido a que distintas especies están adaptadas para desarrollarse en estrechos rangos de microhábitats con particularidades ambientales (Pérez et al. 2014) y atributos biológicos (Smith et al. 2001). Es así que funcionan como indicadores excepcionalmente sensibles a las condiciones de su hábitat y de los impactos en la alteración de las comunidades dulceacuícolas. Goldschmidt (2016) resumió los estudios realizados con ácaros acuáticos como buenos indicadores en casos de acidificación y eutrofización, así como toda actividad antropogénica que conlleva a la degradación de pequeños cuerpos de agua o procesos exitosos de restauración, ya que tienen una alta sensibilidad de respuesta a metales pesados, pesticidas y otras sustancias tóxicas.

La vulnerabilidad en la Península de Yucatán, debido al alto desarrollo urbano y al creciente interés turístico ha incrementado notablemente (Campos-Cámara 2011). La presión se ha concentrado principalmente en ecosistemas acuáticos como cenotes y lagunas (Medina-Moreno et al. 2014). En este contexto, los ácaros acuáticos podrían ser una potencial herramienta de conservación a través de su uso como biomonitores de estos cuerpos de agua. No obstante, Danielopol et al. (2000) han señalado que los primeros esfuerzos de protección se deben enfocar en identificar toda la biodiversidad asociada, antes de conducir alguna estrategia de conservación.

Por esta razón resulta necesario crear sistemas de identificación confiables y rápidos que permitan incorporar a estos organismos en diferentes disciplinas mientras las labores de descripción formal continúan. En este sentido, Pešić et al. (2021) destacan la necesidad de construir bibliotecas de secuencias de ácaros acuáticos con el fin de poder explorar a nivel molecular la diversidad, los patrones de distribución, así como identificar problemas taxonómicos específicos.

Preguntas, objetivos e hipótesis

Con base en la información anterior las preguntas formuladas para este estudio incluyeron las siguientes:

¿Qué géneros y especies de ácaros acuáticos se encuentran en los sistemas cársticos de la Península de Yucatán?

¿Existe una composición de ácaros acuáticos distintiva para cada cuerpo de agua?

¿Existen patrones de distribución para los ácaros acuáticos en la Península de Yucatán?

Derivado de esas preguntas se formuló la hipótesis de que la diversidad de ácaros acuáticos en los cuerpos de agua de la Península de Yucatán es mucho mayor a los registros históricos, y que, dada su alta especificidad a los microhábitats, hospederos y presas, los ensambles en cada uno de los cuerpos de agua muestreados serán característicos y muy probablemente muchas especies exhibirán patrones restringidos de distribución.

Con base en lo antes expuesto, el objetivo general de este estudio fue el siguiente:

Realizar un análisis de la acarofauna acuática encontrada en los ecosistemas cársticos de la Península de Yucatán utilizando datos morfológicos y moleculares.

Los objetivos específicos fueron:

i) Explorar los patrones de distribución y posibles ensambles en los diferentes cuerpos de agua

ii) Generar una línea base de referencia molecular para los ácaros acuáticos en el sur de México, así como

iii) Realizar un estudio morfológico detallado de las especies encontradas del género *Arrenurus* Dugès, 1834.

Estructura del documento

La organización de este documento consta de seis secciones, en el primer capítulo, como ya se vio, se encontrará un breve resumen, introducción, marco teórico, justificación y las preguntas, así como objetivos que dan origen a este documento. En el segundo capítulo se encuentra el artículo: Water mite diversity (Acariformes: Prostigmata: Parasitengonina: Hydrachnidiae) from karst ecosystems in southern of Mexico: A barcoding approach, en el cual se identifican la mayoría de géneros de ácaros presentes en los cuerpos de agua muestreados en la Península de Yucatán haciendo uso de la morfología y la información molecular, asimismo se describe la potencial diversidad de éstos en la región, así como los ensambles por cada cuerpo de agua, finalmente se realizan inferencias sobre la distribución haciendo uso de los códigos de barras generados para cada especie putativa.

En el tercer capítulo se encuentra el artículo: Checklist of Arrenurids (Acari: Hydrachnidia: Arrenuridae) from Mexico with new records from Yucatan Peninsula (Mexico), and the description of four new species of the subgenera *Megaluracarus* and *Dadayella.* En este se listan todas las especies del género *Arrenurus* registradas y descritas en México, se brindan tres registros nuevos para la Península de Yucatán *Arrenurus (?Arrhenuropsis) mexicanus* Cramer & Cook, 1992, *Arrenurus (Megaluracarus) colitus* Cramer & Cook, 1992 y *Arrenurus (Megaluracarus) marshallae* Piersig, 1904. Además, se describen cuatro nuevas especies del subgénero *Megaluracarus: Arrenurus eduardi* **n. sp.***, Arrenurus federici* **n. sp.,** *Arrenurus ecosur* **n. sp.** y *Arrenurus beatrizae* **n. sp.** y una del subgénero *Dadayella*: *Arrenurus cristinae* **n. sp.** haciendo uso de información morfológica y molecular.

En el cuarto capítulo se encuentra el artículo: A new species of *Litarachna* (Acari, Hydrachnidia, Pontarachnidae) from Corozal bay (Belize), described based upon morphology and DNA barcodes en el que se realizó la primera descripción de un ácaro acuático de la familia Pontarachnidae haciendo uso del amalgamiento de información morfológica y molecular. Durante el análisis de esta nueva especie se encontraron similitudes con otra especie ampliamente distribuida (*Litarachna communis)*, el uso de las secuencias del gen COI y el empleo de la base de datos pública BOLD (www.boldsystem.org) permitió comparar ambas especies, diferenciando a la nueva especie, que, si bien no fue descrita para el país, corresponde a la provincia biogeográfica de la Península de Yucatán.

En el quinto capítulo se da información adicional que se obtuvo del primer registro de parasitación de una larva de ácaro sobre un cladócero estrictamente planctónico a través del artículo: First evidence of parasitation of a *Bosmina* (Cladocera) by a water mite larva in a karst sinkhole, in Quintana Roo (Yucatan Peninsula, Mexico), haciendo uso del microscopio electrónico de barrido. En este se destaca la relevancia de la utilización del microscopio electrónico de barrido para documentar detalladamente estructuras implicadas en el reconocimiento y delimitación de especies, pero también en la captura de interacciones biológicas entre dos especies.

Finalmente, en el sexto capítulo se brinda una conclusión general de las investigaciones realizadas, así como una valoración de la relevancia de las mismas.

Estas investigaciones constituyen las primeras exploraciones de los ácaros acuáticos en México, haciendo uso de los datos moleculares utilizando métodos no destructivos

que permiten la recuperación de los ejemplares, mismos que fueron utilizados para la descripción de las especies.

En términos generales, los resultados obtenidos de este trabajo, permiten la posibilidad de incluirlos, a través del uso de herramientas como la secuenciación de siguiente generación o ADN ambiental, en diferentes disciplinas como la ecología y la biología de la conservación o bien de manera aplicada como bioindicadores. Mientras tanto, las labores de descripción formal continuarán para poder asignarles una identidad taxonómica a las especies encontradas.

CAPITULO II

Water mite diversity (Acariformes: Prostigmata: Parasitengonina: Hydrachnidiae) from karst ecosystems in southern of Mexico: A barcoding approach

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(Este artículo pertenece a la edición especial **Biodiversity of Mites**)

Responde a los objetivos específicos i-ii

Water Mite Diversity (Acariformes: Prostigmata: Parasitengonina: Hydrachnidiae) from Karst Ecosystems in Southern of Mexico: A Barcoding Approach

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Abstract: Water mites represent the most diverse and abundant group of Arachnida in freshwater ecosystems, with about 6000 species described; however, it is estimated that this number represents only 30% of the total expected species. Despite having strong biotic interactions with their community and having the potential to be exceptional bioindicators, they are frequently excluded from studies of water quality or ecology, due to actual and perceived difficulties of taxonomic identification in this group. The objective of this study is to use the variations in the sequences of the mitochondrial cytochrome oxidase subunit I (COI), also known as the DNA barcodes region, as a tool to assess the diversity of water mites at 24 sites in the Yucatan Peninsula of Mexico. We found 77 genetic groups or putative species corresponding to 18 genera: *Arrenurus, Atractides, Centrolimnesia, Eylais, Geayia, Hydrodroma, Hydryphantes, Hygrobates, Koenikea, Krendowskia, Limnesia, Limnochares, Mamersellides, Mideopsis, Neumania, Piona, Torrenticola*, and *Unionicola.* This was significant, since there are only 35 species described for this region. Furthermore, this molecular information has allowed us to infer that there are characteristic assemblies per site. These data will facilitate the incorporation of water mites in different studies while the curatorial work continues to assign a Linnaean name.

Keywords: COI**;** Yucatan Peninsula**;** assemblages; richness; Acari

1. Introduction

Water mites belong to the Hydrachnidiae subcohort and represent the most important, abundant, and diverse group of the Arachnida in freshwater ecosystems [1,2]. There are about 6000 named species, with 1300 of them reported from the Neotropics. According to Goldschmidt [3], the neotropical water mite fauna is far from being completely described, and approximately 5440 species could reasonably be expected in this area.

Mexico is a mega-diversity country due to its position in a transition region between the Nearctic and Neotropical zones and its complex physiography [4]. As a result, it is the country in the world with the second highest number of ecosystems and the fourth in terms of biodiversity [4]. In relation to aquatic environments, we know only a small fraction of their biological diversity. Regarding water mites, 317 species have been described and some reported here in the last 40 years [5]. Only 35 were from the Yucatán Peninsula that comprises three Mexican states (Quintana Roo, Yucatan, and Campeche) [6–8].

The Yucatan region includes one of the world's largest karstic aquifers and that represents a mosaic of different geochemistry and hydrogeologic properties on its water ecosystems [9,10]. For example, the Cenote Azul, located in the southern part of the Yucatan Peninsula (18.647 N and 88.412 W, Datum WGS84), is a unique extreme environment, characterized by a high sulfate and strontium content water [10]. Lake Bacalar, also located in the south, hosts the largest living freshwater microbialites in the world [11,12] and has a rich mite fauna, which is still unknown [13].

According to Cook (1980), we were far from knowing all the local water mite diversity in the neotropics, and this situation has not improved significantly over the last 40 years. Other authors have observed that neotropical water mite fauna shows regional diversification, and a high degree of richness and endemism should be expected in this region [1,3].

The taxonomy of water mites is difficult, and systematics is constantly subject to changes [14–16], first, due to the complex life cycle composed of three active stages: parasitic larva, depredatory deutonymph, and adult and three resting stages, namely prelarva, protonymph, and tritonymph, plus the egg [2]. Some groups, such as adults arrenurids, also present a strong sexual dimorphism, where males and females are completely different morphologically. In other cases, this dimorphism is visible in the modification of the legs IV for males. Finally, the diagnostic characteristics, such as setaes, coxal groups, acetabular plate, glandularias, or palps are difficult to identify without taxonomic training. Due to these challenges, many synonyms, cryptic species, subspecies, and "forms" with questionable identity exist in the literature [6,14,17].

The application of molecular biology techniques adds new characters to taxonomy. A particular region of the mitochondrial COI (cytochrome c oxidase I) gene, one of the groups known as DNA

barcode region, is the most common sequence used in water mite taxonomy research. Public databases, including the Barcode of Life Database (www.boldsystems.org) or GenBank (www.ncbi.nlm.nih.gov), and the use of new bioinformatic tools represent a breakthrough in species identification [18,19]. On the other hand, these molecular data allow us to understand, from another perspective, not only the identity of species, but also ecological relationships that exist between these animals. It is also another important character for new species descriptions [20,21] that can solve problems related to cryptic species complexes [14,15], and matching of different development stages from eggs to adult males and females despite their morphological differences [16,21–24].

Additionally, DNA barcoding and the BOLD database (boldsystems.org) can be used to obtain a preliminary approximation of distribution patterns, species assemblages, richness, and diversity among other analysis [25]. The Barcode Index Number (BIN) is a fast-computational algorithm based on differences of the COI fragment. It is a unique Operational Taxonomic Unit (OTU) that highlights a putative species, assigning an exclusive code composed of alphanumeric characters [26]. The BIN system provides information about specimens with their associated metadata (taxonomy, distribution, images, sequences, collector, identifier, and institution where the voucher/specimens is deposited) [26]. This system has been used with success in diverse invertebrate surveys, biodiversity assessments, and species delimitation [13,25,27,28]. Currently there are 77,666 Trombidiformes records in the database where Hydrachnidia is a subcohort.

The aim of this study was to assess water mite diversity in different water bodies from the Center to the Southern Yucatan Peninsula, using DNA barcoding and the subsequent BIN representing each OTU, and their correspondence with identified morphotaxa, as the main approach.

2. Materials and Methods

2.1. Collection of Samples

Data were mined from both BOLD corresponding to previous published studies by the authors [13,23] and unpublished data from a last sampling survey carried out in April and August corresponding to the dry and rainy seasons. By the end, all data represented 24 sites (Table 1) from Yucatan Peninsula (PY), Mexico (Figure 1 and Figure 2). All the samples were collected according to the methods in earlier studies [23], except for two systems: Acapulquito and Palmar, where the collection was carried out by using manual nets with a mesh of 100 μm.

Table 1. Collection locations and Barcode Index Numbers (BINs) associated.

Number	Site	Lat N	Long W	BINs

1	Acapulquito	18.4321	88.5312	11
2	El palmar	18.4407	−88.5273	6
3	Cenote Azul	18.651	−88.4098	14
4	Cenote Cocalitos	18.652	−88.408	21
5	Cenote Escuela Normal	18.651	−88.40g.	9
6	North Bacalar Lake	18.9176	−88.171	14
7	Cenote Pucte 1	19.079	−87.994	11
8	Cenote Pucte 2	19.091	−87.994	9
9	Cenote el Toro	19.098	−88.021	2
10	Ramonal	19.3921	−88.6242	10
11	Cenote Sijil Noh Ha	19.475	−88.052	3
12	Cenote Chancah Veracruz	19.486	−87.988	4
13	Cenote del Padre	19.604	−88.003	6
14	Minicenote	19.607	−87.989	2
15	Cenote Tres Reyes 1	19.668	−87.881	3
16	Cenote Tres Reyes 2	19.692	−87.877	6
17	Santa Teresa	19.723	−87.813	2
18	Chichancanab	19.924	−88.7708	7
19	Cueva de las serpientes	19.93	−88.806	1
20	Cenote km 48	19.943	−87.794	6
21	Chunyaxche Lagoon 1	20.042	−87.581	3
22	Chunyaxche Lagoon 2	20.06	−87.576	12
23	Muyil Lagoon 1	20.069	−87.594	8
24	Muyil Lagoon 2	20.075	−87.607	4

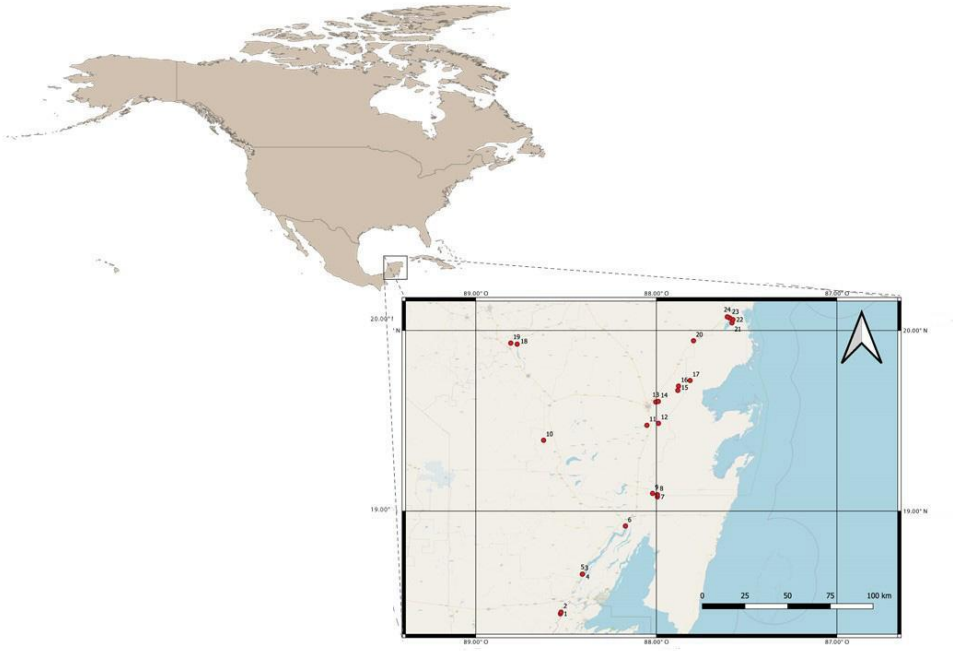

Figure 1. Location of the studied karstic systems. Names and coordinates for each site are in Table 1.

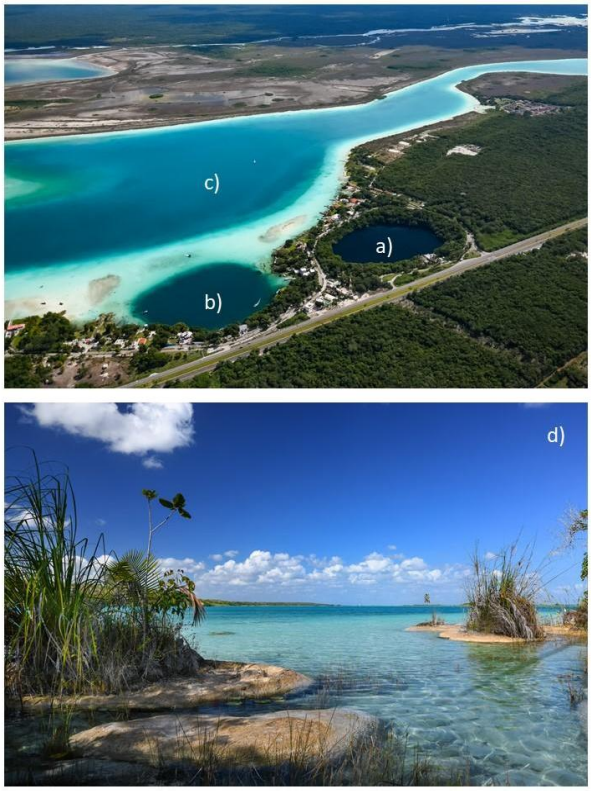

Figure 2. Examples of some sampled localities: (**a**) Cenote Azul, (**b**) Cenote Cocalitos, (**c**) Bacalar lake, and (**d**) large microbialites from Bacalar lake. Photos taken by ©HBahena/ECOSUR.

2.2. Specimen Preparational Analysis

In the laboratory, the fixed samples were viewed under a stereo microscope, and water mites were removed from each one. Representatives of each morphologically distinct group were separated and stored in 5 mL vials with 4 mL of 96° ethanol. All the water mites were identified to genus, using published keys [2,14,28]. All mites were photographed in a stereo microscope Zeizz Stereo Discovery with an Eos Rebel T3i camera.

2.3. DNA Extraction and Amplification

Whenever it was possible, five individuals of each genus were selected for genetic analysis. The whole water mite specimens were placed into 96-well plates, and DNA extraction was carried out by using a standard glass fiber method [29]. After the DNA extraction, the vouchers were recovered and preserved in Koenike´s solution for future curatorial labor and deposited in the Reference Collection at El Colegio de la Frontera Sur, Unidad Chetumal (ECOCH-Z-10339-10364).

The PCR mixtures contained a final volume of 14 μL and were prepared as follows: 2 μL of Hyclone ultra-pure water, 6.25 μL of 10% trehalose (previously prepared: 5 g D-(+)-trehalose dehydrate, in 50 mL of total volume of molecular grade ddH2O), 1.25 μL of 10X PCR buffer, 0.625 μL of MgCl2 (50 mM), 0.0625 μL of dNTP (10 mM), 0.125 μL of each primer (10 μM), 0.06 μL of Platinum Taq DNA polymerase, and 3 μL of DNA template. All specimens were amplified with the zooplankton primers (ZplankF1_tl and ZplankR1_tl). The reactions were cycled at 94 °C for 1 min, followed by five cycles of 94 °C for 40 s, 45 °C for 40 s, and 72 °C for 1 min, followed by 35 cycles of 94 °C for 40 s, 51 °C for 40 s, and 72 °C for 1 min, with a final extension of 72 °C for 5 min. PCR products were visualized on 2% agarose gel (E-Gel 96 Invitrogen); finally, positive PCR products were selected for sequencing.

PCR products were sequenced, using a modified BigDye © Terminator v. 3.1 Cycle Sequencing Kit (Applied Biosystems, Inc. CA, USA), and sequenced bidirectionally on an ABI 3730 capillary sequencer at Eurofins Scientific. Sequences were edited by using Codon Code v.3.0.1 (CodonCode Corporation, Dedham, MA, USA). Sequence data, trace files, collection data, and primer details for all specimens are available within the public dataset DS-YUCWM through the public data portal of the Barcode of Life Data Systems (www.boldsystems.org) and in GenBank (www.ncbi.nlm.nih.gov).

2.4. Sequencing and Data Analysis

All sequences that met minimal quality standards (≥500 bp, without ambiguous bases or stop codons) were assigned to a BIN [19,26]. These BINs are considered putative species or OTUs [13].

The analysis of all sequences with a BIN assignment was conducted by using MEGA v.6. We constructed Neighbor Joining trees for the most families with large numbers of BINs (Arrenuridae, Limnesiidae, Unionicolidae, and Hygrobatidae). The simplified trees were prepared by using Figtree v1 4.4.

A Jaccard index and a dendrogram were calculated with Excel software, to assess beta diversity and the similarity of water mites' BINs among the 24 locations.

3. Results

A total of 607 water mite sequences representing 77 BINs were obtained. These corresponded to 13 families: Anisitsiellidae, Arrenuridae, Eylaidae, Hydrodromidae, Hydryphantidae, Hygrobatidae, Krendowskiidae, Limnesiidae, Limnocharidae, Mideopsidae, Pionidae, Torrenticolidae, and Unionicolidae.

The number of BINs per site varied from one at Cueva de las serpientes to 21 at Cenote Cocalitos (Table 1).

We observed a correspondence between the BINs and the morphospecies for all the mite specimens. In Figure 3, we can see the correspondence between BINs and representatives of the Krendowskiidae family and *Limnesia* genera. In most cases, we matched molecularly and morphologically each BIN to a genus level, except for the following 15 that could only be assigned to families: Torrenticolidae, Limnesiidae, Hygrobatidae, Pionidae, Unionicolidae, and Eylaidae; and three BINs pertaining to Trombidiformes (Table 2).

Figure 3. Members of Krendowskiidae family and *Limnesia* genus. (**A**,**B**) Lateral and ventral view of *Geayia* BIN ACT6195; (**C**,**D**) Dorsal and ventral view of *Krendowskia* BIN ACX8435; (**E**,**F**) dorsal and ventral view of *Limnesia* BIN ACY7380; and (**G**,**H**) dorsal and ventral view of *Limnesia* BIN AEA5595.

Table 2. Summary of taxa identified, BIN, and location.

Family	Genera	BIN	Location
		ADI4862 *	3
Limnocharidae	*Limnochares*	AEA4515 *	14
		AEB4511 *	24

		ACY6840	3, 6, 4
Hydrodromidae	*Hydrodroma*	ADF3732 *	3, 4, 23, 6, 18, 2, 20, 8.
Hydryphantidae	*Hydryphantes*	AEA5005 *	3
Torrenticolidae	*Torrenticola*	AEA7372 *	1
	Unknown genera	AEA4395 *	1
Limnesiidae	*Limnesia*	AEA5595 *	13, 6, 18, 7, 12.
		AEA6471 *	10
		ACX7759	5,4
		ACY7380	19, 5, 22, 4, 2, 6
	Centrolimnesia	AEA3914 *	9, 8, 16, 17
	Unknown genera	AEA4382 *	16, 9
Krendowskiidae	*Krendowskia*	ACX8435	20, 13, 5, 16, 24, 18, 6, 4
	Geayia	ACT6195	1
Mideopsidae	*Mideopsis*	AEA6512 *	1
		ACX8679	20, 13, 18, 5, 4, 24, 11, 23, 22, 8.
		ACY7169	7, 4, 22, 5.
	Unknown genera	AEB4633 *	12
Hygrobatidae	*Hygrobates*	AEA3689 *	1
		AEA3690 *	2
		AEA3924 *	1, 2
		ACX7887	3, 18
		ADO7098	6
	Atractides	ACX7786	5, 4
	Unknown genera	AEA4089 *	23
		AEA5236 *	21, 22, 23
Pionidae	*Piona*	AEB1670 *	6
		ACX8296	13, 12, 3, 6, 4, 23, 24, 7.
	Unknown genera	AEA4809 *	22
Unionicolidae	*Unionicola*	ACX8035 *	4
		AEB4634 *	8
		ACX8034	5, 4, 3, 7, 8, 14
		ACX9008	4, 5, 6
		ADM7936	21, 22, 23, 3
		ADP1665	4, 7, 22, 6
	Koenikea	ADI2928 *	3
		ACY7384	4, 5, 22
		ADI3114	2, 22, 6, 20, 3, 18, 8, 1.
	Neumania	AEA8101 *	20, 7, 10
		AEA5358 *	10
		ACY6829	6, 4
	Unknown genera	AEA4829 *	22, 8, 16

		AEA6062 *	23, 22.
		AEA6668 *	16
		AEA7951 *	16
		AEB1594 *	8
		ACY7381	4
		AEA3726 *	7
		AEA4514 *	1
Eylaidae	*Eylais*	ADD9174 *	4
	Unknown genera	AEA4696 *	15
		AEA5669 *	15
Arrenuridae	*Arrenurus*	ACX8462 *	4
		ACX8780 *	4, 2, 1
		ADI3752 *	3
		AEA3972 *	10
		AEA7182 *	7, 20
		AEA7842	10
		AEA7843 *	10
		AEA7844 *	1
		AEA8234 *	10
		ACL2418	4
		ACX8463	6, 4, 18, 23, 3, 12, 11, 13.
		ACX8464	4, 3, 13, 10, 6, 18.
		ACX8788	5
		ACY6809	7, 4, 21, 22, 24, 4,3
		AEB7095	1
		ADI4458 *	3
		AEA4828 *	17
Anisitsiellidae	*Mamersellides*	AEA6955 *	10
		AEA6956 *	10
Unknown		AEA4343 *	11
		AEA3823 *	15
		AEB1898	8

Localities are the same as Table 1. * Unique BINs in the Barcode of Life Database (BOLD) system.

3.1. Water Mite BINs Richness

Unionicolidae was the most diverse and abundant family, with 20 BINs and 230 sequences distributed among three genera, which were identified as *Unionicola, Koenikea,* and *Neumania,* and unidentified specimens. Fifty percent of the BINs of this family appear to have a restricted distribution inhabiting only one locality, while the other half was found in two to eight localities as *Koenikea* with the BIN ADI3114 (Figure 4 and Table 2).

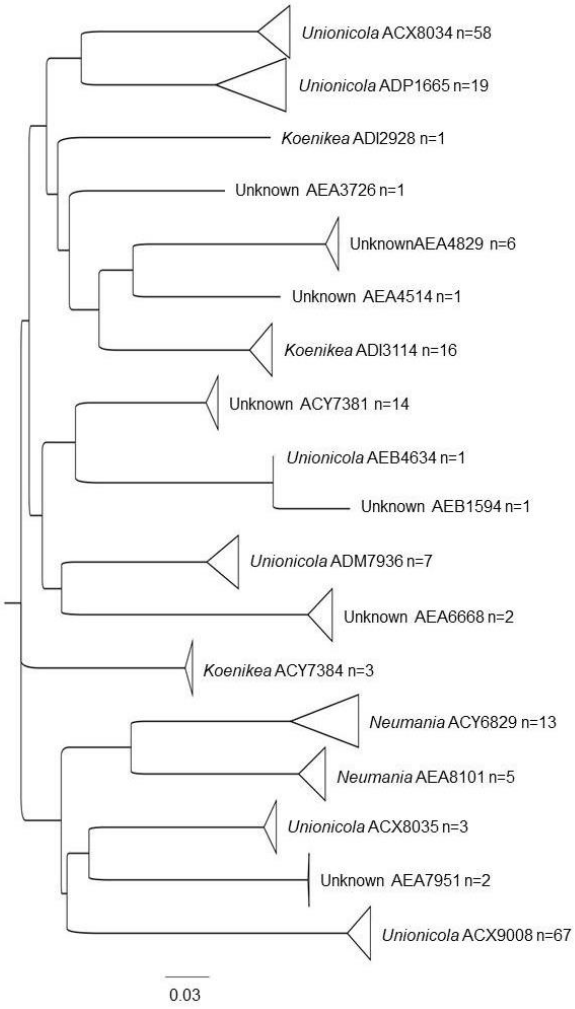

Figure 4. Neighbor Joining (NJ) tree for Unionicolidae family.

The Arrenuridae was the second most diverse family, with 123 sequences and 17 BINs. All of them belonged to the genus *Arrenurus*. For nine BINs from this group, it was possible to correlate males and females and nymphs for three of them (Figure 5. Most of the BINs apparently inhabit only one location, and only three of them seem to have a wide distribution: ACX8463, ACX8464, and ACY6809 (Figure 6 and Table 2).

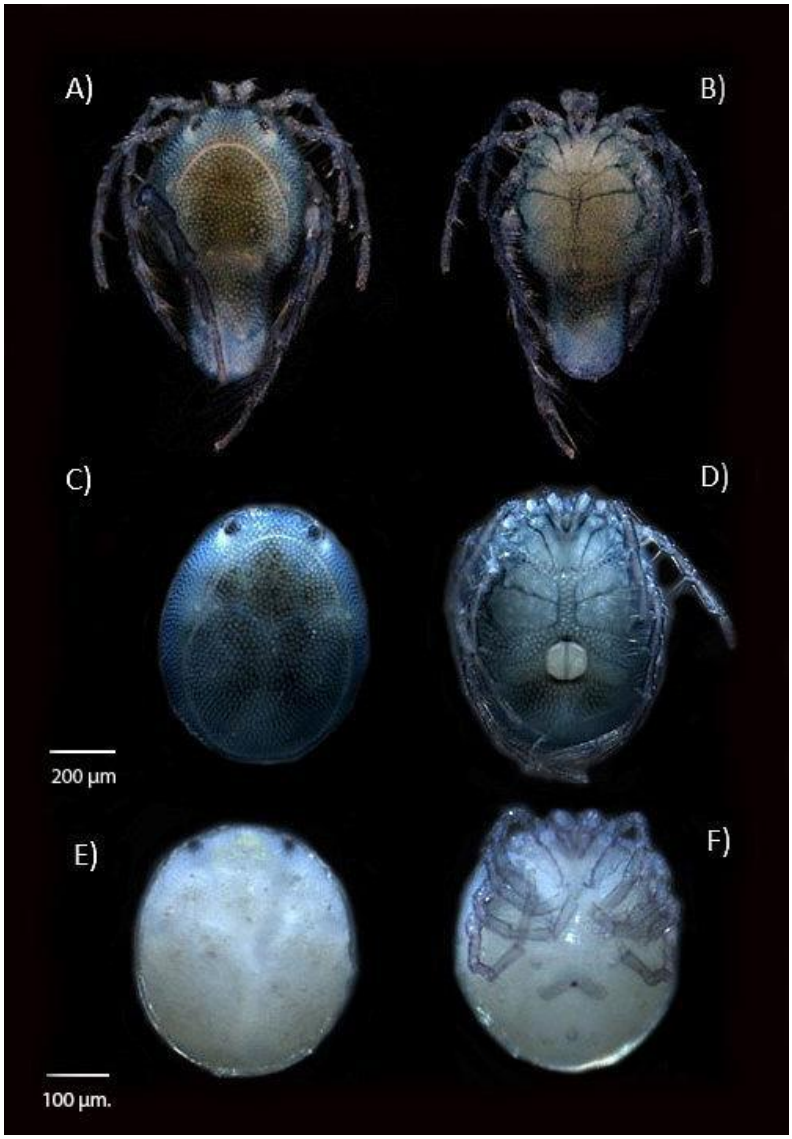

Figure 5. *Arrenurus* sp. BIN ACX8463: (**A**,**B**) dorsal and ventral view of male, (**C**,**D**) dorsal and ventral view of female, and (**E**,**F**) dorsal and ventral view of nymph.

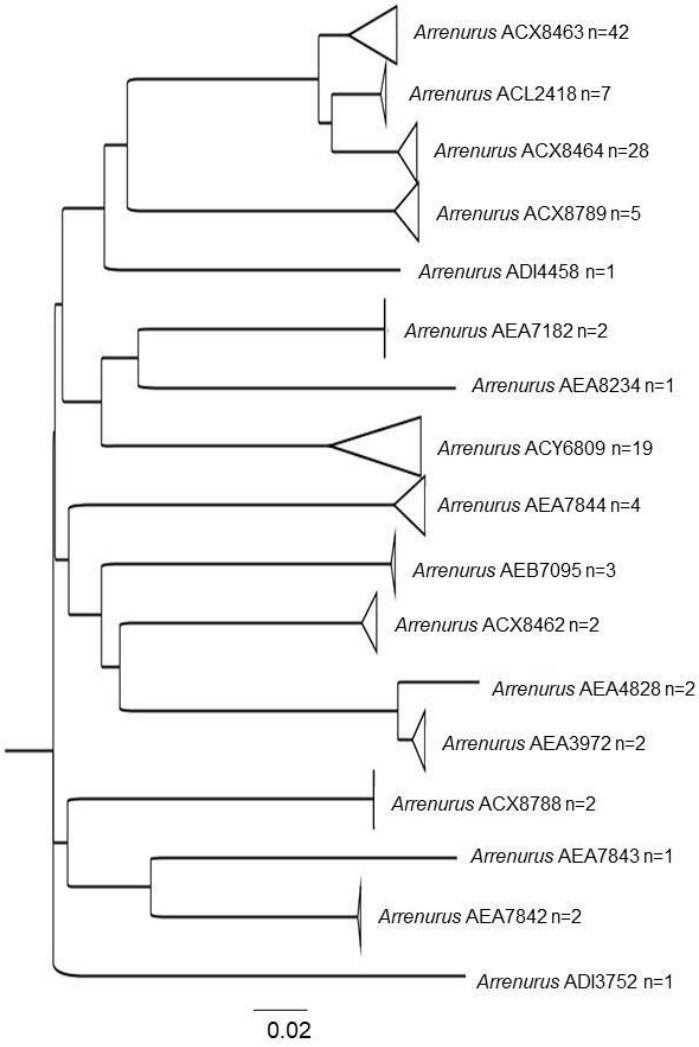

Figure 6. NJ tree for Arrenuridae family.

The Hygrobatidae and Limnesiidae families each had a moderate number of BINs. Hygrobatidae was represented by 48 sequences corresponding to eight BINs; two of them could be identified to genera *Hygrobates* and *Atractides*, and two more BINs could be identified only to family. Most of the Hygrobatidae occur only in one or two localities (Figure 7 and Table 2).

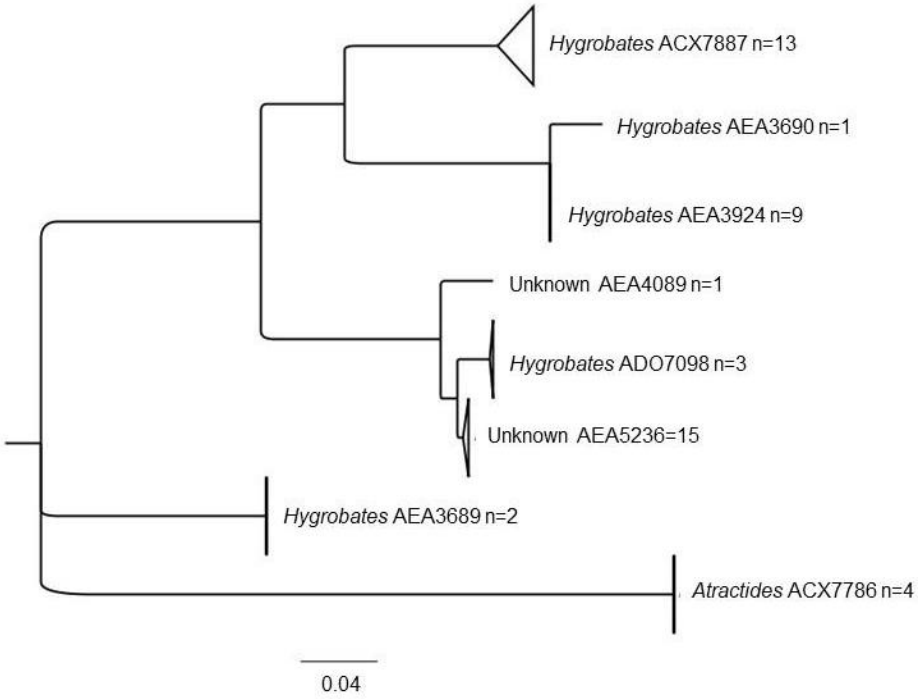

Figure 7. NJ tree for Hygrobatidae family.

The Limnesiidae are represented by 68 sequences and six BINs, with four of them identified as *Limnesia*, one *Centrolimnesia*, and one unidentified genus. More than 80% of the limnesiids occurred in in two or more localities (Figure 8and Table 2).

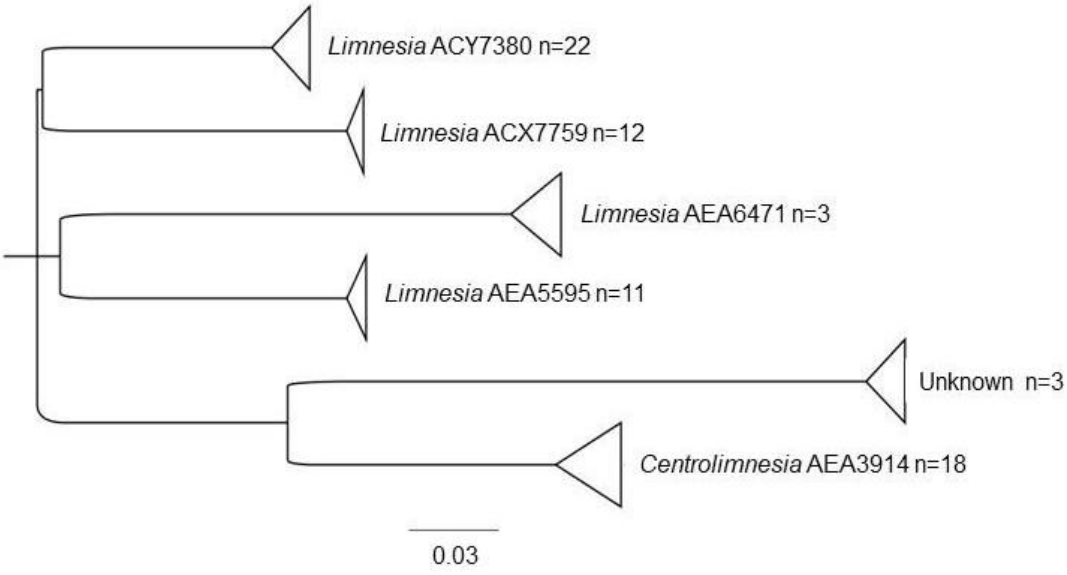

Figure 8. NJ tree for Limnesiidae family.

Other, less diverse families were the Limnocharidae, represented by nine sequences and four BINs, all of them *Limnochares*. Each BIN was found in a single locality, except for ACY6840, which was found

in three close systems: Cenote Azul, Cenote Cocalitos, and North Bacalar Lake. Mideopsidae was represented by 36 sequences clustering in four BINs, with three of them from *Mideopsis* and the other one identified only at the family level; *Mideopsis* BIN ACX8679 seems to have a wide distribution, as it was found in ten localities (Table 2).

Pionidae and Eylaidae were composed of three BINs and were each represented by one genus, *Piona* and *Eylais*, respectively; however, in both families, there were BINs with no genus assignment. In the case of Eylaidae, each BIN inhabited one system, while *Piona* ACX8296 was found in eight localities (Table 2).

Krendowskiidae was represented by two genera, *Geayia* and *Krendowskia*, with 32 sequences and two BINs (Figure 3). Krendowskia ACX8435 was widely distributed. Hydrodromidae was represented by one BIN and 22 sequences belonging to *Hydrodroma* genus. This OTU is widely distributed in eight systems in the sampled area, and all the morphotypes corresponded with one putative species.

Hydryphantidae was a singleton of the genus *Hydryphantes*. Finally, there were five sequences represented by three BINs that belonged to the order Trombidiformes. These individuals were nymphs, which are not included in any taxonomic keys. They cannot be further identified until an adult can be sequenced, as for *Arrenurus* specimens (Figure 5).

From the 77 BINs, 51 were sequenced for the first time and appear as unique in the BOLD database (Table 2). Only four BINs had a wide distribution, from Neotropical Mexico to Eastern– Central Canada. These are the *Unionicola* ADP1665, *Arrenurus* ACL2418, *Geayia* ACT6195, and *Piona* ACX8296.

3.2. BIN Assemblies in the PY

From the total, 58 BINs were present in one or a maximum of three localities, possibly forming unique species assemblages (Tables 1 and 2). The Jaccard index value, in general, for all the localities, was zero or extremely low. However, some systems shared a percentage of their water mite fauna composition as follows: Chichancanab lagoon and Cenote El Padre (44%), Chichancanab lagoon and Cenote Km 48 (44%), Cenote El Toro and Cenote Santa Teresa (33%), Cenote Tres Reyes II and Cenote El Toro (33%), and Cenote Cocalitos and Cenote Escuela Normal (43%). The two latter are important because they are two different water systems inside the Bacalar Lagoon. Despite having such spatial relationship, each system seemed to have a different composition of water mites (Figure 9 and Figure A1, Appendix A).

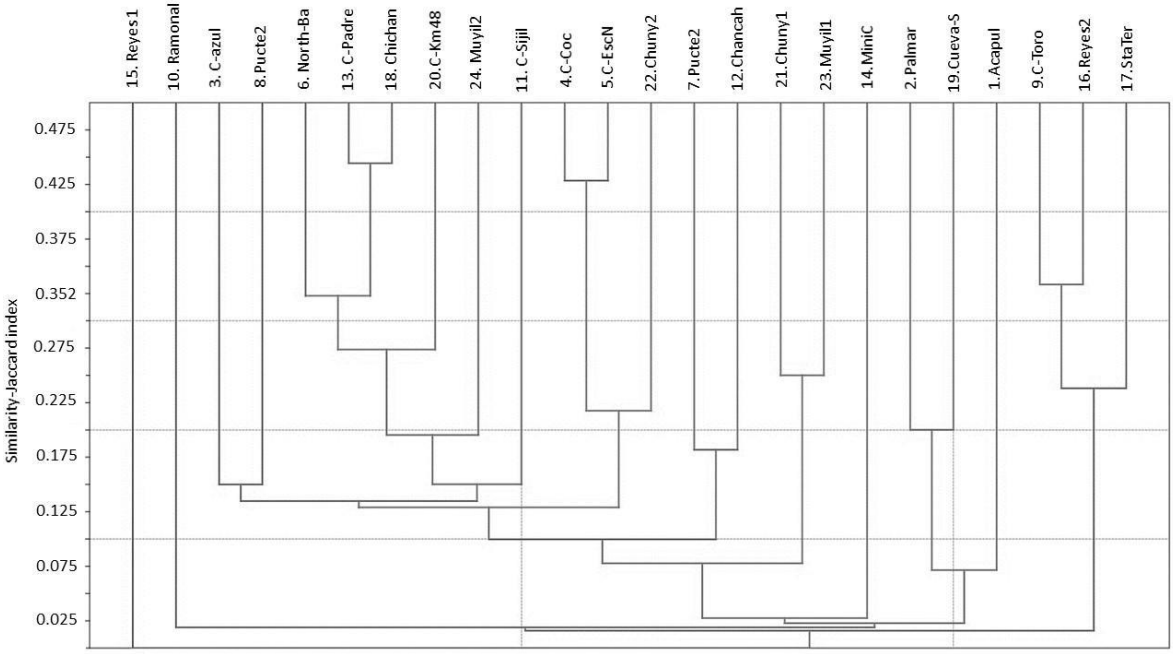

Figure 9. Similarity in water mites' composition between locations. Numbers in front of location name are the same as in Table 1.

4. Discussion

For the first time, a general analysis of the potential richness of water mite fauna in the central–southern part of the Yucatan Peninsula (Mexico), based on DNA barcodes was completed. Our results indicate an 11-fold increase in the number of species found previously in Quintana Roo state and twice the number of species registered in all the PY (in the three states, namely Campeche, Quintana Roo, and Yucatan) [6–8]. Out of 77 BINs, 58 are new in BOLD and seem to have a restricted distribution. This result indicates the presence of a unique set of environmental conditions and a particular water mite fauna composition of which most likely could be undescribed taxa. We need to study mite fauna in a

wider geographic region to support this point; however, we have seen that most species are not widely distributed in our study area.

In the case of *Hydrodroma*, our results indicate the presence of only one morphospecies in eight sampling sites and has a proper correspondence with the unique BIN ADF3732. Previous research identified two species in the PY, *H. peregrina* Cook, 1980, and *H. despiciens* Marshall, 1936. However, for both species, Cook (1980) noticed distinctions from the type specimen. A recent study, using integrative taxonomy [30], compared sequences with the specimens collected from the Cenote Azul (Mexico) (*Hydrodroma* ADF3732), and the authors concluded that it was not *H. despiciens* [30] and probably not *H. peregrina*, due to the differences noticed by Cook [6]. Consequently, *Hydrodroma* BIN ADF3732 is probably a new species that needs to be formally described and is likely endemic to Southern Mexico.

Similarly, in the case of Unionicolidae, we registered 18 BINs (Figure 4 and Table 2). Previous records include ten species for the PY. Three of them correspond to descriptions of *Koenikea indistincta* Marshall, 1936; *Koenikea neopectinifera* Cook, 1980; and *Neumania cenotea* Marshall, 1936. All of them were apparently restricted to this region. The rest are described from other localities in Mexico or different regions. For example, *Unionicola gracilipalpis tenuis* Cook, 1980, was recorded in Campeche, Michigan, and Canada, but the type locality is in Haiti. Nevertheless, *U. gracilipalpis* was originally described from Europe. It is possible that this subspecies could be a full species, but we need to compare the type material to reach such a conclusion. *Unionicola (Pentatax) furculopsis* Cook, 1980, was described from Oaxaca state, but it was found in the Cenote Azul and Bacalar Lagoon by Otero-Colina [7]. Nevertheless, he noticed a similarity with *U. furcula* (Lundblad, 1935) and described some characteristics that the type species did not have, such as denticles in the gnathosoma base. These differences could be critical to identifying a different species, but more detailed research is required. *Neumania (Neumania) diversipalpa* Cook, 1980, was originally described from a single male in a river in Chiapas, based on an adult female, was recorded in the Cenote Azul by Otero-Colina [7]. The match male–female should be made from the same locality or at least after DNA barcodes have been obtained.

These are some examples of the taxonomic uncertainties that exist for water mites from the PY; however, our goal was not to discuss all previous identifications. These are just examples of the taxonomic impediment that still exists about "subspecies", "forms", and species recorded far away from the type locality or in an extremely different habitat from the original site. Some studies have revealed that species previously considered to be cosmopolitan are not really [30]. Many of them could be actually new species or species complexes. We consider that at least 15 OTUs of the Unionicolidae recognized by different BINs are possible new species.

Likewise, for Arrenuridae, there are seven species reported from the PY [8], six *Arrenurus* from the subgenus *Megaluracarus*, and one from the subgenus *Arrenurus*. Most of these reports are from

Campeche and only one from Quintana Roo and Yucatan. We found 17 putative species of *Arrenurus* (Table 2 and Figure 6). Only three of these 17 BINs appear in multiple locations. The remaining 14 were found in only one or two close sampling sites (Table 2). After a comparison with the 135 BINs of arrenurids currently in BOLD, 94% of the BINs that we found appear juts in the south of Mexico. Other studies have previously documented the endemism of this family in other regions of the world [31–33]; however, this cannot be verified until a detailed morphological review of the specimens is made.

Another important achievement of this study is the pairing of males and females in nine BINs of this group that exhibit a high sexual dimorphism. Pairing the nymphal state in another three BINs will also allow us to make more complete formal descriptions if this species turns out to be undescribed (Figure 5).

The Hygrobatidae were the third richest group that we found (Table 2 and Figure 7). These are the first records for the PY. They were common in some locations (personal observation) that were previously surveyed [6,7]. This family seemed to be rare in the 1980s, when the previous studies took place. Some authors [2,34,35] suggest that several members of this family, *Hygrobates* included, are indicators of pollution and environmentally stressed water bodies. They were found in places like Cenote Cocalitos, Palmar, and Cenote Azul, with strong development of tourism (Tables 1 and 2). However, we must clarify the identity and habitat preferences of the species found in order to conclude if they indicate some level of environmental degradation. They may just be adapted to the extreme conditions of these places due to the presence of carbonates [10]. Nevertheless, previous surveys overlooked them.

The uniqueness of each aquatic system is clearly supported by the low values of the Jaccard index between the localities (Figure 9 and Appendix A_Figure A1). For example, Cenote Azul and Cenote Cocalitos (Figure 1 and Figure 2) are two localities with a distance of 160 m, but their similarity index is only 0.13 (Figure 9). This supports previous studies that found a difference in water quality and absence of communication between Cenote Azul and Bacalar [10]. Of the 14 BINS found in Cenote Azul and 21 found in Cocalitos, they only share four: *Limnochares* ACY6840, *Hydrodroma* ADF3732, *Unionicola* ACX8034, and *Arrenurus* ACX8463. These two systems have been extensively sampled, and their differences are also reflected in the composition of their planktonic communities [13,23]. Related studies have found that water mite assemblages are partially explained by environmental parameters such as temperature, conductivity, or pH and can almost be predicted by the potential prey groups, mainly cladocerans, copepods, and chironomids [36].

The PY ecosystems are characterized for being a mosaic of multiple habitats, with extreme differences in hydrogeochemistry conditions [9,10,12]. Their unique configuration that is structured after faults, underground and surface intermittent connections, and sinkholes (the most common

surface water systems) suggests that they could be isolated. Therefore, they exhibit a distinctive diversity. Additionally, the distribution of water mites is known to be influenced by the substrate, type of vegetation, water flow, and depth. For example, El Palmar and Acapulquito present microhabitats with slow flow current combined with pools and submerged vegetation. As a result, we found a mixture of taxa with lotic environment preferences as *Torrenticola* and species with lentic preferences as *Arrenurus* or *Unionicola* [2].

Evidently there are still several unanswered questions in terms of the diversity of water mites in the PY. For example, are there specific assemblies for microhabitats? What causes differences in abundance between species? What are the phylogenetic relationships between them, or how is their evolutive history in the PY? Finally, we consider this analysis as a preliminary step toward the formal description of all the species that we found, including morphological details of the vouchers, in order to assign them a Linnaean name; once this step has been carried out, many of our hypotheses about restricted distributions and new endemic species could be fully tested.

Author Contributions: Conceptualization, L.M.O. and M.E.G.; methodology, M.E.G.; software, L.M.O.; validation, M.E.G.; formal analysis, L.M.O.; investigation, L.M.O.; resources, M.E.G.; data curation, L.M.O.; writing—original draft preparation, L.M.O.; writing—review and editing, M.E.G. and L.M.O.; visualization, L.M.O. and M.E.G.; supervision, M.E.G.; project administration, M.E.G.; funding acquisition, M.E.G.

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Conflicts of Interest: The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript; or in the decision to publish the results.

Appendix A

	AC	PAL	CAZ	COC	CEN	BAN	CP1	CP2	CT	RAM	CSN	CCV	CP	MIC	CR1	CR2	CST	CHI	CS	K48	CH1	CH2	MU1	MU2
AC	-																							
PAL	0.14	-																						
CAZ	0.00	0.06	-																					
COC	0.03	0.08	0.13	-																				
CEN	0.00	0.08	0.05	0.43	-																			
BAN	0.00	0.12	0.22	0.35	0.15	-																		
CP1	0.00	0.00	0.10	0.15	0.13	0.15	-																	
CP2	0.00	0.08	0.15	0.11	0.13	0.10	0.06	-																
CT	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.10	-															
RAM	0.00	0.00	0.05	0.00	0.00	0.05	0.06	0.00	0.00	-														
CSN	0.00	0.00	0.06	0.09	0.09	0.06	0.00	0.09	0.00	0.00	-													
CCV	0.00	0.00	0.06	0.09	0.00	0.20	0.18	0.00	0.00	0.00	0.17	-												
CP	0.00	0.00	0.11	0.17	0.15	0.33	0.15	0.07	0.00	0.07	0.29	0.43	-											
MIC	0.00	0.00	0.07	0.05	0.10	0.00	0.10	0.10	0.00	0.00	0.00	0.00	0.00	-										
CR1	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	-									
CR2	0.00	0.00	0.00	0.04	0.07	0.05	0.00	0.15	0.33	0.00	0.00	0.00	0.09	0.00	0.00	-								
CST	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.10	0.33	0.00	0.00	0.00	0.00	0.00	0.00	0.14	-							
CHI	0.00	0.09	0.24	0.12	0.14	0.31	0.07	0.23	0.00	0.07	0.11	0.10	0.44	0.00	0.00	0.08	0.00	-						
CS	0.00	0.20	0.00	0.05	0.11	0.07	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	-					
K48	0.00	0.10	0.11	0.13	0.15	0.18	0.15	0.25	0.00	0.07	0.13	0.00	0.20	0.00	0.00	0.09	0.00	0.44	0.00	-				
CH1	0.00	0.00	0.13	0.00	0.00	0.00	0.09	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	-			
CH2	0.00	0.14	0.09	0.19	0.25	0.14	0.11	0.18	0.00	0.00	0.08	0.00	0.06	0.00	0.00	0.06	0.00	0.13	0.09	0.13	0.17	-		
MU1	0.00	0.00	0.17	0.12	0.00	0.17	0.07	0.07	0.00	0.00	0.11	0.22	0.18	0.00	0.00	0.00	0.00	0.08	0.00	0.08	0.25	0.20	-	
MU2	0.00	0.00	0.06	0.09	0.18	0.06	0.08	0.08	0.00	0.00	0.17	0.00	0.25	0.00	0.00	0.11	0.00	0.22	0.00	0.25	0.17	0.07	0.00	-

Figure A1. Matrix of Jaccard index values for all pairs of sampled locations, based on water mite BINs. AC = Acapulquito, PAL = Palmar, CAZ = Cenote Azul, COC = Cenote Cocalitos, CEN = Cenote Escuela Normal, BAN = Bacalar Norte, CP1 = Cenote Pucte 1, CP2 = Cenote Pucte 2, CT = Cenote El Toro, RAM = Ramonal, CSN = Cenote Sijil Noh Ha, CCV = Cenote Chancah Veracruz, CP = Cenote del Padre, MIC = Minicetonte, CR1 = Cenote Tres Reyes 1, CR2 = Cenote Tres Reyes 2, CST = Cenote Santa Teresa, CHI = Chichancanab, CS = Cueva de las serpientes, K48 = Cenote Km.48, CH1 = Chunyaxche 1, CH2 = Chunyaxche 2, MU1 = Muyil 1, and MU2 = Muyil 2.

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CAPITULO III

Checklist of Arrenurids (Acari: Hydrachnidia: Arrenuridae) for Mexico with new records from Yucatan Peninsula, and the description of five new species of the subgenera *Megaluracarus and Dadayella.*

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(Este artículo pertenece a la edición especial Aquatic Organisms Research with DNA Barcodes)

Responde a objetivo específico iii

Article

Checklist of arrenurids (Acari: Hydrachnidia: Arrenuridae) of Mexico, with new records from Yucatan Peninsula, and the description of five new species of the subgenera *Megaluracarus* and *Dadayella.*

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Abstract: A checklist of arrenurids of Mexico is presented, including three new records from Yucatan Peninsula. We provide updated descriptions of *Arrenurus mexicanus*, *A. (Megaluracarus*) *colitus* and *A.* (*Megaluracarus*) *marshalli*. Additionally, four new species of the subgenus *Megaluracarus* and one of *Dadayella* are described by using integrative taxonomy: *Arrenurus (Megaluracarus) eduardoi* **n. sp.** characterized by a large thorn-shaped hump in the middle dorsal shield; *Arrenurus (Megaluracaurus) federicoi* **n. sp.** with large pores in the body, including the idiosoma; *Arrenurus (Megaluracarus) ecosur* **n. sp.** with a peculiar pattern of setation in the legs; *Arrenurus (Megaluracarus) beatrizae* **n. sp.** with a short cauda having two pairs of lateral notches, and *Arrenurrus (Dadayella) cristinae* **n. sp.** characterized by a male cauda with two falcate setae. By the first time for the latter, male and female were matched using DNA sequences. Non-destructive methods allowed taking Scanning Electron Microscope images and DNA sequencing of the designed type material. All new species have a divergence using the DNA mitochondrial gene COI from 21.1 to 28.6% within them. With these records and descriptions, the number of *Arrenurus* registered for Mexico increases to 42, most of them from a single locality.

Keywords: taxonomy; morphology; DNA barcodes; COI; karstic; *Arrenurus*.

1. Introduction

Arrenurus Dugés, 1834 is the most species-rich water mite genus, with approximately 1000 species described worldwide and currently divided into 11 accepted subgenera: *Arrenurus, Arrhenuropsides, Arrhenuropsis, Brevicaudaturus, Dadayella, Dividuracarus, Megaluracarus, Micruracarus, Rhomborificias, Rhinophoracarus* and *Truncaturus* [1,2]. In Mexico, 37 species are reported, divided into five subgenera: *Arrenurus, Arrhenuropsis, Dadayella, Megaluracarus,* and *Truncaturus* (Table 1). Six of the total species recorded are only known from the Yucatan Peninsula [3–7]. Likely, this number does not represent the total species number for the genus in this region, considering that Mexico is one of the countries with the greatest biological diversity in the world due to its complex topography, the variety of climates, and the convergence of the two main biogeographic zones of the Americas: the Nearctic and the Neotropics [8]. In particular, the Yucatan Peninsula, one of the worlds' largest karstic aquifer systems, has a great diversity of aquatic ecosystems with unique geohydrological characteristics [9,10].

Recently Montes-Ortiz and Elías-Gutiérrez [11] studied the water mites' diversity from 24 sites in the Yucatan Peninsula using the sequences of the mitochondrial cytochrome subunit I (COI). Their main results are the presence of 77 genetic groups or putative species represented through a Barcode Index Number (BIN), and 17 of them corresponding to the genus *Arrenurus.* This result illustrates the potential water mite diversity for this region since only six species are described for it.

Megaluracarus Viets, 1911 can be considered the most complex subgenus of *Arrenurus* in terms of diversity and the range of morphological characteristics [6]. Another subgenus, *Dadayella* is difficult in taxonomy, since some of their descriptions have been based only on females [1,4]. This study supplies a checklist of Arrenurids from Mexico, providing three new records and describing four new species of the subgenus *Megaluracarus: A. eduardoi* **n. sp.,** *A. federicoi* **n. sp.,** *A. ecosur* **n. sp.,** *A. beatrizae* **n. sp.** and one from *Dadayella* subgenus *A. cristinae* **n**. **sp.** using morphological and molecular data.

2. Materials and Methods

The specimens were collected in five different karst systems from the southern Yucatan Peninsula (Figure 1) during a sampling survey in April and August 2019 [11], using

light traps and a hand net with a mesh size of 50 µm. The mites collected with the light trap were sieved, washed, and fixed in 96° cold ethanol [12]. Specimens collected with a hand net were sorted in the field from the samples using a pipette and fixed in ethanol 96°. All specimens were stored at -18 °C for at least seven days [13].

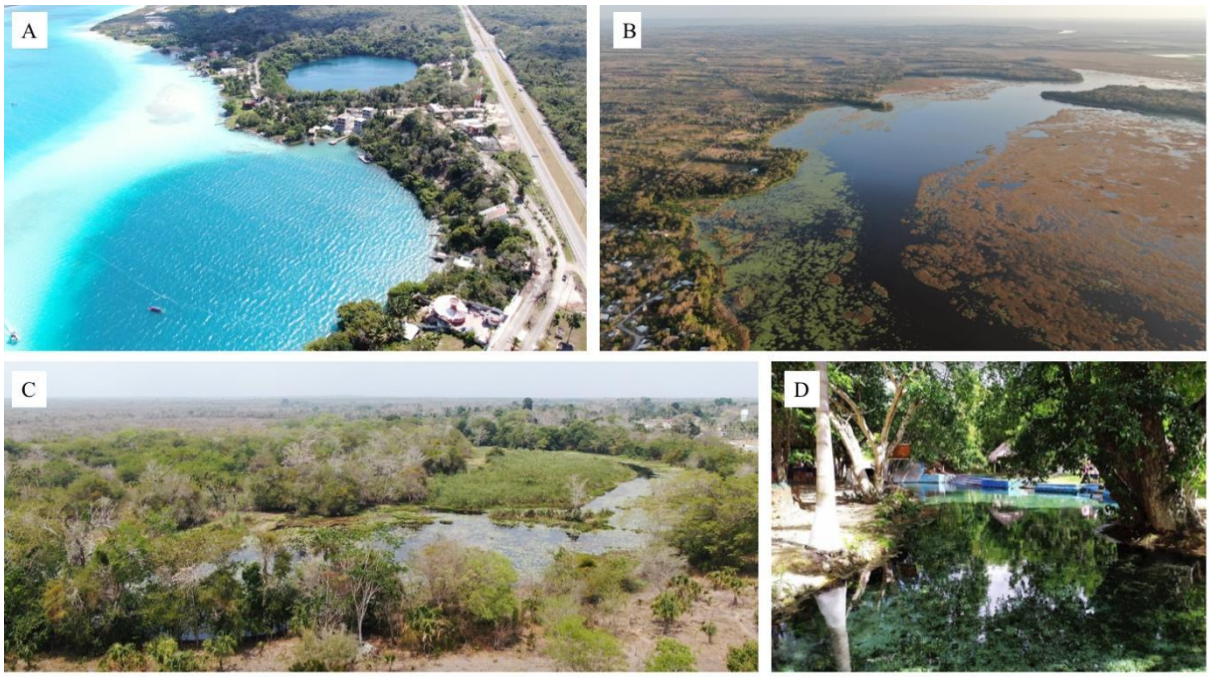

Figure 1. Sampling sites. A – Bacalar lagoon, Cenote Cocalitos (front) and Cenote Azul (back); B – Silvituc lagoon; C – Ramonal wetland; D – Acapulquito stream.

The arrenurids were separated under a stereomicroscope; representative morphospecies were photographed using a Zeiss Discovery stereomicroscope with an attached Eos Rebel T3i camera. Five individuals (when this was possible) from every morphospecies were used for DNA analyses, using a non-destructive extraction method [14]. After the process, most of the specimens were recovered, and the selected type was dissected and mounted in glycerin jelly. In the case of new species from subgenus *Megaluracarus*, after the DNA extraction, detailed images were obtained with a low vacuum and a freezing platina to -31°C attached to a Jeol JSM- 6010 Scanning Electron Microscope (SEM) at the Chetumal Unit of El Colegio de la Frontera Sur. This non-destructive method allows the recovery of the studied specimens, as it does not need the critical drying point and gold coating. Subsequently, whole specimens and the dissected parts were examined and measured under a compound microscope LW Scientific. The drawings were made using a graphic digital tablet on Inkscape V. 0.92.4 (www.inkscape.org) [15].

All specimen preparations recovered were deposited in the Reference Collection of Zooplankton (ECO-CH-Z) at El Colegio de la Frontera Sur (ECOSUR, Chetumal, Mexico), except for two paratypes of *Arrenurus (Megaluracarus) beatrizae* **n. sp. d**eposited in the water mites collection of the Aquatic Zoology Laboratory (AAL) at Facultad de Ciencias, Universidad Nacional Autónoma de México.

Molecular analysis. DNA extraction was performed using a standard glass fiber method [16] modified, following Porco *et al*. [14]. For voucher recovery. Specimens were recovered after the lysis step from the glass fiber filter plates or the 96 well-original plates and preserved in Koenike fluid. For the PCR process see [11,13,17]. PCR products were visualized on 2% agarose gels (E-Gel 96 Invitrogen), and positive PCR products were selected for sequencing bidirectionally at Eurofins Scientific in Louisville, Kentucky.

All sequences were edited using Codon Code Aligner v. 3.0.1 and uploaded to the Barcode of Life Database (BOLD: www.BOLD.org) and are in the public dataset DS_XXXX; DOI: XXXX. The sequences of the new species of *Megaluracarus* were included in a maximum likelihood (ML) tree generated with 1000 replicates using MEGA version X [18]. Two sequences of the *Krendowskia* genus were used to root the three GENWM130-16 and GENWM138-16 (Table 2).

Finally, a total of 1111 good-quality public sequences of the genus *Arrenurus* from the BOLD database were used to build a Neighbor-Joining (NJ) tree for a general comparison with all sequenced specimens from the globe.

We provide the consensus sequence for each species described in this study as an additional character. The resulting tree was included as Supplementary File 1.

All measurements are given in µm. Terminology and abbreviations in the descriptions of the new species follow [1,6,19].

Abbreviations used: BIN = Barcode Index Number; Cxgl-2 = coxoglandularia 2; Cxgl-4 = coxoglandularia 4; Cx-I −IV = first to fourth coxae; Dgl-1−4 = first to fourth dorsoglandularia; L = length; IV-Leg−1− 6 = first to sixth segments of the fourth leg; P1−P5 = first to fifth palp segments; W = width.

Table 1**.** Sequences used in the descriptions for this study

Species	Type locality*	Accession number of the type material	ID in BOLD	Barcode Index Number

Arrenurus (Megaluracarus) eduardoi **n.sp.**	Acapulquito, Riviera del Río Hondo, Quintana Roo (Mexico)*	ECO-CH_000XXXXX	YUCWM195-20, YUCWM087-19 YUCWM085-19 YUCWM084-19	AEA7844
Arrenurus (Megaluracarus) federicoi **n.sp.**	Acapulquito, Riviera del Río Hondo, Quintana Roo (Mexico)*	ECO-CH_000XXXXX	YUCWM198-20 YUCWM196-20 YUCWM197-20	AEB7095
Arrenurus (Megaluracarus) ecosur **n.sp.**	Bacalar Lagoon*, Cenote Cocalitos, Chichancanab, Muyil Lagoon 1, Cenote azul, Cenote Chancah, Cenote Sijil Noh Ha, Cenote del Padre, Quintana Roo (Mexico).	ECO-CH_000XXXXX	BACWM287-16 BACWM016-15 BACWM014-15 BACWM007-15 BACWM005-15 BACWM003-15 BACWM002-15 BACWM244-15 BACWM243-15 BACWM193-15 BACWM133-15 BACWM127-15 BACWM100-15 BACWM083-15 BACWM082-15 BACWM078-15 BACWM074-15 BACWM073-15	ACX8463

			BACWM059-15 YUCWM103-19 YUCWM036-19 YUCWM035-19 YUCWM047-19 YUCWM040-19 YUCWM039-19 YUCWM037-19 YUCWM034-19 YUCWM032-19 YUCWM031-19 CAZUL452-17 SKAAN-079-19 SKAAN-019-19 SKAAN-370-19 BACWM047-15 BACWM046-15 BACWM045-15 BACWM043-15 BACZP2234-16 SKAAN-160-19	
Arrenurus marshallae	Silvituc lagoon*, Escarcega, Campeche (México).	ECO-CH_000XXXXX	EXD479-20 EXD493-20 EXD510-20 EXD567-21	ACL2521
Arrenurus (Dadayella) cristinae **n. sp.**	Ramonal, Quintana Roo* (México)	ECO-CH_000XXXXX	YUCWM012-19 YUCWM017-19	AEA7842

Table 2. List of *Arrenurus* species (Acari: Hydrachnidia: Arrenuridae) known from Mexico

Genus	Subgenus	Specie	Author	Distribution	Habitat
Arrenurus	*Arrenurus*	*dentipetiolatus*	Marshall, 1908	United States of America, Mexico (Oaxaca/Guanajuato)	Pond
		valencius	Marshall, 1919	Venezuela, Cuba, Haití, Guatemala, Mexico (Campeche/Tabasco).	Water filled roadside
		munovus	Cook, 1980	Mexico (Chiapas)	Stream
		wucabus	Cook, 1980	Mexico (Oaxaca)	Pond
		tamaulipensis	Cramer & Cook, 1992	Mexico (Tamaulipas)	Lake
		xochimilcoensis	Cramer & Cook, 1992	Mexico (Mexico City)	Lake
	Megaluracarus	*manubriator*	Marshall, 1903	Marshall, 1903	Standing waters
		birgei	Marshall, 1903	United States of America, Mexico (Tabasco)	Pond
		marshallae	Piersig 1904	United Sates of America, Canada, México (Campeche).	Lagoon
		gricalus	Cook, 1980	Mexico (Campeche)	Water filled ditch
		hartesus	Cook, 1980	Mexico (Veracruz)	Pond
		neoexpansus	Cook, 1980	Mexico (Tabasco)	Pond
		tabascoensis	Cook, 1980	Mexico (Tabasco)	Pond
		trassamus	Cook, 1980	Mexico (Campeche)	Water filled ditch
		zitavus	Cook, 1980	Mexico (Tabasco)	Pond
		campechensis	Cook, 1980	Mexico (Campeche)	Water filled ditch
		wolardus	Cook, 1980	Mexico (Campeche)	Water filled ditch

		costeroae	Cramer & Cook, 1992	Mexico (Veracruz, Colima)	Pond
		alloexpansus	Cramer & Cook, 1992	Mexico (Tamaulipas)	Lake
		apizanus	Cramer & Cook, 1992	Mexico (Colima)	Not specified
		catoi	Cramer & Cook, 1992	Mexico (Tamaulipas)	Lake
		champayanus	Cramer & Cook, 1992	Mexico (Tamaulipas)	Lake
		colitus	Cramer & Cook, 1992	Mexico (Tamaulipas)	Lake
		anae	Cramer & Cook, 1998	Mexico (Tamaulipas)	Lake
		anitahoffmannae	Ramírez-Sánchez & Rivas, 2013	Mexico (Tabasco)	Lake, pond, canal.
		olmeca	Ramírez-Sánchez & Rivas, 2013	Mexico (Tabasco)	Lake, pond, canal.
		maya	Ramírez-Sánchez & Rivas, 2013	Mexico (Yucatán/Quintana Roo)	Cenote
		urbanus	Ramírez-Sánchez & Rivas, 2013	Mexico (Mexico City)	Canal
		eduardoi **n. sp.**	Montes-Ortiz *et. al.*, 2022	Mexico (Quintana Roo)	Pool (in a stream)
		federicoi **n. sp.**	Montes-Ortiz *et. al.*, 2022	Mexico (Quintana Roo)	Pool (in a stream)
		ecosur **n. sp.**	Montes-Ortiz *et. al.*, 2022	Mexico (Quintana Roo)	Cenote, lagoon, wetlands.

		beatrizae **n. sp.**	Montes-Ortiz *et. al.*, 2022	Mexico (Quintana Roo/Tabasco)	Wetland, lagoon.
	Dadayella	*zempoala*	Cook, 1980	Mexico (Mexico state)	Small stream
		adrianae	Cramer & Cook, 1992	Mexico (Colima/Michoacán)	Wetland, lagoon.
		veracruzensis	Cramer & Cook, 1992	Mexico (Veracruz)	Pond
		aztecus	Cramer & Cook, 1992	Mexico (Veracruz)	Wetland, lagoon.
		colimensis	Cramer & Cook, 1992	Mexico (Colima)	Wetland, lagoon.
		cristinae **n. sp.**	Montes-Ortiz *et. al.*, 2022	México (Quintana Roo)	Wetland
	Truncaturus	*plevamus*	Cook, 1980	Costa Rica, Mexico (Guerrero)	Small stream
		zukovus	Cook, 1980	Mexico (Chiapas)	Botton gravels stream
		teoceloensis	Rivas & Cramer, 1998	Mexico (Veracruz)	Stream
	Arrhenuropsis	*mexicanus*	Cramer & Cook, 1992	Mexico (Tamaulipas/Colima)	Lagoon
	?	*nayaritensis*	Cook, 1980	Mexico (Nayarit)	Small stream

Nomenclatural acts

This published work and the nomenclatural acts have been registered in ZooBank, the online registration system for the ICZN. The ZooBank Life Science Identifiers (LSIDs) can be resolved, and the associated information is viewed through any standard web browser by appending the LSID to the prefix http://zoobank.org/. The LSID for this publication is: ulr: XXXXXX. The online version of this work is archived and available from the following digital repositories: Diversity-Basel.

3. Results

Before our study, there were 37 *Arrenurus* species registered for Mexico, grouped into five subgenera: *Megaluracarus, Arrenurus, Dadayella, Truncaturus, Arrhenuropsis,* and one species represented by a female, without subgenus assigned *A. (?) nayaritensis* (Table 2). From these, six species are distributed in the Yucatan Peninsula; with our new records and species descriptions, the total number increases to 42 arrenurids registered for the country, and 11 of them are found in the Yucatan Peninsula.

We obtained four sequences for *Arrenurus* (*Megaluracarus*) *marshallae*, and 14 more are public in the BOLD database with the associated BIN ACL2521. In the case of *A. (?Arrhenuropsis*) *mexicanus* Cramer & Cook, 1992 we could not obtain the genetic information. However, we provide morphological notes. *A.* (*Megaluracarus*) *colitus* Cramer & Cook 1992 was represented by one sequence and the BIN AEA8234. Some measurements and notes are provided for these three species to achieve a more complete record. For *A. (Megaluracarus) eduardoi* **n. sp.,** we obtained four sequences, and the BIN AEA7844 was assigned. *A. (Megaluracarus) federicoi* **n. sp.** has three sequences and the BIN AEB7095. *A. (Megaluracarus) ecosur* **n. sp.** is represented by 39 sequences and the BIN ACX8463. In the case of *A. (Megaluracarus) beatrizae* **n. sp.** we were unable to obtain the genetic information. Nonetheless, all the morphological data are given. Finally, for *A. (Dadayella) cristinae* **n. sp.**, we obtained two sequences, and the BIN assigned was AEA 7842.

In the NJ tree comparing our material with all worldwide sequenced arrenurids (Supplementary File 1), the 1111 specimens represented 148 BINs, of which only 50 have a taxonomical identification. The BINs reported for Mexico (including

those used for descriptions or new records in this study) are separated from those reported for other world regions, except for *A. marshallae* BIN ACL2521 and BIN ACL2418, which are found in Canada as well.

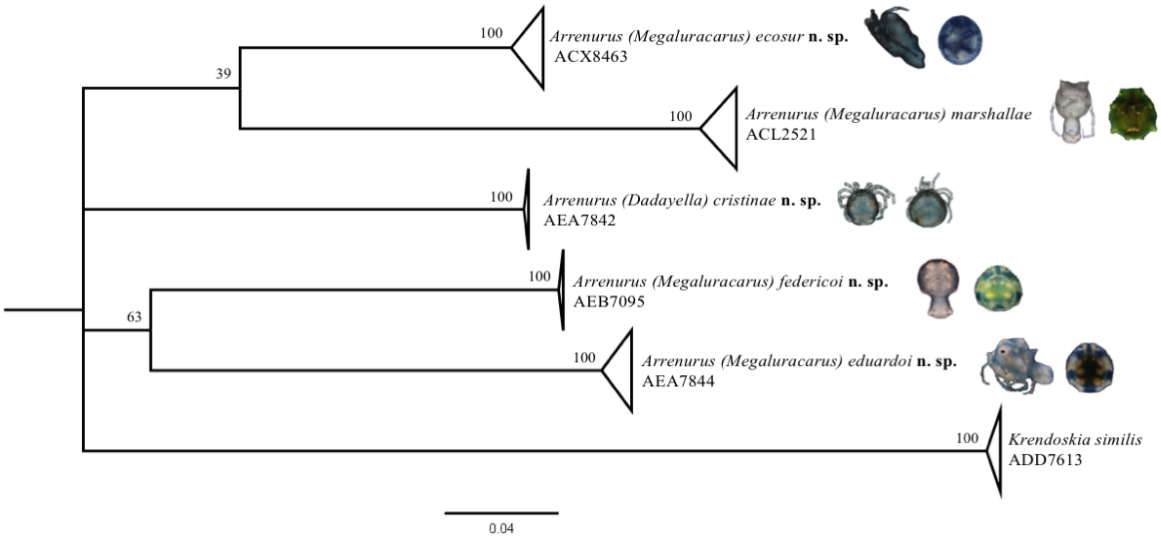

Figure 2. Maximum Likelihood tree, based on COI sequences. Bootstrap support values were generated after 1000 replicates. The name is followed by the Barcode Index Number and corresponding photograph of male and female. *Krendoskia similis* was used as an outgroup.

3.1. **Systematic part**

Family Arrenuridae Thor, 1900

Genus *Arrenurus* Dugés, 1834

Subgenus *Arrhenuropsis* Viets, 1954

***Arrenurus (?Arrhenuropsis) mexicanus* Cramer & Cook, 1992**

(Figure 3)

Material examined: One male from Ramonal pond (Access Number: ECO-CH-Z-XXXX), Quintana Roo, 19°23´31´´ N, -82°37´27´´ W; emergent vegetation, April 14, 2019.

Description. MALE: Idiosoma bluish-green with white areas in the Dgl 1-4 regions, 799 L without petiole, and 493 W. Dorsal shield small, oval, and located in the anterior part of the dorsum, 296 L and 345 W (Figure 3A). Genital field 394 W,

gonopore 69 L, and 48 W (Figure 3B). Dorsal L of palpal segments L: P1 27; P2 74; P3 29; P4 84; P5 84. Dorsal L of fourth leg segments: IV-Leg-1 32; IV-Leg-2 104; IV-Leg-3 101; IV-Leg-4 148; IV-Leg-5 151; IV-Leg-6 109.

Figure 3. *Arrenurus (?Arrhenuropsis) mexicanus* Cramer & Cook, male. A – Habitus, dorsal view; B – Habitus, ventral view. Scale bar: 200 μm.

Remarks. Male and female were described by Cramer and Cook [4]. Therefore, we only give some diagnostic measurements. This record represents the second of this species for the country.

Distribution. Previously known from the Champayan lagoon, Altamira, Tamaulipas state, 22º22´49´´N, -97º58´34´´W (Mexico).

Subgenus *Megaluracarus* Viets, 1911.

***Arrenurus (Megaluracarus) colitus* Cramer & Cook, 1992**

(Figure 4)

Material examined: One female from Ramonal pond, Quintana Roo state, 19°23´31´´ N, -82°37´27´´ W; emergent vegetation, April 14, 2019.

Description. FEMALE: Idiosoma bluish-green with white areas in the Dgl 1-4 regions, 680 L and 552 W; dorsal furrow complete, dorsal shield, 512 L and 483 W (Figure 4A). Genital field 305 W, gonopore 99 L, and 116 W (Figure 4B). Dorsal L of fourth leg segments: IV-Leg-1 74; IV-Leg-2 101; IV-Leg-3 99; IV-Leg-4 119; IV-Leg-5 106; IV-Leg-6 116.

Figure 4. *Arrenurus (Megaluracarus) colitus* Cramer & Cook, female. A – dorsal view; B – ventral view. Scale bar: 200 μm. The sequence of this specimen, recovered after DNA extraction, is represented by the BIN AEA8234.

Sequence:
ATCTATGATACCATTGGAACAGCCCATGCTTTGATTATAATTTTCTTTAT
AGTCATACCCATCATAATTGGAGGATTTGGAAATTGACTCGTTCCCCTC
ATGTTAGAAGCTCCAGATATAGCATTCCCACGAATAAATAACATAAGAT
TTTGATTACTTCCACCCCCCTTAACACTCCTTCTATCAAGATCATTAACT
TCTTCAGGAGCAGGAACTGGATGAACAGTTTACCCTCCTTTATCAAGAA
ATATCGCCCATGGAGGACCTTCAGTAGACCTAGCAATTTTCTCCCTACA
CCTTGCAGGTGTGTCCTCAATTTTAGGAGCAATTAACTTCTTGGCTACAT
TTATAAACATAAAACCTAAACATATAAAATATGACCGAATCCCCCTATT
TGTAATTTCTATTTTTATCACCGTAATCCTCCTTCTTCTTTCCCTCCCCGT
ATTAGCTGGAGCCATTACTATACTTCTTACTGATCGAAATTTTAATACTT
CATTTTTTGACCCGGCGGGGGGAGGAGATCCCATCCTTTACCAACATCT
ATTT

Remarks. Female and male were described by Cramer and Cook [4]. We provide some additional measurements data. The chaetotaxy of the palp and IV-Leg 5-6 as well as the position of ventral and dorsal glandularia agree with the original description. The only noticeable difference is that the first and second coxae tips extend slightly beyond the body proper in our specimen. The associated sequence was obtained, representing a unique BIN (BOLD: AEA8234).

Distribution. Previously known from the Champayan lagoon, Altamira, Tamaulipas state (Mexico). This record represents the second of this species for the country.

***Arrenurus (Megaluracarus) marshalli* Piersig, 1904**

Syn. *A. globator (err)* Marshall, 1903; *A. marshallae* Viets, 1914; *A. marshallae* Marshall, 1940.

(Figure 5)

Material examined: One male, one female, and one nymph from Silvituc lagoon (Access Number: ECO-CH-Z-XXXX), Escarcega municipality, Campeche state, 18°37´26´´ N, -90°17´5.9´´ W, March 18, 2020.

Description. MALE: Idiosoma light bluish, 962 L (including cauda) and 560 W; dorsal shield 776 L (including cauda) and 422 W (Figure 5A). Genital field 281 W, gonopore 47 L and 61 W (Figure 5B). Dorsal L of palpal segments: P1 34; P2 63; P3 33; P4 66; P5 47. Dorsal L of fourth leg segments: IV-Leg-1 86; IV-Leg-2 128; IV-Leg-3 151; IV-Leg-4 178; IV-Leg-5 165; IV-Leg-6 138.

FEMALE: Idiosoma light bluish, 986 L and 907 W; dorsal furrow complete, dorsal shield 719 L and 680 W. Genital field 454 W, gonopore 138 L, and 140 W.

Consensus sequence:

ACATTATACTTCGCATTCGGAGCTTGATCGGGTATAGTAGGAGCAAGAC
TTAGAAGTCTAATCCGACTAGAATTAGGGCAACCAGGAAGACTTTTAGG
AAATGATCAAATTTACAACACCATTGTTACAGCGCATGCTTTCATTATA
ATCTTCTTTATAGTTATACCAATTATAATCGGAGGATTCGGAAACTGATT
AGTACCCCTAATACTAGCCGCCCCTGATATGGCATTCCCACGAATAAAT
AATATAAGATTCTGACTTCTACCGCCAGCCTTAACACTTCTTTTATCAAG
ATCGTTAACTTCAGTAGGAGCAGGAACCGGATGAACAGTCTACCCTCCC
CTATCCAGAAACATTGCACATGGTGGACCTTCAGTAGATATAGCTATCT
TCTCATTACATTTAGCAGGAGTCTCCTCAATTTTAGGAGCTATCAATTTT
CTAGCTACAATTTTAAATATAAAGCCTAAACATATAAAATATGACAGAA
TTCCATTATTTGTAGTTTCAATTTTTATTACAGTAATTCTTCTTTTACTTT
CACTGCCTGTATTAGCAGGAGCTATTACTATACTTCTTACAGATCGAAA
TTTTAACACCTCTTTCTTCGATCCAGCTGGAGGAGGAGATCCTATTTTAT
ACCAA

Figure 5. *Arrenurus (Megaluracarus) marshallae* Piersig, male. A – dorsal view; B – ventral view. Scale bar: 200 μm. The sequence of this specimen, recovered after DNA extraction, is represented by the BIN ACL2521.

Remarks. Our specimens agree with the descriptions given by Marshall (1903) and Wilson (1961). According to Cook [19], the status of *A. marshallae* is complex because the species is a member of a closely related group characterized by the possession of a long cauda and horn-like projections over the eyes. It can be separated from other species (*A. megalurus megalurus, A. megalurus intermedius)* from the slightly indented posterior end of the cauda.

The sequences obtained in this study with the BIN ACL2521 agree with another 14 public sequences from *A. marshallae.* Some of these were identified morphologically by Bruce Smith. Morphological and molecular identification agree (Figure 2). These public sequences in the BOLD database integrated with the morphology will make it possible to verify the records of putative *A. marshallae* in other localities and other members of this complex group.

Distribution. Previously known from the United States and Canada. This record constitutes the first for Mexico.

***Arrenurus (Megaluracarus) eduardoi* n. sp.**

(Figures 6 − 7)

Holotype: Male from Acapulquito stream, Riviera del Río Hondo, Othon P. Blanco municipality, Quintana Roo state (Access Number: ECO-CH-Z-XXXX), 18° 25´ 55´´ N, 88° 31´51´´ W; emergent vegetation and submerged roots, April 11, 2019, coll. L. Montes.

Paratypes: two females and two males, same data as holotype (Access Number: ECO-CH-Z-XXXX).

Diagnosis. Male with a large thorn-shaped hump in the middle of the dorsal shield (Figure 6C-D), falcate setae on Dgl-2 and Dgl-3 (Figure 7A); three pinnate setae on P2 (two in anterolateral position and one in anteromedial position) and one falcate seta on medial position on P3. Bipectinate setae on all lateral IV-Leg-3 segment and serrate setae in the anterolateral position of IV-Leg-2 segment (Figure 7G).

Description. MALE: Idiosoma bluish with yellow spots in the Dgl 1-4 regions (Figure 7A), 1178 L and 785 W; anterior part of idiosoma very wide (Figure 6A, 7A). Dgl-2 and Dgl-3 setae falcate. Dorsal shield 1000 L (cauda included), 571 W. Cauda long, representing almost half of the total body length, 470 L and 478 W, small humps in Lgl-4 region. Dorsal furrow complete, passing ventrally at base of cauda and continuing immediately posterior to the acetabular plates. In lateral view, there is a large thorn-shaped hump centrally on the dorsum (212 height) (Figure 6C, D). Anterior and posterior coxal groups separated, Cxgl-1 between Cx-II and Cx-III, Cx-IV laterally slightly extending beyond the idiosoma, posterior region concave. Cxgl-2 is located between Cx-IV and the acetabular plates (Figure 7C). Genital field 457 W, gonopore 113 L, and 102 W, acetabular plate extending laterally from the gonopore region with two setae posterior to each plate (Figure 7C). Dorsal L of palpal segments: P1 21; P2 73; P3 47; P4 79; P5 47; P3 with a long falcate seta on anterolateral position (Figure 6B, 7D). Dorsal L of fourth leg segments: IV-Leg-1 120; IV-Leg-2 155; IV-Leg-3 196; IV-Leg-4 210; IV-Leg-5 189; IV-Leg-6 172; IV-Leg-2 with three serrate setae in anterolateral position and IV-Leg-3 with ten

bipectinate setae, on IV-Leg-4 and IV-Leg 5 with 10 and 11 small, pinnate setae respectively (Figure 7G). IV-Leg is bearing numerous swimming setae.
FEMALE: Idiosoma 1000 L and 948 W, dorsal shield 800 L and 680 W, bears the postocularia and four pairs of glandularia. Anterior idiosoma margin rounded with distinct posterolateral projections (Figure 6E, 7F). Genital field 514 W, gonopore 182 L, and 187 W. Anterior and the posterior coxal group separated, coxae not extending beyond the anterior margin of idiosoma (Figure 6F, 7F). Idiosoma and legs are bluish with yellow areas on Dgl 1− 4 regions (Figure 7E).

Consensus sequence:
ACTCTATACTTCGCTTTTGGCGCTTGATCAGGCATAATCGGAGCTAGCCTT
AGAAGTCTTATCCGTTTAGAACTTGGACAACCTGGTAATCTTTTAGGAAA
CGATCAAATATACAACACAATTGTCACTGCCCACGCATTTGTTATAATCT
TTTTCATAGTTATGCCAATCATAATCGGAGGATTCGGAAACTGATTAGTT
CCTATTATACTAGCAGCCCCAGATATAGCTTTCCCACGAATAAATAATAT
AAGATTTTGACTTCTTCCCCCCGCTTTAACTCTTCTACTNTCAAGATCTCT
ATCTTCTTCAGGAGCGGGGACTGGCTGAACAGTTTACCCCCCTCTTTCNA
GTAATATCGCTCACGGAGGACCATCTGTCGATATAGCTATTTTCTCACTTC
ACTTAGCAGGAGTTTCGTCTATTCTAGGGGCAATTAACTTCTTAGCCACA
ACTATAAACATAAAGCCAAAATATATAAAATATGACCGAATCCCCTTATT
TGTAGTCTCAATTTTCATCACAGTCATTCTCCTCCTCTTATCATTACCAGT
CTTAGCTGGAGCTATCACAATACTATTAACTGATCGAAACTTTAACACAT
CATTCTTTGACCCTGCCGGAGGGGGAGACCCAATTCTTTACCAA

Etymology. This species is named after Eduardo Montes, brother of the first author, for his empathy, solidarity and for the lovingly provided support.

Discussion. ***A. (Megaluracarus) eduardoi*** **n. sp.** is similar to *A. campechensis* Cook, 1980 and *A. maya* Ramírez-Sánchez & Rivas, 2013 in terms of overall shape and sturdy idiosoma. However, males of ***A. eduardoi*** **n. sp.** presents a distinctive large thorn-shaped hump in the middle of the dorsal shield (in lateral view) that easily separates this species from the latter two. This hump resembles *A. gibberifer* Viets 1933, originally described from Uruguay. Nevertheless, the shape of both species is quite different, especially in the dorsal view of cauda; ***A. eduardoi*** **n. sp.** presents a trapezoidal shape (Figure 6A), while *A. gibberifer* has a quadrangular shape. Additionally, the reported size for *A. gibberifer* is much smaller (742 L and 528 W) than the registered for ***A. eduardoi*** **n. sp**. Furthermore, the palp chaetotaxy of these two species is distinct. The BOLD database assigned the unique BIN AEA7844 (Table 1), used to pair the sexes. The result of the ML tree (Figure 2) and

the NJ tree (Supplementary File 1) separated ***A. eduardoi* n. sp**. from the others registered in the database and strongly supported the status of these new species.

Distribution. So far only known from the type locality, Acapulquito stream, Riviera del Río Hondo, Quintana Roo (Mexico).

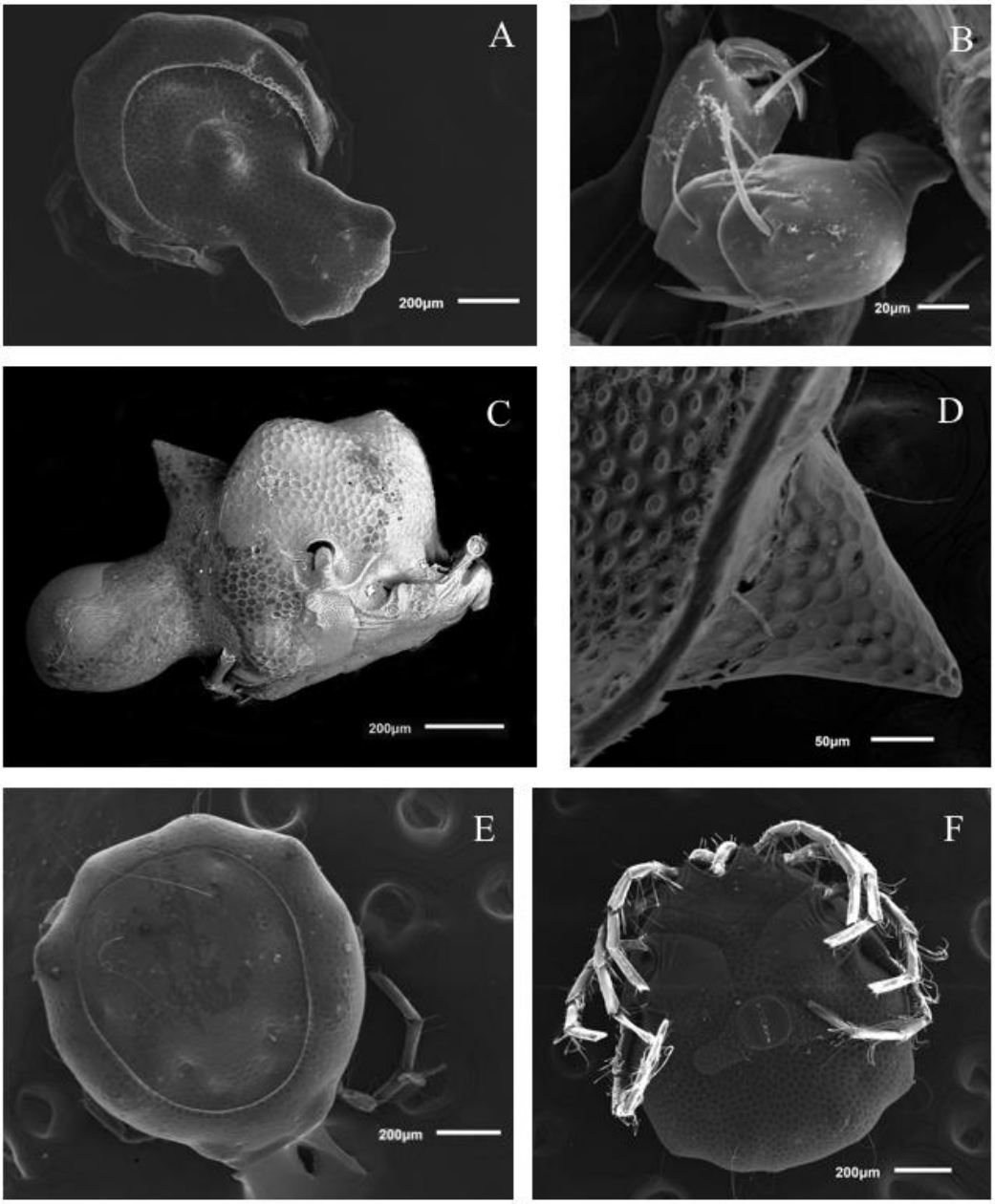

Figure 6. SEM micrograph at a low vacuum of ***Arrenurus (Megaluracarus) eduardoi* n. sp.** Male**.** A – dorsal view; B – palp, medial view; C – lateral view; D – detail on the thorn-shaped hump on the dorsal shield. Female. E – dorsal view; F – ventral view. The sequence of this specimen, recovered after DNA extraction, is represented by the BIN AEA7844.

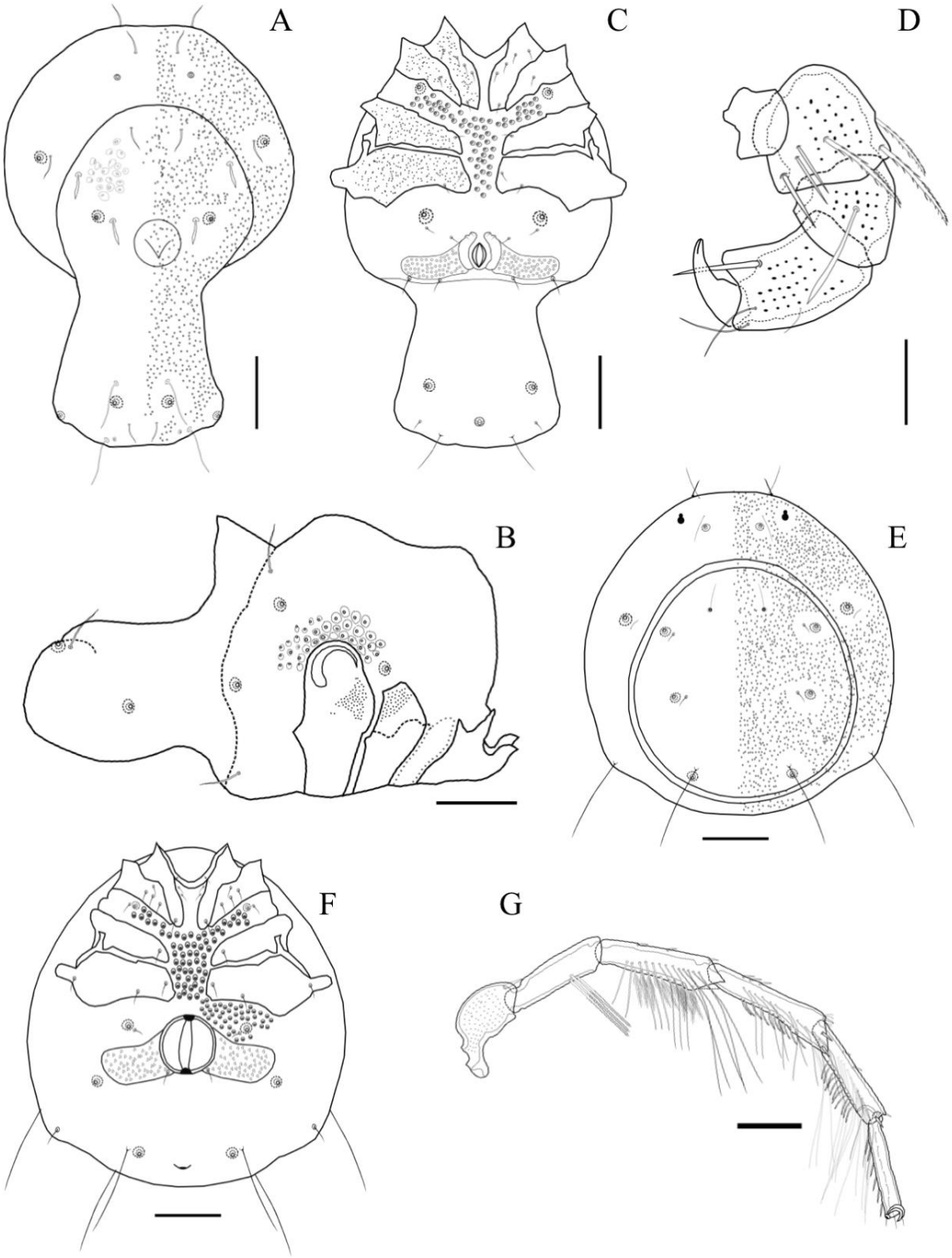

Figure 7. ***Arrenurus (Megaluracarus) eduardoi* n. sp.** Male. A – dorsal view B – lateral view; C – ventral view; D – palp, medial view; G –Leg IV, lateral view; Female. E – dorsal view; F – ventral view. Scale bars: A, B, C, E, F = 200 μm, D = 50 μm, G = 100 μm.

***Arrenurus (Megaluracarus) federicoi* n. sp.**

(Figures 7 – 8)

Type material. Holotype: Male from Acapulquito stream, Riviera del Río Hondo, Othon P. Blanco, Quintana Roo state (Access Number: ECO-CH-Z-XXXX), 18° 25´ 55´´ N, 88° 31´51´´ W; emergent vegetation and submerged roots, April 11, 2019, coll. L. Montes.

Paratypes: two females and one male, same data as holotype (Access Number: ECO-CH-Z-XXXX).

Diagnosis. Pores huge (as well on the idiosoma as the legs and palps), Dgl-1 and Cxgl-2 on distinct humps in males. Numerous setae surround the acetabular field in both sexes.

Description. MALE**:** Idiosoma 1037 L and 693 W, uniformly bluish, with large pores. The anterior part of the idiosoma is wide, with noticeable humps in the Dgl-1 area, which are visible on the lateral view (Figure 8C, 9B). Dorsal shield 718 L (cauda included) and 436 W. Cauda of medium length, representing a third of the total length of the body, 365 L and 394 W (Figure 8A, 9A); with lobes posterolaterally directed and Lgl-4 on small humps. Dorsal furrow complete, passing ventrally at base of cauda and continuing immediately posterior to the acetabular plates. In lateral view, a big hump is visible in the anterior part of the idiosoma in the Dgl-1 region (Figure 8C, 9B). Coxae with a porous surface, anterior and posterior coxal groups, separated, Cxgl-1 located in the middle of Cx-II and Cx-III; Cx-II and Cx-IV slightly extending beyond the anterolateral margin of the idiosoma; Cx-III slightly overlapping Cx-IV (Figure 8B, 9C). Cxgl-2 is located between Cx-IV and the acetabular plates. Genital field 403 W, gonopore 102 L and 75 W. Acetabular plates extending laterally from the gonopore and surrounded by numerous setae (anterior ones small 24 L, posterior ones longer 82 L) (Figure 8D, 9C). Dorsal L of Palpal segments: P1 37, P2 63, P3 41, P4 63, P5 38 (Figure 9D). Dorsal L of fourth leg segments: IV-Leg-1 125, IV-Leg-2 165, IV-Leg-3 209, IV-Leg-4 159, IV-Leg-5 193, IV-Leg-6 165: IV-Leg-5 bears six swimming setae, IV-Leg-4 distal process

bear nine short swimming setae, IV-Leg-3 bear 12 swimming setae, both IV-Leg-2 and IV-Leg-3 bear three tiny spine-like setae on lateral surface (Figure 9G). FEMALE: Idiosoma bearing huge pores, bluish with yellow spots on the region of Dgl 1–4 and eyes (Figure 9E), 1170 L and 1066 W, dorsal shield 714 L and 790 W, bears the postocularia and three pairs of glandularia. Idiosoma rounded in the anterior margin and with posterolateral lobes (Figure 8F, 9E). Genital field 499 W surrounded by small setae (38-52 L), gonopore 190 L, and 204 W. (Figure 8E). The anterior and posterior coxal groups separated, Cx-II and Cx-IV extending slightly beyond the margin of the idiosoma (Figure 8G, 9G).

Consensus sequence:
CTCTATTTCGCCTTTGGAGCATGATCAGGAATAATTGGAGCAAGATTAAG
AAGTCTAATTCGCTTAGAACTAGGACAACCAGGAAGACTATTAGGAAAC
GATCAAATTTATAACACTATTGTTACAGCTCATGCATTCATCATAATTTTC
TTCATAGTAATACCTATCATAATCGGAGGATTTGGAAATTGATTAGTACC
ATTAATACTAGCTGCTCCAGATATAGCTTTTCCTCGAATAAATAATATAA
GATTTTGACTATTACCCCCAGCATTATCCCTTCTACTAGCAAGCTCCCTTT
CTTCATCAGGAGCAGGAACAGGATGAACAGTTTACCCCCCATTATCAAG
AAATATCGCACACGGAGGACCTTCAGTTGATATAGCTATCTTCTCACTCC
ACCTAGCAGGAGTATCTTCAATTCTAGGAGCCATCAATTTTCTAGCAACA
ATCATAAATATAAAACCTAAATACATAAAATATGATCGAATCCCTTTATT
TGTTATCTCTATCTTTATCACAGTAATCTTACTCTTATTATCCTTACCAGTT
TTAGCTGGAGCTATCACTATACTATTAACAGATCGAAACTTTAATACATC
ATTCTTCGACCCAGCAGGAGGAGGAGATCCTATCCTATACCAACAT

Etymology. This species is named after Federico Montes, father of the first author, in the form of gratitude for bringing her closer to science since childhood.

Discussion. ***Arrenurus (Megaluracarus) federicoi*** **n. sp.** is similar to *A. maya* Ramírez-Sánchez & Rivas, 2013, described from a Cenote in Yucatan, in the shape of idiosoma, the pattern of dorsoglandularia position and in the presence of setae surrounding the genital field. The significant difference is in the palp chaetotaxy, *A. maya* presents three long thickened setae while ***A. federicoi*** **n. sp.** does not, in IV-Leg-6 ***A. federicoi*** **n. sp.** presents four spine-like setae while *A. maya* presents ten. Furthermore, *A. maya* has very small Dgl-4 associated setae, while ***A. federicoi*** **n. sp.** Dgl-4 associated setae are at least four times longer than in *A. maya* (Figure 9A). Both *A. catoi* Cramer & Cook, 1992 and *A. campechensis* Cook, 1980 are similar to the new species in the shape of the anterior idiosoma in dorsal view and Dgl-1 over

humps. However, ***Arrenurus federicoi* n. sp.** can be separated from both latter species by the chaetotaxy of the palp, IV- Leg, the distinctive shape of cauda in dorsal view, and especially the cuticle with large pores. The BOLD database assigned the unique BIN AEB7095 (Table 1), used to pair the sexes. The ML tree (Figure 2) and the NJ tree (Supplementary material) separated ***A. federicoi* n. sp**. from the others registered in the database and strongly support the status of these new species.

Distribution. So far only known from the type locality, Acapulquito stream, Riviera del Río Hondo, Quintana Roo (Mexico).

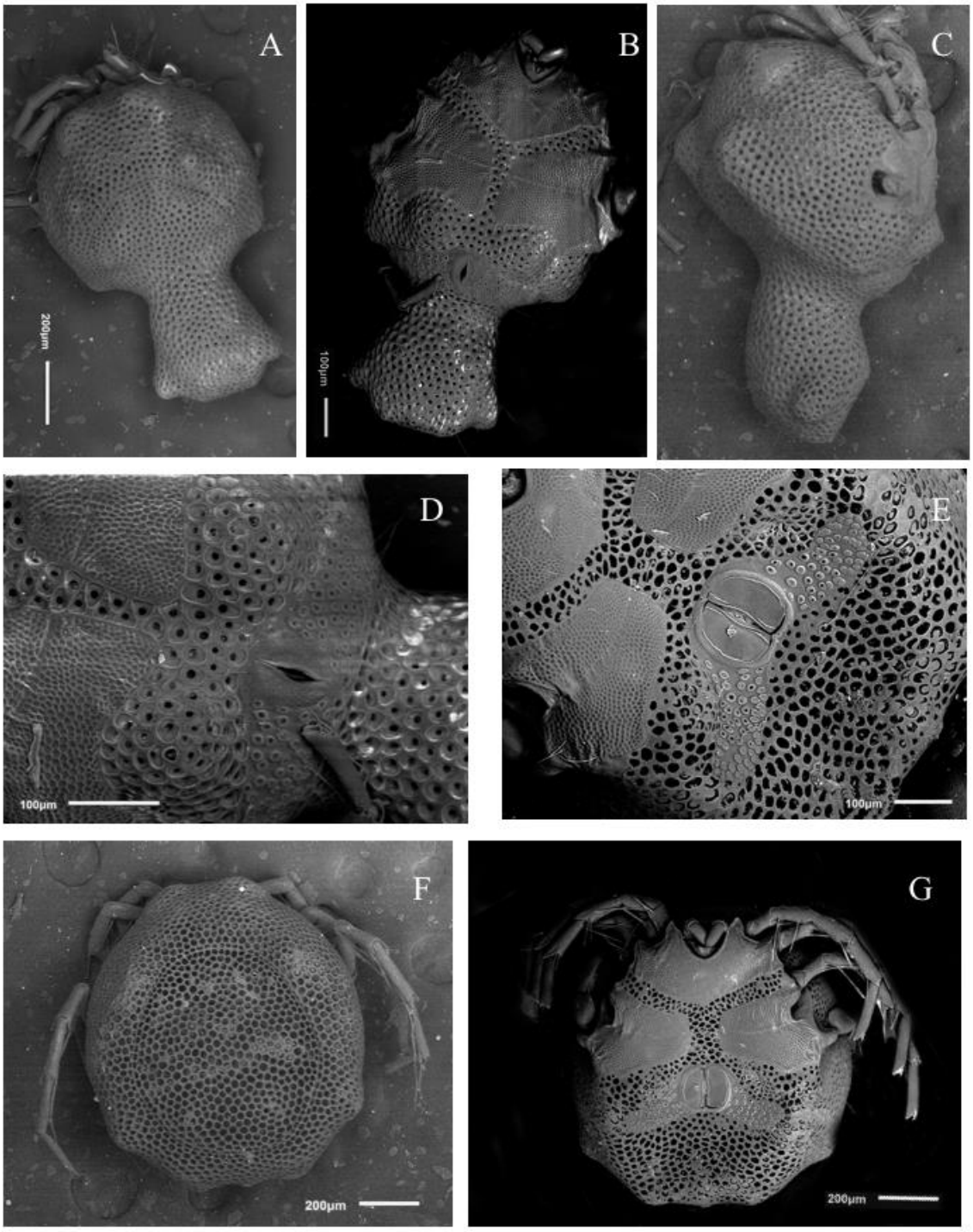

Figure 8. SEM micrograph at a low vacuum of ***Arrenurus (Megaluracarus) federicoi* n. sp.** Male**.** A – dorsal view; B – Ventral view; C – lateral view; D – detail of genital field detail. Female. E – detail of genital field detail; F – dorsal view; G – ventral view. The sequence of this specimen, recovered after DNA extraction, is represented by the BIN AEB7095.

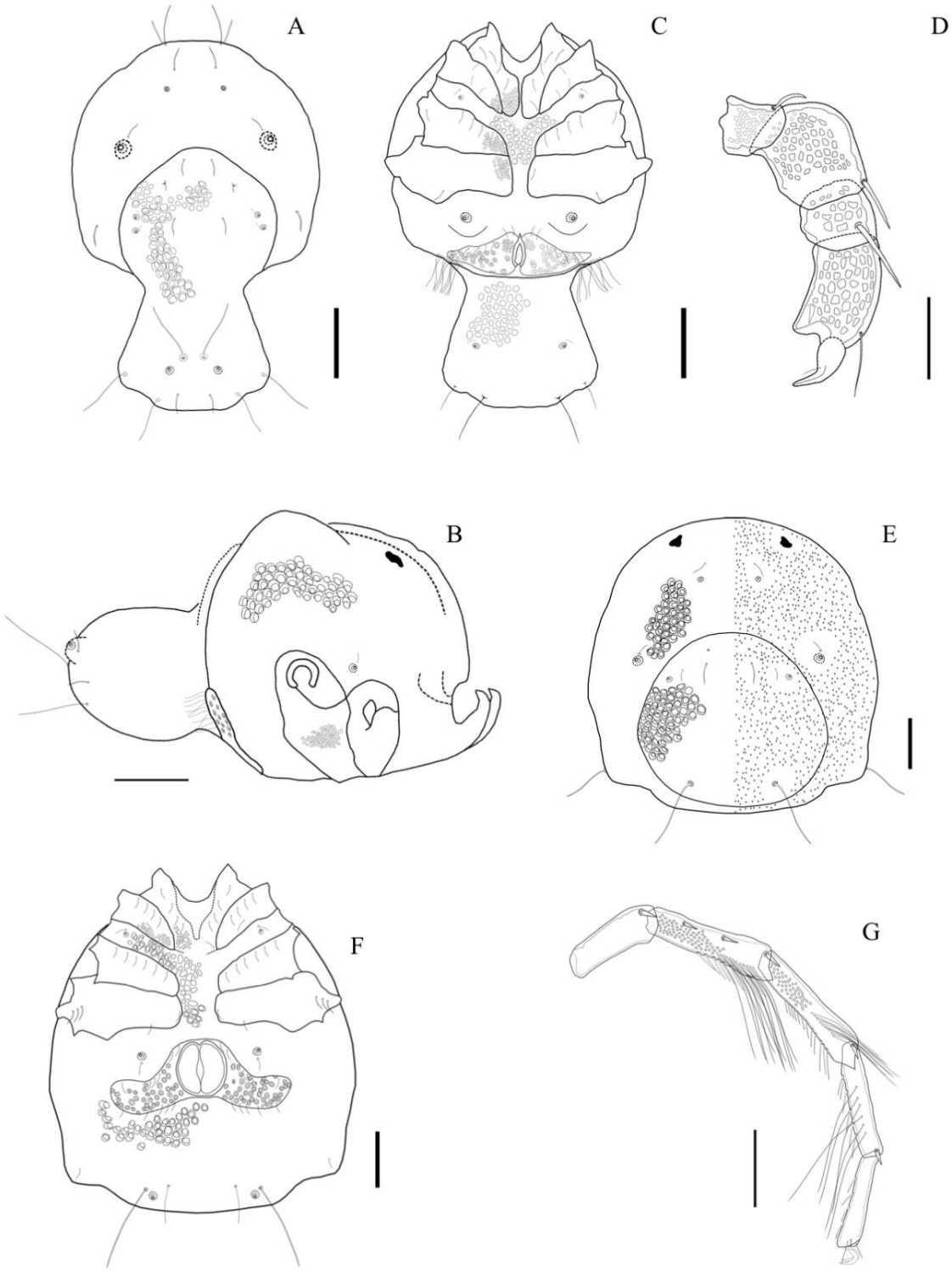

Figure 9**.** ***Arrenurus (Megaluracarus) federicoi*** **n. sp.** Male. A – dorsal view; B – lateral view; C – ventral view; D – palp, medial view; G – IV-Leg, lateral view. Female. E – dorsal view; F – ventral view. Scale bars: A, B, C, E, F = 200 μm, D = 50 μm, G = 100 μm.

***Arrenurus (Megaluracarus) ecosur* n. sp.**

(Figures 10 −11)

Holotype: Male from Mis Casas, Bacalar lagoon, Bacalar, Quintana Roo (Access Number: ECO-CH-Z-XXXX), 18° 25´ 55 N, 88° 31´51 W; littoral, emergent vegetation, April 14, 2019, coll. L. Montes.

Paratypes: Three males and one female, with same data as the holotype. Six females and one male from Chichancanab lagoon, José María Morelos, Quintana Roo (Access Number: ECO-CH-Z-XXXX), 19° 55´26 N, 88° 36´14 W.

Diagnosis. Male with cauda of moderate length (330) with Dgl-3 and Dgl-4 on distinct humps. P2 with three long, pinnate setae laterally and three medial short spine-like setae in the posterior margin; P3 with one thin and long pinnate seta lateromedially situated; IV-Leg-3 with three pilose setae lateromedially situated. Considerably long setae of Cglx-2.

Description. MALE**:** Idiosoma 864 L and 483 W, light blue, some specimens with purple legs. Dorsal shield 729 L (including cauda) and 374 W. Dorsal furrow complete. The non-caudal portion of the dorsal shield bearing two pairs of glandularia, Dgl-3 on distinct humps each one (Figure 10C, 11B). Cauda is relatively short, representing one-third of the total length of idiosoma, with a rounded posterior margin. Dgl-4 on small humps. In lateral view, the base of the cauda is thicker than the anterior idiosoma (Figure 10C, 11B). Anterior and posterior coxal groups separated. Cx-I and Cx-II extend slightly beyond the idiosoma margin. Cxgl-2 between Cx-IV and the acetabular plates, with the associated setae considerably long (146 L) (Figure 11C). Genital field 293 W, gonopore 58 L and 56 W. Acetabular plates extending laterally from the gonopore region with numerous long (50 L) setae along their posterior margin (Figure 11C). Dorsal L of palpal segments: P1 29; P2 58; P3 31; P4 62; P5 25 (Figure 10B, 11D). Dorsal L of fourth leg segments: IV-Leg-1 151, IV-Leg-2 119, IV-Leg-3 112, IV-Leg-4 135, IV-Leg-5 154, IV-Leg-6 109. IV-Leg-3 bears eight swimming setae, three small pilose setae, and six medium-length swimming setae on the dorsal surface.

FEMALE: Idiosoma oval, uniformly bluish, 655 L and 590 W, with the postocularia and four pairs of glandularia (Figure 10D, 11E). Genital field 378 W, gonopore 138 L, and 141 W. Anterior and posterior coxal groups separated, Cx-I slightly reaching the margin of the ventral shield (Figure 10E, 11F).

Consensus sequence:
ACACTTTATTTTGCATTTGGAGCTTGATCAGGTATAGTAGGAGCTAGAC
TAAGAAGTCTAATTCGCCTAGAACTAGGACAACCAGGAAATCTTTTAGG
AAACGATCAAATTTACAACACAATTGTAACAGCTCACGCTTTTATTATA
ATCTTTTTCATAGTTATACCAATCATAATCGGAGGATTCGGAAACTGAC
TAGTTCCATTAATACTAGCAGCCCCAGACATAGCGTTCCCACGAATAAA
CAATATAAGATTCTGACTTTTACCACCTGCCCTTACACTCCTACTATCTA
GATCACTATCATCCACTGGAGCAGGAACAGGGTGAACTGTTTATCCACC
CCTTTCAAGAAACATTGCCCATGGAGGACCGTCAGTAGACATAGCAATC
TTCTCACTACACTTAGCAGGTGTGTCATCAATTTTAGGAGCTATCAACTT
TTTAGCCACAATCATAAACATAAAACCTAAACACATAAAATACGATCG
AATTCCCCTTTTTGTTGTATCAATTTTTATTACTGTTATCCTACTTCTTCT
CTCACTTCCAGTTTTAGCAGGAGCTATTACAATGCTACTAACAGATCGA
AATTTCAATACATCATTCTTTGACCCAGCCGGGGGGGGAGACCCTATCT
TATACCAA

Etymology. This species is named in honor of El Colegio de la Frontera Sur (ECOSUR), the research center where the first author completed her graduate studies.

Discussion. ***Arrenurus ecosur*** **n. sp.** is similar to *A. tabascoensis* Cook, 1980 and *A. birgei* Marshall, 1903, both known from Tabasco (Mexico), mainly in the distinct hump in the area of Dgl-3 (when viewed laterally). However, the principal difference among these species is the chaetotaxy of the palps. The new species presents three distinct pinnate setae on P2. ***A. ecosur*** **n. sp.** is also similar to *A. urbanus* Ramírez-Sánchez & Rivas 2013 in the overall shape of the idiosoma in lateral and dorsal view. Nevertheless, the cauda of the new species is longer and thinner. Additionally, *A. urbanus* possess a characteristic patch of two types of setae medially on P2, which are absent in ***A. ecosur*** **n. sp**. The BOLD database assigned the BIN ACX8463 (Table 1), which was used to pair the sexes. The result of the ML tree (Figure 2) and the NJ tree (Supplementary material) separated ***A. eduardoi*** **n. sp**. from the others registered in the database and supports the status of this new species.

Distribution. Wide regional distribution in Yucatan Peninsula: Bacalar lagoon, Chichancanab lagoon, Muyil lagoon, Cenote azul, Cenote Chancah Veracruz, Cenote Sijil Noh Ha, and Cenote del Padre, Quintana Roo (Table 1).

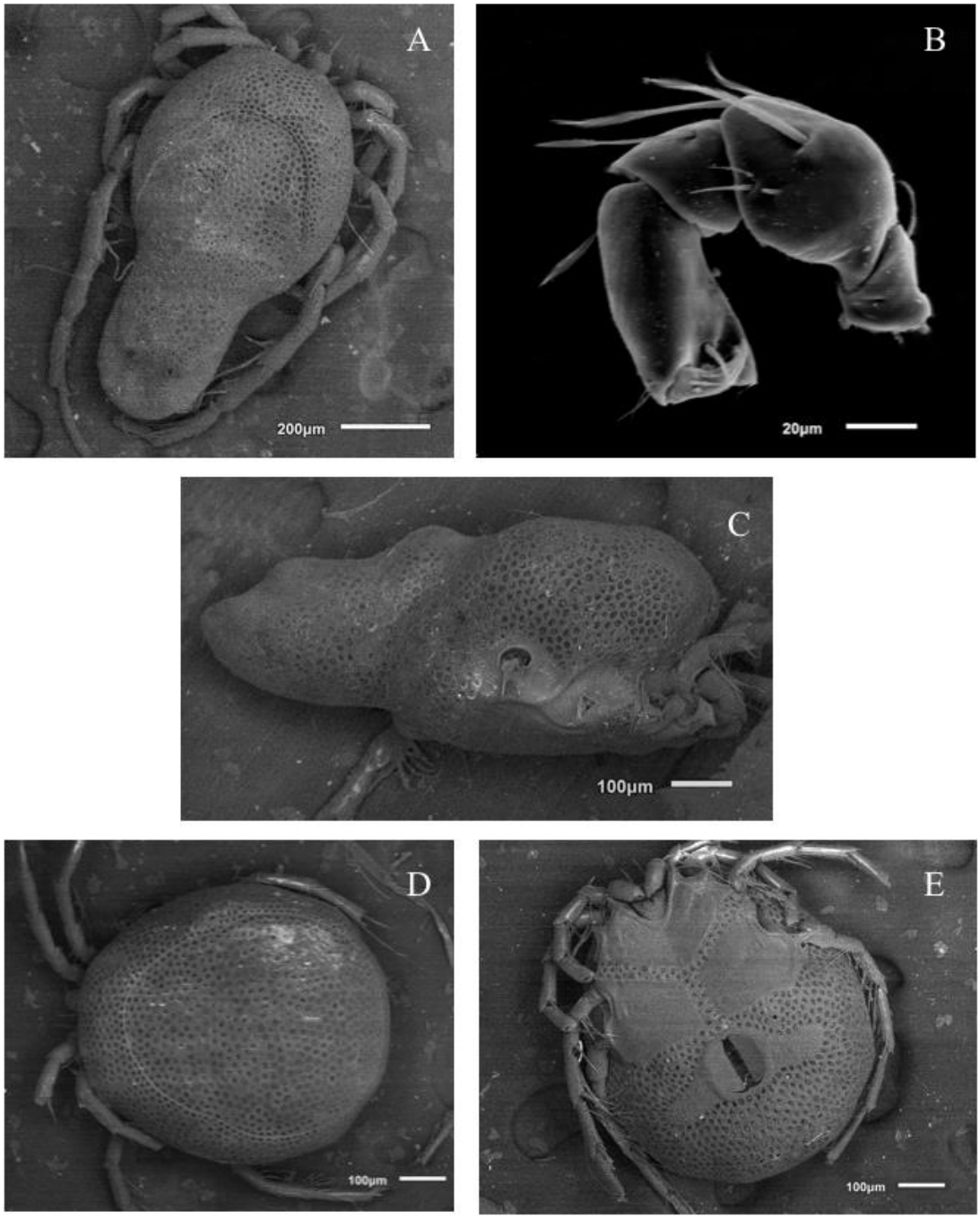

Figure 10. ***Arrenurus (Megaluracarus) ecosur*** **n. sp.** SEM micrograph of **n. sp.** Male**.** A – dorsal view; B – palp; C – lateral view; Female. D – dorsal view; E – ventral view. The sequence of this specimen, recovered after DNA extraction, is represented by the BIN ACX8463.

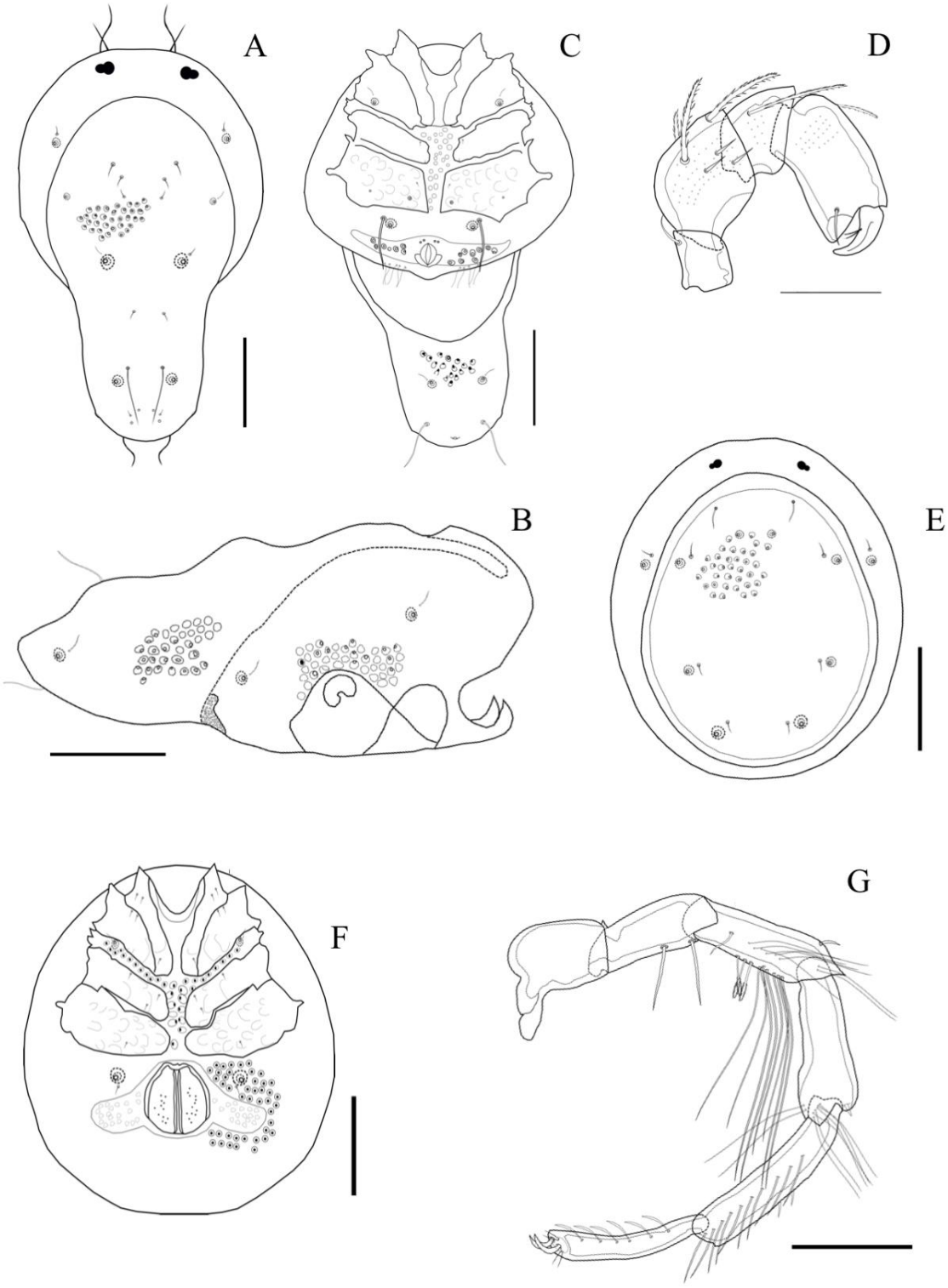

Figure 11. ***Arrenurus (Megaluracarus) ecosur*** **n. sp.** Male. A – dorsal view; B – lateral view; C – ventral view; D – palp, medial view; G – IV-Leg, lateral view. Female. E – dorsal view; F – ventral view. Scale bars: A, B, C, E, F = 200 μm, D = 50 μm, G = 100 μm.

***Arrenurus (Megaluracarus) beatrizae* n. sp.**

(Figures 12, 13)

Holotype: One male from Ramonal wetland, Quintana Roo (Access Number: ECO-CH-Z-XXXX), 19º 23´31´´N -82º 37´27´´W, emergent vegetation, April 14, 2019. Coll. L. Montes and T. Goldschmidt.

Paratypes: Three males, one with the same data as the holotype (Access Number: ECO-CH-Z-XXXX), the other two from San Pedrito lagoon, Pantanos de Centla, Tabasco (Access Number: AAL00273, AAL00274), 18º 21´58.7'' N, -92º 36´03. 6´´O, February 6, 2002. Coll. M. Ramírez-Sánchez.

Diagnosis. Characteristic short cauda with two pairs of lateral notches, tips of Cx-II significantly protruding beyond the anterior margin of the idiosoma, P2 presents a spine-like seta on postero-lateral position, P3 presents a long-pinnate seta located medially.

Description. MALE**:** Idiosoma 640 L, 512 W, dark blue with whitish cauda. Dorsal shield 581 L, 423 W. Dorsal furrow complete, passing ventrally at base of the cauda. Cauda is short 187 L and 285 W, bearing one medial and two pairs of lateral notches (Figure 12A, 13C). The anterior part of the idiosoma is wide and with a slight constriction at the base of the cauda. Dgl-2 and Dgl-3 are close to each other. Dgl-4 is located at the end of the cauda on small humps. The anterior coxal group with complete suture lines, Cx-III and Cx-IV, separated with an incomplete suture line. Tips of Cx-II significantly protrude beyond the idiosoma's anterior margin (Figure 13A). Cxgl-1 is located posteromedially in the margin of Cx-I. Apodemes of Cx-IV protrude slightly beyond the lateral part of the idiosoma. Cxgl-2 with an associated seta posteriorly to Cx-IV (Figure 13A). Genital field 315 W, gonopore 69 L and 27 W. Dorsal L of palpal segments: P1 27; P2 47; P3 41; P4 58; P5 33, P3 with a long-pinnate seta located medially (Figure 13B). Dorsal L of fourth leg segments: IV-Leg-3 104, IV-Leg-4 126, IV-Leg-5 119, IV-Leg-6 116, IV-Leg 4-5, with numerous swimming setae and lateral spine-like setae (seven on IV- Leg 4 and five on IV- Leg 5) (Figure 13D).

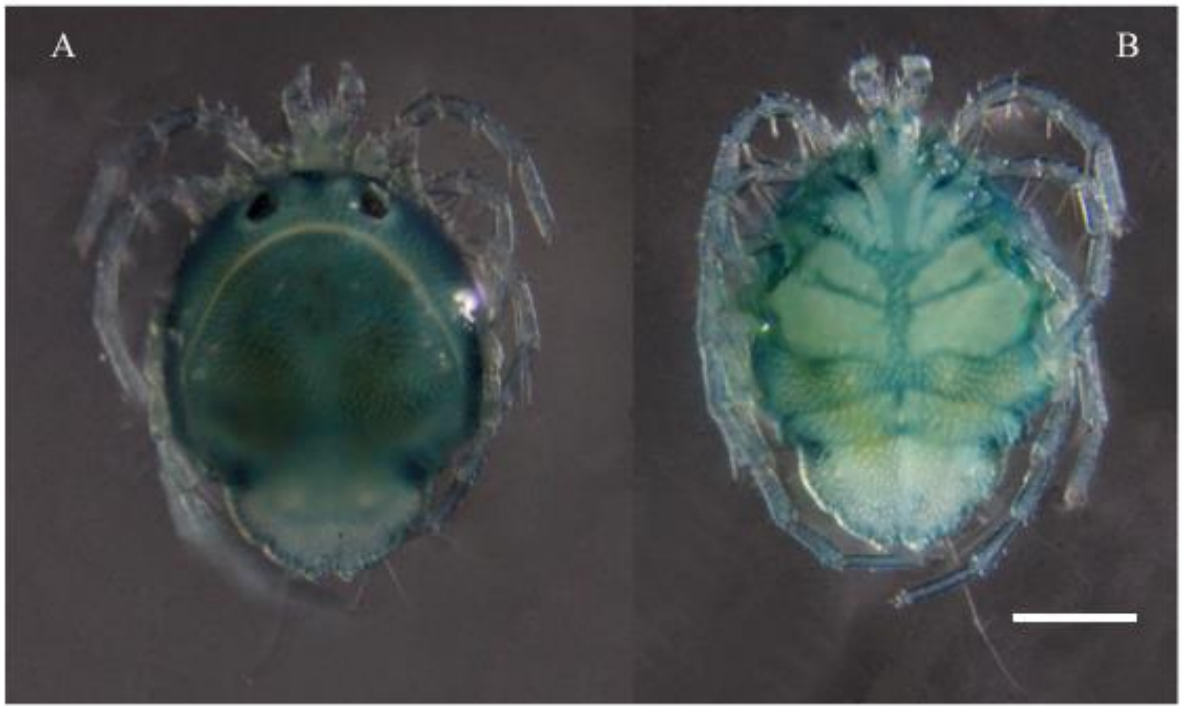

Figure 12. ***Arrenurus (Megaluracarus) beatrizae* n. sp.** Male. A – dorsal view; B – lateral view. Scale bar = 200 μm.

Etymology. This species is named after Beatriz Rosso de Ferradás for her invaluable contributions to water mite acarology in south America.

Discussion. This species belongs to the subgenus *Megaluracarus.* However, the cauda is relatively short compared with other members of the subgenus. The short cauda is a particular characteristic only share by *A. olmeca* Ramírez-Sánchez & Rivas, 2013 from Mexico and *A amazonicus* Viets, 1954 from Brazil. However, both *A. olmeca* and *A. amazonicus* present a patch of spatulate setae on the medial side of P2 while *A. beatrizae* exhibits only one long-pinnate seta. Additionally, the cauda posterior margin in both *A. olmeca* and *A. amazonicus* is smooth. Finally, the number of swimming setae on Leg-4 is reduced in *A. olmeca* compared with ***A. beatrizae* n. sp.**

Distribution. So far only known from el Ramonal, Quintana Roo and San Pedrito lagoon, Tabasco.

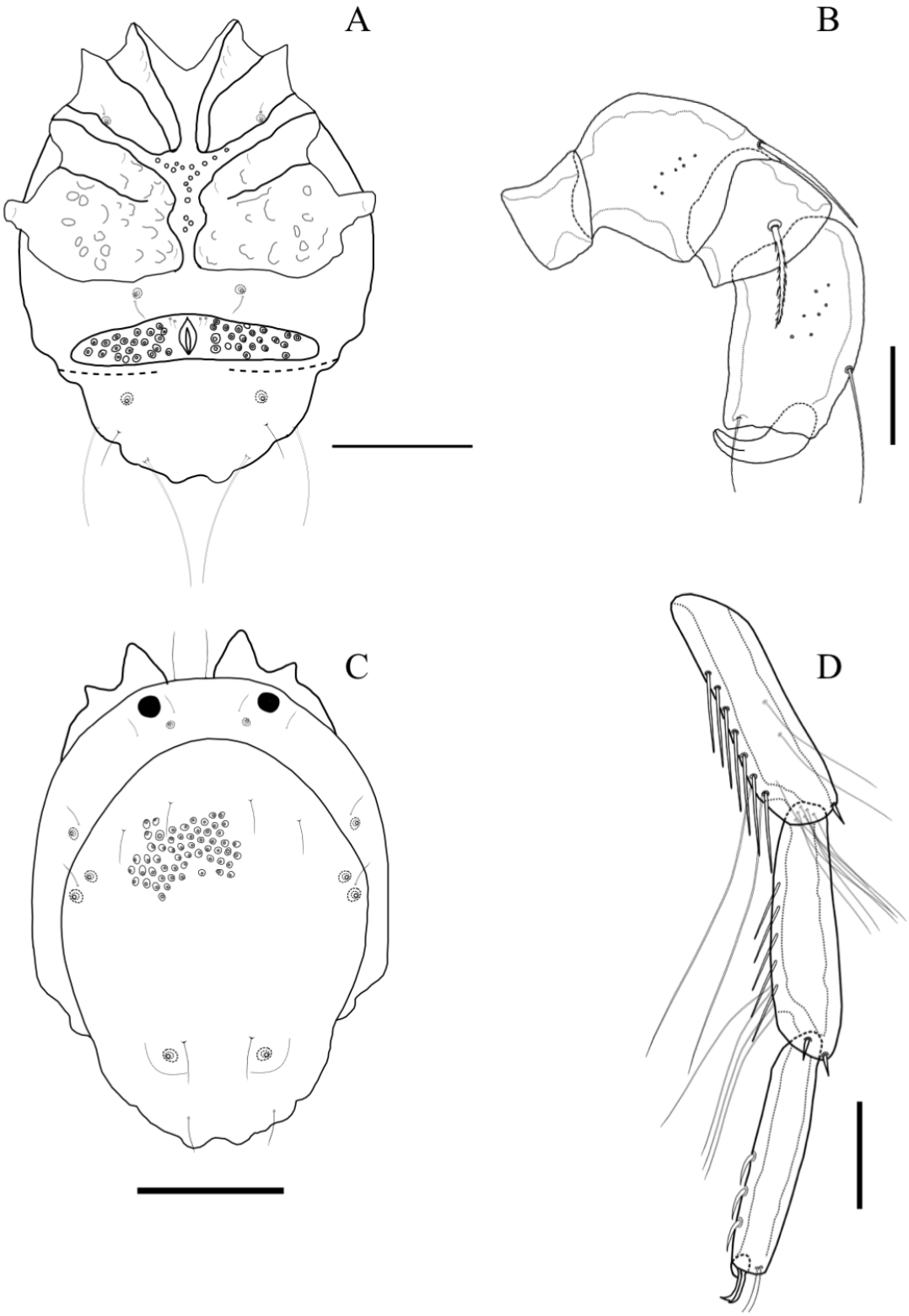

Figure 13. ***Arrenurus (Megaluracarus) beatrizae*** **n. sp.** Male. A – ventral view; B – palp, medial view; C – dorsal view; D – IV-Leg, distal segments. Scale bars: A, C = 200 μm, B = 30 μm, D = 50 μm.

Subgenus *Dadayella* Koenike, 1907.

***Arrenurus (Dadayella) cristinae* n. sp.**

Figures (14 −16)

Holotype: Male from Ramonal wetland, Quintana Roo (Access Number: ECO-CH-Z-XXXX), 19º 23´31´´N, -82º 37´27´´W; emergent vegetation, April 14, 2019. Coll. L Montes and T. Goldschmidt.

Paratypes: One male and two females. Same data as holotype (Access Number: ECO-CH-Z-XXXX).

Diagnosis. Male cauda with two falcate setae located posterolaterally, P2 medially with three simple setae and one pinnate seta on the anterolateral part.

Description. MALE: Idiosoma 364 L and 295 W, uniformly dark blue (Figure 15A). Dorsal furrow incomplete. Dorsal shield 305 L and 207 W, short and relatively square cauda, 49 L. Dgl-4 anteriorly located on the cauda with the associated setae located on small humps and posteriorly in the idiosoma, with two falcate setae on the posterolateral part of the cauda (Figure 16A). Coxae is occupying two-thirds of the ventral region, suture lines complete. CxI-III is diagonally elongated. Cxgl-2 between Cx-II and Cx-IV. Posteriorly to Cx-IV, located the Cxgl-2 (Figure 16B). Genital field 246 W elongated almost reaching the sides of the ventral area, gonopore 59 L and 14 W. Dorsal L of palpal segments L: P1 30; P2 58; P3 38; P4 63; P5 30. P2 with three simple setae medially located and one pinnate seta on the anterolateral part (Figure 16C). L of fourth leg segments: IV-Leg-3 63, IV-Leg-4 73, IV-Leg-5 100, IV-Leg-6 101, IV-Leg-5 with one pinnate seta posteromedially located and four spine-like setae along the dorsal medially surface (Figure 16E).

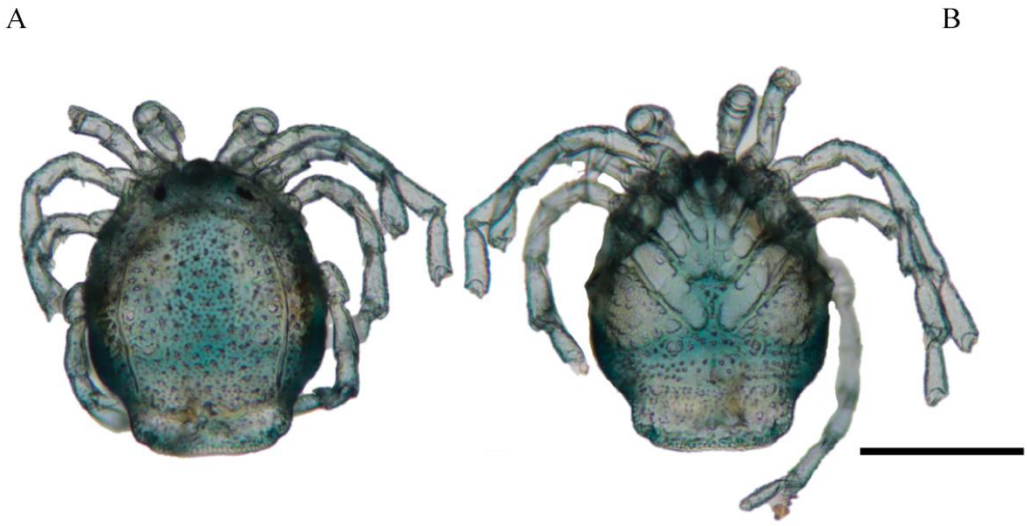

Figure 14. ***Arrenurus (Dadayella) cristinae* n. sp.** Male. A – dorsal view; B – ventral view. Scale bar = 200 μm. The sequence of this specimen, recovered after DNA extraction, is represented by the BIN AEA7842.

FEMALE: Idiosoma 522 L and 483 W, uniformly dark blue (Figure 15), dorsal shield oval, 463 L and 384 W (dorsal furrow complete), with three pairs of glandularia. Dgl-1 (on the ventral plate) is close to Dgl-2 (on the dorsal plate). Dgl-3 setae are located posteriorly and far apart from their respective glandularia (Figure 16D). With complete suture lines, coxae occupy half of the ventral area, Cx-I, and Cx-II, elongated and extended diagonally. Cx-III and Cx-IV separated, Cx-III elongated and diagonally located, Cx-IV triangular. Cxgl-1 is located between Cx-II and Cx-III. Genital field 335 W, wing-shaped and with numerous associated acetabula, gonopore 118 L and 112 W. Cxgl-2 between genital area and Cx-IV (Figure 16F).

Figure 15. ***Arrenurus (Dadayella) cristinae* n. sp.** Female. A – dorsal view; B – ventral view. Scale bar = 200 μm. The sequence of this specimen, recovered after DNA extraction, is represented by the BIN AEA7842. The difference in color is due to the DNA extraction process.

Consensus sequence:
ACTCTTTATTTTGCCTTTGGATTCTGATCAGGTATGGTAGGTGCAAGATT
AAGAAGACTAATTCGCTTAGAATTAGGACAACCAGGGAGACTCTTAGG
GAGAGACCAAATTTACAACACAATCGTAACAGCTCATGCTTTTATCATA
ATCTTTTTTATAGTTATACCTATTATAATTGGAGGTTTCGGAAACTGACT
AGTTCCTCTTATACTAGCAGCTCCAGATATGGCATTCCCACGAATAAAC
AATATAAGATTTTGACTTCTTCCCCCAGCTTTAATTCTCCTTCTATCTAG
ATCTCTCTCCTCAACAGGAGCAGGAACAGGGTGAACAGTATATCCNCCA
CTTTCAAGTAACATTGCACATGGAGGACCTTCAGTTGACATAGCAATCT
TTTCCCTCCATTTAGCAGGAGTCTCATCAATTCTAGGTGCTATCAATTTC
TTAGCTACAACCATCAATATAAAACCAAAATATATAAAATATGATCGTA
TTCCTCTATTTGTCATTTCAATTTTCATCACAGTTATTCTCCTTCTCCTAT
CTTTACCAGTCTTAGCAGGAGCCATTACCATACTTTTAACTGACCGAAA
CTTTAATACATCATTTTTTGATCCAGCTGGAGGAGGAGACCCAATTCTA
TATCAA

Etymology. This species is named after Cristina Cramer Hemkes, for her invaluable contributions to water mite acarology in Mexico.

Discussion. The present species belong to the *Dadayella* subgenus, characterized by males with a small or undifferentiated cauda, with an incomplete dorsal furrow, and P2 with a simple chaetotaxy. ***A. (Dadayella) cristinae* n. sp.** is similar to *A.*

veracruzensis Cramer & Cook, 1992 in the shape and size of the idiosoma, particularly in the quadrangular silhouette of the cauda. The female of *A. veracruzensis* is similar to the new species. However, the chaetotaxy of P2 is quite different ***A. cristinae* n. sp.** presents three simple medial setae and a little pinnate seta in the anterior-lateral part while *A. veracruzensis* presents four medial spine-like setae. Additionally, ***A. cristinae* n. sp.** presents two falcate setae on the postero-lateral part of the cauda, which are absent in *A. veracruzensis*. Most of *the Dadayella* species described are known from females, making comparisons difficult due to their scarce morphological variation. It was possible for ***A. cristinae* n. sp.** to obtain the DNA barcode with the BIN AEA7842. Therefore, we could undoubtedly assign the female to the respective male (Figure 2). These data represent the first sequences obtained for this subgenus.

Distribution. So far only known from the type locality **(**Ramonal, Quintana Roo).

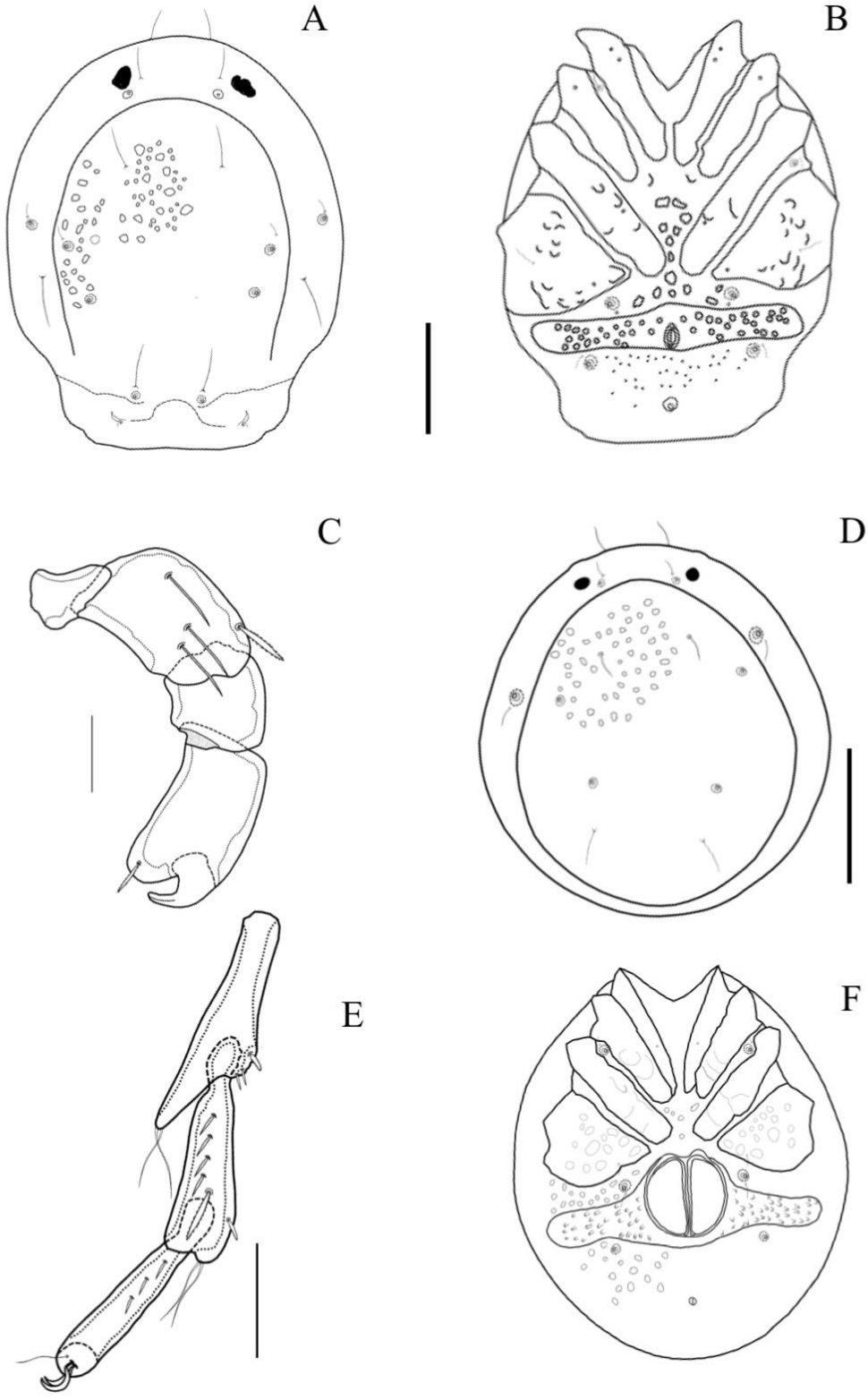

Figure 16. ***Arrenurus (Dadayella) cristinae* n. sp.** Male. A – dorsal view; B – ventral view; C – palp; E – 3-6 IV-Leg. Female. D – dorsal view; F – ventral view. Scale bars: A, B = 100 μm, C = 30 μm, E = 50 μm, D, F = 150 μm.

4. General Discussion

With these new records and species descriptions, the list of Arrenurids from Mexico increases from 37 to 42. The subgenus *Megaluracarus* is the richest in species with 26 known species (as well four of the new species described in the present paper belong to this subgenus). This figure is followed by subgenera *Arrenurus* and *Dadayella* with six species each. The subgenera with fewer representatives are *Truncaturus* and *Arrhenuropsis* with only three and one species, respectively. The case of *Arrenurus (?) nayaritensis* is particular, and the relationships of this species will not be known until the male is described [3].

According to the checklist (Table 2), only five species have a continuous distribution between the USA and Mexico, one between Costa Rica and Mexico, and one with a more extensive range of distribution in the Neotropics and the Caribbean islands, *Arrenurus valencius*, known from Venezuela, Cuba, Haití, Guatemala and Mexico.

The new record of *Arrenurus marshallae* from Mexico is shared with Canada and USA. The remaining species exhibit a restricted distribution to one or two localities (at the present stage of knowledge), and the new records of *Arrenurus colitus* and A. (?*Arrhenuropsis) mexicanus* previously known from Tamaulipas state are now extending the known distribution of these species to Quintana Roo state.

The available molecular information also supports the species' diagnoses. Comparing all available sequences of genus *Arrenurus* from the BOLD database (1111 sequences, see Supplementary information) discriminated the sequences from Mexico, indicating a restricted distribution as only two putative species are shared with Canada. This pattern is repeated in the rest of the tree, where other putative species are recorded in only one country or a maximum of two. However, these inferences are strongly biased due to the few sequences and countries with molecular information available. However, this comparison supports our previous conclusion about the new species presented here.

All the Arrenurids currently known from Mexico have been reported for 14 of the 32 states in the country. From these, Tamaulipas heads the listing with six species, while Mexico state, Michoacán, and Yucatán have only one species recorded. For 18 entities, particularly in the north, there is no information. As stated in the introduction, due to the geographical position of Mexico and its great variety of ecosystems (many unique in the world, e.g., Bacalar lagoon in the tropics,

Cuatrocienegas in the semi-desert), a great diversity of water mites should be expected.

Once we know the diversity of mites, we can make progress to understand their ecological significance and value as water quality indicators.

Author Contributions: Conceptualization, L.M.O; methodology, M.E.G, L.M.O; software, M.E.G, L.M.O; validation, M.E.G and M.M.R.S; formal analysis, L.M.O; investigation, L.M.O; resources, M.E.G; data curation, L.M.O; writing—original draft preparation, L.M.O; writing—review and editing, M.E.G, M.M.R.S and L.M.O; visualization, M.E.G, M.M.R.S and L.M.O; supervision, M.E.G and M.M.R.S; project administration, M.E.G; funding acquisition, M.E.G. All authors have read and agreed to the published version of the manuscript.

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Data Availability Statement: public dataset DS_XXXX; DOI: XXXX

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Goldschmidt, who accompanied and guided LMO during the field collection, as well as for his valuable comments that significantly improved this manuscript.

Conflicts of Interest: The authors declare no conflict of interest

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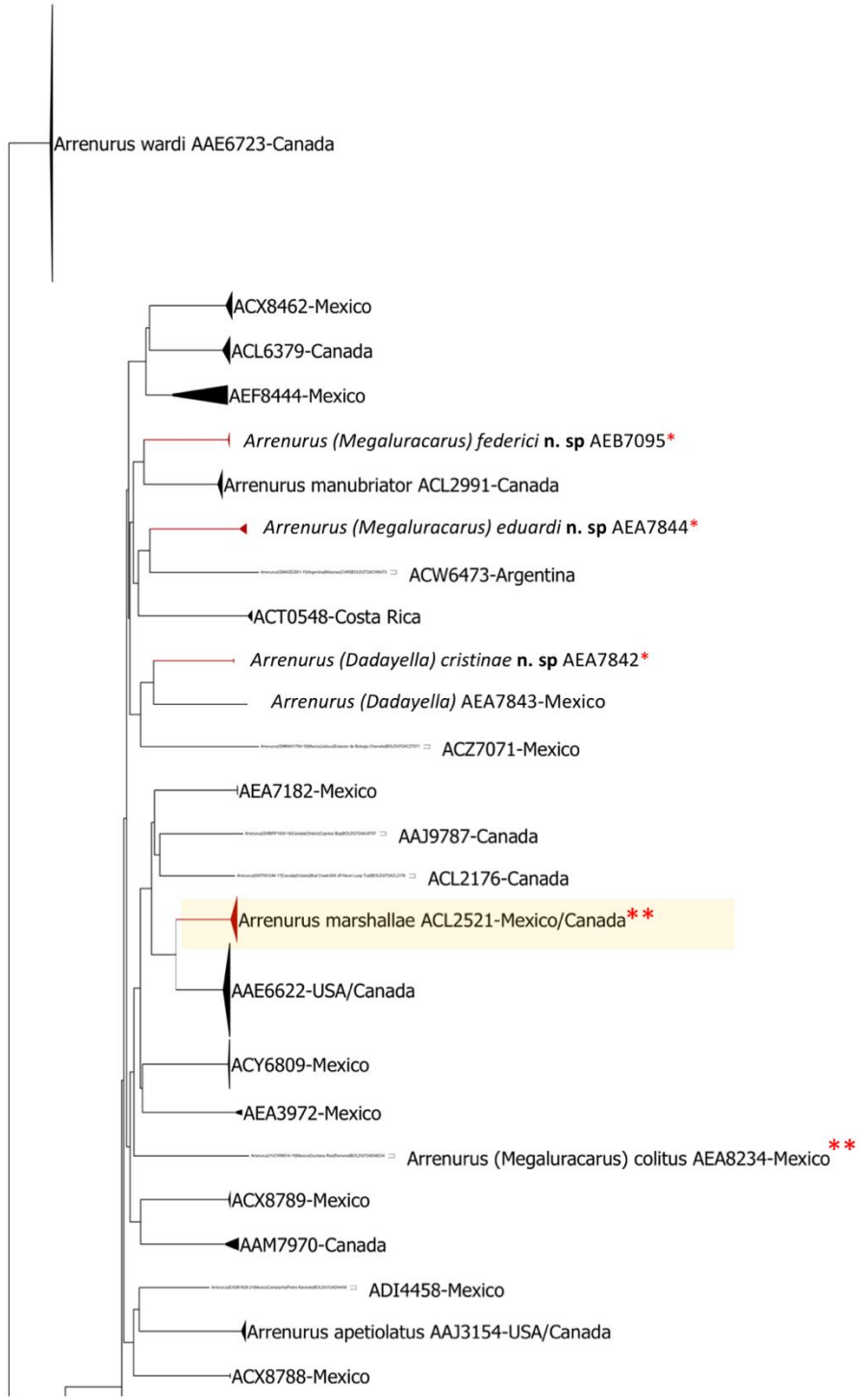
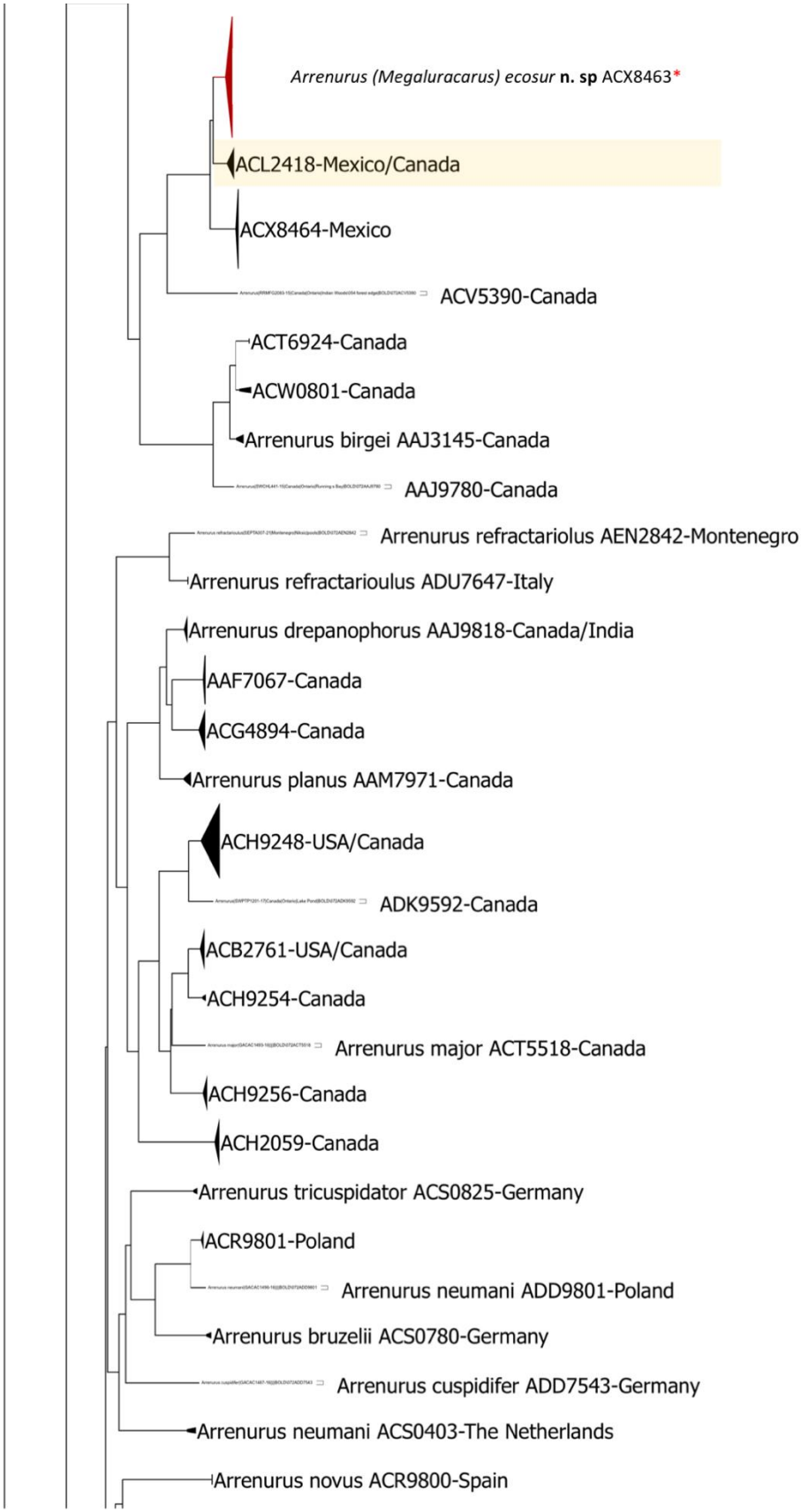
MDPI

Supplementary material. NJ compressed tree based on worldwide COI sequences of *Arrenurus* (In total 1111 sequences, representing 148 putative species). * New species and ** New records. Shared species between Mexico and other countries are highlighted.

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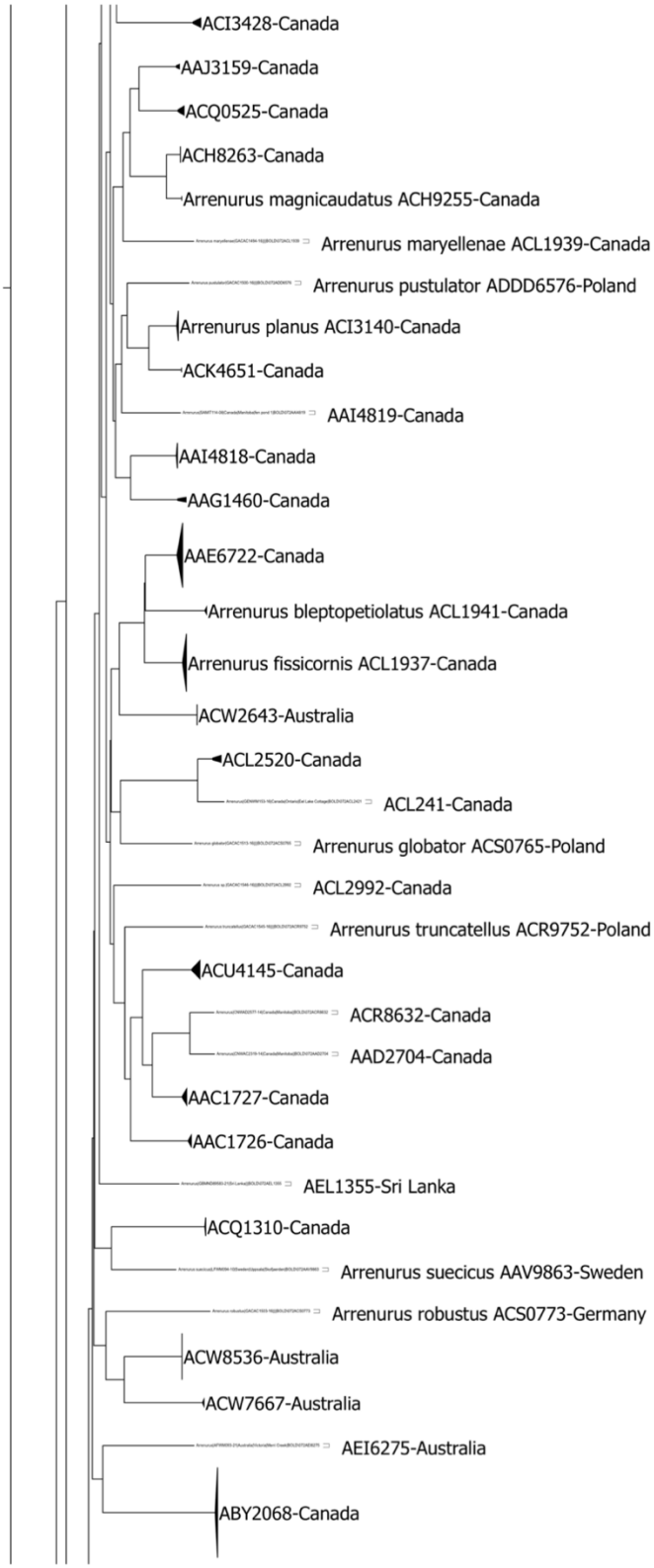
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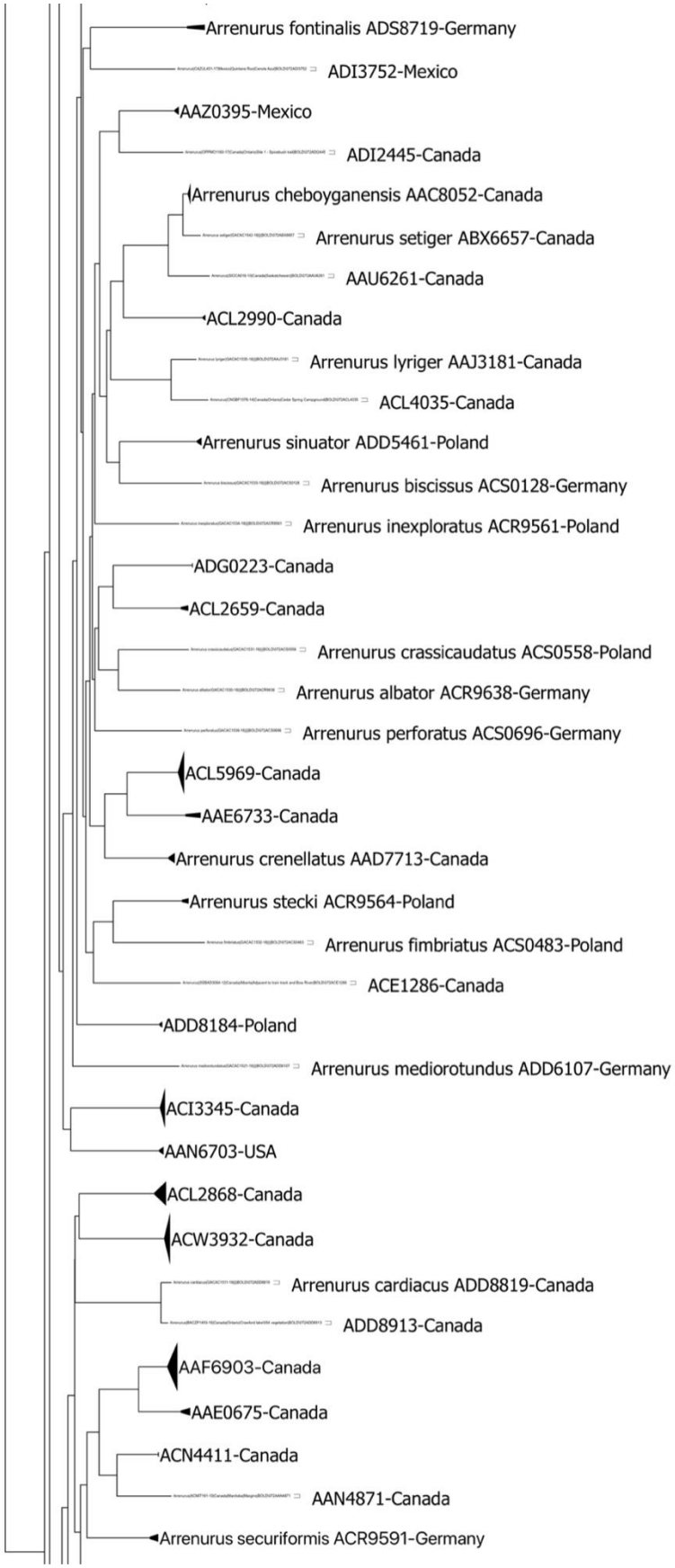
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Arrenurus fimbriatus ACS0483-Poland
ACE1286-Canada
ADD8184-Poland
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ACW3932-Canada
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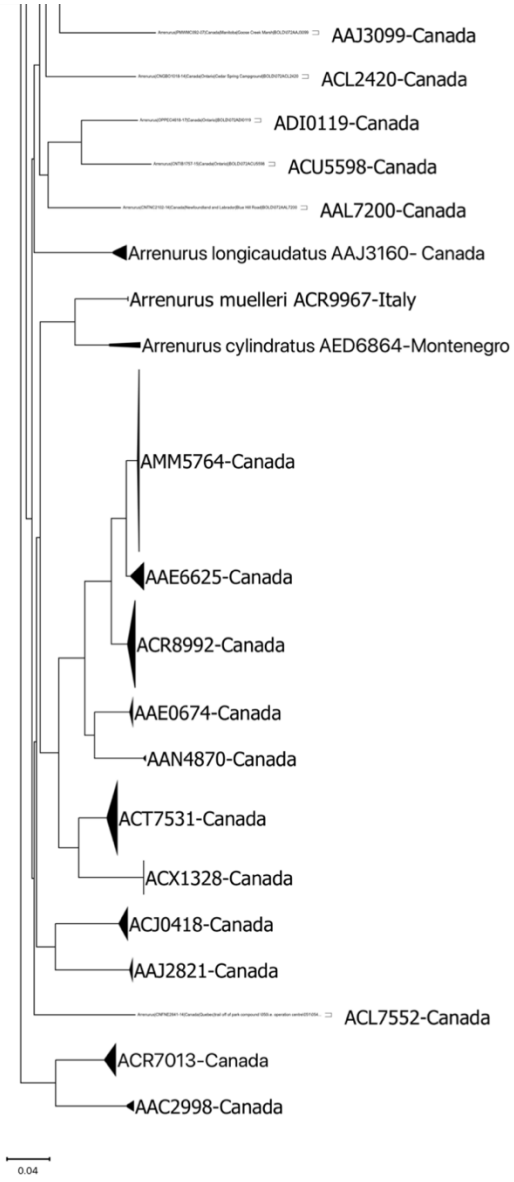
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Arrenurus muelleri ACR9967-Italy
Arrenurus cylindratus AED6864-Montenegro
AMM5764-Canada
AAE6625-Canada
ACR8992-Canada
AAE0674-Canada
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CAPITULO IV

A new species of *Litarachna* (Acari, Hydrachnidia, Pontarachnidae) from Corozal bay (Belize), described based upon morphology and DNA barcodes

Publicado en *Acarologia* **2021**, *61*(3), 602-613; https://doi.org/10.24349/r7no-Ludg

Responde parcialmente al objetivo específico iii

Title: A new species of *Litarachna* (Acari, Hydrachnidia, Pontarachnidae) from Corozal bay (Belize), described based upon morphology and DNA barcodes

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Abstract

A new species of the marine water mite family Pontarachnidae (*Litarachna belicensis* sp. nov.) is described from an estuarine system from Belize. The description is based on morphology, Scanning Electronic Microscope (SEM) images and cytochrome oxidase I (COI) sequences, known as the DNA barcodes. For the first time the DNA barcodes are used for a species description in this group.

Keywords

Scanning Electron Microscopy; Integrative taxonomy; DNA; estuary; Chetumal bay.

Introduction

The Pontarachnidae (known in two genera, *Pontarachna* Philippi, 1840 and *Litarachna* Walter, 1925) is the only water mite family with species occurring in marine habitats. Overall 23 species of the genus *Litarachna* have been recorded (Chatterjee, Schizas and Pešić 2019) mainly from the intertidal zone of the tropical and temperate Pacific, Atlantic, Indian Ocean (Smit 2002; Pešić, Chatterjee and Schizas 2012; Pešić, Durucan and Zawal 2019; Chatterjee, Schizas and Pešić 2019). Only four species are recorded from the tropical west Atlantic Ocean, three of them – *L. caribica, L. degiustii* and *L. lopezae* – for the Caribbean sea (Pešić, Chatterjee and Schizas 2012; Pešić, Chatterjee, Tapas and Schizas 2008; Chatterjee, Schizas and Pešić 2019). Until now, there is a great lack on the data about biology and ecology for this group. For example, some species have been recorded in estuarine systems as well as in freshwater as *Litarachna brasiliensis* Smit, 2007, others have been found in shallow coastal waters as well as at depths of up to 70 m (Pešić et al. 2014), and there is as well one documented planktonic species: *Litarachna kamui* Uchida, 1935. The same happens with respect to distributional patterns and endemism, particularly in the marine provinces corresponding to the American continent (Chatterjee, Schizas and Pešić 2019).

In this paper, a new species of *Litarachna* is described from the Corozal bay, an estuarine system in Belize, in a region corresponding to the Yucatan Peninsula biogeographic zone. This species represents the second record of a planktonic pontarachnid (*Litarachna*) mite. As well it is the first record for this country, and we also provide here the first barcodes in a description for a member of this family.

Materials and methods

The specimens in the present study were collected in open water from Corozal Bay, a binational embayment shared by Mexico and Belize, forming part of Chetumal Bay (Figure 1). A standard plankton net of 0.5m mouth diameter, and a mesh size of 0.333 mm was used to perform a

vertical tow during a night sampling survey. The associated data in the collection site are presented in Table 1. Samples were sieved, washed and fixed using 96% ethanol and stored at -18 °C for seven days (Elías-Gutiérrez et al. 2018).

The mites were sorted under a stereomicroscope. One male and one female were selected for SEM images in a Jeol JSM-SM-6010LA, located at El Colegio de la Frontera Sur, Chetumal Unit. Another five individuals were used for molecular analyses using a non-destructive DNA extraction method (Porco et al. 2010). After the process, we recovered three specimens, and they were mounted in glycerine jelly. All specimens preparations were deposited in the Reference Collection of Zooplankton at El Colegio de la Frontera Sur (ECOSUR, Chetumal, Mexico).

Molecular analysis

DNA extraction was carried out using a standard glass fiber method (Ivanova et al. 2006) modified as Porco et. Al (2010) suggested for voucher recovery. Specimens were recovered after the lysis step from the glass fiber filter plates or the 96 well original plates and preserved in Koenike solution.

After the DNA extraction, the PCR mixtures contained a final volume of 12.5 μL, including 2 μL of Hyclone ultra-pure water, 6.25 μL of 10% trehalose (previously prepared: 5 g D-(+)-trehalose dehydrate, in 50 ml of total volume of molecular grade ddH_2O), 1.25 μL of 10X PCR buffer, 0.625 μL of $MgCl_2$ (50 mM), 0.0625 μL of dNTP (10 mM), 0.125μL of each primer (10μM), 0.06 μL of PlatinumTaq DNA polymerase and 2 μL of DNA template. All specimens were amplified with the Zooplankton primers (ZplankF1_t1 and ZplankR1_t1, see Prosser et al., 2013 for details). The reactions were cycled at 94 °C for 1 min, followed by five cycles of 94 °C for 40 sec, 45 °C for 40 sec and 72 °C for 1 min, followed by 35 cycles of 94 °C for 40 sec, 51 °C for 40 sec and 72 °C for 1 min, with a final extension of 72 °C for 5 minutes. PCR

products were visualized on a 2% agarose gels (E-Gel 96 Invitrogen), and positive PCR products were selected for sequencing bidirectionally.

Sequences were edited using Codon Code v. 3.0.1 and uploaded to BOLD and are available in the public dataset DS-LITBEL (www.boldsystems.org).

A maximum likelihood analysis with 10,000 replicates was conducted using MEGA version X (Kumar et al. 2018); a group of ten sequences from *Atractides* genus were mined from the bold database to root the tree (Table 3); the preference for this genus is based on its belonging to the Hygrobatoidea superfamily, same as the pontarachnid mites, as well they cover the quality requirements of the sequences available in the databases.

The following abbreviations are used:

Morphology body: Cx-I-IV = first to fourth coxae, Cxgl-2 = coxoglandularia 2, Cxgl-4 = coxoglandularia 4, L = length, Vgl = ventroglandularia, Vst = ventral setae, W = width, W-1 = wheel acetabula anterolateral pair, W-2= wheel acetabula anteromedial pair, W-3= wheel acetabula posterior pair.

Appendages (palp, leg): I- to IV-Leg-1-6 = first to sixth segments of leg I to IV, P-1 to P-5 = palp segments 1 to 5.

Molecular: BIN = Barcode Index Number, COI = cytochrome c oxidase subunit I.

All measurements are given in µm.

Results

Systematics

Family Pontarachnidae Koenike, 1910

Genus *Litarachna* Walter, 1925

Litarachna belicensis sp. nov.

(Figures 2-6)

Type series

Holotype: female, Corozal bay, Belize (18°37´27.7´´N 88°28´8.44´´W), 3-5 m with predomination of calcareous rocks and sand, with less presence of sea grass and some spots of fuzzy finger algae (*Batophora oerstedi*), collected from a planktonic sample from 2.8 m depth on 07 may 2019; dissected and slide mounted in glycerin jelly. Paratypes: one male, same collecting data as the holotype; dissected and mounted in a slide with glycerin jelly as mounting medium.

Diagnosis:

Suture between Cx-II and Cx-III incomplete, suture between Cx-III and Cx-IV complete (Figure 6a,6d). Postero-medial apodemes twice the length of postero-lateral apodemes in female as in male. Basal process in P-2 and P-3 (Figure 3b, 5b), in male very long perigenital setae surrounding the genital field in U shape (Figure 5a).

Description:

Female (n=1): Idiosoma L/W 476/357. Anterior coxal group separated medially. Suture lines between Cx-II and Cx-III incomplete; suture lines between Cx-I and Cx-II, as well as between Cx-III and Cx-IV complete. Posterior margin of Cx-IV with two pairs of long apodemes, extending beyond the genital field, postero-lateral apodemes half as long as postero-medial ones (Figures 2, 3, 6a, 6b, 6c)

Genital field L/W 78/47. Pregenital and postgenital sclerite fused, forming a ring around the genital opening. Cxgl-2 and associated seta (sensu Cook, 1974) laying between the genital field and the fourth coxae. Posterior to the genital field a lateral pair of Vgl, and three pairs of wheel-like acetabula (sensu Cook, 1996), W-1 with nine radiating spokes, W-2 with eight and seven radiating spokes (Figures 3, 4).

Posterior to the genital field a pair of platelets bearing two pores. Excretory pore sclerotized in subterminal position.

Palp (Figure 3c) total L 194, dorsal L (% of total L): P-1 27 (14%), P-2 33 (17%), P-3 40 (20%),

P-4 71 (36%), P-5 25 (13%), P-2 and P-3 bearing small ventral projections (Figure 3b). L of I-Leg-3-6: 34, 51, 60, 85; IV-Leg-3-6: 40, 51, 88, 91; III-Leg-5-6 and IV-Leg-5 each with one swimming setae (Figure 3).

One ovigerous female from the collected material contained two eggs with a L/W 170/110 (Figure 8).

Male (n=1): Idiosoma L/W 400/348. As in the female, suture between Cx-II and Cx-III medially incomplete, suture between Cx-I and Cx-II, as well as between Cx-III and Cx-IV complete. Postero-medial apodems twice as long as postero-lateral ones, reaching to posterior end of the genital field (Figure 6d). Between posterior and lateral apodemes Cxgl-4.

Genital field L/W 35/28, genital sclerites forming a complete ring with four pairs of setae (Figure 5c), many long perigenital setae (>95) free in the integument lateral and posterior to the genital field forming a dense U-shaped field (Figure 5b), with less density in the anterior part and considerably increasing density towards the posterior part; in the centre of the perigenital setae, close to the genital opening one pair of tiny wheel-like acetabula (Figure 5c). Genital opening flanked by centrally extended lamellae, posterior to the genital field two pores and two pairs of wheel-like acetabula (sensu Cook, 1996), W-1, W-3 with nine radiating spokes, W-2 with six and four radiating spokes (Figure 5).

DNA sequence (CO1)

Two sequences were obtained, a consensus sequence for this species is:

CTCTATTTTG CTTTAGGAAG ATGATCAGGC ATAATGGGAA CAAGACTTAG 50
AACTTTAATT CGATTAGAAT TAGGTCAACC AGGAGCACTA 100 ATTGGCAATG
AACAAATCTA TAACGTTATC GTAACAGCTC ATGCATTTAT 150 TATAATTTTT
TTCATAGTCA TACCCATAAT AATTGGAGGT TTTGGAAATT 200 GATTAGTTCC
GCTAATAATC AGAGCCCCCG ATATAGCCTT TCCCCGTATA 250 AATAACATAA

GATTCTGACT TTTACCCCCA GCCCTTATCC TTCTTTCAAC 300 AAGATCCATA AGATCAATAG GAGTTGGTAC AGGTTGAACA GTTTACCCTC 350 CCCTCTCAAG AAATTTGGCT CACTCAGGACCA TCCGTTGACT TAACAATCTT 400 CTCTCTCCATT TAGCTGGTAT TTCATCCATC CTTGGGGCCA TCAACTTTAT 450 AGCAACAATT ATAAATATAA AACCTACCCA TATAAAAATG GAACAAGTAC 500 CCCTATTTGT ATGATCAATT TTCATCACAA CCATTCTCCT CCTTCTTTCA 550 CTTCCAGTCT TAGCAGGAGC CATTACTATG CTTTTAACTG ACCGAAACTT 600 CAACACTTCA TTCTTTGATC CAGCCGGTGG AGGTGATCCA ATTTTATACC 650

Etymology

This species is named after the country where it was collected.

Discussion

Litarachna belicensis sp. nov. is quite similar to *L. communis* Walter, 1925 in the complete suture lines between Cx-I and -II as well as Cx-III and -IV and the idiosoma length size (*L. belicensis* 476– *L. communis* 465); the new species differs from *L. communis* in the width of the idiosoma (*L. belicensis* 357 – *L. communis* 430) and the genital field (*L. belicensis* 78/47 – *L. communis* 75/64), as well as the size, shape and width of the postero-medial apodemes (in *L. belicensis* postero-medial apodemes are longer and wider and postero-lateral apodemes are narrow and short and with hook opening inwards while in *L. communis* both postero-medial and -lateral apodemes have similar size and length, with hook opening outwards). *Litarachna belicensis* sp. nov. is similar to *L. degiustii* Cook, 1958 and *L. caribica* Pešić et al., 2008, in the shape and size of postero-medial apodemes. The new species differs from these two in the size of postero-lateral apodemes (strongly reduced in the latter two species) and in the suture lines from the coxal plates (in both *L. caribica* and *L. degiustii* suture lines between Cx-II and Cx-III and between Cx-III and Cx-IV are incomplete).

The mitochondrial information, based on COI (Figure 7), reveals that *L. belicensis* sp. nov represents an independent evolutionary lineage from *L. communis* since it presents a high genetic distance (23% K2P).This result agrees with other studies where different species of *Litarachna* show a big genetic distance (18-30%) (Pešić and Smit 2020; Martin, Dabert and Dabert 2010).

Additionally the bold (www.boldsystems.org) algorithm approach based on the analysis of sequence diversity separates both species (*L. communis* and *L. belicensis* sp. nov) by the assignation of a different BIN (*L. communis* ADD6045 and *L. belicense* sp. nov AEB8019) (Ratnasingham and Hebert 2013). The sum of the morphological and molecular evidence, in addition to the biogeographic location, confirms the identity of *Litarachna belicensis* as a new species.

The integration of molecular information in this study and the future growth of the database with high quality sequences and complete metadata will contribute to the understanding not only of the taxonomic identity of Pontarachnid mites, but also of the taxonomic position of *Litarachna* within the Pontarachnidae family as well as the Pontarachnidae itself within Hydrachnidia, their special biology and ecology together with the distributional patterns and endemism of this enigmatic group.

Acknowledgements

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Captions: Tables and figures

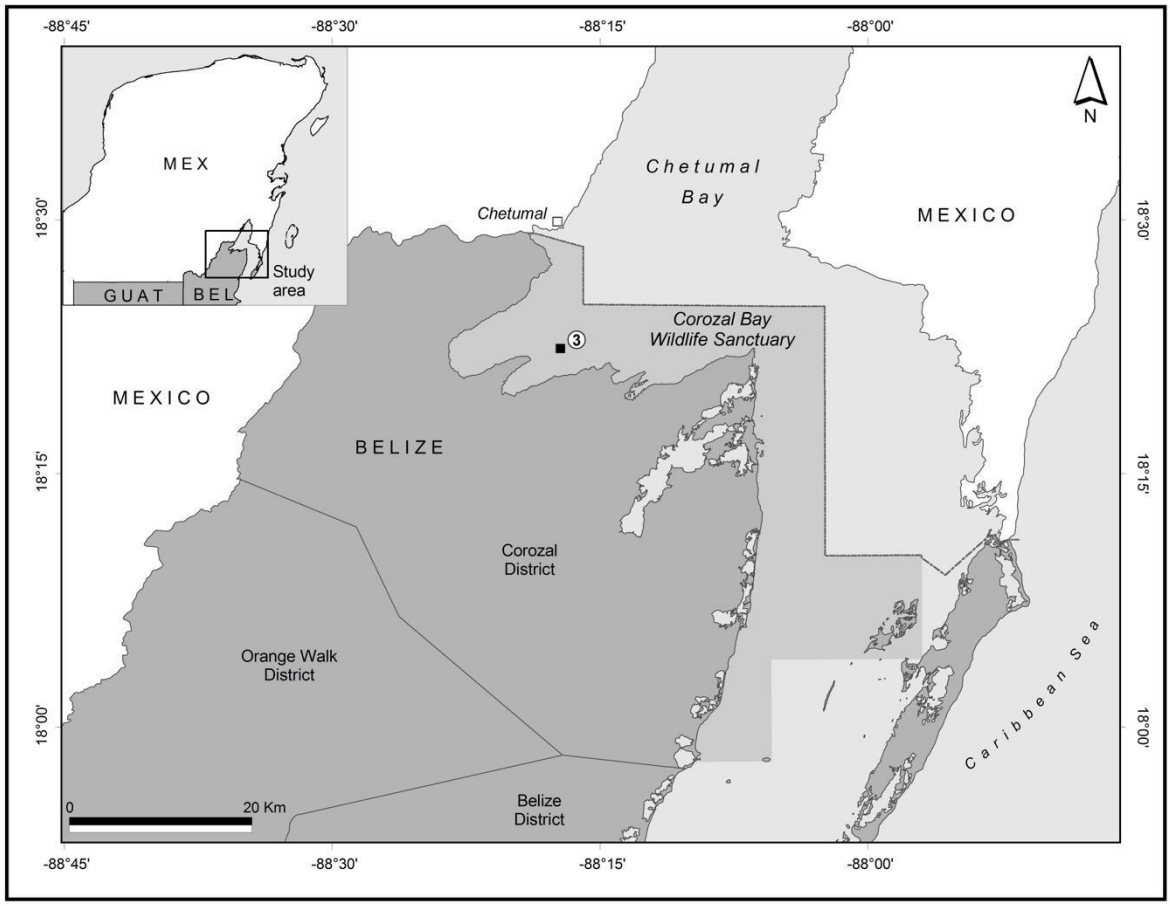

Figure 1. Sample site of *Litarachna belicensis* sp. nov. in Belice Bay.

Table 1. Collection data for the sample site.

Site	**Depth (m)**	**Salinity (ppt)**	**DO%**	**pH**	**Temp (°C)**	**Time in**	**Time out**
Point 3	2.80	20.7	6.71	8.02	26.1	8:46 pm	8:57 pm

Table 3. Specimens sequences used in the molecular study.

Species	**Locality**	**Institution storing**	**Process ID**	**Barcode Index Number (BIN)**

Litarachna comunnis	Marine Litoral Punta Spano, Corsica. (France)	Zoological museum, University of Amsterdam.	NLACA998 NLACA1000	ADD6045
Litarachna belicensis sp. Nov.	Corozal bay (Belize)	El Colegio de la Frontera Sur, unidad Chetumal	YUCWM193 YUCWM192	AEB8019
***Atractides* sp.**	Los Angeles. E.Fork San Gabriel (Estados Unidos)	Stroud Water Research Center.	CFWIA621 CFWIB562 CFWIB563 CFWIB564 CFWIB565	ACE5393
***Atractides* sp.**	Bacalar, Quintana Roo. (México)	El Colegio de la Frontera Sur, unidad Chetumal.	BACWM152 BACWM263 BACWM044 BACWM125	ACX7786

Figure 2. *Litarachna belicensis* sp. nov. Female: a) Dorsal view b) ventral view. Male: c) Dorsal view d) Ventral view. Scalebar = 100 μm

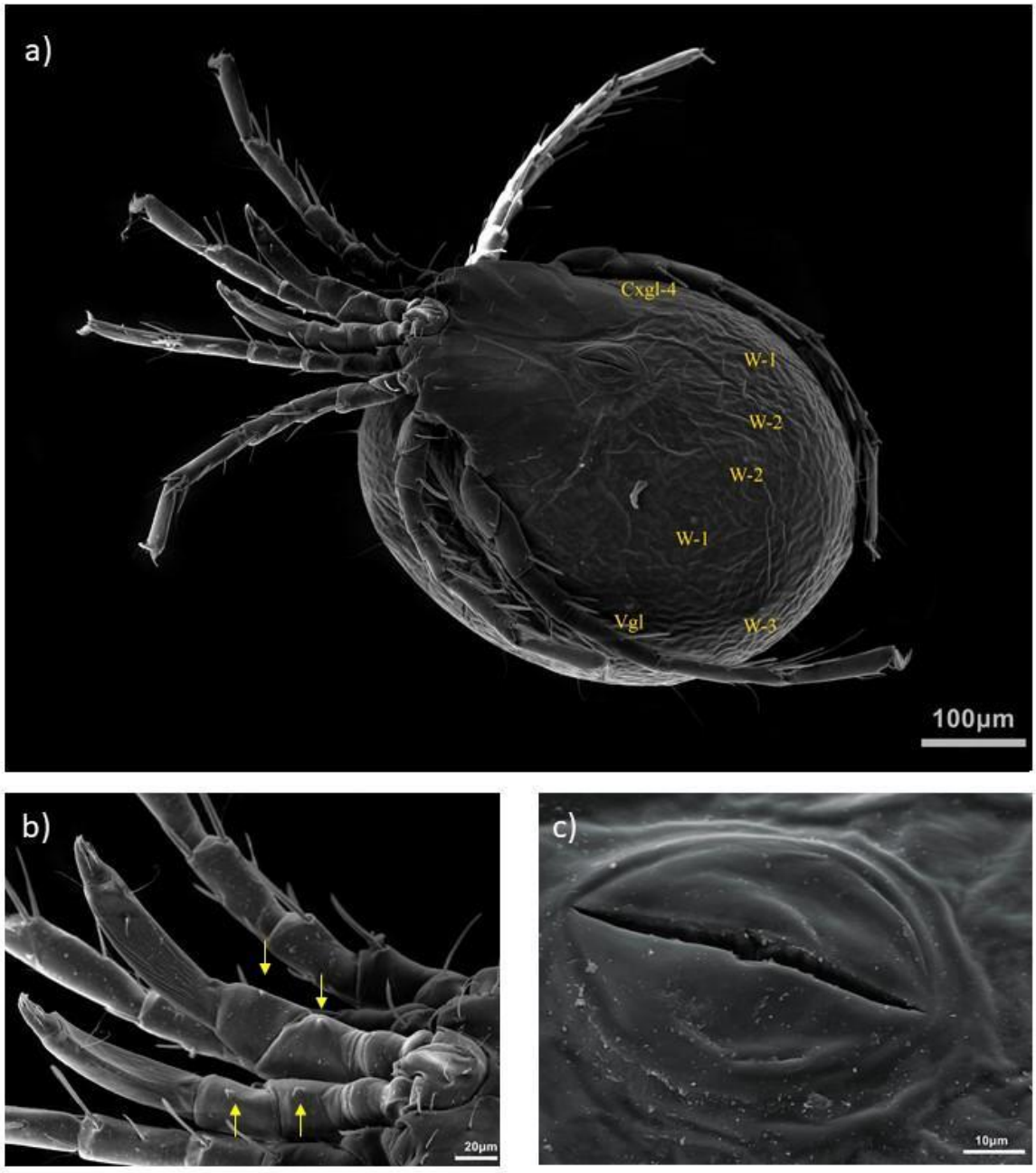

Figure 3. *Litarachna belicensis* a) Female habitus (W-1 wheel acetabula, W-2, W-3) b) palps (arrows point at ventral processes on P-2 and P-3) c) genital field.

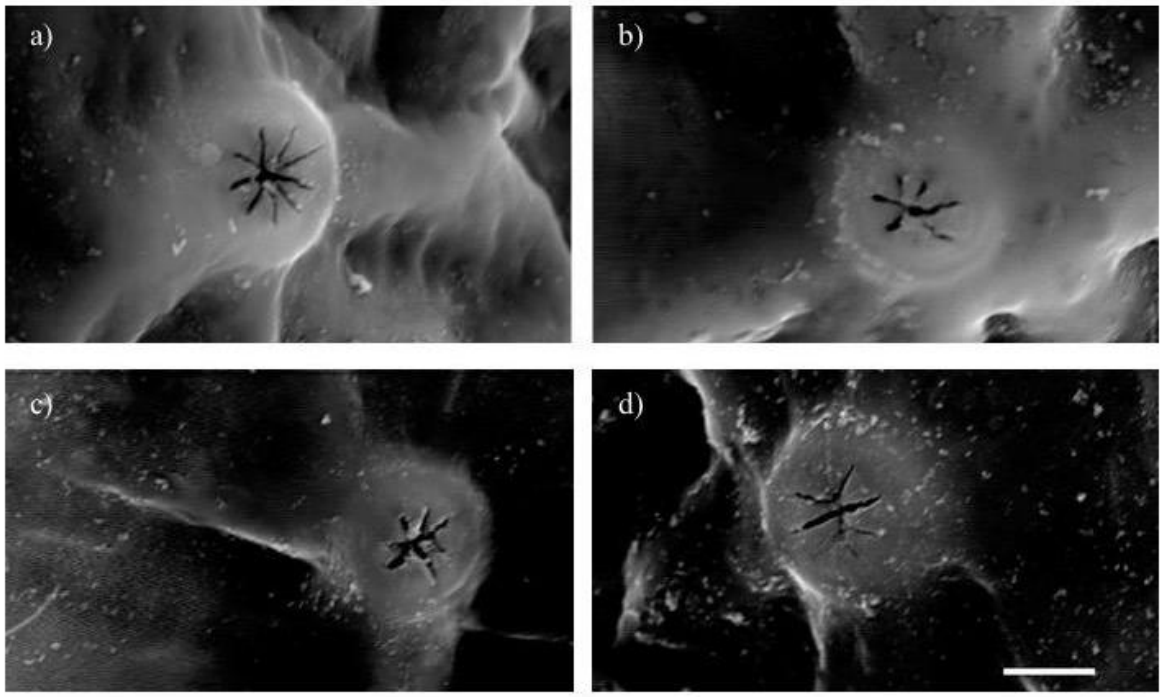

Figure 4. SEM images from wheel acetabula in female of *Litarachna belicensis* sp. nov. a) and d): W-1, b) and c): W-2 Scalebar = 5 µm

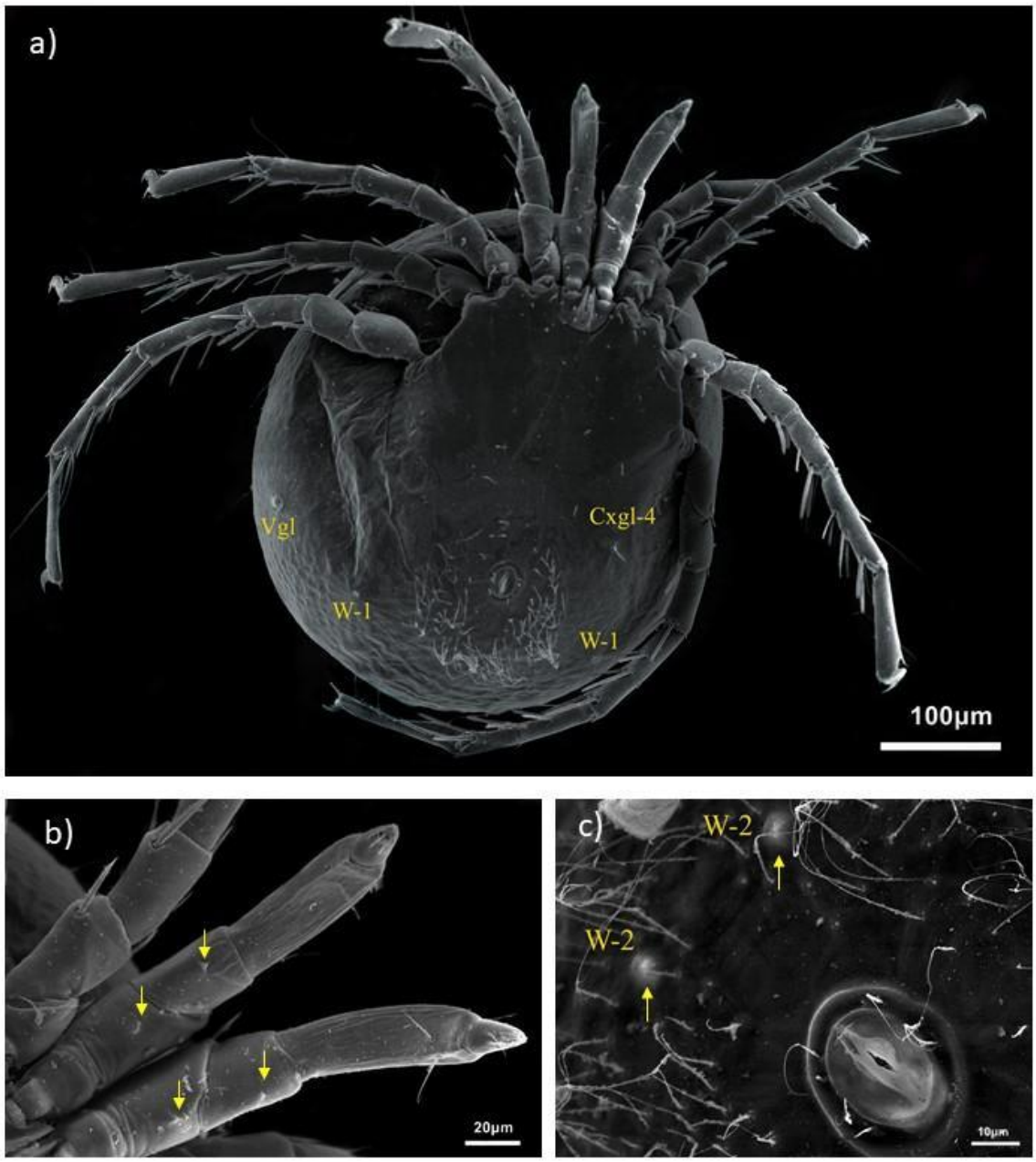

Figure 5. *Litarachna belicensis* sp. nov, male. A) Habitus b) palps (ventral view, arrows point at ventral processes on P-2 and P-3.) c) genital field and area posterior to genital field, including medial pair of wheel-like acetabula (W-2), central part of setal field (note that the image is twisted, the upper left corner oriented to posterior).

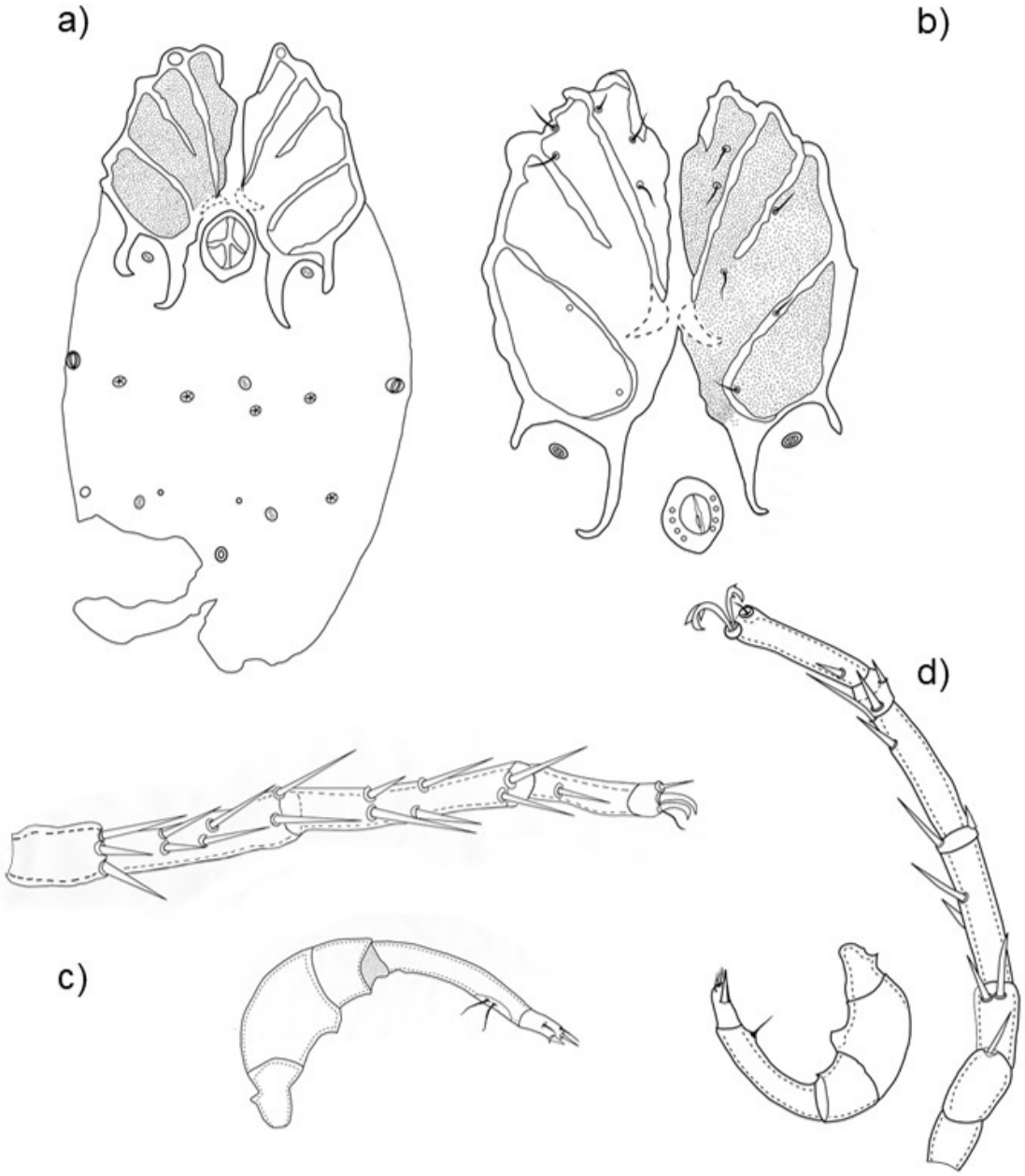

Figure 6. *Litarachna belicensis* sp. nov, female holotype a) idiosoma b) IV leg-3-6 c) palpmale paratype d) idiosoma e) IV leg f) palp.

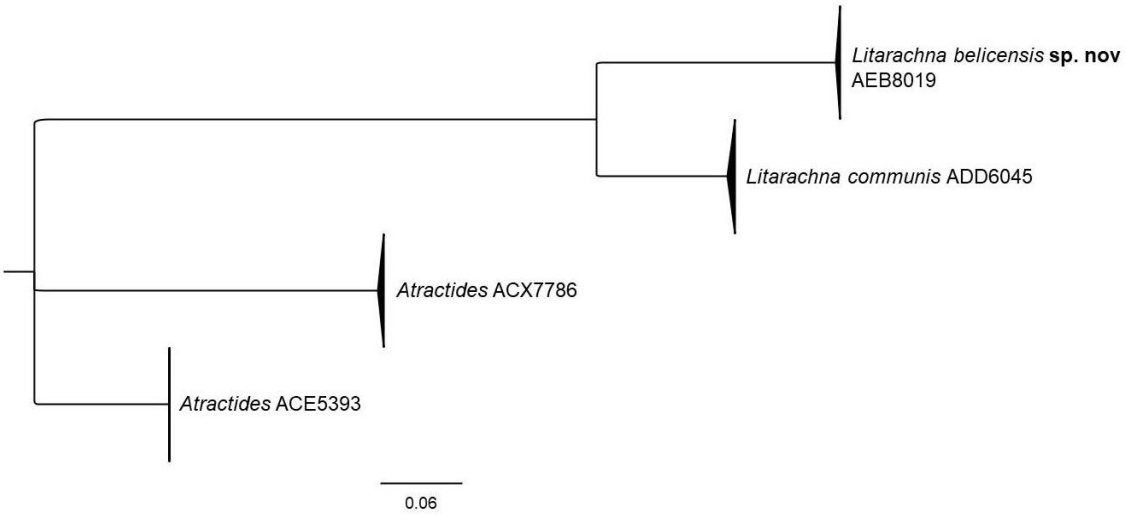

Figure 7. Maximum Likelihood tree, based in COI sequences.

Figure 8. Ovigerous female recovered after DNA extraction, arrows point the eggs. Scalebar = 100 µm.

CAPITULO V

First evidence of parasitation of a *Bosmina* (Cladocera) by a water mite larva in a karst sinkhole, in Quintana Roo (Yucatan Peninsula, Mexico).

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Se presenta este artículo como información adicional derivada de las observaciones de la acarofauna en el Cenote Azul

Title: First evidence of parasitation of a *Bosmina* (Cladocera) by a water mite larva in a karst sinkhole, in Quintana Roo (Yucatan Peninsula, Mexico)

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Abstract

For the first time a parasitic relationship between a water mite larva and a cladocera is found and documented by scanning electron microscope (SEM) imaging. A Unionicolidae larva (cf. *Unionicola*) has been found attached to a *Bosmina tubicen* (Cladocera) collected in a karst sinkhole (cenote) in the southeast of the Yucatan Peninsula (Mexico).

Keywords: water mites; parasitism; cladocera; behavior; SEM

Introduction

Water mites have a complex life cycle compose by three active stages: larva, deutonymph and adult and three resting stages: prelarva, protonymph and tritonymph plus the egg (Smith, 1988; Smith *et al*., 2010). The majority of water mite larvae parasitize adult insects, whereas the free living deutonymphs and adults – with few exceptions – are predators feeding on insect larvae and cladocerans (Smith and Oliver, 1986; Proctor *et al*., 2015; Martin, 2005). In general, the hexapod larvae actively seek an appropriate host and become an ectoparasite, which is passively transported while feeding on host fluids. The parasitic / phoretic phase has great importance not only for nutrition, but as well for dispersal of the water mite larvae (Smith *et al.* 2010; Proctor *et al*., 2015). A host-specific association has been well documented between many water mite larvae and nearly all major groups of aquatic insects as Diptera (mainly Chironomidae), Odonata, Plecoptera, Hemiptera, Coleoptera and Trichoptera (Smith and Oliver, 1986; Martin, 2004). So far, no parasitic relationship with cladocera has been documented, however deutonymphs and adults of several water mite groups (including the Unionicolidae) can be considered as predators of cladocerans (Proctor and Pritchard, 1989; Proctor *et al.*, 2015). However larval and host range of many taxa is still unknown and a lot of undescribed species (and behavioral patterns) can still be expected – especially, but not only in the tropics (Proctor *et al*. 2015).

The observations we present – and document by SEM-images – will certainly contribute to increase the knowledge of larval water mite behavior.

Material and methods

During a faunistic survey of zooplankton composition (Montes-Ortiz and Elías-Gutiérrez, 2018) in the karst sinkhole Cenote Azul (Quintana Roo, Mexico), we found a water mite larva attached to a cladoceran. The specimens were fixed in 96% ethanol and dehydrated subsequently in an ethanol series of 30%, 40%, 50%, 60%, 70%, 80%, 90% and 100% for 15 minutes. The dehydrated sample were critical point dried and gold-coated to be observed under a Scanning Electron Microscope (JEOL-JSM6010) at 10kV (Elías-Gutiérrez *et al*., 2008).

Results and Discussion

The water mite larva was tentatively identified as *Unionicola* sp. (Unionicolidae) (Prasad and Cook, 1972; Smith *et al*. 2010; pers. Comm. M. Vidrine); the 113ladocera could be identified as *Bosmina tubicen* Brehm, 1953 previously found in the area by Elías-Gutiérrez *et al.* (2008). The SEM pictures clearly show that the water mite larva is attached to the lateral side of the valve of the cladoceran (most probably parasitizing) (Figure 1 and 2) – a behavior never reported for water mite larvae so far.

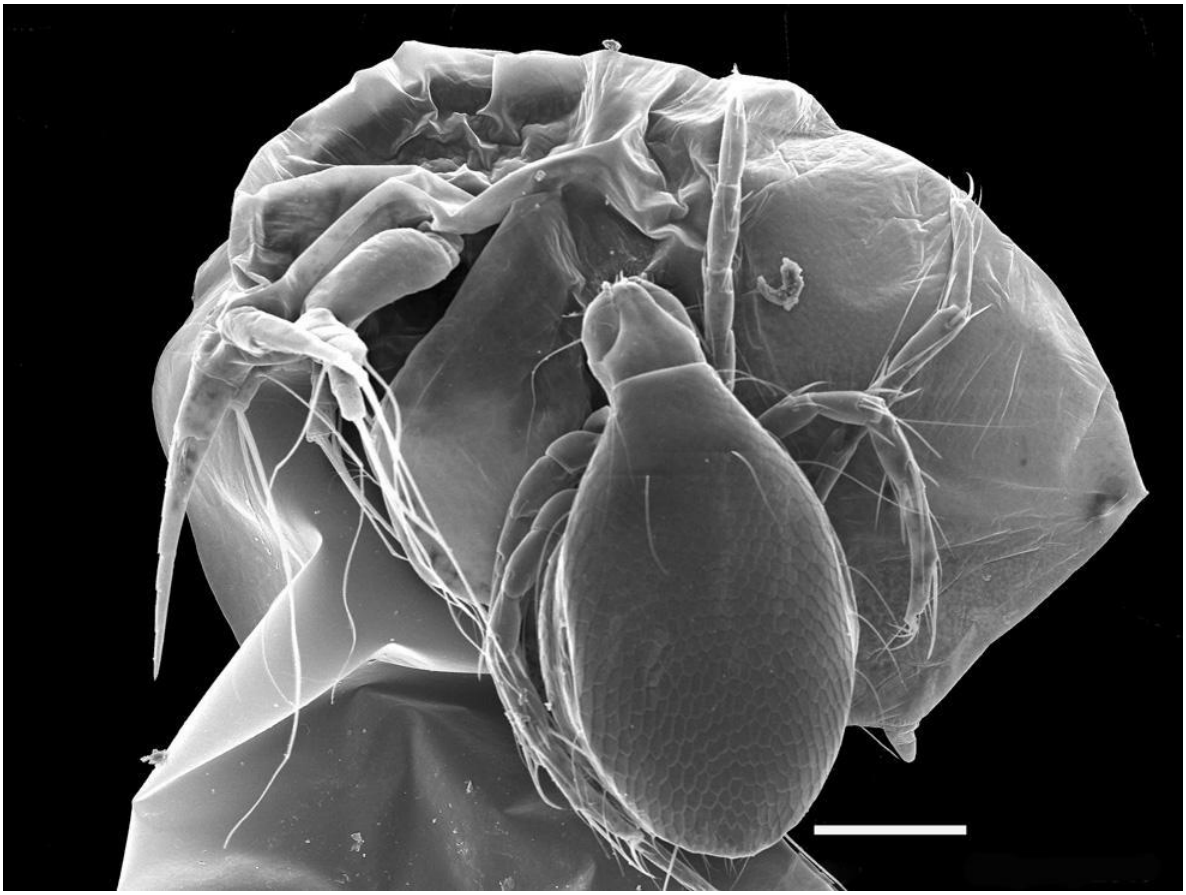

Figure 1

The observation is especially remarkable, as so far, the larvae of Unionicolidae are known to parasitize the adult stages of Diptera (Chironomidae) and Trichoptera, and in most cases investigated up to now a defined host specificity has been found (Proctor *et al*. 2015). Although recently the exceptional case of a trichopteran larva as host of *Unionicola* larvae has been reported by Martin and Tempelman (2014). Rare similar findings have so far been interpreted as accidental, or pure phoretic ("pre-parasitic") associations. However, the authors emphasize that the association they found has to be interpreted as truly parasitic, as the water mite larvae were typically engorged, and suggests evidence for an alternative life cycle of the respective water mite species (Martin and Templemann, 2014).

Buczyńska *et al.*, (2015) reported the finding of water mite larvae (*Tiphys torris*) attached to a Trichoptera pupa. As well in this case the authors pointed out that the water mite larvae were

truly parasitic as they were enlarged. However, in this case the authors interpreted their findings as rather accidental caused by an extended lack of access to a proper host (Buczyńska *et al.*, 2015).

Proctor *et al.* (2015) indicate that the opportunities to contact a host occur irregularly in space and time, in this sense Collins (1975) reported that 75% of *Wandesia thermalis* Viets, 1938 larvae fail to locate a host in a system where the distribution of it is clustered and unpredictable.

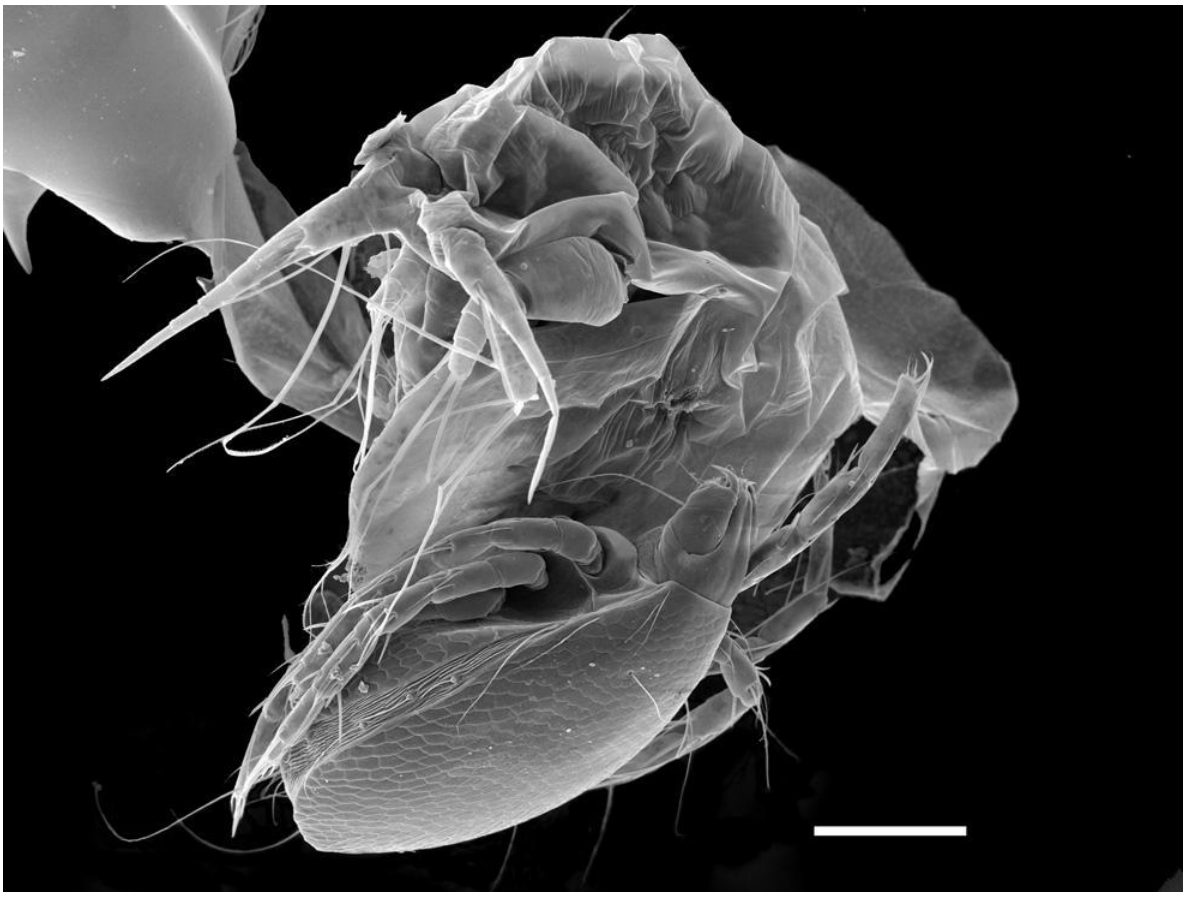

Figure 2a

Consequently, a possible explanation for the unusual association reported here is that the larva did not find an appropriate host and therefore attached to the 115ladocera. Though

Chironomidae (a registered host for *Unionicola*) are an abundant and diverse group in the system (Montes-Ortiz y Elías-Gutiérrez, 2018). Another explanation for the documented finding could be that the larva has attacked the 116ladocera in order to feed on it for a short time before the continuation of its search for a proper host – a behavior as well never documented. Even though the larva is not enlarged, clear traces of the attack are visible (Fig. 2b).

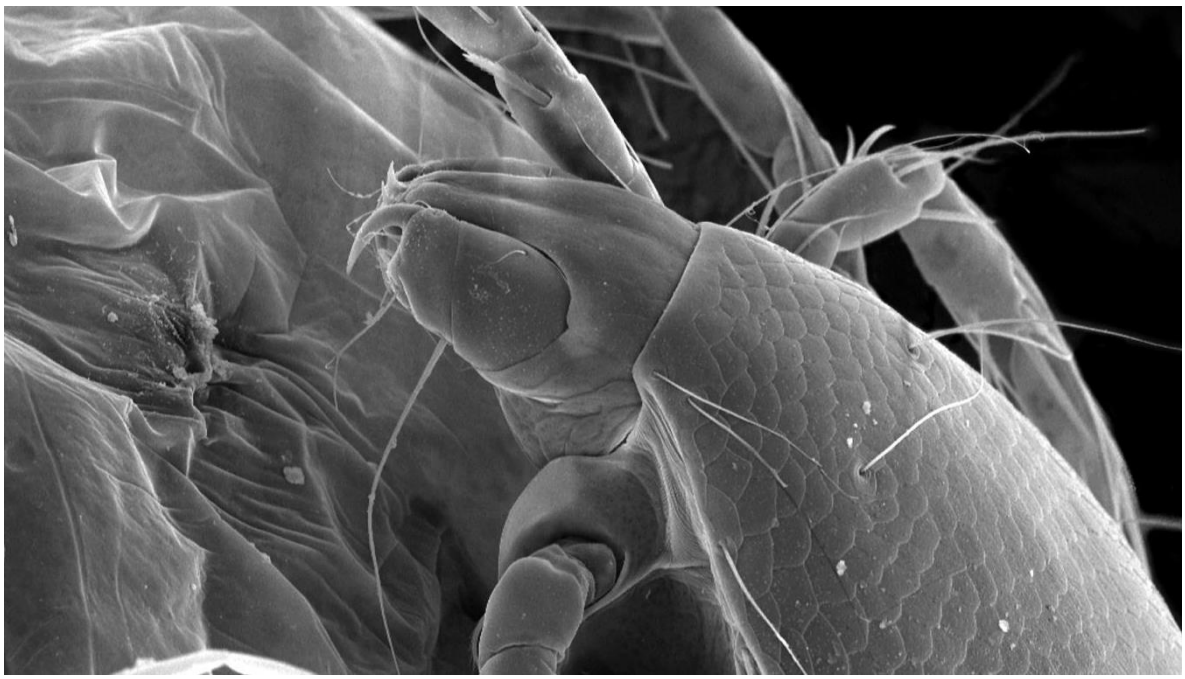

Figure 2b

In both possible cases the discovery reported here provides an important contribution to the extension of the existing concept and knowledge on water mite life cycles and their interaction with other members of the invertebrate fauna. Furthermore, it has to be emphasized, that the knowledge and understanding of water mite life cycles is still fragmentary and even more limited in the tropics.

Acknowledgments

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Malcome Vidrine (USA), Reinhard Gerecke (Germany) and Peter Martin (Germany) contributed valuable information on the water mite larva.

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Figures

Figure legends

Figure 1. Water mite larva (*Unionicola* sp.) attached to a water flea (*Bosmina tubicen*). The scale bar indicates 50 μm.

Figure 2. a) Lateral view of the *Unionicola* larva, frontal view on the *Bosmina.* B) Close up of perforations made by pedipalps and chelicerae of the water mite in the valve of the water flea. The scale bar indicates 50 μm.

CAPITULO VI
CONCLUSIONES

Conclusiones

En este estudio se encontró una alta diversidad de ácaros acuáticos en los 24 sitios de muestreo. En total se identificaron 18 géneros: *Arrenurus, Atractides, Centrolimnesia, Eylais, Geayia, Hydrodroma, Hydryphantes, Hygrobates, Koenikea, Krendowskia, Limnesia, Limnochares, Mamersellides, Mideopsis, Neumania, Piona, Torrenticola* y *Unionicola,* así como 77 grupos genéticos representados por 607 secuencias, de los cuales 51 grupos son nuevos registros para la base de datos (www.boldsystems.org) y 58 están presentes en una o máximo tres localidades.

Los resultados indican que la diversidad de ácaros acuáticos es mucho mayor a los registros históricos y descripciones de especies en la región y muy probablemente la mayoría de grupos genéticos corresponden a especies nuevas. Lo anterior ha sido demostrado a partir del ADN mitocondrial para una especie registrada en la Península de Yucatán y originalmente descrita en Europa, las secuencias que obtuvimos comprobaron que no se trata de la misma especie (Ver Montes-Ortiz y Elías-Gutiérrez, 2018; Więcek et al. 2020).

El análisis detallado del género *Arrenurus* corrobora los resultados previos, ya que, de los especímenes estudiados, cinco especies son nuevas para la ciencia, adicionalmente se incorporan tres nuevos registros para la Península de Yucatán. Asimismo, la comparación de todas las secuencias del género *Arrenurus* existentes en la base de datos bold, permite discernir perfectamente aquellas registradas para México de las que se encuentran registradas para otros países con excepción de dos grupos compartidos con Canadá.

Los datos obtenidos mediante la aproximación molecular en adición a la lista de especies de arrenúridos sugieren que la mayoría de las especies (putativas e identificadas) exhiben una distribución restringida y los diferentes cuerpos de agua presentan un ensamble específico de acarofauna. Sin embargo, es necesario realizar el estudio taxonómico completo de todos los especímenes secuenciados, con el fin de confirmar lo anterior. No obstante esta alta, y al parecer particular diversidad de ácaros acuáticos en los ecosistemas de la Península de Yucatán, evidencian la singularidad de esta región cárstica.

A través de este estudio se demostró que la inclusión de información molecular como las secuencias del gen COI (gen mitocondrial que codifica para la citocromo c oxidasa subunidad I) puede ser un elemento importante de aproximación al reconocimiento de una diversidad incierta como lo es la de los ácaros acuáticos en diferentes regiones del país. Así mismo constituye una herramienta útil para relacionar hembras y machos en especies con dimorfismo sexual marcado. Para el caso del género *Arrenurus,* lo anterior se logró exitosamente en cinco especies.

La biblioteca de información molecular generada permitirá en lo subsiguiente no solo relacionar ambos sexos, sino también la identificación del ciclo de vida completo, además de ser de acceso público, brindando la posibilidad de generar y/o contestar nuevas preguntas de investigación.

Este trabajo demostró, por primera vez, la relevancia de la recuperación de los organismos secuenciados, así como el análisis fino de sus estructuras a través de métodos avanzados no destructivos, como el bajo vacío de la microscopía electrónica. La certeza lograda para las descripciones aquí realizadas representa un hito en el reconocimiento de las nuevas especies presentadas en este trabajo.

Con la contribución de las nuevas especies y registros, la lista de ácaros acuáticos del género *Arrenurus* se incrementa de 39 a 42 y el total de especies reportadas para el país a 264. No obstante, esta lista evidencia una falta considerable de información para distintas regiones, 18 estados permanecen aún sin ningún registro de este grupo y algunos exhiben datos mínimos, como es el caso del estado de Yucatán con solo una especie registrada. A lo anterior podemos sumar los más de dos tercios de grupos genéticos que carecen de una identificación adecuada, tanto en México como en el resto del mundo. Esto, innegablemente, pone de manifiesto la necesidad de continuar con los estudios faunísticos y taxonómicos de los ácaros acuáticos. Para futuros estudios es recomendable realizar muestreos más exhaustivos y sistemáticos con el fin de asegurar la obtención de una mayor proporción de individuos y no obstaculizar la descripción o reconocimiento por una muestra escasa.

Es importante integrar otros ecosistemas a la exploración, como zonas estuarinas y marinas, con el fin de obtener la representatividad de grupos escasamente estudiados como la familia Pontarachnidae, para la cual se obtuvo la primera descripción de una especie del género *Litarachna* para la parte beliceña de la Bahía de Chetumal. De la misma manera, se sugiere explorar las variables que pudieran influenciar la distribución y ensamble de estos organismos, como las características fisicoquímicas y geohidrológicas de los cuerpos de agua, microhábitats, hospederos y disponibilidad de presas.

De forma complementaria, en esta investigación por primera vez se pudo ilustrar, a través de imágenes de microscopía electrónica la parasitación de una larva sobre un cladócero típicamente zooplánctico. Esto abre preguntas relevantes respecto a la biología y a la dinámica que establecen los ácaros con otras especies.

Finalmente, los datos generados durante este estudio reactivan las investigaciones sobre ácaros acuáticos en la Península de Yucatán y en México, ya que solo existen tres publicaciones al respecto en los últimos 20 años. Dicha reactivación no solo tuvo lugar en el área de investigación, sino también en diferentes foros de divulgación de la ciencia en la que se realizaron exhaustivos ejercicios de difusión de este grupo tan importante y tan fuertemente relegado en su estudio.

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