UNIVERSIDAD NACIONAL AUTÓNOMA DE MÉXICO

POSGRADO EN CIENCIAS BIOLÓGICAS

INSTITUTO DE BIOLOGÍA

SISTEMÁTICA

SISTEMÁTICA MOLECULAR DE LA FAMILIA STRIGEIDAE (RAILLIET, 1919)

PARÁSITOS DE AVES ACUÁTICAS EN MÉXICO

TESIS

QUE PARA OPTAR POR EL GRADO DE:

DOCTORA EN CIENCIAS

PRESENTA:

CECILIA ALEJANDRA LÓPEZ JIMÉNEZ

TUTOR PRINCIPAL DE TESIS: DR. JOSÉ MARTÍN GARCÍA VARELA, INSTITUTO DE BIOLOGÍA, UNAM

COMITÉ TUTOR: DR. JOSÉ JAIME ZUÑIGA VEGA, FACULTAD DE CIENCIAS, UNAM
DR. GERARDO PÉREZ PONCE DE LEÓN, INSTITUTO DE BIOLOGÍA, UNAM

Ciudad Universitaria, CD. MX. **Septiembre 2023.**

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COORDINACIÓN GENERAL DE ESTUDIOS DE POSGRADO
COORDINACIÓN DEL POSGRADO EN CIENCIAS BIOLÓGICAS
INSTITUTO DE BIOLOGÍA
OFICIO: CGEP/CPCB/IB/0544/2023
ASUNTO: Oficio de Jurado

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

Me permito informar a usted que en la reunión ordinaria del Comité Académico del Posgrado en Ciencias Biológicas, celebrada el día **24 de abril de 2023** se aprobó el siguiente jurado para el examen de grado de **DOCTORA EN CIENCIAS** de la estudiante **LÓPEZ JIMÉNEZ CECILIA ALEJANDRA** con número de cuenta **308181829** con la tesis titulada **SISTEMÁTICA MOLECULAR DE LA FAMILIA STRIGEIDAE (RAILLIET, 1919) PARÁSITOS DE AVES ACUÁTICAS DE MÉXICO",** realizada bajo la dirección del **DR. JOSÉ MARTÍN GARCÍA VARELA,** quedando integrado de la siguiente manera:

Presidente:	DR. ADOLFO GERARDO NAVARRO SIGÜENZA
Vocal:	DRA. BLANCA ESTELA HERNÁNDEZ BAÑOS
Vocal:	DRA. LAURA ROXANA TORRES AVILÉS
Vocal:	DRA. MARÍA DEL CARMEN GUZMÁN CORNEJO
Secretario:	DR. GERARDO PÉREZ PONCE DE LEÓN

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

A T E N T A M E N T E
"POR MI RAZA HABLARÁ EL ESPÍRITU"
Ciudad Universitaria, Cd. Mx., a 26 de julio de 2023

COORDINADOR DEL PROGRAMA

POSGRADO EN CIENCIAS BIOLOGICAS
COORDINACIÓN

DR. ADOLFO GERARDO NAVARRO SIGÜENZA

c. c. p. Expediente del alumno

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AGRADECIMIENTOS INSTITUCIONALES

Al Posgrado en Ciencias Biológicas de la Universidad Nacional Autónoma de México por el apoyo brindado para concluir el programa de Doctorado.

Al Consejo Nacional de Ciencia y Tecnología (CONACYT) por la beca otorgada para la obtención de grado (CVU 706119).

Al Programa de Apoyo a Proyectos de Investigación e Innovación Tecnológica por financiar este proyecto (PAPIIT No. IN201122).

Al Dr. Martín García Varela, quién fue mi Tutor principal. Gracias por todo el apoyo, motivación y aprendizajes que me brindó durante mi formación académica y personal.

A los miembros del Comité Tutoral conformado por el Dr. Gerardo Pérez Ponce de León y el Dr. José Jaime Zuñiga Vega. Gracias por todo el apoyo, sugerencias y comentarios que ayudaron a enriquecer este proyecto.

AGRADECIMIENTOS A TÍTULO PERSONAL

A la Dra. Laura Márquez Valdelamar y M. en C. Nelly López Ortíz del Laboratorio Nacional de Biodiversidad del Instituto de Biología de la UNAM (LANABIO) por el apoyo en la obtención de secuencias.

Al Dr. Armando Rodríguez Reyes y Rocío González Acosta por el apoyo en los trámites para la obtención de grado.

A la M. en C. Luis García Prieto de la Colección Nacional de Helmintos (CNHE) por el préstamo de los ejemplares, bases de datos e información bibliográfica otorgada.

A la M. en C. Berenit Garfías Mendoza del Laboratorio Nacional de Biodiversidad del Instituto de Biología de la UNAM (LANABIO) por el apoyo en la obtención de las fotografías de microscopia electrónica de barrido (MEB).

A los miembros del jurado, Dr. Adolfo Navarro, Dra. Blanca Hernández, Dr. Gerardo Pérez, Dra. Carmen Guzmán y Dra. Laura Torres por la revisión de la tesis y comentarios que ayudaron al mejoramiento del mismo.

A mis compañeros y amigos del Laboratorio C-104 (Pol, Tona y Mirza) por la amistad y apoyo brindado durante estos años, por hacer más ameno el trabajo de campo y laboratorio.

A Miguel, mi gran compañero de vida.

A mis padres, familia y amigos, quienes siempre me han motivado y confiado en mí.

DEDICATORIA

A mis padres, Armando y Carolina.

Para ver el mundo en un grano de arena,

Y el cielo en una flor silvestre,

Sostén el infinito en la palma de tu mano

Y la eternidad en una hora.

William Blake

ÍNDICE

RESUMEN

La unidad fundamental de la biodiversidad es la especie. La delimitación molecular de especies es una práctica metodológica para identificar linajes evolutivos independientes con nulo flujo genético entre ellos. Actualmente la sistemática dispone de métodos para una taxonomía integrativa. Los miembros que conforman la familia Strigeidae Railliet, 1919 son endoparásitos del intestino de aves acuáticas, a excepción del género *Duboisiella* el cual parasita mamíferos pequeños como tlacuaches y mapaches. La familia está clasificada en 13 géneros con aproximadamente 110 especies distribuidas en todo el mundo. Sin embargo, la clasificación taxonómica dentro de esta familia se ha basado principalmente en caracteres morfológicos de las fases adultas y la asociación con su huésped definitivo. Actualmente, las relaciones filogenéticas dentro de la familia Strigeidae son confusas, ya que es considerado un grupo parafilético con la familia Diplostomidae anidada dentro de esta. Aunado a esto, las diferencias morfológicas entre algunos géneros de la familia son sútiles y las características diagnósticas de cada grupo se comparten entre algunas especies de estos géneros dificultando una correcta clasificación taxonómica del grupo. En la presente tesis se generaron secuencias con dos genes nucleares y un mitocondrial para determinar la posición filogenética de la especie *Parastrigea brasiliana* asociada al intestino de la garza pico de bota (*Cochlearius cochlearius*). Los resultados derivados de los análisis filogenéticos inferidos con métodos probabilísticos como Máxima Verosimilitud (ML) e Inferencia Bayesiana (IB) revelaron que los individuos identificados morfológicamente como *P. brasiliana* colectados del hospedero tipo se anidaron con otras especies del género *Apharyngostrigea.* Con base en los datos moleculares, se transfirio la especie *Parastrigea brasiliana* al género *Apharyngostrigea* formando la nueva combinación *Apharyngostrigea brasiliana*. Morfológicamente, esta especie comparte características diagnósticas de ambos géneros. Adicionalmente, se realizó

un estudio integrativo de especímenes identificados morfológicamente como *Parastrigea macrobursa* y *Strigea* sp., asociadas a cuatro especies de aves rapaces colectadas en nueve localidades del Pacífico y Golfo de México. Los análisis filogenéticos revelaron dos linajes independientes dentro de *Strigea.* El primer linaje corresponde con una especie no descrita previamente colectada en dos especies de aves rapaces (*Rupornis magnirostris* y *Accipiter cooperii*) en seis localidades de México, la cual es nombrada como *Strigea magnirostris* n. sp. La nueva especie se caracteriza morfológicamente por presentar una combinación de caracteres únicos, como son; papilas alrededor de la ventosa oral, seudoventosas bien desarrolladas, diminutas espinas en el segmento anterior del cuerpo, así como un cono genital y bursa copulatoria de gran tamaño. *Strigea magnirostris* n. sp. representa la primera especie de *Strigea* descrita para México y la número 16ava para la región Neotropical.

El segundo linaje reconocido corresponde con especímenes identificados morfológicamente como *Parastrigea macrobursa* del hospedero tipo (*Buteogallus urubitinga*) y *B. anthracinus* en tres localidades de México. Los análisis filogenéticos mostraron que los especímenes identificados morfológicamente como *P. macrobursa* no están relacionadas con otras especies del género *Parastrigea*, ya que se anidan dentro del género *Strigea.* Con base en estos resultados la especie identificada como *Parastrigea macrobursa* es transferida al género *Strigea* para formar la combinación *Strigea macrobursa*, expandiendo su rango de distribución de México hasta Argentina. Los resultados presentados en la tesis revelan que los géneros *Parastrigea*, *Strigea* y *Apharyngostrigea* carecen de caracteres diagnósticos que permitan una adecuada delimitación, por lo que es de suma importancia incorporar información molecular a los registros y descripciones de nuevas especies de estrigeidos. Además, es necesario integrar información de las asociaciones ecológicas huésped-parásito. Por ejemplo, las especies pertenecientes al género *Strigea* se

encuentran asociadas con aves rapaces de las familias Accipitridae y Falconidae, mientras que las especies del género *Apharyngostrigea* están asociadas con aves de la familia Ardeidae, comúnmente conocidas como garzas. Finalmente, las especies del género *Parastrigea* se encuentran asociadas con aves pertenecientes a la familia Threskiornithidae. La actual evidencia generada en este trabajo de tesis sugiere que las asociaciones ecológicas (huésped-parásito) han sido fundamentales en la diversificación de los estrigeidos. Con base en estas asociaciones ecológicas, nosotros inferimos que probablemente las especies identificadas morfológicamente como *Parastrigea* colectadas de aves rapaces o garzas pertenecen a los géneros *Strigea* o *Apharyngostrigea* respectivamente.

Finalmente, en esta tesis estudiamos por primera vez la estructura genética poblacional de un estrigeido que se encuentra distribuido ampliamente, presentando altos valores de prevalencia y abundancia en sus huéspedes definitivos. La especie *Parastrigea diovadena* se encuentra parasitando el intestino del ibis blanco (*Eudocimus albus*) la cual fue colectada en tres provincias biogeográficas de México: Sierra Madre Occidental (SMO), Sierra Madre Oriental (SMOr) y la Sierra Madre del Sur (SMS). Los análisis poblacionales estimados a través de un marcador mitocondrial, citocromo oxidasa subunidad 1 (*Cox 1*) revelaron una alta diversidad haplotípica con una baja diversidad nucleotídica y una nula estructura filogeográfica en las poblaciones de *Parastrigea diovadena*, lo que sugiere que el hospedero definitivo juega un papel central en la estructura genética de sus parásitos, dispersando las poblaciones a lo largo de la República Mexicana. Este estudio constituye un primer esfuerzo para comprender la estructura genética de las poblaciones de parásitos tremátodos con diferentes ciclos de vida en México. Finalmente, los resultados presentados en la tesis permitieron generar un panorama integral sobre la taxonomía, sistemática y filogeografía de tres géneros que conforman la familia Strigeidae asociados con aves acuáticas de México.

ABSTRACT

The fundamental unit of biodiversity is the species. The molecular delineation of species is a methodological practice to identify independent evolutionary lineages with no gene flow among them. Members of the family Strigeidae Railliet, 1919 are endoparasites mainly of the intestine of aquatic birds, with the exception of the genus *Duboisiella* which parasitizes small mammals such as opossums and raccoons. The family is classified into 13 genera with approximately 110 species distributed worldwide. However, the taxonomic classification within this family has been based mainly on morphological characters of the adult stages and on the associations with their definitive host. Currently, the phylogenetic relationships within the family Strigeidae are controversial, due that it is considered as paraphyletic. In addition, the morphological differences among genera of the family are subtle and the diagnostic characteristics of each group overlap among some species of these genera. In the current thesis, sequences of two nuclear genes and one mitochondrial gene were generated to determine the systematic position of the species *Parastrigea brasiliana* associated with the boot-billed heron (*Cochlearius cochlearius*). The phylogenetic analyses inferred with Maximum Likelihood (ML) and Bayesian Inference (BI) revealed that the specimens identified as *P. brasiliana* collected from the type host nested inside of the genus *Apharyngostrigea*. Based on the current molecular evidence, the species *Parastrigea brasiliana* was transferred to *Apharyngostrigea* to form *Apharyngostrigea brasiliana.* Morphologically, this species shares diagnostic characteristics of both genera. Additionally, an integrative study of specimens morphologically identified as *Parastrigea macrobursa* and *Strigea* sp., associated with four species of prey birds were collected in nine localities from Pacific and Gulf of Mexico. Phylogenetic analyses revealed two independent lineages within

Strigea. The first lineage corresponds an undescribed species, which was collected in two species of prey birds (*Rupornis magnirostris* and *Accipiter cooperii*) in six localities in Mexico, which is named as *Strigea magnirostris* n. sp. The new species is morphologically characterized by presenting a combination of unique characters, such as papillae around the oral sucker, well-developed pseudo-suckers, tiny spines on the anterior segment of the body, as well as a large genital cone and copulatory bursa. *Strigea magnirostris* n. sp. represents the first species of *Strigea* described for Mexico and the 16th for the Neotropical region. The second lineage corresponds to specimens morphologically identified as *Parastrigea macrobursa* from its type host (*Buteogallus urubitinga*) and *B. anthracinus* in three localities in Mexico. Phylogenetic analyses showed that the specimens morphologically identified as *P. macrobursa* are not related to other species from the genus *Parastrigea*, and it was nested within *Strigea*. Based on these results, the species *Parastrigea macrobursa* was transferred to *Strigea*, forming a new combination *Strigea macrobursa* expanding its distribution range from Mexico to Argentina. The results generated in the thesis reveal that the genera *Parastrigea*, *Strigea* and *Apharyngostrigea* lack of diagnostic characters that allow an adequate identification. Therefore, is necessary to continue incorporating molecular information in the records and description of new species of strigeids. In addition, the ecological associations are an essential component that should be incorporate to studied. For example, species belonging to the genus *Strigea* are associated with prey birds from the Accipitridae and Falconidae families, and the species of the genus *Apharyngostrigea* show high host specificity, parasitizing ardeids species knowns as herons. In addition, species of the genus *Parastrigea* are associated with birds belonging to the family Threskiornithidae, suggesting that the ecological association played an important role in diversification of the

strigeids. Based on this ecological evidence, appear species of *Parastrigea* collected from prey birds or herons probably belong to the genera *Strigea* or *Apharyngostrigea*, respectively.

Finally, the population genetic structure of *Parastrigea diovadena* a parasite of the white ibis (*Eudocimus albus*) was studied through three biogeographical provinces of Mexico: Sierra Madre Occidental (SMO), Sierra Madre Oriental (SMOr) and the Sierra Mother of the South (SMS). Population analyzes were calculated through the mitochondrial marker, cytochrome oxidase subunit 1 (*Cox 1*) revealed high haplotypic diversity with low nucleotide diversity and null phylogeographic structure in *P. diovadena* populations, suggesting that the definitive host plays a crucial role in the genetic structure, dispersing the populations throughout Mexico. This study constitutes a first effort to understand the genetic structure of populations of parasites with different life cycles in Mexico. The results obtained in this study were published in three manuscripts.

I.INTRODUCCIÓN GENERAL

I.I. DELIMITACION DE ESPECIES

La unidad fundamental de la biodiversidad es la especie. Su definición ha generado debates y controversias, su concepción está íntimamente ligada al nivel de estudio que se desea abordar; por ejemplo, poblacional, morfológico, genético, evolutivo, taxonómico, etc. (Leliaert et al. 2014). Mayden (1997, 1999), llevó a cabo un análisis jerárquico de diferentes conceptos de especie y consideró que el concepto evolutivo es el primario porque presenta el mayor nivel de consiliencia (es decir, aquellas hipótesis que poseen mayor capacidad de unificar conceptos y poder explicativo). Los otros conceptos, secundarios u operacionales, pueden utilizarse para reconocer e identificar entidades biológicas desde diferentes enfoques (p.ej. concepto morfológico, biológico, ecológico, entre otros más). El concepto ontológico o primario se define como un linaje, es decir, una secuencia de una población ancestro-descendiente que evoluciona independientemnte de otros linajes y que tiene una estructura evolutiva única (Wiley y Mayden, 2000).

Actualmente, la propuesta de un Concepto Unificado de Especie (*Unified Species Concept* o *USC*), separa la parte teórica común de los diferentes conceptos y considera que las especies son linajes metapoblacionales separados evolutivamente, entendiéndose como metapoblación, a una población formada por subpoblaciones interconectadas entre ellas (De Queiroz, 2005).

Con base en lo anterior, la delimitación molecular de especies es una práctica metodológica para identificar linajes evolutivos independientes con nulo flujo genético entre linajes (Sites y Marshall, 2003; De Queiroz, 2007). De manera general, las especies deben delimitarse objetivamente y bajo un análisis riguroso. En la actualidad la sistemática dispone

de métodos para una taxonomía integrativa (Miralles y Vences, 2013). Los avances en biología molecular proporcionan, el uso de evidencia directa del genotipo, como son las secuencias de DNA, ofreciendo herramientas para evaluar de forma independiente la transición de caracteres que reflejen la relación ancestro-descendiente. Estas herramientas moleculares han revolucionado la forma en que se abordan preguntas sobre la biodiversidad y son especialmente útiles cuando las características morfológicas son conservadas (Yu et al. 2012).

I.II. UTILIZACIÓN DE DATOS MOLECULARES

El empleo de las secuencias de DNA como caracteres para la delimitación de especies ofrece diversas ventajas, por ejemplo, diferentes regiones del genoma tienen distintas tasas de mutación y por lo tanto pueden ser usadas para identificar y caracterizar poblaciones, especies, géneros etc. Además, de que la secuenciación de DNA es relativamente rápida y la variación en las secuencias no está influenciada por el fenotipo o el ambiente (Galazzo et al. 2002; Avise, 2004; Vilas et al. 2005). De forma general, los marcadores moleculares pueden ser clasificados en tres grupos: 1) Marcadores basados en la hibridación del DNA, por ejemplo, polimorfismos en la longitud de fragmentos de restricción del DNA (*Restriction fragment length polymorphisms,* RFLP); 2) Marcadores moleculares del DNA nuclear y mitocondrial, y 3) Marcadores mixtos, por ejemplo, polimorfismo en la longitud de fragmentos amplificados de DNA, mejor conocidos como AFLP por sus siglas en inglés (*Amplified Fragment Length Polymorphism*). Estos pueden considerarse como una combinación de RFLP y los polimorfismos de DNA amplificados al azar (RAPD´s) (Picca et al. 2002; Rentaría-Alcántara, 2007). Los caracteres moleculares más empleados en la taxonomía integrativa en parásitos han sido los marcadores nucleares y mitocondriales. Estos

marcadores moleculares son pequeños fragmentos de DNA que reflejan variación o polimorfismo entre diferentes individuos (Jiang, 2017). Entre los marcadores mitocondriales más utilizados en los diferentes grupos se encuentran los genes del citocromo oxidasa subunidad 1 (*Cox 1*), compuesto por aproximadamente 648 pares de bases (pb), comúnmente utilizado como un identificador único "código de barras", el cual proporciona una alta resolución para la delimitación a nivel de especie. Seguido por el gen del citocromo b (*cyt b*) con 700 pb y NADH deshidrogenasa subunidad 1 (*NAD1*) con 500 pb. Las propiedades más interesantes que presentan los marcadores mitocondriales en términos filogenéticos y filogeográficos, son su alta tasa de evolución a nivel de secuencias de nucleótidos, su nula recombinación, su variación intraespecífica y herencia materna. Estas características permiten describir la historia matrilineal de organismos coespecíficos y con ello aplicar estimaciones de reloj molecular e inferir análisis de coalescencia (Avise, 1987, 2000; Vázquez-Domínguez et al. 2009).

Los marcadores nucleares que frecuentemente se emplean en los diferentes grupos de organismos son los genes del DNA nuclear ribosomal (DNAr). El DNAr está conformado en repeticiones en tándem y está formado por tres subunidades altamente conservadas (18S, 5.8S y 28S), separadas por dos espaciadores transcritos internos con elevadas tasas de sustitución (ITS1 e ITS2) (Choudhary et al. 2015). Estas repeticiones en tándem se encuentran conservadas a lo largo de todo un genoma y evoluciona concertadamente, lo que se atribuye a eventos de recombinación como entrecruzamiento desigual y conversión génica (Rentaría-Alcántara, 2007; Eickbush y Eickbush, 2007). Tanto los marcadores nucleares como los mitocondriales han sido utilizados para delimitar especies en diferentes grupos de organismos, y en helmintos parásitos no es la excepción (Hernández-Mena et al. 2014; López-Jiménez et al. 2017; Achatz et al. 2022; Pérez Ponce de León et al. 2022).

I.III. FAMILIA STRIGEIDAE RAILLIET, 1919

Los miembros que conforman la familia Strigeidae Railliet, 1919 son parásitos principalmente del intestino de aves acuáticas, a excepción del género *Duboisiella* Baer, 1938 el cual parasita mamíferos pequeños (tlacuaches y mapaches) (Dubois, 1968). Las características diagnósticas que caracterizan a los miembros de esta familia son, 1) cuerpo bisegmentado, 2) segmento anterior en forma de copa y 3) órgano tribocítico bilobulado (uno ventral y otro dorsal) (Dubois, 1938, 1968; Niewiadomska, 2002). El segmento anterior o "*forebody*", contiene los órganos de fijación: ventosa oral, faringe, ventosa ventral, glándula proteolítica y órgano tribocítico. El órgano tribocítico está situado en la parte posterior de la ventosa ventral, el cual refuerza la función adhesiva de las ventosas, además desempeña un papel esencial en la predigestión del tejido del hospedero y locomoción (Dubois, 1938; Niewiadomska, 1973). El segmento posterior o "*hindbody*", contiene los órganos reproductores. Presentan dos testículos ubicados en tándem que pueden ser bilobulados, trilobulados o multibilobulados y carecen de bolsa de cirro. La bursa copulatoria está bien desarrollada y contiene el cono genital. El útero es comúnmente largo y contiene los huevos no embrionados. El ovario es pretesticular y las glándulas vitelógenas se encuentran distribuidas por todo el cuerpo o pueden estar confinadas en el segmento posterior. El poro genital se localiza en posición dorsoterminal (Dubois, 1968; Shoop, 1989; Niewiadomska, 2002).

El género *Strigea* fue descrito por Abildgaard en 1790, posteriormente Railliet en 1919 erigío la familia Strigeidae denominando a *Strigea* como género tipo. Dubois (1938) dividió a la familia Strigeidae en dos subfamilias con base en la especificidad hospedatoria. La subfamilia Strigeinae (parásitos de aves) compuesta por 11 géneros dentro de dos tribus (Strigeini y Cotylurini) y la subfamilia Duboisiellinae (parásitos de mamíferos) compuesta

por un género. Actualmente se han descrito aproximadamente 110 especies de estrigeidos, clasificadas en 13 géneros (Niewiadomska, 2002) (Tabla 1).

Tabla 1. Géneros que componen la familia Strigeidae, Railliet, 1919 (Niewiadomska, 2002), distribución geográfica y familias de hospederos que parasitan.

Género	Distribución	2º H.I	H.D
Subfamilia Strigeinae			
1. *Strigea* Abilgaard, 1790	Cosmopolita	Anfibios, reptiles, aves y mamíferos	**Aves:** Accipitridae, Strigidae, Trogonidae, Cariamidae, Threskiornithidae, Anatidae, Cathartidae, Phalacrocoracidae y Falconidae
2. *Apharyngostrigea* Ciurea, 1927	Cosmopolita	Peces	**Aves:** Ardeidae
3. *Parastrigea* Szidat, 1928	Cosmopolita	Peces y anfibios	**Aves:** Ardeidae, Ciconiidae, Threskiornithidae, Falconidae, Jacanidae, Recurvirostridae, Strigidae, Accipitridae
4. *Apatemon* Szidat, 1928	Cosmopolita	Peces	**Aves:** Anatidae y Rallidae
5. *Australapatemon* Sudarikov, 1959	Cosmopolita	Hirudineos	**Aves:** Anatidae, Strigidae y Accipitridae
6. *Cotylurus* Szidat, 1928	Región Holartica, Neotropical y Oriental	Hirudineos y gasterópodos	**Aves:** Anatidae, Scolopacidae y Rallidae

7. *Ophiosoma* Szidat, 1928	Región Holartica, Neotropical y Oriental	N.I	**Aves:** Ardeidae, Accipitridae y Laridae
8. *Nematostrigea* Sandground, 1934	Europa, Norte América y África	N.I	**Aves de presa:** Pandionidae y Accipitridae
9. *Pseudapatemon* Dubois, 1936	Europa, Norte América y Filipinas	N.I	Scolopacidae
10. *Schwartzitrema* Pérez-Vigueras, 1941	Estados Unidos, Cuba, India y Australia	N.I	**Aves:** Anhingidae, Pelacanidae y Phalacrocoracidae
11. *Cardiocephaloides*, Sudarikov, 1959	Europa, África, América y Australia	Peces	**Aves:** Laridae, Spheniscidae y Rynchopidae
12. *Ichthycotylurus* Odening, 1969	Región Holartica	Peces	**Aves:** Laridae, Sternidae, Spheniscidae y Gaviidae
Subfamilia Duboisiellinae			
13. *Duboisiella* Baer, 1938	Región Neotropical: Brasil, Perú, Argentina, Venezuela, Pánama y México	N.I	**Mamíferos:** Didelphidae

2ºH.I=Segundo hospedero intermediario.
H.D=Hospedero definitivo.
N.I= No identificado.

I.IV. CICLO DE VIDA GENERAL DE STRIGEIDAE

El ciclo de vida de los estrigeidos es indirecto, es decir, requiere de dos o más hospederos para completarlo. El ciclo de vida inicia cuando los huevos operculados se liberan al ambiente acuático a través de las heces de los hospederos definitivos. Posteriormente, del huevo eclosiona la primera fase larval libre nadadora (miracidio) que infectan a caracoles acuáticos pertenecientes a las familias Ampullariidae, Lymnaeidae, Nassariidae, Planorbidae, Physidae y Viviparidae, los cuales fungen como los primeros hospederos intermediarios. En el interior de los caracoles se desarrolla la fase asexual, donde los esporocistos se multiplican hasta formar las redias. De las redias se originan las cercarias, estas últimas emergen del caracol y nadan para penetrar al segundo hospedero intermediario que pueden ser varias especies de invertebrados (hirudíneos) o de vertebrados (peces, anfibios y reptiles). Una vez en el interior del segundo hospedero intermediario, la cercaria se enquista en diferentes órganos tranformándose en metacercarias de tipo “tetracotyle”. El ciclo de vida se completa cuando el hospedero definitivo (aves y mamíferos) consumen al segundo hospedero intermediario infectado (Pearson, 1959, 1972; Odening, 1967) (Fig. 1).

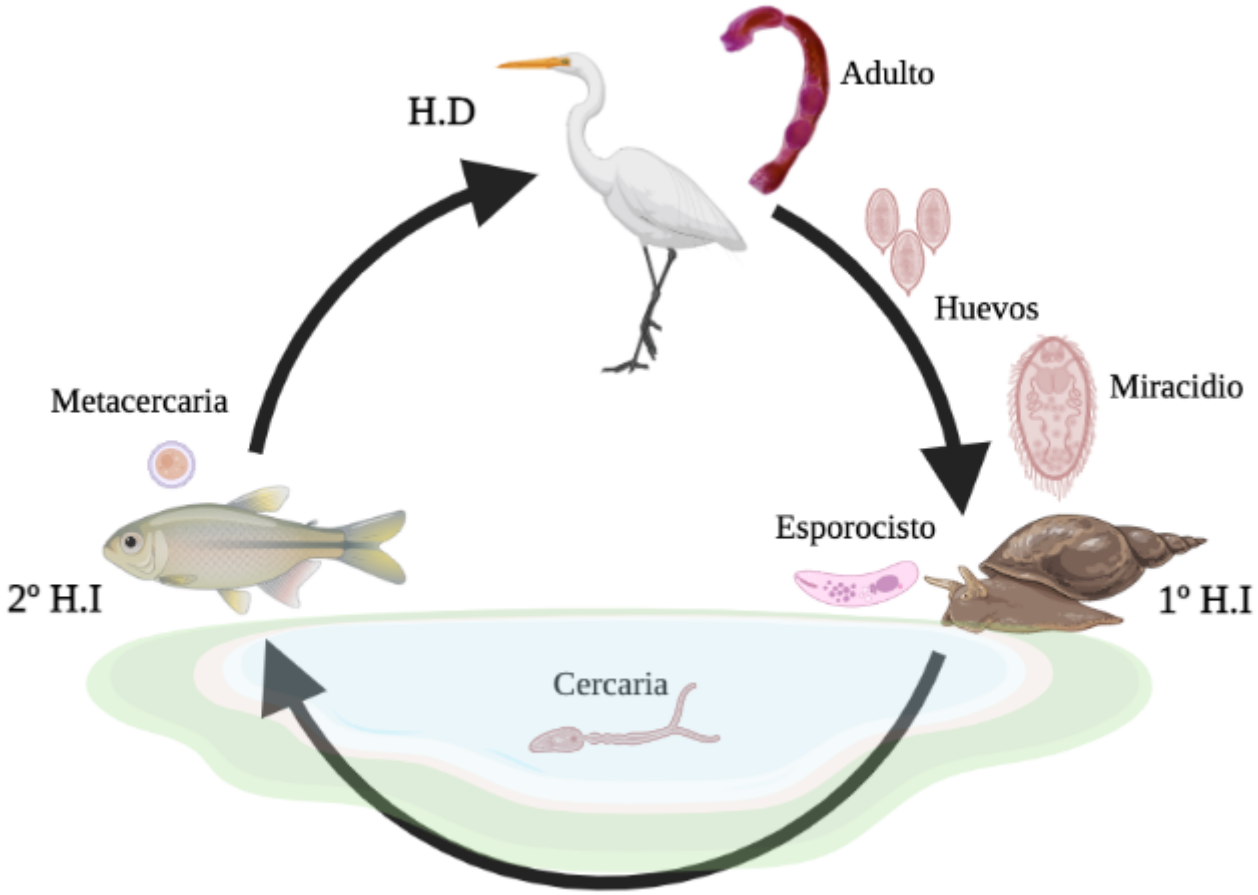

Fig. 1. Ciclo de vida general de la familia Strigeidae. H.D=Hospedero definitivo, H.I=Hospedero intermediario.

I.V. REGISTROS DE ESTRIGEIDOS EN MÉXICO

En México se han registrado un total de 18 especies de estrigeidos y siete especies candidatas pertenecientes a ocho géneros de la familia (*Strigea*, *Parastrigea*, *Apharyngostrigea*, *Apatemon*, *Australapatemon*, *Cotylurus*, *Cardiocephaloides* y *Duboisiella*) en 56 localidades de 19 estados de la República Mexicana. La fase larval (metacercaria) se ha encontrado enquistada en diferentes órganos (mesenterio, cerebro, branquías, hígado, intestino, riñón, músculo y gónadas) de 25 especies de peces pertenecientes a nueve familias (Cichlidae, Cypronodontidae, Poeciliidae, Characidae, Eleotridae, Goodeidae, Tetraodontidae, Serranidae y Belonidae), los cuales fungen como el segundo hospedero intermediario. Mientras que la fase adulta se ha registrado en el intestino de 24 especies de aves acuáticas pertenecientes a ocho familias (Falconidae, Recurvirostridae, Threskiornithidae, Ardeidae, Anatidae, Rallidae, Laridae y Rynchopidae) y en dos especies de mamíferos de la familia Didelphidae (Tabla 2).

Tabla 2. Registros de las especies de la familia Strigeidae (Railliet, 1919) en México.

Especie	Fase de desarrollo	Hospedero	Localidad	Estado	Referencia
Subfamilia: Strigeinae					
Tribu: Strigeini					
***Strigea* Abildgaard, 1790**					
Strigea sp.	Adulto	*Caracara cheriway*	Presa La Angostura	Chiapas	Hernández-Mena et al. 2017
Strigea sp.	Metacercaria	*Mayaheros urophthalmus*	Río Lagartos	Yucatán	Vidal-Martínez, 1995
		Petenia splendida	El Vapor	Campeche	Vidal-Martínez, 1995
		Cyprinodon eximius	Río Conchos	Chihuahua	Salguero-Vargas, 2015
		Gambusia affinis	Río Cuchujaqui	Sonora	Salguero-Vargas, 2015
***Parastrigea* Szidat, 1928**					
Parastrigea mexicana Coil, 1957	Adulto	*Recurvirostra americana*	Salina Cruz	Oaxaca	Coil, 1957
Parastrigea cincta Szidat, 1928	Adulto	*Eudocimus albus*	San Blas	Nayarit	Hernández-Mena et al. 2014
			Caimanero	Sinaloa	Hernández-Mena et al. 2014
			Laguna La Tovara	Nayarit	Ortega-Olivares et al. 2011
			Laguna El Huizache	Sinaloa	Ortega-Olivares et al. 2011
			Tecolutla	Veracruz	Ortega-Olivares et al. 2011
Parastrigea diovadena Dubois y Macko, 1972	Adulto	*Eudocimus albus*	Punta Piedra	Tamaulipas	Hernández-Mena et al. 2014
			Tamiahua	Veracruz	Hernández-Mena et al. 2014
			Tecolutla	Veracruz	Hernández-Mena et al. 2014
			Los Chivos	Veracruz	Hernández-Mena et al. 2014
			Pijijiapan	Chiapas	Hernández-Mena et al. 2014
			Chautengo	Guerrero	Hernández-Mena et al. 2014
			San Blas	Nayarit	Hernández-Mena et al. 2014
			Caimanero	Sinaloa	Hernández-Mena et al. 2014
			Huizache	Sinaloa	Hernández-Mena et al. 2014
Parastrigea plataleae Hernández-Mena, García Prieto y García-Varela, 2014	Adulto	*Platalea ajaja*	Laguna Superior	Oaxaca	Hernández-Mena et al. 2014
		Platalea ajaja	Chautengo	Guerrero	Hernández-Mena et al. 2014
		Platalea ajaja	Huizache	Sinaloa	Hernández-Mena et al. 2014

		Platalea ajaja	Topolobampo	Sinaloa	Hernández-Mena et al. 2014
***Apharyngostrigea* Ciurea, 1927**					
Apharyngostrigea multiovata (Pérez-Vigueras, 1944)	Adulto	*Ardea alba* *Egretta thula* *Nycticorax nycticorax*	Pátzcuaro	Michoacán	Ramos-Ramos, 1994
Apharyngostrigea cornu (Zeder, 1800)	Adulto	*Ardea alba*	Laguna Tres Palos	Guerrero	Violante-González et al. 2012
			Laguna de Coyuca	Guerrero	Violante-González et al. 2012
		Nyctanassa violacea	Laguna Tres Palos	Guerrero	Violante-González et al. 2012
Apharyngostrigea pipientis (Faust, 1918) (Syn. *A. cornu*)	Adulto	*Butorides virescens*	Tamiahua	Veracruz	Hernández-Mena et al. 2014; Locke et al. 2021
		Ardea alba	Río Pánuco	Veracruz	Hernández-Mena et al. 2014; Locke et al. 2021
			Tlacotalpan	Veracruz	Hernández-Mena et al. 2017; Locke et al. 2021
		Nycticorax nycticorax	El Huizache	Sinaloa	Hernández-Mena et al. 2014; Locke et al. 2021
Apharyngostrigea sp.	Adulto	*Nyctanassa violacea*	Cortadura	Veracruz	Hernández-Mena et al. 2014
Apharyngostrigea sp.	Metacercaria	*Astyanax fasciatus*	Noc-Choncunchey	Yucatán	Scholz et al. 1995
			Río Hondo	Quintana Roo	Scholz y Vargas-Vázquez, 1998
		Astyanax aeneus	Río La Fortuna	Chiapas	Salgado-Maldonado et al. 2011
		Bramocharax caballeroi	Río La Palma	Veracruz	CNHE
		Cichlasoma geddesi	Laguna El Vapor	Campeche	Aguirre-Macedo y García-Magaña, 1994; Salgado-Maldonado et al. 1997
		Cichlasoma mayorum	Cenote Xtoloc	Yucatán	Manter, 1936
		Cichlasoma synspilum	Laguna de Términos	Campeche	Vidal-Martínez y Kennedy, 2000

			Camellones-Chontales, Espino	Tabasco	Aguirre-Macedo y García-Magaña, 1994
		Cincelichthys pearsei	El Vapor	Campeche	Salgado-Maldonado et al. 1997
		Dormitator maculatus	Tlacotalpan	Veracruz	Salgado-Maldonado et al. 2005
		Goodea atripinnis	Río La Laja	Guanajuato	Salgado-Maldonado, 2006
		Mayaheros urophthalmus	Camellones-Chontales, Espino	Tabasco	Aguirre-Macedo y García-Magaña, 1994
			Laguna El Espino	Tabasco	Aguirre-Macedo y García-Magaña, 1994
		Petenia splendida	Chiribital	Tabasco	Osorio-Sarabia et al. 1987
		Poecilia mexicana	Río Candelaria, Huejutla	Hidalgo	Salgado-Maldonado et al. 2004
			Río Bonanza	Chiapas	Salgado-Maldonado et al. 2011
		Poeciliopsis gracillis	Río Candelaria, Huejutla	Hidalgo	Salgado-Maldonado et al. 2004
		Priapella compressa	Río Palenque	Chiapas	Salgado-Maldonado et al. 2011
		Thorichthys helleri	RíoTeapa	Tabasco	Aguirre-Macedo y García-Magaña, 1994; Salgado-Maldonado et al. 1997
		Thorichthys meeki	Río Hondo (La Unión)	Quintana Roo	Scholz y Vargas-Vázquez, 1998
			Lago El Chiribital	Tabasco	Vidal-Martínez, 1995
		Sphoeroides testudineus	Chelém	Yucatán	Pech et al. 2009
			Dzilam de Bravo	Yucatán	Pech et al. 2009
		Vieja argentea	San Pedro	Tabasco	López-Jiménez, 2001
		Vieja melanura	El Vapor	Campeche	Salgado-Maldonado et al. 1997
			Camellones-Chontales, Espino	Tabasco	Aguirre-Macedo y García-Magaña, 1994; Salgado-Maldonado et al. 1997
		Xiphophorus variatus	Río La Laja	Guanajuato	Salgado-Maldonado, 2006
Tribu: Cotylurini					
***Apatemon* Szidat, 1928**					
Apatemon gracilis (Rudolphi, 1819) Szidat, 1928	Adulto	*Ardea alba*	Coyuca	Guerrero	CNHE

			Tres Palos	Guerrero	CNHE
		Bucephala albeola	Ojo Caliente	Chihuahua	Gladden y Canaris, 2009
		Fulica americana	Laguna Tecocomulco	Hidalgo	Andrade-Rosales, 2012
Apatemon minor Yamaguti, 1933	Adulto	*Anas diazi*	Chiconahuapan	Estado de México	Soto-Méndez, 2006
***Australapatemon* Sudarikov, 1959**					
Australapatemon burti Miller, 1923	Adulto	*Anas diazi*	Chiconahuapan	Estado de México	Hernández-Mena et al. 2010
		Anas americana	Guerrero Negro	Baja California Sur	Hernández-Mena et al. 2010
		Anas cyanoptera	Chiconahuapan	Estado de México	Hernández-Mena et al. 2010
		Oxyura jamaicensis	Guatimape	Durango	Hernández-Mena et al. 2010
		Anas discors	Laguna Tecocomulco	Hidalgo	Andrade-Rosales, 2012
***Cotylurus* Szidat, 1928**					
Cotylurus brevis Dubois y Rausch, 1950	Adulto	*Anas clypeata*	Chiconahuapan	Estado de México	Soto-Méndez, 2006
		Anas cyanoptera	Laguna Atarasquillo	Estado de México	Martínez-Haro et al. 2012
		Oxyura jamaicensis	Río Lerma	Estado de México	Martínez-Haro et al. 2012
		Anas crecca	Río Lerma	Estado de México	Martínez-Haro et al. 2012
		Anas acuata	Río Lerma	Estado de México	Martínez-Haro et al. 2012
Cotylurus cornutus (Rudolphi, 1808)	Adulto	*Anas clypeata*	Chiconahuapan	Estado de México	Soto-Méndez, 2006
		Anas cyanoptera	Laguna Atarasquillo	Estado de México	Martínez-Haro et al. 2012
Cotylurus gallinulae (Lutz, 1928)	Adulto	*Gallinula chloropus*	Lerma	Estado de México	León-Règagnon, 1992
		Aythya affinis	La Esperanza	Sonora	Hernández-Mena, 2010
Cotylurus magniacetabulus Dubois y Angel, 1972	Adulto	*Anas acuta*	Chiconahuapan	Estado de México	Soto-Méndez, 2006
Cotylurus strigeoides Dubois, 1958	Adulto	*Bucephala albeola*	Ojo Caliente	Chihuahua	Gladden y Canaris, 2009
Cotylurus sp.	Adulto	*Larus occidentalis*	Guerrero Negro	Baja California Sur	Hernández-Mena, 2010
Cotylurus sp.	Metacercaria	*Dormitator maculatus*	Tlacotalpan	Veracruz	Salgado-Maldonado et al. 2005
		Thorichthys helleri	Laguna Yumká	Tabasco	CNHE
		Vieja intermedia	Cedros Lacanjá	Chiapas	CNHE

***Cardiocephaloides* Sudarikov, 1959**					
Cardiocephaloides medioconiger (Dubois y Pérez-Vigueras, 1949	Adulto	*Rynchops niger*	Tecolutla	Veracruz	Hernández-Rodríguez, 1995
		Larus sp.	Laguna de Términos	Campeche	Hernández-Mena et al. 2010
		Larus occidentalis	Guerrero Negro	Baja California Sur	Hernández-Mena et al. 2010
Cardiocephaloides sp.	Metacercaria	*Epinephelus morio*	Chiquila	Quintana Roo	Moravec et al. 1997
			Celestún	Yucatán	Moravec et al. 1997
			Progreso	Yucatán	Moravec et al. 1997
			Chelém	Yucatán	Moravec et al. 1997
			Chuburna	Yucatán	Moravec et al. 1997
		Strongylura notata	Río Lagartos	Yucatán	Tello-Osalde, 1999
		Sphoeroides testudineus	Río Lagartos	Yucatán	Tello-Osalde, 1999
Subfamilia: Duboisiellinae					
***Duboisiella* Baer, 1938**					
Duboisiella proloba Baer, 1930	Adulto	*Didelphis virginiana*	Playa Escondida	Veracruz	Monet-Mendoza et al. 2005
		Philander opossum	Oxolotlán	Tabasco	CNHE

CHNE: Colección Nacional de Helmintos, Instituto de Biología, UNAM.

I.VI. RELACIONES FILOGENÉTICAS DENTRO DE STRIGEIDAE RAILLIET, 1919

La clasificación taxonómica dentro de la familia Strigeidae se ha basado principalmente en caracteres morfológicos de las fases adultas y la asociación con su huésped definitivo (Dubois, 1938, 1968). Dubois (1938) dividió la familia Strigeidae en dos tribus (Strigeinae y Cotylurini) basado en la distribución de las glándulas vitelógenas localizadas en la región anterior del cuerpo. Sin embargo, el emplear sólo las características morfológicas de los organismos tiene algunas limitantes relacionadas con la plasticidad fenotípica, la similitud morfológica entre especies estrechamente relacionadas y la dificultad de asociar distintos estadios larvales con el estado adulto. En este sentido, es indispensable el uso de otras herramientas, como los caracteres moleculares en combinación con análisis morfológicos y ecológicos para determinar la diversidad de especies y las relaciones filogéneticas entre los géneros que conforman esta familia.

Actualmente, Strigeidae es considerada una familia compleja y filogenéticamente inestable. Recientes análisis filogenéticos inferidos con genes nucleares del DNA ribosomal (28S y 18S) y mitocondriales (*Cox 1*) demuestran que la familia Strigeidae es parafilética debido a que algunos géneros clasificados dentro de Strigeidae se anidan dentro de la familia Diplostomidae Poirier, 1886 (Olson et al. 2003; Fraija-Fernández et al. 2015; Hernández-Mena et al. 2017; Blasco-Costa et al. 2016; Blasco-Costa y Locke, 2017; Heneberg et al. 2018). Los análisis filogenéticos resultantes dividieron a la familia en dos clados principales; Strigeidae (I) conformado por especies pertenecientes a cinco géneros (*Strigea*, *Apharyngostrigea*, *Parastrigea, Apatemon* y *Australapatemon*) y Strigeidae (II) conformado por especies pertenecientes a cuatro géneros (*Cotylurus*, *Ichthyocotylurus*, *Cardiocephaloides* y *Nematostrigea*). Los resultados sugieren una subdivisión de la familia,

con el clado I como Strigeidae *sensu stricto* ya que incluye el género *Strigea* (género tipo), mientras que el segundo clado de estrigeidos podrían ser considerados como otra familia independiente (Olson et al. 2003; Blasco-Costa et al. 2016; Blasco-Costa y Locke, 2017; Hernández-Mena et al. 2017; Heneberg et al. 2018).

Aunado a esto, las diferencias morfológicas entre algunos géneros de la familia son sútiles. Por ejemplo, los géneros *Strigea*, *Parastrigea* y *Apharyngostrigea* se distinguen morfológicamente por la ausencia o presencia de una faringe, así como la distribución de las glándulas vitelógenas en el segmento anterior (Niewiadomska, 2002). Sin embargo, las características diagnósticas de cada grupo se sobrelapan entre algunas especies de estos géneros dificultando una correcta clasificación taxonómica del grupo (Dubois, 1968; Heneberg et al. 2018).

Particularmente en México, la mayoría de los registros que componen esta familia se han basado principalmente en análisis morfológicos (Pérez-Ponce de León, 2007). Por lo tanto, para esclarecer la taxonomía y sistemática de los géneros que componen esta familia es indispensable el uso de caracteres moleculares en combinación con análisis morfológicos y ecológicos para determinar la diversidad de especies dentro de estos grupos en México.

II. OBJETIVOS

II.I. OBJETIVO GENERAL

Generar un panorama integral de la taxonomía y sistemática de los géneros que conforman la familia Strigeidae asociados con aves acuáticas de México

II.II. OBJETIVOS PARTICULARES

l. Inferir las relaciones filogenéticas entre géneros y especies de estrigeidos empleando secuencias de genes del DNA nuclear ribosomal (lTSl, 5.8S, ITS2 y los dominios D2+D3 del 28S) y genes mitocondriales, citocromo oxidasa subunidad 1 (*Cox 1*).

2. Evaluar las hipótesis taxonómicas, incluyendo la descripción de nuevas especies.

3. Estimar las divergencias genéticas entre los géneros y especies de la familia Strigeidae.

III. RESULTADOS

Los resultados de la tesis se presentan en tres capítulos. El primer capítulo está conformado por un artículo de investigación publicado en la revista *Parasitology International* el cual forma parte del artículo de requisito, titulado "Molecular and morphological evidence suggest the reallocation from *Parastrigea brasiliana* (Szidat, 1928) Dubois, 1964 to *Apharyngostrigea* Ciurea, 1927 (Digenea: Strigeidae), a parasite of Boat-Billed Heron (*Cochlearius cochlearius*) from the Neotropical region". En este artículo, se generaron secuencias con dos genes nucleares (ITS y 28S) y un gen mitocondrial (*Cox 1*) para determinar las afinidades filogenéticas de una especie de estrigeido, *Parastrigea brasiliana* (Szidat, 1928) colectada en el intestino de la garza pico de bota (*Cochlearius cochlearius*) una especie de ave perteneciente a la familia Ardeidae. Los resultados derivados de los análisis filogenéticos inferidos con Máxima Verosimilitud e Inferencia Bayesiana demostraron que las nuevas secuencias identificadas morfológicamente como *Parastrigea brasiliana* se anida dentro del clado *Apharyngostrigea.* Morfológicamente, *P. brasiliana* comparte características diagnósticas de ambos géneros. La evidencia generada en este proyecto nos permitío tranferir la especie *Parastrigea brasiliana* al género *Apharyngostrigea* para formar la combinación *Apharyngostrigea brasiliana*.

El segundo capítulo está conformado por el artículo titulado "Phylogenetic analyses based on molecular and morphological data reveal a new species of *Strigea* Abildgaard, 1790 (Digenea: Strigeidae), and taxonomic changes in strigeids infecting Neotropical birds of prey" publicado en la revista *Journal of Helminthology*. En este artículo se realizó un estudio integrativo, en el cual se analizaron secuencias de dos genes nucleares (ITS y 28S) y un gen mitocondrial (*Cox 1*), en combinación con caracteres morfológicos y ultraestructurales de dos especies pertenecientes al género *Strigea* colectados de cuatro especies de aves rapaces

(*Rupornis magnirostris*, *Accipiter cooperii*, *Buteogallus urubitinga* y *B. athracinus*) a través de nueve localidades del Pacífico y Golfo de México. La información generada nos permitió describir una nueva especie para la ciencia y la reclasificación de la especie *Parastrigea macrobursa* al género *Strigea* formando la especie *Strigea macrobursa* n. comb.

El tercer capítulo está conformado por un artículo de investigación publicado en la revista *Parasitology Research*, titulado "Exploring the genetic structure of *Parastrigea diovadena* Dubois and Macko, 1972 (Digenea: Strigeidae) an endoparasite of the white ibis, *Eudocimus albus* from the Neotropical region of Mexico". En este artículo se abordó la estructura genética poblacional de una especie perteneciente al género *Parastrigea*, la cual se encuentra asociada al intestino del ibis blanco (*Eudocimus albus*) distribuido en tres provincias biogeográficas de México: Sierra Madre Occidental (SMO), Sierra Madre Oriental (SMOr) y la Sierra Madre del Sur (SMS).

III.I. MOLECULAR AND MORPHOLOGICAL EVIDENCE SUGGEST THE REALLOCATION FROM *PARASTRIGEA BRASILIANA* (SZIDAT, 1928) DUBOIS, 1964 TO *APHARYNGOSTRIGEA* CIUREA, 1927 (DIGENEA: STRIGEIDAE), A PARASITE OF BOAT-BILLED HERON (*COCHLEARIUS COCHLEARIUS*) FROM THE NEOTROPICAL REGION

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Parasitology International (2022) 86: 102468

https://doi.org/10.1016/j.parint.2021.102468

Parasitology International 86 (2022) 102468

Contents lists available at ScienceDirect

Parasitology International

journal homepage: www.elsevier.com/locate/parint

Molecular and morphological evidence suggests the reallocation from *Parastrigea brasiliana* (Szidat, 1928) Dubois, 1964 to *Apharyngostrigea* Ciurea, 1927 (Digenea: Strigeidae), a parasite of boat-billed heron (*Cochlearius cochlearius*) from the Neotropical region

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ARTICLE INFO

Keywords:
Strigeidae
Apharyngostrigea brasiliana
Boat-billed heron
Molecular markers
Taxonomy
Systematics

ABSTRACT

Parastrigea brasiliana (Szidat, 1928) Dubois, 1964, was described from (*Cochlearius cochlearius*) in South America. The taxonomy of this species has been unstable due that it was described as a member of *Strigea* Abildgaard, 1790. However, the same author one year later transferred it to *Apharyngostrigea* Ciurea, 1927 and since then, it has been alternatively placed in the genus *Apharyngostrigea* or *Parastrigea* Szidat, 1928 from Strigeidae. In the current research, specimens identified as *P. brasiliana* were collected from type host in southeastern Mexico. We sequenced three molecular markers: the internal transcribed spacers ITS1 and ITS2 including the 5.8S gene (ITS region), the D1-D3 domains of the large subunit (LSU) from nuclear DNA and cytochrome *c* oxidase subunit I (*cox 1*) from mitochondrial DNA. These sequences were aligned with other sequences available in the GenBank dataset from Strigeidae. Maximum likelihood and Bayesian analyses inferred with three molecular markers consistently showed that *P. brasiliana* is not closely related to other members of the genus *Parastrigea* and are placed in a reciprocal monophyletic clade inside *Apharyngostrigea,* with very low genetic divergence, varying from 0 to 0.09% for the ITS, from 0 to 0.08% for the LSU and from 0.21 to 0.43% for *cox 1*. Consequently, we proposed to reallocate it to *A. brasiliana*. The phylogenetic analyses obtained are key and very useful for re-evaluate the morphology of *A. brasiliana* because this species share morphological characters with the genera *Parastrigea* (concentration of vitelline follicles distributed in two lateral expansions on the forebody) and *Apharyngostrigea* (absence of pharynx). Finally, the current record of *A. brasiliana* expands its distribution range in four countries, namely, the USA, Mexico, Venezuela and Brazil, in the Neotropical region.

1. Introduction

Digeneas of the family Strigeidae are morphologically distinguished by having bodies divided into two segments (a forebody and a hindbody) and a cup-shaped forebody containing a holdfast organ with two lobes (ventral and dorsal) [1,2]. These digeneans are intestinal endoparasites of birds and rarely of mammals and are distributed worldwide. Currently, the family contains 13 genera with approximately 110 nominal species [2]. Recent phylogenetic studies based on sequences of nuclear and mitochondrial molecular markers of only nine of the 13 genera have suggested that Strigeidae was paraphyletic and was clearly divided into two clades: the first clade included the genera *Cardiocephaloides* Sudarikov, 1959, *Ichthyocotylurus* Odening, 1969, *Cotylurus* Szidat, 1928, and *Nematostrigea* Sandground, 1924, and the second clade included the genera *Apatemon* Szidat, 1928, *Australapatemon* Sudarikov, 1959, *Apharyngostrigea* Ciurea, 1927, *Parastrigea* Szidat, 1928, and *Strigea* Abildgaard, 1790. This second clade supported the sister relationship between *Apharyngostrigea* and *Parastrigea* [3–8].

Among strigeids, *Parastrigea* Szidat, 1928 is morphologically characterised by having two symmetrical groups of vitelline follicles in the lateral extensions of the holdfast in the forebody [2]. The 19 described species of this genus are associated mainly with accipitrid, anatid, ardeid, ciconiid, falconid, jacanid, larid, podicipedid, recurvirostrid, threskiornithid and tytonid birds worldwide [9]. In the Americas, 11

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https://doi.org/10.1016/j.parint.2021.102468
Received 28 June 2021; Received in revised form 29 August 2021; Accepted 7 September 2021
Available online 11 September 2021

species of the genus *Parastrigea* have been recorded, with six in South America (*Parastrigea brasiliana* (Szidat, 1928) Dubois, 1964; *Parastrigea caballeroi* Dubois, 1952; *Parastrigea cincta* (Brandes, 1888) Szidat, 1928; *Parastrigea diovadena* Dubois and Macko, 1972; *Parastrigea macrobursa* Drago and Lunaschi, 2011; *Parastrigea buffoni* Drago, Núñez and Lunaschi 2018), whereas five other species of *Parastrigea* are known in Middle America and North America, namely, *Parastrigea campanula* Dubois and Rausch, 1950; *Parastrigea mexicana* Coil, 1957; *Parastrigea ogchnocephala* Dubois and Rausch, 1950; *Parastrigea plataleae* Hernández-Mena, García-Prieto and García-Varela, 2014 and *Parastrigea tulipoides* Miller and Harkema, 1965 [1,9–11].

The boat-billed heron, *Cochlearius cochlearius* (Linnaeus), is a nocturnal species of bird from Ardeidae that inhabits the margins of rivers, lakes, swamps, and marshes, feeding upon amphibians, small fish, crustaceans, insects and small vertebrates, and its distribution range extends from south Mexico down through Middle America and south America as far south as Peru to the west and southeastern Brazil [12,13]. The parasite fauna of boat-billed herons has been documented in some locations from three countries, Mexico, Nicaragua and Brazil, in the Neotropical region. To date, 9 species of helminths have been recorded, with the trematodes exhibiting the highest species diversity with three species, *i.e.*, *Parastrigea brasiliana*; *Irinaia brenesi* Caballero-Caballero and Bravo-Hollis, 1965; *Ascocotyle* sp. and undescribed species of Strigeidae gen. sp., followed by three species of cestodes, *Parvitaenia cochlearii* Coil, 1955; *Valipora spinosa* Fuhrmann, 1908 and *Cyclustera capito* (Rudolphi, 1819) Furhmann, 1901, and two species of nematodes, *Contracaecum multipapillatum* (Drasche, 1882) Baylis, 1920 and *Porrocaecum* sp. [1,14–18].

During a helminthological survey in southeastern Mexico, adult strigeids were collected from the intestine of the boat-billed heron. These specimens were identified morphologically as *Parastrigea brasiliana*. However, the taxonomic history of this species has been controversial because it was originally described within the genus *Strigea* (Szidat, 1928), and then it has alternatively been considered a member of *Apharyngostrigea* or *Parastrigea* because it shares morphological characteristics of both genera: two symmetrical groups of vitelline follicles distributed in the forebody, as in *Parastrigea,* and by the absence of a pharynx, as in *Apharyngostrigea* [1,8,9,19–21]. Therefore, the aims of this study were (1) to provide an update of the morphological description of adult specimens of *Parastrigea brasiliana* recorded in the intestine of the boat-billed heron from the Neotropical region of Mexico and (2) to test the systematic position within strigeids using molecular data from domains D1- D3 from the large subunit (LSU), the internal transcribed spacer (ITS1, ITS2) plus 5.8S from nuclear ribosomal DNA and the cytochrome *c* oxidase subunit I (*cox 1*) from mitochondrial DNA.

2. Materials and methods

2.1. Specimen collection

A total of four boat-billed herons (*C. cochlearius*) were collected between August 2013 and February 2020 in a single locality (Champotón, Campeche; 19° 21′ 49.9″ N, 90° 42′ 56.15″ W) in southeastern Mexico. The digestive tract of birds was dissected and placed in separate Petri dishes containing a 0.75% saline solution and examined using a stereomicroscope. Digeneans collected were fixed in hot 4% formaldehyde solution for morphological studies, and other specimens were preserved in 100% ethanol for DNA analyses. Avian definitive host was identified using the field guide Howell and Webb [22] and the American Ornithologist Union [12] guidelines.

2.2. Morphological analyses

For morphological analysis, specimens were stained with Mayer's paracarmine, dehydrated in a graded ethanol series, cleared with methyl salicylate and mounted in Canada balsam. Specimens were examined and measured using a compound microscope Leica DM 1000 LED equipped with bright field (Leica, Wetzlar, Germany). All measurements are in micrometers and presented as the range followed by the mean in parentheses. Voucher specimens were deposited in the Colección Nacional de Helmintos (CNHE) from Instituto de Biología, Universidad Nacional Autónoma de México (UNAM), México City.

For scanning electron microscopy (SEM), four specimens identified as *P. brasiliana* were dehydrated with an ethanol series, critical point dried, sputter coated with gold, and examined with a Hitachi Stereoscan Model S-2469 N scanning electron microscope operating at 15 kV from the Instituto de Biología, Universidad Nacional Autónoma de México (UNAM).

2.3. DNA isolation, amplification and sequencing

Total genomic DNA was isolated from six specimens identified as *P. brasiliana* preserved in 100% ethanol. Specimens were placed individually in tubes and digested overnight at 56 °C in a solution containing 10 mM Tris–HCl (pH = 7.6), 20 mM NaCl, 100 mM Na2 EDTA (pH = 8.0), 1% Sarkosyl, and 0.1 mg/ml proteinase K. Following digestion, DNA was extracted from the supernatant using the DNAzol reagent (Molecular Research Center, Cincinnati, Ohio) according to the manufacturer's instructions. Two regions of nuclear ribosomal DNA (rDNA) were amplified using the polymerase chain reaction (PCR). The ITS1, 5.8S and ITS2 region was amplified using the forward primer BD1 5′-GTCGTAACAAGGTTTCCGTA-3′ [23] and the reverse primer BD2 5′-ATCTAGACCGGACTAGGCTGTG-3 [24]. Partial fragments of the large subunit (28S) of the ribosomal RNA gene (domains D1-D3) were amplified using the forward primer BD3, 5′-GAACATCG ACATCTTGAACG-3′ [11] and the reverse primer 536, 5′ -CAGCTATCCTGAGGGAAAC-3′ [25]. A fragment of *cox* 1 was amplified using the forward primer Mplat-COX1dF, 5'-TGTAAAACGACGGCCAGTTTWCITTRGATCATAAG-3′ and the reverse primer Mplat-COX1dR, 5′- CAGGAAACAGCTATGACTGAAAYAAYAIIGGATCICCACC -3′ [26].

PCR reactions (25 μl) consisted of 10 μl of each primer, 2.5 μl of 10 × buffer, 2 mM MgCl2, and 1 U of Taq DNA polymerase (Platinum Taq, Invitrogen Corporation, São Paulo, Brazil). PCR cycling parameters for the three molecular markers consisted of denaturation at 94 °C for 1 min, 35 cycles of 94 °C for 1 min, 50 °C for 1 min and 72 °C for 1 min, followed by a post-amplification incubation at 72 °C for 10 min. Sequencing reactions were performed using ABI Big Dye (Applied Biosystems, Boston, Massachusetts) terminator sequencing chemistry, and reaction products were separated and detected using an ABI 3730 capillary DNA sequencer. Contigs were assembled and base-calling differences resolved using Codoncode Aligner version 9.0.1 (Codoncode Corporation, Dedham, Massachusetts) and submitted to GenBank dataset (Table 1).

2.4. Alignments and phylogenetic analysis

Newly generated sequences for the ITS1-5.8S-ITS2, 28S rDNA and *cox 1* gene of *P. brasiliana* were aligned with other sequences of strigeids downloaded from GenBank dataset (Table 1). Sequences of each molecular marker were aligned separately using the software Clustal W [27]. A nucleotide substitution model was selected for each molecular marker using jModelTest v2.1.7 [28] applying the Akaike criterion. The best nucleotide substitution models for each data set were GTR + G + I. Phylogenetic trees were inferred through maximum likelihood (ML) with the program RAxML v7.0.4 [29], and Bayesian inference (BI) analyses were inferred with MrBayes 3.2.2 [30] using the computational resource Cyberinfrastructure for Phylogenetic Research (CIPRES) Science Gateway v3.3 [31]. Trees were edited using FigTree v1.4.4 [32]. Genetic divergences were estimated using *p* uncorrected distances with MEGA v.6 [33].

Table 1
Summary data for the taxa used in the phylogenetic analyses.

Species	Host species	Locality	Life cycle stage	GenBank accesion number			Source
				ITS	28S	COI	
Apharyngostrigea brasiliana	*Cochlearius cochlearius*	Champotón, Campeche, Mexico	A	**MZ614714**	**MZ614708**	**MZ614754**	Current study
			A		**MZ614709**	**MZ614755**	
			A	**MZ614715**	**MZ614710**		
			A	**MZ614716**	**MZ614711**	**MZ614753**	
			A	**MZ614717**	**MZ614712**	**MZ614756**	
			A	**MZ614718**	**MZ614713**	**MZ614757**	
Apharyngostrigea pipientis	*Biomphalaria tenagophila*	Belo Horizonte, Minas Gerais, Brazil	C	MT974151			Locke et al. [7]
Apharyngostrigea pipientis	*Botaurus lentiginosus*	Montreal, area Quebec, Canada	A	MT677870	MT677870	MT679576	Locke et al. [7]
Apharyngostrigea pipientis	*Rana pipiens*	Boucherville, Quebec, Canada	M	HM064966		HM064883	Locke et al. [40]
	Rana pipiens	Boucherville, Quebec, Canada	M	HM064968		HM064884	Locke et al. [40]
Apharyngostrigea pipientis	*Nycticorax nycticorax*	North Dakota, USA	A		JF820597		Pulis et al. [43]
Apharyngostrigea pipientis (as *A. cornu*)	*Ardea alba*	Pánuco, Veracruz, Mexico	A	JX977837		JX977777	Hernández-Mena et al. [11]
Apharyngostrigea pipientis (as *A. cornu*)	*Butorides virescens*	Tamiahua, Veracruz, Mexico	A	JX977838		JX977778	Hernández-Mena et al. [11]
Apharyngostrigea pipientis (as *A. cornu*)	*Nycticorax nycticorax*	Huizache, Sinaloa, Mexico	A	JX977839	MF398345	JX977779	Hernández-Mena et al. [4,11]
Apharyngostrigea sp. (as *A. cornu*)	*Ardea alba*	Tlacotalpan, Veracruz, Mexico	A		MF398344		Hernández-Mena et al. [4]
Apharyngostrigea cornu	*Ardea herodias, Catostomus commersoni, Notemigonus crysoleucas, Pimephales notatus*	Quebec, Canada	A, M	HM064969			Locke et al. [40]
Apharyngostrigea cornu	*Ardea cinerea*	Kherson region, Ukraine	A		AF184264		Tkach et al. [44]
Apharyngostrigea sp. (as *A. cornu*)	*Nyctanassa violacea*	Cortadura, Veracruz, Mexico	A	JX977840		JX977780	Hernández-Mena et al. [11]
Apharyngostrigea simplex	*Egretta thula*	Laguna Juancho, Buenos Aires, Argentina	A	MK510081		MK570088	Locke et al. [7]
Apharyngostrigea simplex	*Poecilia reticulata* (exp.)	Belo Horizonte, Minas Gerais, Brazil	M	MN179273			López-Hernández et al. [8]
Apharyngostrigea simplex	*Biomphalaria straminea*	Belo Horizonte, Minas Gerais, Brazil	C			MN179319	López-Hernández et al. [8]
Apharyngostrigea simplex	*Cnesterodon decemmaculatus*	La Plata, Buenos Aires, Argentina	M			MH777789-91	López-Hernández et al. [8]
Parastrigea plataleae	*Platalea ajaja*	Laguna Superior, Oaxaca, Mexico	A	JX977834		JX977774	Hernández-Mena et al. [11]
	Platalea ajaja	Huizache, Sinaloa, Mexico	A		MF398346		Hernández-Mena et al. [4]
Parastrigea diovadena	*Eudocimus albus*	Tamiahua, Veracruz, Mexico	A	JX977811		JX977751	Hernández-Mena et al. [11]
	Eudocimus albus	Pijijiapan, Chiapas, Mexico	A		MF398348		Hernández-Mena et al. [4]
Parastrigea cincta	*Eudocimus albus*	San Blas, Nayarit, Mexico	A	JX977820		JX977760	Hernández-Mena et al. [11]
	Eudocimus albus	Caimanero, Sinaloa, Mexico	A		MF398347		Hernández-Mena et al. [4]
Strigea falconis	*Circus aeruginosus*	Bartošovice, Czech Republic	A	MF628087			Heneberg et al. [5]
Strigea sp.	*Pelophylax* sp.	Der-Chantecoq, France	M		KT362372		Patrelle et al. [45]
Strigea sp.	*Caracara cheriway*	Presa La Angostura, Chiapas, Mexico	A		MF398343	MF398319	Hernández-Mena et al. [4]
Apatemon sp. '*jamiesoni*'	*Gobiomorphus cotidianus*	Lake Waipori, New Zealand	M	KT334170		KT334182	Blasco-Costa et al. [6]
	Phalacrocorax punctatus	Otago Harbour, New Zealand	A		KT334169		Blasco-Costa et al. [6]
Australapatemon niewiadomski	*Anas platyrhynchos*	Balclutha, New Zealand	A	KT334175	KT334165	KT334180	Blasco-Costa et al. [6]
Australapatemon burti	*Anas diazi*	Estado de Mexico, Mexico	A	JX977787	MF398342	JX977727	Hernández-Mena et al. [4,11]
Australapatemon burti	*Anas cyanoptera*	Estado de Mexico, Mexico	A	JX977786		JX977726	Hernández-Mena et al. [11]
Australapatemon burti	*Anas americana*	Guerrero Negro, Baja California Sur, Mexico	A	JX977785		JX977725	Hernández-Mena et al. [11]
Cardiocephaloides longicollis	*Larus argentatus*	Kherson Oblast, Ukraine	A	MN820662		MN817944	Achatz et al. [39]
	Larus ridibundus	Kherson Region, Ukraine	A		AY222171		Olson et al. [46]
Cardiocephaloides medioconiger	*Larus* sp.	Laguna de Términos, Campeche, Mexico	A	JX977842		JX977782	Hernández-Mena et al. [11]

(*continued on next page*)

Table 1 (*continued*)

Species	Host species	Locality	Life cycle stage	GenBank accesion number			Source
				ITS	28S	COI	
Cardiocephaloides medioconiger	*Thalasseus maximus*	Mississippi, USA	A	MN820664	MN820664	MN817946	Achatz et al. [39]
Cardiocephaloides sp.	*Larus occidentalis*	Guerrero Negro, Baja California Sur, Mexico	A	JX977844	MF398341	JX977784	Hernández-Mena et al. [4,11]
Cardiocephaloides physalis	*Spheniscus magellanicus*	Chile	A		MN820665	MN817947	Achatz et al. [39]
Cotylurus gallinulae	*Aythya affinis*	La Esperanza, Sonora, Mexico	A		MF398340	JX977781	Hernández-Mena et al. [4,11]
Ichthyocotylurus pileatus	*Perca flavescens*	Lawrence River, Quebec, Canada	M	HM064931		HM064725	Locke et al. [40]
Ichthyocotylurus erraticus	*Coregonus autumnalis*	Lough Neagh, Northern Ireland, Ukraine	M		AY222172		Olson et al. [46]
Nematostrigea serpens	*Pandion haliaetus*	Republic of Karelia Russia	A		KF434762		Lebedeva and Yakovleva [47]
Tylodelphys aztecae	*Podilymbus podiceps*	Lago de los Reyes Aztecas, Ciudad de Mexico	A	KT175388	MF398337	KT175323	García-Varela et al. [48]

Sequences in bold were obtained on the current study.
A (adult), M (metacercaria), C (cercaria).

3. Results

3.1. Phylogenetic analyses

The ITS data set included 31 sequences with 1151 characters. The phylogenetic analyses inferred with ML and BI showed that the genus *Parastrigea* is paraphyletic because two subgroups were formed (Fig. 1). The first contained three species of the genus *Parastrigea, i.e.*, *P. plataleae* (JX977834), *P. diovadena* (JX977811) and *P. cincta* (JX977820) parasites of threskiornithid birds recorded in the Neotropical region of Mexico. The second subgroup was formed by the five new sequences of *P. brasiliana* and was nested inside of *Apharyngostrigea*. These five sequences of *P. brasiliana* formed a clade that is sister to another clade that contained nine isolates of *Apharyngostrigea*, four determined to be

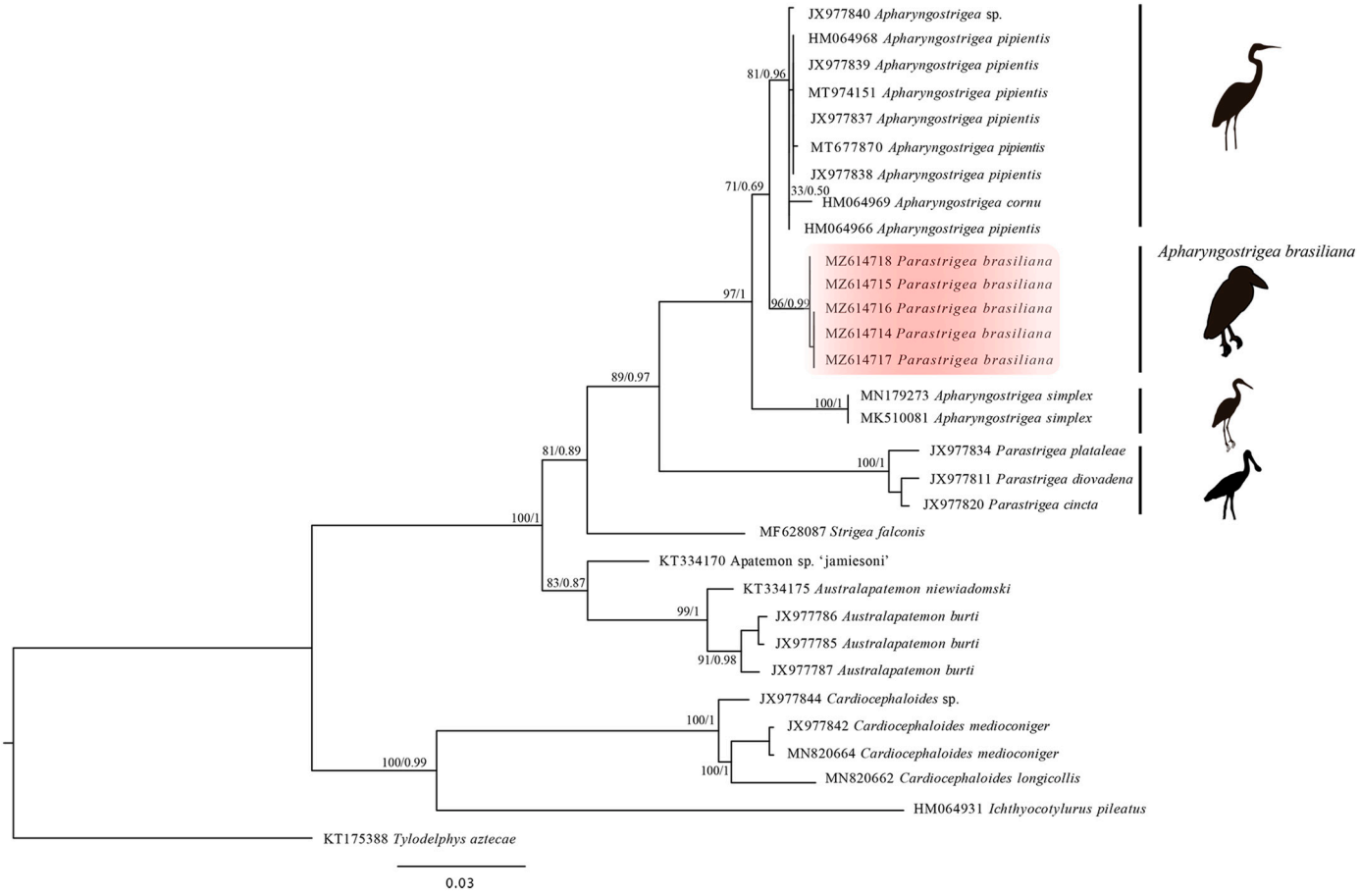

Fig. 1. Phylogenetic trees inferred with maximum likelihood and consensus Bayesian Inference with the ITS1, 5.8S and ITS2 data set. Numbers near internal nodes show ML bootstrap percentage values and Bayesian posterior probabilities (BI).

A. pipientis (Faust, 1918) (HM064968; MT974151; MT677870; HM064966), four sequences were identified originally as *A. cornu* Zeder, 1800 from Mexico and reassigned as *A. pipientis* and *Apharyngostrigea* sp. by Locke et al. [7] (JX977837–840), and one sequence was determined to be *A. cornu* (HM064969). Both clades are sister to two isolates identified as *Apharyngostrigea simplex* (Johnston, 1904) (MK510081; MN179273). All these relationships received well bootstrap values and Bayesian posterior probabilities (Fig. 1). The intraspecific genetic divergence among the five isolates of *P. brasiliana* was very low, ranging from 0 to 0.09% for the ITS sequences. The LSU data included 27 sequences with 1205 characters. The phylogenetic analyses inferred with ML and BI also indicated that *Parastrigea* is paraphyletic (Fig. 2). Three species from *Parastrigea, i.e.*, *P. plataleae* (MF398346), *P. diovadena* (MF398348) and *P. cincta* (MF398347), formed a sister clade to other clades formed by species of the genus *Apharyngostrigea* (Fig. 2). The base of this subclade was formed by a polytomy that contained four isolates, two identified as *A. pipientis* (MT677870; JF820597) plus two isolates originally identified as *A. cornu* and reassigned as *Apharyngostrigea* sp. and *A. pipientis* by Locke et al. [7] (MF398344; MF398345). This subclade is sister to six isolates identified as *P. brasiliana* plus a single sequence identified as *A. cornu* (AF184264) and was supported with strong bootstrap values and Bayesian posterior probabilities (96/1) (Fig. 2). The intraspecific genetic divergence among the six isolates of *P. brasiliana* ranged from 0 to 0.08% for the LSU. Finally, the *cox 1* dataset included 34 sequences with 465 characters. The phylogenetic analyses inferred with ML and BI also showed that the genus *Parastrigea* is paraphyletic (Fig. 3). One clade contained three species of the genus *Parastrigea*., *i.e.*, *P. plataleae* (JX977774), *P. diovadena* (JX977751) and *P. cincta* (JX977760); the second clade was formed by five isolates identified as *P. brasiliana* and was nested inside of *Apharyngostrigea* (Fig. 3). This clade is sister to other subclades formed by five isolates identified as *Apharyngostrigea simplex* from Argentina and Brazil (MN179319, MH777789–91, MK570088). Both subclades were sister to other clades formed by four isolates originally identified as *A. cornu* from Mexico (JX977780, JX977777–779) and reassigned as *Apharyngostrigea* sp. and *A. pipientis,* respectively, by Locke et al. [7], plus three isolates identified as *A. pipientis* (HM064883–884, MT679576) (Fig. 3). All these relationships received high bootstrap values and Bayesian posterior probabilities. Finally, the intraspecific genetic divergence among the five isolates of *P. brasiliana* ranged from 0.21 to 0.43% for *cox 1*.

In summary, the phylogenetic analyses inferred with two nuclear markers and one mitochondrial molecular marker supported the paraphyly of the genus *Parastrigea* with high bootstrap values and Bayesian posterior probabilities (Figs. 1–3). The new ITS, LSU and *cox 1* sequences of specimens identified as *P. brasiliana* formed a clade nested inside *Apharyngostrigea*, and as a result, it should be transferred to *Apharyngostrigea*, *i.e.*, to the genus in which the species was placed by Szidat [34].

3.2. Morphological description

Family Strigeidae Railliet, 1919.
Subfamily Strigeinae Railliet, 1919.
Genus *Apharyngostrigea* Ciurea, 1927.

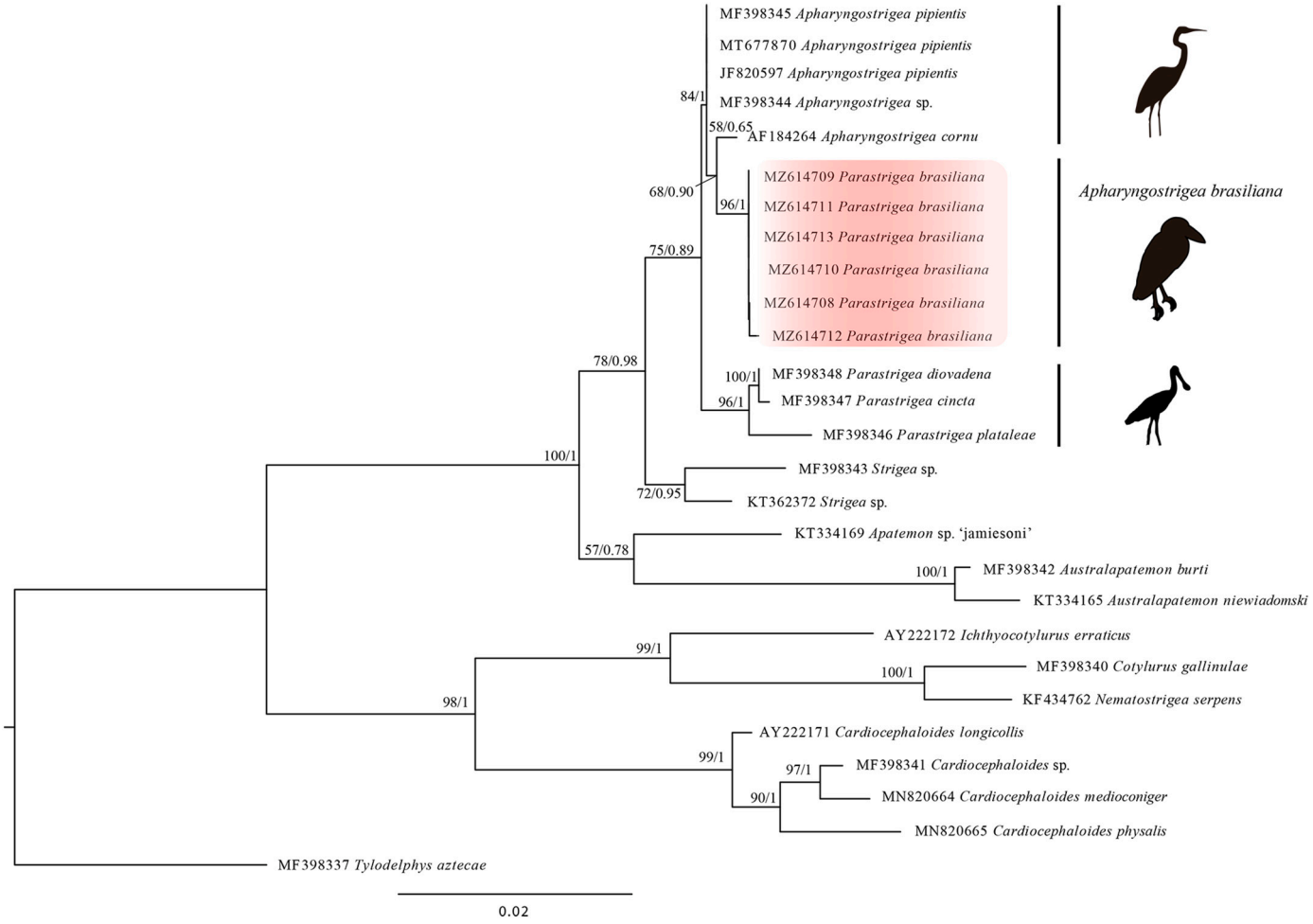

Fig. 2. Phylogenetic trees inferred with maximum likelihood and consensus Bayesian Inference with the LSU data set. Numbers near internal nodes show ML bootstrap percentage values and Bayesian posterior probabilities (BI).

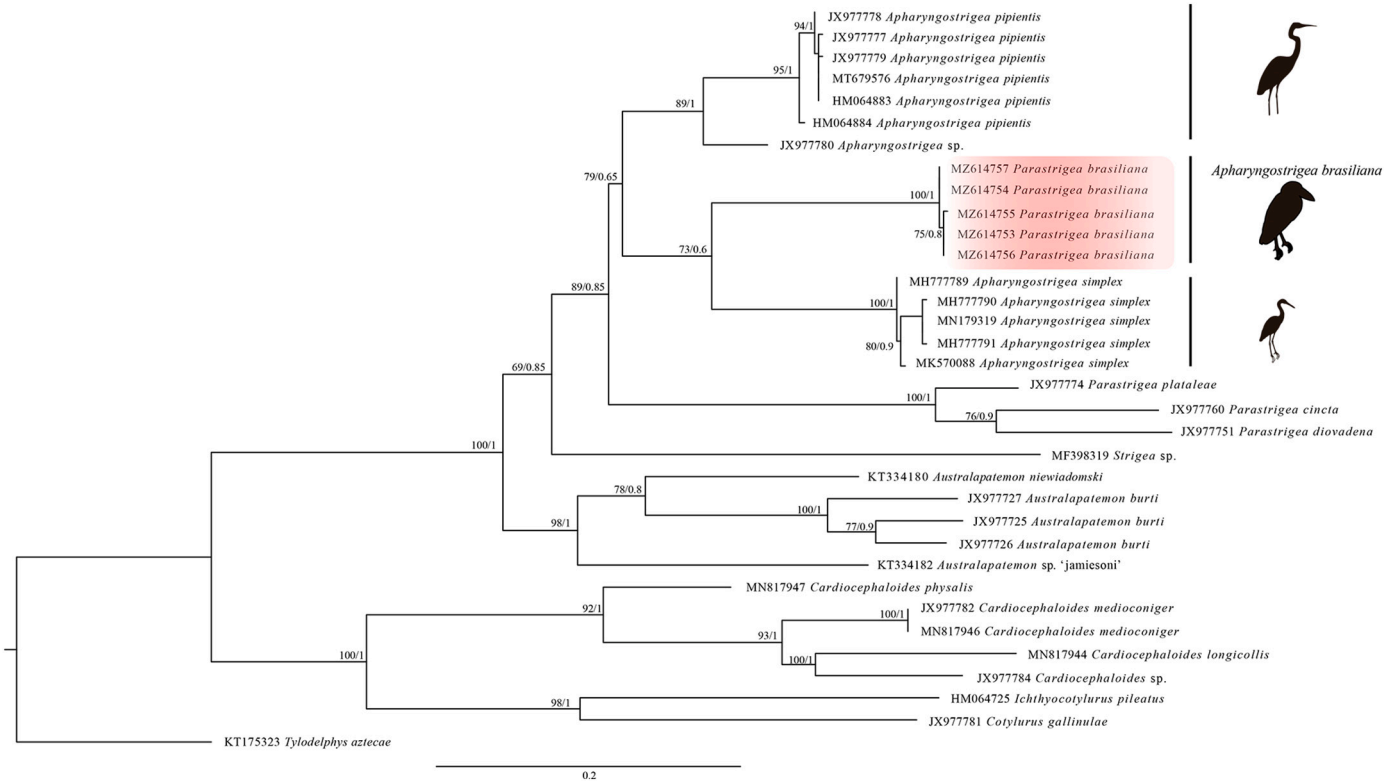

Fig. 3. Phylogenetic trees inferred with maximum likelihood and consensus Bayesian Inference with the *cox 1* data set. Numbers near internal nodes show ML bootstrap percentage values and Bayesian posterior probabilities (BI).

Apharyngostrigea brasiliana Szidat, 1929.
Syn. Strigea brasiliana Szidat, 1928.
Syn. Parastrigea brasiliana (Szidat, 1928) Dubois, 1964.
Host: *Cochlearius cochlearius* Linnaeus (Pelecaniformes: Ardeidae), boat-billed heron.
Locality: Champotón, Campeche, Mexico (19° 21′ 49.9″ N, 90° 42′ 56.15″ W).
Site of infection: Intestine.

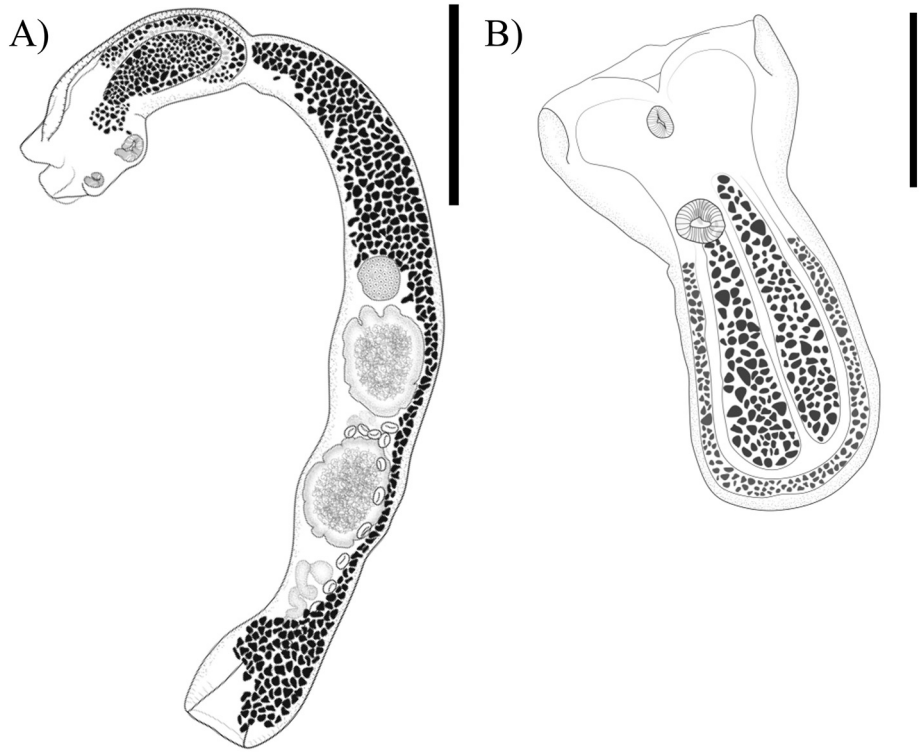

Fig. 4. *Apharyngostrigea brasiliana* from *Cochlearius cochlearius*, (A) Whole worm, lateral view; (B) Forebody, ventral view; Scale bars = 1000 μm (A); 500 μm (B).

Prevalence: 50%.

Voucher specimens: CNHE 8956.

GenBank accession number: MZ614708–18, MZ614753–57.

Description (Figs. 4A–B, 5A–E; Table 2)

Based on 17 gravid adults. Body distinctly bipartite, 2.7–5.4 mm (4.035 mm) in total length (Figs. 4A, 5A). Tegument smooth (Fig. 5A). Forebody covering approximately ¼ of total length of body and are divided in a campaniform anterior region that includes both suckers, and a piriform posterior region with vitelline follicles distributed in two lateral expansions, 616–1536 × 451–696 (1080 × 522) (Figs. 4B, 5B–D). Ratio of forebody length to body length: 1: 3.53–4.41 (3.97). Hindbody claviform, longer than forebody 1867–4405 × 445–718 (2906 × 588), with maximum width in testicular zone. Ratio of forebody length to hindbody length: 1: 2.69–3.03 (2.86). The first third of hindbody is occupying for the neck 587–1720 × 360–643 (987 × 470). Ratio of neck length to hindbody length: 1: 2.56–3.1 (2.94) (Fig. 4). Oral sucker subterminal, weakly developed 77–120 × 61–92 (100 × 72). Ventral sucker spherical to oval, larger than oral sucker: 124–164 × 92–166 (140 × 121), located above constriction. Suckers width ratio: 1: 1.14–1.80 (1.56). Pharynx absent. Holdfast organ well-developed dorsal and ventral lips, with two voluminous symmetrical expansions of vitelline follicles. Proteolytic gland not observed. Testes in tandem: multilobed, situated in second third of hindbody. Anterior testis 283–616 × 300–546 (435 × 445), posterior testis slightly larger than anterior testis 296–654 × 330–570 (471 × 455). Seminal vesicle long, sinuous, postesticular. Ovary oval or reniform, pretesticular 115–289 × 128–344 (170 × 221) situated approximately 31–39/100 of hindbody. Mehlis' gland and vitelline reservoir in intertesticular region. Vitelline follicles distributed in both regions of body: in forebody concentrate two voluminous symmetrical masses, elongated, claviform, disperse laterally up to height of sucker ventral, whereas that in hindbody the vitelline follicles are concentrated in neck (preovarian region), extending ventrally to seminal vesicle or copulatory bursa (Fig. 4). Copulatory bursa large, delimited by moderate constriction. Muscular ring (*Ringnapf*) present (Fig. 5E). Genital cone small, with strong muscular wall, 290–586 × 194–628 (414 × 431). Eggs numerous 5–26 (15), oval 79–93 × 40–61 (85 × 51). Excretory pore terminal.

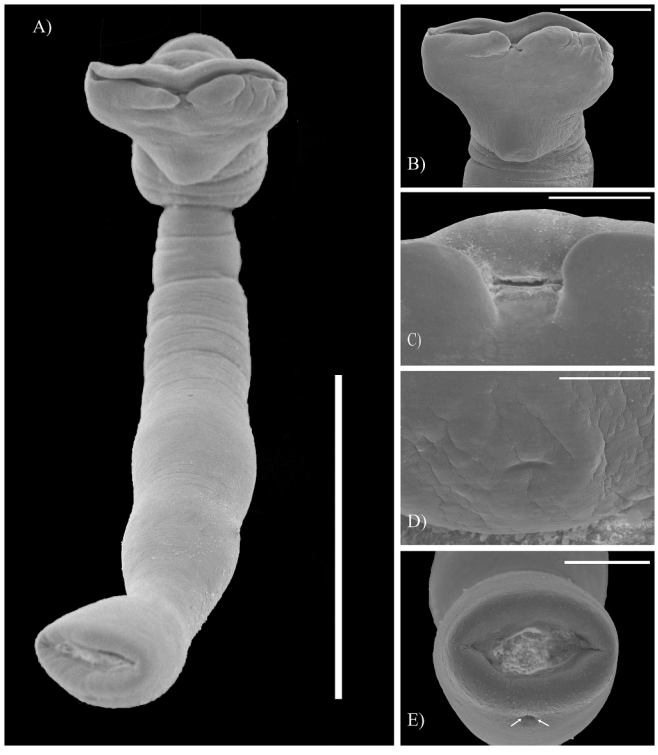

Fig. 5. Scanning electron micrographs of *Apharyngostrigea brasiliana* from *Cochlearius cochlearius*, (A) Whole worm, ventral view; (B) Forebody, ventral view; (C) Oral sucker; (D) Ventral view of the forebody at the level of the ventral sucker; (E) Copulatory bursa, arrows indicate the excretory pore. Scale bars = 1000 μm (A); 300 μm (B); 100 μm (C); 50 μm (D); 200 μm (E).

3.2.1. Remarks

Apharyngostrigea brasiliana was originally described as *Strigea brasiliana* by Szidat [35] from a single specimen recovered from the intestine of the boat-billed heron *Cochlearius cochlearius* from Brazil [1,19,34]. Later Dubois [19], examined a few specimens recovered from the type host in the jardin zoologique, (Artis) Amsterdam and transferred it to genus *Parastrigea*. Morphologically, *A. brasiliana* is distinguished from its congeners by having a forebody divided in a campaniform anterior region that contains both suckers, a pyriform posterior region with vitelline follicles distributed in two lateral expansions, a diagnostic characteristic considered of the genus *Parastrigea* (see Figs. 4B, 5B–D). In addition, it possesses a long neck region occupying approximately one-third of the hindbody and absence of pharynx, a diagnostic feature of the genus *Apharyngostrigea*. Our specimens collected from the intestine of the boat-billed heron (type host) in a single locality (Champotón, Campeche) from the Neotropical region of Mexico are morphologically similar to *A. brasiliana* (Figs. 4 and 5). However, our specimens showed some level of morphological intraspecific variation, and it could be due that Szidat [35], described *A. brasiliana* from a single specimen. Later Dubois [17] expanded the diagnosis of the species by using five specimens (see Table 2). For instance, some metrical data of newly collected material showed some level of morphological intraspecific variation with respect to previous descriptions performed by Szidat [1] and Dubois [17], possess lower limits for the following characteristics: body size (2718–5428 *vs* 7600), forebody width (451–696 *vs* 710–1000), hindbody length (1867–4405 *vs* 4000–6000), ratio of hindbody length to forebody length (2.69–3.03 *vs* 3.1–4.8), anterior testes width (300–546 *vs* 640–820) and posterior testes width (330–570 *vs* 640–850) (see Table 2). *A. brasiliana* has been reported parasitizing ardeid birds in the Americas, such as *Cochlearius cochlearius*, *Nyctanassa violacea* (Linnaeus), *Butorides striatus* (Linnaeus), *Egretta thula* (Molina), and *Tigrisoma lineatum* (Boddaert) from Brazil and *A. alba* from Brazil and Venezuela [9,36]. Finally, in North America, *A. brasiliana* was reported in *A. herodias* and *A. alba* from the USA [37].

4. Discussion

The phylogenetic analyses obtained are very useful and key for the reevaluation of the morphological features of *A. brasiliana* because its taxonomy has been controversial since its description [1,34,35]. Dubois [19] transferred it to *Parastrigea* by having a concentration of vitelline follicles distributed in two lateral expansions on the forebody, and since then, it has been placed alternatively in *Apharyngostrigea* or *Parastrigea* [8,9,21,38].

The ML and Bayesian trees obtained in the current study, inferred with three molecular markers, consistently showed that the samples identified morphologically as *P. brasiliana* are not closely related to other members of the genus *Parastrigea;* consequently, we proposed to reallocate them to *Apharyngostrigea* [1,34]. Our phylogenetic analyses placed all the samples of *A. brasiliana* in a reciprocal monophyletic clade inside *Apharyngostrigea,* with very low genetic divergence, varying from 0 to 0.09% for the ITS, from 0 to 0.08% for the LSU and from 0.21 to 0.43% for *cox 1*. The low level of genetic divergence found with the three molecular markers among specimens is consistent with previous studies in other groups of strigeids. For example, the interspecific genetic divergence among specimens of three species of *Parastrigea* ranged from 0.5 to 1.48% for the ITS [11], and the intraspecific genetics divergence detected among isolates of *A. pipientis* from Nearctic, Neotropical and Afrotropic regions varied from 0 to 1.9% for *cox 1* and among isolates of *A. simplex* from Brazil and Argentina ranged from 0.21

Table 2
Comparative measurements of adults *Apharyngostrigea* spp. recorded in the Americas.

Species	*A. brasiliana*	*A. brasiliana* (syn. *S. brasiliana*)	*A. brasiliana* (syn. *S. brasiliana; P. brasiliana*)	*A. ardearum* (cited as *A. brasiliana*)	*A. pipientis*	*A. simplex*	*A. multiovata*	*A. cornu*
Host	*Cochlearius cochlearius*	*Cochlearius cochlearius*	*Cochlearius cochlearius*	*Ardea alba Bubulcus ibis*	*Ardea alba egretta, Ardea herodias, Botaurus lentiginosus, Nycticorax nycticorax*	*Egretta thula*	*Egretta thula*	Ardeidae
Locality	Champotón, Campeche, Mexico	Brazil	Brazil	Buenos Aires, Argentina	Formosa, Argentina; Quebec, Canada	Buenos Aires, Argentina	Cuba	
Source	Present study	Szidat [34]	Dubois [1]	Labriola and Suriano [38]	Locke et al. [7]	Ostrowski de Núñez [41]	Dubois [1]	Dubois [1]
Body length	2718–5428 (4035)	7000	7600	4370–5970	1575–4000 (2906)	2017– 4881 (2823)	Up to 6500	Up to 6300
Forebody length	616–1536 (1080)	–	1000–1650	1150–1350	650–1000 (823)	462–1215 (690)	680–1400	600–2200
Forebody width	451–696 (552)	–	710–1000	980–1250	476–677 (577)	364–1045 (593)	480– 1370	630–1700
Hindbody length	1867–4405 (2906)	–	4000–6000	2870–3290	850–3000 (2074)	1290–3666 (2153)	2890–5000	1400–4500
Hindbody width	445–718 (588)	900	480–920	670–830	300–551 (412)	316–972 (525)	400–930	270–1320
HL/FL	2.69–3.03 (2.86)	–	3.1–4.8	2.13–2.63 (2.39)	1.2–3.2 (2.5)	2.2–6.9 (5.7)	2.8–4.9	1.6–5.2
Oral sucker length	77–120 (100)	74	80–96	150–210	103–153 (134)	63–185 (129)	126–186	100–260
Oral sucker width	61–92 (72)		100–120	130–210	50–174 (99)	71–168 (113)	100–152	95–260
Ventral sucker length	124–164 (140)	170	145–162	230–250	120–251 (166)	126–268 (191)	179–293	180–340
Ventral sucker width	92–166 (121)	–	175–200	210–310	104–172 (143)	134–252 (186)	124–219	180–360
Proteolytic gland length	–	–	–	270–420	121–320 (212)	151–378 (261)	360–475	160–450
Proteolytic gland width	–	–	–	240–250	183–270 (222)	117–252 (175)	170–210	120–350
Ovary length	115–289 (170)	200	210–260	230–330	160	101–268 (147)	143–440	100–460
Ovary width	128–344 (221)	300	340–360	170–250	87	84–412 (182)	164–499	150–640
Ovary position in HB	31–39/100	–	33–43/100	38–42/100	50/100	near midline	41–54/100	27–49/100
Anterior testes length	283–616 (435)	800	500–740	560–690	238–338 (294)	269–630 (374)	350–960	130–720
Anterior testes width	300–546 (445)	–	640–820	520–720	222–436 (291)	210–840 (384)	330–770	220–1160
Posterior testes length	296–654 (471)	800	550–740	600–630	238–413 (332)	252–630 (387)	350–890	160–880
Posterior testes width	330–570 (455)	–	640–850	580–770	160–436 (283)	252–840 (393)	330–770	220–1200
Genital cone length	290–586 (414)	–	–	270–310	–	–	233–400	240–530
Genital cone width	194–628 (431)	–	–	200–225	–	–	188–330	180–510
Eggs (N)	5–26 (15)	–	–	35[a]	0–40 (12)	9–100	numerous	few
Egg length	79–93 (85)	90	84–94	90–100	64–103 (85)	90–110	81–110	80–118
Egg width	40–61 (51)	55	53–63	50–70	45–60 (54)	50–70	50–70	50–75

[a] Calculated from Fig. 2 in Labriola and Suriano [38].

to 1.30% [8]. In contrast, the interspecific genetic divergence detected in the present study among *Apharyngostrigea* spp., ranged from 1.4 to 3.3% for the ITS, from 0.2 to 0.4% for the LSU and from 10 to 12.2% for *cox 1*. These ranges of divergence are similar to those previously reported in *Cardiocephaloides* spp., which ranged from 1.9 to 6.9% for the ITS and from 0.4 to 1.6% for the LSU, while those for *Apatemon* spp. ranged from 0.9 to 2.1% for the ITS, and that of *Australapatemon* spp. was 1.9% for the ITS [6,11,39]. Finally, the genetic divergence among *Apharyngostrigea* sp., *A. simplex* and *A. pipientis* ranged from 2.78 to 3.42% for ITS and from 6.13 to 14.29% for *cox 1* [8].

To date, the genus *Apharyngostrigea* contains 20 described species and is highly specific ardeid birds worldwide [1,20]. With the reallocation of *A. brasiliana,* the Americas harbour six species: *A. cornu*; *A. pipientis*; *A. simplex*; *A. multiovata* (Vigueras, 1944) Dubois and Vigueras, 1949; and *A. ardearum* (Lutz, 1928) Dubois, 1968 (see Table 2) [7,11,38,40,41].

The current record of *A. brasiliana* from the boat-billed heron in southeastern Mexico represents a new locality record for *A. brasiliana* in Mexico expanding its distribution range in four countries, namely, the USA, Mexico, Venezuela and Brazil, in the Neotropical region. The broad

distribution range of *A. brasiliana* suggests that this species could parasitize multiple intermediate and definitive hosts along its distribution range [36,37]. Although the complete life cycle of *A. brasiliana* is unknown, available evidence from other congeneric species, such as *A. pipientis* and *A. cornu* from the Americas, suggests that the freshwater planorbid snail of the genus *Biomphalaria* (Preston) serves as the first intermediate host, where furcocercous cercariae emerge, searching for vertebrates such as freshwater fishes, hylid and ranid anurans, which serve as second intermediate hosts where the cercaria turn into encysted metacercariae; finally, when the second intermediate hosts are consumed by Ardeid birds, then the life cycle is completed [7,8,41,42]. It is well known that the species *Apharyngostrigea* uses multiple hosts to complete their life cycles with a broad distribution range. For example, Locke et al. [7] found that *A. pipientis* is a species widely distributed in the Nearctic, Neotropical and Afrotropic regions and is associated with different intermediate and definitive hosts. López-Hernández et al. [8] detected cercaria and metacercariae of *Apharyngostrigea simplex* from Argentina and Brazil in South America.

The systematics of strigeid trematodes are complex, and recent molecular evidence suggests that the family is paraphyletic. Previous phylogenies supported the close relationships among *Apharyngostrigea*, *Parastrigea* and *Strigea* [3–8]. Apparently, the distribution of the vitelline follicles in the forebody and hindbody, the absence of a pharynx and the type of definitive host are characteristic keys to delineate the genera. For example, *Parastrigea flexilis* (Dubois, 1934), Dubois 1955, has vitelline follicles distributed in two symmetrical masses on the forebody but lacks a pharynx, and it associates with prey birds of the genus *Circus* (Lacepede) from the Palearctic region [1]. Heneberg et al. [5] performed one of the most exhaustive phylogenetic analyses of strigeid species from central Europe by using multiple molecular markers. The authors recognized that *Parastrigea* and *Strigea* are paraphyletic, and therefore, some species were reclassified; for example, *Parastrigea flexilis, Strigea sphaerula* (Rudolphi, 1803) and *Strigea vandenbrokae* Dubois, 1966 were transferred to the genus *Amphistoma* Rudolphi, 1801. The same authors also transferred *Parastrigea robusta* to *Strigea* because *S. robusta* (Szidat, 1928) Heneberg and Stiko, 2018 is sister to *Strigea strigis* (type species). The current study provides new information about the taxonomy of the genus *Apharyngostrigea*, which allowed us to better understand the evolution of this enigmatic group of parasites.

5. Conclusions

Previous studies have sampled considerable strigeid diversity. The genetic library has increased significantly in the last few years. The nuclear genes from the ITS and the LSU from DNA ribosomes plus cytochrome *c* oxidase subunit I (*cox 1*) from mitochondrial DNA are very helpful molecular markers in resolving taxonomic controversies and establishing phylogenetic relationships at the family level [3–8]. Our phylogenetic trees inferred with more species from *Parastrigea* and *Apharyngostrigea* consistently showed that the samples identified as *P. brasiliana* were not closely related to other members of the genus *Parastrigea,* and in consequence, we proposed to reallocate them to *Apharyngostrigea*. With the reallocation of *A. brasiliana* to *Apharyngostrigea*, the genus contains 20 described species that are highly specific to ardeid birds worldwide. The current record of *A. brasiliana* from the boat-billed heron in southeastern Mexico represents a new locality record, expanding its distribution in four countries, namely, the USA, Mexico, Venezuela and Brazil, in the Neotropical region.

Funding

This research was supported by grants from the Programa de Apoyo a Proyectos de Investigación e Innovación Tecnológica (PAPIIT-UNAM) IN207219.

Acknowledgements

The first author thanks the support of the Programa de Posgrado en Ciencias Biológicas, UNAM and CONACYT (CVU: 706119) for granting a scholarship to complete her PhD program. The specimens collected were under the Cartilla Nacional de Colector Científico (FAUT 0202) issued by the Secretaria dem Medio Ambiente y Recursos Naturales (SEMARNAT). We are grateful to Laura Márquez and Nelly López for their help during the sequencing of the DNA fragments. We also thank Berenit Mendoza Garfias for her help in obtaining the scanning electron microphotographs.

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III.II. PHYLOGENETIC ANALYSES BASED ON MOLECULAR AND MORPHOLOGICAL DATA REVEAL A NEW SPECIES OF *STRIGEA* ABILDGAARD, 1790 (DIGENEA: STRIGEIDAE) AND TAXONOMIC CHANGES IN STRIGEIDS INFECTING NEOTROPICAL BIRDS OF PREY

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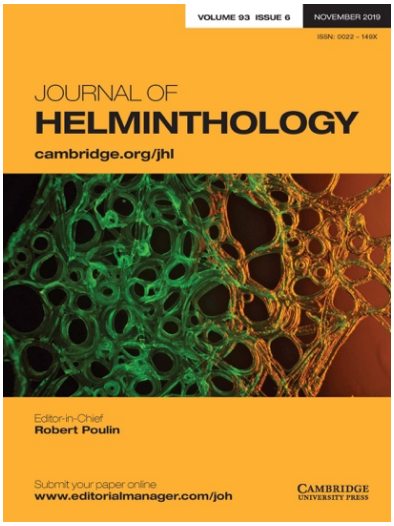
Journal of Helminthology (2023) 97, e35: 1–15.

https://doi.org/10.1017/S0022149X23000196



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Phylogenetic analyses based on molecular and morphological data reveal a new species of *Strigea* Abildgaard, 1790 (Digenea: Strigeidae) and taxonomic changes in strigeids infecting Neotropical birds of prey

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Research Paper

Cite this article: López-Jiménez A, González-García MT, Andrade-Gómez L, García-Varela M (2023). Phylogenetic analyses based on molecular and morphological data reveal a new species of *Strigea* Abildgaard, 1790 (Digenea: Strigeidae) and taxonomic changes in strigeids infecting Neotropical birds of prey. *Journal of Helminthology* **97**, e35, 1–15. https://doi.org/10.1017/S0022149X23000196

Received: 2 March 2023
Revised: 21 March 2023
Accepted: 22 March 2023

Keywords:
Strigeidae; phylogeny; *Strigea*; molecular markers; taxonomy

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Abstract

Members of the genus *Strigea* Abildgaard, 1790 are endoparasites of birds distributed worldwide. Adults of an undescribed species of the genus *Strigea* were collected from the intestines of two hawk species (*Rupornis magnirostris* and *Accipiter coperii*). Other species identified as *Parastrigea macrobursa* that were described in Argentina were also recovered from two hawk species (*Buteogallus urubitinga* and *Buteogallus anthracinus*) in three localities along the coasts of Mexico. Specimens of the two species were sequenced for three molecular markers, the internal transcribed spacers locus (ITS1-5.8S rDNA- ITS2) and the domains D1–D3 from the large subunit from nuclear ribosomal DNA and the cytochrome *c* oxidase subunit 1 from mitochondrial DNA. The newly sequenced specimens were aligned with other strigeids sequences downloaded from GenBank. Maximum likelihood and Bayesian analyses inferred with each molecular marker revealed that our specimens of *Strigea* sp. formed an independent lineage, which is recognized herein as a new species, *Strigea magnirostris* n. sp., representing the first species in Mexico and the 16th in the Neotropical region. Morphologically, the new species is distinguished from other congeneric species from the Americas by having an oral sucker with several papillae around it, well-developed pseudosuckers (118–248 µm), a tegument covered with tiny spines, a larger cone genital (193–361 × 296–637) and a larger copulatory bursa (247–531 × 468–784). Our phylogenetic analyses revealed that *P. macrobursa* is not closely related to other members of the genus *Parastrigea* and is nested within *Strigea*, suggesting that *P. macrobursa* should be transferred to *Strigea* to form *Strigea macrobursa* n. comb., expanding its distribution range from Mexico to Argentina. Finally, the analyses also revealed that the taxonomy and systematics of *Strigea* should be re-evaluated, combining morphological and molecular characteristics.

Introduction

The cosmopolitan family Strigeidae Railliet, 1919 currently contains 13 genera with approximately 110 nominal species distributed worldwide (Niewiadomska, 2002). The type genus *Strigea* was established by Abildgaard, 1790 to accommodate species that have vitellarium uniformly distributed over both parts of the body and the presence of a pharynx (Dubois, 1968; Niewiadomska, 2002). Among strigeids, *Strigea* is considered the most diverse genus within the family, with approximately 47 nominal species associated mainly with strigiform, accipitriform, falconiform, ciconiiform, caprimulgiform, cariamiform, passeriform, gruiform, trogoniform and anseriform birds (Dubois, 1968; Drago *et al.*, 2014). Information on the life cycle of most species of *Strigea* is scarce, but it is thought to involve four hosts. Adult worms live and reproduce sexually in the digestive tracts of birds that serve as definitive hosts. Eggs are expelled into the environment with the faeces of the host. After the ingestion of the eggs by a planorbid snail, which serves as the first intermediate host, the parasites develop into cercariae. The cercariae emerge and swim to find and penetrate the second intermediate host (amphibians), where they develop into mesocercariae and in some cases it may cause severe morphological anomalies as the polydactyly (Sinsch *et al.*, 2019; Svinin *et al.*, 2020, 2023). The amphibian with the mesocercaria is ingested by the third intermediate host (an amphibian, reptile, bird, or small mammal) and then the parasite develops into an encysted, tetracotyle-type metacercaria. Finally, these amphibians, reptiles, birds and mammals

https://doi.org/10.1017/S0022149X23000196 Published online by Cambridge University Press

are some of the principal food resources of prey birds, in which the life cycle is completed (Pearson, 1959, 1972; Odening, 1967).

To date, *Strigea* contains 47 described species, nine of which are distributed in Asia, nine in Africa, five in Oceania and three in Europe (Dubois, 1968, 1988; Dubois & Beverley-Burton, 1971). In the Americas, 21 species have been described, of which six are in North America (*Strigea infundibuliformis* Dubois, 1934; *Strigea macroconophora* Dubois and Rausch, 1950; *Strigea elegans* Chandler and Rausch, 1947; *Strigea sphaerula macrosicya* Dubois and Rausch, 1950; *Strigea gruis* Dubois and Rausch, 1964 and *Strigea macropharynx* Dubois and Rausch, 1965); and 15 species in South America (*Strigea caryophylla* (Diesing, 1850) Mathias, 1925; *Strigea elliptica* (Brandes, 1888) Szidat, 1928; *Strigea bulbosa* (Brandes, 1888) Szidat, 1928; *Strigea nugax* Szidat, 1928; *Strigea vaginata* (Brandes, 1888) Szidat, 1928; *Strigea falconis brasiliana* Szidat, 1929; *Strigea caluri* Dubois, 1962; *Strigea sphaerocephala* (Westrumb, 1823) Dubois, 1937; *Strigea microbursa* Pearson and Dubois, 1985; *Strigea magniova* Dubois, 1988; *Strigea arcuata* Dubois, 1988; *Strigea meridionalis* Lunaschi and Drago, 2009; *Strigea inflecta* Lunaschi and Drago, 2012; *Strigea orbiculata* Lunaschi and Drago, 2013 and *Strigea proteolytica* Drago, Lunaschi and Draghi, 2014) (Dubois, 1968; Lunaschi & Drago, 2006, 2009, 2012, 2013; Drago *et al.*, 2014). The morphological identification of *Strigea* spp. is complex and problematic due to their small size and the difficulty in observing internal and external structures used for taxonomy and differentiation among species (Lunaschi & Drago, 2006, 2009, 2012, 2013; Drago *et al.*, 2014). Additionally, molecular data are scarce and only a few sequences of *Strigea* are currently available (Hernández-Mena *et al.*, 2017; Heneberg *et al.*, 2018; Svinin *et al.*, 2020). In Mexico, the metacercaria of *Strigea* was recorded for the first time by Vidal-Martínez (1995) in two cichlid fish species in south-eastern Mexico (Pérez-Ponce de León *et al.*, 2007). However, the specimens were not deposited, and therefore, the records could not be verified. Hernández-Mena *et al.* (2017) recorded an adult of *Strigea* sp. in crested caracara (*Caracara cheriway* Jacquin) in Presa La Angostura, Chiapas, Mexico. Recently, strigeids in Mexico have started to receive attention and much effort has been made to incorporate morphological and molecular characteristics to describe and delineate the biodiversity of this group of parasites (Hernández-Mena *et al.*, 2014, 2017; López-Jiménez *et al.*, 2021, 2022).

In the current study, adult specimens of the genus *Strigea* were collected from the intestine of roadside hawk (*Rupornis magnirostris* Gmelin) and Cooper's hawk (*Accipiter cooperii* Bonaparte) in six localities from the Neotropical region of Mexico. After a careful morphological examination, the specimens were determined to correspond to an undescribed species of the genus *Strigea*. In addition, other strigeids collected from the intestine of the great black hawk (*Buteogallus urubitinga* Gmelin) and common black hawk (*Buteogallus anthracinus* Deepe) were identified as *Parastrigea macrobursa* Drago & Lunaschi, 2011, a species previously described in South America.

The objectives of the present research were: (a) to provide a morphological description of the new species; and (b) to test the systematic position of *P. macrobursa* by using sequences of the internal transcribed spacers (ITS1-5.8S rDNA- ITS2) and large subunit (LSU) of the nuclear DNA and of the cytochrome *c* oxidase subunit I (*cox 1*) gene of the mitochondrial DNA. We then used the resulting phylogenetic trees as a framework to discuss host–parasite associations and begin to understand the evolutionary history of this group of strigeids.

Materials and methods

Specimen collection

A total of 17 hawks were collected between December 2019 and December 2021 in nine localities from Mexico (fig. 1; table 1). Ten individuals of roadside hawk (*R. magnirostris*), one Cooper's hawk (*A. cooperii*), two individuals of great black hawk (*Buteogallus urubitinga*) and four common black hawks (*B. anthracinus*). Birds were identified following Howell & Webb (1995) and the American Ornithologist' Union (1998) guidelines. Strigeids were removed from the intestines of the birds and examined using a stereomicroscope. Digeneans collected were relaxed in hot distilled water and preserved in 100% ethanol for morphological and molecular analyses.

Morphological analyses

Digeneans preserved in 100% ethanol were stained with Mayer's paracarmine (Merck, Darmstadt, Germany), dehydrated in ethanol series, cleared in methyl salicylate and mounted in Canada balsam for morphological analysis. Specimens were examined using a compound microscope equipped with a bright field Leica DM 1000 light emitting diode microscope (Leica, Wetzlar, Germany). Measurements were taken using Leica Application Suite microscope software (Leica Microsystems GmbH, Wetzlar, Germany) and are given in micrometres and presented with the range followed by the mean in parentheses. Some specimens were dehydrated with an ethanol series, critical point dried, sputter coated with gold and examined with a Hitachi Stereoscan Model S-2469N scanning electron microscope operating at 15 kV. Voucher specimens from the present study were deposited in the Colección Nacional de Helmintos (CNHE) from Instituto de Biología, Universidad Nacional Autónoma de México (UNAM), Mexico City.

DNA isolation, amplification and sequencing

Strigeids preserved in 100% ethanol were placed individually in tubes and digested overnight at 56°C in a solution containing 20 mM sodium chloride, 10 mM Tris–hydrochloride (pH = 7.6), 100 mM ethylenedinitrilotetraacetic acid disodium salt dihydrate (pH = 8.0), 1% Sarkosyl and 0.1 mg/ml proteinase K. Following digestion, DNA was extracted from the supernatant using the DNAzol reagent (Molecular Research Center, Cincinnati, Ohio). The internal transcribed spacers (ITS1-5.8S rDNA- ITS2) of the nuclear ribosomal DNA were amplified using the forward primer BD1 5′-GTCGTAACAAGGTTTCCGTA- 3′ (Bowles & McManus, 1993) and the reverse primer BD2 5′-ATCTAG ACCGGACTAGGCTGTG-3′ (Bowles *et al.*, 1995). The partial fragments of domains D1–D3 of the large subunit of nuclear ribosomal RNA (LSU) were amplified with the forward primer 391 5′-AGCGGAGGAAAAGAAACTAA-3′ (Nadler *et al.*, 2000) and the reverse primer 536, 5′ -CAGCTATCCTGAGGGAAAC-3′ (García-Varela & Nadler, 2005). The complete gene of the cytochrome *c* oxidase subunit 1 (*cox* 1; 850 base pairs (bp)) was amplified using the forward primers AphaF, 5′-TAT GATTTTTTTYTTTTTRATG-3′ and the reverse primer

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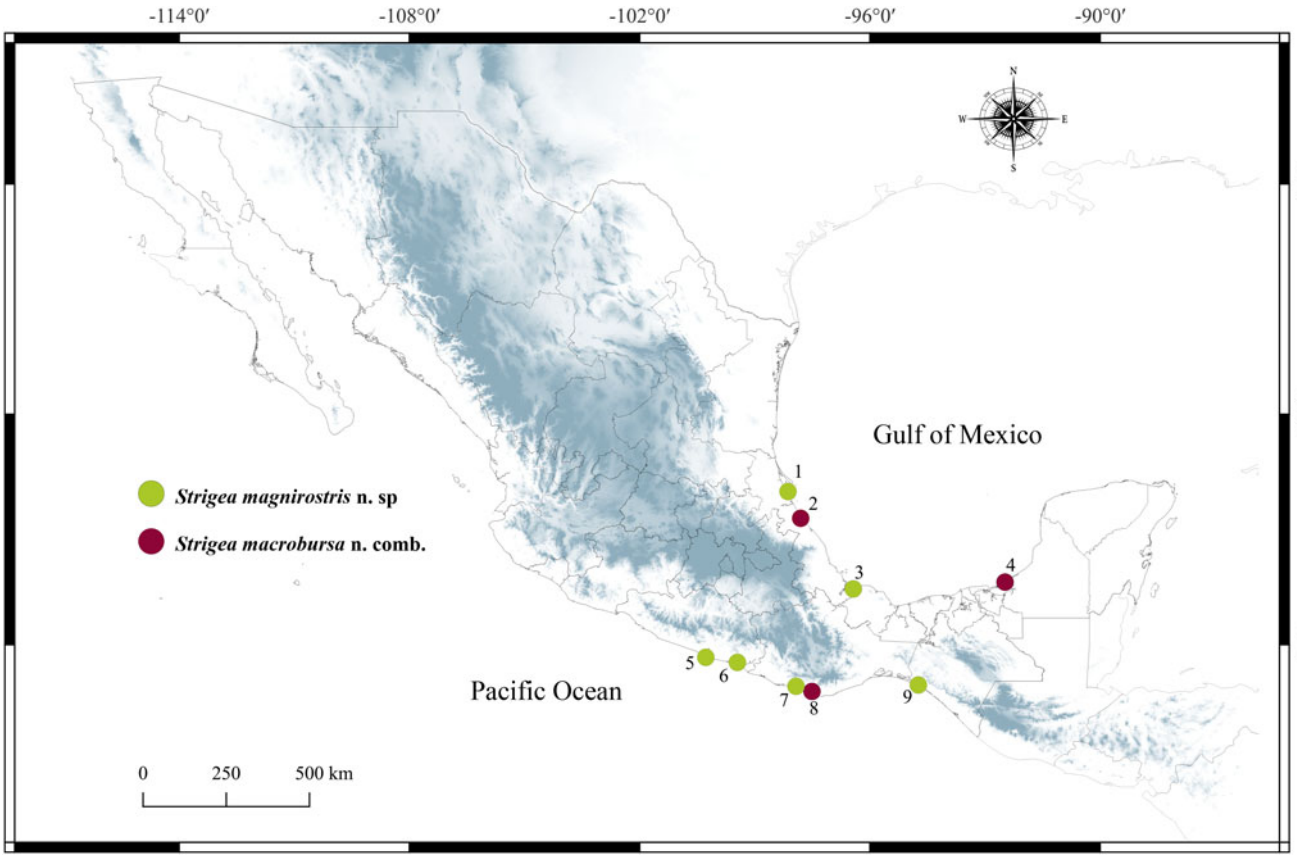

Fig. 1. Map of Mexico showing the sampled sites for the birds. Localities with a circle with colours green and red were positive for the infection with *Strigea magnirostris* n. sp. and *Strigea macrobursa* n. comb., respectively. Localities correspond to those in table 1.

JB4.5′-TAAAGAACATAATGAAATTG3′ (Bowles *et al.*, 1992). Polymerase chain reactions (PCRs) were carried out in 25 µl reactions, consisted of 1 µl of each primer, 2.5 µl of 10 × buffer, 1.5 µl MgCl2, 0.5 µl of dNTP mixture, 0.125 µl of Platinum Taq DNA polymerase (Invitrogen Corporation, São Paulo, Brazil) and 2 µl of genomic DNA. PCR cycling parameters for amplifications consisted of denaturation at 94°C for 1 min, 35 cycles of 94°C for 1 min, 50°C for 1 min and 72°C for 1 min, followed by a post-amplification incubation at 72°C for 10 min. Sequencing reactions were performed using ABI Big Dye (Applied Biosystems, Boston, Massachusetts) terminator sequencing chemistry and reaction products were separated and detected using an ABI 3730 capillary DNA sequencer. Contigs were assembled and base-calling differences resolved using Codoncode Aligner version 9.0.1 (Codoncode Corporation, Dedham, Massachusetts) and submitted to the GenBank dataset (table 1).

Alignments and phylogenetic analysis

Newly-generated sequences of ITS, LSU and *cox* 1 were aligned with other sequences of strigeids available in the GenBank dataset. Sequences of each molecular marker were aligned using the software CLUSTAL_X (Thompson *et al.*, 1997). The best nucleotide substitution model was selected for each molecular marker using jModelTest v2.1.7 (Posada, 2008) and applying the Akaike information criterion. The best nucleotide substitution model for the ITS and LSU dataset were TVM + I + G and for *cox* 1 the dataset was TIM3 + I + G. Phylogenetic trees were reconstructed through maximum likelihood (ML) with the program RAxML v7.0.4 (Silvestro & Michalak, 2012), and Bayesian inference (BI) analyses were inferred with MrBayes 3.2.2 (Ronquist *et al.*, 2012) using the computational resource Cyberinfrastructure for Phylogenetic Research Science Gateway v3.3 (Miller *et al.*, 2010). ML analyses were inferred with the option GTRGAMMAI and 10,000 bootstrap replicates. BI analyses included Markov chain Monte Carlo searches of two simultaneous runs for 10 million generations, sampling every 1000 generations, a heating parameter value of 0.2 and a burn-in of 25%. Trees were drawn and edited using FigTree software v1.4.0 (Rambaut, 2012). Genetic divergences were estimated using *P* uncorrected distances with MEGA v.6 (Tamura *et al.*, 2013).

Results

Molecular characterization and phylogenetic analyses

Nuclear genes

The ITS dataset included 42 sequences with 1042 characters. The phylogenetic analyses performed with ML and BI showed that the genus *Strigea* is monophyletic and is subdivided into two major subclades (fig. 2). The first subclade was formed by 17 isolates of an undescribed species of *Strigea* sp. from the roadside hawk (*R. magnirostris*) and Cooper's hawk (*A. cooperii*) collected from six localities in Mexico. This clade is sister to another subclade formed by eight isolates identified morphologically as *P. macrobursa* recovered from the great black hawk (*Buteogallus urubitinga*) and common black hawk (*B. anthracinus*) from

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Table 1. Specimens' information for *Strigea* spp., locality, state, geographical coordinates, host name, number of host examined/infected (prevalence of infection) and GenBank accession number for specimens studied in the current study.

Locality	State	Coordinates	Host	Host infected/ host revised	Species of *Strigea*	Internal transcribed spacers	Large subunit	Cytochrome *c* oxidase subunit 1
1. Tamiahua	Veracruz	21°18′02″ N 97°26′56.2″ W	*Rupornis magnirostris*	1/1	*S. magnirostris* n. sp.	OQ647944	OQ647911	OQ648146
						OQ647941	OQ647912	OQ648143
2. Tecolutla	Veracruz	20°33′49.8″ N 97°05′57.7″ W	*Buteogallus urubitinga*	1/1	*S. macrobursa* n. comb.	OQ647932	OQ647927	OQ648131
						OQ647933	OQ647928	OQ648130
						OQ647936	OQ647929	OQ648132
						OQ647935	OQ647930	OQ648133
3. Tlacotalpan	Veracruz	18°37′04.15″ N 95°38′56.10″ W	*R. magnirostris*	2/2	*S. magnirostris* n. sp.	OQ647942	OQ647913	OQ648142
						OQ647943	OQ647909	
						OQ647948	OQ647914	OQ648144
						OQ647940	OQ647910	OQ648145
4. Isla Aguada	Campeche	18°48′22.92″ N 91°28′03.68″ W	*B. urubitinga*	1/1	*S. macrobursa* n. comb.	OQ647937	OQ647923	
						OQ647938	OQ647924	
						OQ647939	OQ647925	
								OQ648128
							OQ647926	OQ648129
5. Tres Vidas	Guerrero	16°43′59.85″ N 99°42′48.99″ W	*R. magnirostris*	1/3	*S. magnirostris* n. sp.	OQ647949	OQ647917	OQ648135
			Accipiter cooperii	1/1		OQ647945	OQ647915	OQ648140
						OQ647946		OQ648141
						OQ647947	OQ647916	OQ648147
6. Marquelia	Guerrero	16°35′40.88″ N 98°50′37.90″ W	*R. magnirostris*	1/1	*S. magnirostris* n. sp.	OQ647950	OQ647918	OQ648136
						OQ647951	OQ647919	OQ648137
						OQ647952		OQ648138
7. Villa de Tututepec	Oaxaca	15°56′10.98″ N 97°13′38.87″ W	*R. magnirostris*	1/2	*S. magnirostris* n. sp.	OQ647953	OQ647920	OQ648139
			B. anthracinus	0/3				
8. Santa María, Cocotepec	Oaxaca	15°48′24.56″ N 97°00′49.79″ W	*B. anthracinus*	1/1	*S. macrobursa* n. comb.	OQ647934	OQ647931	OQ648134
9. El Zapotal	Chiapas	15°58′20.26″ N 93°51′23.04″ W	*R. magnirostris*	1/1	*S. magnirostris* n. sp.	OQ647954	OQ647921	
						OQ647955	OQ647922	
						OQ647956		OQ648148
								OQ648149
								OQ648150

The sample number for each locality corresponds with the same number in Figure 1.

three localities in Mexico. All these relationships were supported with well-supported bootstrap values and Bayesian posterior probabilities (fig. 2). The intraspecific genetic divergence among 17 isolates of *Strigea* sp. was low, ranging from 0 to 0.3%, whereas that for *P. macrobursa* ranged from 0 to 0.2% for ITS. The LSU dataset consisted of 21 terminals and 1208 characters. The tree topologies inferred using the LSU dataset from the nuclear DNA showed that the genus *Strigea* is paraphyletic because the genus was subdivided into two major clades. The first major clade was formed by two sequences identified as *Strigea robusta*

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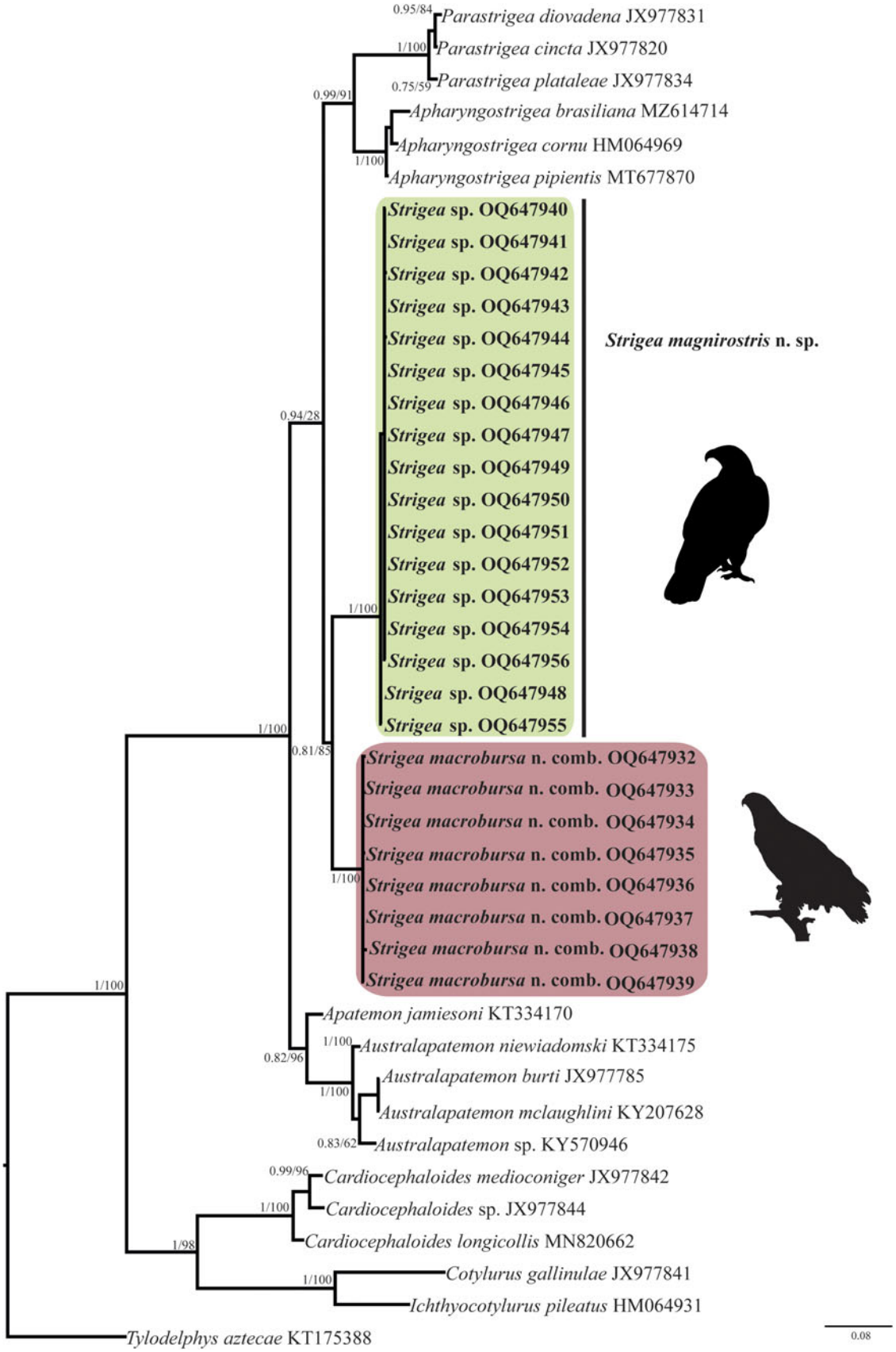

Fig. 2. Phylogenetic trees inferred with Maximum Likelihood (ML) and consensus Bayesian Inference (BI) with the internal transcribed spacers dataset. Numbers near internal nodes show maximum likelihood bootstrap percentage values and Bayesian posterior probabilities.

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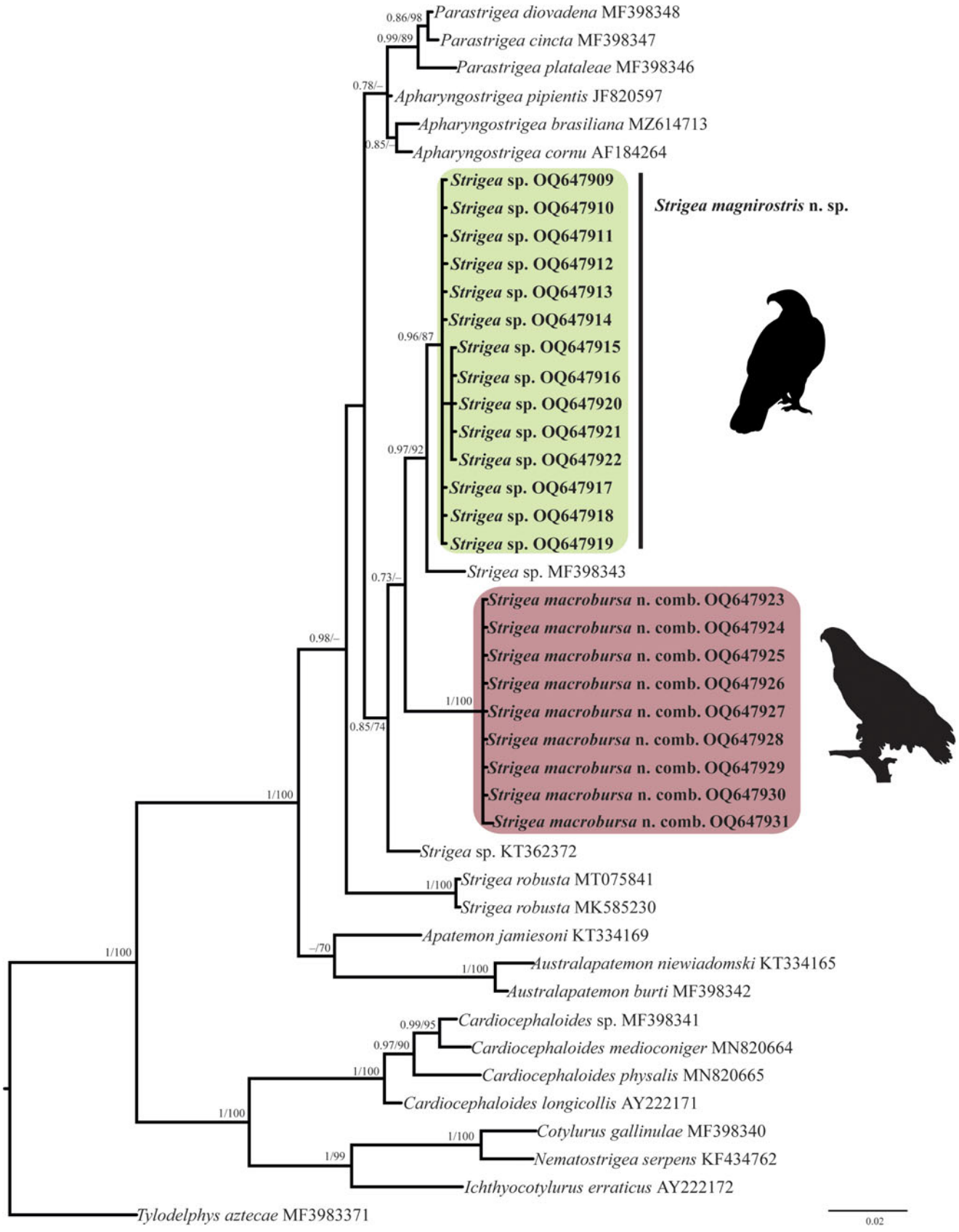

Fig. 3. Phylogenetics trees inferred with Maximum Likelihood (ML) and consensus Bayesian Inference (BI) with the large subunit dataset. Numbers near internal nodes show ML bootstrap percentage values and Bayesian posterior probabilities.

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(MT075841 and MK585230) recovered from the marsh frog (*Pelophylax ridibundus*) and edible frog (*Pelophylax esculentus*), respectively, from Russia, and this clade was sister to a clade formed by species of the genera *Parastrigea* Szidat, 1928 and *Apharyngostrigea* Ciurea, 1927 (fig. 3). The second major clade was formed by four subclades. The first subclade contains an unidentified sequence of *Strigea* sp. (KT362372) from water frog (*Pelophylax* sp.) from France. The second subclade was formed by nine isolates of *P. macrobursa* from Mexico, which is a sister to the third subclade formed by a single sequence of an unidentified sample of *Strigea* sp. (MF398343) recovered from crested caracara (*Caracara cheriway*) from Presa La Angostura, Chiapas, Mexico. The fourth subclade was formed by 14 isolates of *Strigea* sp. recovered from the roadside hawk (*R. magnirostris*) and Cooper's hawk (*A. cooperii*) from six localities in Mexico (fig. 3). Finally, the intraspecific genetic divergence among 14 isolates of *Strigea* sp. was low, ranging from 0 to 0.10%, whereas that for nine isolates of *P. macrobursa* ranged from 0 to 0.08% for LSU.

Mitochondrial gene

The newly completed sequences from *cox 1* were aligned with other partial sequences downloaded from GenBank. The alignment included the first region of *cox 1* with 42 sequences and 374 characters. The phylogenetic analyses performed with ML and BI showed that the genus *Strigea* is monophyletic (fig. 4). The clade was subdivided into three subclades. The first subclade was formed by 16 isolates of an undescribed species of *Strigea* sp. from the Neotropical region of Mexico. The second subclade was formed by an isolate of an unidentified sequence of *Strigea* sp. (MF398319) from Presa La Angostura, Chiapas, Mexico. The third subclade was formed by seven isolates identified morphologically as *P. macrobursa*. All these relationships had high bootstrap values and Bayesian posterior probabilities (fig. 4). The intraspecific genetic divergence ranged from 0 to 1% among isolates of *Strigea* sp. and from 0 to 1.3% for *P. macrobursa* for *cox 1*.

In summary, the phylogenetic analyses performed with two nuclear markers and one mitochondrial molecular marker supported the monophyly of all new isolates of *Strigea* spp. from the Neotropical region (figs 2–4). The new ITS, LSU and *cox 1* sequences revealed that our specimens of *Strigea* sp. recovered from the roadside hawk (*R. magnirostris*) and Cooper's hawk (*A. cooperii*) from six localities in the Neotropical region of Mexico formed an independent lineage, which is recognized herein as a new species and is described next as *Strigea magnirostris* n. sp., representing the first species to Mexico and the 22nd to the Americas. In addition, the specimens identified as *P. macrobursa* collected from the intestine of the great black hawk (*B. urubitinga*) (type host) and common black hawk (*B. anthracinus*) formed a clade nested inside *Strigea*, and as a result, it should be transferred to *Strigea* to form *Strigea macrobursa* n. comb. (figs 2–4).

Morphological description

Family Strigeidae Railliet, 1919

Subfamily Strigeinae Railliet, 1919

Genus *Strigea* Abildgaard, 1790

Strigea magnirostris n. sp.

Type host: *R. magnirostris* (roadside hawk) (Accipitriformes: Accipitridae).

Other host: *A. cooperii* (Cooper's hawk) (Accipitriformes: Accipitridae).

Type locality: Tamiahua, Veracruz, Mexico (21°18′02″ N, 97° 26′56.2″ W).

Other locality: Tres Vidas, Guerrero, Mexico (16°43′59.8″ N, 99°42′48.9″ W).

Site in host: Intestine.

Prevalence: eight of 11 (72%).

Type material: Holotype CNHE 11118; paratypes CNHE 11119; voucher CNHE 11120.

GenBank accession number: ITS, OQ647940–56; LSU, OQ647909–22; *cox 1*, OQ648135–50.

Etymology: The epithet of the species refers to the specific name of the type host.

Description (figs 5 and 6; table 2)

Description (based on 17 adult specimens) (figs 5 and 6): Body 3.03–4.43 mm (3.93 mm) in total length. Tegument spines on the surface of the forebody (fig. 6d). Forebody is longer than is wide, covered with tiny rounded spines, 562–872 (749) long by 400–690 (583) wide, representing 20% of body length (BL) (figs 5 and 6b–d). Hind-body long, strongly curved dorsally with tegument smooth, 2440–3591 (3176) long by 352–632 (492) wide, almost four times longer than the forebody, with a ratio of hind-body length to forebody length of 1: 3.3–5.3 (4.2). Oral sucker terminal, well developed, 77–109 (97) long by 80–115 (100) wide, with several papillae around it (fig. 6c). Ventral sucker well developed, larger than oral sucker, 150–240 (187) long by 124–188 (158) wide. Ratio of ventral sucker length to oral sucker length is 1: 1.45–2.42 (2.0). Pharynx 65–103 (80) long by 64–90 (72) wide. Ratio of pharynx length to oral sucker length is 0.84–1.10 (0.97). Pseudosuckers well developed with conspicuous folds in anterior section, 114–248 (185) long by 77–12 (101) wide (fig. 6b). Holdfast organ lobes can be projected beyond the anterior margin of the forebody, proteolytic gland at base of forebody, 182–225 (200) long by 83–119 (106) wide. Testes in tandem, bilobed, situated near posterior end of the body, anterior testis 272–476 (370) long by 261–496 (378) wide, posterior testis slightly larger than anterior testis at 346–497(420) long by 280–512 (400) wide. Seminal vesicle long, sinuous, postesticular, slightly overlapping with posterior testis. Ovary reniform, pretesticular 139–190 (165) long by 126–214 (170) wide. Mehlis' gland and vitelline reservoir in the intertesticular region. Vitelline follicles of different sizes in both body segments; in the forebody, small follicles extend into the holdfast organ and lateral body wall from the posterior margin of the sucker ventral, while in the hind-body, large follicles are mostly concentrated in the neck (pre-ovarian region) ventrally to the seminal vesicle or copulatory bursa (fig. 5). Copulatory bursa large triangle-shaped broadening in posterior end, 247–531 (390) long by 468–784 (630) wide (figs 5 and 6e). Muscular ring (*Ringnapf*) well developed. Genital cone large and well delimited from body parenchyma, 193–361 (280) long by 380–637 (512) wide, ejaculatory duct and uterus join at base of genital cone, forming hermaphroditic duct. Uterus with large and numerous eggs (20–50) (35), oval, 71–105 long by 40–65 (52) wide. Ratio of genital cone length to egg length is 1: 1.95–3.88 (2.9). Excretory pore terminal.

Remarks

Currently, 21 species of the genus *Strigea* have been described in the Americas that parasitize strigiform, ciconiiform, falconiform, caprimulgiform, passeriform, gruiform, trogoniform and anseriform birds. Of the 21 described species, only five species (*S. falconis brasiliana*, *S. elegans*, *S. microbursa*, *S. magniova* and *S. arcuata*) share morphological characteristics with *S. magnirostris*

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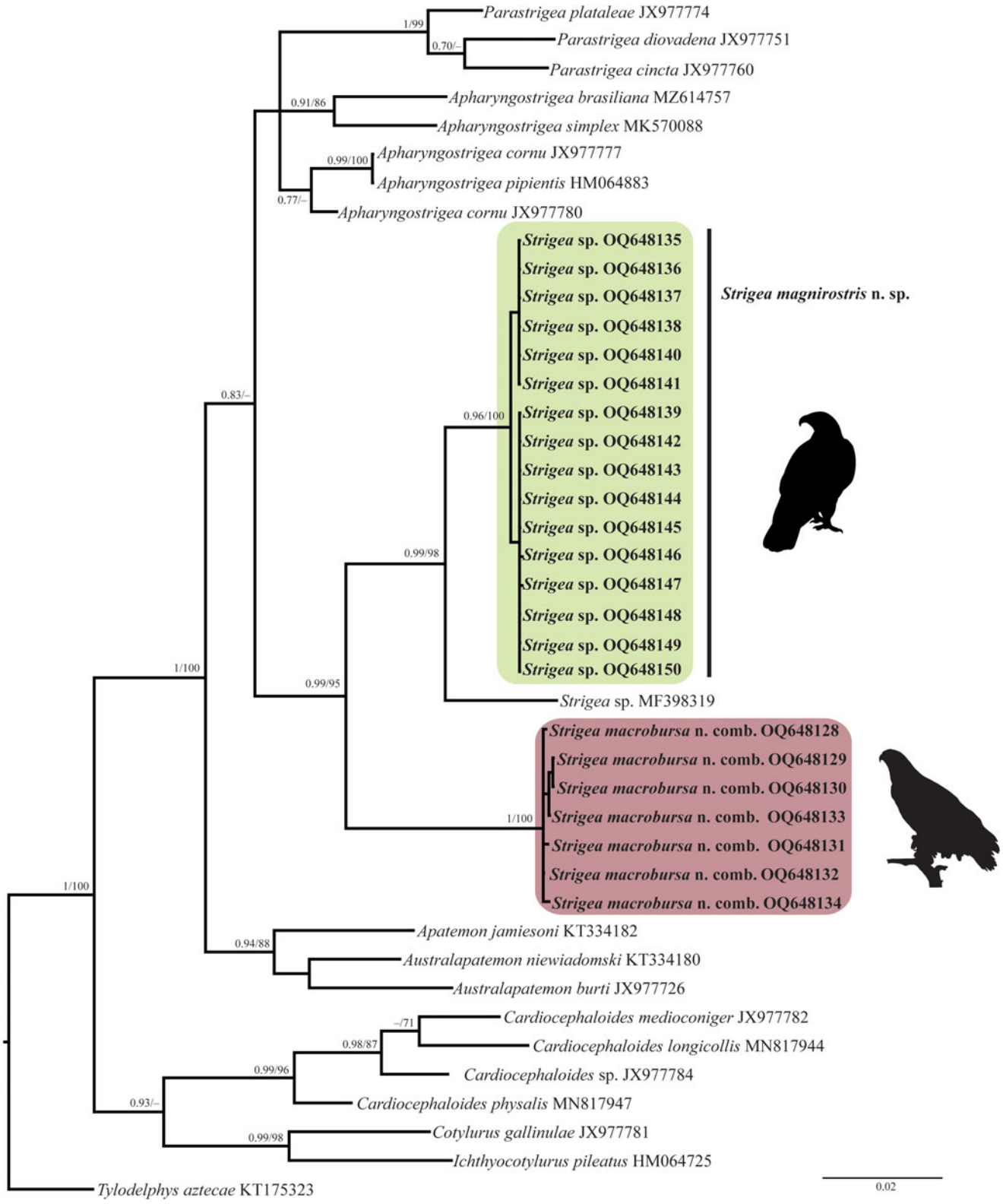

Fig. 4. Phylogenetics trees inferred with Maximum Likelihood (ML) and consensus Bayesian Inference (BI) with the *cox* 1 dataset. Numbers near internal nodes show ML bootstrap percentage values and Bayesian posterior probabilities.

n. sp., such as body shape, presence of a neck region in the hindbody and distribution of vitelline follicles in the forebody, which are scarce and extend into the lobes from the holdfast organ (Chandler & Rausch, 1947; Dubois, 1968, 1988; Pearson & Dubois, 1985; Lunaschi & Drago, 2006, 2009). The new species most closely resembles *S. arcuata*, *S. microbursa* and *S. elegans* by having pseudosuckers that are well developed in the forebody. However, *S. arcuata* can be distinguished from *S. magnirostris*

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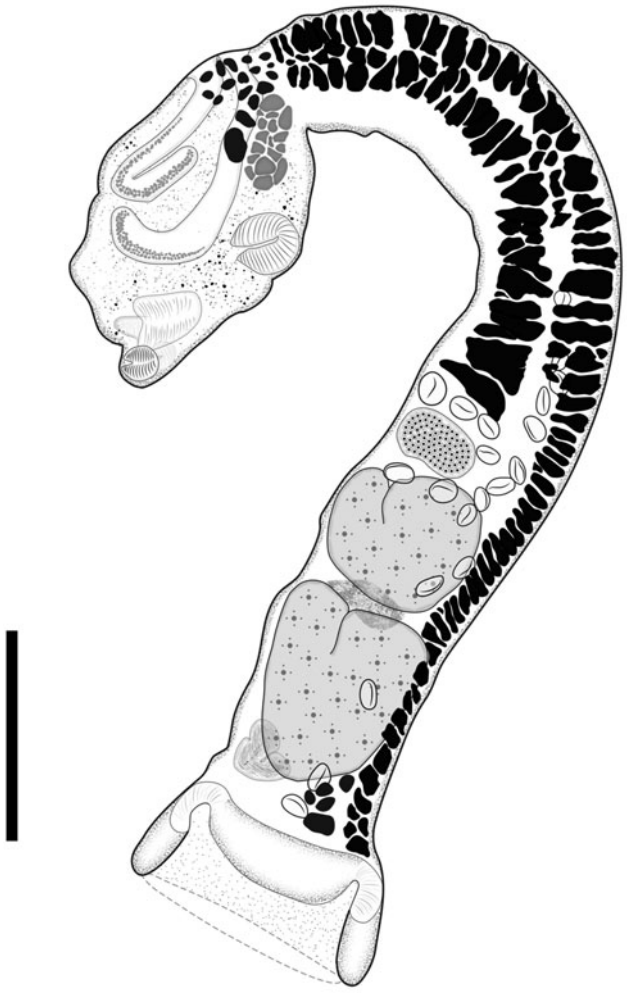

Fig. 5. Adult of *Strigea magnirostris* n. sp. from *Rupornis magnirostris*; whole worm, holotype, lateral view. Scale bars = 500 µm.

n. sp. by having a smaller genital cone included in a circular muscular formation (125 × 145 vs. 193–361 × 296–637 in *S. magnirostris*). In addition, *S. arcuata* possesses lower limits for the following characteristics: pseudosuckers (105 × 80 vs. 118–248 × 64–125 in *S. magnirostris*); hind-body width (180 vs. 352–632); anterior testes (255 × 185 vs. 272–476 × 261–496); and posterior testes (340 × 260 vs. 300–497 × 280–512). The species *S. microbursa* can be distinguished from *S. magnirostris* n. sp. by having a smaller genital cone (100–180 × 80–140 vs. 193–361 × 296–637). In addition, *S. microbursa* possesses lower limits for the following characteristics: pseudosuckers (85–95 × 90–95 vs. 118–248 × 64–125 in *S. magnirostris*); forebody width (230–300 vs. 400–690); ovary length (55–140 vs. 139–190); and ovary width (80–106 vs. 126–214). Finally, *S. elegans* can be distinguished from *S. magnirostris* n. sp. due to its smaller BL (1550–2450 vs. 3030–4437), smaller copulatory bursa (350 diam. vs. 247–531 × 468–784) and larger eggs (115–220 vs. 71–105) (see table 2).

Morphological redescription

Strigea macrobursa n. comb.

Syn. *Parastrigea macrobursa* Drago and Lunaschi, 2011

Host: *B. urubitinga* (great black hawk) (Accipitriformes: Accipitridae).

Other host: *B. anthracinus* (common black hawk) (Accipitriformes: Accipitridae)

Locality: Isla Aguada, Campeche, Mexico (18°48′22.92″ N, 91° 28′03.68″ W).

Other localities: Tecolutla, Veracruz, Mexico (20°33′49.8″ N, 97°05′57.7″ W).

Site in host: Intestine.

Prevalence: 3 of 6 (50%).

Voucher specimens: CNHE 11121, 11122.

GenBank accession number: ITS OQ647932–39; LSU OQ647923–31; *cox 1* OQ648128–34.

Description (figs 7 and 8; table 3)

Description (based on 26 adult specimens) (figs 7 and 8): Body 957–2.88 mm (1920 mm) in total length. Forebody tulip-shaped, 344–775 (560) long by 238–562 (400) wide (fig. 7b). Tegument spines on the surface of the forebody (fig. 8d). Hind-body slightly plump with tegument smooth, two to three times longer than the forebody at 609–2184 (1400) long by 256–759 (508) wide, with some specimens having a neck region (nine individuals) and some specimens lacking a neck region (17 individuals) (fig. 7a, c). Ratio of BL to forebody length is 1: 2.5–4.1 (1: 3.3). Ratio of hind-body length to forebody length is 1: 1.5–3.1 (1: 2.3). Oral sucker subterminal, well developed, 64–95 (80) long by 57–86 (71) wide (fig. 8c). Ventral sucker oval, 74–106 (90) long by 55–98 (76) wide. Prepharynx absent, pharynx 33–66 (52) long by 31–59 (47) wide. Holdfast organ lobes reaching anterior end (fig. 8b, c), proteolytic gland at base of forebody 75 long by 43 wide. Testes in tandem, large, not lobed, anterior testis oval 95–281 (190) long by 132–449 (290) wide, posterior testis slightly larger than anterior testis at 137–392 (260) long by 155–474 (314) wide. Seminal vesicle long, postesticular. Ovary oval, pre-testicular or slightly overlapping anterior testis, 52–188 (120) long by 72–193 (130) wide. Laure's canal, opening dorsally between ovary and anterior testis. Mehlis' gland and vitelline reservoir in the intertesticular region. Vitelline follicles similar in size in both body segments; in the forebody, they are in the dorsal lip of the holdfast organ forming two symmetrical masses situated between the ventral sucker and intersegmental constriction; in the hind-body, the vitelline follicles are concentrated in the pre-ovarian region, extending ventrally to the posterior testis or copulatory bursa (fig. 7a–c). Copulatory bursa large, delimited by pronounced constriction, occupying 30%–45% (40%) of hind-body length, 163–681 (422) long by 246–616 (430) wide (fig. 8e). Muscular ring (*Ringnapf*) absent. Genital cone well delimited from body parenchyma, 89–255 (170) long by 137–230 (180), ejaculatory duct and uterus join at base of genital cone forming hermaphroditic duct. Uterus with large and numerous eggs 3–50 (26) that are 72–117 (95) long by 45–67 (56) wide. Ratio of BL to egg length is 1: 10–28 (1: 19). Genital atrium very deep, genital pore terminal. Excretory pore dorso-subterminal at the level of the copulatory bursa (see table 3).

Remarks

This species was originally described as *P. macrobursa* by Drago & Lunaschi (2011) from the great black hawk (*B. urubitinga*) from Argentina. The specimens collected in the present study are similar to those of the original description by Drago & Lunaschi (2011). For instance, a forebody tulip-shaped and vitelline follicles distributed in two lateral expansions and a large well-delimited copulatory bursa, with a well-delimited genital cone and deep genital atrium. However, the newly collected specimens from the great black hawk (type host) and common black hawk in

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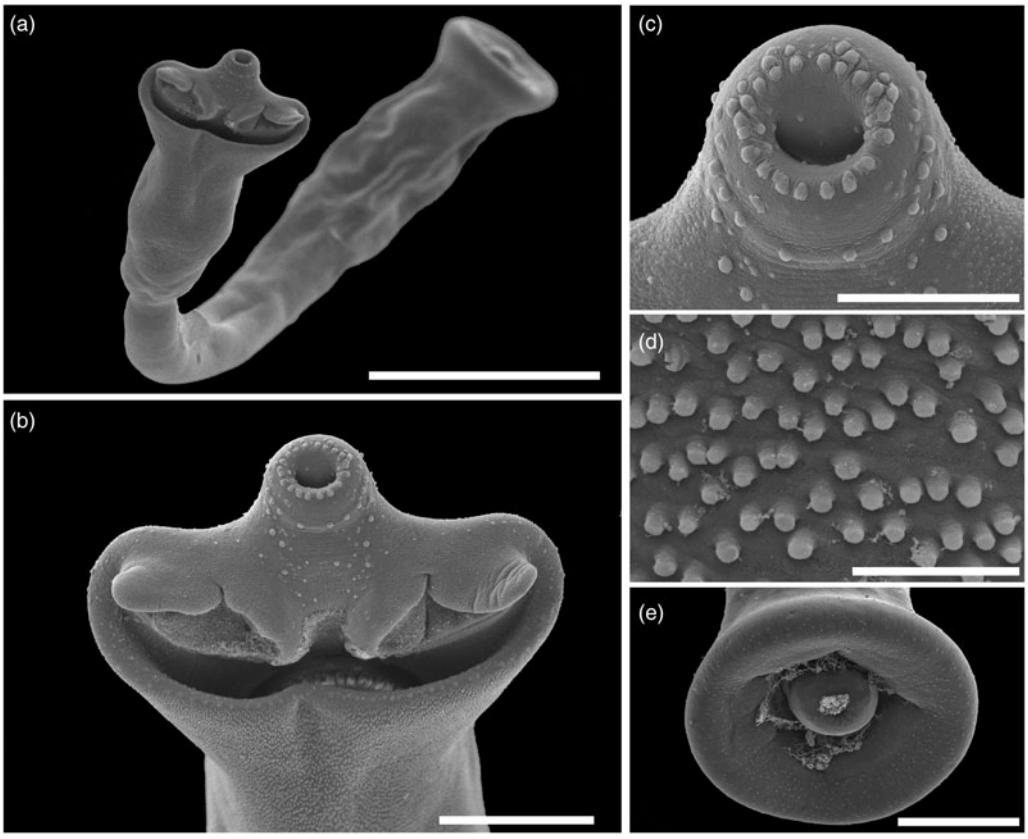

Fig. 6. Scanning electron micrographs of *Strigea magnirostris* n. sp. from *Rupornis magnirostris*. (a) Whole worm, ventral view; (b) forebody, ventral view showing pseudo-suckers; (c) oral sucker with papillae; (d) tegumental spines, ventral view of the forebody; (e) copulatory bursa showing cone genital. Scale bars: (a) 400 μm; (b, e) 100 μm; (c) 50 μm; (d) 10 μm.

three localities from the Neotropical region of Mexico showed some level of morphological intraspecific variation. For example, some of our specimens exhibit a neck in the hind-body, whereas other specimens do not. In addition, our specimens have tegumental spines that gradually diminish in size and number from the anterior to posterior region. However, apparently the presence or absence of spines could be related to the development of the worms. A similar pattern has been observed in specimens of two species, *S. falconis brasiliana* and *S. elliptica*, from the Neotropical region (Lunaschi & Drago, 2006, 2009). Finally, our specimens possess higher limits than original description for the following characteristics: hind-body length (609–2184 vs. 754–1451); ovary length (52–188 vs. 69–131); anterior testes width (132–449 vs. 188–262); and posterior testes width (155–474 vs. 193–304) (see table 3).

Discussion

The taxonomic history and species composition of the family Strigeidae have been complex and unsettled. Recent molecular evidence suggests that the family is paraphyletic. However, the genera *Apharyngostrigea*, *Parastrigea* and *Strigea* share a common ancestor (Blasco-Costa *et al.*, 2016; Blasco-Costa & Locke, 2017; Hernández-Mena *et al.*, 2017; Locke *et al.*, 2021; López-Jiménez *et al.*, 2022). The genetic library of some strigeid species of the genera *Apharyngostrigea*, *Parastrigea* and *Strigea* has recently increased and provides a large opportunity to clarify the taxonomy and species composition of these three genera (Blasco-Costa *et al.*, 2016; Hernández-Mena *et al.*, 2017; Locke *et al.*, 2021; López-Jiménez *et al.*, 2022). In the current study, we combined morphological and molecular characteristics to describe a new species, *S. magnirostris* n. sp. that represents the first species in the Neotropical region of Mexico and the 22nd in the Americas. Morphologically, the new species is distinguished from other congeneric species from the Americas by having an oral sucker with several papillae around it, well-developed pseudosuckers, a tegument covered with tiny spines, a larger cone genital and a larger copulatory bursa. In addition, the phylogenetic trees established with three molecular markers supported that the isolates identified morphologically as *P. macrobursa* from *B. urubitinga* (type host) and *B. anthracinus* collected from three localities in Mexico are not closely related to other members of the genus *Parastrigea* because they were nested inside *Strigea*. Therefore, we transferred it to *Strigea* to form *S. macrobursa* n. comb., expanding its geographical distribution from Mexico to Argentina (Drago & Lunaschi, 2011), representing the first record in Mexico. Interestingly, our phylogenies established that *S. magnirostris* n. sp., *S. macrobursa* n. comb. and *Strigea* sp. were associated with accipitriform and falconiform birds from the Neotropical region on a clade, suggesting that at least two clades could be formed, one represented by the 22 described species from the Neotropical region and the second represented by the six species from the Nearctic region in the Americas. However, this hypothesis should be tested with more species from other biogeographical regions and primarily adult specimens because the sequences from the LSU available in

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Table 2. Comparative measurements of *Strigea magnirostris* n. sp. and related species.

	Strigea magnirostris n. sp.	*Strigea arcuata*	*Strigea microbursa*		*Strigea elegans*	*Strigea magniova*	*Strigea falconis brasiliana*	
Source	Present study	Dubois (1988)	Pearson & Dubois (1985)	Lunaschi & Drago, (2009)	Chandler & Rausch (1947)	Dubois (1988)	Dubois (1968)	Lunaschi & Drago (2006)
Locality	Mexico	Paraguay	Indonesia	Argentina	United States	Paraguay	Brazil, Cuba	Argentina
Host	*Rupornis magnirostris* *Accipiter cooperii*	*Accipiter erythronemius* *Parabuteo unicinctus*	*Spilornis cheela*	*Buteogallus meridionalis*	*Bubo virginianus*	*R. magnirostris*	Accipitridae Falconidae	*R. magnirostris*
Body length	3030–4437	3700	1400–3600	1266–3021	1550–2450	1320	up to 2500	1305–1392
Forebody (Fo)	562–872 × 400–690	900 × 600	420–600 × 230–300	832–1083 × 328–551	560–1050 × 420–560	320–340 × 230–240	380–590 × 420–700	319–415 × 314–367
Hind-body (Hi)	2440–3591 × 352–632	2800 × 180	900–1200 × 170–220	1083–2102 × 232–435	980–1800 × 320–500	850–1000 × 160–260	1110–1830 × 340–580	890–1073 × 362–435
Pseudo-suckers	118–248 × 64–125	105 × 80	85–95 × 90–95	–		–	–	–
Oral sucker	72–109 × 80–115	110 × 105	68–117 × 70–127	69–107 × 74–117	110 × 130	48–68 × 48–57	100–125 × 85–115	76 × 55
Ventral sucker	142–240 × 119–188	200 × 135	65–122 × 73–138	107–143 × 116–143	198 × 220	55–63 × 70–78	160–235 × 140–200	152–162 × 71–105
Proteolytic gland	157–225 × 66–119	185 × 115	–	143–193 × 126–152	150 × 180	–	105–130 × 120–190	–
Pharynx	65–103 × 64–90	95 × 90	57–132 × 52–150	62–83 × 52–64	–	30 × 28	73–95 × 70–95	74 × 48
Ovary	139–190 × 126–214	140 × 170	55–140 × 80–106	105–138 × 88–217	150 × 165	52–65 × 60–80	110–200 × 175–300	59–68 × 101–107
Anterior testis	272–476 × 261–496	255 × 185	110–150 × 130–190	143–280 × 131–343	400 × 425	120–160 × 130–195	235–360 × 235–410	169–227 × 174–190
Posterior testis	300–497 × 280–512	340 × 260	110–190 × 150–190	179–241 × 157–314	400 × 430	130–150 × 140–220	275–370 × 235–420	197–217 × 179–241
Copulatory bursa	247–531 × 468–784	–	–	104–420 × 102–381	350 diameter	–	–	183–241 × 215–226
Genital cone	193–361 × 296–637	125 × 145	100–180 × 80–140	–	–	95–115 × 98–105	240–350 × 220–310	128–167 × 129–143
n eggs	8–50	32[a]	1–3	1–3	1–8	3–7	1–3	3–5
Eggs	71–105 × 40–65	84–96 × 55–63	100–105 × 50–57	83–129 × 52–98	115–220 × 65–73	105–115 × 52–60	67–91 × 42–55	82–88 × 48–52
Ratio Hi/Fo length	3.2–5.3	3.1[a]	2.0–2.1[a]	1.2–2.3	1.4–2.3	2.6–2.9[a]	1.8–3.6	2.1–3.4
Ratio Vs/Os	1.3–1.8	1.2[a]	1[a]	1.2–1.5[a]	1.6[a]	1.3–1.4[a]	1.6–1.7[b]	1.9
Ratio Ph/Os	0.7–0.9	0.8[a]	0.7–1.1[a]	0.7–1	–	0.4–0.5[a]	0.8[b]	0.9[b]
Ratio Hi/Gc	9.2–15.1	22.4[a]	6.6–9[a]	–	–	8.6–8.9[a]	4.6–5.2[b]	6.4–7[b]
Ratio Gc/E	1.9–3.8	1.4	1–1.7[a]	–	–	0.9–1[a]	3–5[b]	1.5–2[b]

Gc/eggs, genital cone length/egg length; Hi/Fo, hind-body length/forebody length; Hi/Gc, hind-body length/genital cone length; Ph/Os, pharynx width/oral sucker width; Vs/Os, suckers width ratio.
[a]Calculated from original descriptions.
[b]Calculated from original descriptions by Drago *et al.* (2014).

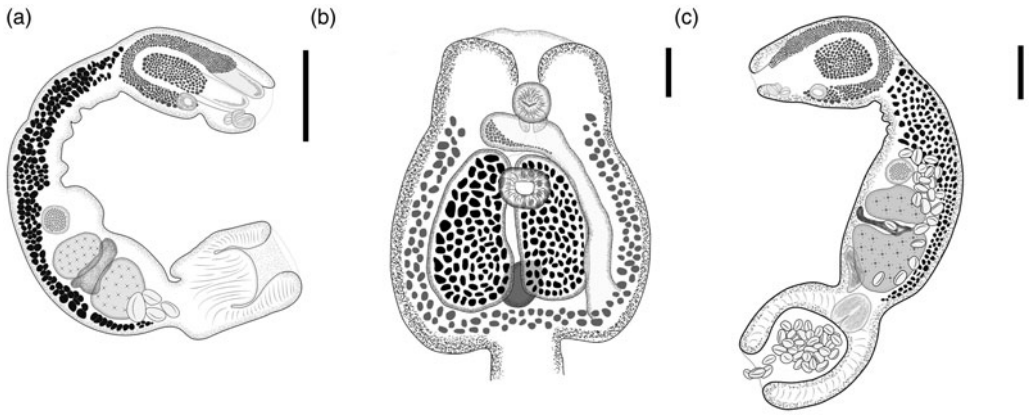

Fig. 7. Adult of *Strigea macrobursa* n. comb. from *Buteogallus urubitinga*. (a) Whole worm; (b) forebody, ventral view; (c) whole worm. Scale bars: (a) 300 μm; (b) 100 μm; (c) 250 μm.

GenBank are from larval forms of *Strigea* spp. (KT362372, MT075841 and MK585230) (Patrelle *et al.*, 2015; Svinin *et al.*, 2020).

Heneberg *et al.* (2018) performed one of the most comprehensive taxonomic reviews of strigeids that included samples of the genera *Strigea*, *Parastrigea*, *Apharyngostrigea, Cotylurus* Szidat, 1928 and *Apatemon* Szidat, 1928 from Central Europe. These authors sequenced the small subunit and the ITS2 from nuclear ribosomal DNA and the second region of the barcode from *cox 1* and nicotinamide adenine dinucleotide dehydrogenase subunit 1 from mitochondrial DNA. However, these authors could not compare their sequences with other sequences of strigeids previously analysed by Hernández-Mena *et al.* (2014, 2017) and Blasco-Costa *et al.* (2016) because these authors sequenced the ITS (ITS1-5.8S rDNA- ITS2), the domains D1–D3 of the LSU from nuclear DNA and the *cox 1* barcode, the first region from

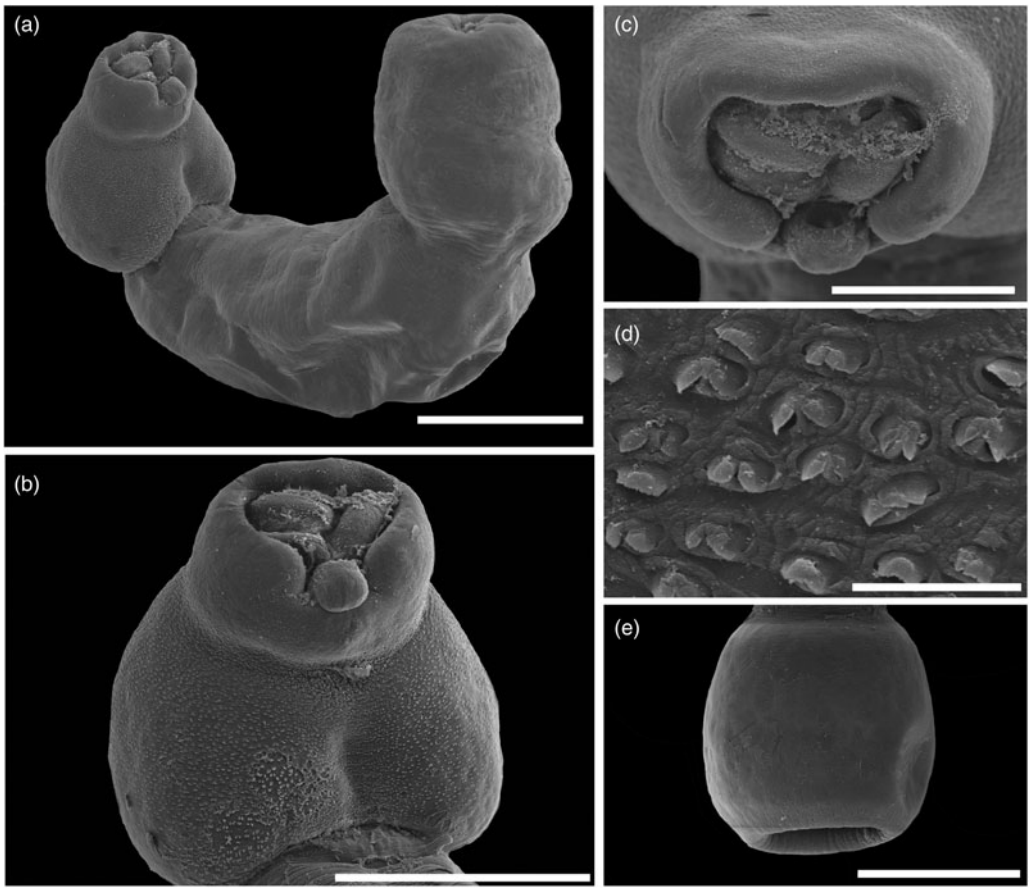

Fig. 8. Scanning electron micrographs of *Strigea macrobursa* n. comb. from *Buteogallus urubitinga*. (a) Whole worm, ventral view; (b) forebody, ventral view; (c) oral sucker; (d) tegumental spines; (e) copulatory bursa. Scale bars: (a) 500 μm; (b, e) 400 μm; (c) 200 μm; (d) 10 μm.

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Table 3. Comparative measurements of adults *Strigea macrobursa* Drago & Lunaschi, 2011 recorded in the Americas.

	Strigea macrobursa n. comb.	*Strigea macrobursa* (syn. *Parastrigea macrobursa*)
Source	Present study	Drago & Lunaschi (2011)
Locality	Mexico	Argentina
Host	*Buteogallus urubitinga* *Buteogallus anthracinus*	*B. urubitinga*
Body length	957–2886	1189–2117
Forebody (Fo)	344–775 × 238–562	435–783 × 348–638
Hind-body (Hi)	609–2184 × 256–759	754–1451 × 391–658
Neck	130–576 × 94–291	–
Oral sucker	64–95 × 57–86	76–87 × 64–99
Ventral sucker	74–106 × 55–98	82–107 × 60–150
Proteolytic gland	75 × 53	64–83 × 60–76
Pharynx	34–66 × 31–59	44–60 × 39–60
Ovary	52–188 × 72–193	69–131 × 109–190
Anterior testis	95–281 × 132–449	97–155 × 188–262
Posterior testis	137–392 × 155–474	102–213 × 193–304
Copulatory bursa	163–681 × 246–616	290–648 × 280–532
Genital cone	89–255 × 137–230	117–179 × 107–176
n eggs	3–50	3–45
Eggs	72–117 × 45–67	92–143 × 57–77
Ratio Hi/Fo	1.5–3.1	1.7–3.1
Ratio BL/Fo	2.5–4.1	2.7–4.1
Ratio BL/E	10–28	10–20

BL/Fo, body length/forebody length; BL/E, body length/eggs length; Hi/Fo, hind-body length/forebody length.

the mitochondrial DNA. These three molecular markers have proven very useful for delineating species and inferring phylogenetic relationships at the genus level within Strigeidae. Herein, we compared the sequences of Heneberg *et al.* (2018) with other sequences available in GenBank and with the newly generated sequences. We generated two new alignments. The first includes 60 sequences of ITS2 with 320 bp, representing 30% (1042 bp) of our original dataset that contains ITS1-5.8S rDNA-ITS2. Our phylogenetic trees established with ITS2 were similar to the tree inferred by Heneberg *et al.* (2018), including the polyphyly of *Strigea*, with weak bootstrap support and posterior probabilities. In addition, the isolates of the new species *S. magnirostris* n. sp. plus *S. macrobursa* formed a clade together with *S. falconis* (MF628087) (see online supplementary fig. S1). The second alignment contained 46 sequences of *cox 1* (including the newly generated sequences in the current study) with 297 bp of the second region of the barcode. The phylogenetic trees placed all the species of *Strigea*, including the new species *S. magnirostris* n. sp. and *S. macrobursa* in a clade. However, the species *Parastrigea flexilis* Dubois, 1934 (MF628065) was nested inside *Strigea*, suggesting that *P. flexilis* should be transferred to *Strigea* (see online supplementary fig. S2). To clarify the taxonomy of the genera *Strigea*, *Parastrigea* and *Apharyngostrigea*, it is necessary to review the taxonomy of the species that share diagnostic characteristics among the three genera. For instance, *Parastrigea* is characterized by the distribution of vitellaria (two symmetrical masses on the forebody), which are present in *S. falconis*, *S. strigis* (Schrank, 1788) Abildgaard, 1790, *S. robusta* (Szidat, 1928) Heneberg and Sitko, 2018, *Apharyngostrigea brasiliana* Szidat, 1928 (Dubois, 1964) and *S. macrobursa* n. comb., (Dubois, 1968; Heneberg *et al.*, 2018; López-Jiménez *et al.*, 2022).

In summary our phylogenetic trees established with ITS and *cox 1* supported the monophyly of *Strigea*. However, the LSU tree showed that *Strigea* is paraphyly because two sequences of larval forms identified as *S. robusta* (MT075841 and MK585230) were nested inside other clades. In addition, the genetic divergence among the species of the first clade of *Strigea*, *S. magnirostris* n. sp., *S. macrobursa* and two isolates of *Strigea* sp. (MF398343 and KT362372), ranged from 0.6% to 1.6% and from 2.4% to 2.8% with respect to *S. robusta*. These high ranges of divergence are similar between *Strigea* and *Apharyngostrigea*, which ranged from 1.9% to 2.2% for the LSU marker. The phylogenetic analyses established with the LSU, in combination with the high genetic divergence, of the two sequences of larval forms identified as *S. robusta* suggests that they do not belong to *Strigea*. However, the ITS2 tree (see online supplementary file S1) placed five isolates of *S. robusta* of adult and larval forms (MF537205, MT075803, MK295777, MF537208 and MF628100) from Germany, Russia and Poland in a single clade that is a sister to the type species.

In the current study, we described a new species of *Strigea*, collected from the intestines of two hawk species (*R. magnirostris* and *A. coperii*) which is named *S. magnirostris* n. sp. In addition, the species *P. macrobursa* was transferred to *Strigea* to form

https://doi.org/10.1017/S0022149X23000196 Published online by Cambridge University Press

S. macrobursa n. comb. To clarify the taxonomy of the genus *Strigea*, it is necessary to sequence more species (including the type species, *S. strigis*) from diverse biogeographical regions with the ITS (ITS1-5.8S rDNA- ITS2), the D1–D3 domains from the LSU and the first region from the *cox 1* gene. Finally, the current integrative study represents a continuation of our effort in describing and understanding the biodiversity of strigeids in the Neotropical region.

Supplementary material. To view supplementary material for this article, please visit https://doi.org/10.1017/S0022149X23000196.

Acknowledgements. We are grateful to Laura Marquez and Nelly López for their help during the sequencing of the DNA fragments. We also thank Berenit Mendoza Garfias for her help in obtaining the scanning electron microphotographs.

Financial support. ALJ and MTGG thank the support of the Programa de Posgrado en Ciencias Biológicas, UNAM and CONACYT (ALJ. CVU. No. 706119; MTGG. CVU. No. 956064) for granting a scholarship to complete her PhD program and his Master program, respectively. LAG thanks the Coordinación de la Investigación Científica and Dirección General de Asuntos de Personal Académico (DGAPA-UNAM) Mexico for the Postdoctoral Fellowship granted. This research was supported by the Programa de Apoyo a Proyectos de Investigación e Innovación Tecnológica (PAPIIT-UNAM) IN201122.

Competing interests. None.

Author contributions. ALJ and MGV conceived and designed the study. ALJ and MTGG conducted data gathering. ALJ, LAG and MTGG performed statistical analyses. ALJ, LAG and MGV wrote and edited the article. ALJ, MTTG, LAG and MGV collected the samples. ALJ performed the methodology.

Ethical standards. The sampling in this work complies with the current laws and animal ethics regulations of Mexico.

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https://doi.org/10.1017/S0022149X23000196 Published online by Cambridge University Press

III.III. EXPLORING THE GENETIC STRUCTURE OF *PARASTRIGEA DIOVADENA* DUBOIS AND MACKO, 1972 (DIGENEA: STRIGEIDAE) AN ENDOPARASITE OF THE WHITE IBIS, *EUDOCIMUS ALBUS* FROM THE NEOTROPICAL REGION OF MEXICO.

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Parasitoloy Research (2021) 120(6): 2065–2075.

https://doi.org/10.1007/s00436-021-07185-w

Parasitology Research
https://doi.org/10.1007/s00436-021-07185-w

GENETICS, EVOLUTION, AND PHYLOGENY - ORIGINAL PAPER

Exploring the genetic structure of *Parastrigea diovadena* Dubois and Macko, 1972 (Digenea: Strigeidae), an endoparasite of the white ibis, *Eudocimus albus*, from the Neotropical region of Mexico

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Received: 9 December 2020 / Accepted: 5 May 2021

Abstract

Parastrigea diovadena Dubois and Macko, 1972, is an allogenic trematode species that infects the intestine of white ibis. This widely distributed Neotropical species has been studied poorly, and nothing is known about its population genetic structure. In the current study, we attempt to fill this gap for the first time and to explore the genetic diversity in *P. diovadena* populations from three biogeographic provinces (Sierra Madre Oriental, Sierra Madre Occidental, and Sierra Madre del Sur) in the Neotropical region of Mexico. Newly generated sequences of the internal transcribed spacers (ITS) from ribosomal DNA and cytochrome c oxidase subunit 1 (*cox 1*) from mitochondrial DNA were compared with sequences available from the GenBank data set. Phylogenetic analyses performed with the ITS and *cox 1* data sets using maximum likelihood and Bayesian inference unequivocally showed that new sequences of *P. diovadena* recovered from the white ibis formed a clade with other sequences of specimens previously identified as *P. diovadena.* The intraspecific genetic divergence among the isolates was very low, ranging from 0 to 0.38% for ITS and from 0 to 1.5% for *cox 1*, and in combination with the phylogenetic trees confirmed that the isolates belonged to the same species. The *cox 1* haplotype network (star-shaped) inferred with 62 sequences revealed 36 haplotypes. The most frequent haplotype (H3, n = 18) corresponded to specimens from all the populations (except Tecolutla, Veracruz). In addition to the common haplotype, we identified four other shared haplotypes (H2, H9, H12, and H14) and 31 unique haplotypes (singlets). In addition, high haplotype diversity (Hd = 0.913), low nucleotide diversity (Pi = 0.0057), and null genetic differentiation or population structure (Fst = 0.0167) were found among the populations from the three biogeographic provinces. The results suggest that the biology of the definitive host has played a key role in the population genetic structure of *Parastrigea diovadena* in the Neotropical region of Mexico.

Keywords Trematoda · *Parastrigea diovadena* · White ibis · Neotropical region · Genetic structure · Molecular markers

Section Editor: Simonetta Mattiucci

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Introduction

The white ibis, *Eudocimus albus* Linnaeus, is a common wading bird widely distributed along the Atlantic and Pacific coasts of the Americas. On the Atlantic side, its distribution range extends from east of Virginia, the Greater Antilles, Middle America, to north of South America. On the Pacific side, its distribution range extends from Baja California Mexico to northwest Peru (Heath et al. 2009; Patten 2012; Ramírez et al. 2014). This wading bird is found in diverse habitats, such as marshes, wetlands, mangroves, lagoons, and shallow lakes, feeding upon crayfish, crustaceans, amphibians, insects, mollusks, and small fishes (American Ornithologist' Union 1998). The parasite fauna of white ibis is relatively well-known in some localities from the southeastern USA and Mexico. To date, 57 species have

Published online: 25 May 2021

been recorded, and trematodes exhibit the highest species diversity, with 29 species, followed by nematodes (20 species), acanthocephalans (5 species), and cestodes (3 species) (Lumsden 1961; Schmidt and Bush 1972; Bush et al. 1973; Schmidt 1973; Bush and Forrester 1976; Dronen and Blend 2008; Pérez-Álvarez et al. 2008; García-Varela et al. 2011; Ortega-Olivares et al. 2011; Hernández-Orts et al. 2016). Particularly, in the Neotropical region of Mexico, four species of trematodes (*Parastrigea cincta* Dubois, 1968; *Parastrigea diovadena* Dubois and Macko, 1972; *Patagifer lamothei* Dronen and Blend, 2008; and *Maritrema corai* Hernández-Orts, Pinacho-Pinacho, García-Varela and Kostadinova, 2016) are considered part of the biogeographical core parasite fauna of white ibis (Ortega-Olivares et al. 2011; Hernández-Mena et al. 2014; Hernández-Orts et al. 2016).

Parastrigea Szidat, 1928, is a genus of endoparasites of the family Strigeidae associated with fish-eating birds, particularly ardeids, ciconiids, threskiornithids, falconids, recurvirostrids, anatids, and podicipedids across the globe. As in other strigedis, members of the genus *Parastrigea* exhibited a complex life cycle involving a freshwater snail (planorbids) as the first intermediate host. The metacercaria is unencysted or encyst parasitizing mainly the cranial cavity and body cavity of fish, and leeches that serve as second intermediate hosts (Niewiadomska 2002). Currently, the genus *Parastrigea* contains 18 nominal species; six of these species have been recorded in the Neotropical region from the Americas, i.e., *P. brasiliana* (Szidat, 1928) Dubois, 1964; *P. caballeroi* Dubois, 1952; *P. cincta* (Brandes, 1888) Szidat, 1928; *P. diovadena* Dubois and Macko, 1972; *P. mexicana* Coil, 1957; and *P. plataleae* Hernández-Mena, García-Prieto and García-Varela 2014 (Ortega-Olivares et al. 2011; Drago and Lunaschi 2011; Hernández-Mena et al. 2014; Heneberg et al. 2018). Over the past few years, many efforts have focused on studying the genetic diversity of trematode species that affect domestic and wild vertebrates with major impacts on human populations worldwide, including *Schistosoma mansoni* (Standley et al. 2010; Shalaby et al. 2011; Blanton et al. 2011), *S. japonicum* (Zhou et al. 2009, 2011), *Paragonimus westermani* (Van Herwerden et al. 1999; Iwagami et al. 2000), *Fasciola hepatica* (Walker et al. 2011; Teofanova et al. 2011; Husch et al. 2020), *F. magna* (Králová-Hromadová et al. 2011; Juhásová et al. 2016), and *Echinostoma* sp. (Saijuntha et al. 2011). However, the study of the genetic diversity of trematode species that infect wild vertebrates has started to be explored (see Stefka et al. 2009; Louhi et al. 2010; Blasco-Costa et al. 2012; Marigo et al. 2013; Razo-Mendivil et al. 2013; Herrmann et al. 2014). Esch et al. (1988) classified the trematodes into two groups depending on their life cycle: autogenic (trematode species whose life cycle is completed among hosts that are all confined to an aquatic system) and allogenic (trematode species, whose life cycle is completed with the participation of multiple host species that inhabit diverse habitats). The genetic structures of autogenic species are characterized by low genetic flow and greater genetic structure due to their aquatic hosts having low dispersal. In contrast, the genetic structures of allogenic species are characterized by high genetic flow and a high dispersal rate, with a null population structure (Blouin et al. 1995; Jarne and Théron 2001; McCoy et al. 2003; Criscione and Blouin 2004; Prugnolle et al. 2005; Blasco-Costa et al. 2012; Blasco-Costa and Poulin 2013).

The main objective of the current study was to characterize the population genetic structure of *Parastrigea diovadena*, an allogenic trematode associated with the white ibis, *Eudocimus albus*, collected in nine localities from three biogeographic provinces in the Neotropical region of Mexico, by using two molecular markers (the internal transcribed spacers, ITS1, ITS2, and 5.8S from nuclear DNA and cytochrome c oxidase subunit I from mitochondrial DNA). This study allowed us to test whether the mountain range from Mexico (i.e., Sierra Madre Oriental, Sierra Madre Occidental, and Sierra Madre del Sur) served as biogeographic barriers that shaped the genetic structure of the populations of *P. diovadena*.

Materials and methods

Parasite samples

A total of 40 white ibis were collected between October 2006 and December 2010 in nine localities across three biogeographic provinces from the Neotropical region of Mexico: four from the Sierra Madre Oriental (n = 25), three from the Sierra Madre Occidental (n = 18), and two from Sierra Madre del Sur (n = 19) (Table 1; Fig. 1). Birds were dissected within the following 4 h, and their viscera were placed in separate Petri dishes containing a 0.75% saline solution and examined under a dissecting microscope. Digeneans were relaxed in a 0.75% saline solution and fixed with a 4% hot-formalin solution for morphological studies, and other specimens were preserved in 100% ethanol for DNA analyses.

DNA isolation, amplification, and sequencing

A total of 35 specimens identified as *Parastrigea diovadena* were placed individually in tubes and digested overnight at 56 °C in a solution containing 10 mM Tris–HCl (pH 7.6), 20 mM NaCl, 100 mM Na_2 EDTA (pH 8.0), 1% Sarkosyl, and 0.1 mg/ml proteinase K. Following digestion, DNA was extracted from the supernatant using the DNAzol reagent (Molecular Research Center, Cincinnati, Ohio) according to the manufacturer's instructions. The cytochrome c oxidase subunit 1 (*cox 1*) of the mitochondrial DNA and the internal transcribed spacers from nuclear ribosomal DNA (ITS1 and

Table 1 Specimen information. Collections sites, geographical coordinates, sample number, and GenBank accession number. Sequences marked with bold were obtained in this study. Localities correspond with the numbers in Fig. 1

Locality	Coordinates	Sample number	GenBank ITS	GenBank cox1
Sierra Madre Occidental				
1. Caimanero, Sinaloa	25° 36′ 30″ N 108° 26′ 25″ W	734	**MW293750**	**MW300491**
		735	JX977791	JX977731
		736	**MW293751**	**MW300492**
		737	JX977790	JX977730
		738	**MW293752**	**MW300493**
		739	**MW293753**	**MW300494**
		740	JX977789	JX977729
		741	**MW293754**	**MW300495**
		742	**MW293755**	**MW300496**
		743	**MW293756**	**MW300497**
2. El Huizache, Sinaloa	23° 05′ 28″ N 106° 15′ 57″ W	267	**MW293757**	**MW300498**
		797	JX977796	JX977736
		798	JX977795	JX977735
		799	JX977797	JX977737
		800	**MW293758**	**MW300499**
3. San Blas, Nayarit	21° 32′ 00″ N 105° 17′ 22″ W	183	JX977807	JX977747
		806	JX977809	JX977749
		807	JX977808	JX977748
Sierra Madre del Sur				
4. Chautengo, Guerrero	16° 38′ 08″ N 99° 08′ 26″ W	836	**MW293759**	**MW300500**
		837	**MW293760**	**MW300501**
		838	**MW293761**	**MW300502**
		839	**MW293762**	**MW300503**
		840	**MW293763**	**MW300504**
		841	**MW293764**	**MW300505**
		842	JX977794	JX977734
		843	JX977793	JX977733
		844	**MW293765**	**MW300506**
		845	JX977792	JX977732
5. Rio Pijijiapan, Chiapas	15° 31′ 54″ N 93° 09′ 39″ W	846	**MW293766**	**MW300507**
		847	JX977804	JX977744
		848	JX977805	JX977745
		849	JX977806	JX977746
		850	**MW293767**	**MW300508**
		851	**MW293768**	**MW300509**
		852	**MW293769**	**MW300510**
		853	**MW293770**	**MW300511**
		855	**MW293771**	**MW300512**
Sierra Madre Oriental				
6. Punta Piedra, Tamaulipas	24° 29′ 00″ N 97° 45′ 00″ W	443	JX977803	JX977743
		444	JX977802	JX977742
		744	**MW293772**	**MW300513**
		745	**MW293773**	**MW300514**
		746	**MW293774**	**MW300515**
		747	**MW293775**	**MW300516**
		748	**MW293776**	**MW300517**
		749	JX977801	JX977741

Table 1 (continued)

Locality	Coordinates	Sample number	GenBank ITS	GenBank cox1
		750	**MW293777**	**MW300518**
		751	**MW293778**	**MW300519**
7. Tamiahua, Veracruz	21° 15′ 49″ N 97° 26′ 41″ W	926	JX977811	JX977751
		927	JX977810	JX977750
		928	JX977812	JX977752
		929	**MW293779**	**MW300520**
		930	**MW293780**	**MW300521**
8. Tecolutla, Veracruz	20° 27′ 35″ N 97° 01′ 44″ W	931	JX977813	JX977753
		932	JX977815	JX977755
		933	**MW293781**	**MW300522**
		934	JX977814	JX977754
		935	**MW293782**	**MW300523**
9. Los Chivos, Veracruz	18° 56′ 13″ N 95° 58′ 08″ W	801	**MW293783**	**MW300524**
		802	JX977798	JX977738
		803	JX977800	JX977740
		804	**MW293784**	**MW300525**
		805	JX977799	JX977739

ITS2 plus 5.8S) were amplified using polymerase chain reaction (PCR). The *cox 1* was amplified using the forward primer Plat-diploCOXF, 5′-CGTTTRAATTATACGGATCC-3′ and the reverse primer Plat-diploCOXR, 5′-AGCATAGTAATMGCAGCAGC-3′ (Moszczynska et al. 2009), and the ITS region was amplified using the forward primer BD1 5′-GTCGTAACAAGGTTTCCGTA-3′ (Bowles and McManus 1993) and the reverse primer BD2 5′-ATCTAGACCGGACTAGGCTGTG-3 (Bowles et al. 1995). PCR reactions (25 µl) consisted of 10 µl of each primer, 2.5 µl of 10×buffer, 2 mM MgCl2, and 1 U of Taq DNA polymerase (Platinum Taq, Invitrogen Corporation, São Paulo, Brazil). PCR cycling parameters consisted of denaturation at 94 °C for 1 min, 35 cycles of 94 °C for 1 min, 50 °C for 1 min, and 72 °C for 1 min, followed by a post-amplification incubation at 72 °C for 10 min. Sequencing reactions were performed using the initial primers described above and two internal primers for ITS, forward BD3 5′-GAACATCGACATCTTGAACG-3′ and reverse BD4 5′ATAAGCCGACCCTCGGC-3′ (Hernández-Mena et al. 2014) with ABI Big Dye (Applied Biosystems, Boston, Massachusetts) terminator sequencing chemistry, and reaction products were separated and detected using an ABI 3730 capillary DNA sequencer. Contigs were assembled and base-calling differences resolved using Codoncode Aligner version 9.0.1 (Codoncode Corporation, Dedham, Massachusetts). Sequences were deposited in the GenBank data set (Table 1).

Alignments and phylogenetic analysis

New sequences of *cox 1* and ITS of *Parastrigea diovadena* were obtained and aligned with other 27 sequences of *P. diovadena* downloaded from GenBank data set (Table 1). Sequences of each molecular marker were aligned separately using the software Clustal W (Thompson et al. 1997). Based on the topologies of a previous study from Strigeidae (Hernández-Mena et al. 2014), sequences of the genera *Apharyngostrigea* and *Parastrigea* were selected as outgroup: *A. cornu* (JX977778 for cox 1; JX977838 for ITS), *P. cincta* (JX977756 and JX977760 for cox 1; JX977816 and JX977820 for ITS), and *P. plataleae* (JX977761 and JX977763 for cox 1; JX977821 and JX977823 for ITS). A nucleotide substitution model was selected for each molecular marker using jModelTest version 2.1.7 (Posada 2008) applying the Akaike criterion. The best nucleotide substitution models for each data set was GTR + G + I. Phylogenetic trees were inferred through maximum likelihood (ML) with the program RAxML version 7.0.4 (Stamatakis 2006). A GTRGAMMAI substitution model was used, and 10,000 bootstrap replicates were run to assess nodal support. We also analyzed our data in a Bayesian framework using MrBayes 3.2.2 (Ronquist et al. 2012), with two Markov chain (MCMC) runs for 10 million generations, sampled every 1000 generations, a heating parameter value of 0.2, and burn-in of (25%). Trees were edited using FigTree version 1.4.0 (Rambaut 2012). Phylogenetic trees were visualized in FigTree v. 1.4.2 (http://tree.bio.ed.ac.uk/software/figtree/). Genetic divergences were estimated using *p* uncorrected distances with MEGA v.5 (Tamura et al. 2011).

Population genetic structure and historical demographic

To analyze the molecular information in the framework of population genetics the *cox 1* was analyzed. We grouped individuals

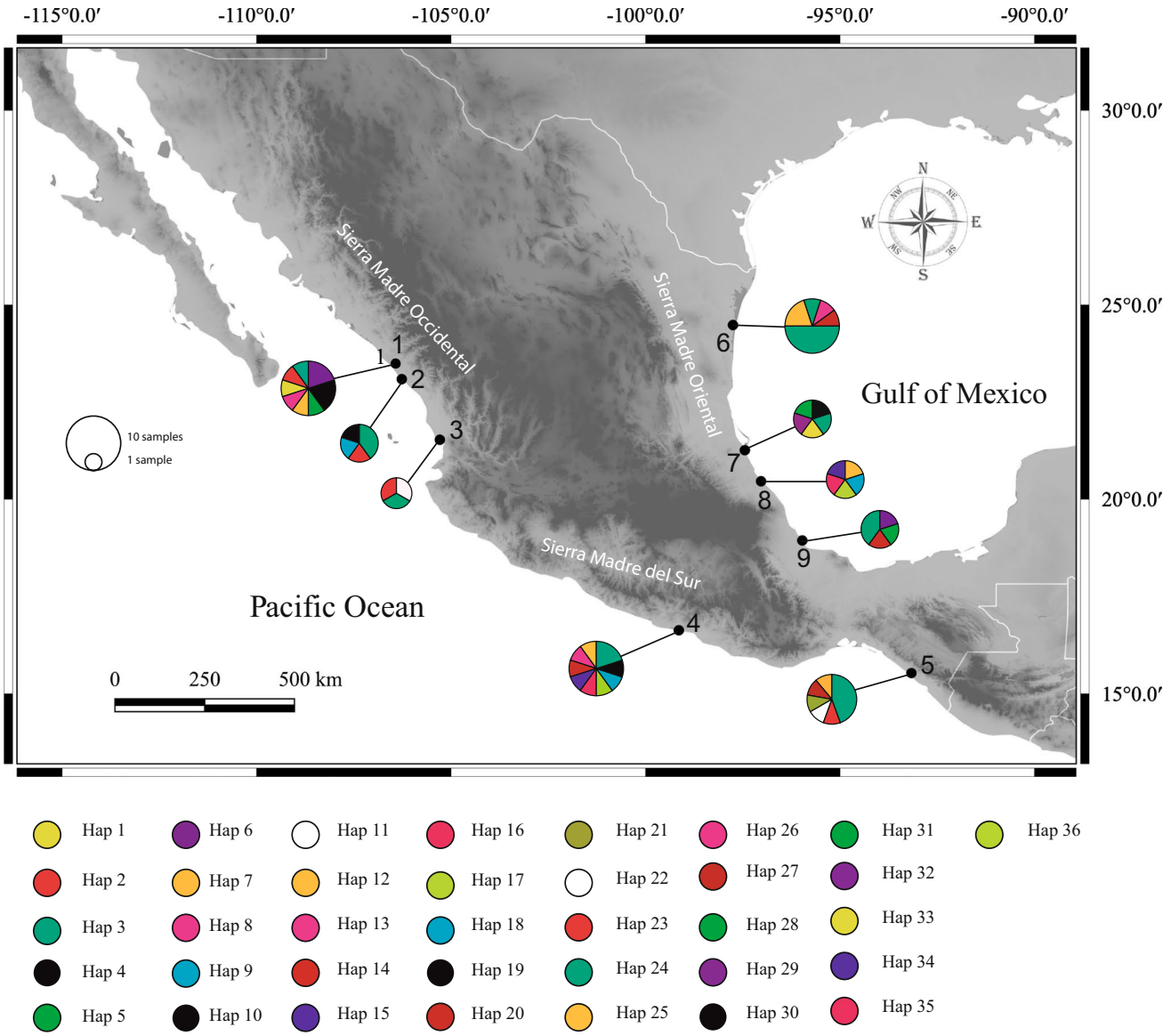

Fig. 1 Sampling sites of specimens of *Parastrigea diovadena* and distribution of haplotypes. Collection sites are numbered in accordance with Table 1

of *P. diovadena* into three populations considering geographic proximity and evidence of mountain ranges; (I) Sierra Madre Occidental (SMOc), (II) Sierra Madre del Sur (SMS), and (III) Sierra Madre Oriental (SMOr). Intrapopulation variation was summarized using standard statistic: number of haplotypes (H), number of segregating sites (S), haplotype diversity (Hd), nucleotide diversity (Pi), and average number of nucleotide differences (K), which were all calculated using the program DnaSP v. 5. 10 (Rozas et al. 2003). Haplotype diversity represents the probability that two randomly sampled alleles are different. Nucleotide diversity is defined as the average number of nucleotide differences per site in pairwise comparison among DNA sequences (Nei 1987). To examine haplotype frequency among the populations of *P. diovadena*, a statistical network was constructed using the program PopART with the median-joining algorithm (Bandelt et al. 1999). The degree of genetic differentiation among the populations was estimated using the fixation indices Fst (Hudson et al. 1992), with the program Arlequin v.3.5 (Excoffier and Lischer 2010). To investigate the genetic variation among populations or within populations, the analysis of molecular variance (AMOVA) was performed, considering genetic distance among the haplotypes using Arlequin v.3.5. To investigate the population history and demography, Tajima's D (Tajima 1989) and Fu's Fs (Fu 1997) test were calculated using DnaSP v. 5. 10 (Rozas et al. 2003). The values were considered significant when the P-values were less than 0.05.

Results

Phylogenetic analyses

The *cox 1* data set included 479 characters and 67 sequences. Nucleotide frequencies were A = 0.163, C = 0.142, G = 0.220, and T = 0.475. The phylogenetic analyses inferred with ML and BI showed that the isolates identified as *P. diovadena* formed a clade within of the genus *Parastrigea*, supported by a strong bootstrap support value and Bayesian posterior probabilities (95/1.0) (Fig. 2A). The intraspecific genetic divergence among the isolates was very low, ranging from 0 to 1.5%. The *ITS* data set included 1090 characters and 68 sequences. Nucleotide frequencies were A = 0.196, C = 0.227, G = 0.272, and T = 0.304. The phylogenetic analyses inferred with ML and BI also showed that all the isolates identified as *P. diovadena* formed a clade supported with strong bootstrap support value and Bayesian posterior probabilities (98/0.99) (Fig. 2B). The intraspecific genetic divergence among the isolates was very low, ranging from 0 to 0.38%.

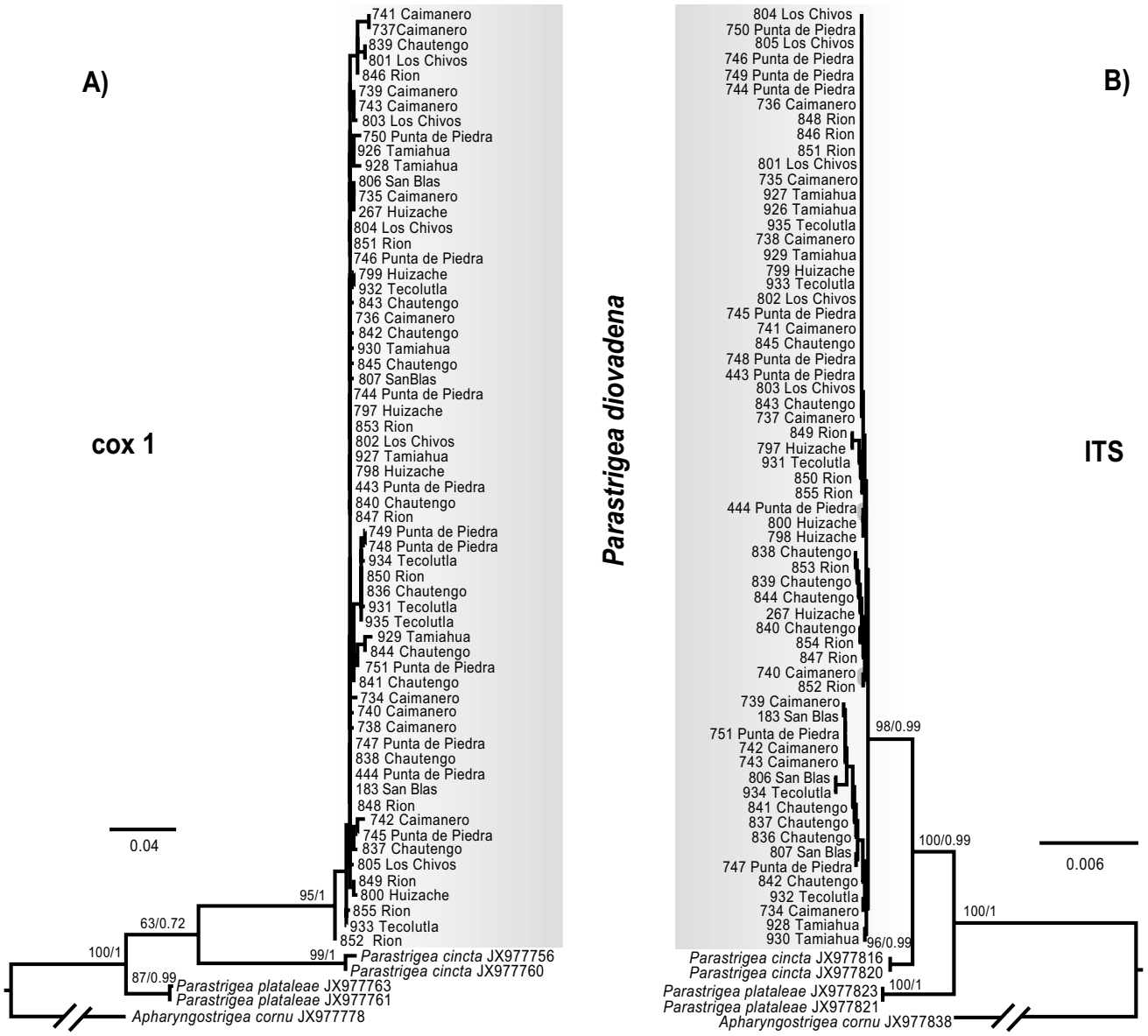

Fig. 2 Phylogenetic trees, using maximum likelihood (−ln of 2437.8416) method and Bayesian inference for the *cox 1* data set (**A**). Phylogenetic trees, using maximum likelihood (−ln of 3965.5207) method and Bayesian inference for the ITS data set (**B**). Numbers near internal nodes show ML bootstrap values and posterior probability clade frequencies. Scale bars represent the branch length. The terminals contain the ID number and locality according with Table 1

In summary, the phylogenetic analyses inferred with each data set placed all the isolates recovered from white ibis from the Neotropical region in a monophyletic clade within of the genus *Parastrigea*, and in combination with the low genetic divergence, these results suggest that the isolates belong to a single lineage identified as *P. diovadena.*

Population genetic structure and demographic analysis

The haplotype network built in this study was inferred with 62 specimens and 462 characters. In total, 36 haplotypes were detected, and 34 polymorphic sites (4 mutations were nonsynonymous and 30 were synonymous) were found. The most frequent haplotype (H3, n = 18) corresponded to specimens from all sampled populations (except in Tecolutla, Veracruz; locality 8 in Fig. 1). This main haplotype was also central to all other haplotypes; therefore, a star-shaped network was formed (Fig. 3). In addition to the common haplotype, we identified four other shared haplotypes (H2, H9, H12, and H14) and 31 unique haplotypes (singlets). Most of the identified haplotypes are separated from each other by one, two, or three substitutions. The level of haplotype diversity (Hd = 0.913) was very high, and nucleotide diversity was low (Pi = 0.00573) among the populations from the three biogeographic provinces sampled (Sierra Madre Oriental, Sierra Madre Occidental, and Sierra Madre del Sur) from the Neotropical region of Mexico. The diversity indices estimated are summarized in Table 2. The results of Tajima's D (−2.15) and Fu's FS (−40.93) tests are shown in the Table 2, indicating an excess of rare alleles over what would be expected under neutrality, suggesting population expansion of *P. diovadena*. The Fst values were estimated to assess genetic differentiation among the populations from the three biogeographic provinces analyzed (Sierra Madre Oriental, Sierra Madre Occidental, and Sierra Madre del Sur). Interregion Fst values were low, ranging from 0.025 to 0.039 (Table 3). The AMOVA between geographic groups indicated that the populations were poorly genetically differentiated from one another, with most of the variation distributed within populations (98.32%) rather than among populations (1.68%) (Table 4).

Discussion

To the best of our knowledge, adult specimens of *Parastrigea diovadena* were described from white ibis from the Peninsula de Zapata, Cuba (Dubois and Macko 1972). Later, *P. diovadena* was recorded on the coasts of the Gulf of Mexico and Pacific Sea slopes (Bush and Forrester 1976; Ortega-Olivares et al. 2011). This trematode is considered part of the biogeographical core parasite fauna of the white

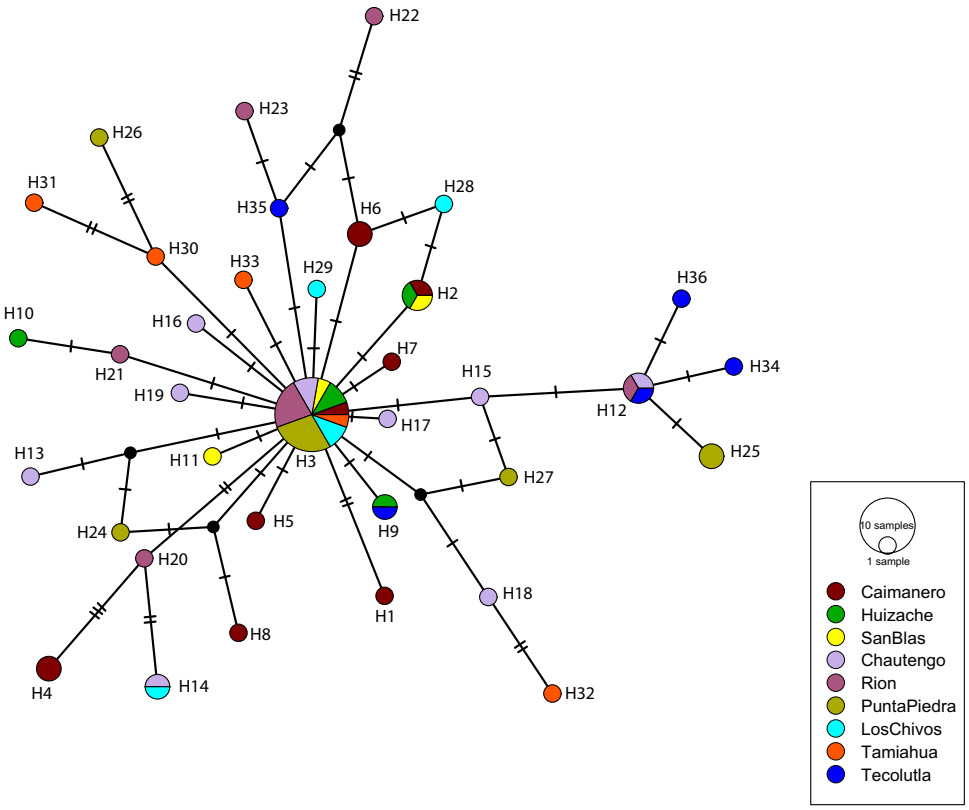

Fig. 3 Median-joining network of samples of *Parastrigea diovadena* built with the gene cytochrome *c* oxidase subunit I (*cox 1*). Each circle represents a haplotype, with size proportional to the haplotype's frequency in the populations

Table 2 Molecular diversity indices and neutrality tests calculated for *cox 1* data sets among the populations of *Parastrigea diovadena* used in this study (*n* number of sequences, *H* number of haplotypes, *S* number of segregating sites, *Hd* haplotype diversity, *Pi* = nucleotide diversity and *K* average number of nucleotide differences, *SMOc* Sierra Madre occidental, *SMS* Sierra Madre del Sur, and SMOr Sierra Madre Oriental)

Region	n	S	H	Hd ± SD	Pi ± SD	K	Tajima's D	Fu's Fs
SMOc	18	15	11	0.928 ± 0.040	0.00538 ± 0.00104	2.568	− 1.70975	− 5.282
SMS	19	20	13	0.906 ± 0.060	0.00576 ± 0.00106	2.748	− 1.99930	− 7.788
SMOr	25	21	17	0.903 ± 0.054	0.00605 ± 0.00091	2.880	− 1.84455	− 12.163
All	62	34	36	0.913 ± 0.031	0.00573 ± 0.00061	2.720	− 2.15238	− 40.932

ibis. In the current study, adult specimens were recovered from three biogeographic provinces (Sierra Madre Oriental, Sierra Madre Occidental, and Sierra Madre del Sur) from the Neotropical region of Mexico. Phylogenetic analyses inferred with the ITS and *cox 1* data sets unequivocally showed that all the specimens identified as *P. diovadena* formed a clade confirming that all the specimens belong to the same lineage. The intraspecific genetic divergence among the isolates was very low, ranging from 0 to 1.5% for *cox 1*. These values of intraspecific genetic divergence are similar to those reported among isolates of *Parastrigea cincta* (Brandes, 1888) Szidat, 1928; and *Parastrigea plataleae* Hernández-Mena, García-Prieto and García-Varela, 2014; ranging from 0 to 0.87% and from 0 to 1.52% for *cox 1*, respectively (Hernández-Mena et al. 2014). The genetic divergence among isolates of *Cardiocephaloides mediconiger* Dubois and Vigueras, 1949, from different localities from Cuba ranged from 0 to 1.5% (Locke et al. 2018), and among different isolates of *Apharyngostrigea cornu* (Zeder, 1800) Ciurea, 1927, from Canada ranged from 0 to 1.6% for *cox 1* (Locke et al. 2011, 2018). With respect to ITS, the intraspecific genetic divergence among the isolates of *P. diovadena* was low, ranging from 0 to 0.38%. These values are also similar to those found among isolates of *Parastrigea cincta* and *Parastrigea plataleae*, ranging from 0 to 0.37% (Hernández-Mena et al. 2014), and *Apharyngostrigea pipientis* Faust, 1918, showed identical sequences and between two isolates of *Cardiocephaloides mediconiger* was 2.3% (Locke et al. 2011, 2018).

The haplotype network was built with *cox 1* sequences of 62 specimens identified as *P. diovadena* from three biogeographic provinces (Sierra Madre Oriental, Sierra Madre Occidental, and Sierra Madre del Sur). The haplotype distribution did not show a relationship between the spatial distribution and its genetic diversity (Figs. 1, 3). Rather, the pattern observed clearly revealed a star-shaped network with a main haplotype (H3, n = 18), central to all other haplotypes, characteristic of expanding populations (Posada and Crandall 2001). The main haplotype was recorded in three localities (6, 7, and 9; Fig. 1; Table 1) from the Sierra Madre Oriental; in three localities (1, 2, and 3; Fig. 1; Table 1) from the Sierra Madre Occidental; and in two localities (4 and 5; Fig. 1; Table 1) from the Sierra Madre del Sur. The fixation index (Fsts) values obtained among populations from the three biogeographic provinces were low (see Table 3), and no genetic structure was detected. The three biogeographical provinces surveyed are separated by geographical barriers as follows: mountains, dry lowlands of the Isthmus of Tehuantepec, the Balsas Depression, and the central Trans-Mexicana Volcanic Belt (Barrier et al. 1998; Ferrari et al. 2012; Morrone et al. 2017). Despite the presence of geographic barriers among the populations of *P. diovadena*, analyses revealed high haplotype diversity (0.913), low nucleotide diversity (0.0057), and a null population structure, which is characteristic of a population undergoing expansion similar to that found in free-living organisms (Goodall-Copestake et al. 2012). In addition, the Fu's Fs statistic values were negative within populations, indicating an excess of low-frequency haplotypes, suggesting that this trematode had experienced rapid population growth in the past. Given that parasites are closely tied to their definitive hosts, it might be expected that parasites and their hosts would share similar phylogeographical patterns (Nieberding et al. 2004; Criscione et al. 2005). Stangel et al. (1991) analyzed the genetic variability in white ibis from two colonies separated by over 600 km in the United

Table 3 Pairwise Fst values estimated for cox (under the diagonal) and Fst p-values (above the diagonal). *Significance level* 0.05. *SMOc* Sierra Madre Occidental (Caimanero, Huizache and San Blas,); *SMS* Sierra Madre del Sur (Chautengo and Río Pijijiapan); and *SMOr* Sierra Madre Oriental (Punta Piedra, Tamihahua, Tecolutla, and Los Chivos)

	SMOc	SMS	SMOr
SMOc	---	0.108±0.022	0.027±0.019
SMS	0.0253	---	0.783±0.043
SMOr	0.0395	-0.0121	---

Table 4 Analysis of molecular variance (AMOVA) inferred with *cox 1* of specimens of *Parastrigea diovadena* collected from nine localities across three regions in Mexico: *SMOc* Sierra Madre Occidental, *SMS* Sierra Madre del Sur, and *SMOr* Sierra Madre Oriental

Source of variation	*df*	Sum of squares	Variance components	Percentage of variation
Among populations	2	3.627	0.02294 Va	1.68
Within populations	59	79.341	1.34476 Vb	98.32
Total	61	82.968	1.363770	

States of North America. Their analyses revealed a lack of genetic differentiation and thereby a null genetic structure. This phenome was due to the establishment of new colonies of individuals from different colonies, reducing the genetic differences among colonies and promoting high gene flow between the populations. In addition, white ibis has a low level of philopatry as well as a nomadic movement pattern and biological characteristics that promote panmictic populations. Although the complete life cycles of species of *Parastrigea* are unknown, available evidence from other members of the family, such as *Cotylurus* Szidat 1928, *Apatemon* Szidat 1928, *Australapatemon* Sudarikov 1959, and *Cardiocephaloides*, suggests that adult worms live and sexually reproduce in the digestive tracts of birds that serve as definitive hosts. Eggs are expelled into the environment with the feces of the host. After the ingestion of the eggs by a snail of the genera *Biomphalaria* Preston, 1910, and *Potamopyrgus* Stimpson, 1865, which serves as the first intermediate host, the parasites develop into cercariae. Cercariae emerge and swim to find fish and leeches that serve as the second intermediate host. If a bird ingests an infected second intermediate host, the parasite develops into the adult stage within the digestive tract of the bird (Niewiadomska 2002; Davies and de Núñez 2012; Blasco-Costa et al. 2016; Pyrka et al. 2020).

The genetic structure of trematode species with different life cycles (autogenic and allogenic) has been studied recently (see Blouin et al. 1995; Jarne and Théron 2001; McCoy et al. 2003; Criscione and Blouin 2004; Prugnolle et al. 2005; Blasco-Costa et al. 2012; Blasco-Costa and Poulin 2013). The genetic structure found in *P. diovadena* a species with an allogenic life cycle, followed the same pattern previously reported as follows: high haplotype diversity, null population genetic structure (a direct result of high gene flow between organisms of different areas), and the lack of a relationship between genetic diversity and geographic distribution, suggesting that the biology of the definitive host has played a key role in the population genetic structure of *Parastrigea diovadena* in the Neotropical region of Mexico.

Acknowledgements The first author thanks the support of the Programa de Posgrado en Ciencias Biológicas, UNAM, and CONACYT (CVU:706119) through granting a scholarship to complete her PhD program. We are grateful to the following colleagues who help during field work: Mirza Ortega-Olivares, Rogelio Rosas-Valdez, and Carlos Daniel Pinacho-Pinacho. The authors express their gratitude to Laura Márquez and Nelly López Ortiz for their help with the automatic sequencer. The birds were collected under the Cartilla Nacional de Colector Científico (FAUT 0202) issued by the Secretaría del Medio Ambiente y Recursos Naturales (SEMARNAT) to MGV.

Funding This research was funded by the Programa de Apoyo a Proyectos de Investigación e Innovación Tecnológica (PAPIIT-UNAM) IN207219.

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IV. DISCUSIÓN GENERAL

La clasificación taxonómica dentro de la familia Strigeidae se ha basado durante muchos años en caracteres morfológicos de los adultos y en las asociaciones ecológicas con sus huéspedes definitivos (Dubois, 1938, 1968). Actualmente las relaciones filogenéticas dentro de la familia Strigeidae son controversiales, debido a que la familia se dividió en dos grupos. El primer grupo Strigeidae (I), está conformado por especies pertenecientes a cinco géneros (*Strigea*, *Apharyngostrigea*, *Parastrigea, Apatemon* y *Australapatemon*) y Strigeidae (II) que está conformado por especies pertenecientes a cuatro géneros (*Cotylurus*, *Ichthyocotylurus*, *Cardiocephaloides* y *Nematostrigea*), entre estos dos grupos se encuentran especies de la familia Diplostomidae, por lo que Strigeidae es considerado un grupo parafilético (Olson et al. 2003; Fraija-Fernández et al. 2015; Hernández-Mena et al. 2017; Blasco-Costa y Locke, 2017; Heneberg et al. 2018). En la presente tesis confirmamos que el grupo Strigeidae (I) es monofilético y está conformado por tres subclados. El primero contiene algunas especies de los géneros *Apatemon* y *Australapatemon*, los cuales están asociados aves acuáticas de las familias Anatidae, Phalacrocoracidae y Rallidae (Andrade-Rosales, 2012; Blasco-Costa et al. 2016). Una diferencia ecológica entre estos dos géneros está relacionada con la fase larval (metacercaria), por ejemplo las especies de *Apatemon* se encuentran enquistadas en el segundo hospedero intermediario que generalmente son peces, mientras que las especies del género *Australapatemon* la metacercaria se encuentra enquistada en sanguijuelas. El segundo subclado está compuesto por especies del género *Strigea* asociado con aves rapaces pertenecientes a la familias Falconidae y Accipitridae. Finalmente el tercer clado está conformado por especies de los géneros *Apharyngostrigea* y *Parastrigea*. Las relaciones filogenéticas recuperadas en nuestros análisis coinciden con

estudios previos, sugiriendo una subdivisión de la familia con el grupo I como Strigeidae *sensu stricto* ya que incluye el género *Strigea* (género tipo), mientras que el segundo grupo de estrigeidos podría considerarse como otra familia (Blasco-Costa et al. 2016; Hernández-Mena et al. 2014, 2017).

Dentro del clado de Strigeidae *sensu stricto*, las diferencias morfológicas entre los géneros *Strigea*, *Parastrigea* y *Apharyngostrigea* son sútiles, ya que se distinguen morfológicamente por la distribución de las glándulas vitelógenas en el segmento anterior del cuerpo, así como la presencia o ausencia de una faringe (Niewiadomska, 2002).

En la presente tesis se generaron secuencias con dos genes nucleares y un mitocondrial para determinar la posición filogenética de la especie *Parastrigea brasiliana* asociada al intestino de la garza pico de bota (*Cochlearius cochlearius*) una especie de ave perteneciente a la familia Ardeidae en la localidad de Champotón en el estado de Campeche. Los resultados derivados de los análisis filogenéticos revelaron que las nuevas secuencias identificadas morfológicamente como *Parastrigea brasiliana* colectadas del hospedero tipo se anidan dentro del clado de *Apharyngostrigea* spp. Con base en los datos moleculares, transferimos la especie *P. brasiliana* al género *Apharyngostrigea*, formando la nueva combinación *Apharyngostrigea brasiliana* (Capítulo 1; Figs. 1–3). Morfológicamente, esta especie comparte características diagnósticas de ambos géneros. Por ejemplo, presenta las características diagnósticas del género *Parastrigea* (dos masas simétricas de glándulas vitelógenas en el segmento anterior) combinadas con características del género *Apharyngostrigea* (ausencia de una faringe) (Capítulo 1; Fig. 4). Este patrón morfológico también ha sido observado en otros estudios. Por ejemplo, Heneberg et al. (2018) estudiaron las relaciones filogenéticas de diferentes especies de la familia Strigeidae en Europa, basado en marcadores nucleares (18S e ITS2) y mitocondriales (*Cox 1* y Nadh). En este estudio

encontraron especies parafiléticas dentro de la familia, reclasificando la especie *Parastrigea robusta* Szidat, 1928 al género *Strigea*. Especie que presenta características morfológicas diagnósticas del género *Parastrigea,* pero con datos moleculares se anida dentro del clado *Strigea*. Así mismo, algunos individuos de *Strigea falconis* Szidat, 1928 y *Strigea strigis* (Schrank, 1788) Abildgaard, 1790 comparten caracteres diagnósticos del género *Parastrigea* (Dubois, 1968).

Adicionalmente, se realizó un estudio integrativo de especímenes identificados morfológicamente como *Parastrigea macrobursa* Drago y Lunaschi, 2011 y *Strigea* sp., asociadas a cuatro especies de aves rapaces (*Rupornis magnirostris*, *Accipiter cooperii*, *Buteogallus urubitinga* y *B. athracinus*) colectadas en nueve localidades del Pacífico y Golfo de México (Capítulo 2; Fig. 1). En este estudio se generaron secuencias con dos genes nucleares del DNA ribosomal (28S e ITS) y la región completa (900 pb) del gen mitocondrial citocromo c oxidasa subunidad 1 (*Cox 1*) en combinación con datos morfológicos (medidas morfométricas) y fotografías de microscopia electrónica de barrido (MEB). Los resultados filogenéticos derivados de los análisis de Máxima Verosimilitud e Inferencia Bayesiana mostraron dos linajes independientes dentro de *Strigea* (Capítulo 2; Figs. 2–4). El primer linaje corresponde con una especie no descrita previamente colectada en dos especies de aves rapaces (*Rupornis magnirostris* y *Accipiter cooperii*) en seis localidades de México, la cual es nombrada como *Strigea magnirostris* n. sp. La nueva especie se caracteriza morfológicamente por presentar una combinación de caracteres únicos, como son; papilas alrededor de la ventosa oral, seudoventosas bien desarrolladas, diminutas espinas en el segmento anterior del cuerpo, así como un cono genital y bursa copulatoria de gran tamaño (Capítulo 2; Figs. 5–6). Cabe resaltar que esta sería la primera especie de *Strigea* descrita para México y la número 16ava para la región Neotropical.

El segundo linaje corresponde con especímenes identificados morfológicamente como *Parastrigea macrobursa* colectadas del hospedero tipo (*Buteogallus urubitinga*) y *B. anthracinus* en tres localidades de México. Los análisis filogenéticos mostraron que los especímenes de *Parastrigea macrobursa* no están relacionadas con otras especies del género *Parastrigea*, ya que se anidan dentro del clado conformado por especies del género *Strigea.* Con base en estos resultados transferimos la especie *Parastrigea macrobursa* al género *Strigea* para formar *Strigea macrobursa* n. comb. (Capítulo 2; Figs. 2–4). Los especímenes colectados en el presente estudio son morfológicamente similares a la descripción original de *Parastrigea macrobursa* en Argentina. Por ejemplo, el segmento anterior o "*forebody*" tiene forma de tulipan, las glándulas vitelógenas se encuentran distribuidas en dos masas simétricas en el segmento anterior (carácter diagnóstico del género *Parastrigea*), además poseen una bursa copulatoria de gran tamaño y cono genital bien delimitado (ver Drago y Lunaschi, 2011). Sin embargo, nuestros especímenes mostraron cierta variación morfológica, por ejemplo, algunos individuos presentan un "*cuello*" en la parte posterior del cuerpo o "*hindbody*" mientras que otros individuos no lo presentan. Así mismo, nuestros especímenes tienen espinas en la parte anterior del cuerpo, las cuales van disminuyendo en número y tamaño (Capítulo 2; Figs. 7–8). Aparentemente la presencia o ausencia de espinas podría estar relacionado al estado de desarrollo del parásito. Un patrón similar ha sido encontrado en especímenes de dos especies de *Strigea* (*S. falconis brasiliana* y *S. elliptica*) de la región Neotropical (Lunaschi y Drago, 2006, 2009, 2013).

Este estudio nos permitió registrar por primera vez la especie *Strigea macrobursa* n. comb. en México, expandiendo su rango de distribución hasta Argentina. Así mismo, se confirma nuevamente la superposición de rasgos diagnósticos entre especies de los géneros *Parastrigea*, *Strigea* y *Apharyngostrigea.* Actualmente estos géneros carecen de caracteres

diagnósticos que permitan una adecuada identificación de los grupos, por lo que es de suma importancia seguir incorporando información molecular en los registros y descripciones de nuevas especies en México. Cabe destacar que hemos detectado un componente ecológico en cada uno de los géneros estudiados. Por ejemplo, las especies pertenecientes al género *Strigea* se encuentran asociadas con aves rapaces de las familias Accipitridae y Falconidae, mientras que las especies del género *Apharyngostrigea* muestran una alta especificidad hospedatoria en aves de la familia Ardeidae, comúnmente conocidas como garzas. Finalmente, las especies del género *Parastrigea* se encuentran asociadas con aves pertenecientes a la familia Threskiornithidae, sugiriendo que el componente ecológico podría jugar un papel principal en las relaciones filogenéticas de los estrigeidos. Con base en estas asociaciones ecológicas, inferimos que probablemente las especies identificadas morfológicamente como *Parastrigea* colectadas de aves rapaces o garzas pertenecen a los géneros *Strigea* o *Apharyngostrigea* respectivamente.

Este patrón ecológico ha sido encontrado en otros géneros de tremátodos. Por ejemplo, los adultos pertenecientes al género *Clinostomum* Leydi, 1856 muestran una estrecha relación ecológica con aves de la familia Ardeidae. Así mismo, su fase larval o metacercaria muestra un patrón de especificidad hospedatoria a nivel del segundo hospedero intermediario parasitando peces pertenecientes a las familias Cichlidae, Poecilidae, Profundulidae, Eleotridae, entre otros (Sereno-Uribe et al. 2013, 2018). Por otro lado, López Jiménez et al. (2017) encontraron la presencia de tres linajes pertenecientes al género *Uvulifer* Yamaguti, 1934 asociados a peces de las familias Characidae, Cyprinidae y Cichlidae respectivamente, distribuidas en México y Centroamérica. Esta asociación ecológica a nivel del segundo hospedero intermediario y definitivo encontrado en diversos grupos de tremátodos sugiere

fuertes patrones coevolutivos entre los parásitos y sus hospederos derivados de diferentes estrategias en sus ciclos de vida.

Finalmente, se evaluó la estructura genética poblacional de *P. diovadena*, una especie de estrigeido que forma parte de la helminto-fauna del ibis blanco (*Eudocimus albus*) una especie de ave perteneciente a la familia Threskiornithidae. En este estudio colectamos especímenes adultos de *P. diovadena* en tres provincias biogeográficas de México (Sierra Madre Oriental, Sierra Madre Occidental y Sierra Madre del Sur) para evaluar sí las barreras geográficas juegan un papel esencial en la estructuración genética de las poblaciones de este parásito.

Para explicar la estructura filogeográfica en parásitos, se ha planteado la hipótesis que los tremátodos con un ciclo de vida autógeno (es decir, el ciclo de vida se completa solo en el ambiente acuático) tendrían una mayor estructura filogeográfica debido a la menor capacidad de dispersión de sus hospederos en comparación de aquellos parásitos con un ciclo de vida alogénico (parásitos que se desplazan a través de hospederos acuáticos y hospederos terrestres, ya sea aves o mamíferos) (Blouin et al. 1995; Nadler, 1995; McCoy et al. 2003). Sin embargo, la mayoría de los estudios que analizan la diversidad genética en parásitos, se han enfocado principalmente en especies de importancia médica, veterinaria y comercial (Attwood, 2001, 2010; Chelomina et al. 2014; Voronova et al. 2017; Humphries et al. 2019) y pocos estudios han explorado la variabilidad genética de los tremátodos que parasitan animales silvestres (Crioscione y Blouin, 2004; Blasco-Costa et al. 2012; Herrmann et al. 2014; López et al. 2015).

Los resultados derivados de los índices de diversidad molecular y los test de neutralidad calculados a través del marcador mitocondrial, citocromo oxidasa subunidad 1 (*Cox 1*) revelaron una alta diversidad haplotípica (Hd=0.913) con una baja diversidad nucleotídica

(Pi=0.0057) y una nula estructura genética (Fst= 0.0167). Además los valores de Fu`s Fs fueron negativos dentro de las poblaciones, indicando un número de alelos mayor a lo observado, sugiriendo una rápida expansión demográfica en las poblaciones de *P. diovadena* (Capítulo 3; Fig. 3, Tabla 2). En conclusión, los resultados presentados en este estudio mostraron que las poblaciones de *P. diovadena* carecen de una estructura filogeográfica lo que sugiere que el hospedero definitivo (*E. albus*) juega un papel crucial en la estructura genética de sus parásitos, dispersando las poblaciones a lo largo de la República Mexicana.

Este patrón ha sido encontrado en otros estudios. Por ejemplo, Crioscione y Blouin (2004) compararon la estructura filogeográfica de tres especies de tremátodos que se encuentran infectando peces salmónidos. Dos especies de tremátodos con ciclo autogénico (*Deropegus aspina* McCauley y Pratt, 1961 y *Plagioporus shawi* Margolis, 1972) y una con ciclo de vida alogénico (*Nanophyetus salmincola* Chapin, 1927). Dichos autores encontraron que las especies autógenas tenían poblaciones altamente estructuradas y niveles bajos de flujo génico en comparación con la especie alogénica muestreada de los mismos lugares. Estos resultados muestran cómo la variación en los ciclos de vida de los parásitos puede dar forma a una estructuración genética diferente, esto se debe a la capacidad de dispersión, ya que los movimientos de los hospederos en un ciclo de vida autogénico están confinados a las conexiones hidrológicas (por ejemplo, peces de agua dulce) mostrando bajos niveles de dispersión y por ende bajos niveles de flujo génico, a comparación de los hospederos con ciclo de vida alogénico, lo que puede influenciar en una estructura filogeográfica marcada en las poblaciones de sus parásitos (Criscione et al. 2005; Barret et al. 2008; Blasco-Costa y Poulin, 2013). Este estudio constituye un primer esfuerzo para comprender la estructura genética de las poblaciones de parásitos tremátodos con diferentes ciclos de vida en México.

Los resultados presentados en la tesis permitieron generar un panorama integral sobre la taxonomía, sistemática y filogeografía de tres géneros que conforman la familia Strigeidae asociados a aves acuáticas de México.

V. CONCLUSIONES

- ◊ Los análisis filogenéticos sugieren una subdivisión de la familia Strigeidae, con el clado I como Strigeidae *sensu stricto* ya que incluye el género tipo, mientras que el segundo clado de estrigeidos podrían ser considerados como otra familia independiente.
- ◊ Dentro del clado de Strigeidae *sensu stricto*, las diferencias morfológicas entre los géneros *Strigea*, *Parastrigea* y *Apharyngostrigea* son sútiles y los caracteres diagnósticos se sobreponen en algunas especies.
- ◊ La especie *Apharyngostrigea brasiliana* comparte características diagnósticas de dos géneros. Por ejemplo, presenta dos masas de glándulas vitelógenas en la parte anterior del cuerpo, caracter diagnóstico del género *Parastrigea* y por otro lado carece de una faringe, carácter diagnóstico del género *Apharyngostrigea.*
- ◊ Se registró por primera vez la especie de estrigeido *Apharyngostrigea brasiliana* n. comb. colectada del pico zapato (*Cochlearius cochlearius*) en la localidad Champotón en el estado de Campeche, expandiendo su rango de distribución de México hasta Argentina.
- ◊ Los miembros pertenecientes al género *Apharyngostrigea* muestran una alta especificidad hospedatoria parasitando aves de la familia Ardeidae, conocidas como garzas.
- ◊ Se describió una especie del género *Strigea* colectada de dos especies de aves rapaces (*Rupornis magnirostris* y *Accipiter cooperii*) en seis localidades de México.
- ◊ *Strigea magnirostris* n. sp. se distingue morfológicamente de otras especies por presentar papilas alrededor de la ventosa oral, seudoventosas bien desarrolladas,

diminutas espinas en el segmento anterior del cuerpo, así como un cono genital y bursa copulatoria de gran tamaño.

- ◊ *Strigea magnirostris* n. sp. representa la primera especie del género descrita para México y la número 16ava para la región Neotropical.
- ◊ Se colectaron por primera vez especímenes identificados morfológicamente como *Parastrigea macrobursa* del hospedero tipo *Buteogallus urubitinga* en tres localidades de México.
- ◊ Los análisis filogenéticos revelaron que los especímenes identificados morfológicamente como *Parastrigea macrobursa* están relacionados con otras especies del género *Strigea*. Por lo tanto, fue reclasificada como *Strigea macrobursa* n. comb. expandiendo su rango de distribución de México hasta Argentina.
- ◊ Con base en los resultados presentados en este estudio los géneros *Parastrigea*, *Strigea* y *Apharyngostrigea* carecen de caracteres diagnósticos que permitan una adecuada identificación de los grupos.
- ◊ Las asociaciones ecológicas podrían ser utilizadas para distinguir entre especies de los géneros *Parastrigea*, *Strigea* y *Apharyngostrigea*.
- ◊ Es de suma importancia continuar incorporando información molecular en el registro y descripción de nuevas especies pertenecientes a la familia Strigeidae.
- ◊ En la presente tesis se analizó la estructura genética de las poblaciones de *P. diovadena* asociadas al ibis blanco (*E. albus*), en tres regiones biogeográficas en México (Sierra Madre Oriental, Sierra Madre Occidental y Sierra Madre del Sur).
- ◊ Los resultados derivados de los análisis mostraron una nula estructura genética en *P. diovadena* a pesar de las barreras biogeográficas que separan las poblaciones , lo que

se sugiere que el hospedero definitivo juega un papel preponderante en la dispersión de sus parásitos.

◊ Los resultados presentados en la tesis permitieron generar un panorama integral sobre la taxonomía, sistemática y filogeografía de tres géneros que conforman la familia Strigeidae asociados a aves acuáticas de México.

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VII. APÉNDICE

Artículos publicados como becario de CONACYT (CVU: 706119) y financiados por el Programa de Apoyo a Proyectos de Investigación e Innovación Tecnológica (PAPIIT-IN201122) que se realizaron de forma paralela y colaborativa durante el periodo de Doctorado.

VII.I.

Sereno-Uribe, A.L., **López-Jiménez, A**., González-García, M.T., Pinacho-Pinacho, C.D., Macip Ríos, R. & García-Varela, M. (2022) Phenotypic plasticity, genetic structure and systematic position of *Neoechinorhynchus emyditoides* Fisher, 1960 (Acanthocephala: Neoechinorhynchidae): a parasite of emydid turtles from the Nearctic and Neotropical regions. *Parasitology* 149, 991–1002.

https:// doi.org/10.1017/S003118202200049X

Parasitology

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Research Article

Cite this article: Sereno-Uribe AL, López-Jiménez A, González-García MT, Pinacho-Pinacho CD, Macip Ríos R, García-Varela M (2022). Phenotypic plasticity, genetic structure and systematic position of *Neoechinorhynchus emyditoides* Fisher, 1960 (Acanthocephala: Neoechinorhynchidae): a parasite of emydid turtles from the Nearctic and Neotropical regions. *Parasitology* **149**, 991–1002. https://doi.org/10.1017/S003118202200049X

Received: 22 December 2021
Revised: 22 March 2022
Accepted: 3 April 2022
First published online: 19 April 2022

Key words:
Acanthocephalans; freshwater turtles; Gulf of Mexico; molecular markers; taxonomy

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CAMBRIDGE UNIVERSITY PRESS

Phenotypic plasticity, genetic structure and systematic position of *Neoechinorhynchus emyditoides* Fisher, 1960 (Acanthocephala: Neoechinorhynchidae): a parasite of emydid turtles from the Nearctic and Neotropical regions

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Abstract

The taxonomy of the 10 recognized *Neoechinorhynchus* species associated with emydid turtles is complex due to the morphological conservatism. In the present study, specimens of *N. emyditoides* from northern and southeastern Mexico exhibit great phenotypic plasticity on its diagnostic characteristics. We sequenced three molecular markers: the internal transcribed spacers ITS1, ITS2 and 5.8S gene, the D2 + D3 domains of the large subunit from nuclear DNA and cytochrome *c* oxidase subunit I (*cox1*) from mitochondrial DNA. Sequences of the nuclear molecular markers were aligned and compared with other congeneric species associated with emydids available in GenBank. Phylogenetic analyses supported the polyphyly of *Neoechinorhynchus*. The species from emydids formed a clade, which was subdivided into five subclades that correspond with each species analysed (*N. pseudemydis*, *N. chrysemydis*, *N. emydis*, *N. schmidti* and *N. emyditoides*). To understand better the genetic structure of *N. emyditoides* a haplotype network was inferred with 29 *cox1* sequences, revealing the presence of 13 haplotypes, two of which were shared and 11 were unique. The high values of fixation index, F_{st} (0.4227–0.8925) detected between the two populations from southeastern and the two from northern Mexico indicated low genetic flow among the populations. Our data suggest that the *Neoechinorhynchus* species associated with emydid turtles diversified in the eastern USA and that of *N. emyditoides* expanded its distribution range reached southeastern Mexico.

Introduction

Phenotypic plasticity is defined as the ability of a genotype to produce multiple phenotypes in response to environmental conditions (Roff, 2002; Miner *et al.*, 2005). In organisms with complex and diverse life cycles such as parasites, different environmental conditions include (1) the host's immune system, (2) different host species and (3) the geographical distribution of the definitive hosts (Poulin, 2007). The recent application of molecular markers has helped to define, recognize, delineate and better understand intraspecific variation that can be attributed to differences in the development and phenotypic plasticity of acanthocephalans (Steinauer *et al.*, 2007; Rosas-Valdez *et al.*, 2012, 2020; Alcántar-Escalera *et al.*, 2013; Perrot-Minnot *et al.*, 2018; Pinacho-Pinacho *et al.*, 2018*a*; Lisitsyna *et al.*, 2019).

The Neoechinorhynchidae (Ward, 1917) Van Cleave, 1928 is a large, globally distributed family of acanthocephalans, which are typically parasites from the intestine of teleost fishes and freshwater turtles. At present, the family includes 14 genera split into four subfamilies (Gibson and Wayland, 2022). The type genus *Neoechinorhynchus* Stiles and Hassall, 1905 represents a hyperdiverse group of endoparasites of marine, freshwater, brackish water fishes and freshwater turtles, with ~117 species distributed worldwide (Amin, 2013; Smales, 2013; Pinacho-Pinacho *et al.*, 2018*a*; Amin *et al.*, 2019, 2020). In the Americas, 50 species have been described: 33 in North America, corresponding to the Nearctic biogeographical region, and 17 in Middle and South America, corresponding to the Neotropical biogeographical region (Amin, 2013; Pinacho-Pinacho *et al.*, 2018*b*). Members of *Neoechinorhynchus* have been the target of numerous studies related to their ecology, host–parasite relationships,

https://doi.org/10.1017/S003118202200049X Published online by Cambridge University Press

VII.II.

Sereno-Uribe, A.L., González-García, M.T., Ortega-Olivares, M.P., **López-Jiménez, A.,** García-Varela, M. & Andrade-Gómez, L. (2022) First record of *Patagifer bilobus* (Rudolphi, 1819) Dietz, 1909 (Digenea: Echinostomatidae), with a morphological and molecular characterization from two threskiornithid species in Mexico. *Parasitology Research* 121, 1921–1935.

https://doi.org/10.1007/s00436-022-07526-3

Parasitology Research (2022) 121:1921–1935
https://doi.org/10.1007/s00436-022-07526-3

FISH PARASITOLOGY - ORIGINAL PAPER

First record of *Patagifer bilobus* (Rudolphi, 1819) Dietz, 1909 (Digenea: Echinostomatidae), with a morphological and molecular characterization from two threskiornithid species in Mexico

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Received: 22 November 2021 / Accepted: 18 April 2022 / Published online: 30 April 2022

Abstract
Patagifer Dietz, 1909 is a small genus of echinostomatids, with 12 recognized species, mostly parasitising threskiornithid birds, distributed worldwide. In the current research, adult specimens of the type species, *Patagifer bilobus* (Rudolphi, 1819) Dietz, 1909 from the white faced ibis (*Plegadis chihi*) and white ibis (*Eudocimus albus*) were re-described, providing new metrical data for the number of head collar spines. Those specimens were recorded from eight localities in Mexico and compared morphologically with specimens previously identified as *Patagifer lamothei*. A total of 19 specimens identified as *P. bilobus* including two *hologenophores* were sequenced with three molecular markers: domains D1–D3 of the large subunit (LSU), the internal transcribed spacer (ITS1, ITS2) plus 5.8S from the nuclear rDNA, and nicotinamide adenine dinucleotide dehydrogenase subunit 1 (*nad1*) from mitochondrial DNA. The new sequences were aligned with other sequences of *Patagifer* spp., downloaded from GenBank. Phylogenetic trees inferred from each data set, placed all the specimens in a clade, confirming that the isolates belonged to the same species. The morphological examination of specimens previously identified as *P. lamothei* by Ortega-Olivares MP, Hernández-Mena DI, Pérez-Ponce de León G, García-Varela M (2011) Helminths of the white ibis, *Eudocimus albus* (Aves Therskiornithidae) in Mexico. (Zootaxa 3088, 15–26. 10.11646/zootaxa.3088.1.2) and in combination with molecular data confirms that those specimens should be reassigned to *P. bilobus*. In addition, this is the first study in *P. bilobus* using an integrative taxonomy approach.

Keywords Morphology · Systematic · Trematoda · LSU · ITS · *nad1* · Molecular phylogeny · Americas · Integrative taxonomy

Introduction

Patagifer Dietz, 1909 is a small genus of echinostomatids with 12 recognized species that mostly parasitize threskiornithid birds distributed worldwide. Only three species have been recorded in other bird families, like Ardeidae, Podicipedidae, and Scolopacidae (Faltýnková et al. 2008; Dronen and Blend 2008). The species of *Patagifer* are characterized mainly by the following morphological traits: an elongated body, with maximum width at the head collar; forebody short concave; a ribbon-like hindbody; collar-spines rod-shaped, 48–64 in single row along edge of collar; angle spines 2 × 3–4, all of comparable size or one pair substantially larger; dorsal spines smallest (Faltýnková et al. 2008). Kostadinova (2005) mentioned that *Patagifer* can be distinguished from other genera of echinostomatids by the presence of a collar strongly developed, muscular, usually broader than body, distinctly bilobed by deep narrow dorsal incision and wider ventral notch. Based on these morphological traits, the taxonomic history and species composition of the genus *Patagifer* have been complex and unstable due to the phenotypic plasticity of diagnostic characteristics that

Section Editor: Matthew Thomas Wayland.

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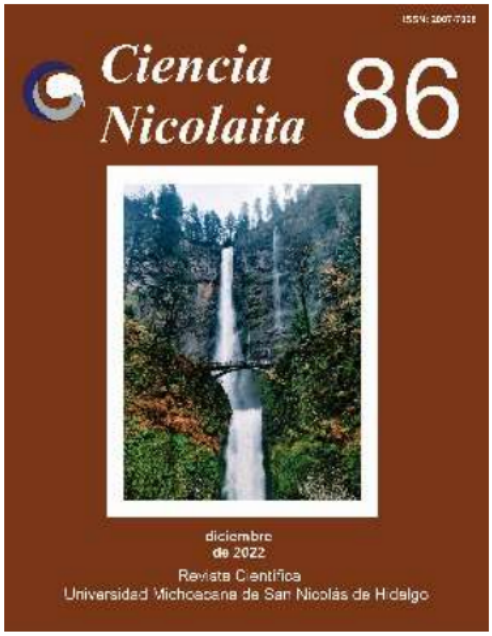
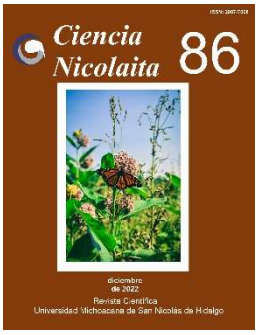
VII.III.

Sereno-Uribe, A.L., **López-Jiménez, A.,** Ortega-Olivares, M.P., Andrade-Gómez, L., González-García, M.T. & García-Varela, M. (2022) Vislumbrando la diversidad de clinostomidos (Platyhelminthes: Digenea), parásitos asociados a peces y aves acuáticas en México y Centroamérica mediante información obtenida de la biología molecular. *Ciencia Nicolaita* 86, 34–44.

DOI: https://doi.org/10.35830/cn.vi86.661





Ciencia Nicolaita 86

ISSN: 2007-7068

Universidad
Michoacana
de San Nicolás
de Hidalgo

Vislumbrando la diversidad de clinostomidos (Platyhelminthes: Digenea), parásitos asociados a peces y aves acuáticas en México y Centroamérica mediante información obtenida de la biología molecular

Looking at the diversity of clinostomids (Platyhelminthes: Digenea), parasites associated with fish and water birds in Mexico and Central America by molecular and biological information

Ana Lucia Sereno-Uribe, Alejandra López-Jiménez, Mirza Patricia Ortega-Olivares, Leopoldo Andrade-Gómez, Marcelo Tonatiuh González-García y Martín García-Varela

Para citar este artículo: Ana Lucia Sereno-Uribe, Alejandra López-Jiménez, Mirza Patricia Ortega-Olivares, Leopoldo Andrade-Gómez, Marcelo Tonatiuh González-García y Martín García-Varela, 2022. Vislumbrando la diversidad de clinostomidos (Platyhelminthes: Digenea), parásitos asociados a peces y aves acuáticas en México y Centroamérica mediante información obtenida de la biología molecular. Ciencia Nicolaita no. 86, 33-44. DOI: https://doi.org/10.35830/cn.vi86.661

Historial del artículo:
Recibido: 23 de mayo de 2022
Aceptado: 28 de septiembre de 2022
Publicado en línea: diciembre de 2022

Ver material suplementario

Correspondencia de autor: garciav@ib.unam.mx

VII. IV.

García-Varela, M., **López-Jiménez, A.,** González-García, M.T., Sereno-Uribe, A.L. & Andrade-Gómez, L. (2023) Contrasting the population genetic structure of a specialist (*Hexaglandula corynosoma*: Acanthocephala: Polymorphidae) and a generalist parasite (*Southwellina hispida*) distributed sympatrically in Mexico. *Parasitology* 150, 348–358.

doi: 10.1017/S0031182023000033.

Parasitology

cambridge.org/par

Research Article

Cite this article: García-Varela M, López-Jiménez A, González-García MT, Sereno-Uribe AL, Andrade-Gómez L (2023). Contrasting the population genetic structure of a specialist (*Hexaglandula corynosoma*: Acanthocephala: Polymorphidae) and a generalist parasite (*Southwellina hispida*) distributed sympatrically in Mexico. *Parasitology* **150**, 348–358. https://doi.org/10.1017/S0031182023000033

Received: 19 November 2022
Revised: 31 December 2022
Accepted: 1 January 2023
First published online: 12 January 2023

Key words:
Acanthocephalans; generalist; Mexico; Polymorphidae; phylogeography; specialist

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Contrasting the population genetic structure of a specialist (*Hexaglandula corynosoma*: Acanthocephala: Polymorphidae) and a generalist parasite (*Southwellina hispida*) distributed sympatrically in Mexico

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Abstract

Polymorphidae is a monophyletic group of acanthocephalans distributed worldwide. Within this family, *Hexaglandula corynosoma* is a specialist species that uses a single bird species as a definitive host. *Southwellina hispida* is a generalist species that uses a broad spectrum of definitive hosts to complete its life cycle. In the current research, sequences of cytochrome c oxidase subunit 1 (*cox1*) from mitochondrial DNA were generated from 44 specimens of *H. corynosoma* and 76 of *S. hispida* distributed sympatrically in 6 biogeographic provinces of Mexico with the objective of characterizing and comparing the population genetic structure of 2 acanthocephalan species with opposing life strategies. The phylogeographic studies indicated that the populations of both species lacked a phylogeographic structure and exhibited high haplotype diversity, low nucleotide diversity and low F_{st} values among the biogeographic provinces; in combination with negative values on the neutrality test, this suggests that the populations of acanthocephalans are expanding. Paratenic hosts are key for the transmission from intermediate to definitive hosts in the generalist species. However, the inclusion of paratenic hosts does not play a principal role in the population genetic structure of *S. hispida* within its distribution along the coasts of Mexico.

Introduction

Parasitism is a highly successful lifestyle and has evolved independently at least 60 times in different groups of metazoans worldwide (Price, 1980; Poulin and Morand, 2004). Parasites have been traditionally divided into 2 major groups depending on their life cycle: generalists and specialists (Thompson, 1994, 2005). The generalist parasites use a wide range of definitive hosts, whereas the specialist parasites use a single definitive host to complete their life cycle. Under these 2 opposing strategies, generalist parasites infect a broad spectrum of hosts resulting in an optimal or suboptimal level of fitness, whereas specialist parasites prioritize a single optimal host in which fitness is maximized (Rigaud *et al.*, 2010; Lievens *et al.*, 2018). Some studies have suggested that parasite life cycle complexity (generalists *vs* specialists) could influence population genetic structure (Nadler, 1995; Criscione and Blouin, 2004; Barrett *et al.*, 2008; Archie and Ezenwa, 2011). According to Li *et al.* (2014), a specialist parasite shows significantly less genetic flow; therefore, populations are less connected and are subdivided into smaller populations, leading to strong genetic differentiation. In some cases, the populations might experience bottlenecks, decreasing the effective population size. Moreover, specialist species are more sensitive to stochastic fluctuations that can cause local extinction. In contrast, a generalist parasite shows a high effective population size, high genetic flow and a population that is structured or panmictic. Moreover, a generalist parasite may show greater persistence of populations over the long term because the generalist may be less sensitive to stochastic fluctuations in any given resource as it is able to replace a scarce resource with another (see Sehgal *et al.*, 2001; Brant and Ortí, 2003; Archie and Ezenwa, 2011; Li *et al.*, 2014).

The recent application of molecular markers has helped establishing a more robust classification scheme in acanthocephalans (Near *et al.*, 1998; García-Varela *et al.*, 2000). In particular, cytochrome c oxidase subunit 1 (*cox1*) from mitochondrial DNA is among the most useful molecular markers for defining, recognizing and delineating species and better understanding the population genetic structure in acanthocephalans (Steinauer *et al.*, 2007; Rosas-Valdez *et al.*, 2012, 2020; Alcántar-Escalera *et al.*, 2013; Goulding and Cohen, 2014; Perrot-Minnot

https://doi.org/10.1017/S0031182023000033 Published online by Cambridge University Press

VII.V.

García-Varela, M., Hernández-Orts, J., **López-Jiménez, A.,** & González-García, M. (2023). Molecular and morphological characterization of *Andracantha gravida* (Alegret, 1941) (Acanthocephala: Polymorphidae) in piscivorous birds from the Gulf of Mexico. *Journal of Helminthology* 97, e31. 1–9.

doi:10.1017/S0022149X22000955

Journal of Helminthology

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Molecular and morphological characterization of *Andracantha gravida* (Alegret, 1941) (Acanthocephala: Polymorphidae) in piscivorous birds from the Gulf of Mexico

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Research Paper

Cite this article: García-Varela M, Hernández-Orts JS, López-Jiménez A, González-García MT (2023). Molecular and morphological characterization of *Andracantha gravida* (Alegret, 1941) (Acanthocephala: Polymorphidae) in piscivorous birds from the Gulf of Mexico. *Journal of Helminthology* **97**, e31, 1–9. https://doi.org/10.1017/S0022149X22000955

Received: 17 October 2022
Revised: 28 December 2022
Accepted: 28 December 2022

Keywords:
Palaeacanthocephala; scanning electron microscopy; *Nannopterum auritus*; *Pelecanus occidentalis*; *cox*1; haplotype network

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Abstract

Adult specimens of *Andracantha gravida* (Alegret, 1941) were recorded from the intestines of the double-crested cormorant *Nannopterum auritus* (Lesson) (type host) and brown pelican *Pelecanus occidentalis* L. in two localities from Mexico: Celestún, Yucatan (south-eastern) and Punta Piedra, Tamaulipas (north-eastern). The specimens of *A. gravida* are morphologically characterized by having a pipe-shaped body without swellings, the absence of small trunk spines between the two fields of spines on the foretrunk and a cylindrical proboscis with 14–16 rows of 10–12 hooks per row. Newly generated partial sequences of the mitochondrial cytochrome *c* oxidase subunit 1 (*cox*1) gene were generated from adult isolates of *A. gravida* from Mexico and compared with one sequence of *A. gravida* and with sequences of other polymorphid acanthocephalans available in GenBank. Phylogenetic analyses based on maximum likelihood and Bayesian inference methods of the *cox*1 dataset placed all the species of *Andracantha* in a single clade, with weak support. The analyses of the *cox*1 dataset placed *Andracantha sigma* Presswell, García-Varela & Smales, 2018, as sister to the clade formed by *A. gravida*, *Andracantha phalacrocoracis* (Yamaguti, 1939), *Andracantha leucocarboi* Presswell, García-Varela & Smales, 2018 and an unidentified species of *Andracantha* from Japan. The newly generated *cox*1 sequences of *A. gravida* from piscivorous birds of Mexico formed a strongly supported clade with the published sequence of *A. gravida* from the double-crested cormorant from the south-eastern coast of Mexico. The intraspecific genetic divergence among isolates identified as *A. gravida* ranged from 0.0% to 2.2%. A *cox*1 haplotype network inferred with 14 sequences revealed the presence of nine haplotypes, two of which were shared between the populations of piscivorous birds from the north-eastern and south-eastern coasts of Mexico and seven of which were unique. The fixation index between the populations from north-eastern and south-eastern Mexico was low (0.06949), which suggests genetic flow. This can be explained by the migration patterns of the brown pelican and the double-crested cormorant along the coasts of the Gulf of Mexico.

Introduction

Members of the genus *Andracantha* (Schmidt, 1975) are considered to be typical components of helminth fauna of cormorants and shags (Phalacrocoracidae) (see Schmidt, 1975; Presswell *et al.*, 2018). However, some *Andracantha* species have been recorded in diverse hosts, such as the brown pelican *Pelecanus occidentalis* L., the red-breasted merganser *Mergus serrator* L., the American bald eagle *Haliaeetus leucocephalus* L, and the little blue penguin *Eudyptula novaehollandiae* Forster (Schmidt, 1975; Richardson & Cole, 1997; Laskowski *et al.*, 2008; Presswell *et al.*, 2018). Morphologically, species of *Andracantha* are identified by the presence of two fields of spines separated by a bare zone in the anterior part of the trunk, a cylindrical proboscis with a slightly swollen region, a cone-shaped neck, six or eight pyriform cement glands, usually arranged in bilateral pairs and eggs with or without polar protrusion in the middle fertilization membrane (Schmidt, 1975; Presswell *et al.*, 2018). Based on these morphological features, the genus *Andracantha* currently comprises nine species: five of them were described in the Americas: *Andracantha gravida* (Alegret, 1941) (type species), *Andracantha phalacrocoracis* (Yamaguti, 1939), *Andracantha mergi* (Lundström, 1941), *Andracantha baylisi* (Zdzitowiecki, 1986) and *Andracantha tandemtesticulata* (Monteiro *et al.*, 2006); three in Oceania: *Andracantha clavata* (Goss, 1941), *Andracantha sigma* (Presswell, García-Varela & Smales, 2018) and

https://doi.org/10.1017/S0022149X22000955 Published online by Cambridge University Press