UNIVERSIDAD NACIONAL AUTÓNOMA DE MÉXICO

# POSGRADO EN CIENCIAS BIOLÓGICAS

POSGRADO EN CIENCIAS BIOLÓGICAS
INSTITUTO DE BIOLOGÍA

## ANÁLISIS FILOGENÉTICO DE LA FAMILIA GRYPORHYNCHIDAE (CESTODA: CYCLOPHYLLIDEA) CON BASE EN CARACTERES MORFOLÓGICOS Y MOLECULARES

# T E S I S

QUE PARA OBTENER EL GRADO ACADÉMICO DE

**DOCTORA EN CIENCIAS**

P R E S E N T A

**M. en C. MIRZA PATRICIA ORTEGA OLIVARES**

**TUTOR PRINCIPAL DE TESIS: DR. JOSÉ MARTÍN GARCÍA VARELA. INSTITUTO DE BIOLOGÍA**
**COMITÉ TUTOR: DR. JUAN PEDRO LACLETTE SAN ROMAN. INSTITUTO DE BIOMÉDICAS**
**DRA. BLANCA ESTELA HERNÁNDEZ BAÑOS. FACULTAD DE CIENCIAS**

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Ciencias Biológicas

COORDINACIÓN

**Dr. Isidro Ávila Martínez**
**Director General de Administración Escolar, UNAM**

**Presente**

Me permito informar a usted que en la reunión ordinaria del Comité Académico del Posgrado en Ciencias Biológicas, celebrada el día 1 de octubre de 2012, se aprobó el siguiente jurado para el examen de grado de **DOCTORA EN CIENCIAS** de la alumna **ORTEGA OLIVARES MIRZA PATRICIA** con número de cuenta **95334824** con la tesis titulada: **"ANÁLISIS FILOGENÉTICO DE LA FAMILIA GRYPORHYNCHIDAE (CÉSTODA: CYCLOPHYLLIDEA) CON BASE EN CARACTERES MORFOLÓGICOS Y MOLECULARES"**, realizada bajo la dirección del **DR. JOSÉ MARTÍN GARCÍA VARELA:**

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

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M. del Coro Arizmendi

**DRA. MARÍA DEL CORO ARIZMENDI ARRIAGA**
**COORDINADORA DEL PROGRAMA**

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

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# Índice

# Presentación

La información generada en el presente proyecto doctoral se encuentra dividida en tres secciones, la primera corresponde a la introducción, antecedentes, objetivos generales, particulares, discusión y conclusiones. La segunda sección corresponde a resultados particulares, y se compone a su vez de tres capítulos que se presentan en formato de artículo; el primero publicado en Zootaxa, el segundo ha sido aceptado para su publicación en Folia Parasitologica y el tercero será sometido para su publicación en una revista internacional del área. Cada uno de los manuscritos contiene a su vez: introducción, material y métodos, resultados, discusión y literatura citada.

# Resumen

La clase Cestoda incluye organismos endoparásitos obligados de vertebrados que se caracterizan por tener un cuerpo aplanado dorso ventralmente y simetría bilateral, además de una distribución cosmopolita. Este grupo es considerado como uno de los más diversos dentro del phylum Platyhelminthes teniendo a la fecha más de 4,000 especies reconocidas. Los grupos de céstodos se distinguen entre sí por caracteres ecológicos (tipo de huésped que parasitan) y caracteres morfológicos tales como la forma del cuerpo, presencia de estructuras en la parte anterior del cuerpo (cabeza), y distribución de los órganos reproductores. Los céstodos han sido clasificados tradicionalmente dependiendo del criterio de cada "especialista" y solo recientemente se ha implementado un sistema de clasificación basado en las relaciones filogenéticas de ciertos grupos. Uno de los grupos más estudiado es el orden Cyclophyllidea, considerado como aquel de aparición más reciente, con 13 familias actualmente reconocidas. Sin embargo, existe una controversia en cuanto a la validez de la familia (Gryporhynchidae), la cual incluye céstodos parásitos de aves ictiófagas. Para llevar acabo el estudio de los céstodos recolectados de aves ictiófagas, el presente trabajo se encuentra dividido en tres capítulos.

El capítulo I incluye un listado de los helmintos parásitos del ibis blanco *Eudocimus albus* (Threskiornithidae) colectado en diversos cuerpos de agua de México. Este listado muestra breves descripciones taxonómicas y comentarios para cada uno de los helmintos recolectados. La revisión de 52 huéspedes establecieron tres registros de especies de tremátodos por primera vez para México, cinco registros de nuevas localidades para distintos helmintos y un registro nuevo de helminto en el ibis blanco. Este trabajo representa el primer estudio realizado en México sobre la helmintofauna de *Eudocimus albus*, el cual incluye el registro de siete especies de helmintos.

En el capítulo II se realizó la descripción del adulto de *Glossocercus cyprinodontis* parásito de aves ictiófagas recolectadas en Tamaulipas, Campeche y Yucatán.

*Glossocercus cyprinodontis*, especie tipo del género, fue descrita originalmente de pleroceroides (larvas) recolectadas de peces en la bahía de Galveston, Texas, Estados Unidos de América. En el presente trabajo se registró el adulto de *G*. *cyprinodontis* en *Pelecanus occidentalis*, *Egretta rufescens* y *Nycticorax nycticorax*, describiéndose por primera vez la morfología interna y distribución de los órganos reproductores de la forma adulta del céstodo. La diagnosis del género *Glossocercus* fue enmendada con base en la morfología del adulto de *G*. *cyprinodontis*.

En el capítulo III se presentan los resultados de los análisis filogenéticos de los céstodos parásitos encontrados en aves ictiófagas. Los análisis fueron inferidos a partir de secuencias de los genes 18S y 28S del DNA ribosomal. Los céstodos recolectados pertenecen a siete géneros (*Cyclustera*, *Dendrouterina*, *Glossocercus*, *Neovalipora*, *Paradilepis*, *Parvitaenia* y *Valipora*), los cuales se ubicaron dentro de la familia Gryporhynchidae, y fueron comparados y analizados junto con otros miembros del orden Cyclophyllidea, con la finalidad de probar su monofilia y ubicar filogenéticamente a sus miembros. Los análisis filogenéticos mostraron que todas las especies de la familia Gryporhynchidae resultaron ser un grupo monofilético, por lo que se apoyó su validez y su reconocimiento dentro del orden Cyclophyllidea.

## Abstract

The class Cestoda includes endoparasites of vertebrate hosts and is characterized by having a flattened body, bilateral symmetry, and a cosmopolitan distribution. This group is considered one of the most diverse in the phylum Platyhelminthes with 4,000 recognized species. Major groups of tapeworms are distinguished among them by ecological characters (type of host) and by morphological traits as body shape, presence of structures in the anterior region of the body (head), and distribution of reproductive organs. However, tapeworms have been traditionally classified according to an authority criterion and only recently a classification system for this group has been implemented based on the phylogenetic relationships of some examined taxa. The order Cyclophyllidea is considered as one of the most derived tapeworms containing a total of 13 families. However, the validity of Gryporhynchidae has been controversial. This study is divided in three chapters.

In the first chapter, I include a survey of the helminth parasites of white ibis *Eudocimus albus* (Threskiornithidae) collected from different localities in Mexico was presented. This study shows taxonomic descriptions and comments for each parasite species. Examination of 52 hosts established three new records of trematode species for the first time for Mexico, five new localities, and a new parasite record for the white ibis. This work represents the first study in Mexico on the helminth fauna of *Eudocimus albus*, which includes records of seven species of helminth parasites.

In the second chapter, we described for the first time the adult of *Glossocercus cyprinodontis* collected from fish-eating birds from Tamaulipas, Campeche and Yucatan. *Glossocercus cyprinodontis* type species was originally described from pleroceroides (larvae) from fishes collected in Galveston Bay, Texas. In this study we recorded tapeworms of *G. cyprinodontis* from *Pelecanus occidentalis*, *Egretta rufescens* and *Nycticorax nycticorax*, and the internal morphology and distribution of reproductive organs

of the adult were described. Based on these new observations the diagnosis of the genus was amended.

Finally in the third chapter, we assessed the phylogenetic relationships among genera of Gryporhynchidae collected from fish-eating birds. The analyses were inferred using sequences of the small (18S) and large subunit (28S) from DNA Ribosomal. The tapeworms collected belonging to seven genera (*Cyclustera*, *Dendrouterina*, *Glossocercus*, *Neovalipora*, *Paradilepis*, *Parvitaenia* y *Valipora*) classified into the family Gryporhynchidae. The members of this family were compared and analyzed with other species of the order Cyclophyllidea to test the monophyly and the systematic position. The phylogenetic analyses showed all the members of Gryporhynchidae as a monophyletic group, therefore it is recognized as a valid family into the Cyclophyllidea.

# Introducción y Antecedentes

Los helmintos son un grupo de parásitos que incluyen cuatro phyla no relacionados filogenéticamente: Platyhelminthes, Acanthocephala, Nematoda y Clase Hirudinea (Phylum Annelida), caracterizados por ser organismos abundantes y ampliamente distribuidos en la naturaleza (Brooks y MacLennan 1993; Brusca y Brusca 2003; Pérez-Ponce de León et al. 2007). En México el estudio de los helmintos parásitos de animales silvestres ha sido de gran interés, llevándose a cabo por más de 100 años. De acuerdo con Pérez-Ponce de León et al. (2007), uno de los grupos más diversos registrados en México son los Platyhelminthes, con más de 1, 241 especies. Sin embargo, de acuerdo con algunos estudios se ha estimado que en la actualidad menos del 21% de la diversidad de especies de helmintos parásitos ha sido descrita.

En particular la clase Cestoda es considerada como una de las más diversas dentro del phylum Platyhelminthes (Schmidt 1934). Los céstodos son un grupo bien reconocido de organismos, caracterizado por ser endoparásitos obligados, de simetría bilateral, aplanados dorso-ventralmente, distribuidos en todo el mundo (Schmidt 1934; Mariaux 1998) y se reconocen más de 4,000 especies parásitas de vertebrados (Mariaux 1998; Olson y Caira 1999). Sin embargo, los céstodos representan un grupo difícil de estudiar debido a tres razones principales: 1) son excepcionalmente frágiles debido a la falta de estructuras firmes; 2) la forma aplanada de los organismos y el tipo de metabolismo obstaculizan la correcta fijación y preparación de los ejemplares, y 3) la estrobilización del cuerpo de los organismos en estado adulto dificulta su colecta (Mariaux 1996, 1998).

Por otro lado, también existen grandes problemas en cuanto a la identificación de las especies, ya que las variaciones intra- e interespecífica son poco conocidas (Mariaux 1998). Adicionalmente a los problemas de identificación, en ocasiones se presentan dificultades para poder obtener grandes cantidades de especímenes, por lo que continuamente especies de céstodos se describen con pocos ejemplares o fragmentos de un solo organismo. Esta insuficiencia de datos ha llevado comúnmente a la incorrecta identificación de especies o en la invalidez de algunos taxones (Hoberg et al. 1997; Mariaux 1998).

*Morfología general de los céstodos*

De acuerdo con Schmidt (1934) y Littlewood (2006), los céstodos usualmente consisten de una cadena de segmentos, llamados proglótidos, en donde se localizan uno o más juegos de órganos reproductores masculino y femenino (organismos hermafroditas). Cada uno de los proglótidos es producido de manera continua por un proceso asexual de gemación. Cada nuevo proglótido formado desplaza hacia el final posterior al proglótido anterior tomando su lugar, de esta forma los proglótidos que son desplazados se vuelven sexualmente maduros. Los últimos proglótidos al final del cuerpo son proglótidos grávidos que poseen los huevos, los cuales son liberados o desintegrados en el ambiente. El cuerpo entero formado por la totalidad de los proglótidos es llamado estróbilo y al proceso por el cual se forman nuevos proglótidos se le conoce como estrobilización. Si el cuerpo se encuentra segmentado se lo conoce como polizóico, y si los proglótidos formados se encuentran encimados uno sobre otro se los conoce como *"craspedota".* La mayoría de los céstodos en su forma adulta poseen una región de fijación, llamado escólex, el cual puede poseer ventosas, surcos, ganchos, espinas, zonas glandulares o una combinación de estas características. En ocasiones entre el escólex y el primer segmento del estróbilo, existe una zona no diferenciada conocida como cuello, el cual puede ser largo, corto o estar ausente. El cuello contiene las células germinales que tienen el potencial de producir nuevos proglótidos (ver Fig. 1)

El estudio taxonómico de los céstodos esta basado principalmente en la anatomía de los diferentes órganos (Schmidt 1934). Para algunos grupos, la forma, tamaño y número de ganchos del escólex, así como el número y disposición de los órganos reproductores en los proglótidos, son características morfológicas diagnósticas para delimitar especies (Khalil et al. 1994). Sin embargo, los céstodos han sido clasificados tradicionalmente dependiendo del criterio de cada "especialista" y solo recientemente se ha implementado un sistema de clasificación basado en las relaciones filogenéticas de ciertos grupos. Un ejemplo de esto, es el número de órdenes de céstodos reconocidos, que varía entre 9 y 19 dependiendo del punto de vista del especialista (Mariaux 1996).

*Orden Cyclophyllidea*

Históricamente, la clasificación de la clase Cestoda se fundamenta principalmente en caracteres morfológicos y ecológicos. Sin embargo, a partir de la década de los 80′s, el estudio de los céstodos comenzó a desarrollarse desde el punto de vista de la sistemática filogenética implementando el uso de caracteres morfológicos y moleculares, con la finalidad de ayudar a resolver problemas de relaciones entre los organismos (Mariaux 1996; Hoberg et al. 1999a; Mariaux y Olson 2001; Littlewood 2006). En particular, el orden Cyclophyllidea ha sido el más estudiado y se han propuesto varias hipótesis filogenéticas de relación entre los organismos con base en caracteres morfológicos y moleculares, con la finalidad de entender algunos patrones generales de evolución de este grupo (Mariaux 1996; Littlewood 2006). Con base en estos estudios se ha propuesto al orden Cyclophyllidea como uno de los grupos de aparición más reciente dentro de la clase Cestoda, con 13 familias reconocidas (Khalil et al. 1994; Hoberg et al. 1999b).

Sin embargo, existe una gran controversia con un grupo de céstodos parásitos de aves ictiófagas (principalmente Pelecaniformes) los cuales fueron clasificados por Spassky y Spasskaya (1973) como miembros de la familia Gryporhynchidae. En clasificaciones recientes del orden Cyclophyllidea, esta familia no es reconocida como válida por algunos autores y todos los céstodos parásitos de aves ictiófagas se ubican dentro de la familia Dilepididae (Bona 1994). La validez de la familia Gryporhynchidae sustentada en análisis filogenéticos y su relación con otras familias del orden

Cyclophyllidea aún no ha sido esclarecida. En años recientes, se ha propuesto a la familia Gryporhynchidae como un grupo independiente a la familia Dilepididae, basado en caracteres morfológicos (Hoberg et al. 1999b) y secuencias parciales del gen 18S (SSU) del DNA ribosomal (Mariaux 1998), e incluso en trabajos recientes la familia es aceptada en la literatura (Beveridge 2001; Chervy 2002; Scholz et al. 2004; Ortega-Olivares et al. 2008; Scholz et al. 2008; Yoneva et al. 2008; Marigo et al. 2010; Presswell et al. 2012). Sin embargo, las hipótesis filogenéticas propuestas por Mariaux (1998) y Hoberg et al. (1999b) tienen que ser tomados con precaución, ya que solo analizaron una especie representante de la familia Gryporhynchidae. Presswell et al. (2012) realizaron una análisis filogenético basado en el gen 18S (SSU) donde se incluían especies de diferentes familias del orden Cyclophyllidea, así como un par de especies de la familia Gryporhynchidae, y sus resultados confirman que los gryporhynchidos son un grupo independiente de Dilepididae. Sin embargo las especies de gryporhynchidos incluidas en este análisis se encuentran en una politomía por lo que aún se desconoce la relación de la familia Gryporhynchidae dentro del orden Cyclophyllidea.

### *Clasificación de la familia Gryporhynchidae*

La familia Gryporhynchidae actualmente está dividida en 10 géneros distribuidos en todo el mundo: *Amyrthalingamia*, *Ascodilepis*, *Cyclustera*, *Dendrouterina*, *Glossocercus*, *Neovalipora*, *Neogryporhynchus*, *Paradilepis*, *Parvitaenia* y *Valipora* (Scholz et al. 2002, 2004; Ortega-Olivares et al. 2008). Estos géneros se distinguen taxonómicamente por caracteres morfológicos como la forma y tamaño de los ganchos del rostelo y distribución de los órganos reproductores. Sin embargo, algunas características morfológicas no han sido claramente definidas y constantemente la validez de ciertos géneros se ha puesto en duda, de tal manera que algunos de ellos han sido sinonimizados (ver Bona 1994; Scholz et al. 2004).

*Ciclo de vida*

Los miembros de la familia Gryporhynchidae en estado adulto son parásitos del tracto digestivo de aves ictiófagas. Su ciclo de vida incluye un huésped intermediario, uno paraténico y uno definitivo. Invertebrados acuáticos (generalmente artrópodos) actúan como huéspedes intermediarios donde se desarrolla una larva (plerocercoide). Los artrópodos infestados son ingeridos por peces, los cuales actúan como huéspedes paraténicos. Los peces infestados son ingeridos por aves ictiófagas (huéspedes definitivos), completando el ciclo de vida. En esta última etapa los céstodos se desarrollan en el intestino de sus huéspedes hasta el estadio adulto, se lleva a cabo la reproducción sexual y los huevos son liberados al ambiente a través de las heces de las aves, esperando ser ingeridos por nuevos artrópodos y comenzar un nuevo ciclo de vida (Baer y Bona 1960; Spassky y Spasskaya 1973; Bona 1975, 1994) (ver Fig. 2).

*Registros de gryporhynchidos en México*

En México se han registrado 8 géneros y 21 especies de céstodos de la familia Gryporhynchidae en peces de diferentes familias y aves ictiófagas de los órdenes Suliformes y Pelecaniformes (Scholz y Salgado-Maldonado 2001; Scholz et al. 2002, 2004; Ortega-Olivares et al. 2008) (Tabla 1). Estos trabajos incluyen registros de especies, estudios taxonómicos, listado de especies y análisis de comunidades de helmintos (Coil 1955; Scholz et al. 2002; Barrera-Guzmán y Guillén-Hernández 2008; Ortega-Olivares et al. 2008, 2011; Violante-González et al. 2011, 2012), principalmente en localidades del sureste de México. Además, Ortega-Olivares (2007) realizó un estudio biogeográfico de la helmintofauna de aves ictiófagas de localidades del Golfo de México, incluyendo registros de siete géneros de la familia Gryporhynchidae. Sin embargo, hasta ahora no existen análisis que incluyan información sobre caracteres morfológicos y moleculares obtenidos de las especies de céstodos parásitos de aves ictiófagas particularmente de la familia Gryporhynchidae.

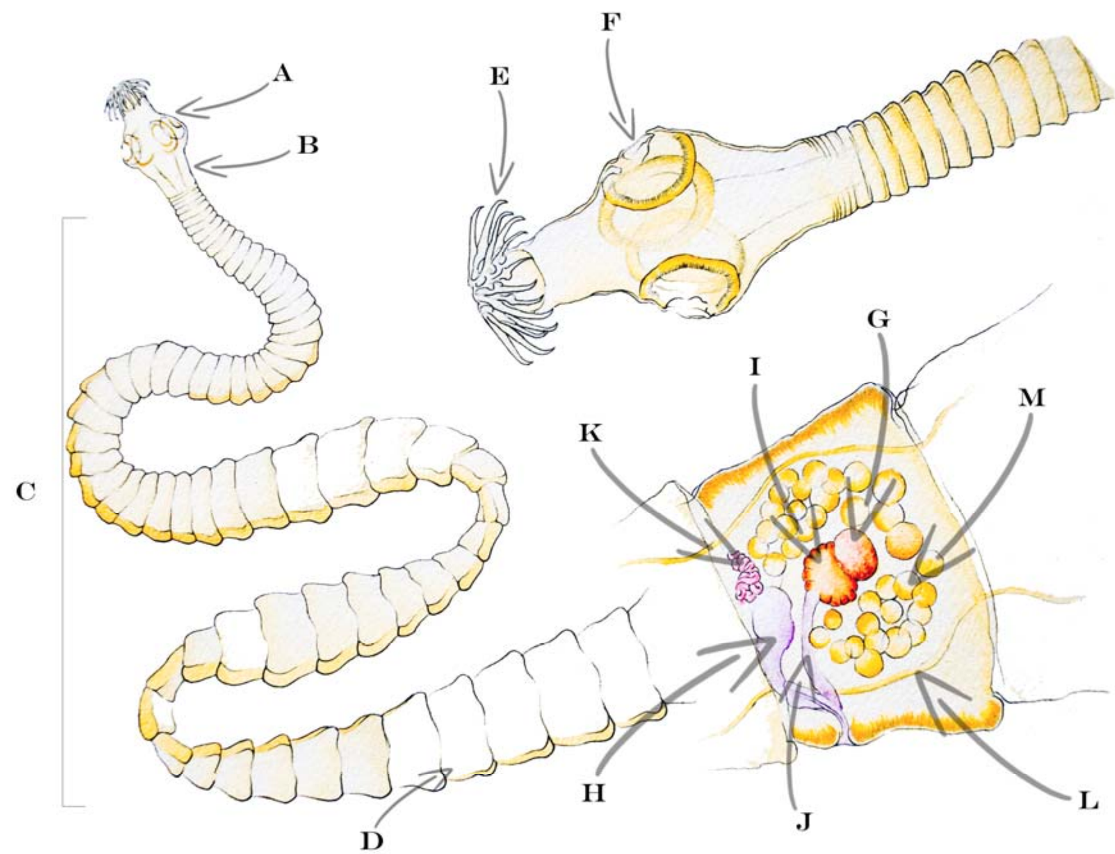


Figura 1. A-M. Morfología general de los céstodos. A. Rostelo. B. Cuello. C. Estróbilo. D. Proglótido. E. Ganchos. F. Ventosas. G. Vesícula seminal. H. Cirro. I. Ovario, J. Vagina. K. Vasos deferentes. L. Canal osmorregulador. M. Testículos.

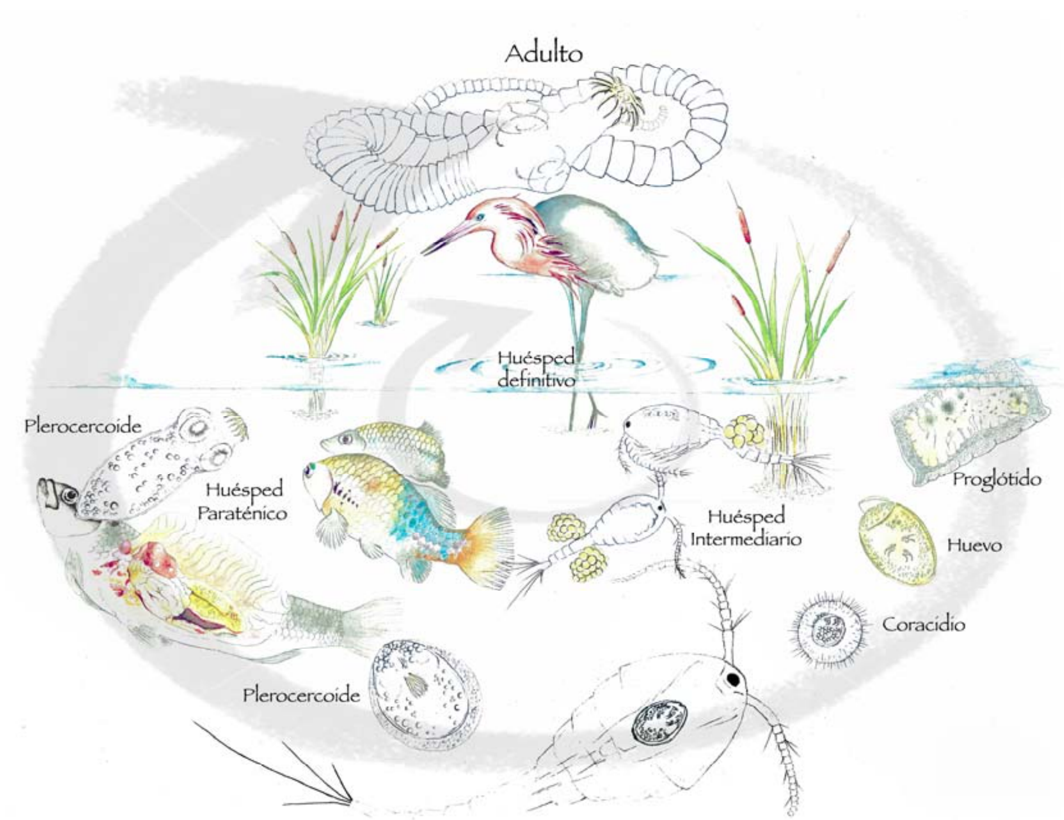


Figura 2. Ciclo de vida de céstodos de la familia Gryporhynchidae.

Tabla 1. Distribución de las especies de céstodos de la familia Gryporhynchidae registrados en huéspedes intermediarios (peces) y definitivos (aves ictiófagas) en México.

| Especie | Huésped | Estadio | Localidad | Referencia |
|---|---|---|---|---|
| ***Cyclustera capito*** (Rudolphi, 1819) Fuhrmann, 1901 | *Floridichthys polyommus* | Plerocercoide | Laguna Chelém, Yucatán | Scholz y Salgado-Maldonado 2001<br>Scholz et al. 2004 |
| | *Phalacrocorax brasilianus* | Adulto | Laguna Chelém, Yucatán | Scholz et al. 2002 |
| | *Egretta thula* | Adulto | Chuburná, Yucatán | Ortega-Olivares et al. 2008 |
| | *Egretta tricolor* | Adulto | Chuburná, Yucatán | Ortega-Olivares et al. 2008 |
| | *Cochlearius cochlearius* | Adulto | Chuburná, Yucatán | Ortega-Olivares et al. 2008 |
| | *Eudocimus albus* | Adulto | Chuburná, Yucatán | Ortega-Olivares et al. 2008 |
| | *Platalea ajaja* | Adulto | Chuburná, Yucatán | Ortega-Olivares et al. 2008 |
| ***Cyclustera ibisae*** (Schmidt y Bush, 1972) Bona, 1975 | *Phalacrocorax brasilianus* | Adulto | Laguna Chelém, Yucatán | Scholz et al. 2002 |
| | *Egretta thula* | Adulto | Chuburná, Yucatán | Ortega-Olivares et al. 2008 |
| | *Cochlearius cochlearius* | Adulto | Chuburná, Yucatán | Ortega-Olivares et al. 2008 |

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| | *Platalea ajaja* | Adulto | Chuburná, Yucatán | Ortega-Olivares et al. 2008 |
| | *Eudocimus albus* | Adulto | La Rivera, Veracruz | Ortega-Olivares et al. 2011 |
| | | Adulto | Los Chivos, Veracruz | Ortega-Olivares et al. 2011 |
| | | Adulto | Laguna Tempoal, Veracruz | Ortega-Olivares et al. 2011 |
| | | Adulto | Laguna Caimanero, Sinaloa | Ortega-Olivares et al. 2011 |
| | | Adulto | Punta Piedra, Tamaulipas | Ortega-Olivares et al. 2011 |
| ***Cyclustera* cf. *ralli*** (Underwood y Dronen, 1986) Bona, 1994 | *Cyprinus carpio* | Plerocercoide | Presa Ignacio Ramírez, Estado de. México | Scholz y -2001<br>Scholz et al. 2004 |
| | *Notropis sallei* | Plerocercoide | Presa Ignacio Allende, Guanajuato | Scholz y Salgado-Maldonado 2001<br>Scholz et al. 2004 |
| | *Alloophorus robustus* | Plerocercoide | Lago Pátzcuaro, Michoacán | Scholz y Salgado-Maldonado 2001<br>Scholz et al. 2004 |
| | *Girardinichthys multiradiatus* | Plerocercoide | Lago Chicnahuapan, Almoloya del Río, Estado de México | Scholz y Salgado-Maldonado 2001<br>Scholz et al. 2004 |

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| | *Xenotoca variata* | Plerocercoide | Presa Ignacio Allende, Guanajuato | Scholz y Salgado-Maldonado 2001<br>Scholz et al. 2004 |
| ***Dendrouterina ardeae*** (Rausch, 1955) Bona, 1975 | *Ardea herodias* | Adulto | Almoloya del Río, Estado de México | Scholz et al. 2002 |
| | | | Río Tamesí, Tamaulipas | Ortega-Olivares et al. 2008 |
| ***Dendrouterina* (?) *fuhrmanni*** (Clerc, 1906) Baer y Bona, 1960 | *Botaurus pinnatus* | Adulto | El Bayo, Veracruz | Ortega-Olivares et al. 2008 |
| ***Dendrouterina papillifera*** (Fuhrmann, 1908) Baer y Bona, 1960 | *Bubulcus ibis* | Adulto | Tabasco, Tabasco | Scholz et al. 2002 |
| | *Egretta thula* | Adulto | Lago Pátzcuaro, Michoacán | Scholz et al. 2002 |
| | *Egretta caerulea* | Adulto | Los Chivos, Veracruz | Ortega-Olivares et al. 2008 |

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| | *Egretta rufescens* | Adulto | Carbonera, Yucatán | Ortega-Olivares et al. 2008 |
| ***Dendrouterina herodiae*** Fuhrmann, 1912 | *Egretta thula* | Adulto | Lago Pátzcuaro, Michoacán. | Scholz et al. 2002 |
| ***Dendrouterina pilherodiae*** Mahon, 1956 | *Rhamdia guatemalensis* | Plerocercoide | Cenote Ixin–há, Yucatán | Scholz y Salgado-Maldonado 2001<br>Scholz et al. 2004 |
| | *Ardea alba* | Adulto | El Bayo, Veracruz | Ortega-Olivares et al. 2008 |
| ***Glossocercus auritus*** (Rudolphi, 1819) Bona, 1994 | *Poecilia catemaconis* | Plerocercoide | Lago de Catemaco, Veracruz | Scholz y Salgado-Maldonado 2001<br>Scholz et al. 2004 |
| | *Poecilia mexicana* | Plerocercoide | Calabozo, Hidalgo | Scholz y Salgado-Maldonado 2001<br>Scholz et al. 2004 |
| | *Poecilia sphenops* | Plerocercoide | Presa Tepecoacuilco, Guerrero | Scholz y Salgado-Maldonado 2001<br>Scholz et al. 2004 |

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| | *Poecilia sphenops* | Plerocercoide | Santiago Xochihuehuetlán, Guerrero | Scholz y Salgado-Maldonado 2001<br>Scholz et al. 2004 |
| | *Poecilia sphenops* | Plerocercoide | Río Huajuapan, Oaxaca | Scholz y Salgado-Maldonado 2001<br>Scholz et al. 2004 |
| | *Poecilia* sp. | Plerocercoide | Lagua Escondida, Veracruz | Scholz y Salgado-Maldonado 2001<br>Scholz et al. 2004 |
| | *Poeciliopsis gracilis* | Plerocercoide | Presa Tepecoacuilco, Guerrero | Scholz y Salgado-Maldonado 2001<br>Scholz et al. 2004 |
| | *Ardea alba* | Adulto | Río Papaloapan, Veracruz | Scholz et al. 2002 |
| | | | El Bayo, Veracruz | Ortega-Olivares et al. 2008 |
| | *Ardea herodias* | Adulto | Los Chivos, Veracruz | Ortega-Olivares et al. 2008 |
| | *Egretta thula* | Adulto | Río Papaloapan, Veracruz | Scholz et al. 2002 |
| | *Egretta caerulea* | Adulto | Los Chivo, Veracruz | Ortega-Olivares et al. 2008 |
| | *Nyctanassa violacea* | Adulto | Los Chivo, Veracruz | Ortega-Olivares et al. 2008 |

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| ***Glossocercus caribaensis*** (Rysavy y Macko, 1973) Bona, 1994 | *Cichlasoma urophthalmus* | Plerocercoide | Mitza, Yucatán | Scholz y Salgado-Maldonado 2001<br>Scholz et al. 2004 |
| | *Fundulus grandissimus* | Plerocercoide | Laguna Chelém, Yucatán | Scholz y Salgado-Maldonado 2001<br>Scholz et al. 2004 |
| | | Plerocercoide | Chuburná, Yucatán | Scholz y Salgado-Maldonado 2001<br>Scholz et al. 2004 |
| | | Plerocercoide | Yucalpetén, Yucatán | Scholz y Salgado-Maldonado 2001<br>Scholz et al. 2004 |
| | *Fundulus persimilis* | Plerocercoide | Laguna Chelém, Yucatán | Scholz y Salgado-Maldonado 2001<br>Scholz et al. 2004 |
| | | Plerocercoide | Chuburná, Yucatán | Scholz y Salgado-Maldonado 2001<br>Scholz et al. 2004 |
| | | Plerocercoide | Yucalpetén, Yucatán | Scholz y Salgado-Maldonado 2001<br>Scholz et al. 2004 |

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| | *Ardea herodias* | Adulto | Los Chivos, Veracruz | Ortega-Olivares et al. 2008 |
| | *Egretta thula* | Adulto | Carbonera, Yucatán | Ortega-Olivares et al. 2008 |
| | *Egretta caerulea* | Adulto | Chuburná, Yucatán | Ortega-Olivares et al. 2008 |
| | *Egretta rufescens* | Adulto | Chuburná, Yucatán | Ortega-Olivares et al. 2008 |
| ***Glossocercus cyprinodontis*** Chandler, 1935 | *Pelecanus occidentalis* | Adulto | Punta Piedra, Tamaulipas | Ortega-Olivares et al. 2012 (en prensa) |
| | *Egretta caerulea* | Adulto | Chuburná, Yucatán | Ortega-Olivares et al. 2008 |
| | *Egretta rufescens* | Adulto | Chuburná, Yucatán | Ortega-Olivares et al. 2008 |
| | | Adulto | Chuburná, Yucatán | Ortega-Olivares et al. 2012 (en prensa) |
| | *Nycticorax nycticorax* | Adulto | Laguna de Términos, Campeche | Ortega-Olivares et al. 2012 (en prensa) |
| | *Cochlearius cochlearius* | Adulto | Chuburná, Yucatán | Ortega-Olivares et al. 2008 |
| ***Glossocercus* sp.** | *Ardea herodias* | Adulto | Los Chivos, Veracruz | Ortega-Olivares et al. 2008 |
| | *Nyctanassa violacea* | Adulto | Los Chivos, Veracruz | Ortega-Olivares et al. 2008 |

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| | | Adulto | Río Máquinas, Veracruz | Ortega-Olivares et al. 2008 |
| ***Neogryporhynchus cheilancristrotus*** (Wedl, 1855) Baer y Bona, 1960 | *Phalacrocorax brasilianus* | Adulto | Río Tecolutla, Veracruz | Scholz et al. 2002 |
| | *Ardea herodias* | Adulto | Río Tamesí, Tamaulipas | Ortega-Olivares et al. 2008 |
| ***Paradilepis caballeroi*** Rysavy y Macko, 1973 | *Chirostoma jordani* | Plerocercoide | La Biznaga, Guanajuato | Scholz y Salgado-Maldonado 2001<br>Scholz et al. 2004 |
| | *Cichlasoma callolepis* | Plerocercoide | Silvituc, Campeche | Scholz y Salgado-Maldonado 2001<br>Scholz et al. 2004 |
| | *Phalacrocorax brasilianus* | Adulto | Río Tecolutla, Veracruz | Scholz et al. 2002 |
| | | Adulto | Río Papaloapan, Veracruz | Scholz et al. 2002 |
| | | Adulto | Laguna Coyuca, Gurrero | Violante-González et al. 2011 |
| | | Adulto | Laguna Tres Palos, Guerrero | Violante-González et al. 2011 |

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| ***Paradilepis* cf. *urceus*** (Wedl, 1855) Joyeux y Baer, 1950 | *Chirostoma jordani* | Plerocercoide | Presa Ignacio Allende, Guanajuato | Scholz y Salgado-Maldonado 2001<br>Scholz et al. 2004 |
| ***Paradilepsis* sp.** | *Chirostoma jordani* | Plerocercoide | La Biznaga, Guanajuato | Scholz y Salgado-Maldonado 2001<br>Scholz et al. 2004 |
| | *Phalacrocorax brasilianus* | Adulto | Río Tecolutla, Veracruz | Scholz et al. 2002 |
| | *Cochlearius cochlearius* | Adulto | Chuburná, Yucatán | Ortega-Olivares et al. 2008 |
| ***Parvitaenia cochlearii*** Coil, 1955 | *Atherinella crystallina* | Plerocercoide | Río Santiago, Nayarit | Scholz y Salgado-Maldonado 2001<br>Scholz et al. 2004 |
| | *Dormitator latifrons* | Plerocercoide | Marismas de Chalacatepec, Jalisco | Scholz y Salgado-Maldonado 2001<br>Scholz et al. 2004 |
| | | Plerocercoide | Salinas de Careyes, Jalisco | Scholz y Salgado-Maldonado 2001<br>Scholz et al. 2004 |
| | | Plerocercoide | Río Sn. Nicolás, Jalisco | Scholz y Salgado-Maldonado 2001<br>Scholz et al. 2004 |

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| | *Gobiomorus maculatus* | Plerocercoide | Río Cuitzmala, Jalisco | Scholz y Salgado-Maldonado 2001 |
| | | | | Scholz et al. 2004 |
| | *Agonostomus montícola* | Plerocercoide | Río Cuitzmala, Jalisco | Scholz y Salgado-Maldonado 2001 |
| | | | | Scholz et al. 2004 |
| | *Poecilipsis gracilis* | Plerocercoide | Presa Tepecoacuilco, Guerrero | Scholz y Salgado-Maldonado 2001 |
| | | | | Scholz et al. 2004 |
| | *Ardea herodias* | Adulto | Río Tamesí, Tamaulipas | Ortega-Olivares et al. 2008 |
| | *Ardea alba* | Adulto | Laguna Coyuca, Guerrero | Violante-González et al. 2012 |
| | *Nyctanassa violacea* | Adulto | Laguna Tres Palos, Guerrero | Violante-González et al. 2012 |
| | *Butorides virescens* | Adulto | Río Tamesí, Tamaulipas | Ortega-Olivares et al. 2008 |
| | *Cochlearius cochlearius* | Adulto | Tututepec, Oaxaca | Coil 1955 |
| | | | | Scholz et al. 2002 |
| ***Parvitaenia macropeos*** (Wedl, 1855) Baer y Bona, 1960 | *Cichlasoma istlanum* | Plerocercoide | Presa Tepecoacuilco, Guerrero | Scholz y Salgado-Maldonado 2001 |
| | | | | Scholz et al. 2004 |

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| ***Valipora campylancristrota*** (Wedl, 1855) Baer y Bona, 1960 | *Chirostoma humboldtianum* | Plerocercoide | Presa Villa Victoria, Estado de México | Scholz y Salgado-Maldonado 2001<br>Scholz et al. 2004 |
| | *Chrirostoma jordani* | Plerocercoide | Presa Ignacio Ramirez, Estado de México | Scholz y Salgado-Maldonado 2001<br>Scholz et al. 2004 |
| | *Chirostoma riojai* | Plerocercoide | Presa Ignacio Ramirez, Estado de México | Scholz y Salgado-Maldonado 2001<br>Scholz et al. 2004 |
| | *Girardinicththys multiradiatus* | Plerocercoide | La Lagunilla, Estado de México | Scholz y Salgado-Maldonado 2001<br>Scholz et al. 2004 |
| | | Plerocercoide | Presa Ignacio Ramirez, Estado de México | Scholz y Salgado-Maldonado 2001<br>Scholz et al. 2004 |
| | | Plerocercoide | Presa Trinidad Fabela, Estado de México | Scholz y Salgado-Maldonado 2001<br>Scholz et al. 2004 |
| | *Rhamdia guatemalensis* | Plerocercoide | Cenote Ixin-há, Yucatán | Scholz y Salgado-Maldonado 2001<br>Scholz et al. 2004 |

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| | *Ardea alba* | Adulto | El Bayo, Veracruz | Ortega-Olivares et al. 2008 |
| ***Valipora minuta*** (Coil, 1950) Baer y Bona, 1960 | *Rhamdia guatemalensis* | Plerocercoide | Cenote Sn. Pedro, Quintana Roo | Scholz y Salgado-Maldonado 2001<br>Scholz et al. 2004 |
| | | Plerocercoide | Cenote Ixin-há, Yucatán | Scholz y Salgado-Maldonado 2001<br>Scholz et al. 2004 |
| | *Poecilia sphenops* | Plerocercoide | Presa Tepecoacuilco, Guerrero. | Scholz y Salgado-Maldonado 2001<br>Scholz et al. 2004 |
| | *Poeciliopsis gracilis* | Plerocercoide | Presa Tepecoacuilco, Guerrero | Scholz y Salgado-Maldonado 2001<br>Scholz et al. 2004 |
| | *Butorides virescens* | Adulto | Laguna Escondida, Veracruz | Ortega-Olivares et al. 2008 |
| | | | Río Máquinas, Veracruz | Ortega-Olivares et al. 2008 |
| ***Valipora mutabilis*** Linton, 1927 | *Cichlasoma beani* | Plerocercoide | Río Santiago, Nayarit | Scholz y Salgado-Maldonado 2001<br>Scholz et al. 2004 |
| | *Cichlasoma geddesi* | Plerocercoide | Laguna Zoh, Campeche | Scholz y Salgado-Maldonado 2001<br>Scholz et al. 2004 |

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| | *Cichlasoma meeki* | Plerocercoide | Laguna Zoh, Campeche. | Scholz y Salgado-Maldonado 2001 |
| | | | | Scholz et al. 2004 |
| | *Rhamdia guatemalensis* | Plerocercoide | Cenote Ixin-há, Yucatán | Scholz y Salgado-Maldonado 2001 |
| | | | | Scholz et al. 2004 |
| | *Butorides virescens* | Adulto | El Bayo, Veracruz | Ortega-Olivares et al. 2008 |
| | *Nycticorax nycticorax* | Adulto | Lago Pátzcuaro, Michoacán | Scholz et al. 2002 |
| | | Adulto | Río Teapa, Tabasco | Scholz et al. 2002 |

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# Objetivos

## Objetivo general

El objetivo central es generar una hipótesis filogenética a nivel genérico de la familia Gryporhynchidae y probar su validez dentro del orden Cyclophyllidea.

## Objetivos particulares

- Poner a prueba la monofilia de la familia Gryporhynchidae dentro del orden Cyclophyllidea empleando caracteres moleculares (secuencias de los genes nucleares 18S y 28S del ADN ribosomal).
- Resolver problemas taxonómicos a nivel específico y genérico en miembros de la familia Gryporhynchidae.
- Realizar redescripciones de especies basadas en organismos adultos.

# Capítulo I

Helminths of the white ibis, *Eudocimus albus* (Aves: Threskiornithidae) in Mexico.

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# Helminths of the white ibis, *Eudocimus albus* (Aves: Therskiornithidae) in Mexico.

MIRZA P. ORTEGA-OLIVARES, DAVID I. HERNÁNDEZ-MENA, GERARDO PÉREZ-PONCE DE LEÓN & MARTÍN GARCÍA-VARELA

*Departamento de Zoología, Instituto de Biología, Universidad Nacional Autónoma de México, Avenida Universidad 3000; Ciudad Universitaria; C.P. 04510; Distrito Federal. México. E-mail: garciav@servidor.unam.mx.*

## Abstract

Eight species of helminths are reported from the intestine of 52 white ibis [(*Eudocimus albus* (Linneaeus)] collected in 14 localities along the Mexican coasts of the Gulf of Mexico, and the Pacific Ocean. Three trematodes, *Parastrigea cincta* Brandes, 1888; *Parastrigea diovadena* Dubois & Macko, 1972, and *Patagifer lamothei* Dronen & Blend, 2008, one cestode *Cyclustera ibisae* Schmidt & Bush, 1972 and four acanthocephalan species *Hexaglandula corynosoma* Travassos, 1915, *Arhythmorhynchus frassoni* Molin, 1858, *Southwellina hispida* Van Cleave, 1925, *Ibirhynchus dimorpha* García-Varela, Pérez-Ponce de León, Aznar & Nadler, 2011, were found. Four of these species were recorded for the first time in the white ibis, raising the number of helminth species that parasitize this bird to 57 along its distributional range. Additionally, 4 of the helminth species are recorded in Mexico for the first time.

**Key words:** white ibis, *Eudocimus albus*, Trematoda, Cestoda, Acanthocephala, new host records, new geographical records, Mexico

## Introduction

The distributional range of the white ibis, *Eudocimus albus* (Linneaeus), extends from Virginia, on the East coast of the U.S.A, southwards through Louisiana, Texas, Florida, the Caribbean, and along both coasts of Mexico and Central America, reaching coastal areas in the north of South America (Howell & Webb 1995; American Ornithologist' Union 1998; Peterson & Chalif 2000). This bird species inhabits marshes, mangroves, lakes, and lagoons feeding upon crustaceans, amphibians, mollusks, small fishes, and insects. Previous reports of the helminth fauna of the white ibis, mainly in Southeastern U.S.A., indicate a high species richness, with 53 species, including 28 trematodes, 20 nematodes, 3 cestodes, and 2 acanthocephalans (Lumsden 1961; Schmidt & Bush 1972; Bush *et al*. 1973; Schmidt 1973; Bush & Forrester 1976; Dronen & Blend 2008; Pérez-Álvarez *et al*. 2008). The white ibis is widely distributed in Mexico, however, only three isolated reports have been published describing a few components of the helminth fauna of this bird (Ortega-Olivares *et al*. 2008; Pérez-Álvarez *et al*. 2008; García-Varela *et al*. 2011). Considering the paucity of information about the helminth fauna of the white ibis in Mexico, the main objective of this paper is to present the results of a survey on the helminth parasite fauna of this bird collected from 14 localities from both coasts of Mexico.

## Material and methods

Fifty-two adult white ibis were collected between October, 2006 and December, 2010 in 14 Mexican localities, 6 located along the Pacific Ocean slope (n=23), and 8 along the Gulf of Mexico (n=29) (Table 1; Fig. 1). Hosts were captured with a shotgun, under the collecting permit FAUT 0202 issued by the Secretaría de Medio Ambiente y Recursos Naturales (SEMARNAT). Birds were dissected, the intestinal tract and other organs were removed from the body, placed in Petri dishes separately, with saline 0.75%, and examined under a dissecting microscope a few

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hours after their capture. Trematodes, cestodes and acanthocephalans were relaxed in saline solution 0.75%, fixed with 4% hot-formalin solution, stained with Mayer's Paracarmin and Shuberg's hydrochloric carmine, and mounted on permanent slides with Canada balsam. Some trematodes were heat-fixed under slight cover slip pressure with 4% formalin. Scoleces of some specimens of tapeworms were squashed and fixed whit a mixture of glycerine-ammonium picrate (see Vidal-Martínez *et al.* 2001; Scholz *et al.* 2004) to enable the observation and measurements of rostellar hooks. Measurements are given in millimeters (mm). Helminths were identified by conventional morphological criteria following keys provided by Yamaguti (1963), Petrochenko (1958), Schmidt (1973), for acanthocephalans, Dubois (1968), Yamaguti (1971), McDonald (1981) and Niewiadomska (2002) for digeneas, and Ortega-Olivares *et al.* (2008) for tapeworms; additionally, original descriptions of each species were also consulted. All the worms were deposited in the Colección Nacional de Helmintos (CNHE), Instituto de Biología, Universidad Nacional Autónoma de Mexico (UNAM), México City. Data on the mean intensity of infection are not presented because it was no possible to quantify the total number of helminths recovered from some individual hosts, particularly those heavily infected. Birds were identified based on Howell & Webb (1995) and the American Ornithologists' Union (1998); some bird specimens were deposited in the Colección de Aves Museo de Zoología Alfonso L. Herrera, Facultad de Ciencias, UNAM, Mexico City (Accession number: MZFC 22524).

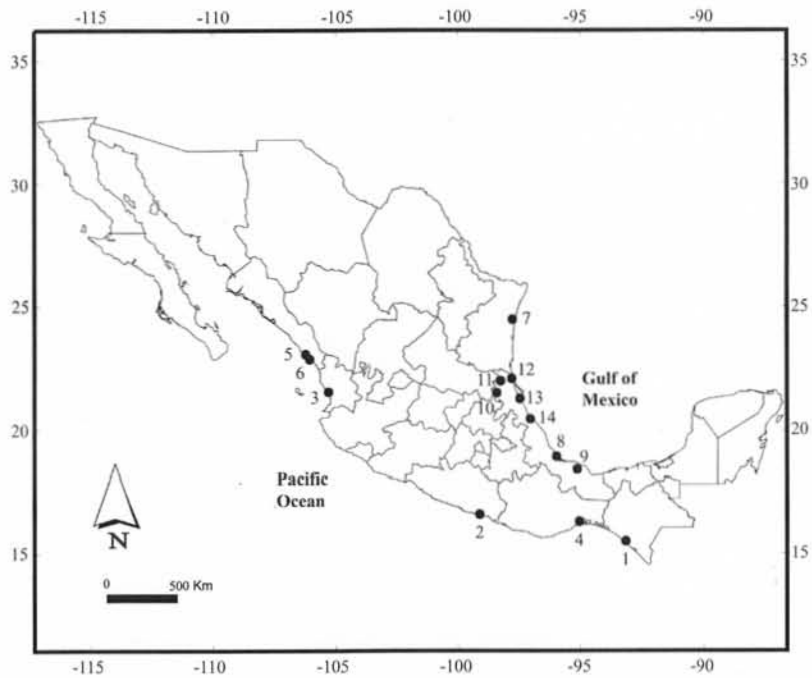



**FIGURE 1.** Birds collection sites (Codes are shown in Table 1). (1) Ríon Pijijiapan; (2) Laguna Chautengo; (3) Laguna la Tovara, San Blas; (4) Laguna Superior; (5) Laguna el Huizache; (6) Laguna el Caimanero; (7) Punta Piedra, Laguna Madre; (8) Humedal los Chivos; (9) Lago de Catemaco; (10) Laguna Tempoal; (11) Río Pánuco; (12) Laguna la Rivera; (13) Laguna Tamiahua; (14) Río Tecolutla.

## Results

Eight species of helminth parasites (3 trematodes, 1 cestode, and 4 acanthocephalans) were recovered in 8 localities along the Gulf of Mexico and 6 localities along Pacific Ocean slope of Mexico, from the intestine of 52 white ibis. The current helminths recorded in this study update the checklist of parasites of *Eudocimus albus* in the Americas.

### DIGENEA

**Family Strigeidae Railliet**

**Genus *Parastrigea* Szidat (Brandes, 1888)**

***Paratrigea cincta* Dubois, 1968**
(Fig. 2)

**Localities.** NAYARIT: Laguna la Tovara, San Blas. SINALOA: Laguna el Huizache, Laguna el Caimanero. VERACRUZ: Río Tecolutla.

**Specimens deposited.** CNHE 7168.

**Remarks.** This specie was described by Dubois (1968) from the heron *Ardea* sp. and the red faced head *Phimosus infuscatus berlepschi* (Lichtenstein) from Brazil, Colombia and Venezuela. The specimens collected in the present study from *Eudocimus albus* were identified as *Parastrigea cincta* because the total length of the organism (varying from 4.7–6.0 mm), as well as other measurements, correspond with the ranges given for the species. The anterior segment length ranges from 2.2–2.5 by 2.6–2.8 width, while the posterior segment length ranges from 2.9–3.4 by 1.8–2.2 width. Characteristically, the anterior segment is wider because of the lateral expansion of the holdfast; the vitellarium is follicular and densely extended into copulatory bursa, and testes are multilobated (see Dubois 1968; McDonald 1981) .This trematode is reported in Mexico for the first time, and *Eudocimus albus* represents a new definitive host for *P. cincta*.

***Parastrigea diovadena* Dubois & Macko, 1972**
(Fig. 3)

**Localities.** CHIAPAS: Ríon Pijijiapan. GUERRERO: Laguna Chautengo. NAYARIT: Laguna la Tovara, San Blas. SINALOA: Laguna el Huizache, Laguna el Caimanero. TAMAULIPAS: Punta Piedra, Laguna Madre. VERACRUZ: Laguna la Rivera, Laguna Tamiahua, Río Tecolutla.

**Specimens deposited.** CNHE 7169, 7170, 7171.

**Remarks.** The trematodes found in the anterior region of the intestine of *Eudocimus albus* in nine localities of Mexico were identified as *Parastrigea diovadena* based on morphological characters. The measurements of some organs of our material are slightly smaller than those reported in the original description by Dubois & Macko (1972). This species of trematode seems to be specific for the white ibis, because it has only been recorded in this host in some localities of Cuba (Dubois & Macko 1972) and Florida (Forrester & Spalding 2003). In the current study, *P. diovadena* is recorded for the first time in Mexico, extending the geographical distribution range of this trematode.

**Family Echinostomatidae Rudolphi**

**Genus *Patagifer* Dietz**

***Patagifer lamothei* Dronen & Blend, 2008**
(Fig. 4)

**Localities.** GUERRERO: Laguna Chautengo. NAYARIT: Laguna la Tovara, San Blas; OAXACA: Laguna Superior. SINALOA: Laguna el Huizache, Laguna el Caimanero. TAMAULIPAS: Punta Piedra, Laguna Madre. VERACRUZ: Humedal los Chivos, Laguna Tempoal.


**FIGURES 2–4. 2.** *Parastrigea cincta* Brandes, 1888 ventral view. **3.** *Parastrigea diovadena* Dubois & Macko, 1972 dorsal view. **4.** *Patagifer lamothei* Dronen & Blend, 2008 ventral view. Scale bars = 1 mm.

**Specimens deposited.** CNHE 7778, 7779, 7780.

**Remarks.** The morphological characters of this material correspond with those presented in the original description of this equinostomid trematode (Dronen & Blend 2008). The presence of a bilobed head collar, bearing a total of 56 spines (28 spines per side) is a major character that distinguishes this species, as well as the position of the ovary and the position of anterior and posterior testes, with respect each other. *Patagifer lamothei* was originally reported as a parasite of the white ibis, *Eudocimus albus* in Galveston, Texas (Dronen & Blend 2008); its presence in the white of ibis in 5 localities from the Pacific Ocean slope and in other 3 localities of the Gulf of Mexico, extends the distribution area of this equinostomatid trematode.

## CESTODA

### Family Gryporhynchidae Spassky & Spasskaya

### Genus *Cyclustera* Fuhrmann

***Cyclustera ibisae* (Schmidt & Bush, 1972) Bona, 1975**
(Figs. 5, 6)

**Localities.** SINALOA: Laguna el Caimanero. TAMAULIPAS: Punta Piedra, Laguna Madre. VERACRUZ: Humedal los Chivos, Laguna Tempoal, Laguna la Rivera.

**Specimens deposited.** CNHE 7712, 7713, 7714.

**Remarks.** The tapeworms recovered in this study were identified as *Cyclustera ibisae* by considering the morphology of the rostellar hooks (Ortega-Olivares *et al.* 2008). This species was previously found in the white ibis, *Eudocimus albus* in Florida (Schmidt & Bush 1972). Later, *C. ibisae* was recorded from the intestine of *Ardea alba* (Linnaeus) (≈ *Casmerodius albus*), *Pelecanus occidentalis* (Linnaeus), *Phalacrocorax auritus* (Lesson), and *P. brasilianus* (Humboldt) from Cuba (see Rysavy & Macko 1973; Bona 1975). *Cyclustera ibisae* is distinguished from other congeneric species by possessing the rostellar hooks arranged in 2 circles of 10 hooks each, with handle and guard distinctively striated lengthwise (see Scholz *et al.* 2002) (Fig. 6). Adults of this species have been reported in numerous groups of fish-eating birds distributed in Southeastern U.S.A. (Florida, Georgia), Cuba and Southeastern Mexico (Yucatan Peninsula) (Schmidt & Bush 1972; Rysavy & Macko 1973; Sepúlveda *et al.* 1994; Kinsella *et al.* 1996; Kinsella & Forrester 1999; Scholz *et al.* 2002; Ortega-Olivares *et al.* 2008). The presence of *C. ibisae* in the white ibis in 3 localities of the Gulf of Mexico and 1 locality of the Pacific Ocean slope, extend the distribution range of this tapeworm.

## ACANTOCEPHALA

### Family Polymorphidae Meyer

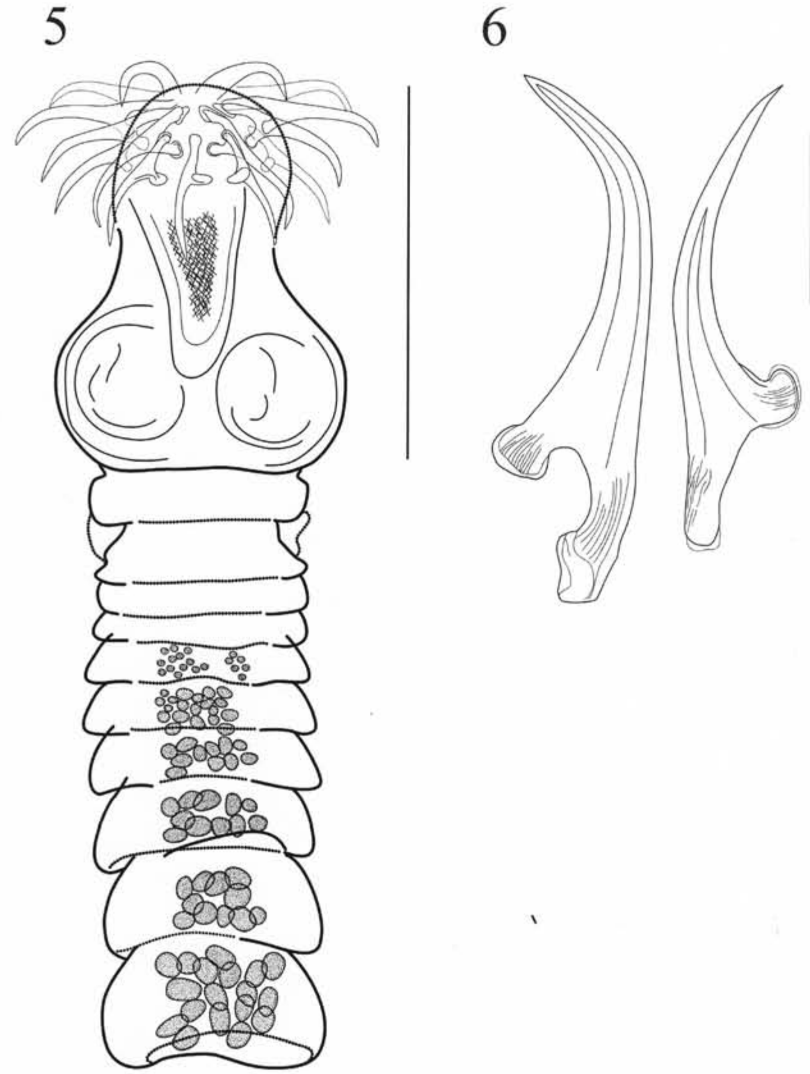
### Genus *Ibirhynchus* García-Varela, Pérez-Ponce de León, Aznar & Nadler

***Ibirhynchus dimorpha* (Schmidt, 1973) García-Varela, Pérez-Ponce de León, Aznar & Nadler, 2011**
(Fig. 7)

**Localities.** VERACRUZ: Humedal los Chivos and Lago de Catemaco.

**Specimens deposited.** CNHE 6164.

**Remarks.** *Southwellina dimorpha* was transferred by García-Varela *et al.* (2011) to the newly described genus *Ibirhynchus* based on morphological and molecular characters. The specimens collected in this study exhibit the diagnostic characters of this species, i.e., a short and barrel-shaped proboscis armed with 17 to 24 longitudinal circles of 9 to 14 hooks each. In addition, our specimens show sexual dimorphism in terms of spine distribution in the anterior region of the trunk. The females are characterized by possessing a single field of spines whereas the males




**FIGURES 5–6. 5.** *Cyclustera ibisae* Schmidt & Bush, 1972. **6.** Hooks of the rostellum (Distal hooks and proximal hooks). Scale bars. FIGURE. 5 = 1mm. FIGURE. 6 = 50μm.

have 2 fields. *Ibirhynchus dimorpha* has been previously reported in the white ibis in localities of Florida and Mexico (Schmidt 1973; Forrester & Spalding 2003; Richardson & Font 2006; García-Varela *et al.* 2011), and in the whooping crane *Grus americana* (Linnaeus) in Florida (Richardson & Font 2006).

**Genus *Arhythmorhynchus* Lühe**

***Arhythmorhynchus frassoni* (Molin, 1858)**
(Fig. 8)

**Localitiy.** SINALOA: Laguna el Caimanero.

**Specimens deposited.** CNHE 7711.

**Remarks.** The 5 specimens collected in the present study correspond to *Arhythmorhynchus frassoni*. This species is characterized by having a long slender body, divided in 2 regions by a constriction, and by having a spined foretrunk, a fusiform proboscis broadening at the base, armed with 18 longitudinal circles of 20 hooks each. The ventral hooks at base of the proboscis are larger than the rest, and the neighboring lateral hooks are smaller than the rest (Petrochenko 1958; Nickol & Heard 1970; Nickol *et al.* 2002). The specimens recovered in this study are immature, possibly indicating that *Eudocimus albus* may represent an accidental host for *A. frassoni*. The distribution area of *A. frassoni* includes Florida, and the Yucatan Peninsula in Mexico. It has also been reported as larval stage in the fiddler crab *Uca rapax* (Smith) (Nickol *et al.* 2002) and the spined fiddler crab *Uca spinicarpa* (Rathbun) (Guillén-Hernández *et al.* 2008). Adults of *A. frassoni* have been previously found in *Rallus longirostris* Boddaert from Southeastern North America (Nickol & Heard 1970; Nickol *et al.* 2002). The present record extends its distributional range into the Pacific Ocean slope, since it was already found in Laguna el Caimanero, Sinaloa, Northwestern Mexico.

**Genus *Hexaglandula* Petrochenko**

***Hexaglandula corynosoma* Travassos, 1915**
(Fig. 9)

**Localitiy.** VERACRUZ: Laguna la Rivera.

**Specimens deposited.** CNHE 7777.

**Remarks.** The 2 specimens collected in the present study correspond to *Hexaglandula corynosoma* by having a ventral curved trunk covered by spines extending into the middle region of the trunk, and by lacking genital spines. The specimens show a cylindrical proboscis armed with 16 longitudinal circles of 11 hooks each, conical neck, and double-walled proboscis receptacle, attached at the base of proboscis, with cerebral ganglion at mid-level (see Petrochenko, 1958). Nickol *et al.* (2002) reported the presence of cystacanths and adults of *H. corynosoma* in Southeastern U.S.A., and recently, Guillén-Hernández *et al.* (2008) reported the cystacanths of this species in the decapod *Uca spinicarpa* and the adult in the Yellow-crowned night heron *Nyctanassa violacea* (Linnaeus) in Mexico. The specimens recovered from the intestine of the white ibis represent a new host record for *H. corynosoma*.

**Genus *Southwellina* Witenberg**

***Southwellina hispida* Van Cleave, 1925**
(Fig. 10)

**Localitiy.** VERACRUZ: Laguna la Rivera.

**Specimens deposited.** CNHE 7776.

**Remarks.** The 2 specimens collected in the present study correspond to *Southwellina hispida* by possessing two fields of spines in the anterior region of the trunk, a proboscis cylindrical armed with 16 to 17 longitudinal circles of 12 to 15 hooks each, conical neck, and a double-walled proboscis receptacle (see Schmidt, 1973). This

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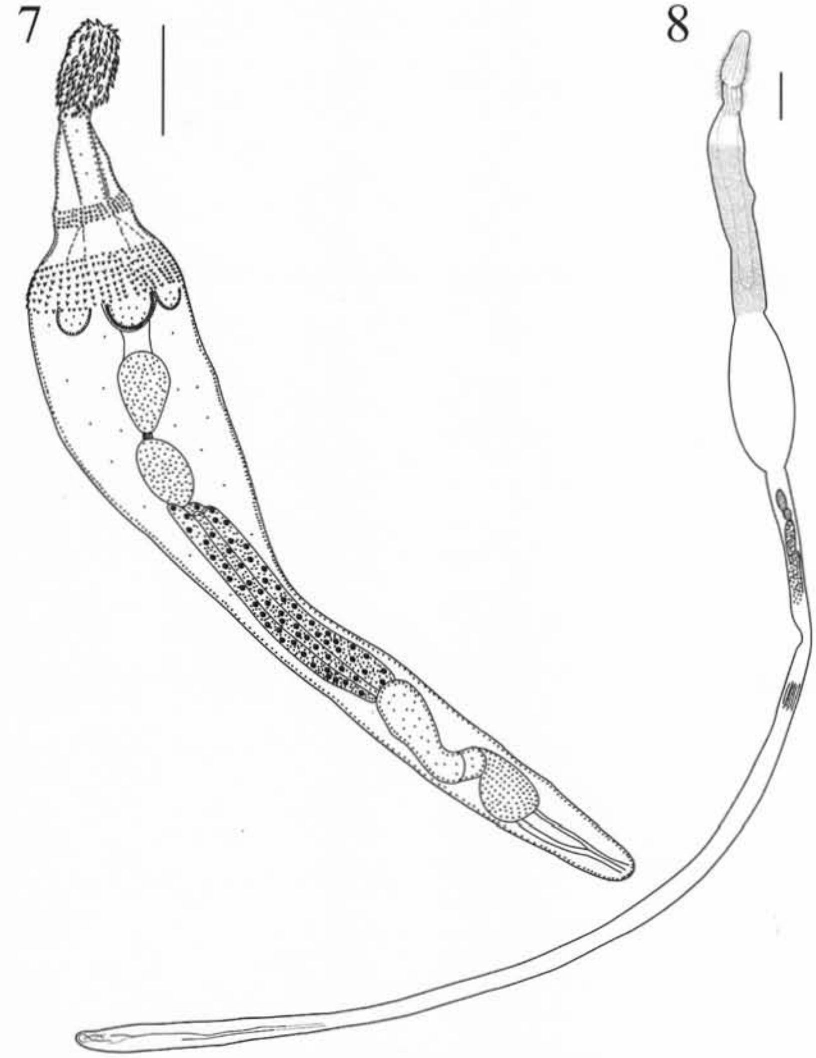
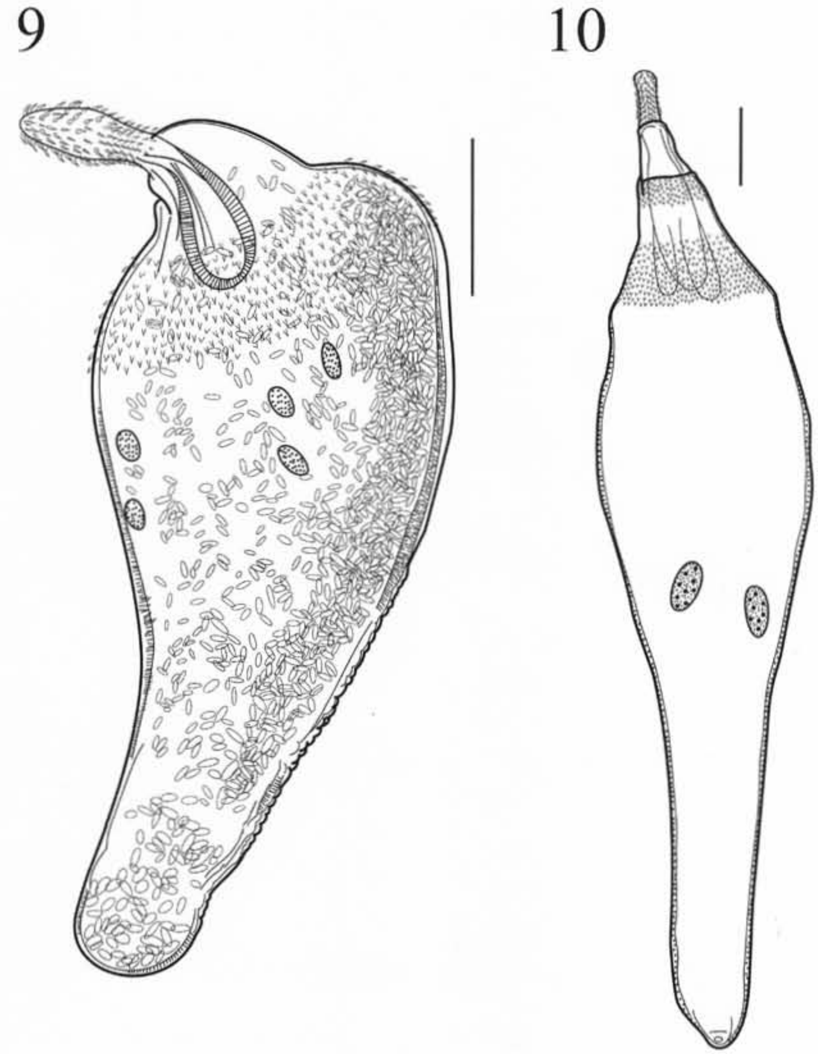


**FIGURES 7–8. 7.** Male of *Ibirhynchus dimorpha* Schmidt, 1973. **8.** Male of *Arhythmorhynchus frassoni* Molin, 1858. Scale bars = 1mm.




**FIGURES 9–10.** 9. Female of *Hexaglandula corynosoma* Travassos, 1915. 10. Female of *Southwellina hispida* Van Cleave, 1925. Scale bars = 1mm.

acanthocephalan species use crustacean decapod (*Procambarus clarkii* Girard) and fish-eating birds to complete their life-cycle. However, snakes, frogs, freshwater and brackish water fish, serve as paratenic hosts (Schmidt 1985). In Mexico, adults of *S. hispida* parasitize several heron species (García-Varela & Pérez Ponce de León 2008; Barrera-Guzmán & Guillén-Hernández 2008; García-Prieto *et al.* 2010), whereas cystacanths have been found in the mesentery of fish (Vidal-Martínez *et al.* 2001; Violante-González *et al.* 2007; García-Prieto *et al.* 2010). The two specimens found in the intestine are immature and this may indicate that the white ibis is an accidental host.

## Discussion

The current study represents the first systematic survey of the helminth fauna of the white ibis, *Eudocimus albus* in Mexico. Of the 52 white ibis analyzed here, eight species of helminths were collected. *Eudocimus albus* represents a new host record for *Parastrigea cincta* and its presence in the white ibis in Mexico extend their known geographic range. This species was previously found in *Phimosus infuscatus berlepschi* from Brazil, Colombia and Venezuela (Dubois 1968). The other two species of trematodes, *Parastrigea diovadena* and *Patagifer lamothei*, were previously found in the white ibis, in localities of Cuba, Florida and Texas (see Dubois & Macko 1972; Forrester & Spalding 2003; Dronen & Blend 2008, respectively). The presence of both species in the white ibis extend their distribution area in Mexico.

The presence of the acanthocephalans *Arhythmorhynchus frassoni*, *Hexaglandula corynosoma* and *Southwellina hispida* in *E. albus* represent new host records and in addition, the distributional range of these species is extended. These 3 acanthocephalan species are common endoparasites of fish-eating birds, i.e, herons, cormorans, pelicans, and eagles (Nickol & Heard 1970; Richardson & Cole 1997; Nickol *et al.* 2002; Guillen-Hernandéz *et al.* 2008; García-Prieto *et al.* 2010). The presence of *S. hispida* and *A. frassoni* in the intestine of the white ibis possibly represent accidental infections, due that specimens collected of both species are immature. The tapeworm *Cyclustera ibisae*, was found in five localities in Mexico, extending its geographical distribution. This tapeworm was previously recorded in Florida, U.S.A. and the Yucatan Peninsula in Mexico (Dronen & Blend 2008; Ortega-Olivares *et al.* 2008).

This study raising the total number of species reported for this bird species to 57. The white ibis feeds upon a wide spectrum of preys, such as crustaceans, mollusks, insects, amphibians, and small fishes. Likewise it has a wide distribution range, extending from North America to South America, and show a migration patterns from North to South(Howell & Webb 1995). The lack of studies in Central and South America as well as in the Caribbean make the inventory of the helminth fauna of this bird far from complete. Therefore new survey in other geographic areas could add more helminth species to the invetory of this wadding bird.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## Capítulo II

First description of adults of the type species of the genus *Glossocercus* Chandler, 1935 (Cyclophyllidea: Gryporhynchidae).

Manuscrito sometido para su publicación en la Revista **Folia Parasitologica** (Fecha de aceptación 12 de agosto 2012)

Dear Dr. Garcia Varela:

It is a pleasure to accept your manuscript entitled "First description of adults of the type species of the genus Glossocercus Chandler, 1935 (Cyclophyllidea: Gryporhynchidae)." in its current form for publication in Folia Parasitologica. The comments of the reviewer(s) who reviewed your manuscript are included at the foot of this letter.

Thank you for your fine contribution. On behalf of the Editors of Folia Parasitologica, we look forward to your continued contributions to the Journal.

Sincerely,

Dr. Tomas Scholz

Editor in Chief, Folia Parasitologica

tscholz@paru.cas.cz

Associate Editor

Comments to the Author:

(There are no comments.)

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**First description of adults of the type species of the genus Glossocercus Chandler, 1935 (Cyclophyllidea: Gryporhynchidae).**

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| Keywords: | morphology, Type species, Strobilar morphology, Glossocercus cyprinodontis, Pelecanus, Egretta, Nycticorax, Gulf of Mexico, Principal component analysis |
| | |

**First description of adults of the type species of the genus *Glossocercus* Chandler, 1935 (Cyclophyllidea: Gryporhynchidae).**

**Mirza P. Ortega-Olivares, Rogelio Rosas-Valdez** and **Martín García-Varela**

Departamento de Zoología, Instituto de Biología, Universidad Nacional Autónoma de México, Avenida Universidad 3000, Ciudad Universitaria, C.P. 04510, Distrito Federal, Mexico

**Running header.** Description of the adult *Glossocercus cyprinodontis*

**Keywords**: Morphology, type species, strobilar morphology, *Glossocercus cyprinodontis*, *Pelecanus*, *Egretta*, *Nycticorax*, Gulf of Mexico, Principal component analysis

**Corresponding author**

Martin García Varela

Departamento de Zoología,

Instituto de Biología, Universidad Nacional Autónoma de México, Avenida Universidad 3000, Ciudad Universitaria, C.P. 04510, Distrito Federal, México.

Phone (525556229130)

Fax (5255500164)

Email: garciav@unam.mx

**Abstract:** The type species of the genus *Glossocercus* Chandler, 1935, *G. cyprinodontis* Chandler, 1935, was described as metacestode (larval stage) from the mesentery of the sheepshead minnow fish (*Cyprinodon variegatus* Lacépède) from Galveston Bay, Texas. The description was based on the morphology of the rostellar hooks; however, the features of the

internal morphology of the proglottides could no be provided. In the present study we describe for the first time the features of the adult *G. cyprinodontis* from the intestine of *Pelecanus occidentalis* Linnaeus, *Nycticorax nycticorax* Linnaeus and *Egretta rufescens* Gmelin in Mexico. *Glossocercus cyprinodontis* possessing similar strobilar morphology with the other two congeneric species, both distributed in the Neartic and Neotropical regions, i.e. *Glossocercus caribaensis* (Rysavy et Macko, 1971) and *Glossocercus auritus* (Rudolphi, 1819). However, *G. cyprinodontis* differs mainly in the shape of the rostellar hooks (those of *G. cyprinodontis* possess the handle and the guard strongly sclerified compared to those of *G. auritus* and *G. caribaensis*) and their size (total length of 175–203 μm in *G. cyprinodontis* compared to 189–211 in *G. caribaensis* and 220–285 in *G. auritus*). Generic diagnosis of *Glossocercus* is provided: rostellar hooks in two rows with ten hooks in each different shape and length, scolex large and globular, proglottides craspedote wider than long, genital pores irregularly alternating, vagina transverse surrounded by epithelial cells, ventral to cirrus sac, uterus with bar-shaped in mature proglottides and in gravid proglottides occupies all space between osmoregulatory ducts with eggs, ovary lobed in middle of proglottis, cirrus sac elongate between osmoregulatory canals, cirrus armed with spinitriches and apical tuft of slender spinitriches.



*Glossocercus* Chandler, 1935 (Gryporhynchidae) is a genus of cyclophyllidean cestodes that includes intestinal parasites of pelicans, herons and freshwater turtles distributed in America, Africa, Australia and Indonesia (Bona 1994; Pichelin et al. 1998). Currently, the genus contains nine species: *Glossocercus cyprinodontis* Chandler, 1935 (type species), *G. auritus* (Rudolphi, 1819), *G. glandularis* (Fuhrmann, 1905), *G. ardeae* (Johnston, 1911), *G. chelodinae* (MacCallum, 1921), *G. clavipera* (Baer and Bona, 1960), *G. paracyclorchida* (Baer and Bona, 1960), *G. megascolecina* (Ukoli, 1967) and *G. caribaensis* (Rysavy and

Macko, 1971) (Bona 1994; Pichelin et al. 1998). Morphologically, these nine species are characterized by the hook pattern 'glossocercoid' defined as hooks with massive sclerification in the handle and guard, long blade and strong to reduced beak, either two discontinuous lines in sclerified structure separate the handle and the guard from the hook body and blade (see Pichelin et al. 1998).

The type species *Glossocercus cyprinodontis* Chandler, 1935 was described from metacestodes found in the mesentery of sheepshead minnow fish (*Cyprinodon variegatus* Lacépède) from Galveston Bay, Texas, USA and a new genus, *Glossocercus*, was proposed to accommodate it (Chandler 1935). Metacestodes are a larval stage of cestodes, and consequently the description of this type species of *Glossocercus* was restricted to morphological features of the scolex, principally to rostellar hooks (see Chandler 1935), and morphological traits of the adult remained unknown. Ortega-Olivares et al. (2008) collected adults of *G. cyprinodontis* from the intestine of herons in the Yucatan, Mexico, but they did not provide morphological description of these tapeworms.

During a helminthological survey on the helminth parasites of aquatic birds in Mexico, we collected adult tapeworms conspecific with *Glossocercus cyprinodontis* in fish-eating birds in lagoons of the Gulf of Mexico. In this paper, the morphological characters of the adult of this tapeworm are described for the first time and the generic diagnosis of *Glossocercus* is provided.

## MATERIALS AND METHODS

**Specimen collection and preparation.** We collected 180 fish-eating birds between June 2006 to April 2011 in 19 localities in Mexico from coasts of the Pacific Ocean and the Gulf of Mexico. However, tapeworms were recorded only in three localities from the Gulf of Mexico (see Fig 1; Table 1). Hosts were killed with a shotgun, under collecting permit FAUT

No. 0202. After capture, the intestinal tract was removed from the body, placed in Petri dishes with saline (0.75%) and examined using a stereoscopic microscope. Tapeworms were removed from the lumen of the intestine, washed in saline , fixed with 4% hot formalin and stored in ethanol (70%). In the laboratory, the cestodes were stained with Schuberg's hydrochloric carmine or Mayer's paracarmin, and mounted on permanent slides with Canada balsam. Specimens were deposited in the Colección Nacional de Helmintos (acronym CNHE), at the Instituto de Biología, Universidad Nacional Autónoma de México, Mexico City and US National Parasite Collection (USNPC), Beltsville, Maryland, USA.

**Morphological examination.** Identification was based on morphological criteria following the original description (Chandler 1935) and features reported by Scholz et al. (2004) and Ortega-Olivares et al. (2008). A paratype of *G. cyprinodontis* deposited in the US National Parasite Collection (No. 39528) was also examined. The present description of the adult is based on 18 stained specimens obtained from different hosts. Drawings were made with the aid of a drawing tube. Measurements of morphological characters are given as minimum and maximum in μm (unless otherwise stated), followed by mean and number of specimens measured (n) in parentheses. Measurements of the testes, cirrus sac, vagina and seminal receptacle were obtained from fully mature proglottides.

**Principal component analysis.** Morphometric comparisons using the principal component analysis (PCA) were carried out to evaluate morphometric differences between tapeworms from different hosts. We used PCA as a purely descriptive tool, with the primary goal being to visualize clustering of specimens in the morphometric space (Agustí et al. 2005). The PCA is a multivariate analysis that has been used to determine the intra- and inter-specific morphological variation among helminthes species with well results (see Bell and Sommerville 2002; Bell et al. 2002; Agustí et al. 2005; Pinacho-Pinacho et al. 2012).

In this study two PCA analyses were performed; the first included 19 morphometric variables (see Table 2) obtained from 11 tapeworms: three from *Pelecanus occidentalis* Linnaeus, five from *Egretta rufescens* Gmelin, and three from *Nycticorax nycticorax* Linnaeus. The second analysis included five morphometric variables of rostellar hooks obtained from eight tapeworms: one specimen from *P. occidentalis*, three of *E. rufescens*, three of *N. nycticorax*, including the paratype (metacestode) of *G. cyprinodontis* (see Table 2). Both analyses were conducted with the statistics packages PAST v. 1.60 (Hammer et al. 2001).

**RESULTS**

**Family Gryporhynchidae Spassky and Spasskaya, 1973**

**Genus *Glossocercus* Chandler, 1935**

***Glossocercus cyprinodontis* Chandler, 1935 (Fig. 2-8)**

**Morphological description of adults:** Most of specimens studied shrinkage due to fixation. Strobila large, 2.0–6.5 mm (4.0, n = 18) long; maximum width 1635. Proglottides craspedote, with convex lateral margins. Immature proglottides usually wider than long, 380–797 (225, n = 85) long and 473-797 (571, n = 85) wide (Fig. 2). Mature proglottides wider than long, 133–530 (310, n = 65) long and 540–1096 (880, n = 65) wide. Gravid proglottides wider than long, 291–550 (410, n = 40) long and 606–1635 (278, n = 40) wide.

Scolex globular (Fig. 3), with maximum width at level of suckers, 302–628 (485, n = 17) in diameter. Suckers rounded, with weakly to moderately developed musculature, 98–177 (140, n = 68) in diameter. Rostellum retractable, protrusible, with thick longitudinal muscular fibres, subglobular pad bearing hooks and short stem; anterior surface of pad slightly concave (forming small anterior depression), entire length of fully extended rostellum 690 (n = 1), maximum width at anterior pad 250 (n = 1); most of rostella folded within rostellar pouch; in few cases, rostella contracted, with stem slightly wider than apical muscular pad; diameter of pad 127–301 (203, n = 17). Rostellar pouch as long as wide, 153–278 (200, n = 18) long, 120–272 (199, n = 18) wide; thick-walled, oval to pyriform, reaching to level of suckers. Rostellar pouch with radially arranged longitudinal fibers. Rostellar hooks 20 (n = 16) in number, arranged in two regular rows; anterior and posterior hooks with different shape. Distal hooks 163–198 (179, n = 21) long; with robust guard directing forward and merges with the handle; blade longer than handle; blade 80–163 (109, n = 57) long; handle 63–88 (80, n = 21) long, tapered to its end of distal hooks; blade /handle ratio 1.05–1.84 (1.40, n = 21) (Fig. 4). Proximal hooks 105–155 (135, n = 34) long; with blade longer than handle; guard forming angle almost 90° to axis of handle; blade and handle of almost equal in length, blade 58-93 (72, n = 61) long; handle 48–80 (65, n = 35) long, tapered to its end of proximal hooks; blade/handle ratio 0.86–1.54 (1.15, n = 34) (Fig. 4).

Strobila protandrous. Genital pores irregularly alternating, situated far anterior. Genital atrium thick-walled, base slightly expanded. Genital ducts between osmoregulatory canals. Ventral osmoregulatory canals 20–78 (34, n = 140) wide, with transverse anastomosis along posterior margin of each proglottis. Dorsal osmoregulatory canals 5–14 (9, n = 90) wide.

Testes circular, 20–39 (30, n = 75) in number, surround ovary completely (Fig. 5); 37–50 in diameter (43, n = 80). External vas deferens strongly coiled, divided into prostate and aprostate parts; prostate part covered by intensely stained cells forming compact body, usually overlapping cirrus sac near anterior proglottis margin; aprostate part forming numerous coils antiporally and posteriorly to cirrus sac and prostate part of vas deferens, often reaching posteriorly to level of seminal receptacle and ovary (Fig. 6). Cirrus sac (Fig. 6) elongate, thick-walled, between osmoregulatory canals; 185–282 (245, n = 80) long, 35–87 (42, n = 80) wide. Evaginated cirrus cylindrical, 16–20 (16, n=24) length, armed with small and delicate spinitriches shorter than 1 μm; apical tuft with slender spinitriches about 25 long (Fig. 7).

Vitelline follicles situated in middle of proglottis. Ovary transversely elongate with fan-shaped lobes (Fig. 5); lobules large, thick, most prominent anteriorly when ripe; lobes contiguous and indistinct that conform a single mass. Mehlis' gland not observed. Seminal receptacle oval, 52–122 (80, n = 56) long and 42–175 (76, n = 56) wide; situated in middle of proglottis, surrounded by ovary. Vagina transverse, straight, surrounded by epithelial cells, internally lined with hyaline layer visible up to thin proximal extremity indicating end of vagina and beginning of seminal receptacle; vaginal canal ventral of equal length with cirrus sac; vagina 18–35 (25, n = 30) wide; lumen of vaginal canal 7–28 (22, n = 25) wide (Fig. 5). Uterine primordium bar-shaped, posterior to ovary in mature proglottides. Fully developed uterus occupies entire space of proglottides between osmoregulatory ducts, with diverticula filled of eggs (Fig. 8). Mature eggs not seen, apparently due to apolytic nature of proglottides.

Type host: *Cyprinodon variegatus* Lacépède (Cyprinodontiformes: Cyprinodontidae) (metacestodes).

Site of infection: Mesentery.

Type locality: Galveston Bay, Texas.

Definitive hosts: *Egretta rufescens*, *Nycticorax nycticorax* (Pelecaniformes: Ardeidae). *Pelecanus occidentalis* (Pelecaniformes: Pelecanidae)

Site of infection: Intestine.

Localities of adults specimens were collected: Campeche: Laguna de Términos. Tamaulipas: Punta Piedra. Yucatan: Chuburná.

Specimens deposited: CNHE 8264–8267; USNPC 105875-105877.

**Remarks.** The adults of *G. cyprinodontis*, *G. caribaensis*, and *G. auritus* have been recorded in North America including Mexico, Brazil, Nicaragua, and the Caribbean (Bona 1994, Ortega-Olivares et al. 2008). *Glossocercus cyprinodontis* differs from other two congeneric species, i.e., *G. caribaensis* and *G. auritus*, in the size and shape of the rostellar hooks, which are smaller in the former species (see Table 3 and Fig. 9). The guard of *G. cyprinodontis* is directed forward and merges with the handle in the distal hooks, whereas that of *G. caribaensis* is inclined posteriorly and separated by two discontinuous lines, and that of *G. auritus* is directed forward, being separated by one sclerified line (Fig. 9).

*Glossocercus cyprinodontis* was described from metacestodes based on the morphology of the rostellar hooks. Bona (1994) provided a generic diagnosis of *Glossocercus* based on the metacestodes of the type species (morphology of rostellar hooks) and other congeneric species (hook morphology and strobilar characteristics). However, the generic diagnosis was incomplete because the morphological features of the adult of the type species were unknown. In the current study adult specimens identified as *G. cyprinodontis* were collected from their definitive hosts for the first time, which made it possible to provide the correct generic diagnosis of *Glossocercus*.



**Generic diagnosis**: Cyclophyllidea: Gryporhynchidae. Strobila large. Proglottides craspedote with convex lateral margins and wider than long. Scolex large and globular. Rostellum retractable. Rostellar pouch as long as wide. Rostellar hooks arranged in two regular rows with ten hooks in each; anterior and posterior hooks of different shape and length. Strobila protandrous. Genital pores irregularly alternating, situated far anterior. Genital ducts between osmoregulatory canals. Testes circular surround ovary completely. Cirrus sac elongate, wall thick, between osmoregulatory canals. Cirrus armed with spinitriches and apical tuft of slender spinitriches. Vitelline follicles in the middle of proglottis. Ovary lobed, slightly transversely elongate, in middle of proglottis; lobes contiguous and indistinct that conform a single mass. Seminal receptacle oval, situated in the middle of proglottis. Vagina transverse, straight, surrounded by epithelial cells, ventral to cirrus sac and equal length. Uterus appears bar-shaped, posterior to ovary in mature proglottides. Fully developed uterus occupies all space between osmoregulatory ducts with diverticula filled of eggs.

**Principal component analysis.** The first analysis includes 19 morphological characters of the adult: total length of body, immature proglottides length, immature proglottides wide, mature proglottides length, mature proglottides wide, scolex length, scolex wide, pad wide, rostellar pouch length, rostellar pouch wide, sucker diameter, number of testes, testes diameter, cirrus length, cirrus wide, seminal receptacle length, seminal receptacle wide, ventral osmoregulatory canals wide, and dorsal osmoregulatory canals wide. The first component explains 39% of the variance and the second component explains 26% of variance (Fig. 10). The second analysis included data of five morphological traits obtained from the rostellar hooks of the adults and the metacestode (paratype): total length, length of blade (B), length of handle (H), the ratio B/H of the distal hooks, and the length of blade of the proximal hooks. The first component explains 64% of variance and the second component explain 22% of variance (Fig. 11). The first analysis shows that morphological characters of adults from different definitive hosts belong to the same taxon. The second analysis clearly shows that the morphological features of the rostellar hooks of adults and the metacestode (paratype) belong to the same species (Figs. 10-11).

## DISCUSSION

Gryporhynchidae is a family of tapeworms classified into 14 genera parasitizing fish-eating birds throughout the world (Bona 1994, Pichelin et al. 1998, Scholz and Salgado-Maldonado 2001, Scholz et al. 2002, 2004, 2008, Ortega-Olivares et al. 2008, Presswell et al. 2012). In Mexico eight genera have been previously recorded, namely *Cyclustera* Fuhrmann, 1901; *Dendrouterina* Fuhrmann, 1912; *Glossocercus* Chandler, 1935; *Neogryporhynchus* Baer and Bona, 1975; *Neovalipora* Baer, 1962; *Paradilepis* Hsu, 1935; *Parvitaenia* Burt, 1940; and *Valipora* Linton, 1927 (Ortega-Olivares et al. 2008, 2011, Pérez-Ponce de León et al. 2010).

Currently *Glossocercus* comprises nine species that are characterized morphologically by having a 'glossocercoid' rostellar hook pattern. Chandler (1935) described *G. cyprinodontis* as type species from metacestodes. However, the diagnosis was based on the morphology of the rostellar hooks. Bona (1994) provided a generic diagnosis of *Glossocercus*, which could not be based on strobilar morphology of the type species known only as larvae (metacestodes) from fish and never found since the original description by Chandler (1935).

In the present study, strobilar morphology of *G. cyprinodontis* is described for the first time, based on specimens from three different definitive hosts (herons). Statistical comparison of measurements of tapeworms from these hosts has shown that they are conspecific and thus the morphological description of adults is based on pooled from three bird species.

To test the phenotypic variation of the adults of *G. cyprinodontis*, 11 specimens from three definitive hosts were analyzed using 19 morphological characters. The PCA strongly supported that the specimens belong to the same species because values for specimens from individual hosts overlapped and were not separated to distinct clusters. The same pattern was found using the morphological character diagnostic (rostellar hooks), including the paratype (metacestode) and adults. This PCA showed the plots inside of the ellipses indicated that all the specimens represent single species (Figs. 10, 11). These analyses indicate that the specimens recollected from fish-eating birds are conspecific with the metacestode of *G. cyprinodontis*. To confirm the conspecific of *G. cyprinodontis,* sequences of the small subunit of DNA Ribosomal were obtained from six adults. All these specimens conformed a single clade, indicated conspecificity of specimens from different definitive hosts (unpublished data).

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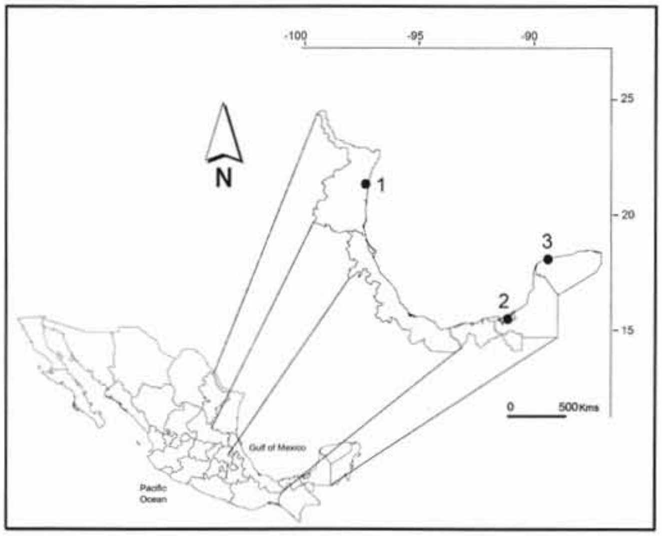
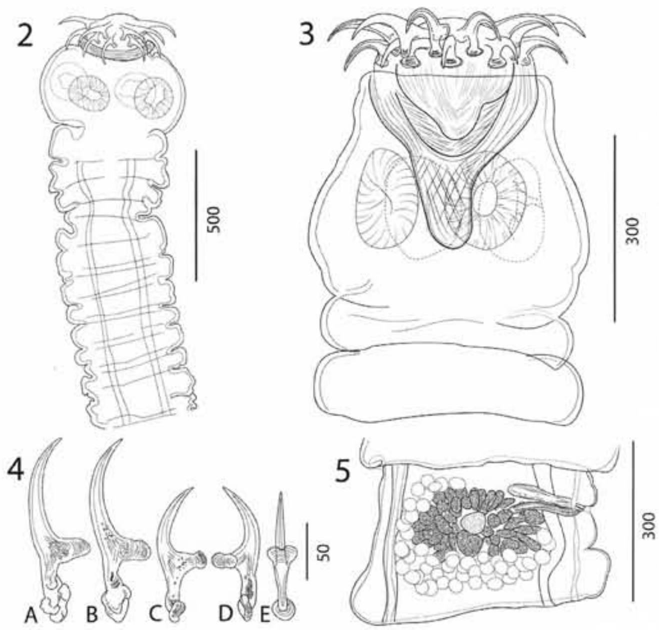
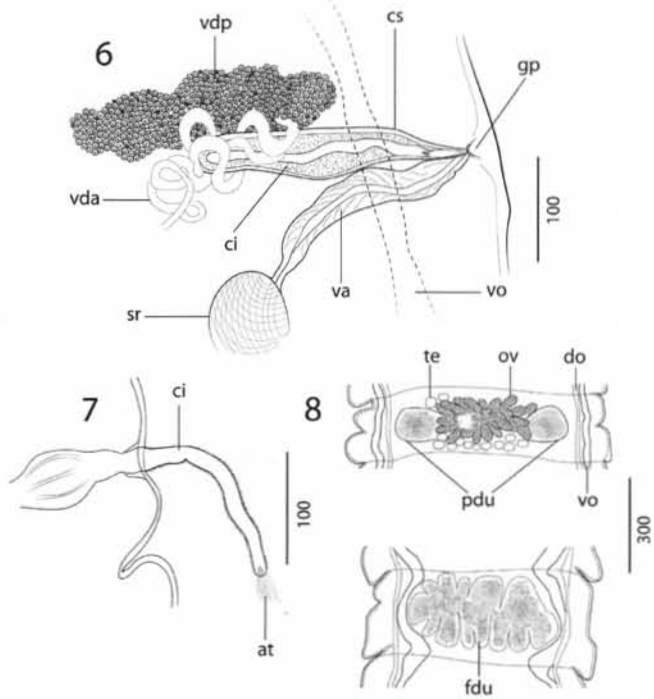


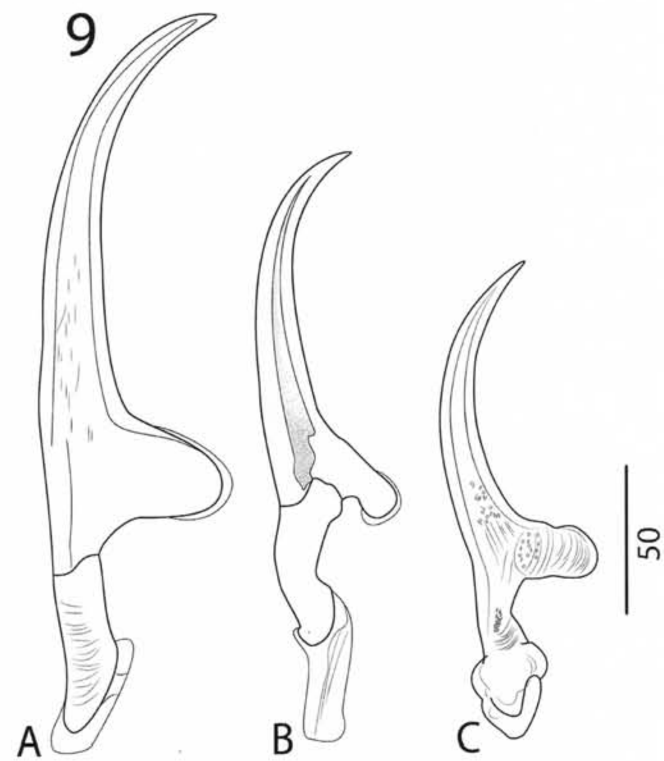

Bird collection sites in the Gulf of Mexico where the adults of Glossocercus cyprinodontis were found. 1. Punta Piedra, Tamaulipas; 2. Laguna de Términos, Campeche; 3. Chuburná, Yucatan.
93x75mm (300 x 300 DPI)



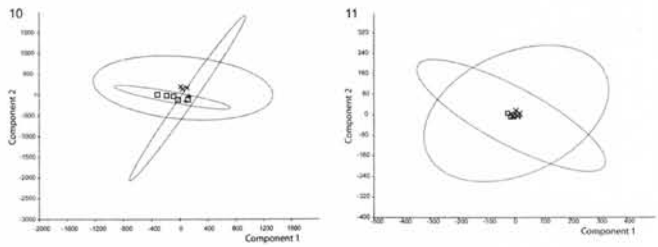

Glossocercus cyprinodontis. Fig. 2. Anterior view of G. cyprinodontis from Nycticorax nycticorax from Laguna de Términos, Campeche. Fig. 3. Scolex of G. cyprinodontis from Pelecanus occidentalis from Punta Piedra, Tamaulipas. Fig. 4. Rostellar hooks of G. cyprinodontis from N. nycticorax from Laguna de Términos, Campeche.. A–B distal hooks, C–E proximal hooks. Fig. 5. Mature proglottis of G. cyprinodontis from P. occidentalis from Punta Piedra, Tamaulipas.
132x125mm (300 x 300 DPI)




Glossocercus cyprinodontis from Pelecanus occidentalis from Punta Piedra, Tamaulipas. Fig. 6. Genital ducts. Fig. 7. Cirrus. Fig. 8. Uterus. Abbreviations: at – apical tuft; ci – cirrus; cs – cirrus-sac; do – dorsal osmoregulatory canal; fdu – fully developed uterus; gp – genital pore; ov – ovary; pdu– partially developed uterus; sr – seminal receptacle; te – testes; va – vagina; vda – external vas deferent aprostate; vdp – external vas deferent prostate; vo– ventral osmoregulatory canal.
149x163mm (300 x 300 DPI)




A-C. Rostellar hooks of the species of Glossocercus in America. A. Glossocercus auritus from Egretta caerulea (modified from Ortega-Olivares et al. 2008). B. Glossocercus caribaensis from Egretta rufescens. C. Glossocercus cyprinodontis from Nycticorax nycticorax.
88x102mm (300 x 300 DPI)




Principal component analyses. Fig. 10. PCA conducted with 19 morphometrical variables of the adults of Glossocercus cyprinodontis from three definitive hosts species. Fig. 11. PCA conducted with five morphometrical variables of the rostellar hooks of the adults from three definitive hosts species and one paratype of G. cyprinodontis. Specimens of G. cyprinodontis from Egretta rufescens are represented by □, Nycticorax nycticorax by ×, Pelecanus occidentalis by +, and Cyprinodon variegatus (paratype) by ϒ.
80x29mm (300 x 300 DPI)

**Table 1**. Survey of examined hosts and localities (see also Fig. 1).

| Hosts (examined/infected) | Localities | Coordinates |
|---|---|---|
| *Egretta rufescens* (5/2) | Chuburná, Yucatán | 21°13'18"N; 89°49'44"W |
| *Egretta rufescens* (1/1) | Punta Piedra, Tamaulipas | 24°29'26"N; 97°45'01"W |
| *Nycticorax nycticorax* (1/1) | Laguna de Términos, Campeche | 18°37'14"N; 91°34'43"W |
| *Pelecanus occidentalis* (10/2) | Punta Piedra, Tamaulipas | 24°29'26"N; 97°45'01"W |

**Table 2.** Comparative measurements *G. cyprinodontis* using for the PCA analyses.

| Characters | *G. cyprinodontis* *P. occidentalis* | *G. cyprinodontis* *E. rufescens* | *G. cyprinodontis* *N. nycticorax* | *G. cyprinodontis* *C. variegatus* |
|---|---|---|---|---|
| Stage | Adult | Adult | Adult | Metacestode |
| Total length body (mm) | 3.57–6.07 (4.06) | 2.68–6.24 (4.49) | 1.99–4.55 (3.28) | – |
| Scolex length | 328.6–406.1 (373.5) | 312.4–387.6 (350) | 454–594 (540.9) | – |
| Scolex wide | 414–566 (486.3) | 357– 508 (447) | 517–549 (535.6) | – |
| Pad wide | 157–210 (189.3) | 153–175 (153.2) | 256301 (253.3) | |
| Rostellum sac length | 156.6–191.9 (178.3) | 152.5–217.7 (191.2) | 175.7–229.4 (202.8) | |
| Rostellum sac wide | 165–272 (228.6) | 120–219 (178.6) | 143–251 (209.3) | |
| Sucker diameter | 210.6–260.6 (242.4) | 196.7–236 (218.2) | 292.4–314.1 (301.6) | |
| Immature proglottides length | 252–350 (297.33) | 190.9–244.02 (226.56) | 164.34–253.98 (219.44) | – |
| Immature proglottides wide | 540–576 (560) | 509.62–783.52 (598.35) | 489.7–585 (530.87) | – |
| Mature proglottides length | 304–502 (378) | 273.9–336.98 (321.14) | 252.32–332.5 (295.64) | – |
| Mature proglottides wide | 924–1000 (966.66) | 567.72–1002.64 (776.72) | 787.5–888.1 (841.28) | – |
| Testes number | 34–36 (35) | 21–35 (29) | 20–31 (26) | – |
| Diameter testes | 41.23–46 (42.96) | 42.09–46 (43.92) | 40.36–43.83 (42.67) | – |
| Cirrus length | 271.25–315.19 (292.58) | 289.69–323.87 (314.10) | 297.29–339.06 (318.08) | – |

| | | | | |
|---|---|---|---|---|
| Cirrus wide | 49.36–54.79 (51.53) | 47.74–56.96 (52.73) | 49.91–51.53 (50.63) | – |
| Ventral osmoregulatory wide | 8.24–10.09 (9) | 6.74–11.49 (9.36) | 7.79–8.28 (8.13) | – |
| Dorsal osmoregulatory wide | 188.64–224.23 (209.48) | 185.30–202.77 (195.83) | 243.18–250.58 (246.33) | – |
| Seminal receptacle length | 65–98.76 (84.81) | 60.83–91.50 (81.04) | 65.92–82.36 (74.77) | – |
| Seminal receptacle wide | 77.5–163.81 (126.76) | 47.47–61.36 (53.69) | 50.39–67.44 (56.27) | – |
| Distal hooks | | | | |
| Length | 197.5 | 162.5–180 (171.66) | 175–190.62 (181.17) | 170 |
| Blade | 109 | 95.83–105 (100.69) | 105.5–122.08 (115.30) | 107 |
| Handle | 87.5 | 62.5–83.33 (73.61) | 74.16–86.25 (80.13) | 87.5 |
| Ratio B/H | 1.2 | 1.2–1.6 (1.36) | 1.2–1.6 (1.4) | 1.2 |
| Proximal hooks | | | | |
| Blade | 72.5 | 62.5–71 (66.44) | 67–84.58 (75.36) | 74 |

**Table 3**. Measures and morphological characters of the rostellar hooks of three species of *Glossocercus* in America.

| Characters | *G. cyprinodontis* [1, 2, 3, 7] | *G. auritus* [2, 3, 5, 6] | *G. caribaensis* [2, 3, 4, 6] |
|---|---|---|---|
| **Distal hooks** | | | |
| Length | 175–203 | 220–285 | 189–211 |
| Blade | 100–128 | 140–195 | 101–132 |
| Handle | 71–96 | 82–160 | 72–97 |
| **Proximal hooks** | | | |
| Length | 128–150 | 160–224 | 120–151 |
| Blade | 71–86 | 93–138 | 52–83 |
| Handle | 60–86 | 83–120 | 56–78 |
| Position of the guard | directed forward | directed forward | posteriorly inclined |
| Guard | merges with handle; strongly sclerified | separates from the handle by one line sinuous; smooth | separate from the handle by two discontinuous lines; smooth |
| Handle | strongly sclerified | sclerified | smooth |

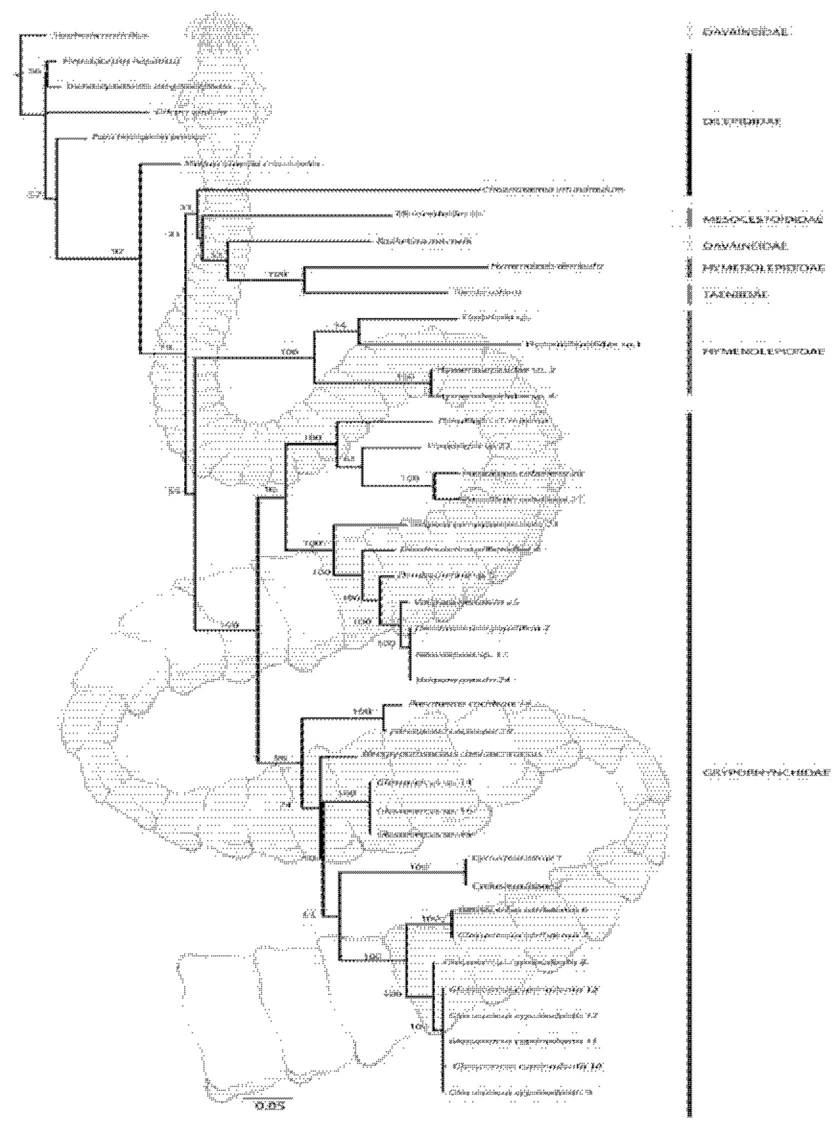
*References: 1. Chandler 1935; 2. Scholz et al. 2004; 3. Ortega-Olivares et al. 2008; 4. Schmidt and Courtney 1973; 5. Scholz et al. 2002; 6. Rysavy and Macko 1973; 7. Current study.

# Capítulo III

## Phylogenetic relationships of the family Gryporhynchidae (Cestoda: Cyclophyllidea) inferred through SSU and LSU rDNA gene sequences.

Manuscrito será sometido para su publicación.

GRAPHICAL ABSTRACT



# PHYLOGENETIC RELATIONSHIPS OF THE FAMILY GRYPORHYNCHIDAE (CESTODA: CYCLOPHYLLIDEA) INFERRED THROUGH SSU AND LSU RDNA GENE SEQUENCES.

Mirza P. Ortega-Olivares and Martín García-Varela

Departamento de Zoología, Instituto de Biología, Universidad Nacional Autónoma de México, Avenida Universidad 3000; Ciudad Universitaria; C.P. 04510; Distrito Federal. México.

**ABSTRACT**

Tapeworms of the family Gryporhynchidae are endoparasites of fish-eating birds and exhibit a cosmopolitan distribution. The 10 genera currently described for this family are classified on basis of their morphology of the rostellar apparatus. However, the phylogenetic relationships among these genera have never been inferred and the validity of this family within the order Cyclophyllidea is controversial. In this study, sequences of the near complete 18S (SSU) and 28S (LSU) ribosomal DNA genes from 11 species of adult gryporhynchids representing 7 genera (*Cyclustera*, *Dendrouterina*, *Glossocercus*, *Neovalipora*, *Paradilepis*, *Parvitaenia*, *Valipora*) were generated. Phylogenetic relationships were inferred using maximum-parsimony (MP), maximum-likelihood (ML), and Bayesian inference of independent and combined (SSU+LSU) data sets. All the phylogenetic trees showed that Gryporhynchidae is a monophyletic group and independent from other families of Cyclophyllidea, with high values of bootstrap. The trees inferred with the combined data set

suggested that the genera *Cyclustera*, *Neogryporhynchus*, *Paradilepis,* and *Parvitaenia* are monophyletic; in contrast, *Dendrouterina*, *Glossocercus*, *Neovalipora*, and *Valipora* are paraphyletic, suggesting that these species that should be reexamined and reclassified using a combination of morphological, ecological and molecular characters.

*Keywords*: Tapeworms, Gryporhynchidae, SSU, LSU, Phylogenetic relationships, Cyclophyllidea.

**1. Introduction**

The Cyclophyllidea van Beneden in Braun, 1900 is considered as a monophyletic group among the most highly derived orders of the Eucestoda and includes the most advanced and specialised tapeworms (Hoberg et al., 1997, 1999b). Adult cyclophyllideans are parasites of both, aquatic and terrestrial, vertebrate hosts (except teleosteans and chondrichthians) and specifically are the dominant group of tapeworms occuring in mammalian and avian hosts (Hoberg et al., 1999b). Historically, this group of tapeworms has been classified based on morphological and ecological characteres, and 13 families of tapeworms are included in the Cyclophyllidea (Khalil et al., 1994; Hoberg et al. 1999b). The first phylogenetic studies of cyclophyllideans were mainly based on comparative morphology of the larval stage and ontogeny (Brooks et al., 1991; Brooks and McLennan, 1993). However, in recent years molecular data have been used to resolve problems about relationships among tapeworms (Mariaux, 1996; Hoberg et al., 1999a; Mariaux and Olson, 2001; Littlewood, 2006).

A group of tapeworm parasites of fish-eating birds is particularly problematic. Spassky and Spasskaya 1973 erected the family Gryporhynchidae to included all the species possessing larval stages in crustaceans, fishes acting as intermediate hosts and adults in the intestine of fish-eating birds. However, some authors have not recognized this family and the phylogenetic position within Cyclophyllidea has been controversial (Yoneva et al., 2008). All the species of tapeworms parasitic in fish-eating birds have been classified as members of the family Dilepididae due to similarity of the rostellar apparatus and the life cycle (Scholz et al., 2004; Yoneva et al., 2008). However, Mariaux (1998) and Hoberg et al. (1999b) analyzed morphological and molecular characters of a single species (*Neogryporhynchus cheilancristrotus* Wedl, 1855), and separated the family Gryporhynchidae from Dilepididae *sensu stricto* (Scholz et al., 2004).

The classification of the members of Gryporhynchidae has been mainly based on the morphology of rostellar hooks number, arrangement, shape, and size (Bona, 1975; Scholz, 2001; Scholz and Salgado-Maldonado, 2001; Scholz et al., 2002, 2004), and have been divided into 10 genera: *Amirthalingamia* Bray, 1974; *Ascodilepis* Guildal, 1960; *Cyclustera* Fuhrmann, 1901; *Dendrouterina* Fuhrmann, 1912; *Glossocercus* Chandler, 1935; *Neogryporhynchus* Baer and Bona, 1975; *Neovalipora* Baer, 1962; *Paradilepis* Hsu, 1935; *Parvitaenia* Burt, 1940; and *Valipora* Linton, 1927 (Bona, 1975; Scholz et al. 2004).

Gryporhynchidae diversity has not been established properly and they have not been included in phylogenetic studies (Mariaux, 1998; Hoberg et al., 1999b; Presswell et al., 2012); however, broader taxonomic sampling is essential to understand patterns of evolutionary diversification in this group. The main objective of the present research was to test the monophyly of Gryporhynchidae by conducting a phylogenetic analysis among 7 of the 10 genera, using sequences of the near-complete 18S (SSU) and near-complete 28S (LSU) rDNA genes.

## 2. Materials and methods

### *2.1 Biological samples*

Between June 2006 and April 2011, individuals of 11 species of gryporhynchids and three species of hymenolepidids were collected from their naturally infected vertebrate hosts from several localities along the Pacific and Gulf of Mexico (Table 1). Hosts were captured with a shotgun under the collecting license FAUT 0202 issued by the Secretaría del Medio Ambiente y Recursos Naturales (SEMARNAT) to MGV. After their capture, the intestine of the dissected birds was removed from its body, and examined with stereoscopic microscope in order to recover the helminth specimens. Worms were washed in 0.75% saline solution, preserved in absolute ethanol, and stored at 4 ºC. Representative specimens were stained with Schuberg's hydrochloric carmine or Mayer's paracarmin, and mounted on permanent slides with Canada balsam. Identification of the tapeworm species was based on morphological criteria of the rostellar hooks compared with the features reported by Scholz et al. (2002, 2004) and Ortega-Olivares et al. (2008). Voucher specimens were deposited in the Colección Nacional de Helmintos (CNHE), Instituto de Biología, Universidad Nacional Autónoma de México (UNAM), Mexico City (accession numbers in Table 1).

*2.2 DNA Extraction, amplification and sequencing*

For molecular analyses, 29 specimens were sequenced for nuclear genes (near complete SSU and LSU). Information on specimens analyzed for the current study and their corresponding GeneBank accession numbers are given in Table 1. A small sample of tissue of each species of tapeworms was digested overnight at 56 ºC in a solution containing 10 mM Tris-base HCl (pH 7.6), 20 mM NaCl, 100 mM $Na_2$ EDTA (pH 8.0), 1% Sarkosyl, and 0.1 mg/ml proteinase K. Genomic DNA was extracted from the supernatant using the DNAzol reagent (Molecular Research Center, Cincinnati, Ohio) according to the manufacturer instructions. Two regions of nuclear ribosomal DNA (rDNA) were amplified using the polymerase chain reaction (PCR). The near-complete SSU rDNA (~2200 bp) was amplified in two overlapping PCR fragments, and the near-complete LSU rDNA (~4500 bp) was amplified in three overlapping PCR fragments (Table 2).

Polymerase Chain Reaction (final volume 25 μl) consisted of 2.5 μl 10x PCR buffer, 1.5 μl $MgCl_2$, 0.5 μl of dNTP´s, 1 μl of each primer (10 pmol), 1-2 μl of genomic DNA, 0.125 μl of Taq DNA polymerase, and 16.375 μl sterile-distilled water. PCR cycling parameters included denaturation at 94 ºC for 3 min, followed by 35 cycles of 94 ºC for 1 min, annealing at 50-58 ºC (optimized for each rDNA region) for 1 min, and extension at 72 ºC for 1 min, followed by a post-amplification incubation at 72 ºC for 7 min. All PCR reactions were visualized on 1% agarose gels and the products were prepared for direct sequencing using Millipore columns (Amicon, Billerica, Massachusetts). When direct sequencing of PCR products yielded poor results (e.g. due to repeated sequences motifs or misreading), products were cloned by ligation into pGEM–T vector (Promega, Madison, Wisconsin) and used to transform competent *Escherichia coli* (JM109). Positive clones were identified by blue/white selection, and target insert of white colonies were confirmed by PCR of bacterial DNA extracts. Liquid cultures for minipreps were grown in Luria broth containing 50 μg/ml of ampicillin. Plasmids for DNA sequencing were prepared using commercial miniprep kits (Qiaprep, Qiagen). Purified products were directly sequenced in both directions using the ABI Big Dye (Applied Biosystems, Boston, Massachusetts) terminator sequencing chemistry, and reaction products were separated and detected using an ABI 3730 capillary DNA sequencer.  PCR, custom internal, and plasmid primers as appropriate to each gene (see Table 2). Contigs were assembled and base-calling differences resolved using Codoncode Aligner version 3.5.4 (Codoncode Corporation, Dedham, Massachusetts). Sequences were aligned using Clustal X 2.0 (Larkin et al., 2007) and then modified in MacClade (Maddison and Maddison, 2003). Sequences and alignment obtained in this study were deposited in GenBank data set.

*2.3 Phylogenetic analyses*

Tree searches of individual and combined molecular data sets were conducted by three reconstruction methods, maximum-parsimony (MP), maximum-likelihood (ML), and Bayesian inference (BI). Sequences of five species of the family Hymenolepididae (sp. 1, sp. 2, sp. 4 obtained in this study, plus *Fimbriaria* sp., *Hymenolepis diminuta*), six of Dilepididae (*Choanotaenia infundibulum*, *Dilepis undula, Hepatocestus hepaticus, Molluscotaenia crassiscolex*, *Paricterotaenia porosa* and *Trichocephaloidis megalocephala*) one of Taeniidae (*Taenia solium*), two of Davaineidae (*Raillietina mitchelli* and *Skrjabinia cesticillus*), and one of Mesocestoididae (*Mesocestoides* sp.) were obtained from GenBank and used as outgroups, and *Mesocestoides* sp. was used to root the trees. MP analyses were performed with PAUP* 4.0b10 (Swofford, 2003), using a heuristic search strategy with 10,000 searches replicates of random addition taxa sampling and tree-bisection-reconnection (TBR) algorithm was used. Characters were treated as unordered, equally weighted and gaps as missing data. Branch support was estimated using 10,000 replicates. ML was carried out in RaxML v7.0.4 (Stamatakis, 2006) with the selected GTR+ $\Gamma$ model of sequence evolution for SSU, LSU, and combined data set. Clade support was examined with 10,000 bootstrap replicates. Prior to BI, the best-fitting evolutionary models were estimated for each data set separately and combined using jModeltest v. 0.1.1 (Posada, 2008). Parameter settings and best-fit models for each data set were identified on the bases of the BIC (Bayesian Information Criterion) (Schwarz, 1978), implemented in jModeltest v. 0.1.1 (Table 3). Bayesian Inference was performed using MrBayes v 3.1.2 (Huelsenbeck and Ronquist, 2001).

Bayesian MCMC analyses were performed for individual and combined data sets using MrBayes v 3.1.2 (Huelsenbeck and Ronquist, 2001). To reduce the chance of analyses becoming trapped in local optima, two independent analyses were run for each data set, each consisting of two chains from random starting trees and using uniform priors. Chains were run for $1.0 \times 10^6$ generations for individual and combined data sets, respectively. Trees were sampled every 200 generations. The combined data set was treated as comprising two separate partitions based on each gene respectively. The burn-in phases for the different analyses were discarded. The relationships obtained from the remaining sampled trees were highly correlated between the two analyses run for each data set. A 50% majority-rule consensus tree representing the posterior probability distribution of clades was produced from the remaining trees. Clade support was regarded as significant if the clade was present in at least 95% of the sampled trees.

## 3. Results

### *3.1 Sequences data*

Nucleotide frequencies for the combined SSU+LSU data set were 0.220 (A), 0.224 (C), 0.307 (G), and 0.247 (T). The heterogeneity of nucleotide frequencies across taxa was tested using the “base frequencies” option implemented in PAUP* ($X^2$ = 70.60, $P$ = 0.064). Total lengths of the alignments and number of constant and parsimony-informative characters for the SSU, LSU, and combined data sets are provided in Table 3.

### *3.2 SSU dataset*

Most previous analyses of cyclophyllidean relationships were based exclusively on SSU rDNA (Mariaux, 1998; Hoberg et al., 1999b). In this study the total alignment to the SSU rDNA included 42 sequences (27 ingroup plus 15 outgroup) and consists of 2, 532 characters, 1,581 of which are constant, 324 variable characters parsimony uninformative, and 627 characters parsimony informative. The MP analysis yielded 441 trees with a C. I. = 0.56 and length of 2, 971 steps. The MP strict consensus tree (Fig. 1A) supported the monophyly of Gryporhynchidae with a bootstrap value of 69%. This tree yielded two major groups. The first group was divided in two subclades that include the genus *Paradilepis* as a monophyletic assemblage, with three species (*Paradilepis* cf. *minima*, *P*. *caballeroi* and *Paradilepis* sp.). These species are morphologically characterized by having a size of the rostellar hook in a range of 95 to 197 μm long, associated in adult stage to pelicans, cormorants, herons and ibises. The second subclade included the genera *Dendrouterina* (*D*. *papillifera*, *D*. *pilherodiae*, *Dendrouterina* sp.), *Neovalipora* (*Neovalipora* sp.), and *Valipora* (*V*. *campylancristrota*, *V*. *minuta*, and *V*. *mutabilis*). The genera are not monophyletic with high bootstrap values ranging from 98 to 100%. These species possess rostellar hooks size varying from 36 to 52 long in *Dendrouterina* spp., from 23 to 35 in *Neovalipora* sp., and from 24 to 40 in *Valipora* spp. All these species are parasites of herons.

The second major group includes four genera: *Cyclustera*, *Glossocercus Parvitaenia* and *Neogryporhynchus.* The genus *Cyclustera* was represented by two isolates of *C*. *ibisae* from the white ibis (*Eudocimus albus*) nest together. The species is characterized by possessing size of the rostellar hooks measuring 207 to 243 long. The genus *Glossocercus* included three species (*G*. *caribaensis G*. *cyprinodontis* and *Glossocercus* sp.,). The size of the rostellar hooks of these species ranged from 77 to 285 long. These species were nested in two independent clades. The genera *Parvitaenia* (included two isolates) and *Neogryporhynchus* represent two independent groups. However the relationships between both genera are unresolved due to the polytomy in the basal node of the tree (Fig. 1A).

The ML analysis yielded a single tree (Fig. 1B) with a likelihood score of -ln 15903.902. This tree also supported the monophyly of Gryporhynchidae and it represent the sister group of Hymenolepididae with a moderate bootstrap support values. The ML tree recovers similar topology than MP tree (Fig 1 A), but the relationships among genera were well resolved with moderate to high bootstrap values. Additionally the relationship between *Neogryporhynchus cheilancristrotus* and *Parvitaenia cochlearii* was well resolved (Fig. 1B). In this tree the genus *Glossocercus* was a paraphyletic with week bootstrap support value.

*3.3 LSU dataset*

The LSU alignment includes 19 taxa with 4,346 characters, 2,848 of which are constant, 421 variable characters parsimony uninformative, and 1,077 characters parsimony informative. The MP analysis yielded two trees with a C. I. = 0.67 and length of 3,365 steps. MP consensus strict supported the monophyly of Gryporhynchidae. This tree yielded two major clades. The first clade contained three genera *Dendrouterina* (with two species *D. papillifera* and *Dendrouterina* sp.,), *Neovalipora* (*Neovalipora* sp.,) and *Valipora* (with two species *V. mutabilis*, and *V. minuta*). The two species of *Dendrouterina* and *Valipora* were nested in two independent groups supporting the paraphyly of both genera. The second clade included four monophyletic genera *Cyclustera*, *Glossocercus*, *Paradilepis*, and *Parvitaenia* with moderate to high bootstrap support (Fig. 2A). The ML analysis of the LSU data set yielded a tree with a likelihood score of -ln 29893.686. This tree show the same branch patter than MP tree inferred with LSU data set, except for the systematic position of *Paradilepis* sp. In contrasts to phylogenetic tree inferred with SSU data set the genera *Glossocercus* was monophyletic in both trees inferred with LSU data set (see Fig 2A).

*3.4 Combined SSU+LSU dataset*

The combined alignment (SSU + LSU) included 19 taxa, with 6, 599 characters, 4, 469 of which are constant, 628 characters parsimony uninformative, and 1, 502 characters parsimony informative. MP analysis of this data set (Fig. 3A) yielded two trees with a C. I. = 0.68 and length of 4, 805 steps. The strict consensus MP tree (Fig. 3A) and the ML tree (Fig. 3B) supported the monophyly of Gryporhynchidae with 100% of bootstrap support in both analyses. The MP and ML trees yielded near identical topologies, except for the position of *Paradilepis* sp. The Bayesian tree yielded similar topology than the tree inferred with ML, with high values of Bayesian posterior probabilities (BPP´s) (Fig. 4). The combined tree inferred with ML method showed identical topology with respect to ML tree inferred with LSU data set (see Figs. 2B and 3B).

## 4. Discussion

The phylogenetic hypotheses inferred with partial sequences from SSU rDNA gene provided some data on the phylogenetic relationships of Cyclophyllidea. Based on these studies 13 families have been were recognized. However no species of Gryporhynchidae was included in these studies (Mariaux, 1996; Hoberg et al., 1999a; Mariaux and Olson, 2001; Littlewood, 2006). Phylogenetic hypotheses inferred with partial sequences from SSU rDNA and morphological characters included a single species of the family (*Neogryporhynchus cheilancristrotus*) along with other species from Dilepididae. The trees obtained in these studied placed *N. cheilancristrotus* as an independent taxon from Dilepididae (Mariaux, 1998; Hoberg et al., 1999b). Hoberg et al. (1999b) argued that Gryporhynchidae was then recognized as a separate family. All these phylogenetic studies failed to solve the systematic position and validity of Gryporhynchidae (Mariaux, 1998; Hoberg et al., 1999b; Presswell et al., 2012), and Bona’s identification keys (1994) did no recognize the family Gryporhynchidae including all the species into Dilepididae. In this paper the emphasis was on increasing the diversity of gryporhynchids species represented in phylogenetic hypotheses, including sequences of two nuclear genes, and testing the monophyly of the family

Gryporhynchidae and the relationship among genera.

All phylogenetic trees inferred with SSU, LSU and combined both (SSU+LSU) data sets support the monophyly of Gryporhynchidae and its validity within Cyclophyllidea (see Figs. 1A-3B and Fig. 4). Additionally sequences from SSU and LSU rDNA were generated for 11 species representing seven of the 10 recognized genera (*Cyclustera*, *Glossocercus*, *Paradilepis*, *Parvitaenia*, *Dendrouterina*, *Neovalipora* and *Valipora*) in the family. The combined analyses inferred with the MP, ML, and Bayesian inference, shows that the genera *Dendrouterina*, *Neovalipora* and *Valipora* are not monophyletic taxa. Morphologically these three genera are differentiated from each other by the size of the rostellar hooks. However our analyses suggested that these genera represent species that should be reclassified using a combination of morphological, ecological and molecular data. Likewise, genera *Cyclustera*, *Neogryporhynchus*, *Paradilepis* and *Parvitaenia* represent independent lineages within the family. All these genera differ mainly in morphological characters as the shape and size of the rostellar hooks, i. e. the genus *Paradilepis* possess a blade longer than handle and the guard slightly protruding, whereas *Parvitaenia* possess straight blade on the same axis as handle and the guard forming angle 90° to axis of handle (see Bona, 1994). However, exists a particular case with the genus *Glosocercus*, which results monophyletic in the combined data set with MP and ML, but was a paraphyletic group in the Bayesian inference. The relationships of the members of this genus should be examined in a future including new species and molecular data.

The present study represents the first phylogenetic analysis from Gryporhynchidae and it recognized within Cyclophyllidea. The inclusion of the genera *Amyrthalingamia* and *Ascodilepis* and more species from genera sampling herein are fundamental to provide a comprehensive phylogenetic framework for this family that allow us understand the evolution of this enigmatic group of tapeworm parasites of fish-eating birds.

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**Figure Legends**

**Figs. 1 A-B**. Trees recovered from analyses of the SSU rDNA data set. (A) Strict consensus of 441 equal parsimonious trees (2, 971 steps) inferred from heuristic MP searches. Numbers near internal nodes show MP bootstrap clade frequencies. (B) Maximum likelihood tree (-ln likelihood 15903.902) obtained from heuristic searches with branch scaled to the expected number of substitutions per site. Numbers near internal nodes show ML bootstrap clade frequencies.

**Figs. 2 A-B.** Trees recovered from analyses of the LSU rDNA data set. (A) Strict consensus of two equal parsimonious trees (3,365 steps) inferred from heuristic MP searches. Numbers near internal nodes show MP bootstrap clade frequencies. (B) Maximum likelihood tree (-ln 29893.686) obtained from heuristic searches with branch scaled to the expected number of substitutions per site. Numbers near internal nodes show ML bootstrap clade frequencies.

**Figs. 3 A-B.** Trees recovered from analyses of the combined (SSU + LSU) rDNA data set. (A) Strict consensus of two equal parsimonious trees (4, 805 steps.) inferred from heuristic MP searches. Numbers near internal nodes show MP bootstrap clade frequencies. (B) Maximum likelihood tree (-ln 29893.686) obtained from heuristic searches with branch scaled to the expected number of substitutions per site. Numbers near internal nodes show ML bootstrap clade frequencies.

**Fig. 4.** Bayesian 50% majority-rule consensus phylogram inferred of combined (SSU+LSU) data set (6, 884 characters), from 11 species of gryporhynchids and 16 outgroups. Phylogram rooted with *Mesocestoides* sp. as outgroup. Bayesian posterior probabilities are given near internal nodes.

Fig. 1

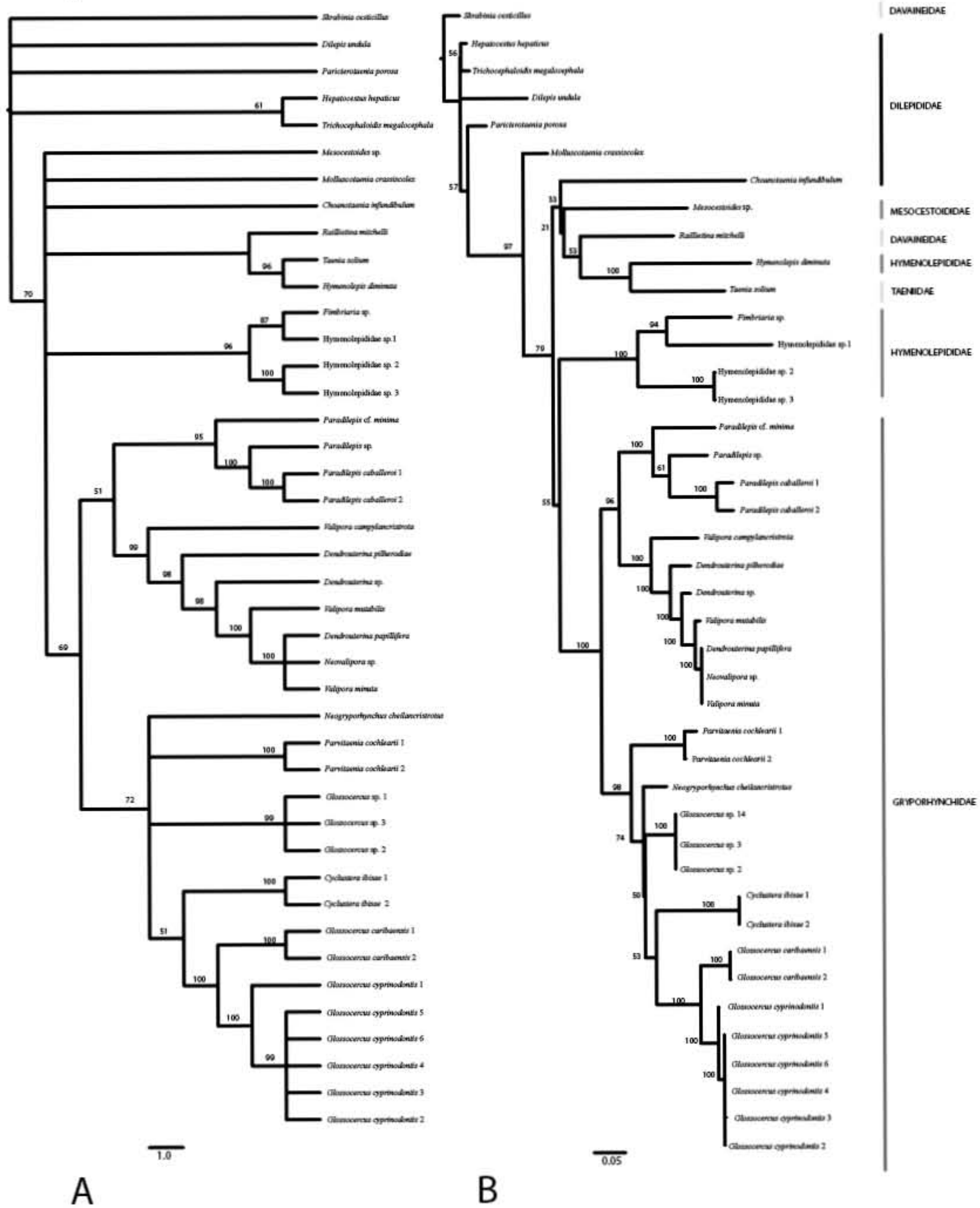


Fig. 2

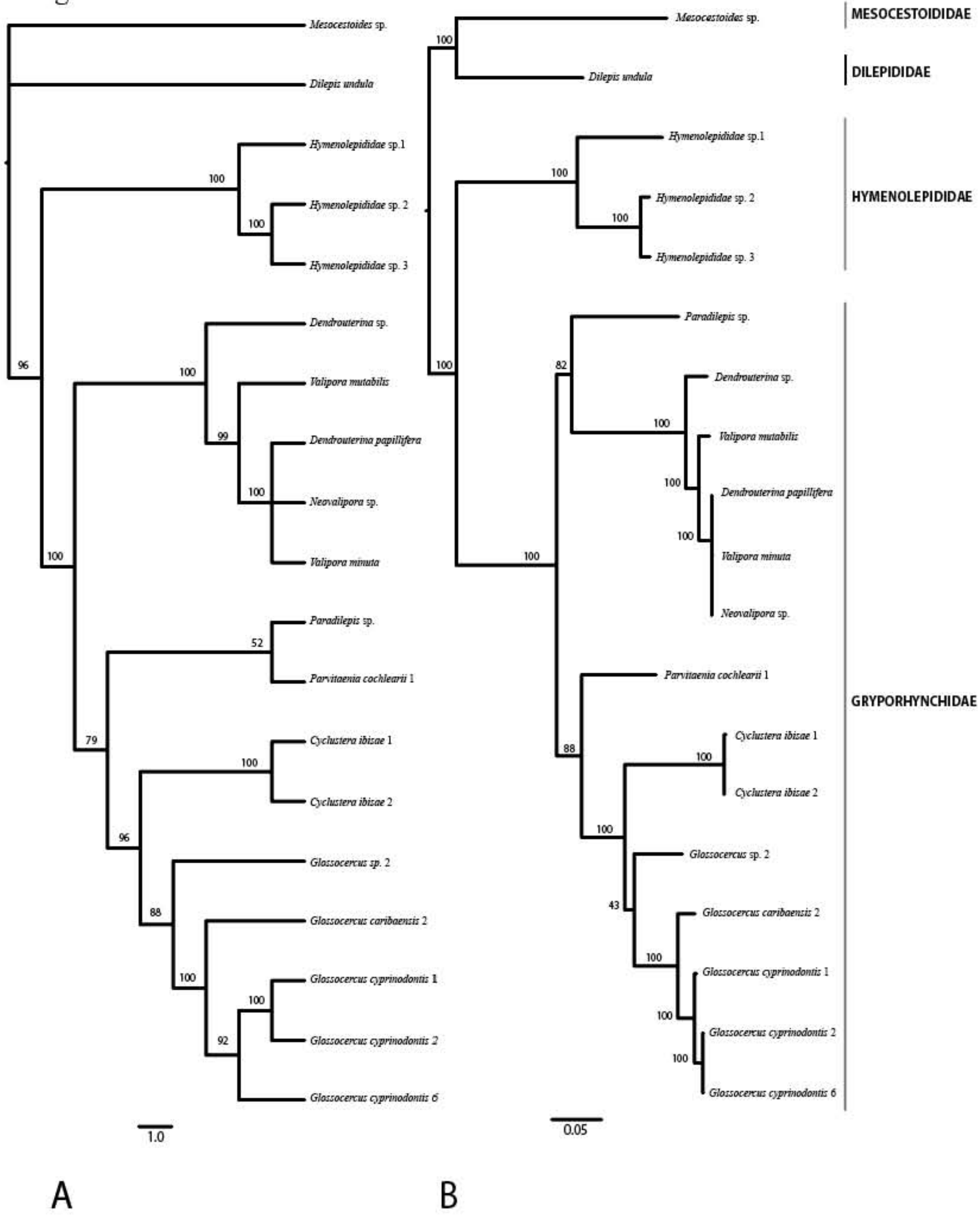

Mesocestoides sp.
Dilepis undula
Hymenolepididae sp.1
Hymenolepididae sp. 2
Hymenolepididae sp. 3
Dendrouterina sp.
Valipora mutabilis
Dendrouterina papillifera
Neovalipora sp.
Valipora minuta
Paradilepis sp.
Parvitaenia cochlearii 1
Cyclustera ibisae 1
Cyclustera ibisae 2
Glossocercus sp. 2
Glossocercus caribaensis 2
Glossocercus cyprinodontis 1
Glossocercus cyprinodontis 2
Glossocercus cyprinodontis 6
1.0
0.05
MESOCESTOIDIDAE
DILEPIDIDAE
HYMENOLEPIDIDAE
GRYPORHYNCHIDAE
A
B

Fig. 3

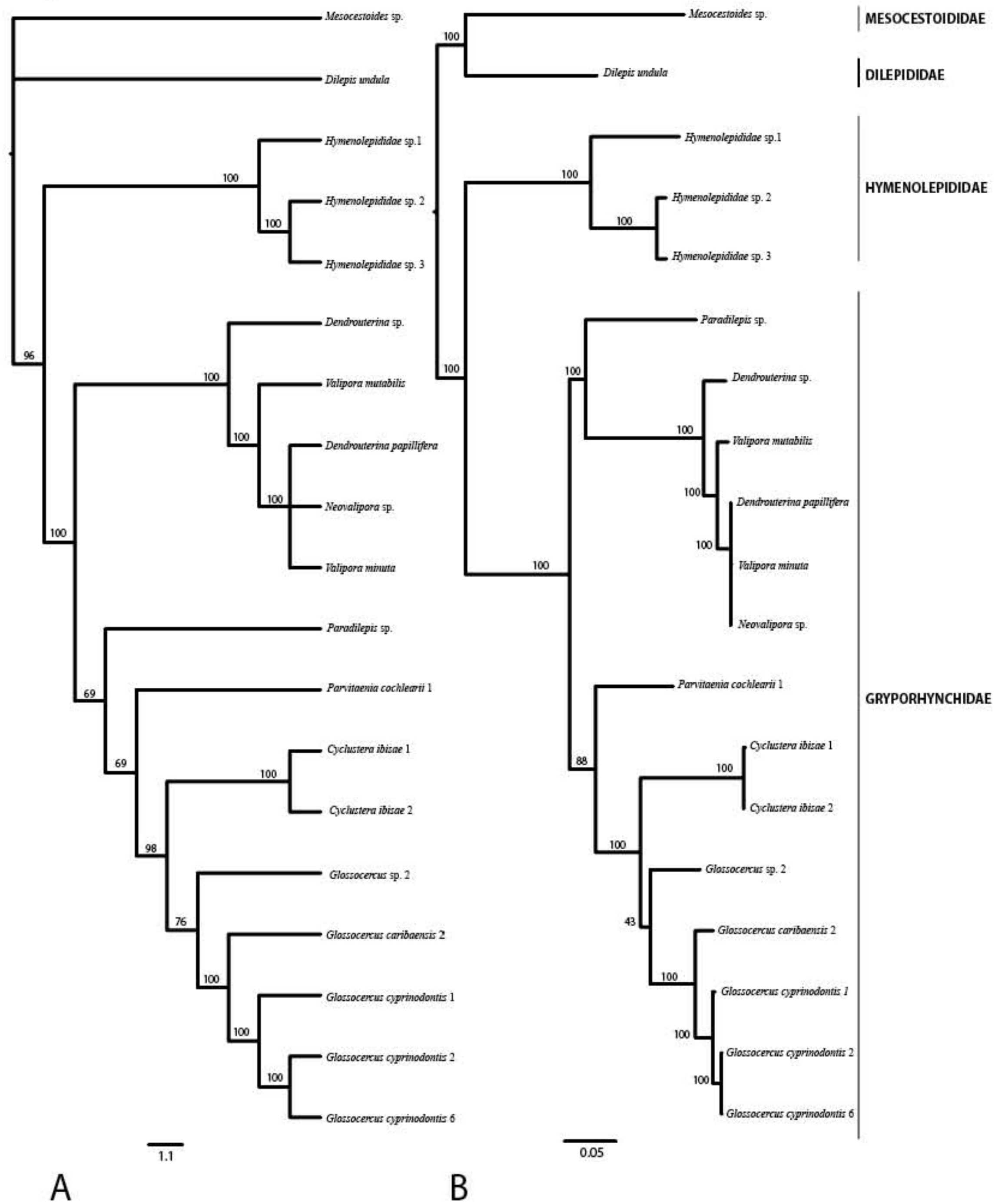
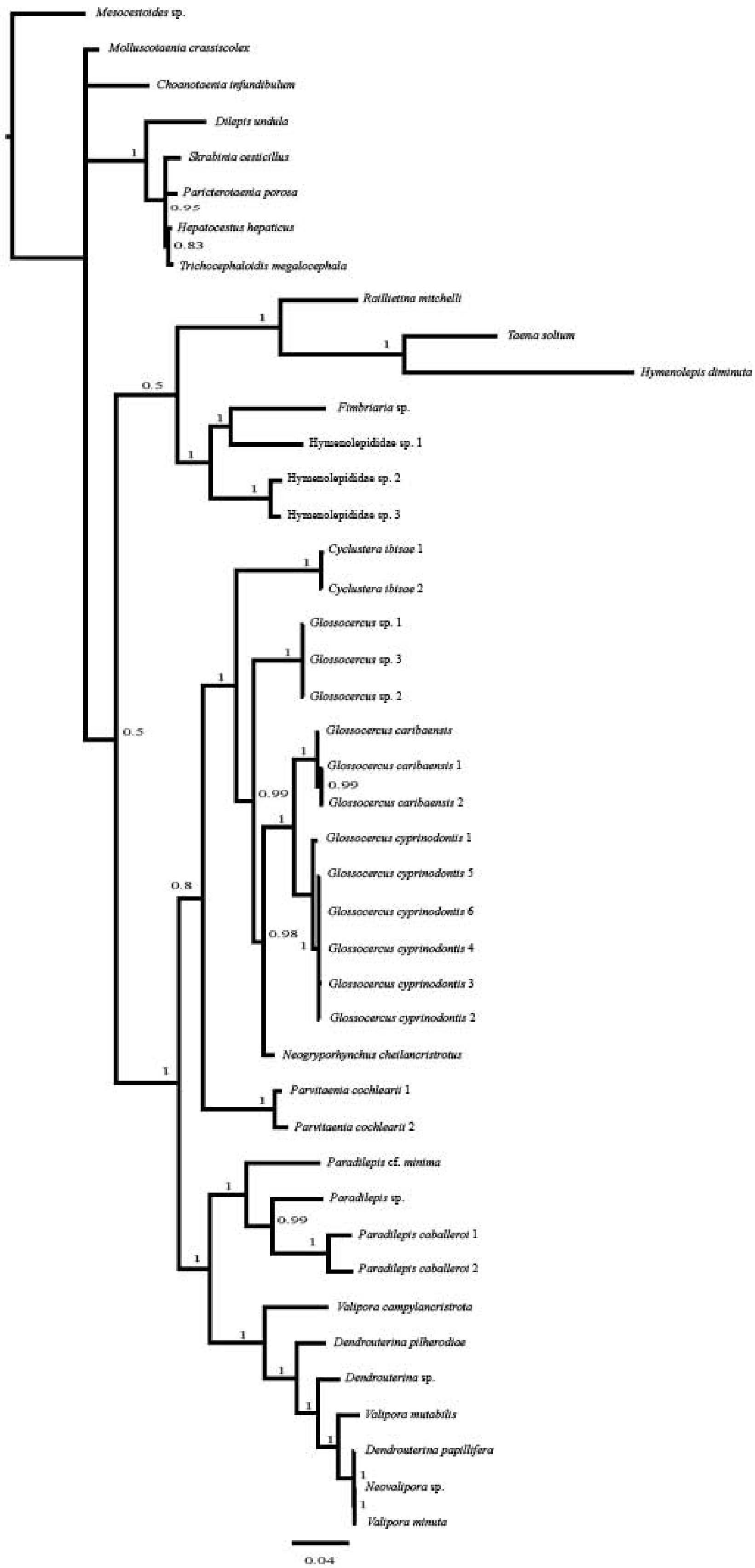

Mesocestoides sp.
Dilepis undula
Hymenolepididae sp.1
Hymenolepididae sp. 2
Hymenolepididae sp. 3
Dendrouterina sp.
Valipora mutabilis
Dendrouterina papillifera
Neovalipora sp.
Valipora minuta
Paradilepis sp.
Parvitaenia cochlearii 1
Cyclustera ibisae 1
Cyclustera ibisae 2
Glossocercus sp. 2
Glossocercus caribaensis 2
Glossocercus cyprinodontis 1
Glossocercus cyprinodontis 2
Glossocercus cyprinodontis 6
1.1
A
0.05
B
MESOCESTOIDIDAE
DILEPIDIDAE
HYMENOLEPIDIDAE
GRYPORHYNCHIDAE

Fig. 4



Mesocestoides sp.
Molluscotaenia crassiscolex
Choanotaenia infundibulum
Dilepis undula
Skrabinia cesticillus
Paricterotaenia porosa
0.95
Hepatocestus hepaticus
0.83
Trichocephaloidis megalocephala
Raillietina mitchelli
Taenia solium
Hymenolepis diminuta
0.5
Fimbriaria sp.
Hymenolepididae sp. 1
Hymenolepididae sp. 2
Hymenolepididae sp. 3
Cyclustera ibisae 1
Cyclustera ibisae 2
Glossocercus sp. 1
Glossocercus sp. 3
Glossocercus sp. 2
0.5
Glossocercus caribaensis
Glossocercus caribaensis 1
0.99
Glossocercus caribaensis 2
Glossocercus cyprinodontis 1
Glossocercus cyprinodontis 5
Glossocercus cyprinodontis 6
Glossocercus cyprinodontis 4
Glossocercus cyprinodontis 3
Glossocercus cyprinodontis 2
0.8
0.98
Neogryporhynchus cheilancristrotus
Parvitaenia cochlearii 1
Parvitaenia cochlearii 2
Paradilepis cf. minima
Paradilepis sp.
0.99
Paradilepis caballeroi 1
Paradilepis caballeroi 2
Valipora campylancristrota
Dendrouterina pilherodiae
Dendrouterina sp.
Valipora mutabilis
Dendrouterina papillifera
Neovalipora sp.
Valipora minuta
0.04

Table 1. Species name, host, sampling localities, voucher number deposited at the Colección Nacional de Helmintos (CNHE), Genbank access numbers. Sequences with an asterisk were obtained in the current study. Nd Not determined.

| **Family** | Species name | Host | Locality | CNHE | GenBank accession number | |
|---|---|---|---|---|---|---|
| | | | | | SSU | LSU |
| **Gryporhynchidae** | | | | | | |
| | *Cyclustera ibisae* 1 | *Eudocimus albus* | Punta Piedra, Tamaulipas | 7713 | XXX | XXX |
| | *Cyclustera ibisae* 2 | *Eudocimus albus* | Los Chivos, Veracruz | 7714 | XXX | XXX |
| | *Dendrouterina papillifera* | *Egretta thula* | Guatimapé, Durango | 8330 | XXX | XXX |
| | *Dendrouterina pilherodiae* | *Ardea alba* | Guatimape, Durango | 8333 | XXX | XXX |
| | *Dendrouterina* sp. | *Botaurus pinnatus* | Los Chivos, Veracruz | – | XXX | XXX |

| | | | | | |
|---|---|---|---|---|---|
| *Glossocercus caribaensis* 1 | *Egretta rufescens* | Ría Lagartos, Yucatan | – | XXX | XXX |
| *Glossocercus caribaensis* 2 | *Egretta caerulea* | Punta Piedra, Tamaulipas | – | XXX | XXX |
| *Glossocercus cyprinodontis* 1 | *Egretta thula* | Rio Panuco, Veracruz | – | XXX | XXX |
| *Glossocercus cyprinodontis* 2 | *Egretta caerulea* | Chuburna, Yucatan | – | XXX | XXX |
| *Glossocercus cyprinodontis* 3 | *Egretta caerulea* | Punta Piedra, Tamaulipas | – | XXX | XXX |
| *Glossocercus cyprinodontis* 4 | *Egretta rufescens* | Chuburna, Yucatan | 8265 | XXX | XXX |
| *Glossocercus cyprinodontis* 5 | *Egretta rufescens* | Punta Piedra, Tamaulipas | 8266 | XXX | XXX |
| *Glossocercus cyprinodontis* 6 | *Egretta thula* | Punta Piedra, Tamaulipas | – | XXX | XXX |
| *Glossocercus* sp. 1 | *Nycticorax nycticorax* | Los Chivos, Veracruz | 8326 | XXX | XXX |

| | | | | | |
|---|---|---|---|---|---|
| *Glossocercus* sp. 2 | *Nyctanassa violacea* | Rio Ulumal, Campeche | 8321 | XXX | XXX |
| *Glossocercus* sp. 3 | *Nyctanassa violacea* | Rio Ulumal, Campeche | 8321 | XXX | XXX |
| *Neovalipora* sp. | *Cyprinodon nasas* | Rio Sofia, Durango | XXX | XXX | XXX |
| *Parvitaenia cochlearii* 1 | *Nyctanassa violacea* | Laguna Tamiahua, Veracruz | 8329 | XXX | XXX |
| *Parvitaenia cochlearii* 2 | *Nyctanassa violacea* | Laguna Tamiahua, Veracruz | 8329 | XXX | XXX |
| *Paradilepis caballeroi* 1 | *Pelecanus occidentalis* | Presa Temascal, Oaxaca | XXX | XXX | XXX |
| *Paradilepis caballeroi* 2 | *Phalacrocorax brasilianus* | Teapa, Tabasco | XXX | XXX | XXX |
| *Paradilepis* cf. *minima* | Nd | Nd | – | JQ042915–JQ042917 | – |
| *Paradilepis* sp. | *Phalacrocorax* | Río Guatimapé, | 8332 | XXX | XXX |

| | | | | | | |
|---|---|---|---|---|---|---|
| | | *brasilianus* | Durango | | | |
| | *Valipora campylancristrota* | *Ardea alba* | Laguna Santiaguillo, Durango | 8284 | XXX | XXX |
| | *Valipora minuta* | *Egretta caerulea* | Rio Tecolutla, Veracruz | 8336 | XXX | XXX |
| | *Valipora mutabilis* | *Botaurus lentiginosus* | San Quintin, Baja California | 8337 | XXX | XXX |
| | *Neogryporhynchus chelilancristrotus* | Nd | Nd | – | Z98320–Z98322 | – |
| **Hymenolepididae** | | | | | | |
| | Hymenolepididae sp. 1 | *Phalacrocorax auritus* | Santa Clara, Sonora | XXX | XXX | XXX |
| | Hymenolepididae sp. 2 | *Nycticorax nycticorax* | Los Chivos, Veracruz | XXX | XXX | XXX |
| | Hymenolepididae sp. 3 | *Nyctanassa violacea* | Los Chivos, | XXX | XXX | XXX |

| | | | | | | |
|---|---|---|---|---|---|---|
| | | | Veracruz | | | |
| | *Hymenolepis diminuta* | Nd | Nd | – | AF124475 | – |
| | *Fimbriaria* sp. | Nd | Nd | – | AF286982 | – |
| **Dilepididae** | | | | | | |
| | *Choanotaenia infundibulum* | Nd | Nd | – | AJ555171–AJ555172 | – |
| | *Trichocephaloidis megalocephala* | Nd | Nd | – | Z98326–Z98328 | – |
| | *Paricterotaenia porosa* | Nd | Nd | – | Z98323–Z98325 | – |
| | *Molluscotaenia crassiscolex* | Nd | Nd | – | Z98314–Z98316 | – |
| | *Hepatocestus hepaticus* | Nd | Nd | – | Z98317–Z98319 | – |
| | *Dilepis undula* | Nd | Nd | – | AF286981 | AF286915 |
| **Taeniidae** | | | | | | |

| | | | | | | |
|---|---|---|---|---|---|---|
| | *Taenia solium* | Nd | Nd | – | GQ260091 | – |
| **Mesocestoididae** | | | | | | |
| | *Mesocestoides* sp. | Nd | Nd | – | EF095248 | EF095263 |
| **Davaineidae** | | | | | | |
| | *Raillietina mitchelli* | Nd | Nd | – | AY382315 | – |
| | *Skrjabinia cesticillus* | Nd | Nd | – | AY382316 | – |

Table 2. Amplify and sequencing primers information. In bold are the internal primers. (F) = Forward, (R) = Reverse.

| Locus Amplicon | Primer name | Primer sequence (5´–3´) | Tm |
|---|---|---|---|
| **SSU** | | | |
| 1 | 18S1A(F) | GGCGATCGAAAAGATTAAGCCATGCA | 50 |
| | 32(R) | CGAAGTCCTATTCCATTATTC | 50 |
| | **635(R)** | **CGCCTGCTGCCTTCCTTGG** | 50 |
| | **Acanto2(R)** | **TAACGGGTAACGGGGAATCA** | 50 |
| 2 | 652(F) | GCAGCCGCGGTAATTCCAGCTC | 50 |
| | 28(R) | AGCGACGGGCGGTGTGT | 50 |
| | **548(F)** | **GTGGTGCATGGCCGTTCTTAG** | 50 |
| | **31(R)** | **CGGAATTAACCAGACAAATC** | 50 |
| | **JRL9(F)** | **GAAACTTAAAGGAATTCACGG** | 50 |
| **LSU** | | | |
| 1 | 502(F) | CAAGTACCGTGAGGGAAA | 50 |
| | 536(R) | CAGCTATCCTGAGGGAAAC | 50 |
| | **504(F)** | **CAAGTACCGTGAGGGAAAGTTG** | 50 |
| | **503(R)** | **CCTTGGTCCGTGTTTCAAGACG** | 50 |
| 2 | 662(F) | ACCCGAAAGATGGTGAACTATG | 58 |
| | 663(R) | CTTCTCCAAC(G/T)TCAGTCTTCAA | 58 |
| | **527(F)** | **CTAAGGAGTGTGTAACAACTCACC** | 58 |

| | | | |
|---|---|---|---|
| 3 | 537(F) | GATCCGTAACTTCGGGAAAAGGAT | 58 |
| | 531(R) | CTTCGCAATGATAGGAAGAGCC | 58 |
| | **533(F)** | **AAGGTAGCCAAATGCCTCGTCATCT** | 58 |
| | **538(R)** | **AGCATATCATTTAGCGGAGG** | 58 |
| | **532(R)** | **AATGACGAGGCATTTGGCTACCTT** | 58 |

Table 3. Tree statistics for rDNA datasets. Small subunit (SSU), Large subunit (LSU), combined rDNA (SSU+LSU) datasets. Number of informative characters, tree length, consistency index (C. I.) and –ln likelihood score.

| Dataset | Total characters | Uninformative characters | Constant characters | Informative characters | Tree length | C. I. | –ln likelihood |
|---|---|---|---|---|---|---|---|
| SSU | 2, 532 | 324 | 1581 | 627 | 2971 | 0.56 | 15903.902 |
| LSU | 4, 346 | 423 | 2845 | 1078 | 3368 | 0.67 | 20457.900 |
| SSU+LSU | 6, 620 | 627 | 4491 | 1502 | 4776 | 0.68 | 29870.907 |

## Discusión y conclusiones

A partir de la integración de los tres capítulos que conforman el presente trabajo de tesis doctoral, se generaron las siguientes conclusiones generales:

Se identificaron taxonómicamente 11 especies de céstodos parásitos de aves ictiófagas. Dos especies de aves ictiófagas: *Phalacrocorax auritus* y *Botaurus lentiginosus* se examinaron por primera vez para céstodos en México, todos los registros que aportamos para estas especies son nuevos. Adicionalmente, se incrementó el registro de céstodos para 12 especies de aves ictiófagas (*Pelecanus occidentalis*, *Phalacrocorax brasilianus*, *Ardea herodias*, *A. alba*, *Egretta caerulea*, *E. rufescens*, *E. thula*, *E. tricolor*, *Nyctanassa violacea*, *Nycticorax nycticorax*, *Botaurus pinnatus* y *Platalea ajaja*) que contaban con registros previos en México. Se identificó un total de 15 especies de céstodos representantes de 7 géneros de la familia Gryporhynchidae, una de ellas registrada como larva (registro del pez *Cyprinodon nazas*). De los datos obtenidos del presente estudio se aportan 14 nuevos registros de huéspedes y 31 nuevos registros de localidad para céstodos en México.

En cuanto al registro de la helmintofauna procedente de 52 ibis blanco (*Eudocimus albus*) colectados en lagunas costeras del Océano Pacífico y Golfo de México, se registraron ocho especies de helmintos (tres tremátodos, un céstodo y cuatro acantocéfalos), de los cuales dos especies de tremátodos (*Parastrigea cincta* y *P*. *diovadena*) se registran por primera vez para México, se amplia la distribución geográfica para cuatro especies de helmintos (*Patagifer lamothei*, *Cyclustera ibisae*, *Ibirhynchus dimorpha* y *Arhythmorhynchus frassoni*), así como el ibis blanco (*E*. *albus*) representa un nuevo registro de huésped para la especie *Hexaglandula corynosoma* y como huésped accidental para *Southwellina hispida*. Este trabajo representa el primer estudio sistemático de la helmintofauna del ibis blanco en México y se adicionan nuevos registro de helmintos conocidos para este huésped. Sin embargo, existen zonas a lo largo de la distribución de *E*.

*albus*, donde no hay registros de helmintos, por lo que el inventario de este huésped aún es incompleto y podría revelar nuevos registros y posiblemente especies nuevas para la ciencia.

Del registro de céstodos presentes en aves ictiófagas, el hallazgo del adulto de *Glossocercus cyprinodontis* resultó de gran relevancia. Esta especie fue descrita como larva (plerocercoide) del pez *Cyprinodon variegatus*, procedente de la bahía de Galveston, Texas, U.S.A. Basado en las características de las larvas, Chandler (1935) erigió el género *Glossocercus*. Sin embargo, desde su descripción original esta especie de céstodo no se había registrado. En el presente estudio la forma adulta de *G. cyprinodontis* fue registrada en las aves *Egretta rufescens*, *Nycticorax nycticorax* y *Pelecanus occidentalis*. En este estudio se describe por primera vez la morfología interna y distribución de los órganos reproductores. Basados en las características morfológicas del adulto se provee una nueva diagnosis para el género *Glossocercus*.

Desde su descripción inicial, históricamente la familia Gryporhynchidae ha estado bajo un debate constante, ya que en clasificaciones recientes este grupo no es reconocido, y todas las especies han sido clasificadas dentro de la familia Dilepididae. La generación de la primera hipótesis filogenética basada en secuencias de 11 especies de gryporhynchidos parásitos de diversas especies de aves ictiófagas, resultó de gran relevancia debido a que los  géneros *Cyclustera*, *Dendrouterina*, *Glossocercus*, *Neogryporhynchus*, *Neovalipora*, *Paradilepis*, *Parvitaenia* y *Valipora* forman en su conjunto un grupo monofilético, e independiente a Dilepididae y por lo tanto basado en está evidencia generada en el presente estudio se reconoce a la familia Gryporhynchidae como válida. Los géneros *Cyclustera*, *Neogryporhynchus*, *Parvitaenia* y *Paradilepis* representan grupos independientes. Sin embargo, los géneros *Dendrouterina*, *Glossocercus*, *Neovalipora* y *Valipora* resultaron ser parafiléticos, por lo que se propone llevar acabo una revisión exhaustiva de los caracteres morfológicos que definen a las especies correctamente, así como la inclusión de más especies y otros caracteres moleculares, que nos permitan definir claramente las relaciones filogenéticas entre géneros. Cabe señalar que la inclusión de especies de los géneros *Amyrthalingamia* y *Ascodilepis* en estudios futuros, resultan de suma importancia para contar con todos los representantes de la familia, permitiendo de esta manera entender mejor la evolución de este enigmático grupo de céstodos parásitos de aves ictiófagas.