UNIVERSIDAD NACIONAL AUTÓNOMA DE MÉXICO

POSGRADO EN CIENCIAS BIOLÓGICAS

INSTITUTO DE BIOLOGÍA

BIOLOGÍA EVOLUTIVA Y SISTEMÁTICA

PATRONES BIOGEOGRÁFICOS Y COFILOGENÉTICOS DE *MARGOTREMA* SPP. (DIGENEA: ALLOCREADIIDAE), PARÁSITOS DE PECES DULCEACUÍCOLAS DE LA SUBFAMILIA GOODEINAE (CYPRINODONTIFORMES: GOODEIDAE) EN EL CENTRO DE MÉXICO

TESIS

QUE PARA OPTAR POR EL GRADO DE:

DOCTOR EN CIENCIAS

PRESENTA:

ANDRÉS MARTÍNEZ AQUINO

TUTOR PRINCIPAL: DR. GERARDO PÉREZ PONCE DE LEÓN (INSTITUTO DE BIOLOGÍA)

COMITÉ TUTOR: DR. LUIS ENRIQUE EGUIARTE FRUNS (INSTITUTO DE ECOLOGÍA)
DRA. ELLA GLORIA VÁZQUEZ DOMÍNGUEZ (INSTITUTO DE ECOLOGÍA)

MÉXICO, D.F. **JUNIO, 2013.**

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UNAM POSGRADO
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 13 de mayo de 2013, se aprobó el siguiente jurado para el examen de grado de **DOCTOR EN CIENCIAS** del alumno **MARTINEZ AQUINO ANDRES** con número de cuenta **95295949** con la tesis titulada: **"PATRONES BIOGEOGRÁFICOS Y COFILOGENÉTICOS DE *Margotrema spp.* (DIGENEA: ALLOCREADIIDAE), PARÁSITOS DE PECES DULCEACUÍCOLAS DE LA SUBFAMILIA GOODEINAE (CYPRINODONTIFORMES: GOODEIDAE) EN EL CENTRO DE MÉXICO"**, realizada bajo la dirección del **DR. GERARDO PEREZ PONCE DE LEON:**

Presidente: DR. DANIEL IGNACIO PIÑERO DALMAU
Vocal: DR. JUAN JOSÉ MORRONE LUPI
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Suplente: DR. OMAR DOMÍNGUEZ DOMÍNGUEZ

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

ATENTAMENTE
"POR MI RAZA HABLARA EL ESPIRITU"
Cd. Universitaria, D.F. a 6 de junio de 2013.

DRA. MARÍA DEL CORO ARIZMENDI ARRIAGA
COORDINADORA DEL PROGRAMA

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

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> La base para consolidar este trabajo se encuentra en mis padres y mis hermanos.

Phylogeny as a cloud of gene histories.
Phylogeny is more like a statistical distribution
than a simple tree of discrete thin branches.
It has a central tendency,
but it also has a variance because
of the diversity of gene trees.
Gene trees that disagree
with the central tendency are not wrong;
rather, they are part of the
diffuse pattern that is the genetic history.

W.P. Maddison. 1997. Gene Tree in Species Tree.

ÍNDICE

RESUMEN

El género *Margotrema*, contiene digéneos parásitos que forman parte de la helmintofauna principal (*core helminth fauna*) de Goodeinae, un grupo monofilético de peces dulceacuícolas endémico del centro de México, debido a que comparten patrones de distribución geográfica y los digéneos exhiben altos niveles de especificidad. Los procesos de diversificación de Goodeinae son el resultado de los cambios hidrogeomorfológicos que modificaron la configuración de la superficie del centro de México. *Margotrema* representa un modelo excelente para realizar análisis filogenéticos, biogeográficos y cofilogenéticos en el sentido de dilucidar, en primera instancia, la historia evolutiva de las dos especies putativas de *Margotrema*: *M. bravoae* y *M. guillerminae*.

Los objetivos de esta tesis fueron los siguientes: a) Realizar la reconstrucción filogenética intraespecífica de las dos especies del género *Margotrema*: *M. bravoae* y *M. guillerminae*. b) Establecer la validez taxonómica de las dos especies del género *Margotrema* (*M. bravoae* y *M. guillerminae*), a través de análisis filogenéticos, basados en coalescencia, para datos moleculares. c) Detectar si la estructura filogenética de *Margotrema* spp. coincide con el patrón de distribución geográfico de las cuatro tribus de Goodeinae. d) Poner a prueba la hipótesis de "La diversificación de los goodeinos, resultado de los eventos de vicarianza-dispersión del centro de México, influye de igual modo en la evolución de las poblaciones de *Margotrema* spp.". Para ello, se realizaron análisis biogeográficos para detectar el patrón de distribución de *Margotrema* spp., en función del tiempo y del espacio, y contrastarlo con la estructura filogenética de Goodeinae y con la historia geológica del centro de México.

Entre agosto de 2008 y julio de 2010 se muestreo en 57 localidades, a lo largo de siete sistemas hidrológicos del centro de México. Se obtuvieron secuencias de dos marcadores moleculares: mitocondrial (*COI*; 118 secuencias) y nuclear (*ITS1;* 98 secuencias). Se realizaron análisis filogenéticos con inferencia bayesiana para bases de datos de genes independientes y combinados. Los árboles con probabilidad posterior más alta fueron utilizados para análisis de delimitación de especies usando modelos de coalescencia (v. gr. *General Mixed Yule Coalescent*; *Species Tree by multispecies coalescent*). La reconstrucción biogeográfica y los procesos cofilogeneticos fueron detectados a través de análisis biogeográficos paramétricos (v.gr. *Dispersal-Extinction-Cladogenesis*).

Con base en el numero de linajes detectado a través de la combinación de los análisis filogenéticos, usando los marcadores *COI* (cuatro linajes) e *ITS1* (dos linajes), se puede evidenciar la distinta tasa de evolución molecular que existe entre ambos marcadores. El Linaje I, apoyado por la resolución filogenética de ambos marcadores más caracteres morfológicos taxonómicamente delimitados, representa una nueva especie para *Margotrema*: *M. resolanae*. Las poblaciones restantes de *Margotrema* pertenecen a *M. bravoae*, y muestran una estructura genealógica que incluye tres linajes con evolución independiente que se refleja en sus patrones de distribución geográfica y especificidad.

Con base en la integración de los resultados de los análisis biogeográficos y cofilogenéticos se detecta que la relación *Goodeinae-Margotrema* representa tres niveles de asociaciones históricas distintas que reflejan que los procesos de divergencia de *Margotrema*, en primera instancia, se deben a eventos vicariantes seguidos de los procesos de diversificación de los grupos monófileticos de goodeinos a los que están asociados. Los tres niveles son: a) *Especie-Especie*. Representado por la asociación histórica *Xenotaenia resolanae-Margotrema resolanae*, exclusivo del Río Cuzalapa. b) *Especie-Linaje* (Codivergencia Tipo I). Representado por la asociación histórica *Characodon audax-Margotrema bravoae* Linaje III, exclusivo del Río Mezquital Medio-Alto. c) *Tribu-Linaje* (Codivergencia Tipo II). Representado, a su vez, por dos tipos de asociaciones históricas distintas: 1) Ilyodontini-*Margotrema bravoae* Linaje I, distribuido sobre los sistemas hidrológicos de los Ríos Ayuquila y Balsas y 2) Girardinichthyini / Chapalichthyini-*Margotrema bravoae* Linaje II, distribuido sobre el Río Lerma. Por tanto, la historia evolutiva de los parásitos *Margotrema* spp. es congruente con la historia filogenética y biogeográfica de sus huéspedes goodeinos.

ABSTRACT

Margotrema is a genus of helminth parasites that form part of the core parasite helmithfauna of goodeines, a group of freshwater cyprinodontiform fish endemic to central Mexico. The diversification of these fish is the result of complex hydrogeomorphological changes that took place in central Mexico. This raises the question of the evolutionary history and biogeography of their parasites, in the case of this study, two putative species of *Margotrema*: *M. bravoae* and *M. guillerminae*.

The objectives of the present thesis were: a) To describe the molecular intraspecific phylogenetic history of *M. bravoae* and *M. guillerminae.* b) To establish species boundaries based on novel phylogenetic species delimitation analyses. c) To test the hypothesis that each subgroup of goodeines (tribes) possesses their own species of *Margotrema* as a result of a similar history of vicariance and dispersal. d) To use recently published phylogenies of Goodeinae and those of their digenena parasite (*Margotrema* spp.) to uncover biogeographical and cophylogenetic patterns and to describe the processes that determined them over 6.5 million years ago. The key question addressed in this study is: Was the evolution of *Margotrema* spp. influenced by the complex geographical scenario of central Mexico, through the close association with their goodeinae hosts, or both?

Between August 2008 and July 2010, samplings were made in 57 localities distributed along seven hydrological systems of Central Mexico. Sequences of two molecular markers –*COI* and *ITS1*– were obtained, and a phylogenetic tree per marker was reconstructed by Bayesian Inference, subjecting them to species delimitation analyses using the *General Mixed Yule Coalescent* algorithm. Subsequently, a *Species Tree* analysis was performed. A *dispersal-extinction-cladogenesis* model was used to describe the historical biogeography of digeneans and their cophylogenetical process with goodein hosts.

The difference in numbers of lineages obtained from phylogenetic trees using *COI* and *ITS1* can be explained by the relative evolutionary rates of these molecular markers (fast and slow, respectively). Lineage I, delimited using both markers plus morphological characters, represents a new species of *Margotrema*: *M. resolanae*. The remaining populations of

Margotrema are postulated to belong to one species (*M. bravoae*), whose populations however display a certain degree of geographic restriction and host specificity.

The biogoegraphical and cophylogenetical results in this study show a response in a geographical context followed by host specificity at three distinct levels of historical association: a) *Species-Species*; historical association represented by *Xenotaenia resolanae-Margotrema resolanae* and exclusive to the Cuzalapa River. b) *Species-Lineage* (Codivergence Type I); historical association represented by *Characodon audax-Margotrema bravoae* Linaje III, exclusive of the Mezquital Medium-Hight River Basin. c) *Tribe-Lineage* (Codivergence Type II); represented, in turn, by two different types of historical associations: 1) Ilyodontini-*Margotrema bravoae Lineage I,* distributed on hydrological systems of the Ayuquila River and Balsas River. 2) Girardinichthyini / Chapalichthyini-*Margotrema bravoae* Lineage II, distributed on the Lerma River. Therefore, the parasite *Margotrema* evolutionary history is congruent with the phylogenetical and biogeographical history and of their goodein hosts.

INTRODUCCIÓN GENERAL

La biología evolutiva de los organismos parásitos puede ser explorada sobre tres tipos de asociaciones históricas distintas; i.e. a) Organismo-Gen, b) Área-Organismo y c) Huésped-Parásito (Page & Charleston, 1998) (Figura 1).

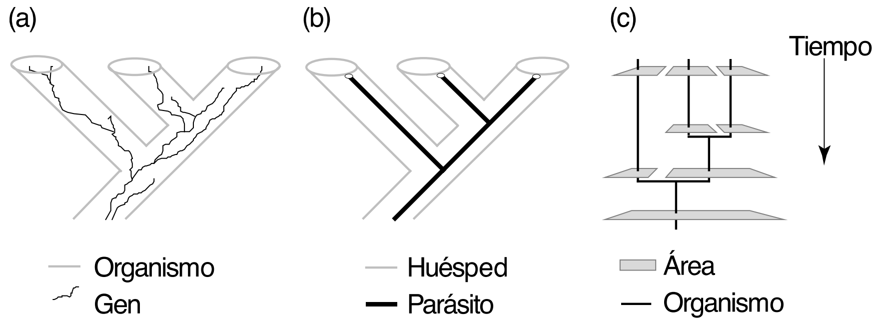

Fig. 1. Asociaciones históricas entre genes, organismos y áreas: (a) un árbol de genes dentro de un árbol de especies, (b) coespeciación entre huésped-parásito y (c) un clado de organismos divergiendo en función de eventos geológicos (vicarianza). En cada esquema, una entidad (el asociado) puede reflejar su estructura en la otra (el huésped). Tomado y modificado de Page & Charleston (1998).

En este contexto, es válido interpretar de manera equitativa la evolución de estas tres asociaciones históricas realizando analogías entre los distintos fenómenos, mecanismos y procesos evolutivos que teóricamente ocurrieron entre ellas; i.e. codivergencia, duplicación, transferencia horizontal y sorteo de linajes (Page, 1994a; Page & Charleston, 1998; Cuadro 1). De este modo, es plausible comparar dos árboles filogenéticos de taxones distintos pero que representan una asociación intrínseca (v. gr., parasitismo), para inferir su historia evolutiva sobre un mismo escenario espacio-temporal capaz de detectar eventos evolutivos que se reflejen en patrones biogeográficos y concordancias filogenéticas (cofilogenéticas) (Page & Holmes, 1998; Charleston, 2011). Estos patrones pueden analizarse sobre las aristas de la teoría coevolutiva (Darwin, 1859; Ehrlich & Raven, 1964; Page, 2003) y, específicamente, reflejarse sobre un *mosaico geográfico* en el que las poblaciones de los asociados (parásitos) difieren de sus características y especializaciones en función a las especies con las que se asocian (huéspedes) (ver Thompson, 1994, 2005).

Cuadro 1. Fenómenos, mecanismos y procesos equivalentes entre diferentes asociaciones históricas. Tomado y modificado de Page & Charleston, 1998.

Asociación histórica	**Codivergencia**	**Duplicación**	**Transferencia horizontal**	**Eventos de sorteo**
Organismo-Gen	Coalescencia interespecífica	Duplicación de genes, sorteo incompleto de linajes	Transferencia de genes	Pérdida de genes, sorteo de linajes
Huésped-Parásito	Coespeciación	Especiación dentro de un mismo huésped	Duplicación de huésped (*host-switch*)	Extinción de parásitos –*missing the boat*–
Áreas-Organismos	Vicarianza	Simpatría	Dispersión	Extinción

Glosario

Asociado. Un linaje que sigue a otro linaje, o un conjunto de entidades históricamente relacionadas.

Codivergencia. Divergencia conjunta tanto del huésped como del asociado.

Duplicación. Divergencia independiente del asociado, con ambos descendientes remanentes asociados con el huésped.

Eventos de sorteo. La ausencia total o aparente de un asociado en los descendientes de un huésped que previamente habían tenido esa asociación.

Transferencia horizontal. La transferencia de un linaje asociado de un huésped ("origen") a otro huésped ("colonizado"), que no es en sí mismo un descendiente inmediato del huésped de origen.

Teoría de la coalescencia. Enfoque matemático que modela la profundidad de árboles de genes de una o varias poblaciones cercanamente relacionadas.

Coalescencia interespecífica. Concordancia topológica para múltiples linajes.

Tiempo de coalescencia. Tiempo para que dos genes homólogos o un conjunto de genes pueda llegar a un ancestro común más reciente (MRCA, por sus siglas en inglés *Most Recent Common Ancestor*).

Sorteo de linajes. Perdida de linajes específicos por cambios debidos a la deriva génica a través del tiempo.

Sorteo incompleto de linajes. Es el fracaso de linajes de haplotipos entre individuos de una misma población para coalescer, permitiendo que al menos uno de los linajes coalezca primero con un linaje de una población menos estrechamente relacionada.

Genealogía de genes. El patrón de similitudes entre secuencias de ADN que contiene información en común acerca de su historia evolutiva.

Transferencia de genes. Proceso en el que un organismo transfiere material genético a otra célula que no es descendiente.

Host switching. Proceso donde el parásito diverge por el cambio o duplicación para colonizar y establecerse en otro linaje de huéspedes.

Missing the boat. Proceso en donde si un asociado se distribuye solo en una parte de la distribución del huésped (por ejemplo, un parásito con distribución irregular), la divergencia dentro de los linajes de huéspedes puede producir descendientes que carezcan de esta asociación. Por tanto, la asociación nunca se extinguió en esos huéspedes sino que nunca estuvo presente.

Los avances en la obtención y métodos de análisis de datos moleculares (secuencias de ADN) han permitido interpretar e integrar la historia evolutiva de los organismos parásitos, misma que es influenciada por las complejas asociaciones que establecen con sus huéspedes (Huelsenbeck *et al*., 2001; Rosenberg & Nordborg, 2002; Morrison, 2006). Por ejemplo, en algunas especies de helmintos parásitos que presentan especificidad se ha registrado variación genealógica codiferenciada por la historia biogeográfica y filogenética de sus huéspedes (Zietara & Lumme, 2002; Plaisance *et al*., 2007; Nieberding *et al*., 2008). Además, recientemente se han propuesto medios de análisis para la reconstrucción biogeográfica usando inferencia filogenética capaz de incorporar de manera directa a la base de datos de análisis la variable *tiempo* a través del uso de árboles de genes, reloj molecular y la relativización de las longitudes de las ramas entre dos árboles filogenéticos de especies (Ree *et al*., 2005; Edwards, 2009; Ronquist & Sanmartin, 2011). En este contexto, es posible poner a prueba hipótesis evolutivas entre asociaciones históricas de tipo huésped-parásito, sobre un espacio geográfico particular a través del tiempo (Sanmartin *et al*., 2008; Crisp *et al*., 2011; Sanmartin, 2012).

En México, un espacio geográfico complejo es la Zona de Transición Mexicana (ZTM) debido a que es una zona de contacto entre dos regiones biogeográficas: Neártica y Neotropical (Luna-Vega *et al*., 2005; Morrone, 2010; Luna-Vega & Contreras-Medina, 2012). La ZTM se ubica en el centro de México, se extiende de costa a costa desde el Océano Pacífico hasta el Golfo de México, entre los paralelos 18º 30-24º 30´ N y los meridianos 98º 30´-105º 00´ W, cubriendo un área de 267 000 km^2 con altitudes que oscilan desde 100-200 m.s.n.m. hasta los 5000 m.s.n.m. (Challenger, 1998; Ferrusquía-Villafranca, 1998; Figura 2). La ZTM es considerada como un área de endemismo para múltiples taxones dulceacuícolas debido a su historia hidrogeomorfologíca compleja. A la fecha se han registrado más de 100 taxones de agua dulce exclusivos de esta área (Miller & Smith, 1986; Huidobro *et al*., 2006; Martínez-Aquino *et al*., 2007a).

Un modelo biológico de asociación histórica para poner a prueba hipótesis de codivergencia en función al escenario geográfico (como por ejemplo la ZTM) y a la historia evolutiva de los huéspedes, puede ser estudiado a través de la denominada fauna principal (*core species*) de helmintos parásitos de peces dulceacuícolas de México (ver Pérez-Ponce de León & Choudhury, 2005), debido a que presenten alta especificidad sobre áreas geográficas restringidas (sistemas hidrológicos epicontinentales), en las que naturalmente no se dispersan por el agua y, por tanto, no tienen flujo génico entre las especies de huéspedes a las que están asociadas o bien, entre los sistemas hidrológicos en los que se distribuyen (Martínez-Aquino *et al*., 2013). Particularmente, la asociación entre *Goodeinae-Margotrema*, grupos biológicos que presumiblemente presentan una estrecha relación evolutiva sobre el centro de México (ver detalles más delante; Pérez-Ponce de León & Choudhury, 2005), es un modelo para poner a prueba hipótesis de codivergencia sobre un escenario geográfico particular.

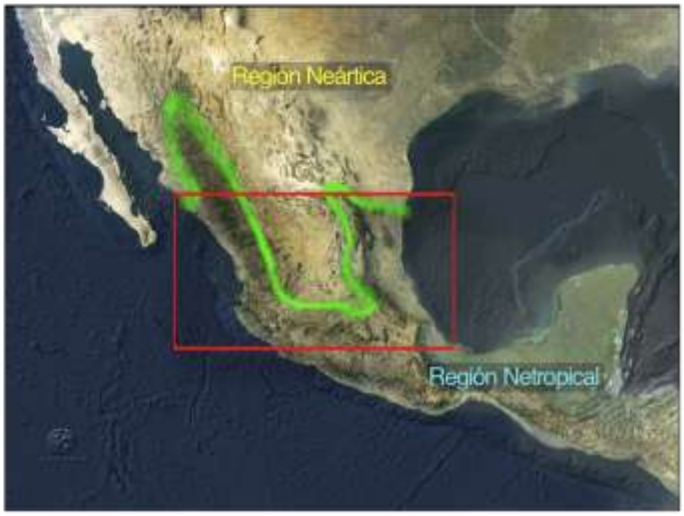

Fig. 2. Panorama fisiogeográfico de México. El rectángulo rojo se refiere al espacio de la Zona de Transición Mexicana sobre el Centro de México. La línea difuminada en verde hace referencia a la delimitación entre dos regiones biogeográficas: Neártica y Neotropical. Mapa tomado de la Comisión Nacional para el Conocimiento y uso de la Biodiversidad (2003).

La subfamilia Goodeinae (Osteichthyes: Cyprinodontiformes: Goodeidae) es un grupo monofilético de peces dulceacuícolas endémico del centro de México (Doadrio & Domínguez-Domínguez, 2004). La subfamilia contiene 41 especies incluídas en cuatro tribus: Girardinichthyini, Chapalichthyini, Ilyodontini y Characodontini; todas presentan un patrón de distribución claramente asociado al escenario geográfico del centro de México (Domínguez-Domínguez *et al*., 2010). Dicho escenario resultado de los cambios hidrogeomorfológicos que modificaron la configuración del área y provocaron procesos de vicarianza, y en algunos casos vicarianza-dispersión, en los distintos linajes de peces (Gesundheit & Macías-García, 2005; Domínguez-Domínguez *et al*., 2006; 2010).

Estudios sobre la helmintofauna de algunas especies de goodeinos (v. gr. Pérez-Ponce de León *et al*., 2000; Martínez-Aquino *et al*., 2004, 2007b, 2009, 2011, 2012; Mejía-Madrid *et al*., 2005), han detectado especies de helmintos parásitos que alcanzan la madurez sexual en los peces y que, al parecer, presentan una estrecha relación evolutiva con sus huéspedes (Pérez-Ponce de León & Choudhury, 2005). Entre otros helmintos, los digéneos del género *Margotrema* Lamothe-Argumedo, 1972 (Digenea: Allocreadiidae) evidencian esta relación.

El género *Margotrema* fue descrito originalmente para incluir a *M. bravoae* Lamothe-Argumedo, 1972 como parásito de *Girardinichthys multiradiatus* en La Lagunilla, Estado de México (Lamothe-Argumedo, 1972). Previo a este trabajo, dos especies de este género fueron descritas: *M. bravoae* y *M. guillerminae* Pérez-Ponce de León, 2001 como parásito de los peces *Notropis calientis* (Cyprinidae) y *Alloophorus robustus* (Goodeinae) en el Lago de Zacapu, Michoacán (Pérez-Ponce de León, 2001). Los caracteres morfológicos diagnósticos que diferenciaron a estas especies fueron la extensión de los ciegos intestinales a lo largo del cuerpo, la distribución de las glándulas vitelógenas y la posición del receptáculo seminal (Pérez-Ponce de León, 2001). Algunos autores cuestionaron la validez taxonómica de *M. guillerminae* sugiriendo que existe una alta variación morfológica en *Margotrema* (Pineda-López *et al*., 2005); sin embargo, no presentaron datos que corroboren tal cuestionamiento, ni plantearon una alternativa metodológica para demostrarlo. Por otra parte, el género *Margotrema* se ha registrado asociado a las cuatro tribus de goodeinos, en 21 especies de peces, y es considerado como restringido para el centro-occidente de México en las cuencas de los ríos Lerma, Santiago, Balsas, Ayuquila y algunos cuerpos de agua del norte de México (Martínez-Aquino *et al*., 2007b, 2011, 2012; Pérez-Ponce de León *et al*., 2007a; 2009, 2013; Aguilar-Aguilar *et al*., 2010). Algunos autores han inferido que *Margotrema* presenta una estrecha relación evolutiva con la de sus huéspedes (Goodeinae), constituyendo parte de su fauna principal (Pérez-Ponce de León & Choudhury, 2005), debido al área de distribución y a la especificidad registrada para peces de esta subfamilia (Mejía-Madrid *et al*., 2005; Pérez-Ponce de León & Choudhury, 2005; Martínez-Aquino *et al*., 2007a, 2007b). Se ha sugerido también que *Margotrema* es un género de afinidad Neártica con base en su posición filogenética y a la historia evolutiva de sus huéspedes (Pérez-Ponce de León *et al*., 2007b; Curran *et al*., 2011). En este sentido, debido a su distribución geográfica fragmentada es posible que exista variación fenotípica interespecífica en *Margotrema* resultado del polimorfismo genético causado por aislamiento geográfico y a la supresión del flujo génico entre sus poblaciones. Además, dicho polimorfismo podría estar relacionado a una asociación específica con los grupos monofiléticos de huéspedes (Tribus) a los que parasitan. De este modo, las poblaciones de *Margotrema* pudieran mostrar un patrón de codistribución y codiferenciación filogenética asociada a la historia evolutiva de las cuatro tribus de goodeinos, reflejado en la historia hidrogeomorfológica del centro de México. En este contexto, analizar la asociación *Goodeinae-Margotrema,* con evidencia de marcadores moleculares, permitirá proponer hipótesis biogeográficas y contrastarlas con hipótesis cofilogenéticas para detectar si existe congruencia o no entre ellas. Así, *Margotrema* es un excelente modelo para analizar la biología evolutiva de parásitos de México sobre tres niveles distintos de asociaciones históricas como se refirió anteriormente: a) Organismo-Gen, genealogía intra e interspecífica de *Margotrema*, b) Área-Organismo, biogeográfica comparada tanto con el escenario geográfico del centro de México como con sus huéspedes y c) Huésped-Parásito, a través del contraste cofilogenético de Goodeinae y *Margotrema* (Page & Charleston, 1998). Por tanto, para realizar estos análisis, en el presente estudio se plantearon las siguientes hipótesis y objetivos (general y particulares)**:**

Hipótesis

1. *Margotrema bravoae* y *M. guillerminae* representan dos especies válidas cuya distinción morfológica está sustentada en su estructura filogenética con base en árboles de genes.

2. La estructura filogenética de *Margotrema* spp. coincide con el patrón de la distribución geográfica de las cuatro tribus de Goodeinae.

3. La diversificación de los goodeinos, resultado de los eventos de vicarianza-dispersión del centro de México, influye de igual modo en la evolución de las poblaciones de *Margotrema* spp.

Objetivo general

Detectar los patrones biogeográficos y cofilogenéticos que influyeron en la asociación histórica de *Goodeinae-Margotrema* en sistemas hidrológicos del centro de México.

Objetivos particulares

1. Establecer la validez taxonómica de las dos especies del género *Margotrema* (*M. bravoae* y *M. guillerminae*), a través de análisis filogenéticos con base en coalescencia, utilizando un marcador molecular mitocondrial (*COX1*) y uno nuclear (*ITS1*).

2. Determinar la estructura genética intra e interpoblacional de *Margotrema* spp.

3. Estimar los tiempos de divergencia filogenética de *Margotrema* spp.

4. Contrastar los tiempos de divergencia filogenética de *Margotrema* spp. con aquellos de los cuatro linajes (tribus) de Goodeinae.

5. Detectar los eventos y procesos biogeográficos espacio-temporales de las poblaciones de *Margotrema* spp. en su área de distribución.

6. Proponer una hipótesis biogeográfico-cofilogenética que explique el patrón de distribución de *Margotrema* spp., en función tanto de los patrones filogenéticos de Goodeinae como de la historia hidrogeomorfológica del centro de México.

Con base en este planteamiento del problema y el modo de abordarlo, se presenta este estudio en tres capítulos. El primero incluye una recopilación de todos los registros helmintológicos para Goodeinae publicados a la fecha, además de algunos que se generaron en este trabajo, con el objetivo de tener un listado lo mas completo posible de todas las localidades donde se han registrado ejemplares de *Margotrema* lo que, en sumatoria, representa su área de distribución actualizada. Los registros efectuados durante el transcurso de este estudio doctoral se presenta en cinco artículos publicados como parte de los primeros resultados derivados de esta investigación. El capítulo dos muestra los resultados filogenéticos de *Margotrema*, el cual representa la primera asociación histórica de tipo Organismo-Gen. El capítulo tres, se muestra en formato de manuscrito para ser sometido para su publicación y, finalmente, este trabajo incorpora una discusión general y conclusiones. Además, con base en el apoyo recibido por el Posgrado en Ciencias Biológicas, UNAM y con la colaboración con distintos grupos de trabajo, se presentan un manuscrito más aceptado para su publicación que se coloca a modo de Apéndice en la parte final del presente trabajo y que se realizó de manera paralela durante este estudio doctoral.

CAPÍTULO I

REGISTROS DE *MARGOTREMA* SPP. PARA PECES DULCEACUÍCOLAS DE MÉXICO: TAXONOMÍA E INVENTARIOS

Para realizar este proyecto el primer objetivo particular fue generar un listado lo mas completo posible de todas las localidades donde se registro *Margotrema* spp., lo que en sumatoria, representa su área de distribución actualizada. Los registros taxonómicos de *Margotrema* spp. aportados durante el transcurso de este proyecto doctoral se presentan en cinco artículos publicados formalmente, como parte de los primeros resultados derivados de esta investigación. Para ello, realizamos muestreos propios que permitieron visitar 30 localidades en donde fueron registrados individuos de *Margotrema* como parásitos asociados a 22 especies de Goodeinae, y en cuatro y una especie más de peces dulceacuícolas de las familias Cyprinidae y Cyprinodontidae, respectivamente. Además, se visitaron 27 localidades nuevas para explorar la posibilidad de recolectar y determinar el área de distribución actual de *Margotrema*. Por último, como resultado de esta exploración de campo se presenta un manuscrito (pág. 43) que será sometido para su publicación en una revista indizada referente al inventario helmintológico más actualizado para Goodeinae – incluyendo el área de distribución de *Margotrema* – el cual representa el grupo de vertebrados mejor estudiado para México en términos de su helmintofauna.

A continuación se presenta una versión de cada documento en orden según la secuencia cronológica en la que cada artículo fué publicado y, por último, un manuscrito en formato de articulo en extenso el cuál será sometido para su publicación en una revista arbitrada e indizada (ISI / SCI).

1) **Helminth parasites of *Xenotaenia resolanae* (Osteichthyes: Cyprinodontiformes: Goodeidae) from the Cuzalapa hydrological system, Jalisco, Mexico** (Martínez-Aquino *et al*., 2009).
2) **Helminth fauna of two cyprinid fish (*Campostoma ornatum* and *Codoma ornata*) from the upper Piaxtla River, Northwestern Mexico** (Aguilar-Aguilar *et al*., 2010).
3) **Endohelminth parasites of the freshwater fish *Zoogoneticus purhepechus* (Cyprinodontiformes: Goodeidae) from two springs in the Lower Lerma River, Mexico** (Martínez-Aquino *et al*., 2011).
4) **Endohelminth parasites of seven goodein species (Cyprinodontiformes: Goodeidae) from Lake Zacapu, Michoacán, Central Mexico Plateau** (Martínez-Aquino *et al*., 2012).

5) **A new species of *Margotrema* (Digenea, Allocreadiidae) from the leopard splitfin *Xenotaenia resolanae* (Cyprinodontiformes, Goodeidae) from west-central Mexico** (Pérez-Ponce de León *et al*., 2013).
6) **Composición taxonómica de helmintos parásitos de Goodeinae (Osteichthys: Cyprinodontiformes: Goodeidae) en México**.

1) HELMINTH PARASITES OF *XEONOTAENIA RESOLANAE* (OSTEICHTHYES: CYPRINODONTIFORMES: GOODEIDAE) FROM THE CUZALAPA HYDROLOGICAL SYSTEM, JALISCO, MEXICO

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Helminth Parasites of *Xenotaenia resolanae* (Osteichthyes: Cyprinodontiformes: Goodeidae) From the Cuzalapa Hydrological System, Jalisco, Mexico

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ABSTRACT: Six helminth species were recorded during the helminthological examination of 35 specimens of the goodeid *Xenotaenia resolanae* from Arroyo Durazno, Jalisco, Mexico, a tributary of the Cuzalapa River. Helminth species identified included: 4 species of digeneans, i.e., *Posthodiplostomum minimum* (metacercariae), *Clinostomum companatum* (metacercariae), *Dendrorchis* sp. (adult), and *Margotrema guillerminae* (adult); and 2 species of nematodes, i.e., *Spiroxys* sp. (larvae) and *Rhabdochona ahuehuellensis* (adult). A very low number of individual larvae were found. The observed species richness, individual parasite abundance, and diversity were low at both component community and infracommunity levels. The values of similarity between infracommunities were relatively high because of the predominance of the digenean *M. guillerminae*, the species that reached the higher values of both prevalence and abundance. High water flow of the collecting site is suggested as the main factor determining the depauperate helminth assemblage in this fish species.

Xenotaenia resolanae Turner 1946, (Cyprinodontiformes: Goodeidae) ranges in small rivers in Central Mexico's Pacific Slope (Domínguez-Domínguez et al., 2005). It is not recorded as an endangered species (Froese and Pauly, 2008), even though some authors have suggested that this fish species is very susceptible to environmental degradation (Lyons and Navarro-Pérez, 1990) and should be regarded as vulnerable (Domínguez-Domínguez et al., 2005). The helminth fauna of this freshwater fish is poorly known. The only published record for *X. resolanae* includes the intestinal nematode *Rhabdochona ahuehuellensis* (Mejía-Madrid et al., 2005). However, this record was established for the Rio Tecolote, Jalisco, a locality different from the one examined in the present study. The aim of this research note is to present the helminthological record of *X. resolanae* from Arroyo Durazno, a tributary of the Cuzalapa River which drains into the Marabasco River Basin in the state of Jalisco, Mexico; to describe the helminth infracommunity structure; and to briefly discuss the possible causes that determine that structure.

On August 2008, 35 specimens of *X. resolanae* were collected by electrofishing from Arroyo Durazno (19°30′32.1″N, 104°17′45.6″W), which is a small affluent of the Cuzalapa River, a tributary of the Marabasco River Basin in Jalisco State, southwestern Mexico. Hosts were taken alive to the laboratory, pithed, and examined individually for intestinal helminths. Other organs (gills, liver, gall, swim and urinary bladders, and spleen) were examined using a stereomicroscope in separate Petri dishes with 0.65% saline. Worms were fixed with 4% hot (steaming) formalin. Platyhelminths were stained with Mayer's paracarmine. Nematodes were cleared with glycerin for light microscopy and stored in 70% ethanol. Voucher specimens of all taxa were deposited in the Colección Nacional de Helmintos (CNHE), Instituto de Biología, Universidad Nacional Autónoma de México, Mexico City. Use of prevalence (% infected), mean intensity (mean number of parasites per infected fish), and abundance (mean number of parasites of a single species per analyzed host) follows Bush et al. (1997). Helminth species were classified from an ecological point of view as dominant (high prevalence and abundance) and rare (low prevalence and abundance) after an Olmstead-Tukey corner test of association (Steel and Torrie, 1981). Infracommunities were described by the mean number of parasite species, the mean number of individual helminths, and the mean value of the Brillouin diversity index that is commonly used in parasitological studies for fully censured communities. To demonstrate numerical dominance, the Berger-Parker index was used (the closer the value to 1.00, the greater the dominance). Similarity among infracommunities was estimated by using 2 indices: the Jaccard index and the Morisita-Horn index, for qualitative and quantitative similarity, respectively, as calculated in Magurran (1988).

A total of 154 individual helminths was collected. Six helminth species were recovered, 4 digeneans and 2 nematodes (Table I). Three species were recovered as larval stages: *Clinostomum complanatum*, *Posthodiplostomum minimum*, and *Spiroxys* sp.; the remaining 3 helminth species were adults. The digenean *Margotrema guillerminae* was the most abundant species, accounting for about 85% of the collected worms, followed by *R. ahuehuellensis*, which accounted for 10.4%. The digenean *Dendrorchis* sp. was the least numerous, with a prevalence of 5.7% and a mean intensity of 1.5 worms per infected host. This digenean was not identified to species level because it appears to represent an undescribed species. Additional samples are necessary to present a proper description. Infection site, number of infected fish, prevalence, abundance, and mean intensity of each helminth species are shown in Table I.

Thirty of the 35 individual hosts were infected with at least 1 species of helminth. Eight of these harbored 2 or more helminth species. The total number of individual helminths of all species per host varied from 1 to 24, with a mean number of 4.4 ± 5.4 individuals per host. The Olmstead-Tukey test showed that only the intestinal digenean *M. guillerminae* was frequent (prevalence >80%) and abundant (abundance >3.7 worms per analyzed host). The other intestinal species, i.e., *R. ahuhuellensis*, exhibited a relatively high prevalence, but it was not abundant, while the *Dendrorchis* sp. was actually a rare species. The remaining species exhibited low prevalence and abundance values (Table I) and were also considered as rare.

The helminth infracommunities were species-poor. Most infracommunities were composed of a single species, 7 of them had 2 helminth species, and only 1 had the maximum of 4 species. The mean number of species per host was 1.1 ± 0.8. Five of the 7 infracommunities with 2 or more species were composed of the intestinal adult worms *M. guillerminae* and *R. ahuehuellensis*. The Brillouin index for all infracommunities varied from 0 to 0.915, with a mean diversity value of 0.113 ± 0.218, while the Berger-Parker dominance index values varied from 0.444 to 1, with a mean of 0.918 ± 0.16. For comparative purposes, we calculated the Brillouin index for the 8 infracommunities with at least 2 species, which varied from 0.23 to 0.915 with a mean diversity value of 0.425 ± 0.215; the Berger-Parker dominance index values varied from 0.444 to 0.900 with a mean of 0.693 ± 0.167. Most of these assemblages were dominated by the digenean *M. guillerminae*. The helminth infracommunities showed a relatively high level of similarity. The corresponding Jaccard index varied from 0 to 1 (mean of 0.654 ± 0.353), and the Morisita-Horn index varied from 0 to 1 (mean of 0.765 ± 0.342).

Three of the 6 helminth species parasitizing *X. resolanae* were larval stages. However, even though they have been previously reported parasitizing other goodeids in Central and Northern Mexico (Peresbarbosa-Rojas et al., 1994; Martínez-Aquino et al., 2004; Mejía-Madrid et al., 2005; Martínez-Aquino et al., 2007), their prevalence and abundance were very low. The remaining 3 taxa have also been reported for other goodeid species and are members of the core endohelminth fauna of this fish family (Mejía-Madrid et al., 2005), particularly *M. guillerminae* and *R. ahuehuellensis*, while congeneric species of *Dendrorchis* have been recorded as parasites of characids in the Neotropical region (Volonterio and Ponce de León, 2005). The record of 3 adult endohelminth species in *X. resolanae*, along with the finding of *Salsuginus* (monogeneans) on the gills (Martínez-Aquino, 2005), conform a particular parasite assemblage where adult native species are predominant. The helminth parasite species composition herein reported for *X. resolanae* is similar to that found in other goodeid fishes in central and northern Mexico (Peresbarbosa-Rojas et al., 1994; Pérez-Ponce de León et al., 2000; Martínez-Aquino et al., 2004; Sánchez-Nava et al., 2004; Mejía-Madrid et al., 2005; Salgado-Maldonado, 2006; Martínez-Aquino et al., 2007; Romero-Tejeda et al., 2008). However, the prevalence and abundance of individual larval forms is very low. This can be explained as a result of the physical conditions of the locality where fishes were collected, particularly water flow. The brook where we conducted our sampling belongs to a small, exoreic river basin

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TABLE I. Helminth parasites of *Xenotaenia resolanae* (n = 35) in Arroyo Durazno, Jalisco, Mexico.

Helminth	Infection site(s)*	Number of infected hosts	Prevalence (%)	Abundance	Mean intensity ± SD	CNHE catalog no.
Adult Digenea						
Margotrema guillerminae	I	28	80	3.74	4.68 ± 5.6	6880
Dendrorchis sp.	U	2	5.71	0.09	1.5 ± 0.71	6881
Larval Digenea						
Clinostomum complanatum	Bc	1	2.86	0.03	1	6882
Posthodiplostomum minimum	M	1	2.86	0.03	1	6883
Adult Nematoda						
Rhabdochona ahuehuellensis	I	7	20	0.46	2.29 ± 1.49	6884
Larval Nematoda						
Spiroxys sp.	M	1	2.86	0.06	2	6885

* I, intestine; U, urinary bladder; Bc, body cavity; M, mesentery.

where water flows very rapidly and, apparently, there are no piscivorous birds nesting in the area because no aquatic vegetation can be established under this permanent water flow and, as a result, the presence of these birds (definitive hosts for several larvae) is not common. This condition is different than that found in endorreic springs, and even in lakes, where most of the goodeid species occur and where a larger number of allogenic parasite species, usually exhibiting high abundance levels, are dispersed by fish-eating reptiles and birds that feed upon goodeids (see Pérez-Ponce de León et al., 2000; Martínez-Aquino et al., 2004, 2007; Martínez-Aquino and Aguilar-Aguilar, 2008; Romero-Tejada et al., 2008).

The data we present here suggest that the helminth parasite species composition and the helminth infracommunity structure in *X. resolanae* are slightly different than those found in freshwater fishes occurring in other water bodies of central and northern Mexico. Our data may indicate that physical conditions in the locality, particularly rapid water flow, could be responsible for determining the helminth community structure. Likewise, the helminth assemblage is consistent with the pattern that shows a depauperate parasite fauna in freshwater fishes inhabiting epicontinental waters in the Nearctic part of Mexico (Espinosa-Huerta et al., 1996; Rojas et al., 1997; Choudhury and Dick, 2000; Pérez-Ponce de León et al., 2000; Martínez-Aquino et al., 2004; Sánchez-Nava et al., 2004; Martínez-Aquino et al., 2007; Martínez-Aquino and Aguilar-Aguilar, 2008; Romero-Tejeda et al., 2008). Another characteristic shared among the helminth community of *X. resolanae* and helminth communities of other freshwater fishes occurring in central and northern Mexico is the numerical dominance exacted by a single species of parasite; in this case, the digenean *M. guillerminae* is the dominant species. Furthermore, the helminth community of *X. resolanae* exhibits low species richness values at both the component community and infracommunity levels; this has been reported for other goodeid fishes in the Nearctic part of Mexico such as *Alloophorus robustus*, *Goodea atripinnis*, *Allotoca diazi*, *Chapalichthys encaustus*, *Characodon audax*, *Skiffia lermae*, *Girardinychthys multiradiatus*, *Xenotoca variata*, and *Zoogoneticus quitzeoensis* (Astudillo-Ramos and Soto-Galera, 1997; Rojas et al., 1997; Pérez-Ponce de León et al., 2000; Sánchez-Nava et al., 2004; Martínez-Aquino et al., 2004, 2007; Romero-Tejeda et al., 2008).

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2) HELMINTH FAUNA OF TWO CYPRINID FISH (*CAMPOSTOMA ORNATUM* AND *CODOMA ORNATA*) FROM THE UPPER PIAXTLA RIVER, NORTHWESTERN MEXICO

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Helminth fauna of two cyprinid fish (*Campostoma ornatum* and *Codoma ornata*) from the upper Piaxtla River, Northwestern Mexico

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Summary

The helminth parasite fauna of 2 species of freshwater fishes from the upper Piaxtla River in northwestern Mexico was studied. A total of 41 cyprinids, corresponding to 20 *Campostoma ornatum* and 21 *Codoma ornata* were analyzed. Six species of platyhelminths were recorded, including 2 species of monogeneans (*Gyrodactylus* sp. and *Dactylogyrus* sp.), 3 species of digeneans (*Posthodiplostomum minimum*, *Clinostomum complanatum*, and *Margotrema* sp.), and 1 species of tapeworm (*Bothriocephalus acheilognathi*). Helminth parasite infracommunities were depauperate, showed low richness and diversity values, and were dominated by 1 or 2 helminth species. This pattern is consistent with that observed for the helminth parasite communities in other freshwater fishes in central and northern Mexico.

Keywords: Platyhelminthes; Digenea; Monogenea; Cestoda; parasite communities; Cyprinidae; Mexico

Introduction

The freshwater fish species *Campostoma ornatum* Girard, 1856 and *Codoma ornata* Girard 1856 (Cypriniformes: Cyprinidae) have a widespread distribution in northern Mexico (Espinosa-Pérez *et al*., 1993; Miller *et al*., 2005; Froese & Pauly, 2009). Both species are relatively common among the freshwater fish fauna of the upper Piaxtla River, which flows from the highlands of the Sierra Madre Occidental to the Pacific Ocean in northwestern Mexico. The helminth fauna of these cyprinids along its distributional range is poorly known. The only available records were recently published by Pérez-Ponce de León *et al*. (2009, 2010) from the upper-Mezquital and the Nazas river basins, respectively. The main objectives of this work are to record the helminth parasite fauna of both species of cyprinids in the upper Piaxtla River by presenting the list of species, and to describe the helminth parasite community structure of each host species through the species richness and diversity atributtes. We briefly compare our findings with data reported by Pérez-Ponce de León *et al*. (2009, 2010) for the same fish species in the Nazas and upper-Mezquital river basins.

Materials and methods

In December, 2008, 20 specimens of *Campostoma ornatum* and 21 of *Codoma ornata* were collected by electrofishing in the upper Piaxtla River (24º 21' 59" N, 105º 31' 7.8" W, altitude 2391 m), located at Municipio San Dimas, Durango State, Northern Mexico. Hosts were taken alive to the laboratory. Once there, hosts were pithed, and examined individually for helminths. Gills and fins were separated in Petri dishes with tap water, and then examined under a stereomicroscope. Other organs (intestine, liver, gall, swim and urinary bladders, and spleen) were examined in separate Petri dishes with 0.65% saline. Platyhelminths were fixed with 4% hot (steaming) formalin, stained with Mayer's paracarmine and mounted as permanent slides in Canada balsam. Several individual monogeneans were fixed in glycerin ammonium-picrate (GAP), to study their sclerotized structures. Voucher specimens of all taxa were deposited in the Colección Nacional de Helmintos (CNHE), Instituto de Biología, Universidad Nacional Autónoma de México, Mexico City (see Table 1). Prevalence (% of infected hosts in a sample), and abundance (mean number of parasites of a single species in the sample) follows definitions by Bush *et al*. (1997). To determine if sample size was sufficient to produce an accurate estimate of the pool of parasites using both host species in the sampled locality, an accumulation species curve and the species richness estimators Chao 1 and Chao 2 were used for each component community. Helminth species were classified from an ecological point of view as dominant (high prevalence and abundance) and rare (low prevalence and abundance) after an Olmstead-Tukey corner test of association (Steel and Torrie, 1981). Infracommunities include all the helminth species in an individual host, and were described by using the mean number of parasite species per host, the mean number of individual helminths, and the mean value of the Brillouin diversity index. Infracommunities were compared qualitatively within the locality using the Jaccard similarity index, and quantitatively using the Morisita-Horn index, as calculated in Magurran (1988).

Results

A total of 148 individual helminths belonging to four species were collected from *Campostoma ornatum*. Helminth species recovered include a fins monogenean *Gyrodactylus* sp., the metacercariae of the digeneans *Posthodiplostomum minimum* from the mesentery and *Clinostomum complanatum* from the body cavity, and the adults of an introduced cestode *Bothriocephalus acheilognathi* from the intestine. The Olmstead-Tukey test for this fish species showed that the monogenean *Gyrodactylus* sp and the metacercariae of *P. minimum* were most frequent (prevalence 95 and 85 %, respectively) and abundant (abundance from 3.1 to 3.25 helminths per analyzed host), while *C. complanatum* and *B. acheilognathi* reached low values for these parameters. Infection site, number of infected fish, prevalence, and abundance for each helminth species are shown in Table 1.

In contrast, a total of 3292 individual helminths belonging to five species were collected from *Codoma ornata*. Helminth species recovered include a gills monogenean of the genus *Dactylogyrus* sp., the adult digenean *Margotrema* sp. from the intestine, the metacercariae of *Posthodiplostomum minimum* and *Clinostomum complanatum*, and the cestode *Bothriocephalus acheilognathi*. The Olmstead-Tukey test showed that only the metacercariae of *P. minimum* was most frequent (prevalence 100 %) and abundant (143.29 worms per analyzed host). The monogenean *Dactylogyrus* sp. and the metacercariae of *C. complanatum* exhibited a high prevalence (100 % and 81%, respectively), but they were not abundant, while *Margotrema* sp. and *B. acheilognathi* showed low prevalence and abundance values (Table 1), and were considered as rare. Infection site, number of infected fish, prevalence, and abundance for each helminth species are shown in Table 1.

In *Campostoma ornatum*, most of the parasite infracommunities (85 %) harbored 2 or more helminth species. In terms of abundance, the total number of individual helminths of all species per analyzed host varied from 1 to 15, with a mean number of 7 ± 3.47. Similarly, parasite infracommunities in *Codoma ornata* harbored 2 or more helminth species, however, the presence of gill monogeneans in 5 hosts was not determined because fish died several hours before examination. For this reason, values of prevalence and abundance for those particular helminth taxa were calculated only for 16 individual hosts (Table 1). The number of helminths of all species per analyzed host was much higher, and varied from 3 to 365, with a mean number of 157 ± 92.39.

Even though, individual hosts of both fish species were infected at least with 1 helminth species, the helminth parasite infracommunities in both host species were relatively species-poor. Most of the infracommunities found in *Campostoma ornatum* (55 %), were composed by three species, and only 2 of them reached a maximum of four species. In terms of species richness, the mean number of species per host was 3 ± 0.88. The Brillouin index for the infracommunities varied from 0 to 0.976, with a mean diversity value of 0.537 ± 0.261, while the Berger-Parker dominance index values varied from 0.333 to 1, with a mean of 0.624 ± 0.194. The helminth infracommunities in *C. ornatum* showed a relatively high level of similarity; the corresponding Jaccard index varied from 0 to 1 (mean of 0.59 ± 0.235) while the Morisita-Horn index varied from 0 to 1 (mean of 0.729 ± 0.215).

In the case of *Codoma ornata*, 42.85% of the infracommunities were also composed by 3 species, and only 2 had a maximum of 5 species. The mean number of species per host was 3.09 ± 0.94. The Brillouin index for the infracommunities varied from 0.043 to 0.571, with a mean diversity value of 0.299 ± 0.163, while the Berger-Parker dominance index values varied from 0.667 to 0.991, with a mean of 0.902 ± 0.078. The helminth infracommunities showed a comparatively higher level of similarity; the corresponding Jaccard index varied from 0.2 to 1 (mean of 0.629 ± 0.224) and the Morisita-Horn index varied from 0.43 to 1 (mean of 0.943 ± 0.15).

Discussion

Helminth taxa

The helminth parasite fauna of the cyprinids *Campostoma ornatum* and *Codoma ornata* in the upper Piaxtla River includes 6 helminth species, 2 monogeneans, 3 digeneans and 1 cestode. The taxonomic composition of the helminth parasite fauna of *Campostoma ornatum* comprises 3 generalist species, which are widely distributed among freshwater fishes in several localities in Mexico (Pérez-Ponce de León *et al.*, 2007; Rojas-Sánchez & García-Prieto, 2008). Species of the monogenean *Gyrodactylus* sp., collected from the fins of their hosts, have been recently recorded from diverse freshwater fishes in central and northern Mexico, where apparently a high species richness of this parasite group is found (Mendoza-Palmero, 2007; Mendoza-Palmero *et al.*, 2009; Pérez-Ponce de León *et al.*, 2010); further studies will allow us to establish the taxonomic identity and potential host specificity of *Gyrodactylus* species occurring in Mexican freshwater fishes. The specimens we collected might be conspecific with an un-

Table 1. Helminth parasites of *Campostoma ornatum* and *Codoma ornata* in the upper Piaxtla River, Durango, Mexico

		Campostoma ornatum (n = 20)				*Codoma ornata* (n = 21)			
Helminth taxa	Infection site	Infected Hosts (n)	Prevalence (%)	Abundance ± SD	CNHE No.	Infected Hosts (n)	Prevalence (%)	Abundance ± SD	CNHE No.
Monogenea									
Gyrodactylus sp.	Fins	19	95	3.1 ± 1.85	7465	-	-	-	-
Dactylogyrus sp. *	Gills	-	-	-	-	16	100	11.2 ± 9.69	7466
Digenea									
Margotrema sp.	Intestine	-	-	-	-	6	28.57	0.33 ± 0.58	7473
Posthodiplostomum minimum	Mesentery	17	85	3.25 ± 2.47	7467	21	100	143.29 ± 85.8	7468
Clinostomum complanatum	Body cavity	8	40	0.45 ± 0.6	7469	17	80.95	4.33 ± 4.49	7470
Cestoda									
Bothriocephalus acheilognathi	Intestine	8	40	0.5 ± 0.76	7471	5	23.81	0.29 ± 0.58	7472

* Values based on16 host

described species, *Gyrodactylus* sp. 4, reported by Pérez-Ponce de León *et al*. (2010) as a parasite of the fins of *Campostoma ornatum* in the Nazas River Basin, however, this species has not yet been described.

Furthermore, the helminth parasite fauna of *Codoma ornata* includes 3 species that were also found in *Campostoma ornatum*; in addition, the adult digenean *Margotrema* sp. and the monogenean *Dactylogyrus* sp. were also found. Currently, 2 species of *Margotrema* spp., an allegedly parasite of goodeid fishes, have been described in freshwater fishes from central Mexico (Pérez-Ponce de León *et al*., 2007). The distinctive morphological character that distinguishes these species is the extent of the ceca. Unfortunately, the specimens we collected from cyprinids in the Piaxtla River had the uteri full of eggs, impeding the observation of the extent of the ceca. Monogeneans of the genus *Dactylogyrus* have also been found parasitizing freshwater fishes in central and northern Mexico (Mendoza-Palmero, 2007; Pérez-Ponce de León *et al*., 2010). In particular, in cyprinids from a nearby river basin (the Nazas River), 6 species of *Dactylogyrus* have been reported (Pérez-Ponce de León *et al*., 2010), 3 of them common parasites of introduced species of cyprinids, and 1 typically found in cichlids. Other 2 species from cyprinids were considered to represent a new species for which no description has been made. As in the case of the fin monogenean, *Gyrodactylus* sp., we may claim conspecificity to some of these undescribed species, however, we preferred to take a conservative position pending proper description of the undescribed species.

Interestingly, the taxonomic composition of the helminth parasite fauna herein reported for both cyprinid species included only platyhelminthes. No nematodes or acanthocephalans were found in present study. This might be due to a relatively small sample size, even though 20 individual hosts are considered a sufficient sample size to detect parasite species richness in a particular locality, and, in addition to that, there are no missing species according with the information provided by the accumulation species curves and the non-parametric species richness estimators Chao 1 and Chao 2. The absence of such parasite groups contrasts with findings we recently made describing the helminth parasite fauna in of freshwater fishes from the Nazas and the upper-Mezquital river basins, including *Campostoma ornatum* (Pérez-Ponce de León *et al*., 2009, 2010). These river basins are located at relatively short distance from the upper Piaxtla River, even though the former runs westwards from high elevation areas of Durango State, through Nayarit State, to the Pacific coast, while the Nazas River represents an endhorreic basin that runs from the Sierra Madre Occidental eastward, through the states of Durango and Coahuila, into the now-dry Laguna del Mayran (Castañeda-Gaytán *et al*., 2005; Návar *et al*., 2006). In the upper-Mezquital River only 4 individuals of *Campostoma ornatum* were sampled, and 2 helminth species were recorded, the cestode *Bothriocephalus acheilognathi*, and the nematode *Rhabdochona canadensis*. We also sampled 23 individuals of *Codoma ornata* and only the tapeworm *B. acheilognathi* was found (see Pérez-Ponce de León *et al*., 2009).

In contrast, in the Nazas River a much larger sample size was analyzed for both cyprinid species (Pérez-Ponce de León *et al*., 2010). Eighty individuals of *Campostoma ornatum* were studied, and the helminth species recorded were: the digeneans *P. minimum* and *Uvulifer* sp., the cestode *B. acheilognathi*, the nematodes *Spiroxys* sp., and *Rhabdochona canadensis* and two species of monogeneans, an undescribed species of *Gyrodactylus* (recorded as *Gyrodactylus* sp. 4) and *Urocleidoides strombicircus*. In 101 analyzed specimens of *Codoma ornata* only 3 helminth species were recorded, the digenean *P. minimum*, the cestode *B. acheilognathi*, and the nematode *R. canadensis* (Pérez-Ponce de León *et al*., 2010). The absence of *R. canadensis* in cyprinids from the Piaxtla River is noteworthy, since this is a common parasite of cyprinids in North America (see Hoffman, 1999). Likewise, the larval digeneans *P. minimum* and *C. complanatum* are commonly found parasitizing freshwater fishes in México (Pérez-Ponce de León *et al*., 2007), while the species of the genus *Margotrema* are allegedly to be members of the helminth parasite core fauna of goodeid fishes in central Mexico (Pérez-Ponce de León & Choudhury, 2005; Mejía-Madrid *et al*., 2005). The adult cestode *B. acheilognathi* is an autogenic and generalist species whose life cycle is completed in the aquatic ecosystem (Hoffman, 1999). This species was introduced into Mexico along with its hosts, grass carps, from Asia. It possesses a large dispersal capability and as a result, it is now found not only in introduced hosts but also in the native freshwater fish fauna (Rojas-Sánchez and García-Prieto, 2008). No carps (*Cyprinus carpio, Ctenopharyngodon idella*), which are commonly used for aquaculture purposes and are disseminated to natural environments on regular basis, were found during our samplings in the Piaxtla River. It could be possible that the endemic cyprinids we studied became infected with the Asian tapeworm through an ecological host extension from other host species, such as poeciliids, but still this needs to be determined with further samplings in the locality.

Helminth communities

Parasite communities of both species of cyprinids are depauperate and are dominated by one helminth species; this, and the fact that a low species richness values at both, the component community and infracommunity levels, were detected, is consistent with the parasite community structure in a diverse array of freshwater fishes inhabiting in epicontinental waters in central and northern Mexico, corresponding with the Nearctic region (Peresbarbosa-Rojas *et al*., 1994; Espinosa-Huerta *et al*., 1996; Rojas *et al*., 1997; Pérez-Ponce de León *et al*., 2000; Martínez-Aquino *et al*., 2004, 2007; Martínez-Aquino & Aguilar-Aguilar, 2008; Romero-Tejeda *et al*., 2008). Helminth parasite communities in the 2 cyprinids we studied herein also show a numerical dominance by a single or a few helminth species. In the case of *Codoma ornata*, dominance is mainly exerted by the metacercariae of *Posthodiplostomum minimum*, which shows a high abundance. In *Campostoma ornatum*, two helminth species were dominant, the digenean *P. minimum* and the monogenean *Gyrodactylus* sp.

Based on the results of the species accumulation curves and the species richness estimators, we are confident about the accuracy of species richness and community structure patterns herein described. Both methods were used as indicators of species richness as a function of sample size, even though it is well-known that addressing sample size is a common problem when dealing with prevalence data (see Jovani & Tella, 2006); likewise, our analysis corroborated that the number of analyzed hosts represents a sufficient sample size to recover most members of the parasite community. Since the number of hosts we analyzed in this study fall within the category of "medium sample size" as proposed by Jovani and Tella (2006), it is possible that the prevalence of infection for each parasite species might have been affected, but not the species richness. We are certain that, by gathering a larger dataset on different helminth parasite communities in freshwater fishes, we will be able to establish a correlation between the sample size and the parasite fauna. As a comparison, the 80 specimens of *Campostoma ornatum* and 101 of *Codoma ornata* that were studied in the Nazas River basin by Pérez-Ponce de León *et al*. (2010), contained 7 (i.e., *Uvulifer* sp., *Posthodiplostomum minimum*,, *Gyrodactylus* sp. 4, *Urocleidoides strombicircus*, *Bothriocephalus acheilognathi*, *Rhabdochona canadensis*, and *Spiroxys* sp.) and 3 helminth species (i.e., *P. minimum*, *B. acheilognathi* and *R. canadensis*, respectively. The data we provide in the present paper represents an additional piece of information on the parasite species composition, and patterns of community structure of the helminth parasites of freshwater fishes in Mexico, particularly from lotic environments of the northwestern region. Still, more data needs to be gathered on these species of cyprinids along its distributional range, as well as on other freshwater fish species, in order to describe general patterns of host-parasite associations, and to fully understand the processes that shaped the historical biogeography and community structure of the freshwater fish parasite fauna in Mexico.

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3) ENDOHELMINTH PARASITES OF THE FRESHWATER FISH *ZOOGONETICUS PURHEPECHUS* (CYPRINODONTIFORMES: GOODEIDAE) FROM TWO SPRINGS IN THE LOWER LERMA RIVER, MEXICO

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Endohelminth parasites of the freshwater fish *Zoogoneticus purhepechus* (Cyprinodontiformes: Goodeidae) from two springs in the Lower Lerma River, Mexico

Endohelmintos parásitos del pez dulceacuícola *Zoogoneticus purhepechus* (Cyprinodontiformes: Goodeidae) en dos manantiales de la cuenca del río Lerma bajo, México

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Abstract. In order to establish the helminthological record of the viviparous fish species *Zoogoneticus purhepechus*, 72 individuals were collected from 2 localities, La Luz spring (n= 45) and Los Negritos spring (n= 27), both in the lower Lerma River, in Michoacán state, Mexico. Twelve helminth taxa were recovered, 5 adults (the digeneans *Margotrema bravoae* and *Phyllodistomum* sp., the cestode *Bothriocephalus acheilognathi*, the nematode *Rhabdochona lichtenfelsi* and the acanthocephalan *Pomphorhynchus* cf. *bulbocolli*), and 7 larvae (the metacercariae of *Clinostomum complanatum*, the cysticercoid of Cyclophyllidea, the nematodes *Rhabdochona* sp., *Eustrongylides* sp., *Contracaecum* sp. and *Spiroxys* sp., and the cysthacanth of *Polymorphus brevis*). Of these, R. lichtenfelsi was the most prevalent and abundant species at La Luz spring with 15.6% and 0.33 individuals per analyzed host. The remaining species were relatively more rare and infrequent. The helminth parasite community of *Z. purhepechus* at Los Negritos spring was remarkably poor and abundance was very low. The omnivorous feeding habits, the position of the host species in the food web, and the environmental characteristics of each locality are suggested as the main factors determining the helminth parasite communities in this freshwater fish.

Key words: Goodeidae, *Zoogoneticus purhepechus,* helminth parasites, community structure, Mexico.

Resumen. Se examinaron 72 individuos del pez vivíparo *Zoogoneticus purhepechus* para establecer el registro helmintológico de la especie. Los huéspedes se recolectaron de los manantiales La Luz (n= 45) y Los Negritos (n= 27), ubicados en la porción baja del río Lerma, en el estado de Michoacán, México. El registro helmintológico consta de 12 especies, incluyendo como adultos los digéneos *Margotrema bravoae* y *Phyllodistomum* sp., el céstodo *Bothriocephalus acheilognathi*, el nemátodo *Rhabdochona lichtenfelsi*, y el acantocéfalo *Pomphorhynchus* cf. *bulbocolli*. Además, como estadios larvarios, se encontraron las metacercarias de *Clinostomum complanatum,* el cisticercoide de Cyclophyllidea, los nemátodos *Rhabdochona* sp., *Contracaecum* sp., *Eustrongylides* sp. y *Spiroxys* sp., y el cistacanto de *Polymorphus brevis*. De éstas, *R. lichtenfelsi* fue la especie más frecuente y abundante en el manantial La Luz, en tanto que las restantes fueron relativamente más raras. La comunidad de helmintos de *Z. purhepechus* en el manantial Los Negritos fue pobre y poco abundante. Se sugiere que los principales factores que determinan la estructura de la comunidad de helmintos son los hábitos alimentarios omnívoros de los huéspedes, la posición que éstos ocupan en la red trófica y las características ambientales de cada localidad.

Palabras clave: Goodeidae, *Zoogoneticus purhepechus***,** helmintos parásitos, estructura de la comunidad, México.

Introduction

The genus *Zoogoneticus* Meek (Cyprinodontiformes: Goodeidae) includes 3 species of livebearing freshwater fishes, *Z. quitzeoensis* Bean, *Z. tequila* Webb and Miller, and the recently described *Z. purhepechus* Domínguez-Domínguez, Pérez-Rodríguez and Doadrio. These fish species are restricted to particular river drainages in central Mexico (Domínguez-Domínguez et al., 2008) and are considered as endangered or critically endangered (De la Vega-Salazar et al., 2003; Domínguez-Domínguez

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et al., 2005, 2008). The helminth fauna of livebearing freshwater fishes of the family Goodeidae has been well documented and the depauperate nature of helminth parasite communities in these fishes has been suggested (Astudillo-Ramos and Soto-Galera, 1997; Rojas et al., 1997; Pérez-Ponce de León et al., 2000; Martínez-Aquino et al., 2004, 2007, 2009; Sánchez-Nava et al., 2004; Martínez-Aquino, 2005; Mejía-Madrid et al., 2005; Romero-Tejeda et al., 2008). However, the only species of *Zoogoneticus* that has been studied to a certain extent is *Z. quitzeoensis* and no records of the helminth parasite fauna of the other 2 congeneric species had been established. The main objective of this work is to record the helminth parasite fauna of *Z. purhepechus* collected in 2 sites (Los Negritos and La Luz springs) located in the lower Lerma River Basin, and to describe the helminth community structure and the processes that determine such structure.

Materials and methods

On July 2009, 72 adult specimens of *Z. purhepechus* were collected using nets in 2 localities, La Luz spring (*n*= 45) (19° 56’ 10.4” N, 102° 17’ 57.8” W; 1 616 m) and Los Negritos spring (*n*= 27) (20° 03’ 23.1” N, 102° 36’ 38.3” W; 1 539 m), in Michoacán state, central Mexico. Hosts were taken alive to the laboratory, pithed and examined individually for intestinal helminths. Other organs (eyes, gall bladder, liver, spleen, swim and urinary bladders) as well as body cavity and musculature, were examined under a stereomicroscope in separate Petri dishes with 0.65% saline. Digeneans, nematodes and cestodes were fixed with 4% (steaming) formalin. Acanthocephalans were maintained at 4°C for 24 hrs. in distilled water, and then fixed in 100% ethanol. Platyhelminths and acanthocephalans were stained with Meyer’s paracarmine. Nematodes were cleared with glycerin for light microscopy and stored in 70% ethanol. Voucher specimens were deposited in the Colección Nacional de Helmintos (CNHE), Instituto de Biología, Universidad Nacional Autónoma de México, Mexico City (Table 1); when accession number is not provided, specimens were collected for DNA work or scanning electron microscopy. Use of prevalence (% infected) and abundance (mean number of parasites of a single species in the sample) follows Bush et al. (1997). The non-parametric species richness estimators Chao 1 and Chao 2, were calculated following Colwell and Coddington (1995) and Escalante (2003), and were

Table 1. Endohelminth parasites of *Zoogoneticus purhepechus* in 2 springs of the Lower Lerma River, Mexico. CNHE= Accession number; HI= Number of infected hosts; %= Prevalence; Ab= Abundance; SD= Standard deviation; MI= Mean intensity

Helminth (Infection site / CNHE)*	*La Luz spring (n= 45)* *HI / % / Ab ± SD / MI ± SD*	*Los Negritos spring (n= 27)* *HI / % / Ab ± SD / MI ± SD*
Adult Digenea		
Margotrema bravoae (I)	1 / 2.2 / 0.02 ± 0.15 / 1	
Phyllodistomum sp. (Ub / 7791)	1 / 2.2 / 0.02 ± 0.15 / 1	
Larval Digenea		
Clinostomum complanatum (Bc, M / 7792)	5 / 11.1 / 0.13 ± 0.4 / 1.2 ± 0.45	
Adult Cestoda		
Bothriocephalus acheilognathi (I, 7793)		2 / 7.4 / 0.07 ± 0.27 / 1
Larval Cestoda		
Cyclophyllidea gen. sp. (Gb)		1 / 3.7 / 0.04 ± 0.19 / 1
Adult Nematoda		
Rhabdochona lichtenfelsi (I, 7794)	7 / 15.6 / 0.33 ± 0.98 / 2.14 ± 1.57	
Larval Nematoda		
Rhabdochona sp. (I, 7108)		1 / 3.7 / 0.04 ± 0.19 / 1
Contracaecum sp. (L, 7105)		1 / 3.7 / 0.07 ± 0.38 / 2
Eustrongylides sp. (L, 7106)		1 / 3.7 / 0.04 ± 0.19 / 1
Spiroxys sp. (M)	4 / 8.9 / 0.13 ± 0.5 / 1.5 ± 1	1 / 3.7 / 0.11 ± 0.58 / 3
Adult Acanthocephala		
Pomphorhynchus cf. *bulbocolli* (I, 7795)	6 / 13.3 / 0.13 ± 0.34 / 1	
Larval Acanthocephala		
Polymorphus brevis (M, 7796)		1 / 3.7 / 0.04 ± 0.19 / 1

*Infection site: Bc= Body cavity; Gb= Gall bladder; Ub= Urinary bladder; I= Intestine; L= Liver; M= Mesentery used to estimate the number of missing species for each component community. Infracommunities include all the helminth species in an individual host, and were described by using the mean number of parasite species per host, the mean number of individual helminths, and the mean value of the Brillouin diversity index. The numerical dominance at the infracommunity level was determined using the Berger-Parker dominance index (Southwood, 1978). Infracommunities were compared qualitatively within the locality using Jaccard similarity index and quantitatively using the Morisita-Horn index, as calculated in Magurran (1988).

Results

Twelve helminth taxa were recovered from the 2 sampled localities. The helminthological record comprises 3 digenean species: *Margotrema bravoae* Lamothe-Argumedo, 1970, *Phyllodistomum* sp., and the metacercariae of *Clinostomum complanatum* (Rudolphi, 1819); 2 cestodes: the cysticercoid of Cyclophyllidea gen. sp., and *Bothriocephalus acheilognathi* Yamaguti, 1934; 2 acanthocephalans: the cystacanth of *Polymorphus brevis* (Van Cleave, 1916) and *Pomphorhynchus* cf. *bulbocolli* Van Cleave, 1919; the adult nematode *Rhabdochona lichtenfelsi* Sánchez-Álvarez, García-Prieto and Pérez-Ponce de León, 1998, and larval forms of *Contracaecum* sp., *Spiroxys* sp., *Eustrongylides* sp., and *Rhabdochona* sp.

La Luz spring. Thirty-four individual helminths were collected from 45 hosts from La Luz spring. These helminths represent 6 species: *M. bravoae*, *Phyllodistomum* sp. (adult), *C. complanatum* (metacercariae), *R. lichtenfelsi* (adult), *Spiroxys* sp. (larvae), and *P.* cf. *bulbocolli* (adult). Infection site, number of infected fish, prevalence, abundance, and mean intensity for each helminth taxa are shown in Table 1. The adult nematode *R. lichtenfelsi* was the most abundant helminth species, accounting for about 42.8% of the worms collected in this locality; this nematode reached a prevalence value of 15.6% and abundance of 0.33 worms per analyzed host. The remaining species were rare, infrequent, and reached very low abundance values (Table 1). Of the 45 analyzed individual hosts, 19 were infected with at least 1 species of helminth in La Luz spring. Only 5 of the 45 hosts harbored 2 or more helminth species. The total number of individuals of all species per host varied from 1 to 7, with a mean intensity of 0.82 ± 1.41. The non-parametric species richness estimators (Chao1 and Chao 2) reached a value of 6. The helminth infracommunities were species-poor. Mean number of species per host was 0.6 ± 0.8. The Brillouin index for all infracommunities varied from 0 to 0.599, with a mean diversity value of 0.099 ± 0.21, while the Berger-Parker dominance index values varied from 0.33 to 1, with a mean of 0.91 ± 0.2. The helminth infracommunities displayed a low level of similarity. The corresponding Jaccard index varied from 0 to 1 (mean of 0.18 ± 0.4) and the Morisita-Horn index varied from 0 to 1 (mean of 0.2 ± 0.37).

Los Negritos spring. Only 11 individual helminths were collected from Los Negritos spring. The 7 species recovered were *B. acheilognathi* (adult), 1 specimen of Cyclophyllidea (cysticercoid), the larval nematodes *Rhabdochona* sp., *Contracaecum* sp., *Eustrongylides* sp., and *Spiroxys* sp., and *Polymorphus brevis* (cystacanth). Infection site, number of infected fish, prevalence, abundance, mean intensity for each helminth species are shown in Table 1. Only *B. acheilognathi* was found in 2 hosts, while the remaining species were even more rare and infrequent (Table 1). The total number of individual helminths of all species per host varied from 1 to 7, with a mean number of 0.4 ± 1.4 individuals per host. The value obtained from the non-parametric species richness estimators (Chao 1= 11 and Chao 2= 25), shows that apparently several missing species remaining to be found at the component of community level. The helminth infracommunities were also species-poor; 2 infracommunities had just 1 species and 1 had a maximum of 4. Mean number of species per host was 0.29 ± 0.87. The Brillouin index for all infracommunities varied from 0 to 0.86, with a mean diversity value of 0.3 ± 0.41, while the Berger-Parker dominance index values varied from 0.43 to 1, with a mean of 0.73 ± 0.31. The helminth infracommunities show a low level of similarity. The corresponding Jaccard index varied from 0 to 0.5 (mean of 0.08 ± 0.2) and the Morisita-Horn index varied from 0 to 0.67 (mean of 0.11 ± 0.27).

The comparison between the helminth parasite fauna of both study sites, exhibits a very low similarity. Table 1 shows that only 1 taxon (*Spiroxys* sp.) is shared between component communities, resulting in a Jaccard value of 0.17, and a Morisita-Horn value of 0.23. However, it is possible that the larval stage of *Rhabdochona* found in Los Negritos might be conspecific with *R. lichtenfelsi*; although, we were unable to identify this larval satge up to species.

Discussion

Most of the helminth species found in this study have been previously recorded in diverse freshwater fish species in central and northern Mexico (Mejía-Madrid et al., 2005; Pérez-Ponce de León et al., 2007, 2009, 2010; Martínez-Aquino and Aguilar-Aguilar, 2008; Romero-Tejeda et al., 2008). Two of these species, the digenean *M. bravoae* and the nematode *R. lichtenfelsi*, are commonly found in goodeid fishes, and have been considered as a part of the core parasite fauna for this fish family (Mejía-Madrid et al., 2005; Pérez-Ponce de León and Choudhury, 2005; Martínez-Aquino et al., 2009).

Other helminth species recorded herein as adults were *Phyllodistomum* sp., *P.* cf. *bulbocolli*, and *B. acheilognathi*. The finding of *Phyllodistomum* sp. in this study represents a new host record for fishes of the family Goodeidae. To date, 6 species of *Phyllodistomum* have been recorded as a parasite of marine and freshwater fishes in Mexico (Pérez-Ponce de León et al., 2007). In this work, we collected 1 single specimen of this digenean, which hinders the accurate taxonomic determination. Considering the host associations of species of *Phyllodistomum* in freshwater fishes of North America (Hoffman, 1999), we may speculate that this represents an undescribed species. Further analysis of this worm and those collected from other goodeids in central Mexico (Martínez-Aquino, unpublished data) will allow the proper taxonomic identification of this digenean by using both, morphological and molecular data.

The acanthocephalan *P.* cf. *bulbocolli* is a parasite of freshwater fishes frequently recorded in North America (Hoffmann, 1999), and it was recently found in Mexico as a parasite of the catostomid *Catostomus nebuliferus* Garman and the cyprinid *Gila conspersa* Garman (Pérez-Ponce de León et al., 2009, 2010). The presence of this acanthocephalan in *Z. purhepechus* from La Luz spring represents the third published record for this species in Mexico, and the first record in freshwater fishes of the family Goodeidae. The cestode *B. acheilognathi* is an introduced species, which currently is widely distributed in freshwater fishes of Mexico (Rojas-Sánchez and García-Prieto, 2008), including several species of goodeids (Peresbarbosa-Rojas et al., 1994; Pérez-Ponce de León et al., 2000; Sánchez-Nava et al., 2004; Romero-Tejeda et al., 2008; Pérez-Ponce de León et al., 2009).

The remaining 7 helminth species found in *Z. purhepechus* in both localities were larval stages frequently recorded in the freshwater fish helminth fauna of Mexico and North America. These species were *C. complanatum*, the cysticercoid of Cyclophyllidea, *Contracaecum* sp., *Eustrongylides* sp. and *P. brevis*, all of them maturing in piscivorous birds, and the nematode *Spiroxys* sp., which reaches the maturity mainly in freshwater turtles. One single immature specimen of *Rhabdochona* sp. was found in Los Negritos spring. This nematode most likely belongs to the species *R. lichtenfelsi*, however, absence of reproductive structures prevent its accurate taxonomic determination.

The data we present here suggest that the helminth parasite species composition, and the helminth infracommunity structure in *Z. purhepechus* is consistent with the pattern that shows a depauperate parasite fauna in goodeid fishes inhabiting freshwaters in the Nearctic part of Mexico such as *Alloophorus robustus* Bean, *Goodea atripinnis* Jordan, *Allotoca diazi* Meek, *Chapalichthys encaustus* Jordan and Snyder, *Characodon audax* Smith and Miller, *Skiffia lermae* Meek, *Girardinychthys multiradiatus* Meek, *Xenotoca variata* Bean, *Xenotaenia resolanae* Turner, and *Z. quitzeoensis* Bean (Astudillo-Ramos and Soto-Galera, 1997; Rojas et al., 1997; Pérez-Ponce de León et al., 2000; Martínez-Aquino et al., 2004, 2007, 2009; Sánchez-Nava et al., 2004; Martínez-Aquino, 2005; Romero-Tejeda et al., 2008). The factors that determine the helminth community structure herein described are concordant with those described in the aforementioned studies (the feeding habits, omnivorous in this case, and the position in the food web of this species of host), i.e., 11 of the 12 helminth species infect their host when it feeds upon some species of crustacean or insect. Likewise, 7 of the 12 helminth species are larval forms that complete their life cycle when the fish is consumed by a definitive host, either a fish-eating bird or a reptile.

On the basis of the values obtained through the species richness estimators used in this study (Chao 1 and Chao 2), it seems that we sampled all the helminth fauna in La Luz spring, since the observed and estimated richness value was very similar; however, values obtained for the same richness estimators in Los Negritos spring indicate that various helminth species apparently remain to be found. This result was surprising, since following the idea of Pérez-Ponce de León and Choudhury (2010) that the inventory of the freshwater fish helminth fauna in Mexico is nearing completion, and not many additional species are expected to be found, and particularly in goodeids, since this is a group of hosts that has been extensively studied for helminths in the last years. Thus, this apparently high number of species remaining to be found at Los Negritos spring, could be the result of an artifact derived from very low abundance and mean intensity values for each helminth taxa. Both nonparametric estimators Chao 1 and Chao2 are sensitive to the presence of rare species (Escalante, 2003), and that is probably the reason of the apparently high number of missing species in that particular locality.

The comparison between the 2 study sites showed that both helminth assemblages had very low numbers of individuals and consequently, remarkable low prevalence and abundance values. However, the helminth community of *Z. purhepechus* at La Luz spring included at least 2 of the species considered specialists for the fish family Goodeidae, the digenean *M. bravoae* and the nematode *R.*

lichtenfelsi (Pérez-Ponce de León and Choudhury 2005). Likewise, the helminth assemblage of Los Negritos spring is comparatively species-poor, specialist species are lacking, and is conformed only by larval stages and the introduced tapeworm *B. acheilognathi*. This pattern results in very low levels of similarity between the component communities. It is noteworthy the absence of digeneans in Los Negritos spring in conjunction with extremely low abundance values for the helminth species found in that locality. On the other hand, in La Luz, helminth species composition includes at least 3 species of digeneans, even though no tapeworms were found, and abundance values of the helminth parasite fauna are slightly higher than in Los Negritos. We may speculate that this is the result of different environmental conditions of the sampling sites, e.g., primary productivity and associated physicochemical parameters (personal observation), and it is possible that the absence of digeneans in Los Negritos could be the result of the lack of molluscs that are the first intermediate host in their life cycle, however this needs to be determined with the proper sampling in the locality.

Of the 3 recognized species of *Zoogoneticus*, the helminth parasite fauna has been studied for 2 of them, *Z. quitzeoensis*, and now, *Z. purhepechus*. We compared the helminth parasite fauna of *Z. purhepechus* and its putative sister species, *Z. quitzeoensis* (see Domínguez-Domínguez et al., 2008), and some differences were found. The endohelminth fauna of both *Zoogoneticus* species comprises 16 taxa, including 7 digeneans (4 adults and 3 metacercariae), 2 cestodes (1 adult and 1 cysticercoid), 5 nematodes (1 adult and 4 larvae), and 2 acanthocephalans (1 adult and 1 larvae) (see Martínez-Aquino, 2005; Mejía-Madrid et al., 2005; Romero-Tejeda et al., 2008). Only 3 of the 16 taxa (*M. bravoae*, *B. acheilognathi*, and *R. lichtenfelsi*) are shared between both species. Considering that they are sister species, and even though they do not occur in sympatry, the differences in helminth parasite fauna are a sampling artifact due to the fact that these species have not been studied along their entire distributional range, in the Lower and Middle Lerma River, respectively. We predict that the result of a detailed survey work in other localities along the distribution range for both species, will allow us to find the species that have not yet been documented, increasing as a result the levels of similarity among the parasite fauna.

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4) ENDOHELMINTH PARASITES OF SEVEN GOODEIN SPECIES (CYPRINODONTIFORMES: GOODEIDAE) FROM LAKE ZACAPU, MICHOACÁN, CENTRAL MEXICO PLATEAU

Endohelminth parasites of seven goodein species (Cyprinodontiformes: Goodeidae) from Lake Zacapu , Michoacán, Central Mexico Plateau

Endohelmintos parásitos de siete especies de godeinos (Cyprinodontiformes: Goodeidae) del lago de Zacapu, Michoacán, en la Mesa Central de México

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ABSTRACT

A total of 11 parasitic endohelminth taxa were found in 7 freshwater fish species of the subfamily Goodeinae in Zacapu Lake, Michoacan, Mexico. Six were adults (*Margotrema* cf. *bravoae*, *Phyllodistomum* sp., *Saccocoelioides sogandaresi, Rhabdochona lichtenfelsi*, *Bothriocephalus acheilognathi* and Caryophillidae gen. sp.), while the remaining 5 taxa (*Clinostomum complanatum*, *Posthodiplostomum minimum*, *Tylodelphis* sp., *Eustrongylides* sp. and *Polymorphus brevis*) were larvae. The taxa *S. sogandaresi*, *Tylodelphis* sp., and *R. lichtenfelsi* reached the highest levels of prevalence and mean abundance among all hosts, while the cestodes *B. acheilognathi* and Caryophillidae gen. sp. showed the lowest values. This study contributes with the inventory of the freshwater fish helminth fauna in Central Mexico Plateau, and particularly with the previous work that has been done with Goodeinae, a subfamily of freshwater fishes endemic to that part of the country.

Key words: Central Mexico Plateau, Goodeinae, helminth parasites.

RESUMEN

En este trabajo se registran 11 taxa de helmintos endoparásitos de 7 especies de peces de la subfamilia Goodeinae del lago de Zacapu, Michoacán, en la mesa central de México. Seis taxa se registraron en estado adulto (*Margotrema* cf. *bravoae*, *Phyllodistomum* sp., *Saccocoelioides sogandaresi, Rhabdochona lichtenfelsi, Bothriocephalus acheilognathi* y Caryophillidae gen. sp.), en tanto que los 5 restantes correspondieron a larvas (*Clinostomum complanatum*, *Posthodiplostomum minimum*, *Tylodelphys* sp. y *Eustrongylides* sp. y *Polymorphus brevis*). Los taxa *S. sogandaresi*, *Tylodelphis* sp. y *R. lichtenfelsi* presentaron los valores más altos de prevalencia y abundancia en los peces estudiados, mientras que los céstodos *B. acheilognathi* y Caryophillidae gen. sp. presentaron los valores más bajos. Este estudio contribuye al inventario de la fauna helmintológica de peces de agua dulce de la mesa central de México y en particular al que ha sido realizado con Goodeinae, una subfamilia de peces dulceacuícolas endémica de esa parte del territorio nacional.

Palabras clave: Mesa central de México, Goodeinae, helmintos parásitos.

Goodeinae (Cyprinodontiformes: Goodeidae) represents a subfamily of viviparous freshwater fishes, which comprises 20 genera and 43 species, entirely endemic to river drainages and basins of central Mexico (Domínguez-Domínguez *et al.,* 2012). The helminth fauna parasitizing these freshwater fishes has been intensively documented during the last two decades. Some papers have addressed the helminth assemblage in one single species of host (i.e., Martínez-Aquino *et al.,* 2004, 2007b, 2009; Sánchez-Nava *et al.,* 2004), while some others described the helminth fauna of three or more goodein species within the same locality (e.g., Peresbarbosa-Rojas *et al*., 1994; Pérez-Ponce de León *et al.,* 2000; Mejía-Madrid *et al*., 2005; Romero-Tejeda *et al.,* 2008).

Zacapu Lake, located in Central Mexico Plateau, in the state of Michoacán, is a medium-size water body fed by several springs, which shows a relatively low level of environmental degradation, and serves as habitat for diverse freshwater taxa. Because of this, it has been argued that this area is important from a conservational point of view (Medina-Nava *et al.,* 2005; Domínguez-Domínguez *et al*., 2006; Martínez-Aquino *et al*., 2007a). The freshwater fish fauna of the lake includes 7 goodein species of which 6 have been partially studied for helminth parasites (Galicia-Guerrero, 2001; Pérez-Ponce de León, 2001; Mejía-Madrid *et al.,* 2005). The aim of this work is to record the endohelminth parasite fauna of all the species of goodeins inhabiting Zacapu Lake, and provide the ecological infection parameters, such as prevalence and abundance.

On July 2009, a total of 161 adult specimens of goodeins belonging to the species *Alloophorus robustus* (Bean, 1892) (n = 17), *Allotoca zacapuensis* Meyer, Radda & Domínguez-Domínguez, 2001 (n = 32), *Goodea atripinnis* Jordan, 1880 (n = 20), *Hubbsina turneri* de Buen, 1940 (n = 20), *Skiffia lermae* Meek, 1902 (n = 19), *Xenotoca variata* (Bean, 1887) (n = 21), and *Zoogoneticus quitzeoensis* (Bean, 1898) (n = 32), were collected in Zacapu Lake (19°49'35'' N; 101°47'10'' W), using seine nets and electrofishing. Fishes were taken alive to the laboratory, sacrificed by pithing, and individually examined for endohelminth parasites. After dissection, gastrointestinal tract was removed and placed in a Petri dish with 0.65% saline. Other organs (eyes, liver, spleen, gall, swim and urinary bladders), and body cavity, were examined under a stereomicroscope in separate Petri dishes with saline 0.65%. Digeneans, nematodes and cestodes were fixed with 4% (steaming) formalin. Acanthocephalans were placed during 24 hrs in distilled water at 4 °C, and preserved in absolute ethanol. Plathyhelminths and acanthocephalans were stained with Mayers paracarmine. Nematodes were cleared with glycerin for light microscopy study. Voucher specimens of all taxa were deposited in the Colección Nacional de Helmintos (CNHE), Instituto de Biología, Universidad Nacional Autónoma de México, Mexico City. The use of prevalence (% infected), and abundance (mean number of parasites of a single species in the sample) follows Bush *et al.* (1997).

In total, 11 endohelminth parasite taxa were collected from the 7 analyzed host species. The endohelminth fauna includes 6 taxa of digeneans (3 adults and 3 larval stages), 2 adult cestodes, 2 nematodes (1 adult and 1 larvae), and 1 acanthocephalan (cystacanth). *Rhabdochona lichtenfelsi* Sánchez-Álvarez, García-Prieto & Pérez-Ponce de León, 1998, *Tylodelphys* sp., and *Polymorphus brevis* (Van Cleave, 1916), were the most widely distributed taxa among the host species. The adult nematode *R. lichtenfelsi* reached the highest levels of prevalence and mean abundance. *Allotoca zacapuensis* harbored 7 endohelminth taxa, whereas the remaining fish hosts were parasitized by 4-5 endohelminth taxa. Endohelminth parasite taxa, prevalence and abundance, as well as the new host and locality records herein established are shown in Table 1.

Four endohelminth taxa are reported in Zacapu Lake for the first time, and in addition to that, 21 new host records are established in this paper. The endohelminth fauna of *Skiffia lermae* is reported for the first time, meanwhile for the goodeins *Allotoca zacapuensis, Hubssina turneri,* and *Zoogoneticus quitezeoensis,* the known endohelminth parasite fauna was duplicated with respect to previous records. Our study brings the total number of helminth parasites of goodeins in Zacapu Lake to 18 (Galicia-Guerrero, 2001; Pérez-Ponce de León, 2001; Mejía-Madrid *et al*., 2005). Additionally, with 64.3% of the fish fauna studied thus far for helminth parasites, our data increase the knowledge about the freshwater fish parasite fauna of this locality in 30%.

The endohelminth fauna of goodeins in Zacapu Lake include at least 2 species which have been considered as specialists to Goodeinae, i.e., *Margotrema* cf. *bravoae* Lamothe-Argumedo, 1970*,* and *Rhabdochona lichtenfelsi* (Pérez-Ponce de León *et al.,* 2000; Mejía-Madrid *et al.,* 2005, 2007; Pérez-Ponce de León & Choudhury, 2005). The adult digenean *Phyllodistomum* sp., could represent a third specialist species; however, this need to be determined once the taxonomic identity of the species is established. Morphologically similar specimens were recently recorded in the goodein fish *Zoogoneticus purhepechus* Domínguez-Domínguez, Pérez-Rodríguez *et* Doadrio, 2008 from a relatively close locality in the lower Lerma River (Martínez-Aquino *et al.,* 2011). These specimens, along with those reported as *Dendrorchis* sp. by Martínez-Aquino *et al.* (2009) have been only recorded in goodeins in central Mexico and may be conspecific. Moreover, they could represent a new species; although until additional taxonomic work based on morphology and molecular markers is carried out, their identities remain uncertain. In contrast, 8 generalist helminth taxa were recorded in the analyzed hosts both as adult or larval stages: *Saccocoelioides sogandaresi* Lumsden, 1963, *Clinostomum complanatum* (Rudolphi, 1819), *Posthodiplostomum minimum* (MacCallum, 1921), *Tylodelphys* sp., *Bothriocephalus acheilognathi* Yamaguti, 1934, Caryophyllidae gen. sp., *Eustrongylides* sp., and *P. brevis.*

For this survey, specimens were collected only in one period during the year, preventing us from establishing a robust comparison of the parasite fauna among analyzed species of hosts, or even between the records we establish in this work, and those previously made for other authors. However, our data correspond with the general pattern described for most species of freshwater fishes occurring in different river basins in central Mexico, i.e., a

Table 1. Number of infected hosts (IH), prevalence (%), abundance (Ab), and infection site of the endohelminth parasites of seven freshwater fish species of goodeins from Zacapu Lake, Michoacán, Central Mexico Plateau.

	Alloophorus robustus (n = 17) IH, %, Ab	*Allotoca zacapuensis* (n = 32) IH, %, Ab	*Goodea atripinnis* (n = 20) IH, %, Ab	*Hubbsina turneri* (n = 20) IH, %, Ab	*Skiffia lermae* (n = 19) IH, %, Ab	*Xenotoca variata* (n = 21) IH, %, Ab	*Zoogoneticus quitzeoensis* (n = 32) IH, %, Ab	CNHE No.
Adult Digenea								
Margotrema cf. *bravoae* (I)		8, 25, 0.44					15, 46.88 0.88φ	7811-12
Phyllodistomum sp.$^{\#}$ (Ub)		3, 9.38, 0.13φ		2, 10 0.1φ			5, 15.63 0.5φ	7813-15
Saccocoelioides sogandaresi$^{\#}$ (I)			15, 75 3.15φ					7816
Larval Digenea								
Clinostomum complanatum (Bc)	2, 11.76 0.18φ					1, 4.76 0.05		7817-18
Posthodiplostomum minimum (Bc, L, M)		9, 28.13, 2.03φ	5, 25 0.50		4, 21.05 1.58φ			7819-21
Tylodelphis sp.$^{\#}$ (Bc)		2, 6.25, 0.09φ*	2, 10 0.3φ	15, 75 9.7φ	13, 68.42 3.47φ	1, 4.76 0.05φ	2, 6.25 0.06φ	7822-26
Adult Cestode								
Bothriocephalus acheilognathi (I)		1, 3.13 0.03						*
Caryophyllidae gen. sp.$^{\#}$ (M)		1, 3.13 0.03φ						7827
Adult Nematode								
Rhabdochona lichtenfelsi (I)	6, 35.29 2.24	21, 65.63 5.03	5, 25 1.25*	15, 75 7.75*	19, 100 4.79φ	15, 71.43 6.76	5, 15.63 0.25	7100, 7102-04, 7107
Larval Nematoda								
Eustrongylides sp. (M)	1, 5.88 0.06φ							7098
Larval Acanthocephalan								
Polymorphus brevis (Bc, M)	1, 5.88 0.06*	3, 9.38 0.16φ		3, 15 0.25φ*	1, 5.26 0.05φ*	4, 19.05 0.29	2, 6.25 0.09φ	7828-30

Infection site: Bc = Body cavity; I = Intestine; L = Liver; M = Mesentery; Ub = Urinary bladder; # = new locality record; φ = new host/locality record; * = No specimens for morphology are available because those were collected for molecular analysis. CNHE No= Catalog number of Colección Nacional de Helmintos.

species-poor helminth fauna, highly dominated by one species of helminth (Pérez-Ponce de León *et al*., 2000). In particular, ecological parameters of the parasitic infections herein reported coincide with those previously reported for other species of goodeins, i.e., the nematode *Rhabdochona lichtenfelsis*, as well as larval digeneans reach the highest prevalence and abundance values (Martínez-Aquino *et al*., 2004, 2007b; Romero-Tejeda *et al.,* 2008),

The results we provide in this paper contribute to our understanding of the helminth parasite fauna of goodeins in Central Mexico Plateau, but also further contribute with the inventory of the Mexican freshwater fish parasite fauna.

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5) A NEW SPECIES OF *MARGOTREMA* (DIGENEA, ALLOCREADIIDAE) FROM THE LEOPARD SPLITFIN *XENOTAENIA RESOLANAE* (CYPRINODONTIFORMES, GOODEIDAE) FROM WEST-CENTRAL MEXICO

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A new species of *Margotrema* (Digenea, Allocreadiidae) from the leopard splitfin *Xenotaenia resolanae* (Cyprinodontiformes, Goodeidae) from west-central Mexico

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A new species of *Margotrema* is herein described from the intestine of the freshwater fish *Xenotaenia resolanae* from the Cuzalapa River, Jalisco State, on the Pacific slope of west-central Mexico. The new species was first recognized after a molecular phylogenetic analysis which explored the genetic variation at the intraspecific level of *Margotrema* spp across central Mexico. Sequences of mitochondrial (COI) and nuclear (ITS1) genes were obtained for 125 individuals, and a general mixed Yule-coalescent analysis (GMYC) for species delimitation of both genes allowed the recognition of an independent evolutionary lineage, representing an undescribed species of *Margotrema*. Detailed morphological observations allowed the species description. The new species is easily distinguished from the other valid species of the genus, *M. bravoae*, by having vitelline follicles restricted to the region between the pharynx and the ventral sucker a wider and more prominent ventral sucker, larger eggs, and symmetrical testes.

The genus *Margotrema* included two species, *M. bravoae* Lamothe-Argumedo, 1970, from the goodeid *Girardinichthys multiradiatus* Meek from La Lagunilla, Estado de México (Lamothe-Argumedo 1970), and *M. guillerminae* Pérez-Ponce de León, 2001 from the cyprinid *Notropis calientis* Jordan and Snyder, and the goodeid *Alloophorus robustus* (Bean) from Lake Zacapu, Michoacán (Pérez-Ponce de León 2001). These species were distinguished mainly on the basis of relative length of cecae. While exploring the genetic diversity of *Margotrema* spp. in their goodeid hosts across central Mexico (in 15 localities representing seven hydrological systems), Martínez-Aquino *et al.* (2013) found evidence to synonymize *M. guillerminae* with the type-species, *M. bravoae*, and to recognize an independent genetic lineage (Lineage I), parasitizing the endemic goodeid *Xenotaenia resolanae* in the Cuzalapa river, a tributary of the Marabasco River Basin. The helminth fauna of *X. resolanae* had been studied by Martínez-Aquino *et al.* (2009); the species of *Margotrema* was erroneously determined as *M. guillerminae* based on the extension of the ceca along body, a character that results from intraspecific morphological variability and is not reliable, in these digeneans, as a taxonomically important trait to distinguish species. After the molecular analyses, a closer look at the morphology of specimens (museum and freshly collected material) was then undertaken for specimens allocated to Lineage I, concluding it represented a new species. We describe the new species in this paper.

Specimens were stained with Mayer´s paracarmine and Gomori´s thrichorome, mounted as permanent slides using Canada balsam and deposited at the CNHE (Colección Nacional de Helmintos). For SEM study, specimens were post fixed in 1 % OsO_4 for 1 hr, dehydrated through a graded series of ethyl alcohol and then critical point dryied with CO_2. Specimens were mounted on metal stubs, coated with gold, and examined in a Hitachi Stereoscan Model S-2469N at 15 kV. Measurements are presented in micrometers, as range followed by the average and standard deviation in parentheses.

Margotrema resolanae n. sp.

(Fig. 1A–C)

Host. *Xenotaenia resolanae* Meek.

Locality. Cuzalapa River, Jalisco State, west-central Mexico. 19°30’32.1” N, 104°17’45.6” W.

Specimens deposition. CNHE, Holotype 6868, paratypes 6869; CNHE 6880.

Etymology. This species in named after the host species (*Xenotaenia resolanae*) to which the digenean exhibits a strong host-specificity.

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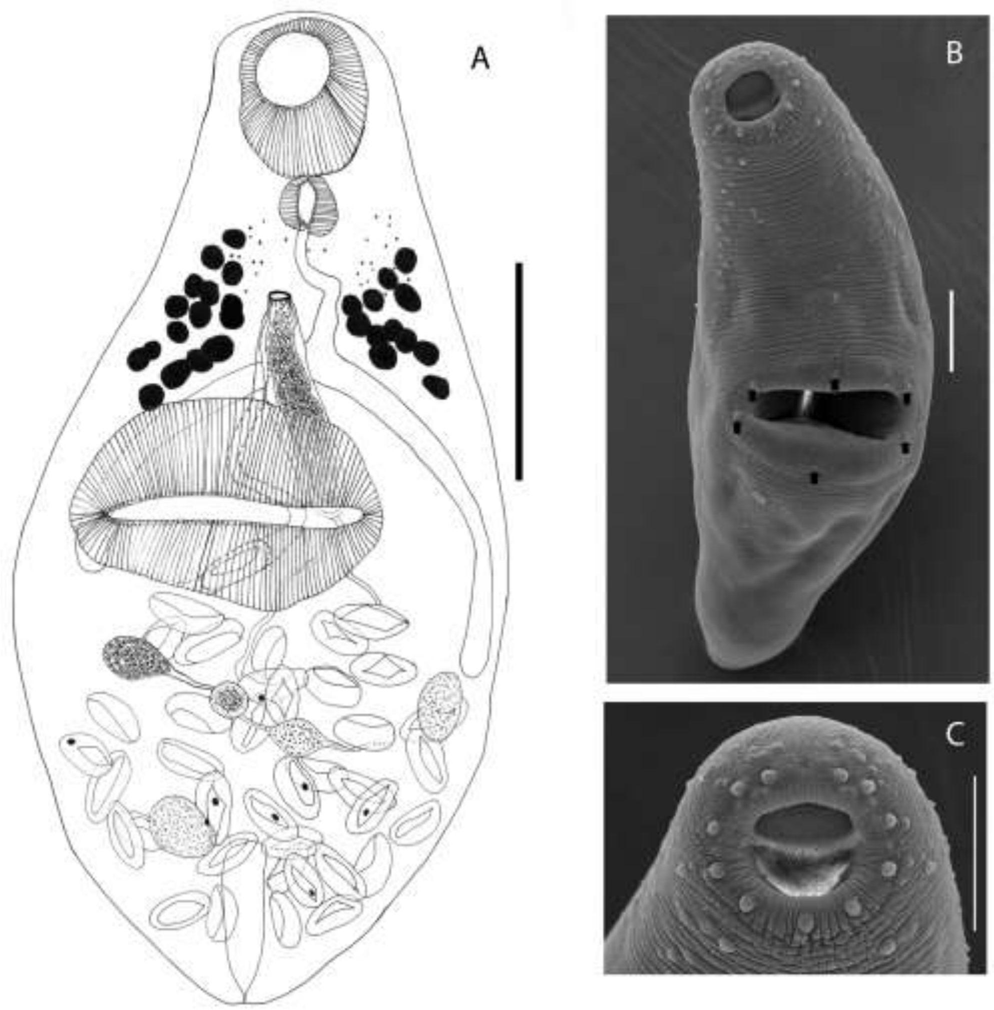

FIGURE 1. *Margotrema resolanae* **n. sp.**, **A.** Line drawing of Holotype, ventral view. Scale Bar 200µm. **B.** Scanning Electron Microscopy micrograph of body. Scale Bar 50 µm, and **C.** Detail of the oral sucker showing the distribution of 11 dome-like papillae. Scale Bar 50 µm.

Description. Based on measurements of 14 specimens. Body elongate, unspined, with scattered eyespot remnants 384–925 (595±135) long, maximum width attained at level of ventral sucker 167–481 (277±71). Oral sucker subterminal 95–143 (114±15) long by 100–133 (115±10) wide. Scanning Electron Microscopy (SEM) micrograph of the oral sucker shows a pattern of 11 dome-like papillae, 4 anterior, 4 lateral (2 on each side), and 3 posterior (Fig. 1C). Mouth 36–77 (56±12) long, 50–88 (66±12) wide. Prepharynx absent. Pharynx 32-59 (43 ± 8) long, 31-59 (44±7) wide. Esophagus relatively short, 46-101 (63 ± 16, n = 6) long. Cecal bifurcation at 156-308 (215±45) from anterior end. Short ceca extending posteriorly to surpass the posterior end of ventral sucker; in some specimens ceca extend into the anterior testis. Ventral sucker wide, very well-developed, located in equatorial area or slightly in posterior third of body 110–230 (156±34) long, 146–296 (216±37) wide, with a pattern of 6 dome-like papillae, 4 lateral (2 on each side), 1 anterior, 1 posterior (Fig. 1B); average oral sucker/ventral sucker ratio 1:1.37 (length), 1: 1.88 (width). Testes relatively small, symmetrical (oblique in few specimens), subspherical. Anterior testis 34–99 (63±17) long, 35–85 (59±13) wide. Posterior testis 27–108 (62±21) long, 40–77 (64±12) wide. Cirrus sac straight, dorsal to ventral sucker 107–216 (151±42) long, containing bipartite seminal vesicle and short cirrus. Genital atrium poorly developed. Genital pore anterior to cecal bifurcation, at esophagus level 143–261 (200±39) from anterior end of body. Ovary pretesticular, relativelly small, subspherical, dextral 24–60 (39±11) long, 49–91 (67±12) wide. Seminal receptacle immediately postovarian. Vitelline reservoir, Laurer´s canal, and Mehlis' gland not observed because the large number of eggs in uterine loops. Vitellarium follicular; vitelline follicles in two lateral fields in anterior end of body, extending mostly between pharynx and anterior margin of ventral sucker or slightly surpassing in some specimens. Uterus highly coiled, occupying most of posterior half of body, ascending sinuously between testes, forming a short metraterm to reach genital atrium dorsally to ventral sucker. Eggs large, embryonated 50–79 (67±7, n = 10) long, 25–47 (35±5, n = 10) wide. Excretory vesicle tubular reaching testes level.

Remarks. The new species closely resembles *M. bravoae* in general morphology of the body however, *M. resolanae* n. sp. may be easily distinguished from the type species by possesing vitelline follicles restricted to the region between the pharynx and the anterior end of ventral sucker. In contrast, *M. bravoae* has vitelline follicles extending from the pharynx level posteriorly to ovary, surpassing ventral sucker. Additionally, the new species posseses a wider and more prominent ventral sucker, larger eggs (measuring 67 by 35 compared with 48 by 33) and symmetrical testes. The new species exhibits a close phylogenetic and biogeographical association with the host species, and can be regarded as a member of the helminth parasite core fauna of *Xenotaenia resolanae* (sensu Pérez-Ponce de León & Choudhury 2005). The description we present herein corroborates that a DNA-based taxonomic approach is necessary to uncover previously unrecognized biodiversity, greatly enhancing our chances to complete the inventory of the freshwater fish parasite fauna in Mexico (Pérez-Ponce de León & Choudhury 2010). The record of Martinez-Aquino et al (2009) of *M. guillerminae* as a parasite of *X. resolanae* should then be referred as the new species we described in this paper. The generic diagnosis should be amended to include the following traits: Vitelline follicles restricted, extending between ovary and pharynx; postovarian testes, oblique or symmetrical; intestinal ceca short (extending to the ventral sucker region), or long (extending to surpass the testes).

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6) COMPOSICIÓN TAXONÓMICA DE HELMINTOS PARÁSITOS DE GOODEINAE (OSTEICHTHYES: CYPRINODONTIFORMES: GOODEIDAE) EN MÉXICO

Manuscrito para ser sometido para su publicación en una revista arbitrada e indizada (ISI / SCI).

Articulo en extenso

Martínez-Aquino *et al*.- Helmintos parásitos de Goodeinae

Composición taxonómica de helmintos parásitos de Goodeinae (Osteichthys: Cyprinodontiformes: Goodeidae) en México

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Resumen. Este estudio es un inventario helmintológico actualizado que representa la composición taxonómica de helmintos parásitos para Goodeinae en el centro de México. El registro helmintológico incluye 19 taxones de digéneos (11 adultos y ocho metacercarias), siete de monogéneos, 12 de céstodos (tres adultos y nueve metacéstodos), 15 de nemátodos (ocho adultos y siete larvas) y dos de acantocéfalos (un adulto y un cistacanto). Se observa un predominio de digéneos, nemátodos y céstodos, respectivamente, así como una escasez de acantocéfalos y un numero relativamente bajo de monógenos. El número de taxones encontrados en estadios adultos (29 taxones, 55%) es ligeramente mayor que el de larvas (25 taxones, 45%). Existe un mayor número de taxones autogénicos (35 taxones, 64%) que alogénicos (20 taxones, 36%). *Margotrema bravoae*, *M. resolanae*, *Phyllodistomum* sp., *Saccocoeloides* sp., *Gyrodactylus lamothei*, *G. mexicanus*, *Gyrodactylus* sp. 1, *Gyrodactylus* sp. 2, *Rhabdochona ahuehuellensis* y *R. lichtenfelsi* son taxones endémicos del centro de México y especialistas de la subfamilia Goodeinae. El dígeneo *Centrocestus formosanus*, el monogéneo *G.* cf. *elegans*, el nemátodo *Pseudocapillaria tomentosa* y el céstodo *Bothriocephalus acheilognathi* son especies introducidas a la región. Se reconocen dos características de la helmintofauna de goodeinos: 1) un predominio de digéneos, nemátodos y céstodos; 2) un mayor número de especies autogénicas. La segunda característica refleja una estrecha relación evolutiva durante el proceso de diversificación de los helmintos asociados a la ictiofauna dulceacuícola mexicana (i.e. *faunas principales*), asociada a Goodeinae. En total, como resultado del trabajo de muestreo realizado en los últimos 5 años se presentan 112 nuevos registros de helmintos, e incluyen 23 taxones de helmintos parásitos asociados a 28 especies de goodeinos, de las cuales seis son nuevos para este grupo de peces. Este inventario representa al grupo de vertebrados – Goodeinane – mejor estudiado en términos helmintoparasitofaunísticos para México, lo que permitirá a especialistas de distintas áreas integrar y expandir interpretaciones acerca de la biodiversidad parasitaria de México.

Introducción

La subfamilia Goodeinae (Osteichthyes: Cyprinodontiformes: Goodeidae), sin duda, es el grupo de vertebrados en México con el mayor número de trabajos donde se describe la fauna helmintológica que los parasita. Los estudios de la última década reconocen que de las 42 especies que se incluyen actualmente en este grupo de peces dulceacuícolas, 33 de ellas cuentan con registros helmintológicos. Esto representa al 80% de esta subfamilia, por lo que Goodeinae, junto con Cichlidae e Ictaluridae, forma parte de los tres grupos de peces dulceacuícolas mejor estudiados en México en términos helmintológicos (Pérez-Ponce de León & Choudhury, 2010). Los trabajos publicados sobre la helmintofauna de goodeinos consisten en cinco categorías: 1) Registros esporádicos o aislados para la descripción de la helmintofauna (v. gr. Lamothe-Argumedo & Cruz-Reyes, 1972; García-Prieto & Osorio-Sarabia, 1991; Guzmán-Cornejo & García-Prieto, 1999; Scholz & Salgado-Maldonado, 2000, 2001; Salgado-Maldonado & Pineda-López, 2003; Alcántar-Escalera *et al*., 2013). 2) Registros derivados de inventarios helminofaunísticos para sistemas hidrológicos particulares (v. gr., Salgado-Maldonado *et al*., 2001a; 2001b; 2004a; 2004b; Salgado-Maldonado, 2006; Pérez-Ponce de León *et al*., 2009). 3) Registros sistematizados en los que se estudia la ecología de comunidades en especies particulares de goodeinos (v. gr. Peresbarbosa-Rojas *et al*., 1994; Pérez-Ponce de León *et al*., 2000; Sánchez-Nava *et al*., 2004; Mejía-Madrid *et al*., 2005; Mendoza-Palmero *et al*., 2007; Romero-Tejeda *et al*., 2008; Martínez-Aquino *et al*., 2004, 2007b, 2009a, 2011, 2012). 4) Descripciones taxonómicas (v. gr. Lamothe-Argumedo, 1970; Sánchez-Alvárez *et al*., 1998; Pérez-Ponce de León, 2001; Mejía-Madrid & Pérez-Ponce de León, 2003; Mendoza Palmero *et al*., 2009; Pérez-Ponce de León *et al*., 2013). 5) Interpretaciones evolutivas entre asociaciones *Goodeinae-faunas principales* (v. gr., Mejía-Madrid *et al*., 2007b; Martínez-Aquino *et al*., 2013).

En sumatoria, de las 204 especies de peces dulceacuícolas de México que cuentan con registros para taxones de helmintos parásitos (Pérez-Ponce de León & Choudhury, 2010), las cinco categorías anteriormente mencionadas representan el 16% del conocimiento de la helmintofauna mexicana generada hasta el momento. Sin embargo, solo existen dos inventarios de taxones de helmintos parásitos para goodeinos: 1) Mejía-Madrid *et al*. (2005), donde presentan un listado de los helmintos en estadio adulto en él categorizan su composición taxonómica y realizan comentarios empírico-biogeográficos para inferir posibles procesos evolutivos entre Goodeinae y sus faunas principales (*sensu* Pérez-Ponce de León & Choudhury, 2005). 2) Pineda-López *et al.*, (2005: 462), intentaron usar los registros de helmintos tanto en estadios larvarios como de adultos para inferir historias evolutivas de tipo *Goodeinae-faunas principales*. Desafortunadamente, esos autores tuvieron una gran confusión al apoyar sus inferencias usando la historia evolutiva de peces no goodeinos, sino mas bien con aquellos del género *Chirostoma* (Atheriniformes), lo que vuelve aún más débiles sus especulaciones. En otro contexto, Mejía-Madrid *et al*. (2007b) y Martínez-Aquino *et al*. (2013), han logrado integrar la historia evolutiva de los goodeinos con la de sus helmintos parásitos e interpretarla sobre la historia hidrogeomorfológica del centro de México con base en evidencia morfológica, molecular, biogeográfica, genealógica y filogenética. Estos autores resaltan la importancia de generar una basta cantidad de información referente al área de distribución de los taxones de helmintos a usar como modelo de estudio para postular inferencias evolutivas entre asociaciones de tipo huésped-parásito. El área de distribución de una especie se define como el área que ocupa (Rapoport, 1975). Se determina a partir de las localidades donde se ha registrado, entendiendo por localidad de registro tanto su descripción de localización y coordenadas geográficas, como sus atributos de hábitat. En este contexto, contar con toda la información generada, publicada, actualizada, organizada de manera sistematizada y colocarla en documentos de acceso libre para la comunidad científica permite integrar e interpretar los procesos y patrones que ocurren en la biodiversidad de parásitos. Por tanto, el objetivo de este trabajo es recabar toda la información bibliográfica especializada y publicada para helmintos parásitos de peces de la subfamilia Goodeinae, así como aportar datos originales de nuestros propios muestreos, para elaborar un inventario actualizado que permita describir su composición taxonómica a modo de describir patrones de diversidad de la fauna parasitaria en el centro de México.

Materiales y métodos

Recolecta de huéspedes y de helmintos parásitos

Entre agosto de 2008 y julio de 2010 se realizaron siete muestreos en 57 localidades ubicadas en siete sistemas hidrológicos del centro de México; Río San Pedro Mezquital, Río Ameca, Río Ayuquila, Río Coahuayana, Río Lerma, Río Cuzalapa y Río Balsas. En total se examinaron 1632 peces de 28 especies de goodeinos (Apéndice 1).

Los peces fueron recolectados con un chinchorro de 3 m de longitud y ½ pulgada de luz de malla, con equipo de electropesca de corriente directa y con trampas tipo nazas; se transportaron vivos al laboratorio para su examen helmintológico, el cuál se realizó dentro de las 24 horas posteriores a su captura. La determinación taxonómica de cada uno de los huéspedes fue apoyada con base en claves taxonómicas directamente en el campo (Miller *et al*., 2005), y verificada posteriormente por especialistas con base en datos moleculares (v.gr. Pérez-Rodríguez *et al*., sometido).

La obtención de helmintos se realizó mediante un examen general de los peces con microscopio estereoscópico. El examen externo incluyó la superficie general del cuerpo, ojos, orificios del cuerpo (boca y ano) y aletas (anal, caudal, dorsal, pectorales y pélvicas). Los peces se sacrificaron, inmediatamente se extrajeron los arcos branquiales y aletas y se colocaron en cajas de Petri con agua del medio, estos órganos se revisaron en busca de helmintos bajo microscopio esteroscópico con ayuda de pinceles finos y agujas de disección. El examen interno se realizó haciendo un corte longitudinal en el pez desde el ano hasta la altura de las aletas pectorales, prolongándose hasta la boca y separando los diferentes órganos de la cavidad del cuerpo, que después fueron colocados por separado en cajas de Petri con solución salina al 0.75%. Todos los helmintos fueron contados y separados *in situ* con ayuda de pinceles; gran parte del material recolectado fue fijado en alcohol absoluto y guardado en criotubos para su posterior análisis molecular.

Los digéneos, monogéneos y céstodos se fijaron en formol al 4% caliente, posteriormente se conservaron en viales con formol al 4% limpio. Además, con los monogéneos y metacéstodos se realizaron preparaciones semipermanentes fijadas de acuerdo al método descrito por Mendoza-Palmero *et al*. (2009), lo que permitió el estudio de las estructuras esclerotizadas. Los ejemplares se colocaron entre porta y cubre objetos aplanando ligeramente y sellando las esquinas de los cubreobjetos con barniz, aplicando picrato-amonio por las orillas del cubreobjetos dejando que penetrara por capilaridad (Mendoza-Palmero *et al*., 2009).

Los nemátodos se fijaron en formol salino al 4% caliente, después se colocaron en viales y se conservaron en este mismo fijador hasta su estudio. Después de ser identificados taxonómicamente se conservaron en alcohol al 70%.

Los acantocéfalos se colocaron en agua destilada y se refrigeraron entre 12 a 24 hrs. para que evertieran la proboscis, después se fijaron en alcohol absoluto. Para su posterior procesamiento se lavaron en alcohol al 96%. Con excepción de los nemátodos, todos los helmintos recolectados se tiñeron con Paracarmín de Meyer, y con ellos se realizaron preparaciones permanentes para su estudio morfológico y determinación taxonómica según las técnicas de tinción descritas por Pérez-Ponce de León *et al*. (2009). Una vez teñidos los ejemplares, se deshidrataron en una serie de alcoholes graduales hasta alcohol etílico absoluto, se aclararon en salicilato de metilo y se montaron en bálsamo de Canadá para hacer preparaciones permanentes con las cuales se llevó a cabo el estudio morfológico.

Los nemátodos se estudiaron mediante aclaramiento en preparaciones semipermanentes. Se aclararon en una serie progresiva de solución glicerina / agua destilada (1:20, 1:15, 1:10, 1:5, 1:2) calentando en una platina de temperatura regulada a 44ºC hasta su evaporación, con dos cambios de glicerina en cada paso (Moravec, 1998).

Los parámetros de infección parasitaria fueron descritos con base en los términos de prevalencia (porcentaje de huéspedes parasitados con una especie dada de parásito) e intensidad promedio (promedio aritmético del total de parásitos de una especie recolectados en una muestra entre el total de huéspedes parasitados) según el criterio de Margolis *et al.* (1982). De acuerdo con Esch *et al.* (1988) se distinguieron especies autogénicas (las que completan su ciclo de vida dentro del cuerpo de agua, en huéspedes acuáticos) y alogénicas (que completan su ciclo de vida fuera del cuerpo de agua, principalmente en aves).

Clasificación del inventario faunístico

Con base en los registros helmintológicos publicados para peces de la subfamilia Goodeinae y datos originales aportados en este trabajo, se preparó un listado organizado según el orden filogenético de cada grupo (Digenea, Monogenea, Cestoda, Nematoda y Acanthocephala). Las especies de huéspedes se ordenaron filogenéticamente siguiendo la propuesta de Domínguez-Domínguez *et al*. (2010).

La lista de especies de cada grupo de helminto se presenta de manera sistematizada por familia, en orden alfabético, antecediendo los representantes en estadio adulto y en segundo los de fase larvaria. Para cada taxones de parásito se indica el autor y el año de su publicación y, a su vez, se refiere a la (s) especie (s) de huésped (es) – en orden alfabético – a la (s) que esta (n) asociado (s) seguido del acrónimo de la (s) localidad (es) en donde se distribuye (n). Los registros se presentan en orden de tipo parásito-huésped-acrónimo de la localidad y corresponden a modo de filas y columnas. En el listado, cuando no se refiere el nombre de la especie huésped o al acrónimo de una localidad en la columna que le precede, se entiende que el registro corresponde a la misma especie de huésped o localidad, según sea el caso. Por último, se muestran los parámetros de infección para cada especie, incluyendo los registros previamente publicados y aquellos aportados por nuestros propios muestreos.

Resultados

Se registró un total de 55 taxones de helmintos parásitos para 36 de las 42 especies descritas a la fecha para Goodeinae (Cuadro 1, 2). En total, 57 cuerpos de agua (localidades) fueron muestreados y corresponden tanto a sistemas loticos (v.gr., ríos y arroyos) como lénticos (v.gr., manantiales, presas y lagos) (Cuadro 3). El registro helmintológico incluye 19 taxones de digéneos (11 adultos y ocho metacercarias), siete de monogéneos, 12 de céstodos (tres adultos y 9 metacéstodos), 15 de nemátodos (ocho adultos y siete larvas) y dos de acantocéfalos (un adulto y un cistacanto). Se observa un predominio de digéneos, nemátodos y céstodos (en ese orden), así como una escasez de acantocéfalos y un relativo incremento en el numero de taxones de monogéneos en comparación con registros publicados previamente.

De los 55 taxones de helmintos registrados, 20 (36%) fueron formas larvarias alogénicas cuyos adultos maduran principalmente en aves, en tanto que los 35 (64%) restantes son autogénicos adultos que en su mayoría maduran en peces. El número de estadios adultos (30 taxones o 55%), es mayor que el de las fases larvarias (25 taxones o 45%). Las prevalencias más altas se registraron en tres taxones autogénicos y dos alogénicos, respectivamente: *Margotrema bravoae*, *Salsuginus angularis* y *Rhabdochona lichtenfelsi* y *Posthodisplostomum minimum* y *Tylodelphys* sp. El dígeneo *M. bravoae* y el nemátodo *R. lichtenfelsi*, fueron los parásitos en estadio adulto con mayor numero de registros asociados a goodeinos, presentándose en 22 y 19 especies de huéspedes, respectivamente (Cuadro 1). Se reconocen once taxones endémicos de México y especialistas para la subfamilia Goodeinae: *M. bravoae, M. resolanae, Phyllodistomum* sp., *Saccocoeloides* sp., *Gyrodactylus lamothei*, *G. mexicanus*, *Gyrodactylus* sp. 1, *Gyrodactylus* sp. 2, *R. ahuehuellensis* y *R. lichtenfelsi*. Se presenta al goodeino *Allotoca goslinei* como un nuevo huésped con registro helmintológico.

En total, 112 registros son aportados por nuestros muestreos e incluyen 23 taxones de helmintos parásitos asociados a 28 especies de goodeinos, de los cuales seis son nuevos para Goodeinae. Asimismo, cinco taxones potenciales requieren ser descritos como especies nuevas para la ciencia (ver Notas en Cuadro 1)~~.~~ Se proporciona el registro helmintológico para nueve nuevas localidades (Cuadro 3). La metacercaria *Centrocestus formosanus*, el céstodo *B.*

acheilognathi y el nemátodo *Pseudocapillaria tomentosa* son especies introducidas por causas antropogénicas.

Discusión

El inventario helmintológico incluye registros para 36 de las 42 especies de goodeinos. Las seis especies de goodeinos que no forman parte de este inventario se encuentra en categoría de en peligro de extinción, estrictamente extirpadas o en cautiverio; v.gr., *Allodontichthys polylepis* Rauchenberger, 1988*; Chapalichthys peraticus* Álvarez del Villar, 1963, *Characodon garmani* Jordan & Evermann, 1898, *Ilyodon lennoni* Meyer & Foerster, 1983, *Skiffia francesae* Kingston, 1978 y *Zoogoneticus tequila* Webb & Miller, 1998 (Domínguez-Domínguez *et al*., 2005, 2008; de la Vega-Salazar, 2006). Con base en los registros helmintológicos publicados para Goodeinae y los aportados en este trabajo, es posible mencionar que la helmintofauna presente en estos peces ha sido documentada casi en tu totalidad. En este contexto, Goodeinae es prácticamente el primer grupo de vertebrados en México al que se le describe su helmintofauna en todas las especies de huéspedes potenciales actuales. De este modo, a continuación se describe brevemente los patrones descriptivos de la biodiversidad de helmintos parásitos de Goodeinae.

Todas las especies de peces muestreadas presentaron por lo menos un taxón de helminto parásito. La riqueza más alta de helmintos registrada en goodeinos se encuentra en *Goodea atripinnis* con 28 taxones de helmintos, seguida de *Xenotoca variata* con 18, en tanto que *Alloophorus robustus* y *Girardinichthys multiradiatus* presentan 17 taxones. El 35% de los taxones de helmintos registrados en este inventario son digéneos; los monogéneos constituyen el 13%, los céstodos el 22%, en tanto que los nemátodos y acantocéfalos el 27% y 4%, respectivamente. La riqueza de especies de digéneos, nemátodos y céstodos y el bajo número de especies de acantocéfalos coincide con los patrones descritos en peces dulceacuícolas para regiones neotropicales del país y para distintas cuencas hidrológicas de México (i.e., Aguilar-Aguilar *et al*., 2008; Garrido-Olvera *et al*., 2012; Pérez-Ponce de Léon *et al*., 2012).

Los digéneos son el grupo taxonómico más numerosos de helmintos para goodeinos. Los digéneos adultos y las metacercarias, *Saccocoelioides* cf. *sogandaresi*, *Magnivitellinum simplex* y *Posthodiplostomum minimum*, son especies de amplia distribución geográfica.

A la fecha, siete taxones de monogéneos han sido registrados para Goodeinae. Se ha sugerido que esta aparente pobreza se debe a una deficiencia taxonómica de muestreo más que a una característica biológica de las comunidades de helmintos en peces dulceacuícolas de México, por lo que, los monogéneos pueden ser más comunes en México de lo que se ha registrado y su inventario esta aún lejos de completarse (Mendoza-Franco *et al*., 2003; Pérez-Ponce de León & Choudhury, 2010). Es evidente que, con base en los esfuerzos recientes de trabajos de inventarios sistematizados, la composición taxonómica en monogéneos en México ha incrementado notoriamente en los últimos 10 años, por lo que se espera en estudios próximos se contraste este aparente patrón de escasa riqueza específica.

La mayor proporción de céstodos esta constituida por estadios larvarios; nueve de 12 taxones registrados. En particular, los metacéstodos de la familia Dilepididae son parásitos relativamente frecuentes en peces dulceacuícolas del centro y sureste de México y se tienen registrados cinco en ocho especies de goodeinos (Cuadro 1).

La composición taxonómica de los nemátodos presenta cierto grado de riqueza alta y equitativa en estadios adultos y larvarios, con una distribución geográfica relativamente extensa. Esto se debe a su amplia capacidad de infección, baja especificidad y a la amplia distribución de sus huéspedes intermediarios y definitivos.

Dos especies de acantocéfalos fueron registrados: *Pomphorhynchus* cf. *bulbocolli* y *Polymorphus brevis*. La escasa presencia de acantocéfalos en Goodeinae constituye un patrón previamente registrado para otras regiones del país (ver García-Prieto *et al*., 2010; Pérez-Ponce de León & Chodhury, 2010; Pérez-Ponce de León *et al*., 2012).

Los taxones autogénicos representan una mayor proporción (64%) que las alogénicas (36%). El alto porcentaje de taxones autogénicos registrados en este trabajo refleja implícitamente la relevancia ecológica y evolutiva que representan los helmintos parásitos de Goodeinae por tener aparentemente estrechas asociaciones históricas, en el que a su vez reflejan un amplio acervo genético presumiblemente especializado tanto para condiciones bióticas como para recursos abióticos, incluyendo ciclos de vida complejos, cierta calidad ambiental y supervivencia en ambientes acuáticos determinados tanto de huéspedes intermediarios invertebrados como de sus huéspedes definitivos vertebrados.

Los taxones alogénicos registrados para goodeinos son generalistas y con una distribución geográfica relativamente amplia. El proceso de dispersión de los taxones alogénicos es debida a los hábitos alimentarios de sus huéspedes definitivos (aves ictiófagas) y, como efecto secundario, a su capacidad de vagilidad intrínseca. En este sentido, los taxones alogénicos registrados para Goodeinae son evidencia de que los peces goodeinos forman parte de la cadena trófica de las aves; esto puede reflejar a lo largo de la posición geográfica de cada uno de los registros de especies alogénicas, posibles rutas de migración de distintos grupos de aves, en particular Anatidae y Ardeidae.

Algunos otros taxones de helmintos se pueden encontrar como adultos en anfibios, como el caso del dígeneo *Apharyngostrigea* sp. que se desarrolla en ranas (Locke *et al*., 2011). Otros taxones se han registrado en estadios adultos en reptiles, como la larva de digéneo *Ochetosoma bravicaecum*, el cuál madura en serpientes acuáticas del género *Thamnophis* (Jiménez-Ruiz *et al*., 2002); o bien, las larvas de nemátodos *Falcaustra* sp., *Serpinema trispinosum*, *Spiroxys* sp., que maduran en tortugas (Garrido-Olvera *et al*., 2006; Pérez-Ponce de León *et al*., 2007).

Cuatro de las especies de helmintos registradas fueron introducidas antropogénicamente a cuerpos epicontinentales de México: *C. formosanus*, *B. acheilognathi* y *P. tomentosa.* En este contexto, se denota que las comunidades de helmintos parásitos de goodeinos están sujetas a invasión por especies exóticas.

La metacercaria de *C. formosanus* fue introducida a través del caracol *Thiara tuberculata* (Aguilar-Aguilar *et al*., 2009). Actualmente ésta especie presenta una capacidad de infección parasitaria amplia en los peces dulceacuícolas de México registrada en 67 especies de 13 familias de peces dulceacuícolas (Aguilar-Aguilar *et al*., 2009; 2013; Pérez-Ponce de León *et al*., 2010).

La presencia de *B. acheilognathi* en peces nativos de México está relacionada con la introducción de la carpa herbívora *Ctenopharyngodon idella* en 1965 (López-Jiménez, 1981). A partir de entonces, se ha extendido su distribución a múltiples localidades infectando a un gran número de huéspedes, tanto de especies nativas como introducidas en México, debido a su gran capacidad de infección, baja especificidad y a la potencialidad de distribución de sus huéspedes intermediarios (ver Rojas-Sanchéz & García-Prieto, 2008). Sin embargo, a pesar de registrar este hecho en la literatura especializada a la fecha no se ha realizado una propuesta seria que promueva una estrategia política para tratamiento de parásitos silvestres introducidos para peces dulceacuícolas (Martínez-Aquino & Aguilar-Aguilar, 2008; Martínez-Aquino *et al*., 2011; Aguilar-Aguilar *et al*., 2013). Por tanto, es importante resaltar que los 52 registros de este céstodo exótico en 39 localidades para 14 especies de goodeinos, representan una amenaza biológica para los distintos programas de conservación de fauna silvestre (Domínguez-Domínguez *et al*., 2010). Evidentemente, un trabajo sistematizado e integrativo, con datos moleculares y con valores de los parámetros de infección para *B. acheilognathi*, permitirá hacer de manera más explícita la necesidad de promover estrategias de manejo de recursos naturales en aguas interiores mexicanas.

El nemátodo *P. tomentosa* también fue introducido a México con un ciprínido, en este caso la carpa común *Cyprinus carpio* (Moravec *et al*., 2001). *Pseudocapillaria tomentosa* actualmente se encuentra registrada en México en 12 especies de huéspedes de tres familias (Garrido-Olvera *et al*., 2006).

Salgado-Maldonado *et al*. (2001) y Sánchez-Nava *et al*. (2004) registraron a "*Gyrodactylus* cf. *elegans"* (Monogenea: Gyrodactylidae) en *G. multiradiatus* en el centro de México; sin embargo, no fue posible corroborar la validez taxonómica de este registro debido a que estos autores no depositaron ejemplares de referencia en ninguna colección científica nacional o internacional. Adicionalmente, en nuestros muestreos ningún monogéneo recolectado mostró similitud morfológica con esta especie de girodactílido, el cuál es específico para *Abramis brama* (Cypriniformes: Cyprinidae) que se distribuyen en Eurasia (Harris *et al*., 2004). Por esta razón, en este trabajo no consideramos el registro de "*Gyrodactylus* cf. *elegans"* como válido.

El género *Salsuginus* Beverly-Burton, 1984 incluye 12 especies registradas en peces de cuatro familias pertenecientes al orden Cyprinodontiformes: Fundulidae, Cyprinodontidae, Poeciliidae y Goodeidae (Mendoza-Franco & Vidal-Martínez, 2001; Martínez-Aquino *et al*., 2004, 2007b). El presente trabajo contiene 14 nuevos registros del género *Salsuginus* en 12 especies de goodeinos: *A. diazi*, *A. dugesi*, *A. splendens*, *G. atripinnis*, *I. furcidens*, *I. whitei*, *S. multipunctata*, *X. resolanae*, *X. melanosoma*, *X. variata* y *Z. quitzeoensis*. Estos registros concuerdan con la especificidad propuesta para especies de *Salsuginus* para peces del orden Cyprinodontiformes y representan una afinidad biogeográfica Neártica.

Se distinguen 13 taxones de helmintos como endémicos para sistemas hidrológicos del centro de México: los digéneos *A. mexicanum*, *Allocreadim* sp., *M. bravoae*, *M. resolanae*, *Phyllodistomum* sp. y *Saccocoeloides* sp.; los monogéneos *G. lamothei*, *G. mexicanus*, *Gyrodactylus* sp. 1 y *Gyrodactylus* sp. 2 y los nemátodos *R. ahuehuellensis*, *R. lichtenfelsi* y *R. xiphophori*. Estos taxones – a excepción de *A. mexicanum*, *Gyrodactylus* sp. y *R. xiphophori* – se consideran como parásitos especialistas para Goodeinae y reflejan una estrecha relación con la historia evolutiva de estos peces. En este contexto, dichos taxones representan una parte de las *faunas principales* de helmintos en un sentido biogeográfico *sensu* Pérez-Ponce de León & Choudhury (2005).

El nemátodo *R. lichtenfelsi* originalmente fue descrito en peces de la subfamilia Goodeinae (Sánchez-Álvarez *et al*., 1998) y su especificidad en peces de esta subfamilia ha sido bien documentada (Pérez-Ponce de León & Choudhury, 2005; Garrido-Olvera *et al*., 2006; Mejía-Madrid *et al*., 2005, 2007b). Los 16 registros aportados en este trabajo en 10 especies de goodeinos, más los 28 publicados previamente en 19 especies de goodeinos, se suman a la propuesta de especificidad de este nemátodo para Goodeinae. El nemátodo *R. ahuehuellensis* fue descrita como asociada a *I. furcidens* en un afluente del río Balsas (Mejía-Madrid y Pérez-Ponce de León, 2003). Los tres registros documentados en este estudio en tres especies de goodeinos, más los 10 publicados previamente en seis especies de goodeinos, apoyan la especificidad propuesta para este nemátodo asociado a Goodeinae (Mejía-Madrid *et al*., 2005, 2007b).

El género *Margotrema* fue descrito originalmente para incluir a *M. bravoae* Lamothe-Argumedo, 1970 como parásito de *Girardinichthys multiradiatus* en La Lagunilla, Estado de México (Lamothe-Argumedo, 1972). Treinta años después, se describió una segunda especie para este género, *M. guillerminae* Pérez-Ponce de León, 2001 como parásito de los peces *Notropis calientis* (Cyprinidae) y *Alloophorus robustus* (Goodeinae) en el Lago de Zacapu, Michoacán (Pérez-Ponce de León, 2001). Martínez-Aquino *et al*. (2013) delimitaron ambas especies *putativas* con base en análisis filogenéticos moleculares a lo largo de su área de distribución, y propusieron la sinonimia de *M. guillerminae* con *M. bravoae*. Posteriormente, con base en un detallado estudio taxonómico, se describió a *M. resolanae* como parásito del goodeino *Xenotaenia resolanae* (Pérez-Ponce de León *et al*., en prensa).

Con base en los avances de los registros helmintológicos publicados para Goodeinae, distintos autores han descrito patrones biogeográficos entre goodeinos y sus helmintofaunas principales, los cuales representan la integración de dicha acumulación de información (ver

Pérez-Ponce de León & Choudhury, 2005; Martínez-Aquino *et al*., 2007; Garrido-Olvera *et al*., 2012). Por otra parte, Mejía-Madrid *et al*. (2007a, b) y Martínez-Aquino *et al*. (2013) han usado de manera explícita los registros de helmintos parásitos específicos para Goodeinae para poner a prueba hipótesis evolutivas acerca de los eventos históricas que ocurrieron entre Goodeinae y sus faunas principales de parásitos, esto es *M. bravoae*, *M. resolanae*, *R. ahuehuellensis* y *R. lichtenfelsi*. En este contexto, se resalta la utilidad, tanto de los registros aislados como de los organizados de manera sistematizada, para la descripción de la helmintofauna de peces dulceacuícolas en México. Aún cuando algunos autores han sugerido que la diversidad de helmintos para peces dulceacuícolas en México presumiblemente esta próxima a ser completada (Pérez-Ponce de León & Choudhury, 2010), los eventos evolutivos que se involucran en los procesos de diversificación de los parásitos quedan aún por descubrir. Por ejemplo, de las 11 especies que se infiere en este trabajo como *helmintofaunas principales* para Goodeinae, solo tres han sido analizadas detalladamente y se ha detectado diferencias genealógicas asociadas al escenario geográfico y a la historia evolutiva de sus huéspedes (Mejía-Madrid *et al*., 2007b, Martínez-Aquino *et al*., 2013). Los otros ocho taxones de helmintos son modelos biológicos potenciales para expandir el conocimiento referente a las asociaciones históricas exclusivas para goodeinos de México; v. gr *A. mexicanum*, *Allocreadim* sp., *Phyllodistomum* sp., *Saccocoeloides* sp., *G. lamothei*, *G. mexicanus*, *Gyrodactylus* sp. 1 y *Gyrodactylus* sp. 2. Futuros estudios en estos taxones ayudaran a comprender los patrones de diversificación parasitaria en peces dulceacuícolas que habitan en el territorio nacional.

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Cuadro 1. Helmintos parásitos de Goodeinae. Los acrónimos y características de las localidades se refieren en el Cuadro 3.
A = Acrónimo de cada localidad; N = Número de huéspedes examinados; P (%) = Prevalencia expresada en porcentaje;
IP ± de = Intensidad promedio ± desviación estandar; ND = No hay datos disponibles; Au = Autogénica; Al = Alogénica;
Gn = Generalista; Es = Especialista. Sitios de infección: A = Aletas; Aa = Aleta anal; B = Branquias; Cb = Cavidad branquial;
Cc = Cavidad del cuerpo; Co = Corazón; G = Grasa; H = Hígado; I = Intestino; M = Mesenterio; Mu = Musculo; O = Ojos;
P = Piel; Pi = Pared intestinal; Vb = Vesícula biliar; Vu = Vejiga urinaria; * = Cyprinidae; ** Cyprinodontidae.

Taxa (Helminto)	**Huésped / Sitio de infección**	**A**	**N**	**P (%)**	**IP ± de**	**Referencia**
Digenea						
Fam. Allocreadiidae Looss 1902						
Allocreadium lobatum$^{\mathrm{Au/Gn}}$	*Allotoca zacapuensis* / I	Zaca	17	5.8	0.1 ± 0.5	Mejía-Madrid *et al*., 2005
Wallin, 1909	*Zoogoneticus quitzeoensis* / I	Zaca	15	13.3	0.4 ± 1.3	Mejía-Madrid *et al*., 2005
Allocreadium mexicanum$^{\mathrm{Au/Gn}}$	*Characodon audax* / I	Tobo	10	20	0.8 ± 1.7	Mejía-Madrid *et al*., 2005
Osorio-Sarabia,	*Characodon lateralis* / I	Amad	15	46.7	0.8 ± 1.3	Mejía-Madrid *et al*., 2005
Pérez-Ponce de León						
y Salgado-Maldonado, 1986						
Allocreadium sp.$^{\mathrm{Au/?}}$	*Characodon audax* / I	Tobo	42	4.7	ND	Pérez-Ponce de León *et al*., 2009
Nota 1		27No	9	44	ND	Pérez-Ponce de León *et al*., 2009
	Characodon lateralis / I	Amad	9	27	ND	Pérez-Ponce de León *et al*., 2009
Fam. Macroderoididae						
McMullem, 1937						
Margotrema bravoae$^{\mathrm{Au/Es}}$	*Allodontichthys zonistius* / I	Siem	6	6	3	Salgado-Maldonado *et al*., 2004b
Lamothe-Argumedo, 1970		Ahua	10	70	3 ± 2.8	Mejía-Madrid *et al*., 2005
Nota 2			11	100	7.18 ± 5.9	Martínez-Aquino *et al*., 2013
	Allodontichthys hubbsi / I	Tule	6	33.3	0.5 ± 0.8	Mejía-Madrid *et al*., 2005

Allodontichthys tamazulae / I	Tama	7	42.9	1 ± 1.4	Mejía-Madrid *et al*., 2005
Alloophorus robustus / I	Mcha	67	ND	ND	Pérez-Ponce de León, 2001
	Mcha	34	14.7	0.3 ± 0.7	Mejía-Madrid *et al*., 2005
	Luz	10	10	1.6	Mejía-Madrid *et al*., 2005
	Mint	27	7.4	ND	Romero-Tejada *et al*., 2008
		14	7.14	1 ± 0.27	Martínez-Aquino *et al*., 2013
	Zaca	ND	ND	ND	Pérez-Ponce de León, 2001
Allotoca diazi / I	Mcha	40	ND	ND	Pérez-Ponce de León, 2001
		34	14.7	0.3 ± 0.7	Mejía-Madrid *et al*., 2005
		21	23.81	6.2 ± 3.47	Martínez-Aquino *et al*., 2013
		9	33.33	7 ± 5.59	Presente trabajo
Allotoca dugesii / I	Mcha	6	100	3.7 ± 1.7	Mejía-Madrid *et al*., 2005
		22	ND	ND	Martínez-Aquino *et al*., 2013
Allotoca maculata / I	Marc	15	66.8	2.1 ± 2.8	Mejía-Madrid *et al*., 2005
Allotoca meeki / I	Opop	19	79	5 ± 9.1	Mejía-Madrid *et al*., 2005
		4	75	15 ± 11.18	Martínez-Aquino *et al*., 2013
Allotoca zacapuensis / I	Zaca	17	35.3	0.6 ± 1	Mejía-Madrid *et al*., 2005
	Zaca	32	25	ND	Martínez-Aquino *et al*., 2012
Chapalichthys pardalis / I	Tocu	11	18.2	0.4 ± 0.9	Mejía-Madrid *et al*., 2005
		38	10.53	1 ± 0.31	Martínez-Aquino *et al*., 2013
Characodon audax / I	Tobo	30	63	1.84 ± 1.21	Martínez-Aquino *et al*., 2007b
	Tobo	10	10	0.3 ± 0.9	Mejía-Madrid *et al*., 2005
	Abra	12	25	ND	Pérez-Ponce de León *et al*., 2001
	Pin1	34	47	ND	Pérez-Ponce de León *et al*., 2001
	Guad	14	14.2	ND	Pérez-Ponce de León *et al*., 2001
*Cyprinella lutrensis** / I	Buen	38	ND	ND	Pérez-Ponce de León *et al*., sometido
Cyprinodon nazas ** / I	Sofí	27	3.7	ND	Pérez-Ponce de León *et al*., 2009
Girardinichthys multiradiatus / I	Lagu	64	ND	ND	Lamothe-Argumedo, 1970
	Vict	5	40	22.5 ± 6.4	Sánchez-Nava *et al.,* 2004
	Porv	36	5.6	1	Sánchez-Nava *et al.,* 2004
Goodea atripinnis / I	Verd	25	12	0.2 ± 0.5	Mejía-Madrid *et al*., 2005
Ilyodon cortesae / I	Cutz	30	6.7	0.25 ± 0.5	Mejía-Madrid *et al*., 2005
Ilyodon furcidens / I	Tama	11	9.1	0.1 ± 0.3	Mejía-Madrid *et al*., 2005
	Ahua	11	100	4.55 ± 4.59	Martínez-Aquino *et al*., 2013
	Tocu	37	2.70	3 ± 0.49	Martínez-Aquino *et al*., 2013
Ilyodon white / I	Ahue	180	72.2	2.7 ± 4.6	Mejía-Madrid *et al*., 2005

			35	34.29	5.75 ± 4.06	Martínez-Aquino *et al*., 2013
		Potg	4	25	0.25 ± 0.5	Mejía-Madrid *et al*., 2005
			10	20	4 ± 2.2	Martínez-Aquino *et al*., 2013
	Neoophorus regalis / I	Marc	11	54.5	1.8 ± 2.6	Mejía-Madrid *et al*., 2005
		Reye	7	14.3	0.1 ± 0.4	Mejía-Madrid *et al*., 2005
		Rico	28	35.71	2.8 ± 1.96	Martínez-Aquino *et al*., 2013
	Notropis calientis * / I	Zaca	ND	ND	ND	Pérez-Ponce de León, 2001
	Xenotoca variata / I	Mint	31	6	1	Salgado-Maldonado, 2006
	Zoogoneticus purhepechus / I	Luz	45	2.2	1	Martínez-Aquino *et al*., 2011
		Mint	30	7	3.5 ± 0.71	Martínez-Aquino, 2005 (datos de tesis)
			ND	ND	ND	Salgado-Maldonado, 2006
			30	6.7	ND	Romero-Tejada *et al*., 2008
			22	13.64	1.67 ± 0.69	Martínez-Aquino *et al*., 2013
	Zoogoneticus quitzeoensis / I	Zaca	32	46.88	ND	Martínez-Aquino *et al*., 2012
Margotrema resolanae$^{\text{Au/Es}}$ Pérez-Ponce de Léon, Martínez-Aquino y Mendoza-Garfías, sometido	*Xenotaenia resolanae* / I	Cuza	35	80	4.68 ± 5.6	Martínez-Aquino *et al*., 2009a
Margotrema sp.$^{\text{Au/Es?}}$	*Codoma ornata* * / I	Piax	21	28.57	ND	Aguilar-Aguilar *et al*., 2010
	Characodon audax / I	Tobo	42	23.8	ND	Pérez-Ponce de León *et al*., 2001
		Pin1	34	28.5	ND	Pérez-Ponce de León *et al*., 2001
	Goodea atripinnis / I	Igna	ND	ND	ND	Salgado-Maldonado *et al*., 2006
	Xenotaenia resolanae / I	Cuza	36	33	5.67 ± 5.76	Martínez-Aquino, 2005 (datos de tesis)
	Notropis sp. / I	Tamo				Pérez-Ponce de León (com. pers.)
		Sand				Pérez-Ponce de León (com. pers.)
Fam. Gorgoderiade (Loss, 1899)						
Loss, 1901						
Dendrorchis sp.$^{\text{Au/Es?}}$	*Ilyodon furcidens* / I	Siem	57	2	1	Salgado-Maldonado *et al.,* 2004b
	Xenotaenia resolanae / Vu	Cuza	35	5.71	1.5 ± 0.71	Martínez-Aquino *et al*., 2009b

Phyllodistomum sp.$^{\text{Au/?}}$	*Allodontichthys zonistius* / I	Tama	32	9.38	1.33 ± 0.42	Presente trabajo
Nota 3	*Allotoca zacapuensis* / I, Vu	Zaca	32	9.38	ND	Martínez-Aquino *et al*., 2012
	Hubbsina turneri / Vu	Zaca	20	10	ND	Martínez-Aquino *et al*., 2012
	Ilyodon furcidens / I	Tama	56	7.14	1.25 ± 0.35	Presente trabajo
	Zoogoneticus purhepechus / Vu	Luz	45	2.2	0.02 ± 0.15	Martínez-Aquino *et al*., 2011
	Zoogoneticus quitzeoensis / Vu	Zaca	32	15.63	ND	Martínez-Aquino *et al*., 2012
Fam. Haploporidae Nicoll, 1914						
Saccocoeliodes cf. *sogandaresi*$^{\text{Au/Gn}}$ Lumsden, 1961	*Allodontichthys zonistius* / I	Siem	16	56	69 ± 68	Salgado-Maldonado *et al.,* 2004b
	Goodea atripinnis / I	Zaca	20	75	ND	Martínez-Aquino *et al*., 2012
	Ilyodon furcidens / I	Siem	51	10	10 ± 1	Salgado-Maldonado *et al.,* 2004b
	Ilyodon whitei / I	Chis	22	5	ND	Salgado-Maldonado *et al.,* 2001a
Saccocoeliodes sp.$^{\text{Au/Es?}}$	*Ameca splendens* / I	Teuc	35	28	3.2 ± 3	Martínez-Aquino, 2005 (datos de tesis)
Nota 4			33	54.55	3.61 ± 2.32	Presente trabajo
Fam. Macroderoididae McMullem, 1937						
Magnivitellinum simplex$^{\text{Au/Gn}}$ Kloss, 1966	*Ilyodon furcidens* / I	Siem	51	2	1	Salgado-Maldonado *et al.,* 2004b
Metacercarias						
Fam. Clinostomidae Lühe, 1901						
Clinostomun cf. *complanatum*$^{\text{Al/Gn}}$ (Ruddolphi, 1814)	*Allodontichthys zonistius* / Cc	Siem	5	20	1	Salgado-Maldonado *et al.,* 2004b
		Tama	32	9.38	1.67 ± 0.57	Presente trabajo
	Alloophorus robustus / N. R.	Cuit	30	90	ND	Guzmán-Cornejo y García-Prieto, 1999
	/ H, M	Pátz	41	ND	ND	Peresbarbosa-Rojas *et al*., 1994

	Pátz	67	27	ND	Pérez-Ponce de León *et al*., 2000
/ Cc	Mint	7	43	4.7 ± 4.7	Martínez-Aquino, 2005 (datos de tesis)
	Zaca	17	11.76	ND	Martínez-Aquino *et al*., 2012
Allotoca diazi / H, M	Pátz	31	ND	ND	Peresbarbosa-Rojas *et al*., 1994
		40	10	ND	Pérez-Ponce de León *et al*., 2000
Chapalichthys encaustus / Cc	Luz	8	12.5	4.7 ± 7.07	Presente trabajo
Characodon audax / H	Tobo	30	6.7	1	Martínez-Aquino *et al*., 2007b
/ Cc, H, M	Pin1	34	28.3		Pérez-Ponce de León *et al*., 2009
	Pin2	21	28.3		Pérez-Ponce de León *et al*., 2009
	27No	9	22.2		Pérez-Ponce de León *et al*., 2009
/ H	Berr		21.7		Pérez-Ponce de León *et al*., 2009
Goodea atripinnis / N. R.	Cuit	30	13	ND	Guzmán-Cornejo y García-Prieto, 1999
/ H	Igna	22	5	9 ± ND	Salgado-Maldonado *et al.,* 2001b
/ H	Pátz	178	0.6	4 ± ND	Salgado-Maldonado y Osorio-Sarabia, 1987
/ M	Mint	27	30	2 ± 1.41	Martínez-Aquino, 2005 (datos de tesis)
/ N. R.	Atot	ND	ND	ND	Salgado-Maldonado, 2006
/ N. R.	Reme	ND	ND	ND	Salgado-Maldonado, 2006
Ilyodon furcidens / Cc	Potg	10	20	1	Martínez-Aquino, 2005 (datos de tesis)
		10	10	1 ± 0.32	Presente trabajo
	Siem	51	6	1	Salgado-Maldonado *et al*., 2004b
	Siem	51	6	1	Salgado-Maldonado *et al.,* 2004b
	Tama	56	3.57	1 ± 0.19	Presente trabajo
Skiffia lermae / Cc	Mint	61	2	2	Martínez-Aquino, 2005 (datos de tesis)
		ND	ND	ND	Salgado-Maldonado, 2006
		30	3.33	ND	Romero-Tejada *et al*., 2008
Xenotoca variata / N. R.	Cuit	41	26.8	ND	Guzmán-Cornejo y Garcia-Prieto, 1999
/ N. R.	Laja	ND	ND	ND	Salgado-Maldonado, 2006
/ M	Mint	31	3	1	Martínez-Aquino, 2005 (datos de tesis)
/ N. R.		ND	ND	ND	Salgado-Maldonado, 2006
/ Cc		7	14.29	ND	Romero-Tejada *et al*., 2008
N. R.	Reme	ND	ND	ND	Salgado-Maldonado, 2006
N. R.	Ign	ND	ND	ND	Salgado-Maldonado, 2006
N. R.	Atot	ND	ND	ND	Salgado-Maldonado, 2006
/ Cc	Zaca	21	4.76	ND	Martínez-Aquino *et al*., 2012
Xenotaenia resolanae / Cc	Cuza	35	2.86	1	Martínez-Aquino *et al*., 2009a
Zoogoneticus purhepechus	Luz	45	11.1	0.13 ± 0.4	Martínez-Aquino *et al*., 2011

	/ Cc, M					
	Zoogoneticus quitzeoensis / Cc	Mint	30	3	1	Martínez-Aquino, 2005 (datos de tesis)
	/ N. RR		ND	ND	ND	Salgado-Maldonado, 2006
	/ Cc		30	6.6	ND	Romero-Tejada *et al*., 2008
Fam. Diplostomidae Poirier, 1886						
Apharyngostrigea sp.$^{\text{Al/Gn}}$	*Goodea atripinnis* / N. R.	Laja	ND	ND	ND	Salgado-Maldonado, 2006
Tylodelphys sp.$^{\text{Al/Gn}}$	*Allodontichthys zonistius* / Cc	Tama	32	3.13	1 ± 0.18	Presente trabajo
	Allotoca zacapuensis / Cc	Zaca	32	6.25	ND	Martínez-Aquino *et al*, 2012
	Girardinichthys multiradiatus	Almo	20	70	5.1 ± 4.5	Sánchez-Nava *et al.,* 2004
	/ Cc, M, O	Sant	49	71.4	8.9 ± 16	Sánchez-Nava *et al.,* 2004
		Atla	15	7	2	Sánchez-Nava *et al.,* 2004
		Chic	94	45	6.4 ± ND	Salgado-Maldonado *et al*., 2001b
			92	45.7	6.2 ± 9.2	Sánchez-Nava *et al.,* 2004
		Huap	1	100	4	Sánchez-Nava *et al.,* 2004
		Ignr	75	15	3 ± 2.4	Sánchez-Nava *et al.,* 2004
		Juan	53	70	4.5 ± 5.2	Sánchez-Nava *et al.,* 2004
			58	3.4	4 ± 4.2	Sánchez-Nava *et al.,* 2004
		Lagu	50	20	2.6 ± 31	Sánchez-Nava *et al.,* 2004
		Mina	21	19	2.6 ± 31	Sánchez-Nava *et al.,* 2004
		Sala	12	67	4.2 ± 3.6	Sánchez-Nava *et al.,* 2004
		Sant	11	73	6 ± 3.9	Sánchez-Nava *et al.,* 2004
		Trin	31	3	1	Sánchez-Nava *et al.,* 2004
		Vent	40	7.5	1	Sánchez-Nava *et al.,* 2004
		Vict	5	20	1	Sánchez-Nava *et al.,* 2004
		Zemp	20	40	4.4 ± 2.4	Sánchez-Nava *et al.,* 2004
	Goodea atripinnis / Cc	Bizn	18	61	2.4 ± ND	Salgado-Maldonado *et al.,* 2001b
		Igna	20	30	9.7 ± ND	Salgado-Maldonado *et al.,* 2001b
		Trin	29	3	1	Salgado-Maldonado *et al.,* 2001b
		Mint	27	15	7 ± 6.98	Martínez-Aquino, 2005 (datos de tesis)
	/ N. R.	Laja	ND	ND	ND	Salgado-Maldonado, 2006
	/ N. R.	Cuit	30	6.6	ND	Guzmán-Cornejo y García-Prieto, 1999
		Zaca	20	10	ND	Martínez-Aquino *et al*., 2012
	Hubbsina turneri / Cb	Zaca	20	15	ND	Martínez-Aquino *et al*., 2012

	Skiffia lermae / Cb, Cc, H, M	Mint	61	24	2.6 ± 3.8	Martínez-Aquino, 2005 (datos de tesis)
	/ N. R.		ND	ND	ND	Salgado-Maldonado, 2006
	/ Cc	Zaca	19	68.42	ND	Martínez-Aquino *et al*., 2012
	Xenotoca variata / Cb, Cc	Mint	31	13	1.5 ± 0.58	Martínez-Aquino, 2005 (datos de tesis)
	/ N. R.		ND	ND	ND	Salgado-Maldonado, 2006
	/ O		7	14.29	ND	Romero-Tejada *et al*., 2008
	/ Cc	Zaca	21	4.76	ND	Martínez-Aquino *et al*., 2012
	Zoogoneticus quitzeoensis / Cc	Mint	30	13	1	Martínez-Aquino, 2005 (datos de tesis)
	/ N. R.		ND	ND	ND	Salgado-Maldonado, 2006
	/ Cc	Zaca	32	6.25	ND	Martínez-Aquino *et al*., 2012
Posthodiplostomum minimum$^{Al/Gn}$ (MacCallum, 1921) Dubois, 1936	*Allodontichthys zonistius* / M	Siem	16	13	1	Salgado-Maldonado *et al.,* 2004b
	/ Cc	Tama	32	3.13	4 ± 0.71	Presente trabajo
	Alloophorus robustus / H, M, Mu, O	Pátz	41	ND	ND	Peresbarbosa-Rojas *et al*., 1994
	/ H, M, Mu,		67	81	ND	Pérez-Ponce de León *et al*., 2000
	/ N. R.	Cuit	30	93.3	ND	Guzmán-Cornejo y García-Prieto, 1999
	/ N. R.		30	93	57.1 ± ND	Salgado-Maldonado *et al.,* 2001b
	/ G	Mint	7	28	1.5 ± 0.71	Martínez-Aquino, 2005 (datos de tesis)
	/ Cc	Pasj	5	60	5 ± 4.64	Presente trabajo
		Ucas	11	54.55	44.83 ± 77.47	Presente trabajo
	Allotoca diazi / H, M, Mu, O	Pátz	31	N. D	N. D	Peresbarbosa-Rojas *et al*., 1994
			40	70	N. D	Pérez-Ponce de León *et al*., 2000
		Mcha	9	88.89	57.1 ± 56.90	Presente trabajo
	Allotoca goslinei / Cc	Potg	2	100	1	Presente trabajo
	Allotoca zacapuensis / Cc, H, M	Zaca	32	28.13	ND	Martínez-Aquino *et al*., 2012
	Chapalichthys encaustus / H, M, O, Cc	Lcha	50	88	77.18 ± 130.3	Martínez-Aquino *et al.,* 2004
	Characodon audax / Cb, Cc, H, M	Tobo	30	100	53.63 ± 41.2	Martínez-Aquino *et al*., 2007b
		27No	9	22.2		Pérez-Ponce de León *et al*., 2009
		Guad	14	42.8		Pérez-Ponce de León *et al*., 2009
		Tobo	42	80.9		Pérez-Ponce de León *et al*., 2009

Girardinichthys multiradiatus / H, M	Chic	118	22	1	Salgado-Maldonado *et al.,* 2001b
		52	1.9	8	Sanchéz-Nava *et al*., 2004
		44	50	1.27 ± 0.89	Presente trabajo
	Ignr	13	8	3	Salgado-Maldonado *et al.,* 2001b
		14	28.57	9 ± 4.83	Presente trabajo
	Lerm	9	ND	ND	León-Règagnon, 1992
	Juan	53	5.7	1.3 ± 0.6	Sánchez-Nava *et al.,* 2004
		58	5.2	1	Sánchez-Nava *et al.,* 2004
		50	2	1	Sánchez-Nava *et al.,* 2004
	Porv	36	2.8	3	Sánchez-Nava *et al.,* 2004
	Sala	32	93.75	29.67 ± 44.38	Presente trabajo
	Sant	11	9.1	1	Sánchez-Nava *et al.,* 2004
		11	9	1	Sánchez-Nava *et al.,* 2004
	Sier	30	6.7	1	Sánchez-Nava *et al.,* 2004
	Vent	40	2.5	1	Sánchez-Nava *et al.,* 2004
	Tica	12	91.67	1 ± 0.29	Presente trabajo
	Ties	6	33.33	6 ± 4	Presente trabajo
	Tipr	2	50	10 ± 7.07	Presente trabajo
Girardinichthys viviparus / H, M	Chap	31	13	1.75 ± 0.96	Martínez-Aquino, 2005 (datos de tesis)
Goodea atripinnis / M	Bizn	25	60	10.8 ± ND	Salgado-Maldonado *et al.,* 2001b
/ H, Mu	Pátz	178	62	13.3 ± ND	Salgado-Maldonado y Osorio-Sarabia, 1987
/ H, M, Mu, O		35	ND	ND	Peresbarbosa-Rojas *et al.,* 1994
/ H, M, Mu		59	83	ND	Pérez-Ponce de León *et al*., 2000
		30	76.67	36.87 ± 36.19	Presente trabajo
/ H, M	Igna	22	55	5.7 ± ND	Salgado-Maldonado *et al.,* 2001b
/ M	Trin	4	25	7 ± ND	Salgado-Maldonado *et al.,* 2001b
/ Cc, H, M, Mu, O	Juam	6	100	ND	Salgado-Maldonado *et al.,* 2001a
/ N. R.	Cuit	30	86.6	ND	Guzmán-Cornejo y García-Prieto, 1999
/ H	Chap	20	15	1	Martínez-Aquino, 2005 (datos de tesis)
/ Cc, H, M	Lcha	8	62	61.4 ± 76.5	Martínez-Aquino, 2005 (datos de tesis)
/ H, M	Mint	27	22	1.67 ± 1.03	Martínez-Aquino, 2005 (datos de tesis)
/ N. R.	Laja	ND	ND	ND	Salgado-Maldonado 2006
/ H, M	Coin	4	75	36.67 ± 30.93	Martínez-Aquino, 2005 (datos de tesis)
/ N. R.		ND	ND	ND	Salgado-Maldonado, 2006

/ N. R.	Rinc	ND	ND	ND	Salgado-Maldonado, 2006
/ N. R.	Marí	ND	ND	ND	Salgado-Maldonado, 2006
/ N. R.	Cien	ND	ND	ND	Salgado-Maldonado, 2006
/ N. R.	Sori	ND	ND	ND	Salgado-Maldonado, 2006
/ N. R.	Laad	ND	ND	ND	Salgado-Maldonado, 2006
/ N. R.	Atot	ND	ND	ND	Salgado-Maldonado, 2006
/ N. R.	Ferr	ND	ND	ND	Salgado-Maldonado, 2006
/ Cc, H, M	Zaca	20	25	ND	Martínez-Aquino *et al*., 2012
/ Cc	Teuc	38	100	140 ± 134	Presente trabajo
/ Cc	Chic	29			Presente trabajo
Ilyodon furcidens / M	Siem	51	33	2 ± 2	Salgado-Maldonado *et al.,* 2004b
/ H, M	Potg	10	70	8.29 ± 10.23	Martínez-Aquino, 2005 (datos de tesis)
		10	30	12.33 ± 6.07	Presente trabajo
/ G	Puen	3	33	2	Martínez-Aquino, 2005 (datos de tesis)
/ Cc	Tama	56	1.79	1 ± 0.13	Presente trabajo
Skiffia lermae / C, Cc, M	Mint	61	39	4.21 ± 3.5	Martínez-Aquino, 2005 (datos de tesis)
/ N. R.		ND	ND	ND	Salgado-Maldonado, 2006
/ C, Cc		30	30	N. D	Romero-Tejada *et al*., 2008
/ C, Cc, M	Zaca	19	21	N. D	Martínez-Aquino *et al*., 2012
/ Cc	Chap	32	100	50.13 ± 34.05	Presente trabajo
Xenotaenia resolanae / M	Cuza	36	8	1.67 ± 1	Martínez-Aquino, 2005 (datos de tesis)
		35	2.86	1	Martínez-Aquino *et al*., 2009a
Ilyodon whitei / Cc	Tlap	35	97.14	5.74 ± 9.25	Presente trabajo
Xenotoca variata / N. R.	Cuit	41	80.4	ND	Guzmán-Cornejo y García-Prieto, 1999
/ N. R.		41	80	80 ± 26.1	Salgado-Maldonado *et al.,* 2001b
/ H, M	Igna	35	57	13.8 ± ND	Salgado-Maldonado *et al.,* 2001b
/ H, M	Mint	31	6,45	3.5 ± 2.12	Martínez-Aquino, 2005 (datos de tesis)
/ N. R.		N. D	N. D	N. D	Salgado-Maldonado, 2006
/ C, Cc, M		7	14.29	N. D	Romero-Tejada *et al*., 2008
/ N. R.	Laja	N. D	N. D	N. D	Salgado-Maldonado, 2006
/ N. R.	Cien	N. D	N. D	N. D	Salgado-Maldonado, 2006
/ N. R.	Rinc	N. D	N. D	N. D	Salgado-Maldonado, 2006
/ N. R.	Atot	N. D	N. D	N. D	Salgado-Maldonado, 2006
/ N. R.	Marí	N. D	N. D	N. D	Salgado-Maldonado, 2006
/ N. R.	Igna	N. D	N. D	N. D	Salgado-Maldonado, 2006

	Zoogoneticus quitzeoensis / Cc, H, M	Mint	30	17	2.4 ± 2	Martínez-Aquino, 2005 (datos de tesis)
	/ N. R.		N. D	N. D	N. D	Salgado-Maldonado, 2006
Uvulifer sp.$^{\text{Al/Gn}}$	*Goodea atripinnis* / N. R.	Laja	ND	ND	ND	Salgado-Maldonado, 2006
	Xenotoca variata / N. R.	Cien	ND	ND	ND	Salgado-Maldonado, 2006
Fam. Heterophyidae Odhner, 1914						
Ascocotyle (Ascocotyle) tenuicollis$^{\text{Al/Gn}}$	*Allodontichthys zonistius* / Co	Siem	16	13	1	Salgado-Maldonado *et al.,* 2004b
Price, 1935	*Ameca splendens* / Ab	Teuc	35	8	2.3 ± 2.3	Martínez-Aquino, 2005 (datos de tesis)
Centrocestus formosanus$^{\text{Al/Gn}}$	*Goodea atripinnis* / Ab	Igna	11	27	5 ± ND	Scholz y Salgado-Maldonado, 2000
(Nishigori, 1924)	/ N. R.	Cien	ND	ND	ND	Salgado-Maldonado, 2006
	/ N. R.	Atot	ND	ND	ND	Salgado-Maldonado, 2006
	/ N. R.	Laja	ND	ND	ND	Salgado-Maldonado, 2006
	Ilyodon whitei / Ab	Amac	4	50	ND	Salgado-Maldonado *et al.,* 2001a
			520	49	5 ± ND	Scholz y Salgado-Maldonado, 2000
		Chis	22	81.81	ND	Salgado-Maldonado *et al.,* 2001a
	Ilyodon furcidens / Ab	Siem	51	14	7 ± 7	Salgado-Maldonado *et al.,* 2004b
		Puen	3	100	26 ± 15.62	Martínez-Aquino, 2005 (datos de tesis)
Fam. Plagiorchiidae Lühe, 1901						
Ochetosoma brevicaecum$^{\text{Au/Gn}}$	*Girardinichthys multiradiatus* / M	Chic	52	7.7	1.3 ± 0.5	Sánchez-Nava *et al.,* 2004
(Caballero y Caballero, 1941)			92	3.3	1.3 ± 0.6	Sánchez-Nava *et al.,* 2004
		Pedr	25	4	2	Sánchez-Nava *et al.,* 2004
		Juan	58	1.7	2	Sánchez-Nava *et al.,* 2004
		Porv	36	2.8	1	Sánchez-Nava *et al.,* 2004
		Sant	49	2	1	Sánchez-Nava *et al.,* 2004
	Allotoca diazi / I	Pátz	31	ND	ND	Peresbarbosa-Rojas *et al*., 1994
	Goodea atripinnis / I	Pátz	59	ND	ND	Pérez-Ponce de León *et al.,* 2000

Monogenea

Fam. Girodactylidae Van Beneden y Hesse, 1863						
Gyrodactylus lamothei$^{\text{Au/Es}}$ Mendoza-Palmero, Sereno-Uribe y Salgado-Maldonado, 2009 Nota 5	*Allotoca diazi* / Ab	Mcha	21	10	1	Presente trabajo
	Allotoca dugesii / Ab	Mcha	22	14	2.66	Presente trabajo
	Goodea atripinnis / A	Zúñi	7	14	1	Presente trabajo
	Girardinichthys multiradiatus / A, Ab, P	Chic	258	24	7 ± ND	Mendoza-Palmero *et al*., 2009
	/ A	Cano	5	20	3 ± ND	Mendoza-Palmero *et al*., 2007
Gyrodactylus mexicanus$^{\text{Au/Es}}$ Mendoza-Palmero, Sereno-Uribe y Salgado-Maldonado, 2009 Nota 6	*Allotoca dugesii* / Ab	Mcha	22	10	1	Presente trabajo
	Girardinichthys multiradiatus / A, Ab, P	Chic	258	14	1.6 ± ND	Mendoza-Palmero *et al*., 2009
	Goodea atripinnis / A	Rinc	ND	ND	ND	Salgado-Maldonado, 2006
			32	12	1	Presente trabajo
		Laja	ND	ND	ND	Salgado-Maldonado, 2006
			24	13	1	Presente trabajo
		Atot	10	10	1	Presente trabajo
		Chap	13	50	1	Presente trabajo
	Skiffia lermae / Ab	Chap	32	5.26	1	Presente trabajo
	Xenotoca variata / Ab	Igna	30	4	4	Presente trabajo
Gyrodactylus sp. 1 $^{\text{Au/Es?}}$ Nota 7	*Allotoca diazi* / A, Ab	Mcha	21	20	2 ± 1.41	Presente trabajo
	Allotoca dugesii / A, Ab	Mcha	22	19	1.5 ± ND	Presente trabajo
	Characodon audax / A	Tobo	42	?	?	Presente trabajo
	Characodon lateralis / A	Berr	23	?	?	Presente trabajo
	Girardinichthys multiradiatus / A	Chic	44	8	0.7 ± ND	Presente trabajo
	Goodea atripinnis / A	Trin	29	3	1 ± ND	Presente trabajo

	/ Ab	Mcha	13	50	1 ± ND	Presente trabajo
	Skiffia lermae / A, Ab	Mcha	32	47	1.66 ± ND	Presente trabajo
	Xenotoca melanosoma / Ab	Nori	38	11	2	Presente trabajo
	Zoogoneticus quitzeoensis / A	Mint	22	13	1.6 ± ND	Presente trabajo
Gyrodactylus sp. 2 $^{\text{Au/Es?}}$	*Allotoca duguesii* / Ab	Mcha	22	10	1 ± ND	Presente trabajo
Nota 8	*Skiffia lermae* / Ab	Mcha	19	26	1.2 ± ND	Presente trabajo
Gyrodactylus sp. $^{\text{Au/?}}$ Nota 9	*Goodea atripinnis* / A	Sori	ND	ND	ND	Salgado-Maldonado, 2006
Fam. Dactylogyridae Bychowsky, 1933						
Salsuginus angularis$^{\text{Au/Es?}}$	*Allotoca diazi* / Ab	Mcha	20	41	2.4± 0.82	Presente trabajo
(Mueller, 1934)	*Allotoca dugesii* / Ab	Mcha	22	41	2.4± 1.77	Presente trabajo
Beverly-Burton, 1984	*Ameca splendens* / Ab	Teuc	33	5.71	1 ± 0	Presente trabajo
Nota 10	*Chapalichthys encaustus* / Ab	Lcha	50	16	3.13 ± 1.55	Martínez-Aquino *et al.,* 2004
	Characodon audax / Ab	Tobo	30	43.3	1.85 ± 0.99	Martínez-Aquino *et al*., 2007b
	Goodea atripinnis / Ab	Mint	27	4	2	Martínez-Aquino, 2005 (datos de tesis)
			ND	ND	ND	Salgado-Maldonado, 2006
			5	4.54	6	Presente trabajo
		Rinc	ND	ND	ND	Salgado-Maldonado, 2006
		Sori	ND	ND	ND	Salgado-Maldonado, 2006
	Ilyodon furcidens / Ab	Puen	3	33.33	4	Presente trabajo
	Ilyodon whitei / Ab	Ahue	51	ND	ND	Presente trabajo
	Skiffia lermae / Ab	Mint	6	33.33	2.5 ± 0.75	Presente trabajo
	Skiffia multipunctata / Ab	Duer	8	12	1	Presente trabajo
	Xenotaenia resolanae / Ab	Cuza	35	69.44	1.46 ± 1.3	Presente trabajo
	Xenotoca melanosoma / Ab	Cuis	20	20	0.2	Presente trabajo
		Nori	38	50	1.78 ± 0.62	Presente trabajo
	Xenotoca variata / Ab	Mint	31	19	1.33 ± 0.82	Martínez-Aquino, 2005 (datos de tesis)
			ND	ND	ND	Salgado-Maldonado, 2006
			40	7.69	1	Presente trabajo

		Rinc	ND	ND	ND	Salgado-Maldonado, 2006
		Cien	ND	ND	ND	Salgado-Maldonado, 2006
			27	5	1	Presente trabajo
	Zoogoneticus quitzeoensis / Ab	Mint	30	13	3.25 ± 2.6	Martínez-Aquino, 2005 (datos de tesis)
			ND	ND	ND	Salgado-Maldonado, 2006
			22	100	2.8	Presente trabajo
Cestoda						
Fam. Bothriocephalidae Blanchard, 1849 *Bothriocephalus acheilognathi*$^{\text{Au/Gn}}$ Yamaguti, 1934	*Alloophorus robustus* / I	Pátz	41	ND	ND	Peresbarbosa-Rojas *et al*., 1994
			67	1.5	ND	Pérez-Ponce de León *et al*., 2000
		Mint	27	7.4	ND	Romero-Tejada *et al*., 2008
	Allotoca diazi / I	Pátz	31	ND	ND	Peresbarbosa-Rojas *et al*., 1994
			40	8	ND	Pérez-Ponce de León *et al*., 2000
	Allotoca zacapuensis / I	Zaca	17	11.8	0.3 ± 0.1	Mejía-Madrid *et al*., 2005
			32	3.13	ND	Martínez-Aquino *et al*., 2012
	Characodon audax / I	Berr	13	7.7	0.3 ± 1.1	Mejía-Madrid *et al*., 2005
		Pin1	34	8.8		Pérez-Ponce de León *et al*., 2009
		Pin2	21	14.2		Pérez-Ponce de León *et al*., 2009
		27No	9	22.2		Pérez-Ponce de León *et al*., 2009
		Guad	14	22.2		Pérez-Ponce de León *et al*., 2009
	Characodon lateralis / I	Juas	11	7.1		Pérez-Ponce de León *et al*., 2009
	Girardinichthys multiradiatus / I	Cler	ND	ND	ND	García-Prieto y Osorio-Sarabia, 1991
		Lerm	9	ND	ND	León-Règagnon, 1992
		Chic	63	3	1	Salgado-Maldonado *et al.,* 2001b
			92	2.2	1	Sánchez-Nava *et al.,* 2004
			52	1.9	1	Sánchez-Nava *et al.,* 2004
		Lagu	50	26	2.5 ± 1.8	Sánchez-Nava *et al.,* 2004
		Atla	15	13.3	1	Sánchez-Nava *et al.,* 2004
		Cimm	7	42.9	1.7 ± 0.6	Sánchez-Nava *et al.,* 2004

	Ignr	75	2.7	1	Sánchez-Nava *et al.,* 2004
	Juan	53	24.5	1.7 ± 1.6	Sánchez-Nava *et al.,* 2004
		58	6.9	1	Sánchez-Nava *et al.,* 2004
		50	8	1	Sánchez-Nava *et al.,* 2004
	Pedr	25	4	1	Sánchez-Nava *et al.,* 2004
	Sala	12	8.3	1	Sánchez-Nava *et al.,* 2004
	Sier	30	10	1	Sánchez-Nava *et al.,* 2004
	Vent	40	12.5	1.2 ± 0.4	Sánchez-Nava *et al.,* 2004
	Trin	ND	ND	ND	Salgado-Maldonado y Pienda-López, 20
	Mara	4	50	0.5 ± 0.6	Mejía-Madrid *et al*., 2005
	F				Astudillo-Ramos y Soto-Galera, 1997
	Cano	14	60	2 ± ND	Mendoza-Palmero *et al*., 2007
	Tica	12	91.67	1 ± 0.29	Presente trabajo
Goodea atripinnis / I	Lcha	ND	ND	ND	García-Prieto y Osorio-Sarabia, 1991
	Bata	41	12	4 ± ND	Pineda-López y González-Enríquez, 199
	Lcha	ND	ND	ND	García-Prieto y Osorio-Sarabia, 1991
	Galv	ND	ND	ND	Salgado-Maldonado y Pineda-López, 20
	Xote	ND	ND	ND	Salgado-Maldonado y Pineda-López, 20
	F				Pineda-López y González-Enríquez, 199
	Laad	ND	ND	ND	Salgado-Madonado, 2006
Ilyodon cortesae / I	Cutz	30	6.7	0.2 ± 0.9	Mejía-Madrid *et al*., 2005
Ilyodon whitei / I	Ahue	51	1.96	1 ± 0.14	Presente trabajo
Neotoca lermae / I	Mint	61	3	1 ± 2.83	Martínez-Aquino, 2005 (datos de tesis)
		ND	ND	ND	Salgado-Madonado, 2006
Xenotoca variata / I	Cons	36	8	3.2 ± ND	Pineda-López y González-Enríquez 199
	Igna	10	1		Salgado-Maldonado *et al.,* 2001b
	Galv	ND	ND	ND	Salgado-Maldonado y Pineda-López, 20
	Xote	ND	ND	ND	Salgado-Maldonado y Pineda-López, 20
	Mint	31	19	2 ± 2.45	Martínez-Aquino, 2005 (datos de tesis)
		ND	ND	ND	Salgado-Madonado, 2006
	Cien	ND	ND	ND	Salgado-Madonado, 2006
	Laad	ND	ND	ND	Salgado-Madonado, 2006
	Atot	ND	ND	ND	Salgado-Madonado, 2006
	Rinc	ND	ND	N.D	Salgado-Madonado, 2006
Skiffia bilineata / I	Quer	15	6.6	0.1 ± 0.3	Mejía-Madrid *et al*., 2005
Zoogoneticus purhepechus / I	Negr	27	7.4	0.07 ± 0.27	Martínez-Aquino *et al*., 2011
Zoogoneticus quitzeoensis / I	Mint	30	3	1	Martínez-Aquino, 2005 (datos de tesis)

			ND	ND	ND	Salgado-Madonado, 2006
Proteocephalus longicollis$^{\text{Au/Gn}}$ (Zeder, 1800) Nota 11	*Alloophorus robustus* / I	Mint	27	16.67	ND	Romero-Tejada *et al*., 2008
	Goodea atripinnis / I	Pátz	ND	ND	ND	Mejía-Madrid *et al*., 2005
		Pátz	178	34	1.5	Salgado-Maldonado y Osorio-Sarabia, 1987
	Skiffia lermae / I	Mint	25	24	0.4 ± 1	Mejía-Madrid *et al*., 2005
Fam. Caryophyllaeidae Leuckart, 1878						
Caryophyllidea gen. sp.$^{\text{Au/Gn?}}$	*Allotoca zacapuensis* / M	Zaca	32	3.13	ND	Martínez-Aquino *et al*., 2012
	Characodon audax / M	Tobo	30	20	1.7 ± 0.8	Martínez-Aquino *et al*., 2007b
Metacéstodos						
Fam. Dilepididae Railliet y Henry, 1909						
Dilepididae gen. sp.$^{\text{Al/Gn}}$	*Allodontichthys zonistius* / M	Siem	16	6	1	Salgado-Maldonado *et al.,* 2004b
	Ilyodon furcidens / M	Siem	51	4	2 ± 1	Salgado-Maldonado *et al.,* 2004b
	Alloophorus robustus / M	Mint	7	14	1	Martínez-Aquino, 2005 (datos de tesis)
Cyclustera cf. *ralli*$^{\text{Al/Gn}}$ (Underwood y Dronnen, 1986) Bona, 1994	*Alloophorus robustus* / M	Pátz	25	8	2.5 ± ND	Scholz y Salgado-Maldonado, 2001
	Girardinichthys multiradiatus / M	Chic	211	10	0.1 ± ND	Scholz y Salgado-Maldonado, 2001
			92	5.4	1	Sánchez-Nava *et al.,* 2004
			52	5.8	1	Sánchez-Nava *et al.,* 2004
			44	2.27	1	Presente trabajo
		Juan	50	28	2.8 ± 1.7	Sánchez-Nava *et al.,* 2004
		Mina	21	4.8	1	Sánchez-Nava *et al.,* 2004
		Porv	36	36.1	1.6 ± 0.6	Sánchez-Nava *et al.,* 2004
		Sier	30	3.3	2	Sánchez-Nava *et al.,* 2004
		Vent	40	5	3 ± 2.8	Sánchez-Nava *et al.,* 2004
		Ties	6	16.67	1 ± 0.41	Presente trabajo

		Ignr	14	14.29	1 ± 0.36	Presente trabajo
	Chapalichthys encaustus / M	Lcha	50	2	1	Martínez-Aquino *et al.,* 2004
	Xenotoca variata / M	Igna	24	8	1	Scholz y Salgado-Maldonado, 2001
Glossocercus auritus$^{Al/Gn}$ (Rudolphi, 1819)	*Goodea atripinnis* / H	Lcha	8	12	1	Martínez-Aquino, 2005 (datos de tesis)
Valipora campylancristrota$^{Al/Gn}$ (Wedl, 1855) Baer y Bona, 1960	*Girardinichthys multiradiatus* / Vb	Lagu	50	1	1	Scholz y Salgado-Maldonado, 2001
		Chic	92	3.1	1.7 ± 0.6	Sánchez-Nava *et al.,* 2004
			52	1.9	1	Sánchez-Nava *et al.,* 2004
		Almo	20	5	1	Sánchez-Nava *et al.,* 2004
		Ignr	75	9.3	1.9 ± 1	Sánchez-Nava *et al.,* 2004
		Juan	53	20.8	1.8 ± 1	Sánchez-Nava *et al.,* 2004
			58	12.1	1.1 ± 0.4	Sánchez-Nava *et al.,* 2004
			50	6	1.7 ± 0.6	Sánchez-Nava *et al.,* 2004
		Sier	30	3	2	Sánchez-Nava *et al.,* 2004
		Sant	49	6.1	1	Sánchez-Nava *et al.,* 2004
			11	9.1	1	Sánchez-Nava *et al.,* 2004
		Mina	21	9.5	1.5 ± 0.7	Sánchez-Nava *et al.,* 2004
		Vent	40	12.5	1	Sánchez-Nava *et al.,* 2004
		Tica	12	91.67	1 ± 0.29	Presente trabajo
	Girardinichthys viviparus / Vb	Chap	31	19	1.33 ± 0.96	Martínez-Aquino, 2005 (datos de tesis)
	Xenotoca variata / Vb	Atot	ND	ND	ND	Salgado-Maldonado, 2006
Valipora mutabilis$^{Al/Gn}$ Linton , 1927	*Xenotoca variata* / Vb	Igna	ND	ND	ND	Salgado-Maldonado, 2006
Fam. Proteocephalidea La Rue, 1911						
Cyclophyllidae gen. sp.$^{Al/Gn}$	*Allotoca diazi* / Vb	Pátz	40	2	ND	Pérez-Ponce de León *et al*., 2000
	Zoogoneticus purhepechus / Vb	Negr	27	3.7	0.04 ± 0.10	Martínez-Aquino *et al*., 2011
Proteocephalidae gen. sp.$^{Au/Gn}$	*Alloophorus robustus* / H, I, M	Pátz	41	ND	ND	Peresbarbosa-Rojas *et al*., 1994

	/ I		67	58	ND	Pérez-Ponce de León *et al*., 2000
	Allotoca diazi / H, I, M	Pátz	31	ND	ND	Peresbarbosa-Rojas *et al*., 1994
	/ I		40	5	ND	Pérez-Ponce de León *et al*., 2000
	Goodea atripinnis / Cb	Pátz	35	ND	ND	Peresbarbosa-Rojas *et al*., 1994
	/ I		59	30	ND	Pérez-Ponce de León *et al*., 2000
	Alloophorus robustus / M	Mint	7	14	2	Martínez-Aquino, 2005 (datos de tesis)
	Skiffia lermae / I	Mint	61	7	1.5 ± 1	Martínez-Aquino, 2005 (datos de tesis)
	Xenotoca variata / Cb	Mint	31	3	1	Martínez-Aquino, 2005 (datos de tesis)
Proteocephalus ambloplitis$^{\text{Au/Gn}}$ (Leidy, 1758)	*Goodea atripinnis* / N. R.	Laja	ND	ND	ND	Salgado-Maldonado, 2006
	Xenotoca variata / N. R	Atot	ND	ND	ND	Salgado-Maldonado, 2006
		Cien	ND	ND	ND	Salgado-Maldonado, 2006
Fam. Diphyllobothriidae Lühe, 1910						
Ligula intestinalis$^{\text{Au/Gn}}$ (Linneo, 1758) Bloch, 1782	*Goodea atripinnis* / Cc	Pátz	ND	ND	ND	García-Prieto *et al*., 1998
			59	1	ND	Pérez-Ponce de León *et al*., 2000
	Girardinichthys multiradiatus / Cc	Lagu	50	2	3 ± ND	Salgado-Maldonado *et al.,* 2001b
			50	4	1.5 ± 0.7	Sánchez-Nava *et al.,* 2004
		Lerm	223	9.2	ND	Lamothe-Argumedo y Cruz-Reyes, 197
		Trin	563	16	1.6 ± ND	Astudillo-Ramos y Soto-Galera, 1997
		Ignr	5	80	1.7	Salgado-Maldonado *et al*., 2001b
			75	1.3	1	Sánchez-Nava *et al.,* 2004
		Juan	53	1.9	1	Sánchez-Nava *et al.,* 2004
		Lagu	50	4	1.5 ± 0.7	Sánchez-Nava *et al.,* 2004
		Zemp	20	55	2.7 ± 1.7	Sánchez-Nava *et al.,* 2004
		Sala	32	25	1.38 ± 0.79	Presente trabajo
Nematoda						
Fam. Capillariidae Neveau-Lemaire, 1936						

Capillaria cyprinodonticola$^{Al/Gn}$ Huffman y Bullock, 1973	*Ilyodon furcidens* / I	Siem	21	5	1	Salgado-Maldonado *et al.,* 2004b
Capillaridae gen. sp.$^{Al/Gn}$	*Goodea atripinnis* / I	Ignr	20	5	2	Salgado-Maldonado *et al*., 2001b
		Bizn	25	4	1	Salgado-Maldonado *et al*., 2001b
Pseudocapillaria tomentosa$^{Au/Gn}$ (Dujardin, 1843)	*Alloophorus robustus* / I	Pátz	20	5	3 ± ND	Moravec *et al.,* 2001
		Igna	ND	ND	ND	Salgado-Maldonado, 2006
	Goodea atripinnis / I	Pátz	178	10	2.7 ± ND	Salgado-Maldonado y Osorio-Sarabia, 1987
			18	11.1	0.11 ± 0.3	Mejía-Madrid *et al*., 2005
		Bizn	25	4	1 ± ND	Salgado-Maldonado *et al.,* 2001b
		Mara	5	20	0.2 ± 0.4	Mejía-Madrid *et al*., 2005
		Igna	20	5	2 ± ND	Salgado-Maldonado *et al.,* 2001b
	Goodea gracilis / I	Jesú	14	7.1	0.1 ± 0.3	Mejía-Madrid *et al*., 2005
	Skiffia lermae / I	Mcha	51	5.8	0.2 ± 0.8	Mejía-Madrid *et al*., 2005
	Xenotoca variata / I	Laja	ND	ND	ND	Salgado-Maldonado, 2006
Fam. Rhabdochonidae Travassos, Artigas y Pereira, 1928						
Rhabdochona ahuehuellensis$^{Au/Es}$ Mejía-Madrid y Pérez-Ponce de León, 2003	*Allodontichthys hubbsi* / I	Pihu	5	60	1 ± 1.1	Mejía-Madrid *et al*., 2005
	Allodontichthys tamazulae / I	Tama	7	42.9	0.6 ± 0.8	Mejía-Madrid *et al*., 2005
	Allodontichthys zonistius / I	Ahue	32	9.38	1.67 ± 0.57	Presente trabajo
	Ataeniobius toweri / I	Luna	19	84.2	4.8 ± 3.6	Mejía-Madrid *et al*., 2005
	Ilyodon furcidens / I	Guac	12	8.3	0.08 ± 0.3	Mejía-Madrid *et al*., 2005
		Pihu	8	62.5	0.08 ± 0.3	Mejía-Madrid *et al*., 2005
		Tama	11	36.4	0.5 ± 0.7	Mejía-Madrid *et al*., 2005
			56	10.71	1.67 ± 0.64	Presente trabajo
	Ilyodon whitei / I	Ahue	40	58.3	ND	Mejía-Madrid y Pérez-Ponce de León, 2003
			180	63.9	1.14 ± 2.4	Mejía-Madrid *et al*., 2005
			51	68.63	3.23 ± 2.54	Presente trabajo

	Xenotaenia resolanae / I	Teco	14	28.6	0.4 ± 0.6	Mejía-Madrid *et al*., 2005
		Cuza	35	20	2.29 ± 1.49	Martínez-Aquino *et al*., 2009a
			36	14	1.2 ± 0.4	Martínez-Aquino, 2005 (datos de tesis)
Rhabdochona kidderi$^{\mathrm{Au/Gn}}$ Pearse, 1936	*Ilyodon whitei* / I	?	ND	ND	ND	Mejía-Madrid y Pérez-Ponce de León, 2003
Rhabdochona lichtenfelsi$^{\mathrm{Au/Es}}$ Sánchez-Álvarez, García-Prieto y Pérez-Ponce de León, 1998	*Alloophorus robustus* / I	Cuit	360	40	18 ± ND	Sánchez-Álvarez *et al*., 1998
		Pátz	41	ND	ND	Peresbarbosa-Rojas *et al*., 1994
			67	15	ND	Pérez-Ponce de León *et al*., 2000
			19	63.2	5.4 ± 8	Mejía-Madrid *et al*., 2005
		Luz	10	80	1.9 ± 1.2	Mejía-Madrid *et al*., 2005
		Mint	4	50	1.5 ± 2.4	Mejía-Madrid *et al*., 2005
			7	14	2	Salgado-Maldonado 2006
			27	3.7	ND	Romero-Tejada *et al*., 2008
			14	7.14	1 ± 0.27	Presente trabajo
		Oran	13	7.7	0.1 ± 0.3	Mejía-Madrid *et al*., 2005
		Lcha	2	50	6	Martínez-Aquino, 2005 (datos de tesis)
			ND	ND	ND	Salgado-Maldonado, 2006
		Zaca	17	35.29	ND	Martínez-Aquino *et al*., 2012
		Came	2	100	4.5 ± 3.54	Presente trabajo
		Pasj	5	40	5.5 ± 3.9	Presente trabajo
		Ucas	11	45.45	4.80 ± 3.82	Presente trabajo
	Allotoca diazi / I	Pátz	31	ND	ND	Peresbarbosa-Rojas *et al*., 1994
			40	1	ND	Pérez-Ponce de León *et al*., 2000
			10	40	6.1 ± 12.2	Mejía-Madrid *et al*., 2005
		Mcha	9	44.44	8.5 ± 5.67	Presente trabajo
	Allotoca dugesii / I	Mcha	22	ND	ND	Presente trabajo
	Allotoca zacapuensis / I	Zaca	17	76.5	9 ± 13.8	Mejía-Madrid *et al*., 2005
			32	65.63	ND	Martínez-Aquino *et al*., 2012
	Ameca splendens / I	Teuc	35	80	4.8 ± 5	Martínez-Aquino, 2005 (datos de tesis)
			17	23.5	0.2 ± 0.4	Mejía-Madrid *et al*., 2005
			33	27.27	2.11 ± 1.17	Presente trabajo
	Chapalichthys encaustus / I	Lcha	50	28	3.93 ± 2.92	Martínez-Aquino *et al.,* 2004
		Luz	17	58.8	1 ± 1.2	Mejía-Madrid *et al*., 2005

Chapalichthys pardalis / I	Tocu	11	36.4	0.9 ± 2.1	Mejía-Madrid *et al*., 2005
		38	2.63	1 ± 0.16	Presente trabajo
Characodon audax / I	Berr	13	38.5	0.7 ± 1.4	Mejía-Madrid *et al*., 2005
Goodea atripinnis / I	Cuit	20	40	18 ± ND	Sánchez-Álvarez *et al*., 1998
	Pátz	178	8	7.8 ± ND	Salgado-Maldonado y Osorio-Sarabia, 1987
		35	ND	ND	Peresbarbosa-Rojas *et al*., 1994
		59	33	ND	Pérez-Ponce de León *et al*., 2000
		30	43.33	19 ± 13.62	Presente trabajo
		18	94.4	9 ± 7.7	Mejía-Madrid *et al*., 2005
	Juam	6	83.33	ND	Salgado-Maldonado *et al.,* 2001a
	Esto	4	1	4	Salgado-Maldonado *et al.,* 2004a
	Luz	19	78.9	12 ± 15.17	Mejía-Madrid *et al*., 2005
	Mint	5	80	13 ± 12	Mejía-Madrid *et al*., 2005
		27	19	15.85 ± 13.76	Salgado-Maldonado, 2006
	Oran	17	11.8	0.8 ± 2.7	Mejía-Madrid *et al*., 2005
	Tapi	15	93.3	24.3 ± 21.7	Mejía-Madrid *et al*., 2005
	Merc	15	53.3	2.4 ± 3.5	Mejía-Madrid *et al*., 2005
	Quer	15	6.7	0.1 ± 0.2	Mejía-Madrid *et al*., 2005
	Cris	15	93.3	5.7 ± 3.1	Mejía-Madrid *et al*., 2005
	Tocu	9	66.7	7.2 ± 8.4	Mejía-Madrid *et al*., 2005
	Coro	15	80	2.3 ± 2.1	Mejía-Madrid *et al*., 2005
	Verd	25	60	3.8 ± 6	Mejía-Madrid *et al*., 2005
	Teuc	7	100	9.6 ± 6.2	Mejía-Madrid *et al*., 2005
		4	50	2.5 ± 0.71	Martínez-Aquino, 2005 (datos de tesis)
	Chiq	5	100	6 ± 2	Martínez-Aquino, 2005 (datos de tesis)
	Lcha	8	50	8.75 ± 11.03	Martínez-Aquino, 2005 (datos de tesis)
		ND	ND	ND	Salgado-Maldonado, 2006
	Rinc	ND	ND	ND	Salgado-Maldonado, 2006
	Igna	ND	ND	ND	Salgado-Maldonado, 2006
	Laja	ND	ND	ND	Salgado-Maldonado, 2006
	Cien	ND	ND	ND	Salgado-Maldonado, 2006
	Sori	ND	ND	ND	Salgado-Maldonado, 2006
	Laad	ND	ND	ND	Salgado-Maldonado, 2006
	Atot	ND	ND	ND	Salgado-Maldonado, 2006
	Zaca	20	25	ND	Martínez-Aquino *et al*., 2012
	Idel	1	100	ND	Presente trabajo

		9	44.44	7.5 ± 6.65	Presente trabajo
	Opop	4	25	1 ± 0.5	Presente trabajo
Goodea gracilis / I	Quem	24	92	4 ± 3.4	Mejía-Madrid *et al*., 2005
Hubbsina turneri / I	Zaca	14	92.9	13.9 ± 12.4	Mejía-Madrid *et al*., 2005
		20	75.7	ND	Martínez-Aquino *et al*., 2012
Ilyodon furcidens / I	Siem	52	2	1	Salgado-Maldonado *et al.,* 2004b
Ilyodon whitei / I	Tocu	37	13.51	2.40 ± 0.97	Presente trabajo
Neoophorus regalis / I	Rico	28	46.43	6.62 ± 6.23	Presente trabajo
Skiffia bilineata / I	Quer	15	6.7	0.1 ± 0.3	Mejía-Madrid *et al*., 2005
Skiffia lermae / I	Mint	25	88	9.6 ± 8.4	Mejía-Madrid *et al*., 2005
		61	85	5.12 ± 6.76	Salgado-Maldonado 2006
		30	96.67	ND	Romero-Tejada *et al*., 2008
	Zaca	19	100	ND	Martínez-Aquino *et al*., 2012
Skiffia multipunctata / I	Duer	8	12	1	Martínez-Aquino, 2005 (datos de tesis)
	Luz	22	86.4	5.3 ± 6.1	Mejía-Madrid *et al*., 2005
	Cupa	13	100	2 ± 3.06	Presente trabajo
Xenotoca variata / I	Mint	31	13	3.25 ± 2.22	Martínez-Aquino, 2005 (datos de tesis)
		ND	ND	ND	Salgado-Maldonado, 2006
	Igna	29	21	1.33 ± 0.52	Martínez-Aquino, 2005 (datos de tesis)
		ND	ND	ND	Salgado-Maldonado, 2006
	Oran	26	34.6	1.2 ± 2.1	Mejía-Madrid *et al*., 2005
	Tapi	12	66.75	3 ± 3.4	Mejía-Madrid *et al*., 2005
	Cris	21	42.9	1.5 ± 2.6	Mejía-Madrid *et al*., 2005
	Cien	ND	ND	ND	Salgado-Maldonado, 2006
	Rinc	ND	ND	ND	Salgado-Maldonado, 2006
	Zaca	21	71.43	ND	Martínez-Aquino *et al*., 2012
Zoogoneticus purhepechus / I	Luz	45	15.6	2.14 ± 1.57	Martínez-Aquino *et al*., 2011
Zoogoneticus quitzeoensis / I	Mint	30	87	8.54 ± 4.9	Martínez-Aquino, 2005 (datos de tesis)
		ND	ND	ND	Salgado-Maldonado, 2006
		30	6.67	ND	Romero-Tejada *et al*., 2008
	Zaca	15	46.7	1.5 ± 3.1	Mejía-Madrid *et al*., 2005
		32	15.63	ND	Martínez-Aquino *et al*., 2012
	Came	11	9.09	1 ± 0.30	Presente trabajo
	Cupa	16	31.25	2.40 ± 1.77	Presente trabajo

Rhabdochona xiphophori$^{\text{Au/Es?}}$ Caspeta-Mandujano, Moravec y Salgado-Maldonado, 2001	*Allotoca catarinae* / I	Calt	16	18.8	2.8 ± 6.4	Mejía-Madrid *et al*., 2005
	Xenotoca eiseni / I	Ener	14	35.7	0.6 ± 1.2	Mejía-Madrid *et al*., 2005
Rhabdochona sp.$^{\text{Au/?}}$	*Ilyodon furcidens* / I	Oro	15	13.33	1 ± 0.35	Presente trabajo
	Skiffia multipunctata / I	Adju	2	100	1	Presente trabajo
		Chil	10	100	3.8 ± 2.49	Presente trabajo
	Zoogoneticus purhepechus / I	Negr	27	3.7	2	Martínez-Aquino *et al*., 2011
Larvas de Nematoda						
Fam. Acuariodidea Railliet, Henry y Sisoff, 1912						
Streptocara sp.$^{\text{Al/Gn}}$	*Characodon lateralis* / I	Juas	11	9.1	ND	Pérez-Ponce de León *et al*., 2009
Fam. Anisakidae Railliet y Henry, 1912						
Contracaecum sp.$^{\text{Al/Gn}}$	*Alloophorus robustus* / M	Pátz	67	1	ND	Pérez-Ponce de León *et al.,* 2000
	Chapalichthys encaustus / Mu, M, G, CC	Lcha	50	40	4.05 ± 4.9	Martínez-Aquino *et al.,* 2004
	Characodon audax / CC, M	Pin1	34	2.9	ND	Pérez-Ponce de León *et al*., 2009
		Guad	14	7.1	ND	Pérez-Ponce de León *et al*., 2009
		27No	9	33.3	ND	Pérez-Ponce de León *et al*., 2009
		Tobo	42	2.3	ND	Pérez-Ponce de León *et al*., 2009
	Girardinichthys multiradiatus / M	Almo	20	5	1	Sánchez-Nava *et al.,* 2004
		Atla	25	8	1	Sánchez-Nava *et al.,* 2004
		Chic	52	1.9	1	Sánchez-Nava *et al.,* 2004
		Ignr	75	1.3	1	Sánchez-Nava *et al.,* 2004
		Juan	58	2	2	Sánchez-Nava *et al.,* 2004
			50	4	1	Sánchez-Nava *et al.,* 2004
		Pedr	25	8	1	Sánchez-Nava *et al.,* 2004
		Sier	30	10	1	Sánchez-Nava *et al.,* 2004
		Vent	40	5	2.5 ± 2.1	Sánchez-Nava *et al.,* 2004

		Vict	5	20	1	Sánchez-Nava *et al.,* 2004
	Goodea atripinnis / Cc, I	Igna	22	5	1	Salgado-Maldonado *et al.,* 2001b
		Laja	ND	ND	ND	Salgado-Maldonado, 2006
		Marí	ND	ND	ND	Salgado-Maldonado, 2006
		Cien	ND	ND	ND	Salgado-Maldonado, 2006
		Rinc	ND	ND	ND	Salgado-Maldonado, 2006
	Xenotoca variata / M	Igna	35	31	1.4 ± ND	Salgado-Maldonado *et al.,* 2001b
		Laja	ND	ND	ND	Salgado-Maldonado, 2006
		Rinc	ND	ND	ND	Salgado-Maldonado, 2006
		Marí	ND	ND	ND	Salgado-Maldonado, 2006
	Zoogoneticus purhepechus / H	Negr	27	3.7	2	Martínez-Aquino *et al*., 2011
	Alloophorus robustus / M	Mint	7	14	1	Martínez-Aquino, 2005 (datos de tesis)
	Characodon audax / Cc, M	Tobo	30	6.7	2 ± 1.41	Martínez-Aquino *et al*., 2007b
	Goodea atripinnis / M	Lcha	8	12	2	Martínez-Aquino, 2005 (datos de tesis)
	Ilyodon furcidens / Cc	Puen	3	33	1	Martínez-Aquino, 2005 (datos de tesis)
Fam. Camallanidae Raillet y Henry, 1915						
Serpinema trispinosum$^{Au/Gn}$ (Leidy, 1852)	*Alloophorus robustus* / I	Mint	27	3.3	ND	Romero-Tejada *et al*., 2008
	Characodon audax / I	Tobo	30	3.3	1	Martínez-Aquino *et al*., 2007b
Fam. Dioctophymatidae Railliet, 1915						
Eustrongylides sp.$^{Al/Gn}$	*Alloophorus robustus* / Cc, M	Pátz	41	ND	ND	Peresbarbosa-Rojas *et al*., 1994
	/ M		67	15	ND	Pérez-Ponce de León *et al*., 2000
			ND	ND	ND	Salgado-Maldonado, 2006
		Igna	ND	ND	ND	Salgado-Maldonado, 2006
		Zaca	17	5.88	ND	Martínez-Aquino *et al*., 2012
		Ucas	11	9.09	1 ± 0.3	Presente trabajo
	Allotoca diazi / M	Pátz	40	6	ND	Pérez-Ponce de León *et al*., 2000
	Characodon audax / Cc, M	Tobo	42	11.9		Pérez-Ponce de León *et al*., 2009
	Goodea atripinnis / Mu	Pátz	178	2	1.3 ± ND	Salgado-Maldonado y Osorio-Sarabia, 1987
			30	6.67	1 ± 0.25	Presente trabajo
		Bizn	10	10	1	Salgado-Maldonado *et al.,* 2001b
		Atot	ND	ND	ND	Salgado-Maldonado, 2006

	Girardinichthys multiradiatus / Cc	Almo	ND	ND	ND	Sánchez-Nava *et al.,* 2004
	Girardinichthys viviparus / Cc	Chap	31	35	2.36 ± 1.27	Martínez-Aquino, 2005 (datos de tesis)
	Goodea atripinnis / Mu	Chap	20	ND	ND	Martínez-Aquino, 2005 (datos de tesis)
	Skiffia lermae / Cc	Mint	61	3	1	Martínez-Aquino, 2005 (datos de tesis)
			ND	ND	ND	Salgado-Maldonado, 2006
	Zoogoneticus purhepechus / H	Negr	27	3.7	1	Martínez-Aquino *et al*., 2011
Fam. Gnathostomatidae Railliet, 1895						
Gnathostoma sp.$^{Au/Gn}$	*Alloophorus robustus* / H	Pátz	20	5	1	Salgado-Maldonado *et al.,* 2001b
Spiroxys sp.$^{Au/Gn}$	*Allodontichthys zonistius* / Cc	Ahua	11	9.09	1 ± 0.3	Presente trabajo
	Alloophorus robustus / M, I	Pátz	41	ND	ND	Peresbarbosa-Rojas *et al*., 1994
			67	26	ND	Pérez-Ponce de León *et al*., 2000
	/ M	Mint	7	86	2.33 ± 1.03	Martínez-Aquino, 2005 (datos de tesis)
	/ Cc, M, I	Mint	27	3.7	ND	Romero-Tejada *et al*., 2008
	Allotoca diazi / M, I	Pátz	31	ND	ND	Peresbarbosa-Rojas *et al*., 1994
			40	1		Pérez-Ponce de León *et al*., 2000
	Characodon audax / M	Tobo	30	50	1.87 ± 1.51	Martínez-Aquino *et al*., 2007b
	/ M, Pi	Pin1	34	2.9		Pérez-Ponce de León *et al*., 2009
		Abra	12	16.6		Pérez-Ponce de León *et al*., 2009
		27No	9	33.3		Pérez-Ponce de León *et al*., 2009
		Pin2	21	9.5		Pérez-Ponce de León *et al*., 2009
	Characodon lateralis / H, M	Abra	27	29.6	1.38 ± 1.06	Martínez-Aquino *et al*., 2007b
	/ M, Pi	Juas	11	11.1		Pérez-Ponce de León *et al*., 2009
		27No	9	3.5		Pérez-Ponce de León *et al*., 2009
	Goodea atripinnis / I	Bizn	18	6	1	Salgado-Maldonado *et al*., 2001b
	/ M, I	Pátz	35	ND	ND	Peresbarbosa-Rojas *et al*., 1994
	/ I		178	1	1	Salgado-Maldonado y Osorio-Sarabia, 1987
			59	27	ND	Pérez-Ponce de León *et al*., 2000

		Trin	29	3	1 ± ND	Salgado-Maldonado *et al.,* 2001b
		Mint	27	4	1	Martínez-Aquino, 2005 (datos de tesis)
	/ N. R.	Mint	ND	ND	ND	Salgado-Maldonado, 2006
	/ N. R.	Laja	ND	ND	ND	Salgado-Maldonado, 2006
	/ N. R.	Igna	ND	ND	ND	Salgado-Maldonado, 2006
	/ N. R.	Atot	ND	ND	ND	Salgado-Maldonado, 2006
	Girardinichthys multiradiatus					
	/ I	Ignr	13	15	1	Salgado-Maldonado *et al.,* 2001b
	/ M	Porv	36	2.8	1	Sánchez-Nava *et al.,* 2004
	Ilyodon furcidens / M	Potg	10	20	2	Martínez-Aquino, 2005 (datos de tesis)
	Xenoophorus captivus / Cc, M	Moct	30	10	1	Martínez-Aquino, 2005 (datos de tesis)
	Xenotaenia resolanae / M	Cuza	2.86	0.06	2	Martínez-Aquino *et al*., 2009b
	Xenotoca variata / I	Igna	21	5	1	Salgado-Maldonado *et al.,* 2001b
	/ M	Mint	31	3	1	Martínez-Aquino, 2005 (datos de tesis)
			ND	ND	ND	Salgado-Maldonado, 2006
		Rinc	ND	ND	ND	Salgado-Maldonado, 2006
	Zoogoneticus purhepechus / M	Luz	45	8.9	1.5 ± 1	Martínez-Aquino *et al*., 2011
		Negr	27	3.7	3	Martínez-Aquino *et al*., 2011
		Cupa	16	18.75	1.33 ± 0.58	Presente trabajo
	Zoogoneticus quitzeoensis / M	Mint	30	3	1	Martínez-Aquino, 2005 (datos de tesis)
			30	3.3	ND	Romero-Tejada *et al*., 2008
		Came	11	9.09	3 ± 0.9	Presente trabajo
Fam. Kathlaniidae Lane, 1914						
Falcaustra sp.$^{Au/Gn}$	*Girardinichthys multiradiatus*					
	/ I, M	Lagu	50	12	10 ± 13.8	Sánchez-Nava *et al.,* 2004
		Tepe	1	100	4	Sánchez-Nava *et al.,* 2004
Acanthocephala						
Fam. Pomphorhynchidae Yamaguti, 1939						
Pomphorhynchus cf. *bulbocolli*$^{Au/Gn}$	*Alloophorus robustus* / I	Luz	34	5.88	7 ± 1.67	Presente trabajo
	Zoogoneticus purhepechus / I	Luz	45	13.3	1	Martínez-Aquino *et al*., 2011
		Adju	5	100	1	Presente trabajo

Cistacantos

Fam. Polymorphidae Meyer, 1931						
Polymorphus brevis$^{Al/Gn}$	*Alloophorus robustus* / M, Mu	Pátz	41	ND	ND	Peresbarbosa-Rojas *et al*., 1994
Van Clave, 1916	/ M		67	16	ND	Pérez-Ponce de León *et al*., 2000
	/ Cc, M		11	ND	ND	Alcántar-Escalera *et al*., 2013
			2	100	7 ± 1.41	Presente trabajo
	/ M	Mint	7	14	3	Martínez-Aquino, 2005 (datos de tesis)
	/ Cc, M	Zaca	17	5.88	ND	Martínez-Aquino *et al*., 2012
		Ucas	11	54.44	4.33 ± 3.35	Presente trabajo
	Allotoca catarinae / Cc. M	Calt	6	ND	ND	Alcántar-Escalera *et al*., 2013
	Allotoca diazi / M, Mu	Pátz	31	ND	ND	Peresbarbosa-Rojas *et al*., 1994
	/ M		40	5	ND	Pérez-Ponce de León *et al*., 2000
	Allotoca zacapuensis / Cc, M	Zaca	32	9.38	ND	Martínez-Aquino *et al*., 2012
	Ameca splendenss / Cc	Teuc	35	6	1	Martínez-Aquino, 2005 (datos de tesis)
	Chapalichthys encaustus / M	Lcha	50	6	1	Martínez-Aquino *et al.,* 2004
	/ Cc, M	Luz	2	ND	ND	Alcántar-Escalera *et al*., 2013
			8	12.5	1 ± 0.71	Presente trabajo
	Characodon lateralis / M	Juas	11	27.2	ND	Pérez-Ponce de León *et al*., 2009
	Girardinichthys multiradiatus / M	Sala	12	8.3	1	Sánchez-Nava *et al.,* 2004
			1	ND	ND	Alcántar-Escalera *et al*., 2013
			32	3.13	1 ± 0.18	Presente trabajo
		Sant	11	18.2	1	Sánchez-Nava *et al.,* 2004
			3	ND	ND	Alcántar-Escalera *et al*., 2013
		Ignr	ND	ND	ND	Salgado-Maldonado, 2006
	Goodea atripinnis / H, M	Pátz	178	3	1	Salgado-Maldonado y Osorio-Sarabia, 1987
	/ H, M		59	27	ND	Pérez-Ponce de León *et al*., 2000
	/ Cc, M		2	ND	ND	Alcántar-Escalera *et al*., 2013
			30	10	3 ± 1.02	Presente trabajo
	/ M	Lcha	8	12	1	Martínez-Aquino, 2005 (datos de tesis)
	/ Cc	Ucas	1	100		Presente trabajo
	Hubbsina turneri / Cc, M	Zaca	20	15	ND	Martínez-Aquino *et al*., 2012

Skiffia lermae / Cc, M	Zaca	19	5.26	ND	Martínez-Aquino *et al*., 2012
Skiffia multipunctata / Cc, M	Cupa	2	ND	ND	Alcántar-Escalera *et al*., 2013
		13	15.38	1 ± 0.38	Presente trabajo
Xenotoca variata / H	Igna	35	6	1	Salgado-Maldonado *et al.,* 2001b
/ M	Mint	31	3	1	Martínez-Aquino, 2005 (datos de tesis)
/ H		ND	ND	ND	Salgado-Maldonado, 2006
/ H	Zaca	21	19.05	ND	Martínez-Aquino *et al*., 2012
Zoogoneticus purhepechus / M	Negr	27	3.7	1	Martínez-Aquino *et al*., 2011
/ Cc, M	Adju	1	ND	ND	Alcántar-Escalera *et al*., 2013
		5	100	1	Presente trabajo
	Came	5	ND	ND	Alcántar-Escalera *et al*., 2013
		11	27.27	3.67 ± 2.68	Presente trabajo
	Cupa	13	ND	ND	Alcántar-Escalera *et al*., 2013
		16	43.75	2.86 ± 2.52	Presente trabajo
	Magd	7	14.29	3 ± 1.13	Presente trabajo
Zoogoneticus quitzeoensis / Cc, M	Zaca	32	6.25	ND	Martínez-Aquino *et al*., 2012

Nota 1. Este taxón corresponde a una nueva especie, la cual será descrita posteriormente (Choudhury A., com. pers.).

Nota 2. De acuerdo con Martínez-Aquino et al., 2013, *M. guillerminae* es sinónimo de *M. bravoae,* por lo cual los registros aqui presentados consideran la distribución de la especie actualmente válida únicamente.

Nota 3. Este taxón corresponde a una nueva especie, la cual será descrita posteriormente (Pérez-Ponce de León, com. pers.).

Nota 4. Este taxón corresponde a una nueva especie, la cual será descrita posteriormente (Pérez-Ponce de León, com. pers.).

Nota 5. Mendoza-Palmero *et al*. (2007) registaron *Gyrordactylus* sp. en la Cantera Oriente (Cano), Ciudad Universitaria, UNAM, Ciudad de México, D.F; sin embargo, con base en un datos morfológicas más detallados, Mendoza-Palmero (en este trabajo) detectó que la clasificación taxonómica correcta es *G. lamothei*.

Nota 6. Salgado-Maldonado (2006) registro *Gyrodactylus* sp. de las localidades de Rinc y Laja (ver cuadro TAL para más información); sin embargo, Martínez-Aquino *et al*. (en este trabajo), detectaron con base en distintas medidas morfológicas que la clasificación taxonómica correcta de este taxón es *Gyrodactylus mexicanus*.

Nota 7. Este taxón corresponde a una nueva especie, la cual será descrita posteriormente (Mendoza-Palmero, com. pers.).

Nota 8. Este taxón corresponde a una nueva especie, la cual será descrita posteriormente (Mendoza-Palmero, com. pers.).

Nota 9. Salgado-Maldonado *et al*., (2001) y Sánchez-Nava *et al*., (2004) registraron *Gyrodactylus elegans* en Atla, Cimm, Chic, Juan, Pedr, Sala, Sant y Sier (ver Cuadro 3 para más información referente a las localidades). Mendoza-Palmero *et al*., (2009) mencionan que estos autores no depositaron ejemplares de referencia en ninguna colección científica que valide dichos registros. Harris *et al*., (2004) han sugerido que *G. elegans* es específico de *Abramis brahama* (Linnaeus, 1758) (Cyprinidae). Mendoza-Palmero *et al*., (2009) sugirieron que la determinación realizada por Salgado-Maldonado *et al*., (2001) y Sánchez-Nava *et al*., (2004) es dudosa. Por tanto, debido a la no existencia de vouchers depositados en ninguna colección científica, en este trabajo nosotros tomamos la postura conservadora de (según el Código Internacional de Nomenclatura Zoológica; Ride, 1985) y no consideramos validos los registros de Salgado-Maldonado *et al*. (2001) y Sánchez-Nava *et al*. (2004) ya que carecen de rigor científico.

Nota 10. Martínez-Aquino *et al* (2004; 2007b) registraron este taxón como posible nueva especie; sin embargo, Mendoza-Palmero (en este trabajo), detectó, con base distintas observaciones morfológicas más detalladas, que la determinación y clasificación taxonómica corresponde con *Salsuginus angularis.*

Nota 11. *Proteocephalus longicollis* es considerada un sinónimo de *P. pusillus* (sin. *P. exiguus*) La Rue, 1911 (Scholz y Hanzelová, 1998) que parasita exclusivamente peces de la familia Salmonidae de distribución holártica por lo que la presencia de esta especie en peces de la familia Goodeidae de México requiere una revisión más detallada.

Cuadro 2. Listado huésped-helminto parásito de Goodeinae (con base en el Cuadro 1). DA = Digéneo Adulto; LD = Larva de Digéneo; M = Monogéneo; CA = Céstodo Adulto; LC = Larva de Céstodo; NA = Nemátodo Adulto; LN = Larva de Nemátodo; AA = Acantocéfalo; LA = Larva de Acantocéfalo.

Especie de huésped	**Helmintos**
Allodontichthys hubbsi Miller y Uyeno, 1980	*Margotrema bravoae*^{DA} *Rhabdochona ahuehuellensis*^{NA}
Allodontichthys tamazulae Turner, 1946	*Margotrema bravoae*^{DA} *Rhabdochona ahuehuellensis*^{NA}
Allodontichthys zonistius Hubbs, 1932	*Ascocotyle (Ascocotyle) tenuicollis*^{LD} *Clinostomun complanatum*^{LD} Dilepididae gen. sp.^{LC} *Margotrema bravoae*^{DA} *Phyllodistomum* sp.^{DA} *Posthodiplostomum minimum*^{LD} *Rhabdochona ahuehuellensis*^{NA} *Saccocoeliodes* cf. *sogandaresi*^{DA} *Spiroxys* sp.^{LN} *Tylodelphys* sp.^{LD}
Alloophorus robustus Bean, 1892	*Bothriocephalus acheilognathi*^{CA} *Clinostomun complanatum*^{LD} *Contracaecum* sp.^{LN} *Cyclustera* cf. *ralli*^{LC} Dilepididae gen. sp.^{LC} *Eustrongylides* sp.^{LN} *Gnathostoma* sp.^{LN} *Margotrema bravoae*^{DA} *Polymorphus brevis*^{LA} *Pomphorhynhcus* cf. *bulbocolli*^{AA} *Posthodiplostomum minimum*^{LD} Proteocephalidae gen. sp.^{LC} *Proteocephalus longicollis*^{CA} *Pseudocapillaria tomentosa*^{NA} *Rhabdochona lichtenfelsi*^{NA} *Serpinema trispinosum*^{LN}

	Spiroxys sp.^{LN}
Allotoca catarinae de Buen, 1942	*Polymorphus brevis*^{LA}
	Rhabdochona xiphophori^{NA}
Allotoca diazi Meek, 1902	*Bothriocephalus acheilognathi*^{CA}
	Clinostomun complanatum^{LD}
	Cyclophyllidae gen. sp.^{LC}
	Eustrongylides sp.^{LN}
	Margotrema bravoae^{DA}
	Ochetosoma brevicaecum^{LD}
	Polymorphus brevis^{LA}
	Posthodiplostomum minimum^{LD}
	Proteocephalidae gen. sp.^{LC}
	Rhabdochona lichtenfelsi^{NA}
	Spiroxys sp.^{LN}
Allotoca dugesii Bean,1887	*Gyrodactylus lamothei*^{M}
	Gyrodactylus mexicanus^{M}
	Gyrodactylus sp. 1 ^{M}
	Margotrema bravoae^{DA}
	Rhabdochona lichtenfelsi^{NA}
	Salsuginus angularis^{M}
Allotoca goslinei Smith y Miller, 1987	*Posthodiplostomum minimum*^{LD}
Allotoca maculata Smith y Miller, 1980	*Margotrema bravoae*^{DA}
Allotoca meeki Álvarez del Villar, 1959	*Margotrema bravoae*^{DA}
Allotoca zacapuensis Meyer, Radda y Domínguez, 2001	*Allocreadium lobatum*^{DA}
	Bothriocephalus acheilognathi^{CA}
	Caryophyllidea gen. sp.^{LC}
	Margotrema bravoae^{DA}
	Phyllodistomum sp.^{DA}
	Polymorphus brevis^{LA}
	Posthodiplostomum minimum^{LD}
	Rhabdochona lichtenfelsi^{NA}
	Tylodelphys sp.^{LD}
Ameca splendens Miller y Fitzsimons, 1971	*Ascocotyle (Ascocotyle) tenuicollis*^{LD}
	Polymorphus brevis^{LA}
	Rhabdochona lichtenfelsi^{NA}

	Saccocoeliodes sp$^{\text{DA}}$
	Salsuginus angularis$^{\text{M}}$
Ataeniobius toweri Meek, 1904	*Rhabdochona ahuehuellensis*$^{\text{NA}}$
Chapalichthys encaustus Jordan y Snyder, 1899	*Clinostomun complanatum*$^{\text{LD}}$
	Contracaecum sp.$^{\text{LN}}$
	Cyclustera cf. *ralli*$^{\text{LC}}$
	Polymorphus brevis$^{\text{LA}}$
	Posthodiplostomum minimum$^{\text{LD}}$
	Rhabdochona lichtenfelsi$^{\text{NA}}$
	Salsuginus angularis$^{\text{M}}$
Chapalichthys pardalis Álvarez del Villar, 1963	*Margotrema bravoae*$^{\text{DA}}$
	Rhabdochona lichtenfelsi$^{\text{NA}}$
Characodon audax Smith y Miller, 1986	*Allocreadium mexicanum*$^{\text{DA}}$
	Allocreadium sp.$^{\text{DA}}$
	Bothriocephalus acheilognathi$^{\text{CA}}$
	Caryophyllidea gen. sp.$^{\text{LC}}$
	Clinostomun complanatum$^{\text{LD}}$
	Contracaecum sp.$^{\text{LN}}$
	Gyrodactylus sp. 1$^{\text{M}}$
	Eustrongylides sp.$^{\text{LN}}$
	Margotrema bravoae$^{\text{DA}}$
	Margotrema sp.$^{\text{DA}}$
	Posthodiplostomum minimum$^{\text{LD}}$
	Rhabdochona lichtenfelsi$^{\text{NA}}$
	Salsuginus angularis$^{\text{M}}$
	Serpinema trispinosum$^{\text{LN}}$
	Spiroxys sp.$^{\text{LN}}$
Characodon lateralis Gunther, 1866	*Allocreadium mexicanum*$^{\text{DA}}$
	Allocreadium sp.$^{\text{DA}}$
	Bothriocephalus acheilognathi$^{\text{CA}}$
	Gyrodactylus sp. 1 $^{\text{M}}$
	Polymorphus brevis$^{\text{LA}}$
	Spiroxys sp.$^{\text{LN}}$
	Streptocara sp.$^{\text{LN}}$
Girardinichthys multiradiatus Meek, 1904	*Bothriocephalus acheilognathi*$^{\text{CA}}$
	Contracaecum sp.$^{\text{LN}}$
	Cyclustera cf. *ralli*$^{\text{LC}}$

	Eustrongylides sp.^{LN}
	Falcaustra sp.^{LN}
	Gyrodactylus cf. *elegans* ^{M}
	Gyrodactylus lamothei^{M}
	Gyrodactylus mexicanus^{M}
	Gyrodactylus sp. 1^{M}
	Ligula intestinalis^{LC}
	Margotrema bravoae^{DA}
	Ochetosoma brevicaecum^{LD}
	Polymorphus brevis^{LA}
	Posthodiplostomum minimum^{LD}
	Spiroxys sp.^{LN}
	Tylodelphys sp.^{LD}
	Valipora campylancristrota^{LC}
Girardinichthys viviparus Bustamante, 1837	*Eustrongylides* sp.^{LN}
	Posthodiplostomum minimum^{LD}
	Valipora campylancristrota^{LC}
Goodea atripinnis Jordan, 1880	*Apharyngostrigea* sp.^{LD}
	Bothriocephalus acheilognathi^{CA}
	Capillaria gen. sp.^{NA}
	Centrocestus formosanus^{LD}
	Clinostomun complanatum^{LD}
	Contracaecum sp.^{LC}
	Eustrongylides sp.^{LN}
	Glossocercus auritus^{LC}
	Gyrodactylus lamothei^{M}
	Gyrodactylus mexicanus^{M}
	Gyrodactylus sp.^{M}
	Gyrodactylus sp. 1 ^{M}
	Ligula intestinalis^{LC}
	Margotrema bravoae^{DA}
	Margotrema sp.^{DA}
	Ochetosoma brevicaecum^{LD}
	Polymorphus brevis^{LA}
	Posthodiplostomum minimum^{LD}
	Proteocephalidae gen. sp.^{LC}
	Proteocephalus ambloplitis^{LC}
	Proteocephalus longicollis^{CA}
	Pseudocapillaria tomentosa^{NA}
	Rhabdochona lichtenfelsi^{NA}
	Saccocoeliodes cf. *sogandaresi* sp.^{DA}

	Salsuginus angularis^{M}
	Spiroxys sp.^{LN}
	Tylodelphys sp.^{LD}
	Uvulifer sp.^{LD}
Goodea gracilis Hubbs y Turner, 1939	*Pseudocapillaria tomentosa*^{NA}
	Rhabdochona lichtenfelsi^{NA}
Hubbsina turneri de Buen, 1941	*Phyllodistomum* sp.^{DA}
	Polymorphus brevis^{LA}
	Rhabdochona lichtenfelsi^{NA}
	Tylodelphys sp.^{LD}
Ilyodon cortesae Paulo-Maya y Trujillo-Jiménez, 2000	*Bothriocephalus acheilognathi*^{CA}
	Margotrema bravoae^{DA}
Ilyodon furcidens Jordan y Gilbert, 1882	*Centrocestus formosanus*^{LD}
	Capillaria cyprinodonticola^{NA}
	Clinostomun complanatum^{LD}
	Contracaecum sp.^{LN}
	Dendrorchis sp.^{DA}
	Dilepididae gen. sp.^{LC}
	Magnivitellinum simplex^{DA}
	Margotrema bravoae^{DA}
	Phyllodistomum sp.^{DA}
	Posthodiplostomum minimum^{LD}
	Rhabdochona ahuehuellensis^{NA}
	Rhabdochona lichtenfelsi^{NA}
	Rhabdochona sp.^{NA}
	Saccocoeliodes cf. *sogandaresi* sp.^{LD}
	Salsuginus angularis^{M}
	Spiroxys sp.^{LN}
Ilyodon whitei Meek, 1904	*Bothriocephalus acheilognathi*^{CA}
	Centrocestus formosanus^{LD}
	Eustrongylides sp.^{LN}
	Margotrema bravoae^{DA}
	Posthodiplostomum minimum^{LD}
	Rhabdochona ahuehuellensis^{NA}
	Rhabdochona kidderi^{NA}
	Saccocoeliodes cf. *sogandaresi*^{DA}
	Salsuginus angularis^{M}
Neoophorus regalis Álvarez del Villar, 1959	

	Gyrodactylus sp. 1^{M}
	Margotrema bravoae^{DA}
	Rhabdochona lichtenfelsi^{NA}
	Salsuginus angularis^{M}
Neotoca bilineata Bean, 1887	*Bothriocephalus acheilognathi*^{CA}
	Rhabdochona lichtenfelsi^{NA}
Skiffia lermae Meek, 1902	*Bothriocephalus acheilognathi*^{CA}
	Clinostomun complanatum^{LD}
	Eustrongylides sp.^{LD}
	Gyrodactylus mexicanus^{M}
	Gyrodactylus sp. 1^{M}
	Gyrodactylus sp. 2^{M}
	Polymorphus brevis^{LA}
	Posthodiplostomum minimum^{LD}
	Proteocephalidae gen. sp.^{LC}
	Proteocephalus longicollis^{CA}
	Pseudocapillaria tomentosa^{NA}
	Rhabdochona lichtenfelsi^{NA}
	Salsuginus angularis^{M}
	Tylodelphys sp.^{LD}
Skiffia multipunctata Pellegrin, 1901	*Polymorphus brevis*^{LA}
	Rhabdochona lichtenfelsi^{NA}
	Rhabdochona sp.^{NA}
	Salsuginus angularis^{M}
Xenoophorus captivus Hubbs, 1924	*Spiroxys* sp.^{LN}
Xenotaenia resolanae Turner, 1946	*Clinostomun complanatum*^{LN}
	Dendrorchis sp.^{DA}
	Margotrema resolanae^{DA}
	Margotrema sp.^{DA}
	Posthodiplostomum minimum^{LD}
	Rhabdochona ahuehuellensis^{NA}
	Salsuginus angularis^{M}
	Spiroxys sp.^{LN}
Xenotoca eiseni Rutter, 1896	*Rhabdochona xiphophori*^{NA}
Xenotoca melanosoma Fitzsimons, 1972	*Gyrodactylus* sp. 1^{M}
	Margotrema bravoae^{DA}

	Salsuginus angularis^{M}
Xenotoca variata Bean, 1887	*Bothriocephalus acheilognathi*^{CA}
	Clinostomun complanatum^{LD}
	Contracaecum sp.^{LN}
	Cyclustera cf. *ralli*^{LC}
	Gyrodactylus mexicanus^{M}
	Margotrema bravoae^{DA}
	Polymorphus brevis^{LA}
	Posthodiplostomum minimum^{LD}
	Proteocephalidae gen. sp.^{LC}
	Proteocephalus ambloplitis^{LC}
	Pseudocapillaria tomentosa^{NA}
	Rhabdochona lichtenfelsi^{NA}
	Salsuginus angularis^{M}
	Spiroxys sp.^{LN}
	Tylodelphys sp.^{LD}
	Uvulifer sp.^{LD}
	Valipora campylancristrota^{LC}
	Valipora mutabilis^{LC}
Zoogoneticus purhepechus Domínguez-Domínguez, Pérez-Rodríguez y Doadrio, 2008	*Bothriocephalus acheilognathi*^{CA}
	Clinostomun complanatum^{LD}
	Contracaecum sp.^{LN}
	Cyclophyllidae gen. sp.^{LC}
	Eustrongylides sp.^{LN}
	Margotrema bravoae^{DA}
	Phyllodistomum sp.^{DA}
	Polymorphus brevis^{LA}
	Pomphorhynhcus cf. *bulbocolli*^{AA}
	Rhabdochona lichtenfelsi^{NA}
	Rhabdochona sp.^{NA}
	Spiroxys sp.^{LN}
Zoogoneticus quitzeoensis Bean, 1898	*Allocreadium lobatum*^{DA}
	Bothriocephalus acheilognathi^{CA}
	Clinostomun complanatum^{LD}
	Gyrodactylus lamothei^{M}
	Gyrodactylus sp. 1 ^{M}
	Gyrodactylus sp. 2 ^{M}
	Margotrema bravoae^{DA}
	Phyllodistomum sp.^{DA}
	Polymorphus brevis^{LA}

Posthodiplostomum minimum$^{\mathrm{LD}}$
Rhabdochona lichtenfelsi$^{\mathrm{NA}}$
Salsuginus angularis$^{\mathrm{M}}$
Spiroxys sp.$^{\mathrm{LN}}$
Tylodelphys sp.$^{\mathrm{LD}}$

Cuadro 3. Localidades de registros de helmintos parásitos de Goodeinae. Los asteriscos (*) hacen referencia las localidades nuevas aportadas por nuestros propios muestreos y aportadas al presente estudio.

Acrónimo	**Nombre de la localidad y estado**	**Tipo de hábitat**	**Georreferencias**
Abra	Abraham González, Durango	Canal	24° 12' 50.7" N; 104° 36' 25.5" W
Adju	Las Adjuntas, Michoacán	Río	19° 54′ 39.3″ N, 102° 12′ 20.0″ W
Ahua	Ahuacapán, Jalisco	Arroyo	19º 39' 44.7" N; 104º 19' 13.7" W
Ahue	Ahuehuello, Puebla	Río	18° 45' 19.0" N; 98° 34' 20.4" W
Almo	Almoloya, Almoloya del Río, Estado de México	Canal	19° 11' 20" N; 99° 29' 30" W
Amac	Amacuzac, Morelos	Río	18° 38' 47" N; 99° 27' 02" W
Amad	Amado Nervo, Durango	Manantial	23º 50' 32.0" N; 104º 11' 13.7" W
Atla	Atlacomulco, Estado de México	Bordo/Lago	19° 47' N; 99° 51' W
Atot	Río La Laja en Atotonilco, Guanajuato	Río	21º 00' 07" N; 100º 47' 42" W
Bata	El Batán, Querétaro	Presa	20° 13' 13" N; 100° 24' 39" W
Berr	Los Berros, Durango	Manantial	23º 56" 18.2" N, 104º 16' 26.4" W
Bizn	Biznaga, Guanajuato	Presa	21° 25' 30" N; 100° 52' 52.7" W
Buen	Buenaventura, Chihuahua	?	29° 50' 16.94" N; 107° 28' 24.86" W
Calt	Presa Caltzonzin, Michoacán	Presa	19º 25' 14.8" N; 102º 07' 05.8" W
Came	Lago de Camécuaro, Michoacán	Lago	19° 54′ 2.3″ N, 102° 12′ 24.4″ W
Cano	Cantera Oriente, Ciudad Universitaria, UNAM, Ciudad de México, D.F.	Cantera	19º 19' 3" N; 99º 10' 22.2" W
Chap	Parque Chapultepec, Ciudad de México, D.F.,	Lago artificial	19° 25' 21.1" N; 99° 11' 02.7" W
Chic	Lago de Chicnahuapan, ("Almoloya del Río"), Estado de México	Lago	19° 11' 20"N; 99° 29' 30" W
Chil	Chilchota, Michoacán*	?	
Chiq	Chiquimitio, Michoacán	Arroyo	19° 47' 56.4" N; 101° 14' 45.9" W
Chis	El Chisco, Morelos	Río	18° 33' 00" N; 99° 13' 00" W
Cien	Río La Laja en La Cieneguita, Guanajuato	Río	20° 57' 08" N; 100° 47' 42" W
Cimm	"El CIMMYT" Metepec, Estado de México	Bordo	19° 13' 55" N; 99° 33' 05 " W
Cler	Cienega del Lerma	Cienega	N. R.
Coin	Cointzio, Michoacán	Presa	19° 37' 7"N; 101° 16' 31" W
Cons	Constitución de 1917, Querétaro	Presa	20° 25' 00" N; 100° 05' 00" W
Coro	La Coronilla, Jalisco	Manantial	20º 28' 9.4" N; 104º 04' 10.6" W
Cris	San Cristóbal, Michoacán	Manantial	19º 57' 41.6" N; 101º 18' 57.3"W

Cuis	Pueblo de Cuisillos, Rancho de Don Ramón Simón, Jalisco*	Manantial	20º 35" 44' N; 103º 46' 33" W
Cuit	Cuitzeo, Guanajuato-Michoacán	Lago	20° 04' 34"-19° 53' 25" N; 101° 19' 34"-100° 50' 20" W
Cupa	Cupatziro, Michoacán	Manantial	19° 52′ 51.3″ N, 102° 12′ 34.7″ W
Cutz	Cutzaróndiro, Durango	Manantial	19º 10" 59.0' N; 101º 30' 13.0" W
Cuza	Arroyo El Durazno, Jalisco	Arroyo	19° 30.550' N; 104° 17.665' W
Duer	Río Duero, Michoacán	Río	19° 53' 03.6" N; 102° 08' 53.1" W
Ener	Colonia 6 de enero, Nayarit	Lago	21º 31' 31.7" N; 104º 48' 14.8" W
Estó	Río Estórax, Querétaro	Río	21° 02' 11" N; 99° 50' 45" W
Ferr	Río La Laja en Ferrocarrileros, Guanajuato	Río	21º 48' 45" N; 100º 49' 07" W
Galv	Los Galvanes, Guanajuato	Río	21° 03' N; 100° 48' W
Guac	Guachinango, Michoacán	N.D.	20º 32' 0.5" N; 104º 24' 8.7" W
Guad	Manantial en la UMA de caza, poblado de Guadalupe Aguilera, Durango	Manantial	24º 25' 59.5" N; 104º 38' 29" W
Idel	Tributario de Río San Idelfonso, Querétaro*	Tributario	
Igna	Ignacio Allende, Guanajuato	Presa	20° 55' N; 100° 50' W
Ignr	Ignacio Ramírez, Estado de México	Presa	19° 26' 54" N; 99° 54' 39" W
Jesú	Jesús María, San Luis Potosí	?	21º 55' 31.0"N; 100º 54' 38.3" W
Juam	San Juanico, Michoacán	Presa	19° 50' 36" N; 102° 40' 41" W
Juan	San Juanico, Estado de México	Presa	19° 55' N; 99° 46' W
Juas	Ojo de agua de San Juan, Durango	Manantial	23° 57' 11.6" N; 104° 16' 15" W
Laad	Río La Laja en Las Adjuntas, Guanajuato	Río	21° 07' 29" N; 100° 52' 12" W
Lagu	La Lagunilla , Estado de México	Bordo	19° 08' 30" N; 99° 30' 12" W
Laja	Río La Laja, Guanajuato	Río	21° 20' 26" N; 100° 55' 20" W
Lcha	Chapala, Jalisco	Lago	20° 14' N; 103° 10' W
Lerm	Ciénega del Lerma, Estado de México	Humedal	19° 22' 41" N; 99° 59' 39" W
Luna	Lago de la Media Luna, San Luis Potosí	Lago	21º 51' 18.6" N; 100º 01' 22.3" W
Luz	La Luz, Michoacán	Manantial	19º 56' 08.1" N; 102º 18' 0.2" W
Magd	Canal La Magdalena, Jalisco*	Canal	
Mara	Maravatío, Michoacán	Lago?	19º 52' 56.1" N; 100º 26' 51.9" W
Marc	Río San Marcos, Jalisco	Río	20º 46' 35.7" N; 104º 09' 52.6" W
Marí	Río La Laja en Presa Jesús María, Guanajuato	Presa	21º 21' 16" N; 101º 12' 49" W
Mcha	Pueblo de Chapultepec, Manantial de Chapultepec, Michoacán	Manatial	19º 34' 20" N; 101º 31' 18.7" W
Merc	Presa Aristeo Mercado, Michoacán	Presa	19º 55' 34.6" N; 101º 39' 38" W
Mina	Mina, Toluca, Estado de México	Bordo	N. D.
Mint	La Mintzita, Michoacán	Manantial	19° 38' 40.3"-19° 38' 52.3" N; 101° 16' 28.20"-101° 16' 13.0" W
Moct	Moctezuma, San Luis Potosí	Arroyo	22° 44.673' N; 101° 05.802' W

Negr	Los Negritos, Michoacán	Manantial	20º 03' 23.1" N; 102º 36'38.3" W
Nori	La Noria, Jalisco*	Manantial	20º 35' 45" N; 103º 46' 54" W
Opop	Opopeo, Michoacán	Lago	19º 24' 16.7" N; 101º 36' 09.1" W
Oran	Orandino, Michoacán	Lago	19º 57' 21.8" N; 102º 19' 29.7" W
Pásj	San Jerónimo en Lago de Pátzcuaro, Michoacán*		
Pátz	Lago de Pátzcuaro, Michocacán	Lago	19° 41' - 19° 32' N; 101° 27' - 101° 53' W
Pedr	San Pedro del Rosal, Atlacomulco, Estado de México	Bordo	N. D.
Piax	Río Piaxtla, Municipio de San Dimas, Durango	Río	24º 21' 59" N; 105º 31' 7.8" W
Pihu	Pihuamo, Jalisco	Río	19º 15' 23.5" N; 103º 22' 37.3" W
Pin1	Puente en el poblado de Pino Suárez, carretera Durango-Mezquital, Durango	?	23º 52' 43.5" N; 104º 31' 54.7" W
Pin2	Arroyo Pino Suárez 2, Durango	?	23º 52' 12.4" N; 104º 29' 39.3" W
Porv	Canal el Porvenir, Michoacán	Canal	19° 40' 29" N; 100° 38' 25" W
Potg	Potrero Grande, Jalisco	Río	20° 31' 17.2" N; 104° 07' 29.2" W
Puen	Puente la Rosa, Jalisco	Río	19° 27.766' N; 104° 19.134' W
Quem	Tierra Quemada, San Luis Potosí	Manantial	21º 42' 39.1" N; 100º 41' 32.6" W
Quer	Puente Río Queréndaro, Michoacán	Río	19º 53' 09.6" N; 100º 57' 06.9" W
Reye	Los Reyes, Michoacán	Lago	19º 33' 43.5" N; 102º 27' 39" W
Rico	Manantial Rico, Michoacán	Manantial	19°49'51.85"N; 102°30’7.98’’W
Rinc	Río La Laja en el Rincón de los Remedios, Guanajuato	Río	20º 47' 20" N; 100º 48' 25" W
Sala	Salazar, Estado de México	Lago	19° 18' 34" N; 99° 23' 45" W
Sand	Rio Santa Isabel en Sandoval, Chihuahua	Río	28° 33' 28.36" N; 106° 31' 38.4" W
Sant	Santiago Tiacaque, Ixtlahuaca, Estado de México	Presa	19° 40' 22" N; 99° 42' 28" W
Siem	Sierra de Manantlán, Jalisco	Río	19° 39' N; 104° 14' 24" W
Sier	Parque Sierra Morelos, Estado de México	Bordo	19° 18' 31" N; 99° 41' 18" W
Sofi	Río Guatimape en el poblado de Sofía, Durango	Río	24° 54' 41.1" N; 104° 32' 7.4" W
Sori	Río La Laja en Soria La Huerta, Guanajuato	Río	20° 48' 45" N; 100° 49' 07" W
Tama	Río Tamazula, Jalisco	Río	19º 43' 22.7" N; 103º 12' 08.5" W
Tamo	Rio Tamochi, Chihuahua	Río	28° 21' 6.8" N; 107° 51' 10.44" W
Tapia	Naranja de Tapia, Michoacán	Lago	19º 16' 58.2" N; 101º 45' 50.3" W
Teco	Río Tecolote, Jalisco	Río	19º 27' 40.1" N; 104º 19' 12.3" W
Tepe	Tepetitlan, Estado de México	Presa	19° 37' 50" N; 99° 58' 27" W
Teuc	Balneario "El Rincón", Teuchintlán, Jalisco	Manantial	20° 41.537' N; 103º 50.685' W

Tica	Canal Santiago Tiacaque, Estado de México	Canal	
Ties	Estanque de río en Canal Santiago Tiacaque, Estado de México*	Estanque	
Tipr	Presa en Canal Santiago Tiacaque, Estado de México	Presa	
Tlap	Pueblo Tlapetlahuaya, Puebla	“Manantial”	18° 45' 20.4" N; 98° 34’ 28.8’’ W
Tobo	El Toboso, Durango	Manantial	24° 16' 30.7" N; 104° 34' 52.8" W
Tocu	Tocumbo, Michoacán	Manantial	19º 42' 70" N; 102º 30' 58.4" W
Trin	Trinidad Fabela, Estado de México	Presa	19° 49' 27" N; 99° 47' 12" W
Tule	El Tule, Jalisco	Lago	19º 19" 34.2" ; 103º 22' 15.0" W
Ucas	Ucasanastacua, Michoacán*	¿?	¿?
Vent	Rancho la Venta, Acambay, Estado de México	Bordo	N. D.
Verd	Río Verde, Jalisco	Río	21º 49" 12.0" N; 101º 46' 21.3" W
Vict	Villa Victoria, Estado de México	Presa	19° 27' 30" N; 99° 59' 39" W
Xote	Río Xote, Querétaro	Río	N. D.
Zaca	Zacapu, Michoacán	Lago	19° 49' N; 101° 47' W
Zemp	Zempoala, Estado de México-Morelos	Lago	19° 03' 00" N; 99° 18' 42" W
Zúñi	Río Las Zúñigas, Querétaro*	Río	20°16’N, 100°48’45’’W
27No	Manantial en el pubelo 27 de Noviembre, Durango	Manantial	24° 12' 16.5" N; 104° 29' 38" W

Apéndice 1. Cuadro de localidades de nuestros muestreos para helmintos parásitos de Goodeinae en México. Las georreferencias de cada localidad se detallan en el cuadro 3. N = tamaño de muestra de cada especie de huésped por localidad.

Localidad	**Especie de huésped**	**N**
1. Manantial en Ahuacapán, Jalisco	*Allodontichthys zonistius*	11
	Ilyodon furcidens	11
2. Manantial en Canal La Magdalena, Michoacán	*Chapalichthys pardalis*	3
3. Canal la Magdalena, Michoacán	*Ilyodon furcidens*	2
	Zoogoneticus purhepechus	7
4. Canal de Santiago Tiacaque, Estado de México	*Girardinichthys multiradiatus*	12
5. Manantial Chapultepec, Michoacán	*Allotoca diazi*	21
	Allotoca diazi	9
	Allotoca dugesii	22
	Goodea atripinnis	13
	Skiffia lermae	32
6. Manantial Chilchota, Michoacán	*Skiffia multipunctata*	10
7. Manantial Cupatziro, Michoacán	*Skiffia multipunctata*	13
	Zoogoneticus purhepechus	16
8. Arroyo El Durazno en el Río Cuzalapa, Jalisco	*Xenotaenia resolanae*	35
9. El Tule (Río), Jalisco	*Allodontichthys tamazulae*	19
10. Estanque en el Río del Canal Santiago Tiacaque, Estado de México	*Girardinichthys multiradiatus*	6
11. La Angostura, lago Zacapu (balneario), Michoacán	*Skiffia lermae*	11
	Allotoca zacapuensis	32
	Goodea atripinnis	20
	Hubbsina turneri	16
	Skiffia lermae	19
	Zoogoneticus quitzeoensis	30
12. Manantial La Luz, en Jacona de Plancarte, Michoacán	*Zoogoneticus purhepechus*	32
	Zoogoneticus purhepechus	12
	Chapalichthys encaustus	8
	Skiffia multipunctata	4
	Alloophorus robustus	35
13. Manantial La Mintzita, Michoacán, Michoacán	*Zoogoneticus quitzeoensis*	22
	Alloophorus robustus	14
	Goodea atripinnis	5

	Skiffia lermae	6
	Xenotoca variata	40
14. Lago de Camecuaro, Michoacán	*Alloophorus robustus*	2
	Zoogoneticus purhepechus	11
15. Lago de Opopeo, Michoacán	*Goodea atripinnis*	4
	Allotoca meeki	4
16. Las Adjuntas (Río), Michoacán	*Skiffia multipunctata*	2
	Zoogoneticus purhepechus	5
17. Manantial Los Negritos, Michoacán	*Chapalichthys encaustus*	2
	Zoogoneticus purhepechus	19
	Zoogoneticus purhepechus	9
	Zoogoneticus purhepechus	8
18. Manantial Cutzaróndiro, Michoacán	*Ilyodon cortesae*	31
19. Manantial La Estancia, Michoacán	*Zoogoneticus purhepechus*	21
20. Parque Ecológico Chicnahuapan (Lago), Estado de México	*Girardinichthys multiradiatus*	44
	Girardinichthys multiradiatus	29
21. Lago de Pátzcuaro, Michoacán	*Goodea atripinnis*	31
	Alloophorus robustus	2
22. Predio Porfirio, La Angustura, Lago de Zacapu, Michoacán	*Allotoca zacapuensis*	3
	Xenotoca variata	1
	Alloophorus robustus	2
23. Bordo en el Canal de Santiago, Tiacaque, Estado de México	*Girardinichthys multiradiatus*	2
24. Bordo en Ignacio Ramírez, Estado de México	*Girardinichthys multiradiatus*	14
25. Bordo en Salazar, Estado de México	*Girardinichthys multiradiatus*	35
26. Manantial Rico, Michoacán	*Neophorus regalis*	39
26. Río Ahuehuello en Santo Domingo, Puebla	*Ilyodon whitei*	51
28. Río Ángulo cerca del lago de Zacapu, Michoacán	*Alloophorus robustus*	9
	Xenotoca variata	20
	Zoogoneticus quitzeoensis	11
	Allotoca sp.	2
29. Río Tamazula, Jalisco	*Allodontichthys zonistius*	32
	Ilydon furcidens	56
30. Tributario del Río San Idelfonso, Querétaro	*Goodea atripinnis*	10
31. San Jerónimo, Lago Pátzcuaro, Michoacán	*Alloophorus robustus*	5
32. Santa María del Oro (Río), Jalisco	*Ilydon furcidens*	15

33. Bordo en Tlapetlahuaya, Puebla	*Ilyodon whitei*	34
34. Manantial en el parque de Tocumbo, Michoacán	*Chapalichthys pardalis*	38
	Goodea atripinnis	16
	Ilydon whitei	18
35. Ucasanastacua, Pátzcuaro, Michoacán	*Alloophorus robustus*	11
	Goodea atripinnis	
36. Puente en el poblado de Pino Suárez, carretera Durango-Mezquital, Durango	*Characodon audax*	21
37. Manantial en Abraham González, Durango	*Characodon audax*	12
38. Manantial en la Unidad de Manejo Ambiental de caza, Guadalupe Aguilera, Durango	*Characodon audax*	14
39. Manantial el Toboso, Durango	*Characodon audax*	42
40. Manantial en el poblado Amado Nervo, Durango	*Characodon lateralis*	9
41. Ojo de Agua de San Juan, Durango	*Characodon lateralis*	11
42. Ojo de Agua del poblado de los Berros, Durango	*Characodon lateralis*	23
43. Río Potrero Grande, Jalisco	*Ilydon furcidens*	10
	Allotoca goslinei	2
44. Río Piaxtla, Municipio San Dimas, Durango	*Codoma ornata*	21
45. Manantial en Teuchitlán (balneario), Jalisco	*Ameca splendens*	33
46. Manantial Veneros, Jalisco	*Ameca splendens*	6
47. Río Zúñigas, Queretaro	*Goodea atripinnis*	7
48. Río La Laja en el Rincón de los Remedios, Guanajuato	*Goodea atripinnis*	32
49. Río La Laja, Guanajuato	*Goodea atripinnis*	24
50. Río La Laja en Atotonilco, Guanajuato	*Goodea atripinnis*	10
51. Ignacio Allende, Guanajuato	*Xenotoca variata*	30
52. Trinidad Fabela, Estado de México	*Goodea atripinnis*	29
53. Puente La Rosa, Jalisco	*Ilydon furcidens*	3
54. Río Duero, Michoacán	*Skiffia multipunctata*	8
55. Pueblo de Cuisillos, Rancho de Don Ramón Simón, Jalisco	*Xenotoca melanosoma*	20
56. La Noria, Jalisco	*Xenotoca melanosoma*	38
57. Río La Laja en La Cieneguita, Guanajuato	*Xenotoca variata*	31

CAPÍTULO II

MOLECULAR PHYLOGENY OF THE GENUS *MARGOTREMA* (DIGENEA: ALLOCREADIIDAE), PARASITIC FLATWORMS OF GOODEID FRESHWATER FISHES ACROSS CENTRAL MEXICO: SPECIES BOUNDARIES, HOST-SPECIFICITY, AND GEOGRAPHICAL CONGRUENCE

En este proyecto se planteo como segundo objetivo particular reconstruir las relaciones filogenéticas intraespecíficas del género *Margotrema* para esclarecer la validez taxonómica de sus dos especies putativas (*M. bravoae* y *M. guillerminae*), con base en marcadores moleculares. Para ello, se reconstruyeron las relaciones genealógicas de 125 ejemplares de *Margotrema*, asociados a 14 especies de goodeinos (incluídas en las cuatro tribus de la subfamilia), además de una especie huésped de la familia Cyprinidae (*Codoma ornata*). Estos ejemplares estaban distribuidos en 15 localidades de siete sistemas hidrológicos del centro de México (Martínez-Aquino *et al*., 2013). La reconstrucción filogenética se realizó usando una base de datos de secuencias de ADN, obtenidos de dos marcadores moleculares: *COX-1*, mitocondrial e *ITS1*, nuclear. Las bases de datos fueron analizadas, tanto de manera independiente como combinada, a través de análisis probabilísticos para la reconstrucción filogenética de secuencias de ADN, es decir, de probabilidad posterior (Huelsenbeck & Ronquist, 2001), teoría de la coalescencia (GMYC, por sus siglas en ingles *General Mixed Yule Coalescent* (Pons *et al*., 2006; Fontaneto *et al*., 2007) y árboles de genes y árboles de especies (*Species Tree multispecies coalescent*; Maddison, 1997; Edwards, 2009). Con base en las relaciones filogenéticas de *Margotrema*, se propusieron las primeras hipótesis biogeográficas y cofilogenéticas observadas para la asociación *Goodeinae-Margotrema*.

A continuación se presenta una versión del documento publicado referente a las relaciones filogenéticas intraespecificas de *Margotrema* spp., intitulado:

> **Molecular phylogeny of the genus *Margotrema* (Digenea: Allocreadiidae), parasitic flatworms of goodeid freshwater fishes across central Mexico: species boundaries, host-specificity and geographical congruence** (Martínez-Aquino *et al*., 2013).

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Molecular phylogeny of the genus *Margotrema* (Digenea: Allocreadiidae), parasitic flatworms of goodeid freshwater fishes across central Mexico: species boundaries, host-specificity, and geographical congruence

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We explored the genetic variation at the intraspecific level of two putative species of *Margotrema*, *M. bravoae* and *M. guillerminae*, to establish the species boundaries. Sequences of a mitochondrial (*COI*) and nuclear (*ITS1*) gene were obtained for 125 specimens distributed in 15 localities from seven hydrological systems. An alignment of 750 and 831 bp including gaps of *COI* and *ITS1*, respectively, was assembled. We analysed the gene fragments separately as well as together by using Bayesian inference for phylogenetic reconstruction. Based on the phylogenetic analyses, an ultrametric tree was built for each gene, and a general mixed Yule-coalescent model for species delimitation was carried out. A multispecies coalescent analysis was performed in *BEAST using both molecular markers. The results show four independent evolutionary lineages that we interpret as two valid species of *Margotrema*; the first is represented by an independent lineage, and the second is composed of three lineages. Because the species *M. bravoae* and *M. guillerminae* nested together within these three lineages, the validity of *M. guillerminae* as an independent species is questioned, and it is proposed to represent a junior synonym of *M. bravoae*. Each lineage shows a congruent geographical pattern with respect to the hydrological system where they occur. Additionally, each lineage shows congruence with respect to the hosts they parasitize, either at the species or at higher taxonomical levels (tribe). The parasite evolutionary history is congruent with the evolutionary and biogeographical history of their hosts.

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ADDITIONAL KEYWORDS: COI – GMYC – goodeinae – ITS1 – species tree.

INTRODUCTION

Our understanding of the evolution of parasitic organisms, as well as the complex and intricate association they establish with their hosts, has greatly improved with the use of molecular tools and a range of analytical methods that are available for analysing these data sets. Advancements in methodological tools has resulted in establishing more robust species delimitation criteria for parasitic organisms and understanding the influence of host specificity and

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*Corresponding author. E-mail: maandres@ibiologia.unam.mx geography on speciation mechanisms in parasites (see Poulin, Krasnov & Mouillot 2011a; Poulin *et al*., 2011b). Species limits based on molecular results are sometimes not congruent with limits established by using traditional characters such as morphology. This discrepancy is because each gene has a phylogenetic history that is intimately connected with, but not necessarily identical to, the history of the organism in which the genes exist (i.e. Maddison, 1997; Page & Charleston, 1997). Molecular phenomena such as gene duplication, incomplete lineage sorting, and horizontal gene transfer can produce complex gene trees that differ from species trees (i.e. Funk & Omland, 2003; Maddison & Knowles, 2006). The apparent incongruence between gene trees and our conception of what a parasite species is (based only on morphological criteria) is still poorly understood. Likewise, host–parasite associations might be the result of a long evolutionary history that may be reflected in similarities in their evolutionary trees (Page, 2003, and references therein). Few studies have been conducted to address the evolutionary history of freshwater fish helminth parasites. For example, Choudhury & Dick (2001) analysed the historical biogeography of a geologically old group of fishes, sturgeons, and their parasite assemblage. Apparently, this host–parasite association was historically structured by colonization and subsequent coevolution, and these authors concluded that vicariance and the dispersal of acipenserids resulted in small but widespread and highly distinct monophyletic parasite lineages.

In this paper, we have made use of relatively novel methodological approaches, and we have chosen a host–parasite system that involves a genus of digeneans that are allegedly host-specific to goodein freshwater fishes, i.e. that form part of the biogeographical helminth parasite core fauna (*sensu* Pérez-Ponce de León & Choudhury, 2005). Goodeines are an endemic monophyletic group of freshwater cyprinodontiforms that are restricted to central Mexico (Domínguez-Domínguez *et al*., 2010). The subfamily Goodeinae is represented by 42 species included in four monophyletic groups: Girardinichthyini, Chapalichthyini, Ilyodontini, and Characodontini (each recognized taxonomically as a tribe); the phylogenetic relationships and historical biogeography of this group has been studied to a certain extent (see Domínguez-Domínguez *et al*., 2010, and references therein). The diversification of this fish group is the result of complex geological events that have been taking place since the Miocene in central Mexico, as a result of hydro-geomorphological changes that influenced the orography of this area. Goodeinae represents one of the freshwater fish groups that have been studied intensively from the parasitological point of view (Peresbarbosa-Rojas, Pérez-Ponce de Léon & García-Prieto, 1994; Pérez-Ponce de León *et al*., 2000; Martínez-Aquino *et al*., 2004, 2007, 2009a, 2011, 2012; Sánchez-Nava *et al*., 2004; Mejía-Madrid, Domínguez-Domínguez & Pérez-Ponce de León, 2005). Their helminth parasite faunas consist of 40 species (Pérez-Ponce de León & Choudhury, 2010; Martínez-Aquino *et al*., 2011, 2012). Among the helminth species that infect goodeines in central Mexico, two putative species of *Margotrema*, *M. bravoae* Lamothe-Argumedo, 1972 and *M. guillerminae* Pérez-Ponce de León, 2001, are apparently specific to goodein hosts. Subtle morphological differences, i.e. extension of the intestinal ceca, being shorter and extending posteriad to reach the region of the anterior testis in *M. guillerminae*, vitelline follicles primarily lateral to the ceca, and the seminal receptacle located immediately posterior to the ovary, are used to distinguish *Margotrema* species, and some authors have discussed the need for a study of the intraspecific morphological variability (Pineda-López *et al*., 2005). Species of *Margotrema* have been recorded along 13 independent hydrological systems in central Mexico, parasitizing 22 species of goodeines included in the four recognized tribes, although scattered records are available for two species of cyprinids, *Codoma ornata* and *Notropis calientis*, and one species of cyprinodontid, *Cyprinodon nazas*.

In this paper, we study this host–parasite system in order to: (1) describe the molecular phylogenetic history of populations of the two species of parasites; (2) delimit species boundaries based on phylogenetic reconstructions and novel algorithms that use maximum likelihood as a means of providing more objective outcomes; and (3) test the hypothesis that each subgroup of goodeines (tribes) possesses their own species of *Margotrema* as a result of a similar history of vicariance and dispersal. This paper is based on three main premises: (1) full characterization of parasite biodiversity requires finding all species, including those that have formed relatively recently and may therefore have minimal levels of genetic and morphological divergence; (2) two different species may show no morphological divergence because the speciation event is very recent (and structural changes have not yet evolved) or, conversely, due to morphological stasis over long periods of evolutionary time (Nadler & Pérez-Ponce de León, 2011); and (3) the evolutionary history of the parasite reflects that of their hosts due to establishing a host-specific association and congruent geographical and host distribution patterns, at the species or population levels. While addressing species delimitation and the discovery of independent evolutionary lineages at the intraspecific level as the main question, this study further intends to decipher the relative role of geographical distribution and host-specificity in current patterns of parasite genetic variability.

MATERIAL AND METHODS

Collection of hosts and parasites

Between August 2008 and July 2010, a total of 1213 individual hosts representing 24 goodein species (included in the four tribes of Goodeinae) were collected. Samples were collected from 44 localities distributed along seven hydrological systems in central Mexico: the San Pedro Mezquital River, Ameca River, Ayuquila River, Coahuayana River, Lerma River, Cuzalapa River, and Balsas River. Specimens of *Margotrema* spp. were found in 15 of the 44 localities and in 15 of the 24 goodein species (see Supporting Information Appendix S1; Fig. 1). We also sampled a few specimens of *Margotrema* spp. in the upper Piaxtla River in north-western Mexico (Aguilar-Aguilar *et al*., 2010) (Appendix S1). Additionally, in January 2010, we collected specimens of two species of digeneans in tributaries of the Chagres River in Panama: *Wallinia chavarriae* Choudhury, Hartvigsen-Daverin & Brooks, 2002 (Digenea: Allocreadiidae) as a parasite of *Gephyrocharax* sp. (Characidae) at the locality of the Frijolito River (09°08′58.1″N, 79°43′53.8″W), and specimens of *Prosthenhystera* sp. (Digenea: Callodistomidae) as a parasite of another species of characid at the locality of Quebrada Juan Grande (09°08′50.5″N, 79°43′21.1″W); these worms were used as outgroups for the phylogenetic analysis in this study. Fishes were sampled using minnow traps, seine nets, and electrofishing, taken alive to the laboratory, killed, and individually examined for parasites. Internal organs were removed, placed in a Petri dish with 0.65% saline, and examined for helminths under a stereomicroscope. Digeneans were removed from the intestine of their hosts and placed in 0.65% saline. For molecular analysis, some specimens of *Margotrema* spp. (from 14 localities) were cleaned with saline and preserved in 100% ethanol. Of all the specimens preserved in ethanol and used for molecular analysis, digital vouchers (i.e. microphotography and videotape) were obtained for 23.3%. Another set of specimens were collected and fixed in hot (steaming) 4% formalin for morphological identification. Unflattened specimens were stained with Mayer's paracarmine and mounted as permanent slides using

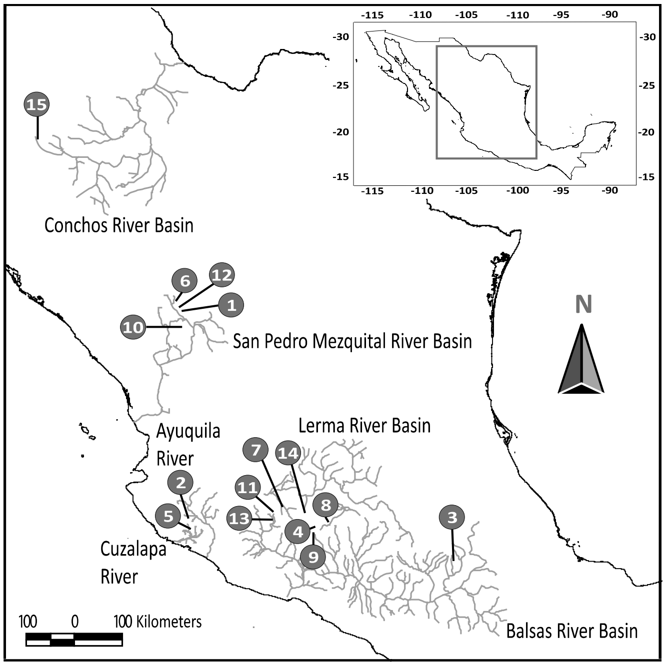

Figure 1. Hydrological systems and collection sites for *Margotrema* spp. in Mexico. Numbers correspond to each locality where specimens of *Margotrema* spp. were collected (for more information see Supporting Information Appendix S2).

Canada balsam. Digeneans were identified by conventional morphological criteria following Caira & Bogéa (2005) and Cribb (2005) and the original species descriptions (Lamothe-Argumedo, 1972; Pérez-Ponce de León, 2001). All specimens collected for morphological analysis were deposited as voucher specimens of *Margotrema* spp. in the Colección Nacional de Helmintos (CNHE), Instituto de Biología, Universidad Nacional Autónoma de México (UNAM) [National Helminth Collection, Biology Institute, National Autonomous University of Mexico], Mexico City (Appendix S2).

DNA extraction, PCR amplification, and sequencing

Sequences from two markers were obtained: 750 bp of the *COI* gene and a range of 666–810 bp from the *ITS1* gene. These markers have proven to be useful for establishing species delimitation among digeneans (Blair, 2006; Miura *et al.*, 2006; Razo-Mendivil, Rosas-Valdez & Pérez-Ponce de León, 2008). To obtain a range of the *Margotrema* species' genetic variability (while avoiding sequencing individuals from the same host), DNA was extracted from one individual of *Margotrema* spp. for each host, per locality. In addition, specimens of *W. chavarriae*, *Prosthenhystera* sp., and *Allocreadium lobatum* Wallin, 1909 (from *Semotilus atromaculatus* from Tobacco Creek, Manitoba, Canada) were used for DNA extraction. DNA was obtained by using the DNAeasy blood and tissue extraction kit (Qiagen, Valencia, CA, USA) following the manufacturer's instructions. Amplifications of the selected DNA fragments were carried out in a total volume of 25 μL, with 2.5 μL of 10 × PCR buffer, 1.25 μL of $MgCl_2$, 1 μL of dNTPs (0.25 mM), 1 μL of each primer, 0.4 μL of Taq polymerase (OMEGA; 0.625 U), 1–5 μL of DNA template and 13.85 or 17.85 μL of ddH_2O. PCRs were carried out using primers JB3 fwd (5′-TTTTTTGGGCATC CTGAGGTTTAT-3′) (Morgan & Blair, 1998) and CO1-R trema rev (5′-CAACAAATCATGATGCAAA AGG-3′) (Miura *et al.*, 2005) for the *COI* fragment, and Glyp1 fwd (5′-GCTGAGAAGACGACCAAACTT GAT-3′) (Razo-Mendivil *et al.*, 2010) and BD2 rev (5′-TATGCTTAAATTCAGCGGGT-3′) (Luton, Walker & Blair, 1992) for *ITS1*. To obtain DNA sequences of *ITS1*, we used the following internal primers: 5.8S rev (5′-AATGTGCGTTCAAGATGTCGAT-3′) and ReInt BD1 (5′-ATGTTCATAAGACAACCCAGCTC-3′), specifically designed for this study. PCR cycling conditions were as follows: for *COI*, an initial denaturing step of 5 min at 94 °C, followed by 35 cycles of 92 °C for 30 s, 47 °C for 45/50 s, and 72 °C for 90 s, and a final extension step at 72 °C for 10 min; for *ITS1*, an initial denaturing step of 2 min at 94 °C, followed by 30 cycles of 94 °C for 1 min, 50 °C for 15 s, and 60 °C for 4 min, and a final extension step at 60 °C for 5 min. PCR products were either purified using Millipore columns (Millipore, Bedford, MA, USA) and then sequenced on an ABI Prism 3100 Genetic Analyser, or sent directly to the High-Throughput Genomics Unit at the University of Washington (http://www.htseq.org/index.html).

Molecular data sets

All sequences were edited using the platform Geneiuos Pro v5.1.7 (Drummond *et al.*, 2010). *COI* and *ITS1* alignments were assembled using an interface available with MAFFT v6.717b (Katoh & Toh, 2008) within Geneiuos Pro, with a final edition by eye in the same platform. For the *COI* sequences, we checked the nucleotide alignment and for the presence of pseudogenes in Geneiuos Pro, using the translated amino acid sequences based on the flatworm mitochondrial genetic code. In the *ITS1* marker sequences, we detected two 5′–3′ regions with different short tandem repeats (STRs). The first region was detected after 88 bp in all sequences of *Margotrema* spp., and the second region of 345 bp was only found in sequences of individuals from Cuzalapa River, Jalisco. As the first region of STRs possesses a total of 97 variants and second region posseses two variants, where homology cannot be established based on model substitution (Li *et al.*, 2002; Ellegren, 2004; Guy-Franck, Kerrest & Dujon, 2008), we did no use this molecular information in the phylogenetic analysis.

Phylogenetic reconstruction and species delimitation analyses

The most appropriate DNA sequence evolution model was selected by using jModelTest 0.1.1. (Posada, 2008) and applying the Bayesian Information Criterion (BIC) (Schwarz, 1978) for each data set separately (*COI* and *ITS1*). The *COI* data set was partitioned into first-, second- and third-codon positions with the appropriate nucleotide substitution model implemented for each codon position [HKY+G for the first (Hasegawa, Kishino & Yano, 1985); TPM2uf+G for the second (Kimura, 1981); and HKY+I+G for the third codon position (Hasegawa *et al.*, 1985)]. The nucleotide substitution model that fit the *ITS1* best was HKY+G. Sequences from two additional species, *A. lobatum* and *W. chavarriae*, were used to root the trees because they belong to the same family as *Margotrema* (Allocreadiidae). An additional taxon, *Prosthenhystera* sp. (Callodistomidae), was used as an outgroup because it is a sister group of the family Allocreadiidae (Curran, Tkach, &

Ovestreet, 2006, 2011). We inferred the phylogenetic relationships using Bayesian inference (BI) through MrBayes v. 3.1.2. (Huelsenbeck & Ronquist, 2001). The phylogenetic trees were reconstructed for both genes separately using two parallel analyses of Metropolis-coupled Markov chain Monte Carlo (MCMC) for 20×10^6 generations each to estimate the posterior probability (PP) distribution. Topologies were sampled every 1000 generations. Once the average standard deviation of split frequencies was less than 0.01, as suggested by MrBayes 3.1.2., the convergence between runs was checked. The robustness of the clades was assessed using Bayesian PP for the BI analysis, where $PP > 0.95$ was considered strongly supported. A majority consensus tree with branch lengths was reconstructed for each run after discarding the first 15 000 sampled trees. All phylogenetic analyses in this study were carried out using the supercomputer *HERCULES* (http://www.ub.edu/molevol/) at the Universitat de Barcelona, Spain.

We used general mixed Yule-coalescent (GMYC) modelling for estimating species boundaries directly based on the phylogenetic tree topology (Pons *et al*., 2006; Fontaneto *et al*., 2007). The GMYC algorithm detects differences in the rate of lineage branching at the species and population levels, recognizable as a sudden increase in the apparent diversification rate when the ultrametric node height (distances to tips) is plotted against the log number of nodes in a lineage-through-time plot (Nee, Mooers & Harvey, 1992). Analyses were conducted with each data set separately (*COI* and *ITS1*). The consensus trees from the BI analyses were passed through the program Tree Edit v1.0a10 (Rambaut & Charleston, 2002) to obtain an ultrametric tree with branch lengths using non-parametric rate smoothing (NPSR), with the model weight rate differences across the root (Sanderson, 1997). A single threshold value for the input tree was used (Monaghan *et al*., 2009), a method that has already been applied successfully to certain groups of organisms (i.e. Ahrens, Monaghan & Vogler, 2007; Monaghan *et al*., 2009). The ultrametric phylogenies recovered with the MrBayes/Tree Edit were subjected to the GMYC analyses script (freely available as part of the splits package from http://r-forge.r-project.org/projects/splits/) with the platform R 2.12.0 (R Development Core Team, 2009). A list of delimited GMYC species (described in the file's output as maximum-likelihood entities) was compiled from the graphical output of the GMYC analysis in R.

We performed a *Species Tree* (multispecies coalescent) analysis using a multiple marker approach applied to the combined data set of *COI+ITS1* to infer a species tree from separate genes trees (i.e. Maddison, 1997; Edwards, 2009). We used the Bayesian MCMC method implemented in *BEAST (star BEAST) software (version 1.7.2, Drummond & Rambaut, 2007; Heled & Drummond, 2010) to infer the genealogical relationship between the lineages of *Margotrema* spp. We performed that analysis on a data set that combined both *COI* and *ITS1* data sets, implementing the gene-specific substitution models of GTR available in the BEAST package (BEAUti v.1.7.0; Drummond & Rambaut, 2007). Specifically, we used *BEAST to infer the *Species Tree* of combined data sets assuming a Yule speciation process tree prior and ran two independent tree searches of 20×10^6 generations each, retaining one in every 1000 samples from the posterior distribution of the model parameter log files and tree files. Branch support for the different tree topologies was evaluated by the PP of the inferred relationships, where $PP > 0.95$ was considered to provide strong nodal support. The trees with the highest lineage PP were chosen from the *BEAST output files using the program TreeAnnotator v. 1.7.2 (Drummond & Rambaut, 2007). The *Species Tree* analysis was run in the aforementioned programs in the Bioportal of the University of Oslo (http://www.bioportal.uio.no/). Furthermore, the proportion (p) of absolute nucleotide sites (p-distance) (Nei & Kumar, 2000) was obtained to compare the genetic distance among and between lineages, with and without outgroups. We generated two data sets for *COI* and *ITS1* as follows: (1) a data set including all *Margotrema* individuals together with sequences of species of the same family Allocreadiidae, and (2) a data set that included only all individuals of *Margotrema*. The p-value matrices were obtained for each data set gene using MEGA version 5 (Tamura *et al*., 2011), with variance estimation with the bootstrap method (100 replicates) and with a nucleotide substitution (transitions + transversions) uniform rate.

RESULTS

We obtained DNA sequences from a total of 134 individuals assigned to *Margotrema* spp. (127 specimens, ingroup), *Allocreadium lobatum* (one specimen, outgroup), *Prosthenhystera* sp. (three specimens, outgroup), and *Walliniae chavarriae* (three specimens, outgroup). In total, for the ingroup, we obtained sequences of *COI* and *ITS1* from 14 localities that were associated with 15 goodein and one cyprinid species, respectively (Appendix 2; Fig. 1). In the next section, we show the results obtained for each molecular marker.

COI

We sequenced 125 specimens corresponding to 118 individuals of *Margotrema*, one of *A. lobatum*, three of *Prostenhystera* sp., and three of *W. chavarriae*. This data set consisted of 750 bp, except for two individuals that had 698 bp (one from *Chapalichthys pardalis* and one from *Ilyodon furcidens* in Tocumbo), and one individual with 621 bp (from *Alloophorus robustus*). For the outgroups, *COI* sequences of *W. chavarriae* also had 750 bp, *Prostenhystera* sp. had 445 bp, and those of *A. lobatum* had 687 bp. The level of variation in the single nucleotide polymorphisms (SNPs) for each partition (first, second, and third codon positions) was 97/116/49 conserved, 153/134/201 variable, 140/131/171 parsimony-informative, and 13/3/30 singleton sites, respectively. In the partition of the protein-coding gene, the third codon position was the most variable, followed by the first and then the second position.

We inferred the Bayesian phylogenetic relationships for 118 individuals of *Margotrema* to assess species limits using GMYC analyses. The results of these analyses are presented in Table 1. The number of *species* – independent evolutionary lineages – detected by the GMYC analyses with the *COI* data set was 4 (Fig. 2). The genetic distance values among the four lineages of *Margotrema* with *COI* ranged from 2.87 to 9.39% (Table 2), and when the ingroup was compared with the outgroups, from 13.65 to 14.57% with *W. chavarriae*, from 20.09 to 21.25% with *A. lobatum*, and from 72.47 to 73.07% with *Prosthenhystera* sp.

Table 1. Number of GMYC species recovered and outputs obtained from the single-threshold GMYC analyses performed for the two data sets examined

Dataset	T	NC	NS	CI	L0	LGMYC	LR
COI	−0.32	5	9	4–15	590.8	596.8	12.0*
ITS1	−1.40	4	5	4–14	310.1	320.9	8.3*

Ultrametric tree reconstruction with the following parameters: T, threshold genetic distance from the branch tips where the coalescent-speciation transition occurred; NC, number of clusters (GMYC species with more than one individual); NS, number of GMYC species discriminated; CI, confidence intervals of GMYC species; L0, likelihood of null model; LGMYC, likelihood of GMYC model; LR, likelihood ratio with significance indicated by an asterisk ($^*P < 0.01$).

ITS1

We successfully obtained a range of 799–810 bp for 98 individuals of *Margotrema*, except for one individual from *Alloophorus robustus* from La Mintzita spring and one from *Characodon audax* from the Abraham González spring, for which 365 and 722 bp, respectively, were obtained. The variation in SNPs of the ingroup was from 365 to 810 bp, with 715 conserved sites, 99 variable sites, 45 parsimony-informative sites, and 54 singleton sites. Meanwhile, for outgroups, the size of *ITS1* was shorter, with 487, 630, and 606 bp for one specimen of *A. lobatum*, two specimens of *Prosthenhystera* sp., and two specimens of *W. chavarriae*, respectively.

The number of *species* – independent evolutionary lineages – detected by GMYC using the topology of the Bayesian inference of 98 individuals of *Margotrema* of *ITS1* was 2 (Fig. 3). The results of the GMYC analysis are presented in Table 1. The genetic distance value between the two lineages of *Margotrema* with *ITS1* was 3.48%. Lineage I and Lineage II varied by 46.11 and 47.02% with respect to *W. chavarriae*, 43.93 and 44.86% with *A. lobatum*, and 52.3 and 54.44% with *Prosthenhystera* sp., respectively. Lineage I, delimited using GMYC based on the *ITS1* ultrametric tree, corresponds to Lineage I from the *COI*-based GMYC analysis as far as individuals, hosts, and geographical locations are concerned. On the other hand, *ITS1*-based Lineage II contains individuals that were grouped into Lineages II, III and IV based on the ultrametric tree obtained with *COI*.

COMBINED DATA SET (*COI*+*ITS1*)

The complete alignment using both *COI* and *ITS1* gene fragments consisted of 93 sequences, of which 88 corresponded to individuals of *Margotrema*, two of *Prosthenhystera* sp., one of *A. lobatum*, and two of *W. chavarriae*, with 1711 bp including gaps. The combined data set was used to perform a multispecies coalescent analysis as implemented in *BEAST, resulting in a *Species Tree* (ST) (Fig. 4).

The ST analysis represents the most clear-cut summary of the data. A brief description of the host–parasite association and geographical distribution of each of the four lineages recovered by analysis of the combined data set of *COI* and *ITS1* gene trees is presented next: Lineage I is composed of one single

Figure 2. Bayesian ultrametric tree inferred from the *COI* data set and subjected to GMYC analysis. Names of terminal taxa include a code referring to the locality (four upper-case letters), the host species (three letters), and numbers indicating the isolate (for more information see Supporting information Appendix S2). The scale bar represents the number of nucleotide substitutions per site. Filled circles above/below branches represent Bayesian posterior probability ≥ 0.95.

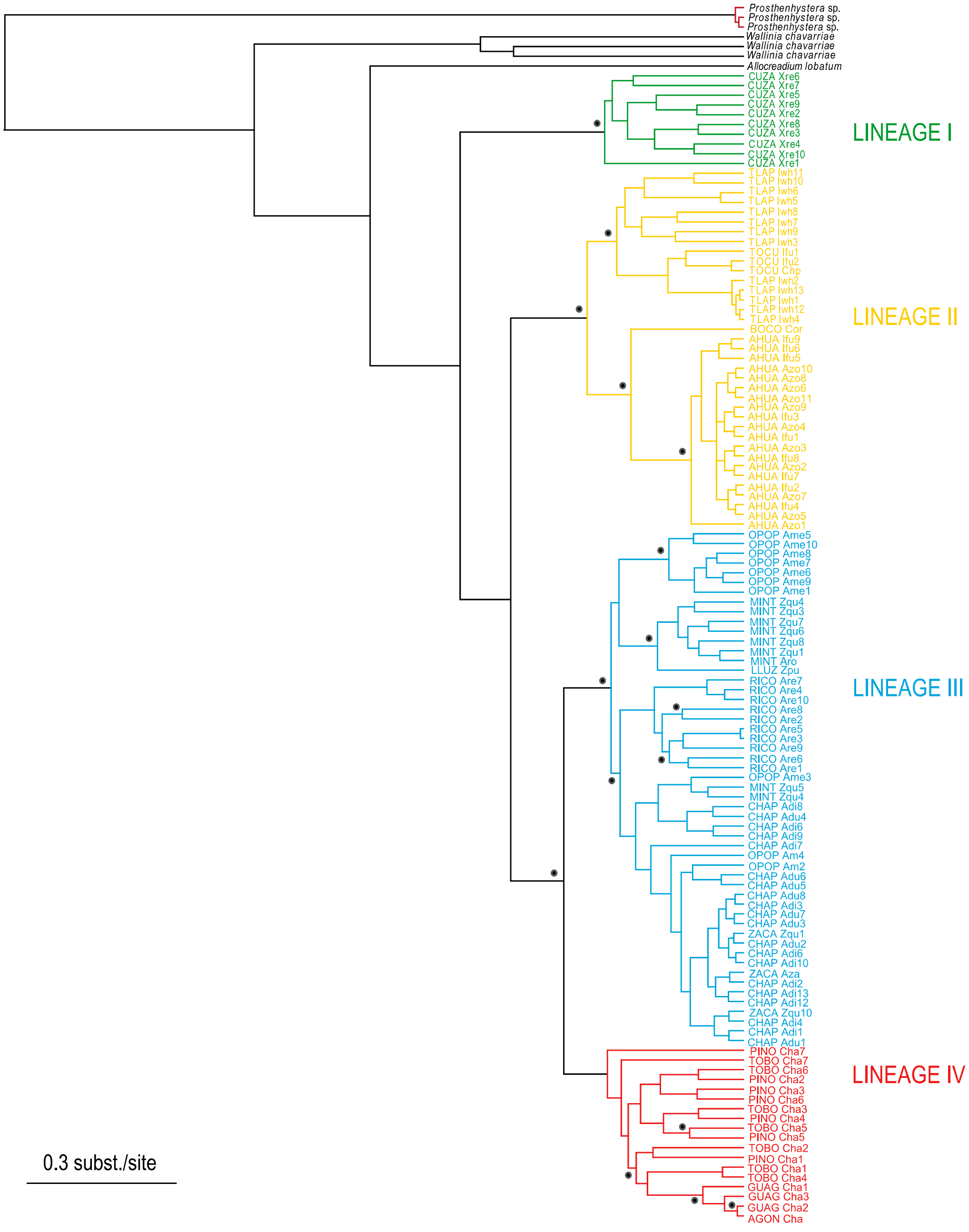
Prosthenhystera sp.
Prosthenhystera sp.
Prosthenhystera sp.
Wallinia chavarriae
Wallinia chavarriae
Wallinia chavarriae
Allocreadium lobatum
CUZA Xre6
CUZA Xre7
CUZA Xre5
CUZA Xre9
CUZA Xre2
CUZA Xre8
CUZA Xre3
CUZA Xre4
CUZA Xre10
CUZA Xre1
LINEAGE I
TLAP Iwh11
TLAP Iwh10
TLAP Iwh6
TLAP Iwh5
TLAP Iwh8
TLAP Iwh7
TLAP Iwh9
TLAP Iwh3
TOCU Ifu1
TOCU Ifu2
TOCU Chp
TLAP Iwh2
TLAP Iwh13
TLAP Iwh1
TLAP Iwh12
TLAP Iwh4
BOCO Cor
LINEAGE II
AHUA Ifu9
AHUA Ifu6
AHUA Ifu5
AHUA Azo10
AHUA Azo8
AHUA Azo6
AHUA Azo11
AHUA Azo9
AHUA Ifu3
AHUA Azo4
AHUA Ifu1
AHUA Azo3
AHUA Ifu8
AHUA Azo2
AHUA Ifu7
AHUA Ifu2
AHUA Azo7
AHUA Ifu4
AHUA Azo5
AHUA Azo1
OPOP Ame5
OPOP Ame10
OPOP Ame8
OPOP Ame7
OPOP Ame6
OPOP Ame9
OPOP Ame1
MINT Zqu4
MINT Zqu3
MINT Zqu7
MINT Zqu6
MINT Zqu8
MINT Zqu1
MINT Aro
LLUZ Zpu
RICO Are7
RICO Are4
RICO Are10
LINEAGE III
RICO Are8
RICO Are2
RICO Are5
RICO Are3
RICO Are9
RICO Are6
RICO Are1
OPOP Ame3
MINT Zqu5
MINT Zqu4
CHAP Adi8
CHAP Adu4
CHAP Adi6
CHAP Adi9
CHAP Adi7
OPOP Am4
OPOP Am2
CHAP Adu6
CHAP Adu5
CHAP Adu8
CHAP Adi3
CHAP Adu7
CHAP Adu3
ZACA Zqu1
CHAP Adu2
CHAP Adi6
CHAP Adi10
ZACA Aza
CHAP Adi2
CHAP Adi13
CHAP Adi12
ZACA Zqu10
CHAP Adi4
CHAP Adi1
CHAP Adu1
PINO Cha7
TOBO Cha7
TOBO Cha6
PINO Cha2
LINEAGE IV
PINO Cha3
PINO Cha6
TOBO Cha3
PINO Cha4
TOBO Cha5
PINO Cha5
TOBO Cha2
PINO Cha1
TOBO Cha1
TOBO Cha4
GUAG Cha1
GUAG Cha3
GUAG Cha2
AGON Cha
0.3 subst./site

Table 2. Distance matrix of uncorrected p-distances of among and within lineages derived from *COI* analysis by general mixed Yule-coalescence model

	I	II	III	Intralineage
Lineage I				0
Lineage II	8.78			1.15
Lineage III	9.39	6.62		1.13
Lineage IV	9.08	6.04	2.87	0.08

population distributed in Arroyo Durazno in the Cuzalapa River, Jalisco State. This lineage is also restricted to a single species of goodein, *Xenotaenia resolanae*, a member of the Tribe Ilyodontini (Figs 1, 2). The intralineage genetic distance (for *COI*) is null. Lineage II was found in four localities, two of them corresponding to the Balsas River Basin (Tlapetlahuaya spring, Puebla State, and Tocumbo spring, Michoacan State) parasitizing *Allodontichthys zonistius*, *Ilyodon whitei*, *I. furcidens* (Tribe: Ilyodontini), and *Chapalichthys pardalis* (Tribe: Chapalichthyini), one in the Armeria River (stream in Ahuacapán, Jalisco State), and the last locality was further north in a tributary of the Conchos River Basin (Bocoyna, Chihuahua State) parasitizing *Codoma ornata* (Cyprinidae) (Figs 1, 2). Interestingly, no species of goodeines are currently distributed in that river basin. The intralineage genetic distance in Lineage II (for *COI*) is 1.15%. Lineage III is the most widespread within *Margotrema* and contains six localities along four hydrological systems included in the Lerma River Basin: Opopeo Lake, La Mintzita spring, Chapultepec spring, La Luz spring, La Angostura at Zacapu Lake, and Rico spring, all in Michoacán State (Figs 1, 2). The hosts where Lineage III was found correspond to members of the Tribe Chapalichthyini (i.e. *A. robustus*, *Zoogoneticus purhepechus*, and *Z. quitzeoensis*), as well as members of the Tribe Girardinichthyini (i.e. *Allotoca diazi*, *A. duguesi*, *A. meeki*, *A. zacapuensis*, and *Neoophorus regalis*). The intralineage genetic distance in Lineage III (for *COI*) is also very low, 1.13%. Finally, Lineage IV is found in four localities whose distribution is restricted to the Mezquital River Basin (Figs 1, 2), and the specimens are found parasitizing *Characodon audax*, a member of the basal tribe of Characodontini goodeines. The intralineage genetic distance in Lineage IV (for *COI*) is also very low, 0.08%.

The distinction of the two putative species of *Margotrema* (*M. bravoae* and *M. guillerminae*) is not supported by the molecular data. As previously mentioned, these two species are separated by having intestinal ceca that extend to different levels of the body's length. Our molecular analysis clearly shows that the two morphs representing such conditions, i.e. the long and short ceca, are nested together in what is recognized as Lineages II, III and IV (see Figs 2, 3).

DISCUSSION

General patterns of species diversification

The results of the methodological approach we conducted in this paper to test species boundaries and as a subsequent result to uncover the number of genetic lineages of *Margotrema* based on separate or combined data sets (i.e. GMYC analyses) show that only Lineage I is consistently recovered by all three analyses (Figs 2, 3). An important difference between the *COI* and *ITS1* species delimitation analyses using the GMYC model is the number of lineages recovered, as *COI* recovers four lineages (Fig. 2), while *ITS1* actually recovers only two (Fig. 3). Apparently, *ITS1* possesses a weaker phylogenetic signal, resulting in two species based on coalescent resolution. This finding might be explained by the relatively slower rate of evolution (i.e. nucleotide substitution) of this gene. In contrast, *COI* exhibits a higher resolution because, as a mitochondrial gene, it is considered to be a rapidly evolving gene (Avise, 2008; Hickerson *et al*., 2010). Reciprocal monophyly was found only in Lineage I when the data sets were analysed separately. This result strongly supports the idea that Lineage I represents an undescribed species of *Margotrema*.

The GMYC model represents an approach designed for the identification of independently evolved lineages (species) as the most likely point of transition from coalescence to speciation branching patterns on an ultrametric phylogenetic tree with branch lengths scaled to time (Pons *et al*., 2006; Fontaneto *et al*., 2007). This approach recovers highly diverse biological groups whose boundaries are determined by using molecular markers; it has been used in taxonomically neglected taxa such as weevils and wasps (see Astrin *et al*., 2012; Ceccarelli, Sharkey & Zaldívar-Riverón, 2012). Likewise, recent studies have used this approach to determine species boundaries at the intraspecific level, i.e. using subpopulations that seem to represent only one morphologically valid species (Marshall *et al*., 2011). In this study, we used GMYC for establishing species boundaries in populations of two putative species of *Margotrema* and for providing data to support the idea that species can be delimited by using shifts in the phylogenetic branching rate that are found by a single-threshold mixed Yule-coalescent model (Marshall *et al*., 2011; Powell, 2012). In our study, we found contrasting results between the two molecular markers in the number of lineages delimited because the rates of molecular evolution

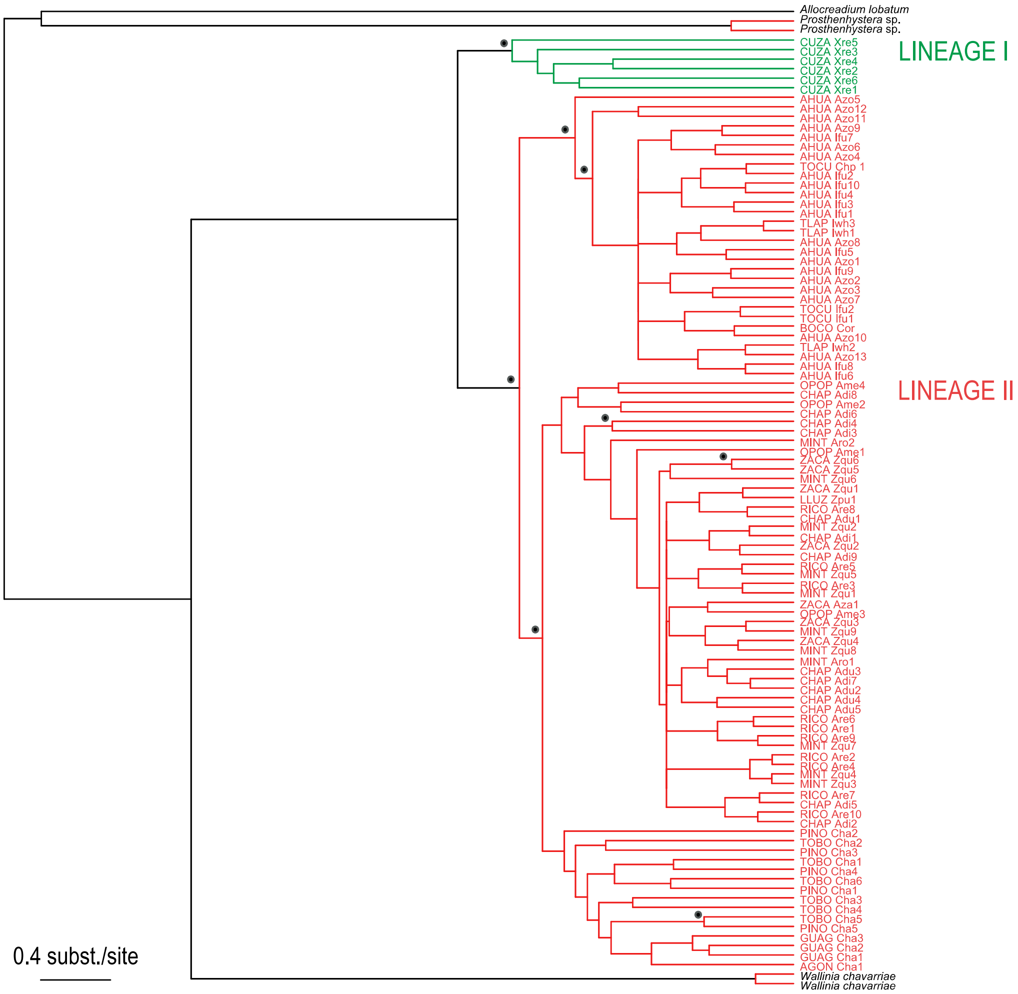

Figure 3. Bayesian ultrametric tree inferred from the *ITS1* data set and subjected to GMYC analysis. Names of terminal taxa include a code referring to the locality (four upper-case letters), the host species (three letters), and numbers indicating the isolate (for more information see Supporting information Appendix S2). The scale bar represents the number of nucleotide substitutions per site. Filled circles above/below branches represent Bayesian posterior probability ≥ 0.95.

differ between the two markers. We chose to follow a conservative approach by considering Lineages II, III, and IV as a single species with three independently evolving lineages with a strong phylogenetic structure, suggesting a case of incipient speciation that results from the potential lack of gene flow among lineages due to geographical separation. The branching pattern of the *ITS1* phylogenetic tree, i.e. short branches for all terminals, is a strong indicator of insufficient time to accumulate polymorphisms and to complete the speciation process, even with geographical separation of their populations (Figs S1 and S2). For instance, Bueno-Silva, Boeger & Pie (2011) found similar results while studying a monogenean parasite species with freshwater and brackish fish hosts using the same molecular markers.

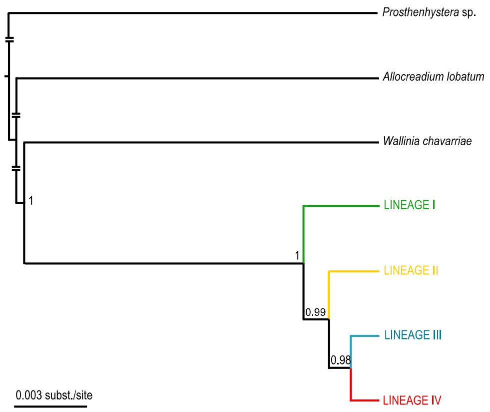

Figure 4. Coalescent-based phylogenetic tree obtained from the Species Tree analysis of the combined data set (*COI*+*ITS1*). The scale bar represents the number of nucleotide substitutions per site. Bayesian posterior probabilities > 0.95 are shown above the node.

We took a second step in our analysis and tested the genealogical relationships for the combined data set of *COI* and *ITS1* by using an ST approach with a consensus method that allowed us to eliminate the effect of the weight of a particular gene (Edwards, Liu & Pear, 2007; Liu *et al*., 2008). This analysis has the ability to jointly infer a summary tree from different gene trees (i.e. genealogies) sampled from multiple individuals. In this context, a *BEAST analysis works by embedding separate genealogies inside a summary tree through coalescence backwards in time, starting from the tips of the tree (Rannala & Yang, 2003). The model assumes that there is no gene flow among the focal groups (in our case, the terminal ingroup taxa representing independent evolutionary lineages), regardless of whether the lineages are taxonomically or geographically related, and therefore any incongruence between the genealogies and the summary tree can be explained by the retention of ancestral polymorphisms. This assumption is particularly appropriate for autogenic digenean parasites with some level of host specificity that are distributed in a restricted geographical area (i.e. water bodies) that do not naturally disperse over water and that therefore have no gene exchange among host species or hydrological systems. For this reason, the *BEAST approach has been used recently to analyse all information in the mtDNA and nuclear DNA data sets in a single analysis, given the ability of *BEAST to account for differences in the time to coalesce between haploid (mitochondrial) and diploid (nuclear) markers (Heled & Drummond, 2010). This approach can improve the accuracy of tree reconstruction compared with the more traditional approaches that do not account for the coalescent process, assuming that the condition of no gene flow among lineages is met (Heled & Drummond, 2010). The *BEAST approach is especially useful in cases involving recent divergence events, where retention of ancestral polymorphisms among lineages is more likely to mislead phylogenetic reconstruction. This observation may explain the results of our analysis as a case of either incipient speciation for Lineages II, III, and IV or the lack of gene flow among them. For this reason, we followed a conservative position and consider them as a single species. The phylogenetic relationships detected with the ST approach and the high values of PP support these relationships (PP > 98) (Fig. 4), providing unambiguous genetic evidence of the discovery of four independent evolutionary lineages among *Margotrema* that represent two monophyletic species, irrespective of the outgroups analysed. Additionally, our results reinforce the idea that species limits can be established by using not just the results of a phylogenetic inference but also the shifts in the phylogenetic branching rate revealed by the single-threshold mixed Yule-coalescent model; furthermore, the ST approach was used for combining information of two molecular markers as suggested by Marshall *et al*. (2011) and Satler *et al*. (2011), obtaining a more reliable phylogenetic tree of species' relationships.

TAXONOMIC IMPLICATIONS

Two species of *Margotrema* have been described thus far, *M. bravoae* as a parasite of *Girardynychthys multiradiatus* from the Ciénega de Lerma (Lamothe-Argumedo, 1972), and *M. guillerminae* as a parasite of *Notropis* (= *Hybopsis*) *calientis* and *Alloophorus robustus* from Zacapu Lake (Pérez-Ponce de León, 2001), both in the Lerma River Basin in central Mexico. These species were distinguished solely on morphological grounds, and the major difference was the extension of the intestinal ceca along the body, with *M. guillerminae* representing a morphotype with short ceca, while *M. bravoae* exhibited long ceca extending posteriorly to half the distance between the testes and the posterior end of the body (Pérez-Ponce de León, 2001: 1113). Additional records of these two species were established in several published accounts after they were originally described, even though observation of the distinguishing character was particularly difficult in gravid specimens because the uterus is normally full with large-sized embryonated eggs. *Margotrema bravoae* had been recorded in at least 14 species of goodeines in 14 localities, while *M. guillerminae* had been found in 13 goodeines in 15 localities. Actually, in some localities, both species had been recorded in independent publications. For instance, in Zacapu Lake, the type locality for *M. guillerminae*, other authors have reported the presence of *M. bravoae*. Mejía-Madrid *et al*. (2005) studied 35 species of goodeines. Of the species examined, 18 were infected with *Margotrema* spp., ten were infected with *M. bravoae*, and eight were infected with *M. guillerminae*; only *M. bravoae* was found in Zacapu Lake. More recently, Martínez-Aquino *et al*. (2012) conducted a study of the helminth parasite fauna of goodeines from Zacapu Lake, and they only reported the presence of *M.* cf. *bravoae*. In all of those cases, identification was based solely on morphology. Some authors have argued about the validity of *M. guillerminae*; however, they provided no empirical evidence and no reliable arguments to demonstrate that this species was not valid (Pineda-López *et al*., 2005). The results we provide in this study are based on molecular evidence and demonstrate that, irrespective of the tree topologies obtained from either mitochondrial or nuclear genes, *M. guillerminae* should be synonymized with *M. bravoae*. In all analyses, it can be seen that *M. guillerminae*, i.e. morphotypes with short ceca, do not comprise a monophyletic assemblage. This finding means that in Lineages II, III, and IV, representative specimens with long ceca are nested together with those exhibiting short ceca. Therefore, *M. guillerminae* is synonymized with *M. bravoae*, and it is concluded that the ceca extension in this group of digeneans should not be used as a taxonomic character to establish species distinction. We are currently preparing a strictly taxonomic paper in which the synonymy will be formally presented by emending the diagnosis of *M. bravoae* and by describing, in a formal way, the new species uncovered by the molecular data. Furthermore, this synonymy can explain the fact that both species have been recorded as occurring in sympatry, i.e. in the same localities and host species. For instance, both species were recorded as parasites of *Characodon audax* in the Toboso spring, Durango (Mejía-Madrid *et al*., 2005; Martínez-Aquino *et al*., 2007).

As previously stated, our results provide unambiguous evidence that *Margotrema* currently consists of two putative species, *M. bravoae* (represented by three well-defined lineages) and an undescribed species found exclusively in *X. resolanae* in the Cuzalapa River, represented in all of our analyses consistently as Lineage I. Martínez-Aquino *et al*. (2009a) previously recorded *M. guillerminae* as a parasite of *X. resolanae*; however, this identification was based solely on morphological grounds. In the present study, we demonstrate that these specimens are an independent evolutionary entity, which was described as Lineage I in all analyses, either through GMYC (for both molecular markers analysed separately) or through ST with the combined data set. This species represents an undescribed species, which requires further taxonomic work to establish the proper species description. Because traditional morphological traits would not be useful, other sources of information need to be explored.

HOST ASSOCIATION AND BIOGEOGRAPHY

In the literature, phylogenetic analyses of several parasite taxa have been used to uncover patterns of close association between both the host species/parasitic taxa and the geographical distribution of both associates, resulting, in some cases, in incongruent patterns due to instances of speciation via host-switching or ecological host extensions (Nieberding *et al*., 2008, and references therein). In the case of *Margotrema* spp. and their goodein hosts, it seems plausible to postulate, based on the phylogenetic information obtained herein, that each lineage shows some level of congruence with the hosts they infect and the river basin these hosts inhabit. Lineage I shows a restricted distributional range in the Cuzalapa River, in Jalisco State, on the Pacific slope of Mexico, and it is only found, as previously mentioned, in *X. resolanae*. Likewise, even though *M. bravoae sensu lato* contains three well-supported genetic lineages, it appears that each one of these lineages also shows some level of congruence with the hosts and geographical region (hydrological system) in which they live, as shown in Fig. 5A, which represents a summary of the results obtained in this study. The geographical range of Lineage II is wide and consists of three independent hydrological systems, and it is primarily found in members of the tribe Ilyodontini, even though some isolates were found in a member of the tribe Chapalichthyini, and one was even found infecting a member of the family Cyprinidae. Lineage III is apparently restricted to water bodies of the Lerma River Basin and is only associated with members of the tribes Girardinichthyini and Chapalichthyini. Finally, Lineage IV also shows a restricted geographical distribution, in four localities along the San Pedro Mezquital River Basin in north-western Mexico. This lineage is only associated with *Characodon audax*, a member of the tribe Characodontini, allegedly representing the basal members in the phylogenetic history of goodeines (Domínguez-Domínguez *et al*., 2010) (Fig. 5B, C). In this context, and based on the diversification and biogeographical patterns of Goodeinae in central Mexico, we argue that the evolutionary history and the historical biogeography of the four genetic lineages of *Margotrema* are closely linked to that of their hosts, reflecting the three types of historical associations as described by Page &

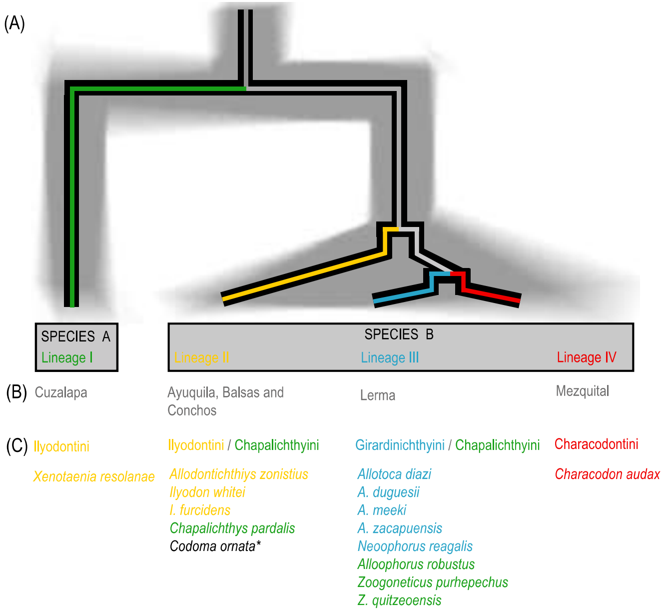

Figure 5. Schematic representation depicting the types of historical associations among genes, areas, and hosts of *Margotrema*. A, phylogenetic inferences (organisms and gene) (diffuse grey, *ITS1*; black lines, *COI*). Each colour represents independent evolutionary lineages. B, hydrological systems representing the distribution area of each lineage (area and organism). C, host association for each lineage of *Margotrema* at tribe or species level (host and parasite). Colours in the scientific names are used to correlate the name of the tribe with the species contained in each tribe. An asterisk (*) indicates a species of host belonging to the family Cyprinidae (for more information see Supporting Information Appendix S2).

Charleston (1998: 356): (1) organism and gene, (2) area and organisms, and (3) host and parasites (Fig. 5A–C). Interestingly, each of the *Margotrema* lineages show some host-specificity with a particular tribe of goodein, and in the particular case of Lineages I and IV, this host-specificity is even narrower because these lineages are found at the fish species level (Fig. 5C). This host-specificity pattern might be explained as a result of a parasite speciation via host-switching of the most recent common ancestor or host–parasite co-divergence events of each *Margotrema* lineage. It has been argued that several factors have shaped the evolutionary and biogeographical history of goodeines in central Mexico (Parenti, 1981; Miller & Smith, 1986; Domínguez-Domínguez *et al*., 2010). If tecto-volcanic activity as well as river capture promoted the diversification of goodeinae in central Mexico, then we hypothesize that the distribution patterns as well as host associations of each lineage of *Margotrema* will be concordant with the hydro-geomorphological events that occurred in central Mexico and that the vicariant and dispersal events that caused the goodein diversification promoted at the same time the diversification of each *Margotrema* lineage.

Other molecular phylogenetic studies of parasite taxa of freshwater fish, including extensive sampling of populations along their distributional range, have shown similar patterns of diversification, in that independent evolutionary lineages are detected even in the absence of morphological data supporting those patterns. For instance, Martínez-Aquino *et al*. (2009b) and Rosas-Valdez, Choudhury & Pérez-Ponce de Léon (2011) uncovered a diversification process in species belonging to the acanthocephalan genus *Neoechinorhynchus* and the digenean genus *Phyllodistomum*, respectively, where the evolutionary history of the parasite lineages is also closely tied to that of their hosts, showing clear instances of speciation via host-switching.

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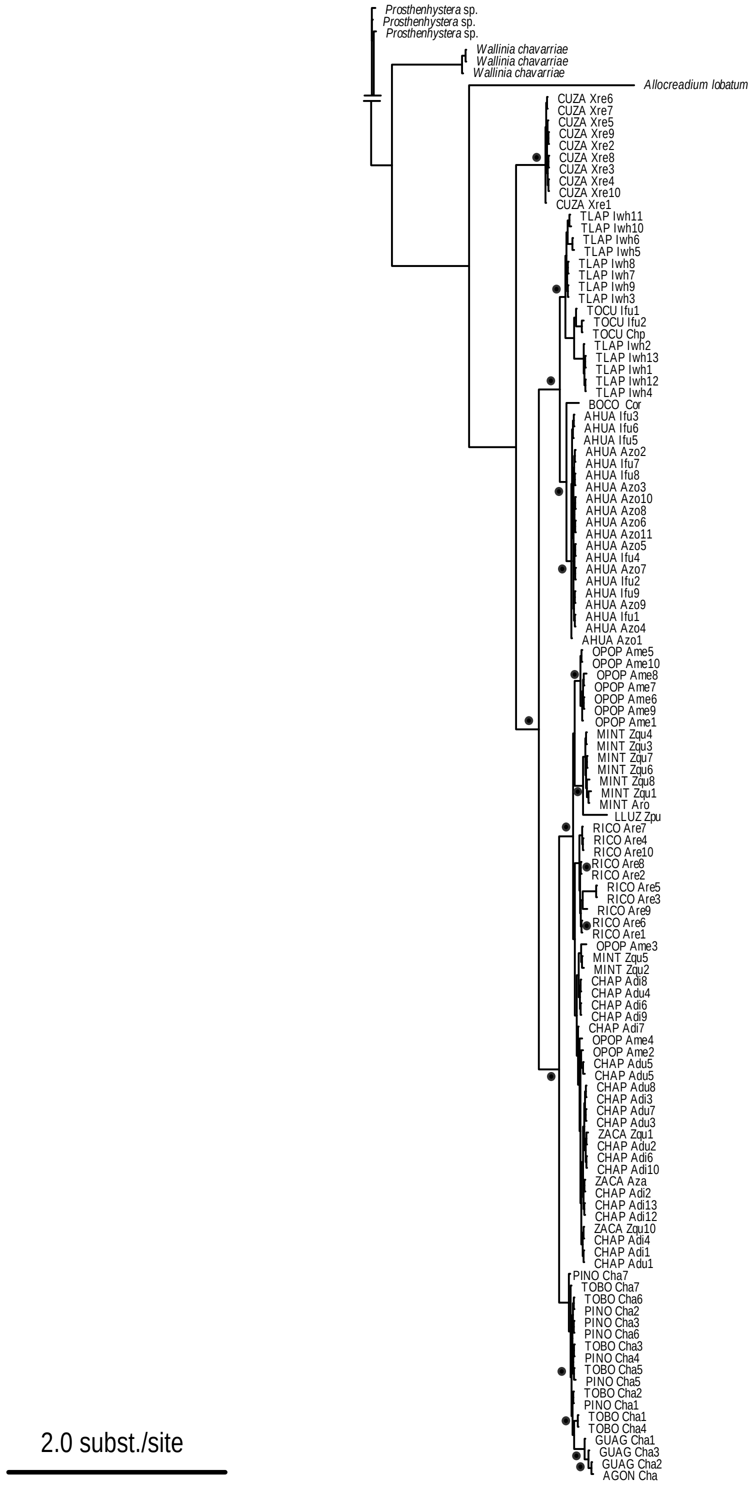
SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article at the publisher's web-site:

Figure S1. Phylogenetic tree obtained with MrBayes for *COI*.
Figure S2. Phylogenetic trees obtained with MrBayes for *ITS1*.
Appendix S1. Localities sampled for freshwater fishes (Goodeinae), host species of *Margotrema* from México.
Appendix S2. *Margotrema* and hosts species, localities analysed, with codes referring to each terminal taxa in the phylogenetic analyses, voucher reference, and accession numbers for each specimen and sequence.

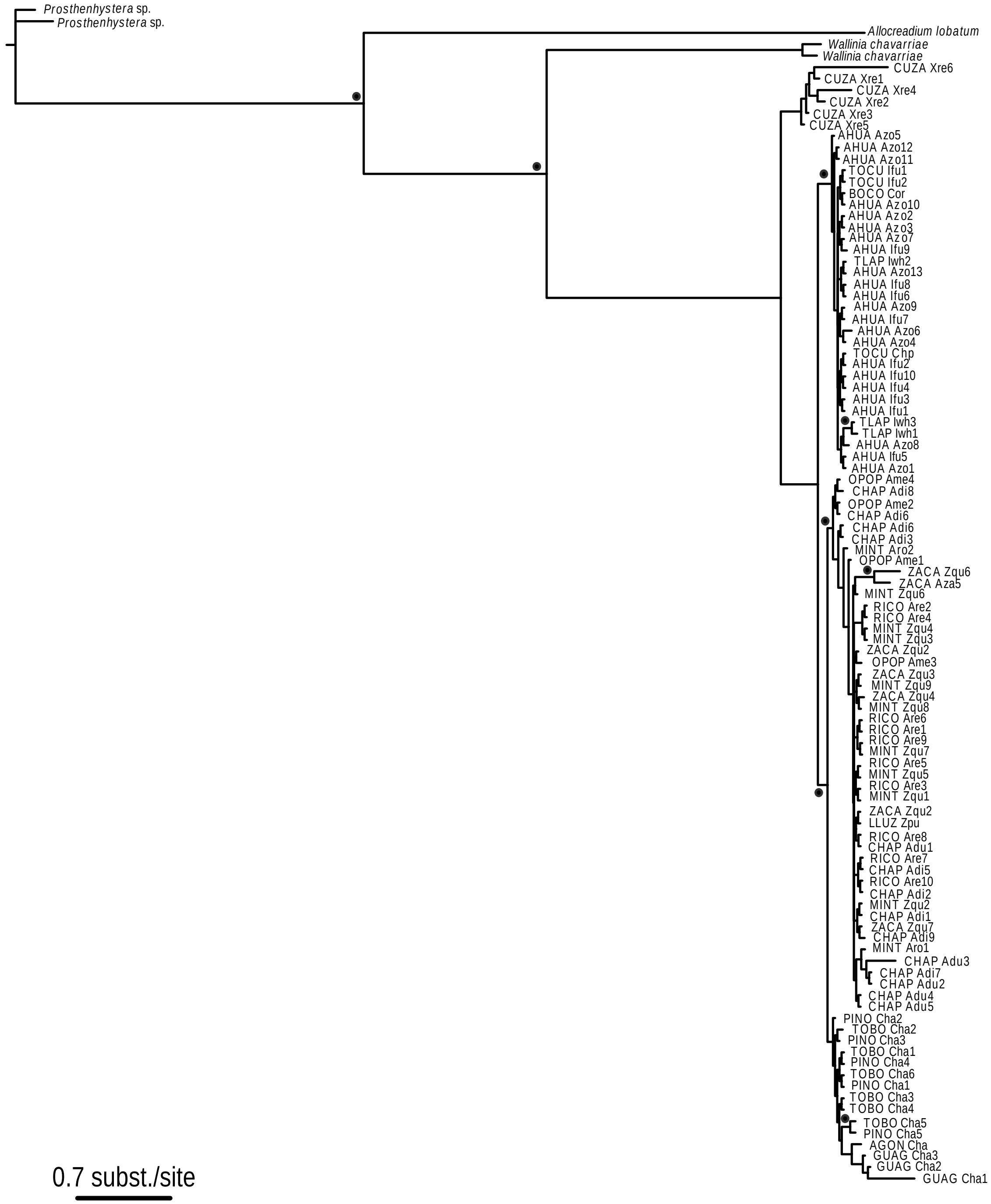
SUPPORTING INFORMATION

Supporting Figure S1
COI



Supporting Figure S2

ITS1



Appendix S1. Localities sampled for freshwater fishes (Goodeinae), host species of *Margotrema* from México. NH = sample size of hosts collected; NM = sampled size of individuals of *Margotrema* collected by locality; M = total number of individuals of *Margotrema used* for morphological analyses; DNA = total number of individuals of *Margotrema* used for molecular analyses; DV = total number of digital vouchers of individuals of *Margotrema* obtained by locality. The asterisks (*1, *2) refer to previously published data by Pérez-Ponce de León *et al*. (2009) and Aguilar-Aguilar *et al*. (2010), respectively, in studies of helminth parasites of freshwater fishes from Mexico, but correspond to the same samplings. The host from locality 44 corresponds to a species of the family Cypriinidae.

Locality	**Host species**	**NH**	**NM**	**M**	**DNA**	**VD**
1. Stream in Ahuacapán, Jalisco	*Allodontichthys zonistius*	11	80	37	43	0
	Ilyodon furcidens	11	50	17	33	0
2. Spring in Canal Magdalena, Michoacán	*Chapalichthys pardalis*	3	0	–	–	–
3. Channel Magdalena, Michoacán	*Ilyodon furcidens*	2	0	–	–	–
	Zoogoneticus purhepechus	7	0	–	–	–
4. Channel Santiago Tiacaque, Estado de México	*Girardinichthys multiradiatus*	12	0	–	–	–
5. Spring Chapultepec, Michoacán	*Allotoca diazi*	21	31	9	22	1
	Allotoca duguesi	22	6	1	5	1
6. Spring Chilchota, Michoacán	*Skiffia multipunctata*	10	0	–	–	–
7. Spring Cupatziro, Michoacán	*Skiffia multipunctata*	13	0	–	–	–
	Zoogoneticus purhepechus	16	0	–	–	–
8. Arroyo Durazno in Cuzalapa River, Jalisco	*Xenotaenia resolanae*	35	131	68	63	0
9. El Tule (River), Jalisco	*Allodontichthys tamazulae*	19	0	–	–	–
10. Estanque en Río del Canal Santiago Tiacaque, Estado de México	*Girardinichthys multiradiatus*	6	0	–	–	–
11. La Angostura, Zacapu Lake (balneario), Michoacán	*Skiffia lermae*	11	0	–	–	–
	Allotoca zacapuensis	32	1	0	1	1
	Goodea atripinnis	20	0	–	–	–
	Hubbsina turneri	16	0	–	–	–
	Skiffia lermae	19	0	–	–	–
	Zoogoneticus quitzeoensis	30	18	4	14	8
12. La Luz spring, Jacona de Plancarte, Michoacán	*Zoogoneticus purhepechus*	32	1	0	1	1
	Zoogoneticus purhepechus	12	0	–	–	–
	Chapalichthys encaustus	8	0	–	–	–
	Skiffia multipunctata	4	0	–	–	–
	Alloophorus robustus	35	0	–	–	–
13. La Mintzita spring, Michoacán, Michoacán	*Zoogoneticus quitzeoensis*	22	5	1	4	3
	Alloophorus robustus	14	3	0	3	–
14. Camecuaro Lake, Michoacán	*Alloophorus robustus*	2	0	–	–	–

	Zoogoneticus purhepechus	11	0	–	–	–
15. Opopeo Lake, Michoacán	_Goodea atripinnis_	4	0	–	–	–
	Allotoca meeki	4	45	29	16	4
16. Las Adjuntas (River), Michoacán	_Skiffia multipunctata_	2	0	–	–	–
	Zoogoneticus purhepechus	5	0	–	–	–
17. Los Negritos spring, Michoacán	_Chapalichthys encaustus_	2	0	–	–	–
	Zoogoneticus purhepechus	19	0	–	–	–
	Zoogoneticus purhepechus	9	0	–	–	–
	Zoogoneticus purhepechus	8	0	–	–	–
18. Cutzaróndiro spring, Michoacán	_Ilyodon whitei_	31	0	–	–	–
19. La Estancia spring, Michoacán	_Zoogoneticus purhepechus_	21	0	–	–	–
20. Parque Ecológico Chicnahuapan (Lake), Estado de México	_Girardinichthys multiradiatus_	44	0	–	–	–
	Girardinichthys multiradiatus	29	0	–	–	–
21. Pátzcuaro Lake, Michoacán	_Goodea atripinnis_	31	0	–	–	–
	Alloophorus robustus	2	0	–	–	–
22. Predio Porfirio, La Angustura, Zacapu Lake, Michoacán	_Allotoca zacapuensis_	3	2	–	–	–
	Xenotoca variata	1	0	–	–	–
	Alloophorus robustus	2	0	–	–	–
23. Dam in Santiago Chanel, Tiacaque, Estado de México	_Girardinichthys multiradiatus_	2	0	–	–	–
24. Dam Ignacio Ramírez, Estado de México	_Girardinichthys multiradiatus_	14	0	–	–	–
25. Dam Salazar, Estado de México	_Girardinichthys multiradiatus_	35	0	–	–	–
26. Rico spring, Michoacán	_Allotoca regalis_	39	61	30	31	–
26. Ahuehuello River in Santo Domingo, Puebla	_Ilyodon whitei_	51	0	–	–	–
28. Ángulo River close to Zacapu Lake, Michoacán	_Alloophorus robustus_	9	0	–	–	–
	Xenotoca variata	20	0	–	–	–
	Zoogoneticus quitzeoensis	11	0	–	–	–
	Allotoca goslinei	2	0	–	–	–
29. Tamazula River, Jalisco	_Allodontichthys zonistius_	32	0	–	–	–
	Ilydon furcidens	56	0	–	–	–
30. Tributary of San Idelfonso River, Querétaro	_Goodea atripinnis_	10	0	–	–	–
31. San Jerónimo, Pátzcuaro Lake, Michoacán	_Alloophorus robustus_	5	0	–	–	–
32. Santa Maria del Oro (River), Jalisco	_Ilydon furcidens_	15	0	–	–	–
33. Bordo or man-made pond in Tlapetlahuaya, Puebla	_Ilyodon whitei_	34	62	29	33	7
34. Spring in park of Tocumbo, Michoacán	_Chapalichthys pardalis_	38	4	2	2	1
	Goodea atripinnis	16	0	–	–	–
	Ilydon furcidens	18	3	1	2	2
35. Ucasanastaua, Pátzcuaro, Michoacán	_Alloophorus robustus_	11	0	–	–	–
36. Puente en el poblado de Pino Suárez, carretera Durango-Mezquital, Durango	_Characodon audax_*[1]	21	45	30	15	–
37. Spring in Abraham González, Durango	_Characodon audax_*[1]	12	16	11	5	0
38. Spring in the Unidad de Manejo Ambiental de caza, Guadalupe Aguilera, Durango	_Characodon audax_*[1]	14	8	5	3	0
39. Spring el Toboso, Durango	_Characodon audax_*[1]	42	28	15	13	0

40. Manantial en el poblado Amado Nervo, Durango	_Characodon lateralis_*[1]	9	0	–	–	–
41. Ojo de Agua de San Juan, Durango	_Characodon lateralis_*[1]	11	0	–	–	–
42. Ojo de Agua del poblado de los Berros, Durango	_Characodon lateralis_*[1]	23	0	–	–	–
43. Río Potrero Grande, Jalisco	_Ilydon furcidens_	10	1	0	1	–
	Allotoca goslinei	2	0	–	–	–
44. Upper Piaxtla River, Municipio San Dimas, Durango	_Codoma ornata_*[2]	21	6	–	–	–

Appendix 2. *Margotrema* and host species. Tribe of each host species. Code for each individual of *Margotrema* sequenced, as shown in the terminal taxa names of figures 2, 3, S1 and S2 (4 capitals for locality, 3 letters for species host and number, respectivelity). Country, state / región, name of locality, type of habitat, altitude (m) of sampled locality, GPS location, date collected, collection method for fishes, names of collectors of fishes, names of collector of digeneans, additional info (digital vouchers of sequencing individuals), vouchers deposited numbers at Colección Nacional de Helmintos (CNHE) [National Helminth Collection]. The asterisks (*, **, ***) after the voucher numbers refer to previously published data by Martínez-Aquino *et al*. (2009a, 2012) and Pérez-Ponce de León *et al*. (2009), respectively, in studies of helminth parasites of freshwater fishes from Mexico, but correspond to the same samplings. Number of sequences (in ascending order) and GenBank accession for *COI* and *ITS1* for *Margotrema* spp.

Part A

Species	**Host species**	**Tribe of Host**	**Taxa Terminal Code**	**Country**	**State/Region**
Margotrema bravoae sensu lato	*Characodon audax*1	Characodontini	AGON Cha	México	Durango
Margotrema bravoae sensu lato	*Allodontichthys zonistius*1	Ilyodontini	AHUA Azo1	México	Jalisco
Margotrema bravoae sensu lato	*Allodontichthys zonistius*2	Ilyodontini	AHUA Azo2	México	Jalisco
Margotrema bravoae sensu lato	*Allodontichthys zonistius*3	Ilyodontini	AHUA Azo3	México	Jalisco
Margotrema bravoae sensu lato	*Allodontichthys zonistius*4	Ilyodontini	AHUA Azo4	México	Jalisco
Margotrema bravoae sensu lato	*Allodontichthys zonistius*5	Ilyodontini	AHUA Azo5	México	Jalisco
Margotrema bravoae sensu lato	*Allodontichthys zonistius*6	Ilyodontini	AHUA Azo6	México	Jalisco
Margotrema bravoae sensu lato	*Allodontichthys zonistius*7	Ilyodontini	AHUA Azo7	México	Jalisco
Margotrema bravoae sensu lato	*Allodontichthys zonistius*8	Ilyodontini	AHUA Azo8	México	Jalisco
Margotrema bravoae sensu lato	*Allodontichthys zonistius*9	Ilyodontini	AHUA Azo9	México	Jalisco
Margotrema bravoae sensu lato	*Allodontichthys zonistius*10	Ilyodontini	AHUA Azo10	México	Jalisco
Margotrema bravoae sensu lato	*Allodontichthys zonistius*11	Ilyodontini	AHUA Azo11	México	Jalisco
Margotrema bravoae sensu lato	*Allodontichthys zonistius*12	Ilyodontini	AHUA Azo12	México	Jalisco
Margotrema bravoae sensu lato	*Allodontichthys zonistius*13	Ilyodontini	AHUA Azo13	México	Jalisco
Margotrema bravoae sensu lato	*Ilyodon furcidens*1	Ilyodontini	AHUA Ifu1	México	Jalisco
Margotrema bravoae sensu lato	*Ilyodon furcidens*2	Ilyodontini	AHUA Ifu2	México	Jalisco

Margotrema bravoae sensu lato	*Ilyodon furcidens*3	Ilyodontini	AHUA Ifu3	México	Jalisco
Margotrema bravoae sensu lato	*Ilyodon furcidens*4	Ilyodontini	AHUA Ifu4	México	Jalisco
Margotrema bravoae sensu lato	*Ilyodon furcidens*5	Ilyodontini	AHUA Ifu5	México	Jalisco
Margotrema bravoae sensu lato	*Ilyodon furcidens*6	Ilyodontini	AHUA Ifu6	México	Jalisco
Margotrema bravoae sensu lato	*Ilyodon furcidens*7	Ilyodontini	AHUA Ifu7	México	Jalisco
Margotrema bravoae sensu lato	*Ilyodon furcidens*8	Ilyodontini	AHUA Ifu8	México	Jalisco
Margotrema bravoae sensu lato	*Ilyodon furcidens*9	Ilyodontini	AHUA Ifu9	México	Jalisco
Margotrema bravoae sensu lato	*Ilyodon furcidens*10	Ilyodontini	AHUA Ifu10	México	Jalisco
Margotrema bravoae sensu lato	*Allotoca diazi*1	Girardinichthyini	CHAP Adi1	México	Michoacán
Margotrema bravoae sensu lato	*Allotoca diazi*2	Girardinichthyini	CHAP Adi2	México	Michoacán
Margotrema bravoae sensu lato	*Allotoca diazi*3	Girardinichthyini	CHAP Adi3	México	Michoacán
Margotrema bravoae sensu lato	*Allotoca diazi*4	Girardinichthyini	CHAP Adi4	México	Michoacán
Margotrema bravoae sensu lato	*Allotoca diazi*5	Girardinichthyini	CHAP Adi5	México	Michoacán
Margotrema bravoae sensu lato	*Allotoca diazi*6	Girardinichthyini	CHAP Adi6	México	Michoacán
Margotrema bravoae sensu lato	*Allotoca diazi*7	Girardinichthyini	CHAP Adi7	México	Michoacán
Margotrema bravoae sensu lato	*Allotoca diazi*8	Girardinichthyini	CHAP Adi8	México	Michoacán
Margotrema bravoae sensu lato	*Allotoca diazi*9	Girardinichthyini	CHAP Adi9	México	Michoacán
Margotrema bravoae sensu lato	*Allotoca diazi*10	Girardinichthyini	CHAP Adi10	México	Michoacán
Margotrema bravoae sensu lato	*Allotoca diazi*11	Girardinichthyini	CHAP Adi11	México	Michoacán
Margotrema bravoae sensu lato	*Allotoca diazi*12	Girardinichthyini	CHAP Adi12	México	Michoacán
Margotrema bravoae sensu lato	*Allotoca duguesi*1	Girardinichthyini	CHAP Adu1	México	Michoacán
Margotrema bravoae sensu lato	*Allotoca duguesi*2	Girardinichthyini	CHAP Adu2	México	Michoacán
Margotrema bravoae sensu lato	*Allotoca duguesi*3	Girardinichthyini	CHAP Adu3	México	Michoacán
Margotrema bravoae sensu lato	*Allotoca duguesi*4	Girardinichthyini	CHAP Adu4	México	Michoacán
Margotrema bravoae sensu lato	*Allotoca duguesi*5	Girardinichthyini	CHAP Adu5	México	Michoacán
Margotrema bravoae sensu lato	*Allotoca duguesi*6	Girardinichthyini	CHAP Adu6	México	Michoacán
Margotrema bravoae sensu lato	*Allotoca duguesi*7	Girardinichthyini	CHAP Adu7	México	Michoacán
Margotrema bravoae sensu lato	*Allotoca duguesi*8	Girardinichthyini	CHAP Adu8	México	Michoacán
Margotrema resolanae	*Xenotaenia resolanae*1	Ilyodontini	CUZA Xre1	México	Jalisco
Margotrema resolanae	*Xenotaenia resolanae*2	Ilyodontini	CUZA Xre2	México	Jalisco
Margotrema resolanae	*Xenotaenia resolanae*3	Ilyodontini	CUZA Xre3	México	Jalisco

Margotrema resolanae	*Xenotaenia resolanae*4	Ilyodontini	CUZA Xre4	México	Jalisco
Margotrema resolanae	*Xenotaenia resolanae*5	Ilyodontini	CUZA Xre5	México	Jalisco
Margotrema resolanae	*Xenotaenia resolanae*6	Ilyodontini	CUZA Xre6	México	Jalisco
Margotrema resolanae	*Xenotaenia resolanae*7	Ilyodontini	CUZA Xre7	México	Jalisco
Margotrema resolanae	*Xenotaenia resolanae*8	Ilyodontini	CUZA Xre8	México	Jalisco
Margotrema resolanae	*Xenotaenia resolanae*9	Ilyodontini	CUZA Xre9	México	Jalisco
Margotrema resolanae	*Xenotaenia resolanae*10	Ilyodontini	CUZA Xre10	México	Jalisco
Margotrema bravoae sensu lato	*Characodon audax*1	Characodontini	GUAG Cha1	México	Durango
Margotrema bravoae sensu lato	*Characodon audax*2	Characodontini	GUAG Cha2	México	Durango
Margotrema bravoae sensu lato	*Characodon audax*3	Characodontini	GUAG Cha3	México	Durango
Margotrema bravoae sensu lato	*Zoogoneticus purhepechus*1	Chapalichthyini	LLUZ Zpu	México	Michoacán
Margotrema bravoae sensu lato	*Alloophorus robustus*1	Chapalichthyini	MINT Aro1	México	Michoacán
Margotrema bravoae sensu lato	*Alloophorus robustus*2	Chapalichthyini	MINT Aro2	México	Michoacán
Margotrema bravoae sensu lato	*Zoogoneticus quitzeoensis*1	Chapalichthyini	MINT Zqu1	México	Michoacán
Margotrema bravoae sensu lato	*Zoogoneticus quitzeoensis*2	Chapalichthyini	MINT Zqu2	México	Michoacán
Margotrema bravoae sensu lato	*Zoogoneticus quitzeoensis*3	Chapalichthyini	MINT Zqu3	México	Michoacán
Margotrema bravoae sensu lato	*Zoogoneticus quitzeoensis*4	Chapalichthyini	MINT Zqu4	México	Michoacán
Margotrema bravoae sensu lato	*Zoogoneticus quitzeoensis*5	Chapalichthyini	MINT Zqu5	México	Michoacán
Margotrema bravoae sensu lato	*Zoogoneticus quitzeoensis*6	Chapalichthyini	MINT Zqu6	México	Michoacán
Margotrema bravoae sensu lato	*Zoogoneticus quitzeoensis*7	Chapalichthyini	MINT Zqu7	México	Michoacán
Margotrema bravoae sensu lato	*Zoogoneticus quitzeoensis*8	Chapalichthyini	MINT Zqu8	México	Michoacán
Margotrema bravoae sensu lato	*Zoogoneticus quitzeoensis*9	Chapalichthyini	MINT Zqu9	México	Michoacán
Margotrema bravoae sensu lato	*Allotoca meeki*1	Girardinichthyini	OPOP Ame1	México	Michoacán
Margotrema bravoae sensu lato	*Allotoca meeki*2	Girardinichthyini	OPOP Ame2	México	Michoacán
Margotrema bravoae sensu lato	*Allotoca meeki*3	Girardinichthyini	OPOP Ame3	México	Michoacán
Margotrema bravoae sensu lato	*Allotoca meeki*4	Girardinichthyini	OPOP Ame4	México	Michoacán
Margotrema bravoae sensu lato	*Allotoca meeki*5	Girardinichthyini	OPOP Ame5	México	Michoacán
Margotrema bravoae sensu lato	*Allotoca meeki*6	Girardinichthyini	OPOP Ame6	México	Michoacán
Margotrema bravoae sensu lato	*Allotoca meeki*7	Girardinichthyini	OPOP Ame7	México	Michoacán
Margotrema bravoae sensu lato	*Allotoca meeki*8	Girardinichthyini	OPOP Ame8	México	Michoacán
Margotrema bravoae sensu lato	*Allotoca meeki*9	Girardinichthyini	OPOP Ame9	México	Michoacán

Margotrema bravoae sensu lato	*Allotoca meeki*10	Girardinichthyini	OPOP Ame10	México	Michoacán
Margotrema bravoae sensu lato	*Characodon audax*1	Characodontini	PINO Cha1	México	Durango
Margotrema bravoae sensu lato	*Characodon audax*2	Characodontini	PINO Cha2	México	Durango
Margotrema bravoae sensu lato	*Characodon audax*3	Characodontini	PINO Cha3	México	Durango
Margotrema bravoae sensu lato	*Characodon audax*4	Characodontini	PINO Cha4	México	Durango
Margotrema bravoae sensu lato	*Characodon audax*5	Characodontini	PINO Cha5	México	Durango
Margotrema bravoae sensu lato	*Characodon audax*6	Characodontini	PINO Cha6	México	Durango
Margotrema bravoae sensu lato	*Characodon audax*7	Characodontini	PINO Cha7	México	Durango
Margotrema bravoae sensu lato	*Neoophorus regalis*1	Girardinichthyini	RICO Nre1	México	Michoacán
Margotrema bravoae sensu lato	*Neoophorus regalis*2	Girardinichthyini	RICO Nre2	México	Michoacán
Margotrema bravoae sensu lato	*Neoophorus regalis*3	Girardinichthyini	RICO Nre3	México	Michoacán
Margotrema bravoae sensu lato	*Neoophorus regalis*4	Girardinichthyini	RICO Nre4	México	Michoacán
Margotrema bravoae sensu lato	*Neoophorus regalis*5	Girardinichthyini	RICO Nre5	México	Michoacán
Margotrema bravoae sensu lato	*Neoophorus regalis*6	Girardinichthyini	RICO Nre6	México	Michoacán
Margotrema bravoae sensu lato	*Neoophorus regalis*7	Girardinichthyini	RICO Nre7	México	Michoacán
Margotrema bravoae sensu lato	*Neoophorus regalis*8	Girardinichthyini	RICO Nre8	México	Michoacán
Margotrema bravoae sensu lato	*Neoophorus regalis*9	Girardinichthyini	RICO Nre9	México	Michoacán
Margotrema bravoae sensu lato	*Neoophorus regalis*10	Girardinichthyini	RICO Nre10	México	Michoacán
Margotrema bravoae sensu lato	*Ilyodon whitei*1	Ilyodontini	TLAP Iwh1	México	Puebla
Margotrema bravoae sensu lato	*Ilyodon whitei*2	Ilyodontini	TLAP Iwh2	México	Puebla
Margotrema bravoae sensu lato	*Ilyodon whitei*3	Ilyodontini	TLAP Iwh3	México	Puebla
Margotrema bravoae sensu lato	*Ilyodon whitei*4	Ilyodontini	TLAP Iwh4	México	Puebla
Margotrema bravoae sensu lato	*Ilyodon whitei*5	Ilyodontini	TLAP Iwh5	México	Puebla
Margotrema bravoae sensu lato	*Ilyodon whitei*6	Ilyodontini	TLAP Iwh6	México	Puebla
Margotrema bravoae sensu lato	*Ilyodon whitei*7	Ilyodontini	TLAP Iwh7	México	Puebla
Margotrema bravoae sensu lato	*Ilyodon whitei*8	Ilyodontini	TLAP Iwh8	México	Puebla
Margotrema bravoae sensu lato	*Ilyodon whitei*9	Ilyodontini	TLAP Iwh9	México	Puebla
Margotrema bravoae sensu lato	*Ilyodon whitei*10	Ilyodontini	TLAP Iwh10	México	Puebla
Margotrema bravoae sensu lato	*Ilyodon whitei*11	Ilyodontini	TLAP Iwh11	México	Puebla
Margotrema bravoae sensu lato	*Ilyodon whitei*12	Ilyodontini	TLAP Iwh12	México	Puebla
Margotrema bravoae sensu lato	*Ilyodon whitei*13	Ilyodontini	TLAP Iwh13	México	Puebla

Margotrema bravoae sensu lato	*Characodon audax*1	Characodontini	TOBO Cha1	México	Durango
Margotrema bravoae sensu lato	*Characodon audax*2	Characodontini	TOBO Cha2	México	Durango
Margotrema bravoae sensu lato	*Characodon audax*3	Characodontini	TOBO Cha3	México	Durango
Margotrema bravoae sensu lato	*Characodon audax*4	Characodontini	TOBO Cha4	México	Durango
Margotrema bravoae sensu lato	*Characodon audax*5	Characodontini	TOBO Cha5	México	Durango
Margotrema bravoae sensu lato	*Characodon audax*6	Characodontini	TOBO Cha6	México	Durango
Margotrema bravoae sensu lato	*Characodon audax*7	Characodontini	TOBO Cha7	México	Durango
Margotrema bravoae sensu lato	*Chapalichthys pardalis*1	Chapalichthyini	TOCU Chp1	México	Michoacán
Margotrema bravoae sensu lato	*Ilyodon furcidens*1	Ilyodontini	TOCU Ifu1	México	Michoacán
Margotrema bravoae sensu lato	*Ilyodon furcidens*2	Ilyodontini	TOCU Ifu2	México	Michoacán
Margotrema bravoae sensu lato	*Allotoca zacapuensis*1	Girardinichthyini	ZACA Aza	México	Michoacán
Margotrema bravoae sensu lato	*Zoogoneticus quitzeoensis*1	Chapalichthyini	ZACA Zqu1	México	Michoacán
Margotrema bravoae sensu lato	*Zoogoneticus quitzeoensis*2	Chapalichthyini	ZACA Zqu2	México	Michoacán
Margotrema bravoae sensu lato	*Zoogoneticus quitzeoensis*3	Chapalichthyini	ZACA Zqu3	México	Michoacán
Margotrema bravoae sensu lato	*Zoogoneticus quitzeoensis*4	Chapalichthyini	ZACA Zqu4	México	Michoacán
Margotrema bravoae sensu lato	*Zoogoneticus quitzeoensis*5	Chapalichthyini	ZACA Zqu5	México	Michoacán
Margotrema bravoae sensu lato	*Zoogoneticus quitzeoensis*6	Chapalichthyini	ZACA Zqu6	México	Michoacán
Margotrema bravoae sensu lato	*Codoma ornata*1		BOCO Cor	México	Chihuahua
Allocreadium lobatum	*Semotilus atromaculatus*			Canada	
Prostenhystera sp.	*Gephyrocharax* sp.			Panama	Gamboa
Prostenhystera sp.	*Gephyrocharax* sp.			Panama	Gamboa
Prostenhystera sp.	*Gephyrocharax* sp.			Panama	Gamboa
Wallinia chavarriae	*Gephyrocharax* sp.			Panama	Gamboa
Wallinia chavarriae	*Gephyrocharax* sp.			Panama	Gamboa
Wallinia chavarriae	*Gephyrocharax* sp.			Panama	Gamboa

Part B

Locality	**Habitat**	**Altitude (m)**	**GPS Location**
Spring in Abraham González	Spring	1865	24º12´45´´N; 104º31´48´´W

Stream in Ahuacapán	Stream	842	19º39´52´´N; 104º19´19.1´´W
Stream in Ahuacapán	Stream	842	19º39´52´´N; 104º19´19.1´´W
Stream in Ahuacapán	Stream	842	19º39´52´´N; 104º19´19.1´´W
Stream in Ahuacapán	Stream	842	19º39´52´´N; 104º19´19.1´´W
Stream in Ahuacapán	Stream	842	19º39´52´´N; 104º19´19.1´´W
Stream in Ahuacapán	Stream	842	19º39´52´´N; 104º19´19.1´´W
Stream in Ahuacapán	Stream	842	19º39´52´´N; 104º19´19.1´´W
Stream in Ahuacapán	Stream	842	19º39´52´´N; 104º19´19.1´´W
Stream in Ahuacapán	Stream	842	19º39´52´´N; 104º19´19.1´´W
Stream in Ahuacapán	Stream	842	19º39´52´´N; 104º19´19.1´´W
Stream in Ahuacapán	Stream	842	19º39´52´´N; 104º19´19.1´´W
Stream in Ahuacapán	Stream	842	19º39´52´´N; 104º19´19.1´´W
Stream in Ahuacapán	Stream	842	19º39´52´´N; 104º19´19.1´´W
Stream in Ahuacapán	Stream	842	19º39´52´´N; 104º19´19.1´´W
Stream in Ahuacapán	Stream	842	19º39´52´´N; 104º19´19.1´´W
Stream in Ahuacapán	Stream	842	19º39´52´´N; 104º19´19.1´´W
Stream in Ahuacapán	Stream	842	19º39´52´´N; 104º19´19.1´´W
Stream in Ahuacapán	Stream	842	19º39´52´´N; 104º19´19.1´´W
Stream in Ahuacapán	Stream	842	19º39´52´´N; 104º19´19.1´´W
Stream in Ahuacapán	Stream	842	19º39´52´´N; 104º19´19.1´´W
Stream in Ahuacapán	Stream	842	19º39´52´´N; 104º19´19.1´´W
Stream in Ahuacapán	Stream	842	19º39´52´´N; 104º19´19.1´´W
Stream in Ahuacapán	Stream	842	19º39´52´´N; 104º19´19.1´´W
Spring Chapultepec	Spring	2072	19º34´20´´N; 101º31´18.7´´W
Spring Chapultepec	Spring	2072	19º34´20´´N; 101º31´18.7´´W
Spring Chapultepec	Spring	2072	19º34´20´´N; 101º31´18.7´´W
Spring Chapultepec	Spring	2072	19º34´20´´N; 101º31´18.7´´W
Spring Chapultepec	Spring	2072	19º34´20´´N; 101º31´18.7´´W
Spring Chapultepec	Spring	2072	19º34´20´´N; 101º31´18.7´´W
Spring Chapultepec	Spring	2072	19º34´20´´N; 101º31´18.7´´W
Spring Chapultepec	Spring	2072	19º34´20´´N; 101º31´18.7´´W

Spring Chapultepec	Spring	2072	19º34´20´´N; 101º31´18.7´´W
Spring Chapultepec	Spring	2072	19º34´20´´N; 101º31´18.7´´W
Spring Chapultepec	Spring	2072	19º34´20´´N; 101º31´18.7´´W
Spring Chapultepec	Spring	2072	19º34´20´´N; 101º31´18.7´´W
Spring Chapultepec	Spring	2072	19º34´20´´N; 101º31´18.7´´W
Spring Chapultepec	Spring	2072	19º34´20´´N; 101º31´18.7´´W
Spring Chapultepec	Spring	2072	19º34´20´´N; 101º31´18.7´´W
Spring Chapultepec	Spring	2072	19º34´20´´N; 101º31´18.7´´W
Spring Chapultepec	Spring	2072	19º34´20´´N; 101º31´18.7´´W
Spring Chapultepec	Spring	2072	19º34´20´´N; 101º31´18.7´´W
Spring Chapultepec	Spring	2072	19º34´20´´N; 101º31´18.7´´W
Spring Chapultepec	Spring	2072	19º34´20´´N; 101º31´18.7´´W
Arroyo Durazno in Cuzalapa River	Stream	825	19º30´32.1´´N; 104º17´45.6´´W
Arroyo Durazno in Cuzalapa River	Stream	825	19º30´32.1´´N; 104º17´45.6´´W
Arroyo Durazno in Cuzalapa River	Stream	825	19º30´32.1´´N; 104º17´45.6´´W
Arroyo Durazno in Cuzalapa River	Stream	825	19º30´32.1´´N; 104º17´45.6´´W
Arroyo Durazno in Cuzalapa River	Stream	825	19º30´32.1´´N; 104º17´45.6´´W
Arroyo Durazno in Cuzalapa River	Stream	825	19º30´32.1´´N; 104º17´45.6´´W
Arroyo Durazno in Cuzalapa River	Stream	825	19º30´32.1´´N; 104º17´45.6´´W
Arroyo Durazno in Cuzalapa River	Stream	825	19º30´32.1´´N; 104º17´45.6´´W
Arroyo Durazno in Cuzalapa River	Stream	825	19º30´32.1´´N; 104º17´45.6´´W
Arroyo Durazno in Cuzalapa River	Stream	825	19º30´32.1´´N; 104º17´45.6´´W
Spring in the Unidad de Manejo Ambiental de caza, Guadalupe Aguilera	Spring	1941	24º25´59.5´´N; 104º38´29´´W
Spring in the Unidad de Manejo Ambiental de caza, Guadalupe Aguilera	Spring	1941	24º25´59.5´´N; 104º38´29´´W
Spring in the Unidad de Manejo Ambiental de caza, Guadalupe Aguilera	Spring	1941	24º25´59.5´´N; 104º38´29´´W
La Luz spring, Jacona de Plancarte	Spring	1616	19º56´10.4´´N; 102º17´57.8´´W
La Mintzita spring	Spring	1895	19°38'40.3"-19°38'52.3"N; 101°16’28.20’’-101°16’13.0’’W
La Mintzita spring	Spring	1895	19°38'40.3"-19°38'52.3"N; 101°16’28.20’’-101°16’13.0’’W
La Mintzita spring	Spring	1895	19°38'40.3"-19°38'52.3"N; 101°16’28.20’’-101°16’13.0’’W
La Mintzita spring	Spring	1895	19°38'40.3"-19°38'52.3"N; 101°16’28.20’’-101°16’13.0’’W
La Mintzita spring	Spring	1895	19°38'40.3"-19°38'52.3"N; 101°16’28.20’’-101°16’13.0’’W

La Mintzita spring	Spring	1895	19°38'40.3"-19°38'52.3"N; 101°16’28.20’’-101°16’13.0’’W
La Mintzita spring	Spring	1895	19°38'40.3"-19°38'52.3"N; 101°16’28.20’’-101°16’13.0’’W
La Mintzita spring	Spring	1895	19°38'40.3"-19°38'52.3"N; 101°16’28.20’’-101°16’13.0’’W
La Mintzita spring	Spring	1895	19°38'40.3"-19°38'52.3"N; 101°16’28.20’’-101°16’13.0’’W
La Mintzita spring	Spring	1895	19°38'40.3"-19°38'52.3"N; 101°16’28.20’’-101°16’13.0’’W
La Mintzita spring	Spring	1895	19°38'40.3"-19°38'52.3"N; 101°16’28.20’’-101°16’13.0’’W
Opopeo Lake	Lake	2268	19°24'20.2"N; 101°36’08.2’’W
Opopeo Lake	Lake	2268	19°24'20.2"N; 101°36’08.2’’W
Opopeo Lake	Lake	2268	19°24'20.2"N; 101°36’08.2’’W
Opopeo Lake	Lake	2268	19°24'20.2"N; 101°36’08.2’’W
Opopeo Lake	Lake	2268	19°24'20.2"N; 101°36’08.2’’W
Opopeo Lake	Lake	2268	19°24'20.2"N; 101°36’08.2’’W
Opopeo Lake	Lake	2268	19°24'20.2"N; 101°36’08.2’’W
Opopeo Lake	Lake	2268	19°24'20.2"N; 101°36’08.2’’W
Opopeo Lake	Lake	2268	19°24'20.2"N; 101°36’08.2’’W
Opopeo Lake	Lake	2268	19°24'20.2"N; 101°36’08.2’’W
Puente en el poblado de Pino Suárez, carretera Durango-Mezquital	Spring	1911	23°52'43.5"N; 104°31’54.7’’W
Puente en el poblado de Pino Suárez, carretera Durango-Mezquital	Spring	1911	23°52'43.5"N; 104°31’54.7’’W
Puente en el poblado de Pino Suárez, carretera Durango-Mezquital	Spring	1911	23°52'43.5"N; 104°31’54.7’’W
Puente en el poblado de Pino Suárez, carretera Durango-Mezquital	Spring	1911	23°52'43.5"N; 104°31’54.7’’W
Puente en el poblado de Pino Suárez, carretera Durango-Mezquital	Spring	1911	23°52'43.5"N; 104°31’54.7’’W
Puente en el poblado de Pino Suárez, carretera Durango-Mezquital	Spring	1911	23°52'43.5"N; 104°31’54.7’’W
Puente en el poblado de Pino Suárez, carretera Durango-Mezquital	Spring	1911	23°52'43.5"N; 104°31’54.7’’W
Spring Rico	Spring	1842	19°49'51.85"N; 102°30’7.98’’W
Spring Rico	Spring	1842	19°49'51.85"N; 102°30’7.98’’W
Spring Rico	Spring	1842	19°49'51.85"N; 102°30’7.98’’W
Spring Rico	Spring	1842	19°49'51.85"N; 102°30’7.98’’W
Spring Rico	Spring	1842	19°49'51.85"N; 102°30’7.98’’W
Spring Rico	Spring	1842	19°49'51.85"N; 102°30’7.98’’W
Spring Rico	Spring	1842	19°49'51.85"N; 102°30’7.98’’W
Spring Rico	Spring	1842	19°49'51.85"N; 102°30’7.98’’W

Spring Rico	Spring	1842	19°49'51.85"N; 102°30’7.98’’W
Spring Rico	Spring	1842	19°49'51.85"N; 102°30’7.98’’W
Spring in Tlapetlahuaya	Spring	1681	18°45'20.40"N; 98°34’28.8’’W
Spring in Tlapetlahuaya	Spring	1681	18°45'20.40"N; 98°34’28.8’’W
Spring in Tlapetlahuaya	Spring	1681	18°45'20.40"N; 98°34’28.8’’W
Spring in Tlapetlahuaya	Spring	1681	18°45'20.40"N; 98°34’28.8’’W
Spring in Tlapetlahuaya	Spring	1681	18°45'20.40"N; 98°34’28.8’’W
Spring in Tlapetlahuaya	Spring	1681	18°45'20.40"N; 98°34’28.8’’W
Spring in Tlapetlahuaya	Spring	1681	18°45'20.40"N; 98°34’28.8’’W
Spring in Tlapetlahuaya	Spring	1681	18°45'20.40"N; 98°34’28.8’’W
Spring in Tlapetlahuaya	Spring	1681	18°45'20.40"N; 98°34’28.8’’W
Spring in Tlapetlahuaya	Spring	1681	18°45'20.40"N; 98°34’28.8’’W
Spring in Tlapetlahuaya	Spring	1681	18°45'20.40"N; 98°34’28.8’’W
Spring in Tlapetlahuaya	Spring	1681	18°45'20.40"N; 98°34’28.8’’W
Spring in Tlapetlahuaya	Spring	1681	18°45'20.40"N; 98°34’28.8’’W
Spring El Toboso	Spring	1890	24° 16' 30.7" N; 104° 34' 52.8" W
Spring El Toboso	Spring	1890	24° 16' 30.7" N; 104° 34' 52.8" W
Spring El Toboso	Spring	1890	24° 16' 30.7" N; 104° 34' 52.8" W
Spring El Toboso	Spring	1890	24° 16' 30.7" N; 104° 34' 52.8" W
Spring El Toboso	Spring	1890	24° 16' 30.7" N; 104° 34' 52.8" W
Spring El Toboso	Spring	1890	24° 16' 30.7" N; 104° 34' 52.8" W
Spring El Toboso	Spring	1890	24° 16' 30.7" N; 104° 34' 52.8" W
Spring in park of Tocumbo	Spring	1010	19°42'8.9"N; 102°30’55.6’’W
Spring in park of Tocumbo	Spring	1010	19°42'8.9"N; 102°30’55.6’’W
Spring in park of Tocumbo	Spring	1010	19°42'8.9"N; 102°30’55.6’’W
La Angostura, Zacapu Lake (balneario)	Lake with spring	1990	19°49'35"N; 101°47’10’’W
La Angostura, Zacapu Lake (balneario)	Lake with spring	1990	19°49'35"N; 101°47’10’’W
La Angostura, Zacapu Lake (balneario)	Lake with spring	1990	19°49'35"N; 101°47’10’’W
La Angostura, Zacapu Lake (balneario)	Lake with spring	1990	19°49'35"N; 101°47’10’’W
La Angostura, Zacapu Lake (balneario)	Lake with spring	1990	19°49'35"N; 101°47’10’’W
La Angostura, Zacapu Lake (balneario)	Lake with spring	1990	19°49'35"N; 101°47’10’’W

La Angostura, Zacapu Lake (balneario)	Lake with spring	1990	19°49'35"N; 101°47’10’’W
Tributary of Conchos River, Bocoyna	River	2261	27°51'30.32"N; 107°36’3.01’’W
Tobacco Creek, Manitoba	River		
Quebrada Juan Grande	Stream		9°8'50.5"N; 79°43’21.1’’W
Quebrada Juan Grande	Stream		9°8'50.5"N; 79°43’21.1’’W
Quebrada Juan Grande	Stream		9°8'50.5"N; 79°43’21.1’’W
Frijolito River	Stream		9°8'58.1"N; 79°43’53.8’’W
Frijolito River	Stream		9°8'58.1"N; 79°43’53.8’’W
Frijolito River	Stream		9°8'58.1"N; 79°43’53.8’’W

Part C

Date collected	Collection method of fishes	Collector of fishes
1-7.12.2008	Seine nets	A. Martínez-Aquino & R. Pérez-Rodríguez
18-24.08.2008	Electrofishing	A. Martínez-Aquino, R. Aguilar-Aguilar & R. Pérez-Rodríguez
18-24.08.2008	Electrofishing	A. Martínez-Aquino, R. Aguilar-Aguilar & R. Pérez-Rodríguez
18-24.08.2008	Electrofishing	A. Martínez-Aquino, R. Aguilar-Aguilar & R. Pérez-Rodríguez
18-24.08.2008	Electrofishing	A. Martínez-Aquino, R. Aguilar-Aguilar & R. Pérez-Rodríguez
18-24.08.2008	Electrofishing	A. Martínez-Aquino, R. Aguilar-Aguilar & R. Pérez-Rodríguez
18-24.08.2008	Electrofishing	A. Martínez-Aquino, R. Aguilar-Aguilar & R. Pérez-Rodríguez
18-24.08.2008	Electrofishing	A. Martínez-Aquino, R. Aguilar-Aguilar & R. Pérez-Rodríguez
18-24.08.2008	Electrofishing	A. Martínez-Aquino, R. Aguilar-Aguilar & R. Pérez-Rodríguez
18-24.08.2008	Electrofishing	A. Martínez-Aquino, R. Aguilar-Aguilar & R. Pérez-Rodríguez
18-24.08.2008	Electrofishing	A. Martínez-Aquino, R. Aguilar-Aguilar & R. Pérez-Rodríguez
18-24.08.2008	Electrofishing	A. Martínez-Aquino, R. Aguilar-Aguilar & R. Pérez-Rodríguez
18-24.08.2008	Electrofishing	A. Martínez-Aquino, R. Aguilar-Aguilar & R. Pérez-Rodríguez
18-24.08.2008	Electrofishing	A. Martínez-Aquino, R. Aguilar-Aguilar & R. Pérez-Rodríguez
18-24.08.2008	Electrofishing	A. Martínez-Aquino, R. Aguilar-Aguilar & R. Pérez-Rodríguez
18-24.08.2008	Electrofishing	A. Martínez-Aquino, R. Aguilar-Aguilar & R. Pérez-Rodríguez
18-24.08.2008	Electrofishing	A. Martínez-Aquino, R. Aguilar-Aguilar & R. Pérez-Rodríguez

18-24.08.2008	Electrofishing	A. Martínez-Aquino, R. Aguilar-Aguilar & R. Pérez-Rodríguez
18-24.08.2008	Electrofishing	A. Martínez-Aquino, R. Aguilar-Aguilar & R. Pérez-Rodríguez
18-24.08.2008	Electrofishing	A. Martínez-Aquino, R. Aguilar-Aguilar & R. Pérez-Rodríguez
18-24.08.2008	Electrofishing	A. Martínez-Aquino, R. Aguilar-Aguilar & R. Pérez-Rodríguez
18-24.08.2008	Electrofishing	A. Martínez-Aquino, R. Aguilar-Aguilar & R. Pérez-Rodríguez
18-24.08.2008	Electrofishing	A. Martínez-Aquino, R. Aguilar-Aguilar & R. Pérez-Rodríguez
18-24.08.2008	Electrofishing	A. Martínez-Aquino, R. Aguilar-Aguilar & R. Pérez-Rodríguez
15.07.2009	Minnow traps and seine nets	A. Martínez-Aquino, D.I.Hernández-Mena & R. Pérez-Rodríguez
15.07.2009	Minnow traps and seine nets	A. Martínez-Aquino, D.I.Hernández-Mena & R. Pérez-Rodríguez
15.07.2009	Minnow traps and seine nets	A. Martínez-Aquino, D.I.Hernández-Mena & R. Pérez-Rodríguez
15.07.2009	Minnow traps and seine nets	A. Martínez-Aquino, D.I.Hernández-Mena & R. Pérez-Rodríguez
15.07.2009	Minnow traps and seine nets	A. Martínez-Aquino, D.I.Hernández-Mena & R. Pérez-Rodríguez
15.07.2009	Minnow traps and seine nets	A. Martínez-Aquino, D.I.Hernández-Mena & R. Pérez-Rodríguez
15.07.2009	Minnow traps and seine nets	A. Martínez-Aquino, D.I.Hernández-Mena & R. Pérez-Rodríguez
15.07.2009	Minnow traps and seine nets	A. Martínez-Aquino, D.I.Hernández-Mena & R. Pérez-Rodríguez
15.07.2009	Minnow traps and seine nets	A. Martínez-Aquino, D.I.Hernández-Mena & R. Pérez-Rodríguez
15.07.2009	Minnow traps and seine nets	A. Martínez-Aquino, D.I.Hernández-Mena & R. Pérez-Rodríguez
15.07.2009	Minnow traps and seine nets	A. Martínez-Aquino, D.I.Hernández-Mena & R. Pérez-Rodríguez
15.07.2009	Minnow traps and seine nets	A. Martínez-Aquino, D.I.Hernández-Mena & R. Pérez-Rodríguez
15.07.2009	Minnow traps and seine nets	A. Martínez-Aquino, D.I.Hernández-Mena & R. Pérez-Rodríguez
15.07.2009	Minnow traps and seine nets	A. Martínez-Aquino, D.I.Hernández-Mena & R. Pérez-Rodríguez
15.07.2009	Minnow traps and seine nets	A. Martínez-Aquino, D.I.Hernández-Mena & R. Pérez-Rodríguez
15.07.2009	Minnow traps and seine nets	A. Martínez-Aquino, D.I.Hernández-Mena & R. Pérez-Rodríguez
15.07.2009	Minnow traps and seine nets	A. Martínez-Aquino, D.I.Hernández-Mena & R. Pérez-Rodríguez
15.07.2009	Minnow traps and seine nets	A. Martínez-Aquino, D.I.Hernández-Mena & R. Pérez-Rodríguez
15.07.2009	Minnow traps and seine nets	A. Martínez-Aquino, D.I.Hernández-Mena & R. Pérez-Rodríguez
15.07.2009	Minnow traps and seine nets	A. Martínez-Aquino, D.I.Hernández-Mena & R. Pérez-Rodríguez
18-24.08.2008	Electrofishing	A. Martínez-Aquino, R. Aguilar-Aguilar & R. Pérez-Rodríguez
18-24.08.2008	Electrofishing	A. Martínez-Aquino, R. Aguilar-Aguilar & R. Pérez-Rodríguez
18-24.08.2008	Electrofishing	A. Martínez-Aquino, R. Aguilar-Aguilar & R. Pérez-Rodríguez
18-24.08.2008	Electrofishing	A. Martínez-Aquino, R. Aguilar-Aguilar & R. Pérez-Rodríguez

18-24.08.2008	Electrofishing	A. Martínez-Aquino, R. Aguilar-Aguilar & R. Pérez-Rodríguez
18-24.08.2008	Electrofishing	A. Martínez-Aquino, R. Aguilar-Aguilar & R. Pérez-Rodríguez
18-24.08.2008	Electrofishing	A. Martínez-Aquino, R. Aguilar-Aguilar & R. Pérez-Rodríguez
18-24.08.2008	Electrofishing	A. Martínez-Aquino, R. Aguilar-Aguilar & R. Pérez-Rodríguez
18-24.08.2008	Electrofishing	A. Martínez-Aquino, R. Aguilar-Aguilar & R. Pérez-Rodríguez
18-24.08.2008	Electrofishing	A. Martínez-Aquino, R. Aguilar-Aguilar & R. Pérez-Rodríguez
1-7.12.2008	Seine nets and electrofishing	A. Martínez-Aquino, R. Aguilar-Aguilar, R. Rosas-Valdez & R. Pérez-Rodríguez
1-7.12.2008	Seine nets and electrofishing	A. Martínez-Aquino, R. Aguilar-Aguilar, R. Rosas-Valdez & R. Pérez-Rodríguez
1-7.12.2008	Seine nets and electrofishing	A. Martínez-Aquino, R. Aguilar-Aguilar, R. Rosas-Valdez & R. Pérez-Rodríguez
23.07.2009	Seine nets	A. Martínez-Aquino, D.I.Hernández-Mena & R. Pérez-Rodríguez
30.07.2009	Electrofishing	A. Martínez-Aquino, D.I.Hernández-Mena & R. Pérez-Rodríguez
30.07.2009	Electrofishing	A. Martínez-Aquino, D.I.Hernández-Mena & R. Pérez-Rodríguez
30.07.2009	Electrofishing	A. Martínez-Aquino, D.I.Hernández-Mena & R. Pérez-Rodríguez
30.07.2009	Seine nets and electrofishing	A. Martínez-Aquino, D.I.Hernández-Mena & R. Pérez-Rodríguez
30.07.2009	Seine nets and electrofishing	A. Martínez-Aquino, D.I.Hernández-Mena & R. Pérez-Rodríguez
30.07.2009	Seine nets and electrofishing	A. Martínez-Aquino, D.I.Hernández-Mena & R. Pérez-Rodríguez
30.07.2009	Seine nets and electrofishing	A. Martínez-Aquino, D.I.Hernández-Mena & R. Pérez-Rodríguez
30.07.2009	Seine nets and electrofishing	A. Martínez-Aquino, D.I.Hernández-Mena & R. Pérez-Rodríguez
30.07.2009	Seine nets and electrofishing	A. Martínez-Aquino, D.I.Hernández-Mena & R. Pérez-Rodríguez
30.07.2009	Seine nets and electrofishing	A. Martínez-Aquino, D.I.Hernández-Mena & R. Pérez-Rodríguez
30.07.2009	Seine nets and electrofishing	A. Martínez-Aquino, D.I.Hernández-Mena & R. Pérez-Rodríguez
16.07.2009	Seine nets and electrofishing	A. Martínez-Aquino, D.I.Hernández-Mena & R. Pérez-Rodríguez
16.07.2009	Seine nets and electrofishing	A. Martínez-Aquino, D.I.Hernández-Mena & R. Pérez-Rodríguez
16.07.2009	Seine nets and electrofishing	A. Martínez-Aquino, D.I.Hernández-Mena & R. Pérez-Rodríguez
16.07.2009	Seine nets and electrofishing	A. Martínez-Aquino, D.I.Hernández-Mena & R. Pérez-Rodríguez
16.07.2009	Seine nets and electrofishing	A. Martínez-Aquino, D.I.Hernández-Mena & R. Pérez-Rodríguez
16.07.2009	Seine nets and electrofishing	A. Martínez-Aquino, D.I.Hernández-Mena & R. Pérez-Rodríguez
16.07.2009	Seine nets and electrofishing	A. Martínez-Aquino, D.I.Hernández-Mena & R. Pérez-Rodríguez
16.07.2009	Seine nets and electrofishing	A. Martínez-Aquino, D.I.Hernández-Mena & R. Pérez-Rodríguez
16.07.2009	Seine nets and electrofishing	A. Martínez-Aquino, D.I.Hernández-Mena & R. Pérez-Rodríguez
16.07.2009	Seine nets and electrofishing	A. Martínez-Aquino, D.I.Hernández-Mena & R. Pérez-Rodríguez

1-7.12.2008	Seine nets and electrofishing	A. Martínez-Aquino, R. Aguilar-Aguilar, R. Rosas-Valdez & R. Pérez-Rodríguez
1-7.12.2008	Seine nets and electrofishing	A. Martínez-Aquino, R. Aguilar-Aguilar, R. Rosas-Valdez & R. Pérez-Rodríguez
1-7.12.2008	Seine nets and electrofishing	A. Martínez-Aquino, R. Aguilar-Aguilar, R. Rosas-Valdez & R. Pérez-Rodríguez
1-7.12.2008	Seine nets and electrofishing	A. Martínez-Aquino, R. Aguilar-Aguilar, R. Rosas-Valdez & R. Pérez-Rodríguez
1-7.12.2008	Seine nets and electrofishing	A. Martínez-Aquino, R. Aguilar-Aguilar, R. Rosas-Valdez & R. Pérez-Rodríguez
1-7.12.2008	Seine nets and electrofishing	A. Martínez-Aquino, R. Aguilar-Aguilar, R. Rosas-Valdez & R. Pérez-Rodríguez
1-7.12.2008	Seine nets and electrofishing	A. Martínez-Aquino, R. Aguilar-Aguilar, R. Rosas-Valdez & R. Pérez-Rodríguez
25.07.2009	Seine nets and electrofishing	D.I.Hernández-Mena & R. Pérez-Rodríguez
25.07.2009	Seine nets and electrofishing	D.I.Hernández-Mena & R. Pérez-Rodríguez
25.07.2009	Seine nets and electrofishing	D.I.Hernández-Mena & R. Pérez-Rodríguez
25.07.2009	Seine nets and electrofishing	D.I.Hernández-Mena & R. Pérez-Rodríguez
25.07.2009	Seine nets and electrofishing	D.I.Hernández-Mena & R. Pérez-Rodríguez
25.07.2009	Seine nets and electrofishing	D.I.Hernández-Mena & R. Pérez-Rodríguez
25.07.2009	Seine nets and electrofishing	D.I.Hernández-Mena & R. Pérez-Rodríguez
25.07.2009	Seine nets and electrofishing	D.I.Hernández-Mena & R. Pérez-Rodríguez
25.07.2009	Seine nets and electrofishing	D.I.Hernández-Mena & R. Pérez-Rodríguez
25.07.2009	Seine nets and electrofishing	D.I.Hernández-Mena & R. Pérez-Rodríguez
02.07.2010	Seine nets	A. Martínez-Aquino, R. Aguilar-Aguilar, F.J. Alcantar & R. Pérez-Rodríguez
02.07.2010	Seine nets	A. Martínez-Aquino, R. Aguilar-Aguilar, F.J. Alcantar & R. Pérez-Rodríguez
02.07.2010	Seine nets	A. Martínez-Aquino, R. Aguilar-Aguilar, F.J. Alcantar & R. Pérez-Rodríguez
02.07.2010	Seine nets	A. Martínez-Aquino, R. Aguilar-Aguilar, F.J. Alcantar & R. Pérez-Rodríguez
02.07.2010	Seine nets	A. Martínez-Aquino, R. Aguilar-Aguilar, F.J. Alcantar & R. Pérez-Rodríguez
02.07.2010	Seine nets	A. Martínez-Aquino, R. Aguilar-Aguilar, F.J. Alcantar & R. Pérez-Rodríguez
02.07.2010	Seine nets	A. Martínez-Aquino, R. Aguilar-Aguilar, F.J. Alcantar & R. Pérez-Rodríguez
02.07.2010	Seine nets	A. Martínez-Aquino, R. Aguilar-Aguilar, F.J. Alcantar & R. Pérez-Rodríguez
02.07.2010	Seine nets	A. Martínez-Aquino, R. Aguilar-Aguilar, F.J. Alcantar & R. Pérez-Rodríguez
02.07.2010	Seine nets	A. Martínez-Aquino, R. Aguilar-Aguilar, F.J. Alcantar & R. Pérez-Rodríguez
02.07.2010	Seine nets	A. Martínez-Aquino, R. Aguilar-Aguilar, F.J. Alcantar & R. Pérez-Rodríguez
02.07.2010	Seine nets	A. Martínez-Aquino, R. Aguilar-Aguilar, F.J. Alcantar & R. Pérez-Rodríguez
02.07.2010	Seine nets	A. Martínez-Aquino, R. Aguilar-Aguilar, F.J. Alcantar & R. Pérez-Rodríguez
1-7.12.2008	Seine nets	A. Martínez-Aquino, R. Aguilar-Aguilar, R. Rosas-Valdez & R. Pérez-Rodríguez

1-7.12.2008	Seine nets	A. Martínez-Aquino, R. Aguilar-Aguilar, R. Rosas-Valdez & R. Pérez-Rodríguez
1-7.12.2008	Seine nets	A. Martínez-Aquino, R. Aguilar-Aguilar, R. Rosas-Valdez & R. Pérez-Rodríguez
1-7.12.2008	Seine nets	A. Martínez-Aquino, R. Aguilar-Aguilar, R. Rosas-Valdez & R. Pérez-Rodríguez
1-7.12.2008	Seine nets	A. Martínez-Aquino, R. Aguilar-Aguilar, R. Rosas-Valdez & R. Pérez-Rodríguez
1-7.12.2008	Seine nets	A. Martínez-Aquino, R. Aguilar-Aguilar, R. Rosas-Valdez & R. Pérez-Rodríguez
1-7.12.2008	Seine nets	A. Martínez-Aquino, R. Aguilar-Aguilar, R. Rosas-Valdez & R. Pérez-Rodríguez
22.07.2009	Seine nets	A. Martínez-Aquino, D.I.Hernández-Mena & R. Pérez-Rodríguez
22.07.2009	Seine nets	A. Martínez-Aquino, D.I.Hernández-Mena & R. Pérez-Rodríguez
22.07.2009	Seine nets	A. Martínez-Aquino, D.I.Hernández-Mena & R. Pérez-Rodríguez
17-24.07.2009	Seine nets and electrofishing	A. Martínez-Aquino, D.I.Hernández-Mena & R. Pérez-Rodríguez
17-24.07.2009	Seine nets and electrofishing	A. Martínez-Aquino, D.I.Hernández-Mena & R. Pérez-Rodríguez
17-24.07.2009	Seine nets and electrofishing	A. Martínez-Aquino, D.I.Hernández-Mena & R. Pérez-Rodríguez
17-24.07.2009	Seine nets and electrofishing	A. Martínez-Aquino, D.I.Hernández-Mena & R. Pérez-Rodríguez
17-24.07.2009	Seine nets and electrofishing	A. Martínez-Aquino, D.I.Hernández-Mena & R. Pérez-Rodríguez
17-24.07.2009	Seine nets and electrofishing	A. Martínez-Aquino, D.I.Hernández-Mena & R. Pérez-Rodríguez
17-24.07.2009	Seine nets and electrofishing	A. Martínez-Aquino, D.I.Hernández-Mena & R. Pérez-Rodríguez
-	-	R. Rosas-Valdez
15-17.01.2010	Seine nets	A. Choudhury, A. Martínez-Aquino, & G. Pérez-Ponce de León
15-17.01.2010	Seine nets	A. Choudhury, A. Martínez-Aquino, & G. Pérez-Ponce de León
15-17.01.2010	Seine nets	A. Choudhury, A. Martínez-Aquino, & G. Pérez-Ponce de León
15-17.01.2010	Seine nets	A. Choudhury, A. Martínez-Aquino, & G. Pérez-Ponce de León
15-17.01.2010	Seine nets	A. Choudhury, A. Martínez-Aquino, & G. Pérez-Ponce de León
15-17.01.2010	Seine nets	A. Choudhury, A. Martínez-Aquino, & G. Pérez-Ponce de León

Part D

Collector of digeneans	**Additional info**	**Vouchers number**	Genebank ***ITS1***	Genebank ***COI***
A. Martínez-Aquino, R. Aguilar-Aguilar & R. Rosas-Valdez	-	Akin CNHE 6493***	KC900074	KC899971

A. Martínez-Aquino & R. Aguilar-Aguilar	-	Akin CNHE 8320	KC899983	KC899864
A. Martínez-Aquino & R. Aguilar-Aguilar	-	Akin CNHE 8320	KC899984	KC899865
A. Martínez-Aquino & R. Aguilar-Aguilar	-	Akin CNHE 8320	KC899985	KC899866
A. Martínez-Aquino & R. Aguilar-Aguilar	-	Akin CNHE 8320	KC899986	KC899867
A. Martínez-Aquino & R. Aguilar-Aguilar	-	Akin CNHE 8320	KC899987	KC899868
A. Martínez-Aquino & R. Aguilar-Aguilar	-	Akin CNHE 8320	KC899988	KC899869
A. Martínez-Aquino & R. Aguilar-Aguilar	-	Akin CNHE 8320	KC899989	KC899870
A. Martínez-Aquino & R. Aguilar-Aguilar	-	Akin CNHE 8320	KC899990	KC899871
A. Martínez-Aquino & R. Aguilar-Aguilar	-	Akin CNHE 8320	KC899991	KC899872
A. Martínez-Aquino & R. Aguilar-Aguilar	-	Akin CNHE 8320	KC899992	KC899873
A. Martínez-Aquino & R. Aguilar-Aguilar	-	Akin CNHE 8320	KC899993	KC899874
A. Martínez-Aquino & R. Aguilar-Aguilar	-	Akin CNHE 8320	KC899994	-
A. Martínez-Aquino & R. Aguilar-Aguilar	-	Akin CNHE 8320	KC899995	-
A. Martínez-Aquino & R. Aguilar-Aguilar	-	Akin CNHE 8321	KC899996	KC899875
A. Martínez-Aquino & R. Aguilar-Aguilar	-	Akin CNHE 8321	KC899997	KC899876
A. Martínez-Aquino & R. Aguilar-Aguilar	-	Akin CNHE 8321	KC899998	KC899877
A. Martínez-Aquino & R. Aguilar-Aguilar	-	Akin CNHE 8321	KC899999	KC899878
A. Martínez-Aquino & R. Aguilar-Aguilar	-	Akin CNHE 8321	KC900000	KC899879
A. Martínez-Aquino & R. Aguilar-Aguilar	-	Akin CNHE 8321	KC900001	KC899880
A. Martínez-Aquino & R. Aguilar-Aguilar	-	Akin CNHE 8321	KC900002	KC899881
A. Martínez-Aquino & R. Aguilar-Aguilar	-	Akin CNHE 8321	KC900003	KC899882
A. Martínez-Aquino & R. Aguilar-Aguilar	-	Akin CNHE 8321	KC900004	KC899883
A. Martínez-Aquino & R. Aguilar-Aguilar	-	Akin CNHE 8321	KC900005	-
A. Martínez-Aquino & D.I.Hernández-Mena	Digital Voucher_223	Akin CNHE 8323	KC900010	KC899898
A. Martínez-Aquino & D.I.Hernández-Mena	-	Akin CNHE 8323	KC900011	KC899899
A. Martínez-Aquino & D.I.Hernández-Mena	-	Akin CNHE 8323	KC900012	KC899900
A. Martínez-Aquino & D.I.Hernández-Mena	-	Akin CNHE 8323	KC900013	KC899901
A. Martínez-Aquino & D.I.Hernández-Mena	-	Akin CNHE 8323	KC900014	KC899902
A. Martínez-Aquino & D.I.Hernández-Mena	-	Akin CNHE 8323	KC900015	KC899903
A. Martínez-Aquino & D.I.Hernández-Mena	-	Akin CNHE 8323	KC900016	KC899904
A. Martínez-Aquino & D.I.Hernández-Mena	-	Akin CNHE 8323	KC900017	KC899905

A. Martínez-Aquino & D.I.Hernández-Mena	-	Akin CNHE 8323	KC900018	KC899906
A. Martínez-Aquino & D.I.Hernández-Mena	-	Akin CNHE 8323	-	KC899907
A. Martínez-Aquino & D.I.Hernández-Mena	-	Akin CNHE 8323	-	KC899908
A. Martínez-Aquino & D.I.Hernández-Mena	-	Akin CNHE 8323	-	KC899909
A. Martínez-Aquino & D.I.Hernández-Mena	Digital Voucher_261	Akin CNHE 8324	KC900019	KC899910
A. Martínez-Aquino & D.I.Hernández-Mena	-	Akin CNHE 8324	KC900020	KC899911
A. Martínez-Aquino & D.I.Hernández-Mena	-	Akin CNHE 8324	KC900021	KC899912
A. Martínez-Aquino & D.I.Hernández-Mena	-	Akin CNHE 8324	KC900022	KC899913
A. Martínez-Aquino & D.I.Hernández-Mena	-	Akin CNHE 8324	KC900023	KC899914
A. Martínez-Aquino & D.I.Hernández-Mena	-	Akin CNHE 8324	-	KC899915
A. Martínez-Aquino & D.I.Hernández-Mena	-	Akin CNHE 8324	-	KC899916
A. Martínez-Aquino & D.I.Hernández-Mena	-	Akin CNHE 8324	-	KC899917
A. Martínez-Aquino & R. Aguilar-Aguilar	-	Akin CNHE 6880*	KC899977	KC899854
A. Martínez-Aquino & R. Aguilar-Aguilar	-	Akin CNHE 6880*	KC899978	KC899855
A. Martínez-Aquino & R. Aguilar-Aguilar	-	Akin CNHE 6880*	KC899979	KC899856
A. Martínez-Aquino & R. Aguilar-Aguilar	-	Akin CNHE 6880*	KC899980	KC899857
A. Martínez-Aquino & R. Aguilar-Aguilar	-	Akin CNHE 6880*	KC899981	KC899858
A. Martínez-Aquino & R. Aguilar-Aguilar	-	Akin CNHE 6880*	KC899982	KC899859
A. Martínez-Aquino & R. Aguilar-Aguilar	-	Akin CNHE 6880*	-	KC899860
A. Martínez-Aquino & R. Aguilar-Aguilar	-	Akin CNHE 6880*	-	KC899861
A. Martínez-Aquino & R. Aguilar-Aguilar	-	Akin CNHE 6880*	-	KC899862
A. Martínez-Aquino & R. Aguilar-Aguilar	-	Akin CNHE 6880*	-	KC899863
A. Martínez-Aquino, R. Aguilar-Aguilar & R. Rosas-Valdez	-	Akin CNHE 6498***	KC900071	KC899968
A. Martínez-Aquino, R. Aguilar-Aguilar & R. Rosas-Valdez	-	Akin CNHE 6498***	KC900072	KC899969
A. Martínez-Aquino, R. Aguilar-Aguilar & R. Rosas-Valdez	-	Akin CNHE 6498***	KC900073	KC899970
A. Martínez-Aquino & D.I.Hernández-Mena	Digital Voucher_480	-	KC900024	KC899918
A. Martínez-Aquino & D.I.Hernández-Mena	-	-	KC900025	KC899919
A. Martínez-Aquino & D.I.Hernández-Mena	-	-	KC900026	-
A. Martínez-Aquino & D.I.Hernández-Mena	Digital Voucher_208	Akin CNHE 8325	KC900027	KC899920
A. Martínez-Aquino & D.I.Hernández-Mena	Digital Voucher_214	Akin CNHE 8325	KC900028	KC899921
A. Martínez-Aquino & D.I.Hernández-Mena	Digital Voucher_222	Akin CNHE 8325	KC900029	KC899922

A. Martínez-Aquino & D.I.Hernández-Mena	-	Akin CNHE 8325	KC900030	KC899923
A. Martínez-Aquino & D.I.Hernández-Mena	-	Akin CNHE 8325	KC900031	KC899924
A. Martínez-Aquino & D.I.Hernández-Mena	-	Akin CNHE 8325	KC900032	KC899925
A. Martínez-Aquino & D.I.Hernández-Mena	-	Akin CNHE 8325	KC900033	KC899926
A. Martínez-Aquino & D.I.Hernández-Mena	-	Akin CNHE 8325	KC900034	KC899927
A. Martínez-Aquino & D.I.Hernández-Mena	-	Akin CNHE 8325	KC900035	-
A. Martínez-Aquino & D.I.Hernández-Mena	Digital Voucher_246_a	Akin CNHE 8326	KC900036	KC899928
A. Martínez-Aquino & D.I.Hernández-Mena	Digital Voucher_246_b	Akin CNHE 8326	KC900037	KC899929
A. Martínez-Aquino & D.I.Hernández-Mena	Digital Voucher_247	Akin CNHE 8326	KC900038	KC899930
A. Martínez-Aquino & D.I.Hernández-Mena	Digital Voucher_249	Akin CNHE 8326	KC900039	KC899931
A. Martínez-Aquino & D.I.Hernández-Mena	-	Akin CNHE 8326	-	KC899932
A. Martínez-Aquino & D.I.Hernández-Mena	-	Akin CNHE 8326	-	KC899933
A. Martínez-Aquino & D.I.Hernández-Mena	-	Akin CNHE 8326	-	KC899934
A. Martínez-Aquino & D.I.Hernández-Mena	-	Akin CNHE 8326	-	KC899935
A. Martínez-Aquino & D.I.Hernández-Mena	-	Akin CNHE 8326	-	KC899936
A. Martínez-Aquino & D.I.Hernández-Mena	-	Akin CNHE 8326	-	KC899937
A. Martínez-Aquino, R. Aguilar-Aguilar & R. Rosas-Valdez	-	Akin CNHE 8327	KC900066	KC899961
A. Martínez-Aquino, R. Aguilar-Aguilar & R. Rosas-Valdez	-	Akin CNHE 8327	KC900067	KC899962
A. Martínez-Aquino, R. Aguilar-Aguilar & R. Rosas-Valdez	-	Akin CNHE 8327	KC900068	KC899963
A. Martínez-Aquino, R. Aguilar-Aguilar & R. Rosas-Valdez	-	Akin CNHE 8327	KC900069	KC899964
A. Martínez-Aquino, R. Aguilar-Aguilar & R. Rosas-Valdez	-	Akin CNHE 8327	KC900070	KC899965
A. Martínez-Aquino, R. Aguilar-Aguilar & R. Rosas-Valdez	-	Akin CNHE 8327	-	KC899966
A. Martínez-Aquino, R. Aguilar-Aguilar & R. Rosas-Valdez	-	Akin CNHE 8327	-	KC899967
A. Martínez-Aquino & D.I.Hernández-Mena	-	Akin CNHE 8328	KC900040	KC899938
A. Martínez-Aquino & D.I.Hernández-Mena	-	Akin CNHE 8328	KC900041	KC899939
A. Martínez-Aquino & D.I.Hernández-Mena	-	Akin CNHE 8328	KC900042	KC899940
A. Martínez-Aquino & D.I.Hernández-Mena	-	Akin CNHE 8328	KC900043	KC899941
A. Martínez-Aquino & D.I.Hernández-Mena	-	Akin CNHE 8328	KC900044	KC899942
A. Martínez-Aquino & D.I.Hernández-Mena	-	Akin CNHE 8328	KC900045	KC899943
A. Martínez-Aquino & D.I.Hernández-Mena	-	Akin CNHE 8328	KC900046	KC899944
A. Martínez-Aquino & D.I.Hernández-Mena	-	Akin CNHE 8328	KC900047	KC899945

A. Martínez-Aquino & D.I.Hernández-Mena	-	Akin CNHE 8328	KC900048	KC899946
A. Martínez-Aquino & D.I.Hernández-Mena	-	Akin CNHE 8328	KC900049	KC899947
A. Martínez-Aquino, R. Aguilar-Aguilar & F.J. Alcantar	Digital Voucher_1269_21-25	Akin CNHE 8322	KC900006	KC899884
A. Martínez-Aquino, R. Aguilar-Aguilar & F.J. Alcantar	Digital Voucher_1269_26-29	Akin CNHE 8322	KC900007	KC899885
A. Martínez-Aquino, R. Aguilar-Aguilar & F.J. Alcantar	Digital Voucher_1269_11-20	Akin CNHE 8322	KC900008	KC899886
A. Martínez-Aquino, R. Aguilar-Aguilar & F.J. Alcantar	Digital Voucher_1277_30-31	Akin CNHE 8322	-	KC899887
A. Martínez-Aquino, R. Aguilar-Aguilar & F.J. Alcantar	Digital Voucher_1277_34-35	Akin CNHE 8322	-	KC899888
A. Martínez-Aquino, R. Aguilar-Aguilar & F.J. Alcantar	Digital Voucher_1280	Akin CNHE 8322	-	KC899889
A. Martínez-Aquino, R. Aguilar-Aguilar & F.J. Alcantar	-	Akin CNHE 8322	-	KC899890
A. Martínez-Aquino, R. Aguilar-Aguilar & F.J. Alcantar	-	Akin CNHE 8322	-	KC899891
A. Martínez-Aquino, R. Aguilar-Aguilar & F.J. Alcantar	-	Akin CNHE 8322	-	KC899892
A. Martínez-Aquino, R. Aguilar-Aguilar & F.J. Alcantar	-	Akin CNHE 8322	-	KC899893
A. Martínez-Aquino, R. Aguilar-Aguilar & F.J. Alcantar	-	Akin CNHE 8322	-	KC899894
A. Martínez-Aquino, R. Aguilar-Aguilar & F.J. Alcantar	-	Akin CNHE 8322	-	KC899895
A. Martínez-Aquino, R. Aguilar-Aguilar & F.J. Alcantar	-	Akin CNHE 8322	-	KC899896
A. Martínez-Aquino, R. Aguilar-Aguilar & R. Rosas-Valdez	-	Akin CNHE 8329	KC900060	KC899954
A. Martínez-Aquino, R. Aguilar-Aguilar & R. Rosas-Valdez	-	Akin CNHE 8329	KC900061	KC899955
A. Martínez-Aquino, R. Aguilar-Aguilar & R. Rosas-Valdez	-	Akin CNHE 8329	KC900062	KC899956
A. Martínez-Aquino, R. Aguilar-Aguilar & R. Rosas-Valdez	-	Akin CNHE 8329	KC900063	KC899957
A. Martínez-Aquino, R. Aguilar-Aguilar & R. Rosas-Valdez	-	Akin CNHE 8329	KC900064	KC899958
A. Martínez-Aquino, R. Aguilar-Aguilar & R. Rosas-Valdez	-	Akin CNHE 8329	KC900065	KC899959
A. Martínez-Aquino, R. Aguilar-Aguilar & R. Rosas-Valdez	-	Akin CNHE 8329	-	KC899960
A. Martínez-Aquino & D.I.Hernández-Mena	Digital Voucher_532	-	KC900050	KC899948
A. Martínez-Aquino & D.I.Hernández-Mena	Digital Voucher_541_a	Akin CNHE 8330	KC900051	KC899949
A. Martínez-Aquino & D.I.Hernández-Mena	Digital Voucher_541_b	Akin CNHE 8330	KC900052	KC899950
A. Martínez-Aquino & D.I.Hernández-Mena	Digital Voucher_181	Akin CNHE 7811**	KC900053	KC899951
A. Martínez-Aquino & D.I.Hernández-Mena	Digital Voucher_118	Akin CNHE 7812**	KC900054	KC899952

A. Martínez-Aquino & D.I.Hernández-Mena	Digital Voucher_121	Akin CNHE 7812**	KC900055	KC899953
A. Martínez-Aquino & D.I.Hernández-Mena	Digital Voucher_124	Akin CNHE 7812**	KC900056	-
A. Martínez-Aquino & D.I.Hernández-Mena	Digital Voucher_131.1	Akin CNHE 7812**	KC900057	-
A. Martínez-Aquino & D.I.Hernández-Mena	Digital Voucher_135	Akin CNHE 7812**	KC900058	-
A. Martínez-Aquino & D.I.Hernández-Mena	Digital Voucher_139	Akin CNHE 7812**	KC900059	-
R. Rosas-Valdez	-	Akin CNHE 8331	KC900009	KC899897
A. Choudhury			KC899972	KC899847
A. Choudhury, A. Martínez-Aquino, & G. Pérez-Ponce de León			KC899973	KC899848
A. Choudhury, A. Martínez-Aquino, & G. Pérez-Ponce de León			KC899974	KC899849
A. Choudhury, A. Martínez-Aquino, & G. Pérez-Ponce de León			-	KC899850
A. Choudhury, A. Martínez-Aquino, & G. Pérez-Ponce de León			KC899975	KC899851
A. Choudhury, A. Martínez-Aquino, & G. Pérez-Ponce de León			KC899976	KC899852
A. Choudhury, A. Martínez-Aquino, & G. Pérez-Ponce de León			-	KC899853

CAPÍTULO III

DOES THE HISTORICAL BIOGEOGRAPHY AND COPHYLOGENY OF THE DIGENEAN *MARGOTREMA* SPP. ACROSS CENTRAL MEXICO MIRRORS THAT OF THEIR FRESHWATER FISH HOSTS (GOODEINAE)?

Para concretar este proyecto, se planteo como objetivo integrar la historia evolutiva de *Margotrema* spp., influenciada por la evolución de Goodeinae y la geología del centro de México, a través de la identificación de patrones biogeográficos y cofilogenéticos. Para ello, se pusieron a prueba las hipótesis biogeográficas y cofilogenéticas detectadas con base en la reconstrucción genealógica de *Margotrema* propuestas por Martínez-Aquino *et al*. (2013), a través de análisis biogeográficos paramétricos (Ree & Sanmartín, 2009; Sanmartín, 2010; Ronquist & Sanmartín, 2011). Para evaluar hipótesis biogeográficas y cofilogenéticas considerando variables espacio-temporales, se realizó un análisis de *Species Tree multispecies coalescent* con el objetivo de estimar las edades de divergencia de los linajes de *Margotrema*, usando métodos de reloj molecular bayesianos (Drummond *et al*., 2006) calibrado a partir de eventos geológicos sobre la región occidental de la Faja Volcánica Transmexicana (FVT) (Ferrari *et al*., 1999; Mateos *et al*., 2002; Ferrari, 2004).

Los patrones de distribución geográfica de *Margotrema* fueron estudiados a través de análisis de DEC (por sus siglas en inglés *Dispersal-Extinction-Cladogenesis*; Ree *et al*., 2005; Ree & Smith, 2008), usando como unidades de estudio los sistemas hidrológicos previamente considerados para análisis biogeográficos de Goodeinae por Domínguez-Domínguez *et al*. (2010). Como primer paso, evaluamos la similitud de las áreas a utilizar a partir de los datos filogenéticos de *Margotrema* (Martínez-Aquino *et al*., 2013), usando métodos binarios bayesianos a través de la reconstrucción de estados ancestrales en filogenias (RASP, por sus siglas en inglés *Reconstruct Ancestral State in Phylogenies*; Nylander *et al*., 2008; Yu *et al*., 2010, 2011). Este análisis fue realizado para describir la concordancia biogeográfica entre las unidades de análisis hidrológicas tanto para peces como para sus digéneos parásitos. Propiamente, la reconstrucción de la biogeografía histórica de *Margotrema* se realizó con base en medios biogeográficos paramétricos tomando en cuenta tres variables: a) distancia geográfica; b) eventos geológicos; c) patrones y fechas de diversificación del ancestro común más reciente (MRCA, por sus siglas en inglés *Most Recent Common Ancestor*), para cada especie de huésped con respecto a cada una de las tribus de Goodeinae, es decir, Chapalichthyini, Characodontini, Girardinichthyini e Ilyodontini.

Como es posible realizar analogías entre los procesos biogeográficos y cofilogenéticos (Page, 1994a; Page & Charleston, 1998; Matzke, 2010), seleccionamos como unidades de análisis a las especies de goodeinos en los que están asociados los digéneos *Margotrema*, implementando un modelo de DEC. Para este análisis también consideramos a) la probabilidad de dispersión de las individuos de *Margotrema,* en función a la vagilidad y dispersión registrada para las especies explícitas de goodeinos a los que están actualmente asociados en cada una de las localidades muestreadas, y tomando en cuenta la distancia geográfica entre las áreas analizadas (sistemas hidrológicos); b) las edades de divergencia de cada especie / género de huésped, así como las relaciones genealógicas entre *Margotrema* y sus áreas de distribución. Se realizó una prueba estadística ("*p*") implementada en el programa TreeMap 3b (Charleston, 2013), para proveer de una medida de confianza (intervalo de confianza = 95%) a aquellos grupos pertenecientes a Goodeinae y *Margotrema* que reflejaban cierto grado de congruencia filogenética (correspondencia topológica entre dos o más arboles filogenéticos). Con base en los patrones biogeográficos y cofilogenéticos observados para *Margotrema*, se interpretó la historia evolutiva de este taxón para comprender como es el origen de los procesos de diversificación de los organismos parásitos en peces dulceacuícolas endémicos de México.

A continuación se presenta un manuscrito en formato de articulo en extenso intitulado "**Does the historical biogeography and evolutionary history of the digenean *Margotrema* spp. across central Mexico mirrors that of their freshwater fish hosts (Goodeinae)?"**, el cuál será sometido para su publicación en una revista arbitrada e indizada (ISI / SCI), referente a los patrones biogeográficos y cofilogenéticos de *Margotrema.*

Manuscrito para ser sometido para su publicación en la revista *Journal of Biogeography*

Original Article

Running header: *Margotrema* biogeography: cophylogeny and history

Does the historical biogeography and evolutionary history of the digenean *Margotrema* spp. across central Mexico mirrors that of their freshwater fish hosts (Goodeinae)?

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ABSTRACT

Aim To use recently published phylogenies of Goodeinae and those of their digenena parasite (*Margotrema* spp.) to uncover biogeographical and cophylogenetic patterns and to describe the processes that determined them over 6.5 Ma. The key questions addressed in this study is: Was the evolution of *Margotrema* spp. influenced by the complex geographical scenario of central Mexico, by the close association with their goodeinae hosts, or both?

Location Hydrological systems of central Mexico.

Methods A species tree for *Margotrema* spp. was obtained using DNA sequence data from two molecular markers, and a molecular dating looking for divergence events within the genus was performed. A dispersal-extinction-cladogenesis (DEC) model was used to describe the historical biogeography of digeneans. Meanwhile, DEC was used to uncover cophylogenetic events between *Margotrema* and their goodeines hosts.

Results This study shows a response of the parasite taxa in a geographical context, followed by establishing host specificity at three distinct levels of the historical association with their hosts: a) *Species-Species*, i.e., a historical association represented by *Xenotaenia resolanae-M. resolanae* exclusively at the Cuzalapa River Basin; b) *Species-Lineage*, i.e., a historical association represented by *Characodon audax-M. bravoae* Linaje III, exclusively distributed along the Upper and Middle Mezquital River Basin and c) *Tribe-Lineage*, i.e., two instances of historical associations among parasites and hosts at taxonomical level of Tribe, one represented by Ilyodontini-*M. bravoae* Lineage I, which are mainly distributed across the Ayuquila and Balsas River Basins, and the second represented by Girardinichthyini / Chapalichthyini-*M. bravoae* Lineage II, only distributed in the Lerma River Basin.

Main conclusions The parasite evolutionary history is congruent with the phylogenetic and biogeographical history and of their hosts, showing instances of coespeciation and several colonization events via host-switching and vicariant plus dispersal events at different times during the diversification history of both associates, related with tecto-volcanic events that occurred in the area.

Keywords

Codivergence, cospeciation, DEC model, divergence times, host switch, host-parasite evolution, lineage loss, Parametric biogeography, vicariance, dispersal.

INTRODUCTION

The evolutionary biology of host-parasite associations can be analogized to biogeographical processes in terms of events such as codivergence, duplication, horizontal transfer and lineage sorting (Page, 1994a; Page & Charleston, 1998; Matzke, 2010). The study of a host-parasite association focuses in the effects of geographical distribution in combination with the phylogenetic relationships of the hosts, on the evolution of the parasitic organisms. These two elements can be analysed by event-based biogeography methods (Ronquist & Sanmartín, 2011), as well as through evolutionary tangled trees of hosts and parasites (Page, 2003). Parametric biogeography methods (Ree *et al*., 2005; Ree & Smith, 2008, Sanmartín *et al*., 2008), coupled with dated species-tree estimation (Maddison, 1997; Edwards, 2009; Heled & Drummond, 2009), and contrasting the phylogenetic relationships between two groups (Charleston, 2011) with a narrow biological association in a particular geographical area, allow for hypotheses of evolutionary history and biogeography to be tested explicitly in time and space (see Sanmartin *et al*., 2008; Cowman & Bellwood, 2013; Ceccarelli & Zaldivar-Riverón, 2013).

Biogeographical reconstruction with parametric methods is implemented through models such as dispersal-extinction-cladogenesis (DEC) (Ree *et al*., 2005; Ree & Smith, 2008), a method that permit uncovering dispersal and extinction events along branches of a phylogeny, while estimating the ranges of the most recent common ancestor (MRCA) and descendent species at each node. DEC can be used to test hypotheses of origin of areas as well as geographical species within lineage formation scenarios, and dispersal (expansion routes), by incorporating both, historical and contemporary information for a particular region and the organisms associated to that region.

Previous studies have shown that geographical features may be influential in shaping genealogical relationships by causing a codifferentiation of parasitic organisms in relation to the evolutionary history of their hosts (Nieberding *et al*., 2008, Mizukoshi *et al*., 2012). However, it is difficult to find a biological model of parasitism capable to explain the process of diversification between hosts and parasites. The biogeographical "core" parasite fauna, i.e. species widely distributed and characteristically associated with and restricted to a monophyletic group of hosts species, in freshwater fishes across central Mexico (see Pérez-Ponce de León & Choudhury, 2005) offers a unique opportunity to test diversification processes between parasites and hosts, because they show high levels of host-specificity, are distributed in a restricted geographical area, and do not disperse naturally across water; therefore, there is no gene flow among individuals parasitising host species other than goodeines, or among goodeines occurring in different hydrological systems (Martínez-Aquino *et al*., 2013).

Additionally, central Mexico, and particularly the so-called Trans Mexican Volcanic Belt (TMVB), has been considered as a model of biodiversification, as it is a transitional area between the Neotropical and Nearctic biogeographical regions (Luna-Vega *et al*., 2005; Morrone, 2010; Luna-Vega & Contreras-Medina, 2012). The TMVB is considered an area of endemism for several taxa, as a result of a complex hydro-geomorphological history; particularly, this is a remarkable area of endemism for the freshwater fish fauna (Miller *et al*., 2005).

Among the endemic elements of the central Mexico freshwater fish fauna are the goodeines, a monophyletic group of cyprinodontiforms that experienced a diversification in the area (Domínguez-Domínguez *et al*., 2010 and references therein). On the other hand, the genus *Margotrema* represents the model of this study since their species are relatively common as parasites of goodeines across Mexico (Martínez-Aquino *et al*., 2013). Since the evolutionary and biogeographical history of goodeines in central Mexico was influenced by the tecto-volcanic activity and events such as river piracy (Parenti, 1981; Miller & Smith, 1986, Domínguez-Domínguez *et al*., 2010), Martínez-Aquino *et al*. (2013) recently demonstrated that the genealogical structure of *Margotrema* (i.e., *M. resolanae* and three independent genetic lineages of *M. bravoae*) shows a particular geographical distribution pattern across hydrological systems in central Mexico, and also that each lineage of *Margotrema* apparently shows some specific association with their hosts, at the goodeine tribe level, as monophyletic group. These results shed light on the hypothesis that the distribution patterns, as well as host associations of each lineage of *Margotrema*, would be concordant with the hydrogeomorphological events that occurred in central Mexico, and that the vicariant and dispersal events that caused the goodein diversification promoted at the same time the diversification of each *Margotrema* lineage. In this context, two general patterns in the evolution of *Margotrema* were uncovered: 1) Restricted geographical distribution in hydrological systems, i.e., *M. resolanae* is exclusively found in the Cuzalapa River Basin, meanwhile *M. bravoae* Lineage I is only found in the Ayuquila, Balsas and Conchos rivers, *M. bravoae* Lineage II is only found in the Lerma River, and *M. bravoae* Lineage III is exclusive to the Mezquital River. And the second pattern: 2) Host specificity at host-species and host-tribe level, e.g., *M. resolanae* is associated only to *Xenotaenia resolanae* (Tribe: Ilyodontini) while *M. bravoae* Lineage I is found in hosts belonging to Ilyodontini; *M. bravoae* Lineage II is associated to hosts of Girardinichthyini and Chapalichthyini, and finally, *M. bravoae* Lineage III is exclusive to *Characodon audax* (Tribe: Characodontini).

In the present study, biogeographical and phylogenetic analyses of goodeines, as well as the phylogenetic relationships of *Margotrema* spp. were explicitly used to explore the evolutionary process that may have driven the diversification of the host-parasite association, and to determine the potential role that geographical distribution had on the evolutionary history of both, goodeines and their digenean parasites. To accomplish that goal, phylogenetic reconstructions of both fish and parasites, along with novel probabilistic algorithms as a means of providing more objective outcomes, were used. This study was then designed to answer the following four questions: 1. What is the divergence time for each lineage of *Margotrema*? 2. Is there a biogeographical congruence between the genealogical history of *Margotrema* and the hydro-geomorphological history of central Mexico which is supported by the historical biogeography of their goodein hosts? 3. Is there a congruence (i.e., cophylogeny) between the evolutionary histories of the tribes of Goodeinae and those of *Margotrema*?, and finally, 4. Is there a congruence between the divergence time among the main clades of Goodeinae and each species (or Lineage) of *Margotrema*?

MATERIALS AND METHODS

Taxa, molecular dataset and phylogenetic analyses

The taxa used for phylogenetic analyses comprised samples of *Margotrema* spp. used already in a previously study; two molecular markers were sequenced, fragments of *COI* (mitochondrial DNA) and *ITS1* (nuclear DNA). Analyses included two outgroups (i.e. *Allocreadium lobatum* and *Walliniae chavarriae*). The software RASP version 2.1 (Nylander *et al*., 2008; Yu *et al*., 2010, 2011) was used for biogeographic analyses of the first phylogeny (see below). We used the same combined dataset (*COI* + *ITS1*) to perform a multispecies coalescent analysis as implemented in *BEAST (version 1.7.2, Drummond & Rambaut, 2007; Heled & Drummond, 2010), resulting in a *Species Tree* (ST) to infer the genealogical relationship between *M. resolanae* and the three lineages of *M. bravoae* (see Martínez-Aquino *et al*., 2013).

Divergence Dating

To propose an accurate time frame for phylogenetic divergence processes between the areas (localities) for each lineage of *Margotrema* spp. (see Appendix 1 in Supporting Information), we estimated mean node ages and their 95% highest posterior densities (HPDs) using a Bayesian relaxed molecular clock method (Drummond *et al*., 2006) as implemented in *BEAST v. 1.7.4 (Drummond *et al*., 2012). In this method, test of evolutionary hypotheses are not conditioned to a single tree topology, which allows for simultaneous evaluation of topology and divergence times while incorporating uncertainty in both. A uniform Yule tree prior was specified, as appropriate for hierarchical rather than reticulate relationships, and a sub-sampling of one representative specimen for every lineage was included to avoid over-representation of certain individual lineages. We applied the same optimal model as obtained by Martínez-Aquino *et al*., (2013), of data partitioning and DNA substitution, identified by BIC for using jModelTest 0.1.1. (Posada, 2008), for each gene *COI* with HKY+I+G and *ITS1* with HKY+G invariant sites in *BEAST package (BEAUti v 1.7.4; Drummond *et al*., 2012). An uncorrelated relaxed log normal molecular clock was applied to model rate variation across branches. Were introduced uniform prior distributions because of the lack of evidence to justify a specific distribution of rates in our data. Based on geographical distribution (localities where with published records) of *Margoterma* (Pérez-Ponce de León *et al*., 2007, 2009, 2013; Martínez-Aquino *et al*., 2007, 2009, 2011, 2012, 2013; Aguilar-Aguilar *et al*., 2010), a geological calibration was applied, based on the uplifting of the western part of the TMVB, which began around 11 million years ago (Ma) (Ferrari *et al*., 1999; Ferrari, 2004). This age was set, as a maximum for the MRCA of *Margotrema* spp. Monophyly was not enforced for any of the other nodes. Analyses were run for 50 million generations with a sampling frequency of one in every 1000 generations. Branch support for the different tree topologies was evaluated by Posterior Probability (PP) of the inferred relationships, where PP > 0.95 was considered to provide strong nodal support. The trees with the highest lineage PP were chosen from *BEAST output files using the program TreeAnnotator v.1.7.4 (Drummond *et al*., 2012). The phylogenetic divergence processes were run in the aforementioned programs in the Bioportal of the University of Oslo (http://www.bioportal.uio.no/).

Historical biogeography

To test the naturalness of 12 hydrological systems as a single biogeographical unit we explored – as a first step – the PP values of the ancestral areas of the main clades recovered within the lineages of *Margotrema* spp. using a Bayesian method for biogeographical and ancestral state reconstructions implemented in the program RASP version 2.1. Bayesian binary MCMC analyses performed on the last 10, 000 trees obtained from both MrBayes runs, were set to 10, 000, 000 cycles, coding the geographic distribution of the terminal taxa into 12 different areas following Domínguez-Domínguez *et al*. (2006, 2010), delimited based on the Mexican hydrological basins and sub-basins map produced by the Comisión Nacional para el Conocimiento y Uso de la Biodiversidad (CONABIO 1998; www.conabio.gob.mx). Furthermore, for this first analysis we used two plus areas correspond to distribution area of outgroups (see Appendix 1).

To uncover the events that influenced the historical biogeography and diversification processes (i.e. Ronquist & Sanmartín, 2011; Sanmartín, 2012) of *M. resolanae* and *M. bravoae* (the three lineages), we performed Dispersal-Extinction-Cladogenesis (DEC) analyses using the program Lagrange v. 20120508 (Ree & Smith, 2008) on the dated ultrametric tree of areas obtained from *BEAST (see below). Once we obtained the probability values from the Bayesian binary MCMC analyses (not show results), we selected a new set of hydrological systems and built a second matrix of eight areas (basins and sub-basin rivers), without outgroups, to tested a DEC model (see Appendix S2A-C). The input file for the analysis was constructed using the web-based *Lagrange configurator* (http://www.reelab.net/lagrange/configurator/index). The maximum range size for ancestral areas was set to two. Dispersal probabilities were constrained based on the following three factors. I) Geographical distance. II) Geological events, i.e. uplifting of the western part of the TMVB (6 – 10 Ma) (Ferrari *et al*., 1999; Ferrari, 2004), Tamazula failure (5 – 4.2 Ma) (Rosas-Elguera *et al*., 1996; Ferrari & Rosas-Elguera, 1999), connection / disconnection of Cuitzeo-Aztlán paleolakes (5.8 Ma) (Israde-Alcántara and Garduño-Monroy, 1999; Silva-Romo *et al*., 2002), main course of the ancient El Naranjo-Verde River (De Cserna & Álvarez, 1995), El Salto waterfall failure (1.5 Ma) (Albitron, 1958). III) Patterns and dates of diversification of the most recent common ancestor (MRCA) of particular taxa of each tribe of goodein hosts; i.e. *Xenotaenia resolanae* (5.1 Ma) and the genus *Allodontichthys* (5.6 Ma), both of the tribe Ilyodontini (6.9 Ma); *Neoophorus regalis* (6.9 Ma), of the tribe Girardinichthyni (8 – 7 Ma) and to *Characodon audax* (1.8 Ma) of the tribe Characodontini (15.5 Ma) (for more details see Domínguez-Domínguez *et al*., 2006, 2010).

A second DEC was carried out to detect the biogeographical events in several localities of *M. bravoae* Lineage III (Martínez-Aquino *et al*., 2013), in the area and particular timing of the plio-pleistocenic lakes from central Mexico (< 1 Ma). For this analysis, we built a third matrix of six areas (i.e. sub-basin) (Appendix S3A-B). The maximum range size for ancestral areas was set to two. Also, dispersal probabilities were constrained based on the three factors mentioned above; i.e. geographical distances, dates of connection / disconnection of Zacapu-Villa Morelos-Cuitzeo paleolakes (Plio-Pleistocene < 1 Ma) (Israde-Alcántara, 1999), patterns and dates of diversification of the MRCA of the tribes Girardinichthyini and Chapalichthyini, respectively (for more details see Domínguez-Domínguez *et al*., 2010).

Cophylogeny

To test the evolutionary associations, i.e., codivergence between goodein fishes and their digenean parasites, a DEC model using the software Lagrange was implemented. Trees involving the association of two taxa are used to infer their common evolutionary history, both in a geographic (area and organism trees) and in a cophylogenetic scenario (host and parasite) (Matzke, 2010). Since the biogeographical interpretations can be analogized to cophylogenetic ones, the following analogies, as study units, were used in accordance with Matzke (2010): area / host; organism / parasite, dispersal / host switch; vicariance / cospeciation; sympatric speciation / parasite speciation on one host; extinction / parasite extinction (lineage loss). Also, in this study the following terms were used following Charleston (2011): Codivergence, which also referred as cospeciation, implies an event where a parasite lineage (e.g., species) infecting a host lineage diverges into two new lineages at the same moment that its host does. Duplication is the event where the parasite lineage diverges into two new lineages, independently of its host, and both new lineages remain on that host lineage. Host switching is defined as the event where the parasite diverges by switching to establishing on another host lineage (Charleston, 2011); following Choudhury (2002), parasite speciation may either be concomitant with and resulting from host speciation (cospeciation) or follow the colonization of a ‘‘new’’ host from an existing one (host-switching).

In this context, to test when cophylogenetic events in a particular time and space occurred between association *Goodeinae-Margotrema* spp., we performed a DEC using the species' hosts as areas and the parasites as organisms distributed in the *areas*. For this analysis, we built a third matrix of 15 areas as a single biogeographical unit (host species) (Appendix S4A-B). The maximum range size for ancestral areas was set to two. The dispersal probabilities were constrained considering geographical distances, similar events as the ones for the geographical DEC analyses, plus divergence timing of genus / species of hosts (Appendix S4B) and the genealogical relationships between *Margotrema* spp. and their geographical areas.

Additionally, to determine whether there is a significant match between host and parasite trees, the phylogenies of the subfamily Goodeinae and *Margotrema* were compared using a statistic test of *p* values with a 95% of confidence intervals through TreeMap 3b (Charleston, 2011). We employed the tree topologies obtained from the cytochrome b gene sequence from the hosts and *COI+ITS1* sequences from four lineages of *Margotrema* spp. The consensus trees from the Bayesian Inference analyses (dated with Maximum Likelihood approach) of Goodeinae (see Domínguez-Domínguez *et al.*, 2010), were edited through the program Mesquite Modular System for Evolutionary Analysis (MESQUITE) version 2.72 (Maddison & Madisson, 2011) to select the terminal taxa that can be found in a host-parasite association with *Margotrema*, and to remove the remaining terminal taxa from the tree. Similarly, the dated molecular phylogeny of *Margotrema* spp. was also edited in MESQUITE, in that samples of each locality were trimmed to a single terminal taxon. This was done because TreeMap 3b only reconciles strictly dichotomous trees.

RESULTS

Divergence times

The divergence time estimates, based on the tree reconstructed using *BEAST, for the MRCA of *M. resolanae* + *M. bravoae* clade was 6.53 Ma. The divergence time between the ancestor of Lineage I and the ancestor of lineages II and III was dated 3.20 Ma, and finally, lineages II and III diverged 1.03 Ma. (Appendix S5).

Historical Biogeography

The results of the reconstruction of ancestral areas through the Bayesian MCMC algorithm in RASP, considering the same dataset for the main lineages of *Margotrema* spp. uncovered eigth of the 18 river sub-basins across central Mexico (not show results), as defined by Domínguez-Domínguez *et al*. (2006, 2010). The same areas were used for carrying out the DEC analysis.

We used two distinct matrices of areas to test the biogeographical events of the Lineages of *Margotrema* spp. in each locality. The most clear-cut summary of the data of the geographical distribution of each *Margotrema* lineage (Fig. 1), was recovered by analysis of the combined dataset matrix of DEC to 0 – 6.5 Ma (i.e. Appendix S2C) and DEC to 0 – 1 Ma (i.e. Appendix S3C) and is presented next.

The analysis in Lagrange show that the MRCAs of *M. resolanae* and *M. bravoae* were distributed in a geographical range which apparently spanned the North-Western Central in Cuzalapa River, Upper and Middle Mezquital River and Armería-Ayuquila Rivers (areas I, B and H, respectively). The first diversification event occurred when this MRCA was isolated by a vicariant event in the Cuzalapa River, from the ancestor of *M. bravoae* (B, H) producing an allopatric speciation for *M. resolanae* in this river (Fig. 1a). Once in areas B and H, the MRCA of the three lineages of *M. bravoae* underwent dispersion to the Cotija area [J] and the Lower Balsas River [K], and apparently it was lost in the Armería-Ayuquila River [H]. This MRCA, through a vicariant event, experienced cladogenesis separating the MRCA of Lineage I (in Area K) from the MRCA of Lineages II and III in areas B and J (Fig. 1a).

Margotrema bravoae Lineage I

The MCRA of Lineage I (distributed in the Lower Balsas River, K) exhibited two dispersal events, in one, the ancestor dispersed back into the Armería and Ayuquila Rivers (H), and in the second, to a tributary of Conchos River (A). A vicariant event then separated areas H and A from K and finally, the areas H and A, and K and L (Upper Balsas River) were separated by vicariant events, respectively (Fig. 1b).

Margotrema bravoae Lineage II & III

Once in areas B and J, the MRCA of Lineages II and III dispersed into Lower Lerma River (C). Later, a vicariant event separated the Upper and Middle Mezquital River (B) from the Lower Lerma River (C) + Cotija (J) (Fig. 1a). Lineage III of *M. bravoae* is exclusively found in the Mezquital River (B).

Margotrema bravoae Lineage II

The MRCA of *M. bravoae* Lineage II distributed in C and J (Fig. 1a) deserved further consideration, since it experienced several vicariant and dispersal events. This ancestor dispersed to Cuitzeo Lake (E) and Zirahuén Lake (G) (Fig. 1c). A vicariant event separated Cotija (J) from the other areas of the Lerme River (C, E, and G). A posterior vicariant event isolated Zirahuén Lake (G) from Cuitzeo Lake and Lower Lerma River which were subsequently separated by another vicariant event. A population from Zirahuén Lake (G) dispersed into Zacapu Lake (D) and Pátzcuaro Lake (F) and later, these areas were geographically separated.

Cophylogenetic patterns

A DEC analysis was performed using all host species – areas – of *Margotrema* in all recorded localities in our phylogenetic study (Fig. 2). These analyses show that the MRCA of *M. resolanae* and *M. bravoae* was associated to *Allodontichthys zonistius* (g) and *Codoma ornata* (h), giving rise to a lineage duplication in one host (i.e. into "g") (akin to a allopatric speciation event) and a posterior cospeciation in *Xenotaenia resolanae* (m), plus lineage loss in *A. zonistius* because to extinction of this host (Fig. 2a). The MRCA of the three lineages of *M. bravoae sensu lato* (Martínez-Aquino *et al*., 2013) was associated to *A. zonistius* and *C. ornata* since 6.53 – 3.20 Ma (Fig. 2a). In total, in the three lineages of *M. bravoae* was found five events of duplication following of host-switching + lineage loss, and only one more without lineage loss; plus six of codivergence events (Fig. 2).

Margotrema bravoae Lineage I

Particularly, the MRCA of the *M. bravoae* Lineage I was associated to *A. zonistius* (g) + *C. ornata* (h) (Fig. 1a), and posteriorly, colonised several host species belonging to the Ilyodontini (Fig. 2b). For example the parasite associated with *A. zonistius* experienced a host switching event into *Ilyodon furcidens* (k) (in Armería Ayuquila Rivers [H]) (Fig. 1, 2), followed by dispersal into *I. whitei* (l) (hypothetically, in a same area as Lower and Upper Balsas Rivers [K] and [L]). After, a codivergence event separated the populations associated to *I. furcidens* (k) (in the Lower Balsas River [K]) and *I. whitei* (l) (in the Upper Balsas River [K]). Finally, *M. bravoae* Lineage I occurring in the Lower Balsas River (K) (Fig. 1b) experienced a host switching event into *Chapalichthys pardalis* (j) with both hosts occurring in sympatry (Fig. 2b).

Margotrema bravoae Lineages II & III

The possible differentiation of the MRCA of Lineages II and III of *M. bravoae* was due to a host switching event that separated Lineage II from III. Previously, DEC uncovers a dispersal event of the ancestor into *Characodon audax*, and a concomitant vicariant event separated the populations of *C. audax* in the Mezquital River from the other ancestor that colonised Chapalychthyini and Girardinychthyini in the Lerma River (Fig. 2a, 2c).

Margotrema bravoae Lineage II

Particularly, in Lineage II, the ancestor experienced colonisation via host switching into *Neoophorus regalis* (d) and *Zoogoneticus quitzeoensis* (n) in the Lerma River Basin (Fig. 2c). Posteriorly, other host switching events occurred once the ancestor was in that particular geographical area. For instance a host switching event allowed the colonisation of *Alloophorus robustus* (e), while another five host switching events allowed the colonisation of *Allotoca meeki* (c), *A. duguesi* (b), *A. diazi* (a), and *A. zacapuensis* (f) (Fig. 1c).

Finally, cophylogenetic results show three general patterns bewteen the two associated taxa, through a reconciliation phylogenetic analysis using TreeMap 3b. These patterns are shown in the form of a Tanglegram, were the topology of the phylogenetic trees of both groups can be compared (Fig. 3). The resulting three shows all three levels of host-parasite association between *Goodeinae* and *Margotrema*: Level 1 is *Species-Species*, interpreted as *cospeciation*; Level 2 is *Species-Lineage,* interpreted as *Codivergence Type I,* when the host experience a speciation process and a parasite lineage colonised posteriorly reaching some level of intraspecific genetic divergence; and Level 3 is *Tribe-Lineage,* interpreted as *Codivergence Type II,* when divergence occurs at deeper level of the phylogenetic history of the hosts (tribes) and parasites colonised these hosts acquiring some level of intraspecific genetic divergence (Fig. 3).

DISCUSSION

Most Recent Common Ancestor of the genus *Margotrema*

The divergence date of the most recent common ancestor (MRCA) of the genus *Margotrema* was estimated in 6.53 Ma, and apparently it was distributed in northern Mexico in the Upper and Middle Mezquital and in the Cuzalapa and Armería-Ayuquila Rivers (Fig. 4a). This MRCA was associated with *A. zonistius* (Goodeinae) and *C. ornata* (Cyprinidae) (Fig. 2a). Recent reports on the presence of *Margotrema* spp. in hydrological systems of Northern Mexico parasitising freshwater fish species belonging to the Cyprinodontidae and Cyprinidae are strong evidence of the possibility that these parasites colonised goodeids from other freshwater fish groups, either from the Nearctic cyprinids or the Neotropical cyprinodontids. For instance, records of *Margotrema* spp, albeit with low relative prevalence and abundance values, have been established for *Cyprinodon nazas* (Cyprinodontidae) in the Nazas River Basin, and more frequently, in the following cyprinids: *C. ornata* in the Piaxtla River Basin and *Cyprinella lutrensis* in the Santa María River Basin (Pérez-Ponce de León *et al*., 2009, 2013; Aguilar-Aguilar *et al*., 2010) (Fig. 4a, Appendix 6).

Based on the recovered ancestral area through the analyses conducted in this study, some support is provided for the Nearctic affinity of this digenean, as previously suggested by Pérez-Ponce de León *et al*. (2007) and Curran *et al*. (2011), even though these authors found a close sister group relationship between *Margotrema* and *Crepidostomum*, both members of the Allocreadiidae, with the latter being a common parasite of centrarchids. The divergence date, ancestral associations and distribution of *Margotrema,* in addition to the biogeographical and cophylogenetic patterns uncovered by the DEC-model, allows us to propose that the ancestral area of the MRCA extended across hydrological systems currently occupied by the Sierra Madre Occidental (SMOc), and western regions of the Altiplano Mexicano (AM) (biogeographical provinces *sensu lato*, Morrone, 2005) (Fig. 4a).

In terms of host-association, two competing historical scenarios could have occurred:

a) *Goodeidae-Margotrema*. This scenario is explained by an ancestral relationship between *Margotrema* and goodeines ocurring in northern Mexico. The ancestral area of *Margotrema*, as shown in this study, is similar to that of the MRCA´s for Goodeinae (divergence dated as 15.5 to 8 Ma, see Domínguez-Domínguez *et al*., 2010). A goodeid fossil (*Empetrichthys erdisi*) was found in the Yaqui River, Sonora, in Northwestern Mexico, a locality close to the area where goodeids are currently distributed (Uyeno & Miller, 1962; Parenti, 1981; Minckley *et al*., 1986). The sister group of Goodeinae is Empetrichthyinae, and the diversification event that gave rise to both subfamilies is recognized to have occurred in the Great Basin in southwestern USA (Parenti, 1981). The result of the overlap on the distribution range of both ancestors was the establishment of a close evolutionary relationship with strong host-specificity, and it was latter dispersed into other goodeines that inherited the association by vertical transmission (Rannala. Michalakis, 2003), even though goodeines experienced a diversification in river basins of northern Mexico, and more intensively in central Mexico. An important question that remain unresolved is where the goodeids inherited the ancestral *Margotrema* from, since no records of the helminth fauna are available for empethrychthyines, and no record of an allocreadiid like *Margotrema* has been established for other cyprinodontiforms.

b) *Cyprinidae-Margotrema*. The ancestral association of *Margotrema* with the cyprinid *C. ornata*, as shown in this study, could have been established in hydrological systems of northern Mexico at 6.5 Ma (Figs. 1a, 2a). The implication of this scenario is that goodeines began the association with the ancestor of *Margotrema* in northern areas of Mexico, previous to the diversification, as a result of a host sharing (ecological host extension) event from other freshwater fish groups, such as cyprinids, with a subsequent specialization in goodeines, where the digenean developed a strong host specificity. We recently collected a few specimens of *Margotrema* in a species of cyprinid in three river basins of northern Mexico (Santa María River, Conchos River and Pagigóchic River, unpublished data) and clearly this may reflect the shadow of the ancestral distribution of extinct goodeids in northern hydrological systems, where cyprinids are common and very abundant (Fig. 4a). The same results of low prevalence and abundance values of *Margotrema* in cyprinids from Chihuahua (and even in *Cyprinodon* from Durango) may indicate the transfer of the ancestor between cyprinids and goodeids. Considering that phylogenetic hypothesis of allocreadiids place *Margotrema* as the sister taxa of *Crepidostomum* (a genera predominantly parasitic in centrarchids, but also found in other freshwater fish groups such as cyprinids, see Hoffman, 1999), it is likely that the origin of *Margotrema* is the result of a host sharing event from centrarchids to cyprinids, and from there to goodeines, or from centrarchids to goodeines. Once in goodeines, *Margotrema* experienced events such as cospeciation and several instances of host switching. Clearly, the diversification rate of the parasite was slower that that of their host, an idea originally proposed by Manter (1966).

It is noteworthy that distinct aquatic taxa currently distributed in central Mexico possessing a Nearctic biogeographical affinity, show an ancestral distribution pattern similar to that proposed in this study for the MRCA of *Margotrema*, including Cyprinidae (Chernoff & Miller 1986: Schönhuth *et al*., 2008: Pérez-Rodríguez *et al*., 2009); and crayfish of the subfamily Cambarellinae (Pedraza-Lara *et al*., 2012). Clearly, future collections of specimens of *Margotrema* in other host species across northern Mexico, and also survey work for helminths in Empetrychthynae in Southwestern USA will be instrumental in providing further support for one of the competing scenarios.

***Xenotaenia resolanae*-*Margotrema resolanae*: an allopatric cospeciation model**

According to our results, the first cladogenetic event during the evolutionary history of *Margotrema* was the separation of *M. resolanae* from the ancestor of *M. bravoae* an event dated 6.53 Ma. The restricted distribution range of both species seem to be the result of an allopatric speciation by peripheral isolates, where populations of both, hosts and parasites, were isolated at the edge of the distributional range of the MRCA, in the Cuzalapa River Basin, resulting in a strong host-specificity pattern of association illustrating a classical model of cospeciation reflecting reciprocal selection, i.e., coevolution. This hypothesis is supported by the divergence date of the MRCA of *A. zonistius* (6.9 Ma) and the posterior diversification process of *X. resolanae*, exclusive to the Purificación-Mascota River basin (between 5.6 to 5.1 Ma) (see Domínguez-Domínguez *et al*., 2010). Unfortunately, geological information of this region is scarce and only the volcanic activity of the Talpa-Mascota graben dated ca. 4.6 Ma has been documented (Carmichael *et al*., 1996; Bandy *et al*., 2001).

Patterns of regional codivergence of *Goodeinae-Margotrema bravoae* across central Mexico

Most Recent Common Ancestor of Margotrema bravoae

The MRCA of *M. bravoae*, after the split from *M. resolanae* underwent phylogenetic codivergence through a dispersal process in areas such as Upper and Middle Mezquital River, Cotija and the Lower Balsas River (Fig. 4b) and subsequently, this ancestor experienced a secondary lineage loss in goodeines of the the Armería and Ayuquila Rivers. Interestingly, the pattern of regional codivergence seems to be closely tied to the evolutionary history of Goodeinae regarding the formation of what currently represents each tribe of the subfamily, i.e., Chapalichthyini, Characodontini, Girardinichthyni and Ilyodontini (Domínguez-Domínguez *et al*., 2010).

The historical biogeography of the Goodeinae, as described by Domínguez-Domínguez *et al*., (2006, 2010), was instrumental to explain the diversification process of the parasite fauna in this complex area. For instance, the erosion of the river tributaries from the Pacific and Atlantic slopes (i.e., Balsas, Ameca, and Pánuco River Basins) promoted the capture of water bodies of the Central Altiplano, with the subsequent dispersal of species that were characteristic of that region. Apparently, the Tamazula fault and the Sayula graben (dated 5 – 4.2 Ma), are two of the main causes that promoted the diversification of the MRCA´s of the each Tribe of goodeines by a series of vicariance and dispersal events represented by isolation processess, and connection-disconnection of river basins with ancestral paleolakes such as Cuitzeo-Aztlán (dated 6 – 3.2 Ma) (Smith *et al*., 1975; Rosas-Elguera *et al*., 1996; Ferrari & Rosas-Elguera, 1999; Domínguez-Domínguez *et al*., 2006, 2010). In this context, the differentiation process of each of the three lineages of *M. bravoae* resulted from geological events that changed the geographical configuration of the area and, apparently, with a posterior diversification of their hosts. Next, we describe the hypothetical scenarios that explain the evolutionary history of the three *M. bravoae* lineages.

Ilyodontini-Margotrema bravoae Lineage I

The differentiation process of the MRCA of *M. bravoae* Lineage I was dated 3.2 Ma. A geographical barrier caused the split the ancestral area of the MRCA of *M. bravoae* (Upper and Middle Mezquital River, Cotija and Lower Balsas River), and what currently represents Lineage I was isolated in the Lower Balsas River. The origin of *M. bravoae* Lineage I is apparently associated with the isolation and posterior diversification of the MRCA of *Allodontichthys* (dated 3.6 to 2.9 Ma) in the Ameca, Ayuquila-Armería, and Coahuayana-Tamazula River Basins (see Domínguez-Domínguez *et al*., 2010). The entire tribe Ilyodontini diversified in these river basins, and Lineage I of *M. bravoae* codiversified through a horizontal transmission pattern (see Rannala & Michalakis, 2003) into other illiodontins, in addition to a member of the Chapalichthyini inhabitant of the same river drainages. According with Domínguez-Domínguez *et al*. (2010) the MRCA of *Allodontichthys* and *Ilyodon* (dated 5.6 Ma), dispersed from the Ayuquila-Armería river into several hydrological systems in central Mexico such as Ameca, Balsas, Coahuayana-Tamazula, Armería-Ayuquila and Purification-Mascota river basins. Previous published records of *Margotrema* (see Mejía-Madrid *et al*., 2005) in ilyodontins in the Coahuayana River Basin (in *Allodonthichthys hubbsi* in El Tule, Jalisco, and *A. tamazulae* in Río Tamazula, Jalisco, are congruent with the hypotheses of area expansion of *M. bravoae* Lineage I related with the diversification process of the Tribe Ilyodontini. Similarly, these records also support the ancestral connection between the Armería-Ayuquila and Coahuayana hydrological systems where the MRCA of the Ilyodontini was distributed (Webb, 2002; Domínguez-Domínguez *et al*., 2010).

The evolutionary history and divergence date of *M. bravoae* Lineage I (3.2 Ma), is congruent with the hypothesis of the origin of the diversification of the genus *Allodontichthys* (3.6 – 2.9 Ma) influenced by vicariance and dispersal events (Domínguez-Domínguez *et al*., 2010) associated with geological events that shaped the biogeographical history of several freshwater fish taxa, i.e., the uprising of the Sierras de Manantlán and Cacoma, the volcanic activity of the Talpa-Mascota graben (dated 3.6 Ma), and the reactivation of the Colima and Tamazula graben in the Pliocene (Allan, 1986; Carmichael *et al*., 1996; Garduño-Monroy *et al*., 1998; Mateos *et al*., 2002; Pérez-Rodríguez *et al*., 2009; Domínguez-Domínguez *et al*., 2010).

Girardinichthyini / Chapalichthyini-Margotrema bravoae Lineage II: a pleistocenic model?

The genealogical differentiation of the MRCA of *M. bravoae* Lineages II and III that occurred between 3.2 and 1.03 Ma presumably in the Transmexican Volcanic Belt, particularly in the

Lower Lerma River and Cotija (Fig. 4c), is related with the patterns of dispersal and isolation of the MRCAs of the goodeids *Zoogoneticus quitzeoensis* and *Allotoca zacapuensis* in Cuitzeo and Zacapu Lakes dated at 3.3 – 2.8 Ma, and the divergence date and expansion process of *Z. purhepechus* into the river basins of the Lower Lerma and the Ameca Rivers as well as Chapala Lake (2.8 Ma and 2 – 1.9 Ma, respectively) (Domínguez-Domínguez *et al*., 2006, 2007c, 2008, 2010).

The evolutionary history of Lineage II occurred between 1 and 0.0167 Ma (Appendix S5), which corresponds with events that took place during the Pleistocene, and because of that, a DEC model was specifically conducted to describe the separation events for this lineage. (Appendix S3). The results of the DEC model, support the idea of the dispersion of *M. bravoae* Lineage II during Pleistocene across hydrological systems of the Lower Lerma River, the Cuitzeo Lake and the Zirahuén Lake, and apparently the dispersion and vicariant events occurred in concordance with the diversification processes of their hosts. Two area relationships are recovered from this model, the first one between the Zacapu, Pátzcuaro and Zirahuén Lakes which further support the idea of ancestral connections between these water bodies (see Domínguez-Domínguez *et al*., 2006, 2010) (Fig. 4e), and that of Cuitzeo Lake and the Lower Lerma River which explains the ancestral connection of this river basin. These area relationships are also supported by the current distribution patterns of several freshwater fish taxa, an idea originally proposed by Alvarez del Villar (1972) and more recently by particular phylogenetic and biogeographical analyses (Domínguez-Domínguez *et al*., 2007a; 2010; Pérez-Rodríguez *et al*., 2009).

Characodon audax-Margotrema bravoae Lineage III

Our results indicate that the MCRA of *M. bravoae* Lineages II and III was associated with the ancestor of the Characodontini in the Upper and Middle Mezquital Rivers, Cotija and Lower Lerma River, an event dated between 3.2 and 1.03 Ma. The posterior isolation caused the codiversification of Lineage III with *Ch. audax*. The presence the ancestor of the Characodontini in Cotija and the Lower Lerma River is incongruent with the distribution patterns established for the genus *Characodon*, which occupies a basal position in the phylogeny of the Goodeinae (Domínguez-Domínguez *et al*., 2010). Even tough, the phylogenetic history of goodeines shows that the vicariant event that caused the allopatric speciation of *Ch. audax* and *Ch. lateralis* dated 1.8 Ma was the formation of the Salto waterfall (Domínguez-Domínguez *et al*., 2006, 2010) (Fig.

4d), however, no records of *Margotrema* spp. have been established for *C. lateralis*, even though several surveys have been conducted in the area (Mejía-Madrid et al., 2005; Martínez-Aquino et al., 2007; Pérez-Ponce de León et al., 2009). Apparently, the codiverence process detected in this study, related with the association of *Ch. audax* and *M. bravoae* Lineage III (exclusive to the Upper and Middle Mezquital River) can be explained by an extinction event of non-viable ancestral populations of the Lineage III in the Lower Mezquital River, following the event of cladogenesis in the host caused by the Salto waterfall.

The results of this study show that the diversification process of the genus *Margotrema* is the result of a combination of geography and host specificity at three distinct levels: a) *Species-Species* (cospeciation), b) *Species-Lineage* (Codivergence Type I); c) *Tribe-Lineage* (Codivergence Type II) (Fig. 3). In this context, the allopatric cospeciation process proposed between *X. resolanae-M. resolanae* is a classic model of cospeciation of type *one by one* (Ehrlich and Raven, 1969). On the other hand, the cophylogenetic process we uncovered between *Goodeinae* and *Margotrema bravoae* (Lineages I, II and III), support Manter´s parasitological rule in that "*the parasites evolve more slowly than their hosts"* (Brooks, 1979). As a result, a general pattern we discovered is that parasites are relatively younger than their hosts, i.e., divergence times do not coincide for parasites and their hosts. For example, divergence date of *C. audax* is dated 1.5 Ma, meanwhile for *M. bravoae* Lineage III it was estimated in 1.03 Ma. Nevertheless, the coevolutionary scenary follows a geographical mosaic in which the populations differ in their characteristics and specializations with respect to the species with which they interact (see Thompson, 2005). Therefore, the patterns of codivergence uncovered for the Goodeinae-*M. bravoae* association occur at three aforementioned levels, and are thus congruent with the geographical mosaic theory.

CONCLUSION

This study provides empirical evidence that demonstrates that the historical biogeography and evolutionary history of the digenean *Margotrema* spp. across central Mexico mirrors that of their goodein freshwater fish hosts. Evidence of coespeciation and a series of vicariant and dispersal events were found as the main causes that explain the codivergence patterns, first between *Margotrema resolanae* and *Xenotaenia resolanae*, second between *Margotrema bravoae* Lineages I, II (codivergence type I at goodein tribe level), and finally, Lineage III (codivergence type II at goodein species level). These results clearly show a cophylogenetic pattern for this host-parasite association, even though these taxa show independent evolutionary histories. Divergence times for each host and parasite lineage are relatively congruent and demonstrate the concordance of the evolutionary and biogeographical history between the two taxa, both in time and space. In this context, the geographical scenario molded by the complex geological and climatic history of the region, is the most important determinant that drives the evolution of the digenean, followed by the cophylogenetic association that this parasite established with their goodein hosts.

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FIGURE LEGENDS

Figure 1 Biogeographical events of the evolution of *Margotrema* spp. from Central Mexico associated to Goodeinae; (a) shows a general pattern of the evolution of *M. resolanae* and of each of the Lineages of *M. bravoae*; (b) shows the specific events that influenced the evolution of *M. bravoae* Lineage I; (c) shows the specific events that influenced the evolution of *M. bravoae* Lineage II.

Figure 2 Host-parasite events of the evolution of *Margotrema* spp. from Central Mexico associated to Goodeinae; (a) shows a general pattern of the evolution of *M. resolanae* and of each of the Lineages of *M. bravoae*; (b) shows the specific events that influenced the evolution of *M. bravoae* Lineage I; (c) shows the specific events that influenced the evolution of *M. bravoae* Lineage II.

Figure 3 Tanglegram show the levels three of parasitic associations between *Goodeinae-Margotrema*. Level 1 (L1-L1, green = *Species-Species*; represent with the association *Xenotaenia resolanae-M. resolanae*). Level 2 (L2-L2, red = *Species-Lineage*; represent with the association *Characodon audax-M. bravoae* Lineage III), Level 3 (L3-L3, blue = *Tribe-Lineage*; represent with the associations between Ilyodontini-*M. bravoae* Lineage I and Girardinichthyini / Chapalichthyini-*M. bravoae* Lineage II). Colours patterns in the curvigram of Goodeinae correspond to each tribe; i.e. blue = Girardinichthyini; green = Chapalichthyini; yellow = Ilyodontini; red = Characodontini. Colours patterns in the curvigram of *Margotrema* correspond to each independent evolutionary lineage; i.e. yellow = *M. bravoae* Lineage I; blue = *M. bravoae* Lineage II; red = *M. bravoae* Lineage III. The gray polygons under the curvigrams correspond to specific lineage of Goodeinae and *M. bravoae*, respectively. The capital letters correspond to the each locality where specimens of *Margotrema* were collected, for details see Appendix S1.

Figure 4 Schematic description of the areas and dispersal routes of generating alternative biogeographic / cophylogenetic hypotheses using dispersal-extinction-model (DEC) analysis of *Margotrema* spp., in function an evolutionary time and hydrogeomorphology historical from Central Mexico and Goodeinae. (a) Ancestral area, green shadow, of *Margotrema* genus detected based on ours results. The red shadow represents an ancestral hypothetical area potential associate to north records from Mexico of *Margotrema* and the distribution area of fossil records of species of Goodeidae. (b) Fragmentation of the ancestral area of most recent common ancestral (MRCA) of *Margotrema* spp. represent by two green shadow. Diversification process of the MRCA of the tribe Ilyodontini and *Margotrema*, respectively, influence for events of vicariance-dispersal on Cuzalapa River (I) and Armería Ayuquila River (H). (c) Geographic scenery of the diversification of *M. bravoae* Lineage I [L1] and *M. bravoae* Lineage II [L2] associate to Goodeinae tribes, based on hypothetical expansion routes. Yellow arrows due reference to dispersal of L1 influence by the dispersal of the Ilyodontini [I]; blue arrows due reference to dispersal of L2 influence by the dispersal of the Chapalichthyini [Ch] and Girardinichthyini [G]. (d) Geographic scenery of the diversification of *M. bravoae* Lineage III associate to a vicariant event (El Salto waterfall) that caused the diversification of *Characodon audax*. Red shadow covers the hypothetical area of the pleistocenic paleolakes. (e) Hypothetical area of the pleistocenic paleolakes when is distributed *M. bravoae* Lineage II; the orange, blue and green shadows correspond to phylogenetic relationships of areas. Polygons in intensity different of gray correspond to biogeographic provinces (*sensu lato* Morrone, 2005): SMOc = Sierra Madre Occidental; AM = Altiplano Mexicano; MTVB = Mexican Transvolcanic Belt; BRB = Balsas River Basin; hydrological systems in green and yellow correspond to the records of *Margotrema* collected and no collected in this study, respectively; filled circles green with a capital letter correspond to localities analysed in this study; filled circles red together with the number, indicate *Margotrema* records not collected in this study, for details see Appendix S1 and S6.

SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

Appendix S1 Table with the areas and area codes, localities and locality codes and parasite species and terminal codes used in the RASP analyses (MCMC), Dispersal-Extinction-Cladogenesis (DEC) analyses and divergence date of *Margotrema* spp. Also show each area, locality and terminal lineage associate to host species.

Appendix S2 Supplementary tables and figures of DEC analyses, Modelo I, general geographic areas.

Appendix S3 Supplementary tables and figures of DEC analyses, Modelo II: pleistocenic events.

Appendix S4 Dispersal matrix used for Lagrange, Modelo III: cophylogenetical events.

Appendix S5 Divergence date of *Margotrema* spp.

Appendix S6. Additional *Margotrema* records in freshwater fish species from Mexico.

BIOSKETCH

Andrés Martínez-Aquino is interested in parasite evolutionary biology using molecular and distributional data analysis, with a particular focus on helminth parasite taxa of wildlife.

Author contributions: All authors conceived and discussed the ideas. A.M-A. and F.S.C. performed the analyses. L.E.E., E.V-D. and F.S.C. contributed to data interpretation. A.M-A. and G.P-PL led the writing. All authors contributed to led the writing.

Figure 1

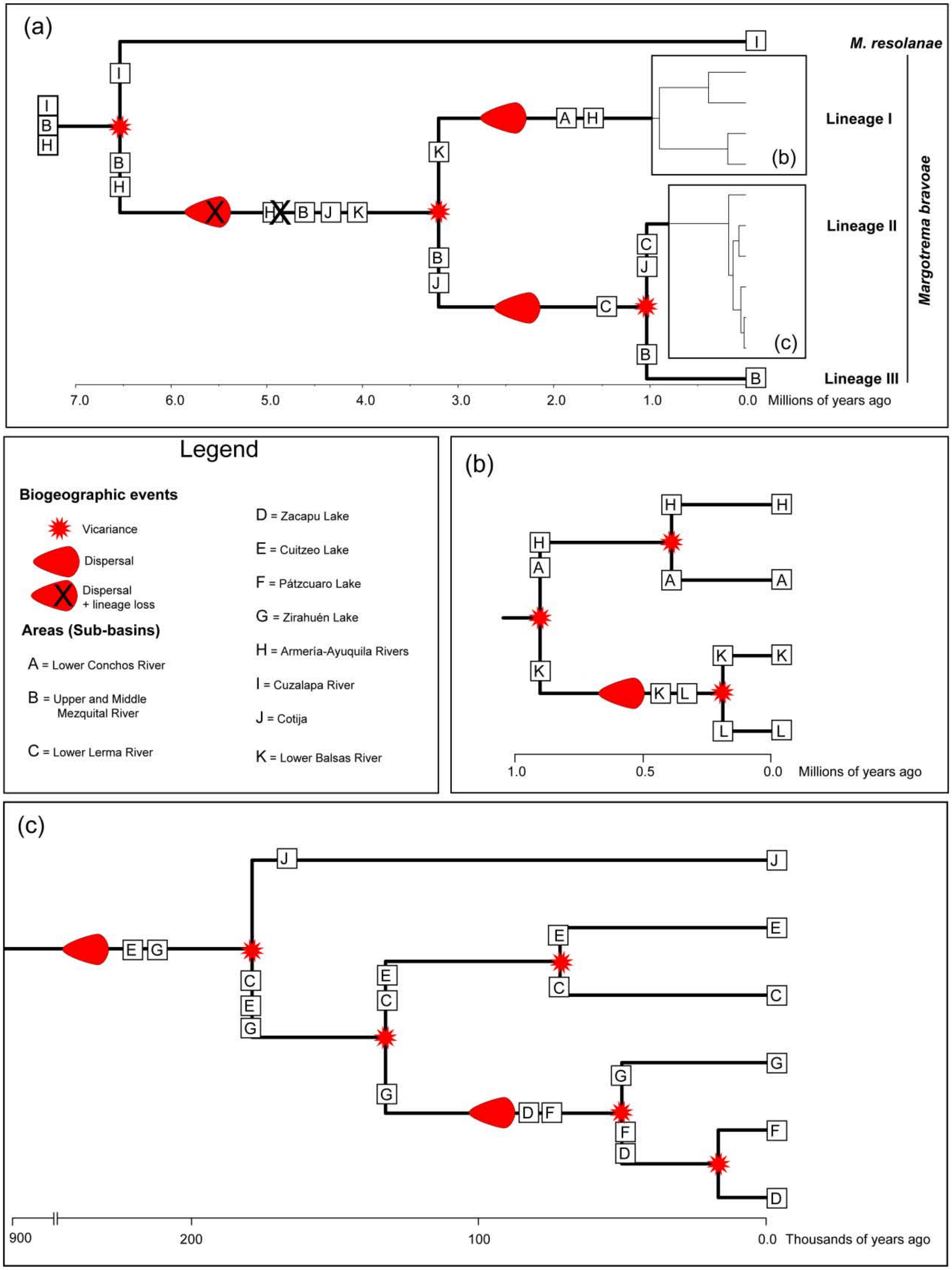

(a)
I
B
H
M. resolanae
Lineage I
Lineage II
Lineage III
Margotrema bravoae
K
J
A
C
(b)
(c)
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0 Millions of years ago
Legend
Biogeographic events
Vicariance
Dispersal
Dispersal + lineage loss
Areas (Sub-basins)
A = Lower Conchos River
B = Upper and Middle Mezquital River
C = Lower Lerma River
D = Zacapu Lake
E = Cuitzeo Lake
F = Pátzcuaro Lake
G = Zirahuén Lake
H = Armería-Ayuquila Rivers
I = Cuzalapa River
J = Cotija
K = Lower Balsas River
(b)
H
A
K
L
1.0
0.5
0.0 Millions of years ago
(c)
J
E
G
C
D
F
900
200
100
0.0 Thousands of years ago

Figure 2

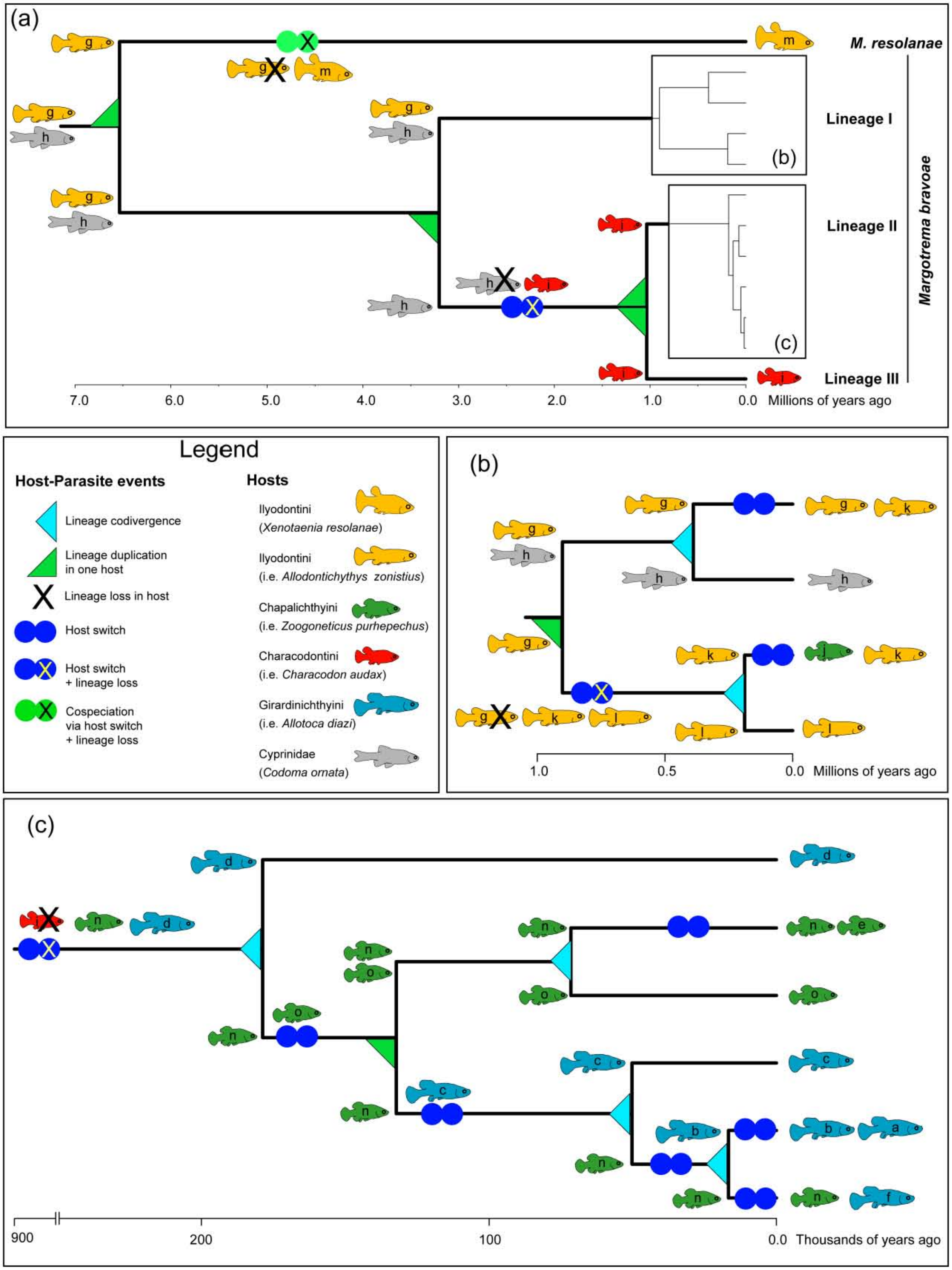

(a)
g
g
h
g
h
g
m
m
M. resolanae
g
h
Lineage I
(b)
Lineage II
i
h
i
h
(c)
i
i
Lineage III
Margotrema bravoae
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0
Millions of years ago
Legend
Host-Parasite events
Lineage codivergence
Lineage duplication in one host
Lineage loss in host
Host switch
Host switch + lineage loss
Cospeciation via host switch + lineage loss
Hosts
Ilyodontini (Xenotaenia resolanae)
Ilyodontini (i.e. Allodontichythys zonistius)
Chapalichthyini (i.e. Zoogoneticus purhepechus)
Characodontini (i.e. Characodon audax)
Girardinichthyini (i.e. Allotoca diazi)
Cyprinidae (Codoma ornata)
(b)
g
h
g
h
g
g
k
h
g
k
j
k
g
k
l
l
l
1.0
0.5
0.0
Millions of years ago
(c)
d
d
i
n
d
n
n
e
n
o
o
o
n
c
c
c
n
b
b
a
n
n
n
f
900
200
100
0.0
Thousands of years ago

Figure 3

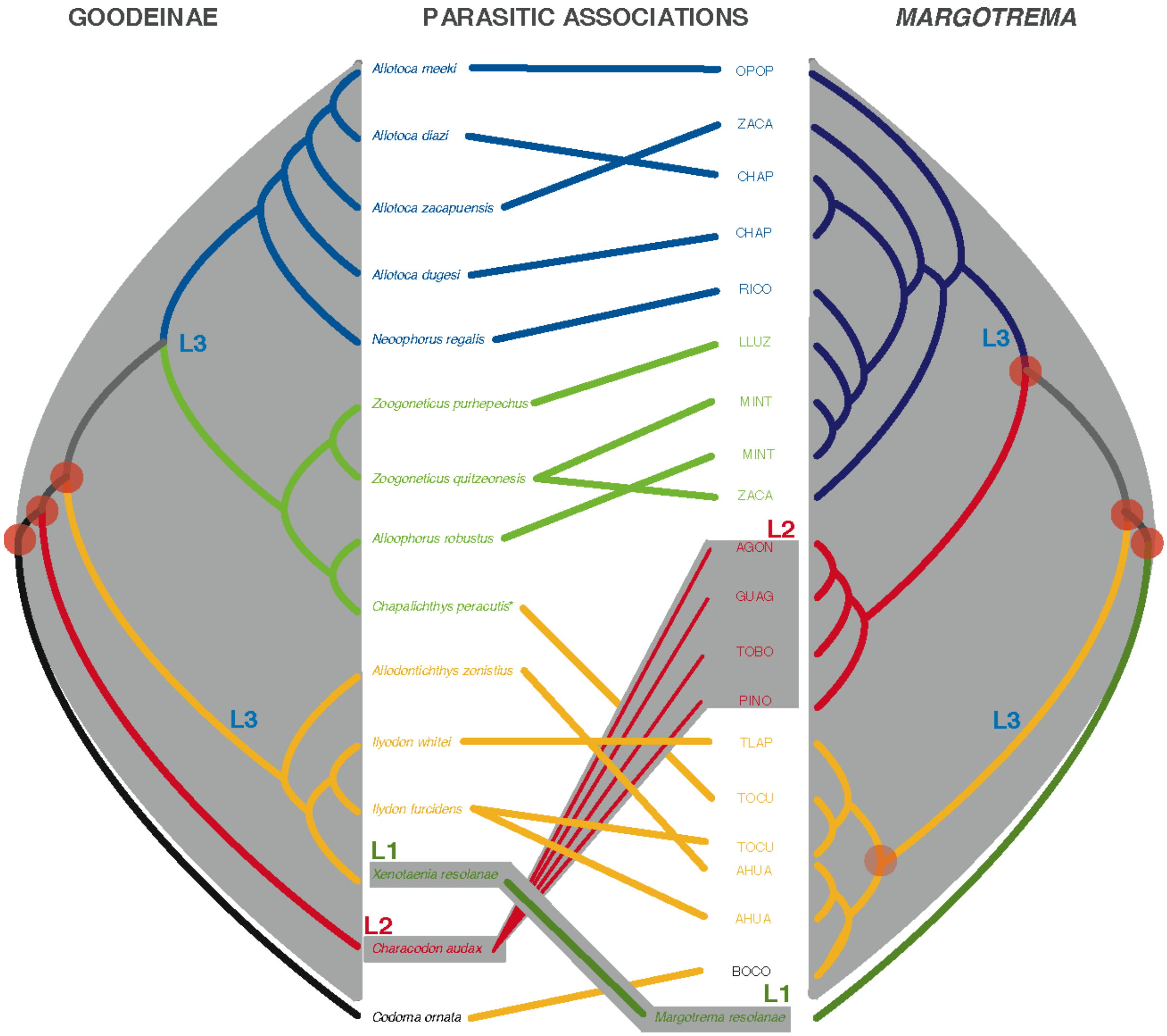
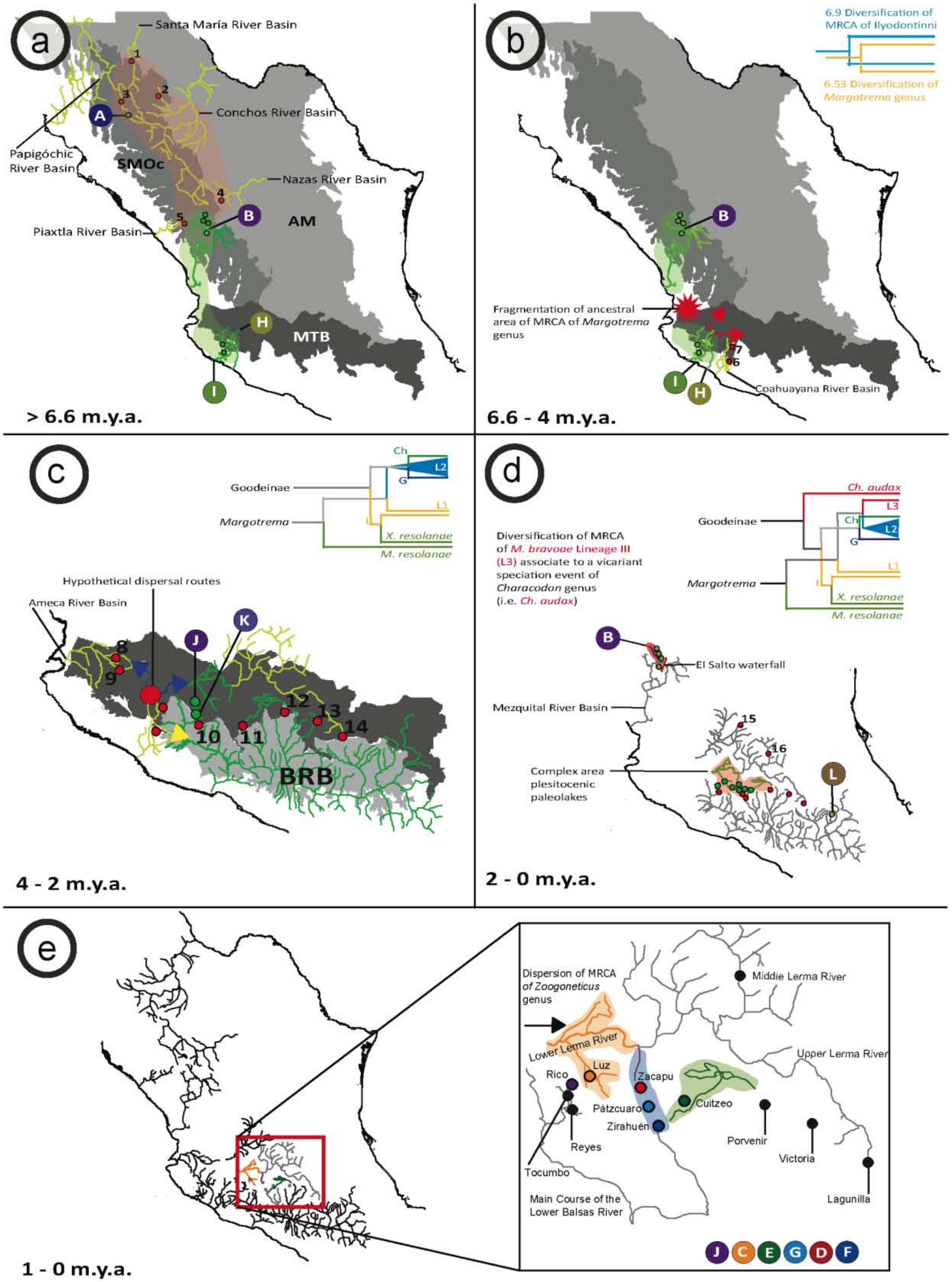

GOODEINAE
PARASITIC ASSOCIATIONS
MARGOTREMA
Allotoca meeki
Allotoca diazi
Allotoca zacapuensis
Allotoca dugesi
Neoophorus regalis
Zoogoneticus purhepechus
Zoogoneticus quitzeonesis
Alloophorus robustus
*Chapalichthys peracutis**
Allodontichthys zonistius
Ilyodon whitei
Ilydon furcidens
L1
Xenotaenia resolanae
L2
Characodon audax
Codoma ornata
L3
L3
OPOP
ZACA
CHAP
CHAP
RICO
LLUZ
MINT
MINT
ZACA
L2
AGON
GUAG
TOBO
PINO
TLAP
TOCU
TOCU
AHUA
AHUA
BOCO
L1
Margotrema resolanae
L3
L3

Figure 4


a
Santa María River Basin
1
2
3
A
Conchos River Basin
Papigóchic River Basin
SMOc
Nazas River Basin
4
5
B
AM
Piaxtla River Basin
H
MTB
I
> 6.6 m.y.a.
b
6.9 Diversification of MRCA of Ilyodontinni
6.53 Diversification of *Margotrema* genus
B
Fragmentation of ancestral area of MRCA of *Margotrema* genus
7
6
I
H
Coahuayana River Basin
6.6 - 4 m.y.a.
c
Ch
L2
G
Goodeinae
L1
Margotrema
I
X. resolanae
M. resolanae
Hypothetical dispersal routes
Ameca River Basin
J
K
8
9
10
11
12
13
14
BRB
4 - 2 m.y.a.
d
Ch. audax
L3
Ch
L2
G
Goodeinae
Diversification of MRCA of *M. bravoae* Lineage III (L3) associate to a vicariant speciation event of *Characodon* genus (i.e. *Ch. audax*)
L1
Margotrema
I
X. resolanae
M. resolanae
B
El Salto waterfall
Mezquital River Basin
15
16
Complex area plesitocenic paleolakes
L
2 - 0 m.y.a.
e
1 - 0 m.y.a.
Dispersion of MRCA of *Zoogoneticus* genus
Middle Lerma River
Lower Lerma River
Upper Lerma River
Luz
Rico
Zacapu
Pátzcuaro
Cuitzeo
Zirahuén
Reyes
Porvenir
Victoria
Tocumbo
Main Course of the Lower Balsas River
Lagunilla
J
C
E
G
D
F

Appendix S1 Codes employed for the RASP analyses (MCMC) (not show results), Dispersal-Extinction-Cladogenesis (DEC) analyses (akin Fig. 1), following Domínguez-Domínguez *et al.* (2006, 2010) hydrological systems (areas). Areas delimited based on the Mexican hydrological basins and sub-basins map produced by the Comisión Nacional para el Conocimiento y uso de la Biodiversidad (CONABIO, 1998; www.conabio.gob.mx). Also show species and lineages of Margotrema included found in each area, locality (and code) and host taxa following Martínez-Aquino *et al.*, 2013. The localities was used to test the divergence date of each lineage of *Margotrema* spp., at locality level (akin Appendix S5).

Locality	**Locality code**
Tributary of Conchos River, Bocoyna, Chihuahua, México	BOCO
Spring in Abraham González, Durango, México	AGON
Spring in the Unidad de Manejo Ambiental de caza, Guadalupe Aguilera, Durango, México	GUAG
Puente en el poblado de Pino Suárez, carretera Durango-Mezquital, Durango, México	PINO
Spring El Toboso, Durango, México	TOBO
La Luz spring, Jacona de Plancarte, Michoacán, México	LLUZ
La Angostura, Zacapu Lake (balneario), Michoacán, México	ZACA
La Mintzita spring, Michoacán, México	MINT
Spring Chapultepec, Michoacán, México	CHAP
Opopeo Lake, Michoacán, México	OPOP
Stream in Ahuacapán, Jalisco, México	AHUA
Arroyo Durazno in Cuzalapa River, Jalisco, México	CUZA
Spring Rico, Michoacán, México	RICO
Spring in park of Tocumbo, Michoacán, México	TOCU
Spring in Tlapetlahuaya, Puebla, México	TLAP

River Chagres at the Frijolito River, Panama

Tobaco creek, Canada

Continued appendix 1.

Area (sub-basin)	Area code
Lower Conchos River	A
Mezquital River Upper and Medium	B (1)
Mezquital River Upper and Medium	B (2)
Mezquital River Upper and Medium	B (3)
Mezquital River Upper and Medium	B (4)
Lower Lerma River	C
Zacapu Lake	D
Cuitzeo Lake	E
Pátzcuaro Lake	F
Zirahuén Lake	G
Armería-Ayuquila Rivers	H
Cuzalapa River	I
Cotija	J
Lower Balsas River	K
Upper Balsas River	L
River Chagres	
Tobaco creek	

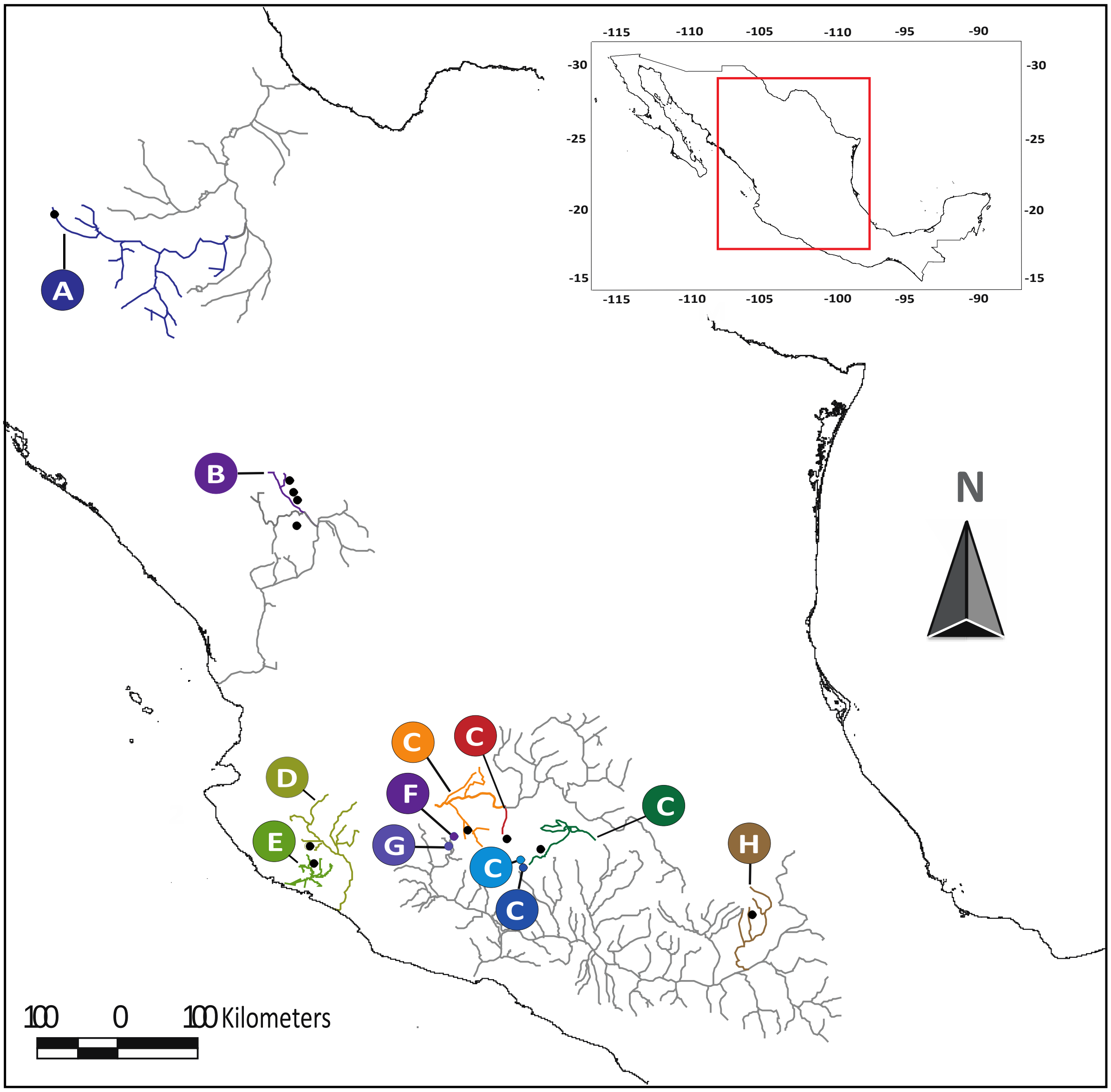
Continued appendix 1.

Parasite species	**Terminal code**	**Hosts taxa**
Margotrema bravoae Lineage I	IA	*Codoma ornata*
Margotrema bravoae Lineage III	IIIB	*Characodon audax*
Margotrema bravoae Lineage III	IIIB	*Characodon audax*
Margotrema bravoae Lineage III	IIIB	*Characodon audax*
Margotrema bravoae Lineage III	IIIB	*Characodon audax*
Margotrema bravoae Lineage II	IIC	*Zoogoneticus purhepechus*
Margotrema bravoae Lineage II	IID	*Allotoca zacapuensis*, *Zoogoneticus quitzeoensis*
Margotrema bravoae Lineage II	IIE	*Alloophorus robustus*, *Zoogoneticus quitzeoensis*
Margotrema bravoae Lineage II	IIF	*Allotoca diazi*, *Allotoca duguesi*
Margotrema bravoae Lineage II	IIG	*Allotoca meeki*
Margotrema bravoae Lineage I	IH	*Allodontichthys zonistius*, *Ilyodon furcidens*
Margotrema resolanae	*M. resolanae*	*Xenotaenia resolanae*
Margotrema bravoae Lineage II	IIJ	*Neoophorus regalis*
Margotrema bravoae Lineage I	IK	*Chapalichthys pardalis*, *Ilyodon furcidens*
Margotrema bravoae Lineage I	IL	*Ilyodon whitei*
Walliniae chavarriae		*Gephyrocharax* sp.
Allocreadium lobatum		*Semotilus atromaculatus*

Appendix S2A Table with codes employed for the Dispersal-Extinction-Cladogenesis (DEC; Model I: 0-6.5 Ma), analyses following Domínguez-Domínguez *et al*. (2006, 2010) hydrological systems (areas). Areas delimited based on the Mexican hydrological basins and sub-basins map produced by the Comisión Nacional para el Conocimiento y uso de la Biodiversidad (CONABIO, 1998; www.conabio.gob.mx). Each lineage of *Margotrema* included found in each area, locality and host species.

Area (sub-basin)	**Code area**	**Lineage *Margotrema* spp. by area**	**Locality by area**	**Host species by area**
Lower Conchos River	A	*Margotrema bravoae* Lineage I	Tributary of Conchos River, Bocoyna, Chihuahua, México	*Codoma ornata*
Mezquital River Upper and Medium	B	*Margotrema bravoae* Lineage III	Spring in Abraham González, Durango, México	*Characodon audax*
Mezquital River Upper and Medium	B	*Margotrema bravoae* Lineage III	Spring in the Unidad de Manejo Ambiental de caza, Guadalupe Aguilera, Durango, México	*Characodon audax*
Mezquital River Upper and Medium	B	*Margotrema bravoae* Lineage III	Puente en el poblado de Pino Suárez, carretera Durango-Mezquital, Durango, México	*Characodon audax*
Mezquital River Upper and Medium	B	*Margotrema bravoae* Lineage III	Spring El Toboso, Durango, México	*Characodon audax*
Lower and Medium Lerma and Central Lakes	C	*Margotrema bravoae* Lineage II	La Luz spring, Jacona de Plancarte, Michoacán, México	*Zoogoneticus purhepechus*
Lower and Medium Lerma and Central Lakes	C	*Margotrema bravoae* Lineage II	La Angostura, Zacapu Lake (balneario), Michoacán, México	*Allotoca zacapuensis*, *Zoogoneticus quitzeoensis*
Lower and Medium Lerma and Central Lakes	C	*Margotrema bravoae* Lineage II	La Mintzita spring, Michoacán, México	*Alloophorus robustus*, *Zoogoneticus quitzeoensis*
Lower and Medium Lerma and Central Lakes	C	*Margotrema bravoae* Lineage II	Spring Chapultepec, Michoacán, México	*Allotoca diazi, Allotoca duguesi*
Lower and Medium Lerma and Central Lakes	C	*Margotrema bravoae* Lineage II	Opopeo Lake, Michoacán, México	*Allotoca meeki*
Armería Ayuquila River	D	*Margotrema bravoae* Lineage I	Stream in Ahuacapán, Jalisco, México	*Allodontichthys zonistius*, *Ilyodon furcidens*
Cuzalapa River	E	*Margotrema resolanae*	Arroyo Durazno in Cuzalapa River, Jalisco, México	*Xenotaenia resolanae*
Cotija	F	*Margotrema bravoae* Lineage II	Spring Rico, Michoacán, México	*Neoophorus regalis*
Lower Balsas River	G	*Margotrema bravoae* Lineage I	Spring in park of Tocumbo, Michoacán, México	*Chapalichthys pardalis*, *Ilyodon furcidens*
Upper Balsas River	H	*Margotrema bravoae* Lineage I	Spring in Tlapetlahuaya, Michoacán, México	*Ilyodon whitei*

Appendix 2SB Figure of the hydrological systems (areas) and collection sites for *Margotrema* spp. in Mexico. The capital letters correspond to each hydrological system for each locality (black point) where specimens of *Margotrema* spp. were collected from (for details see Table of Appendix S2A).



Appendix S2C Matrix used for Lagrange dispersal constrains for different time slices (Model I: 0-6.5 Ma).

Time: 0 – 2 Ma.

	A	B	C	D	E	F	G	H
A	-	1×10^{-6}	1×10^{-6}	1×10^{-6}	1×10^{-6}	1×10^{-6}	1×10^{-6}	1×10^{-6}
B	1×10^{-6}	-	1×10^{-6}	1×10^{-6}	1×10^{-6}	1×10^{-6}	1×10^{-6}	1×10^{-6}
C	1×10^{-6}	1×10^{-6}	-	1×10^{-6}	0.5	1×10^{-6}	1×10^{-6}	1×10^{-6}
D	1×10^{-6}	1×10^{-6}	1×10^{-6}	-	1×10^{-6}	1×10^{-6}	1×10^{-6}	1×10^{-6}
E	1×10^{-6}	1×10^{-6}	1×10^{-6}	1×10^{-6}	-	1×10^{-6}	1×10^{-6}	1×10^{-6}
F	1×10^{-6}	1×10^{-6}	0.5	1×10^{-6}	1×10^{-6}	-	0.95	0.5
G	1×10^{-6}	1×10^{-6}	1×10^{-6}	1×10^{-6}	1×10^{-6}	0.95	-	1
H	1×10^{-6}	1×10^{-6}	1×10^{-6}	1×10^{-6}	1×10^{-6}	0.5	1	-

Time: 2 – 4 Ma.

	A	B	C	D	E	F	G	H
A	-	0.1	1×10^{-6}	1×10^{-6}	1×10^{-6}	1×10^{-6}	1×10^{-6}	1×10^{-6}
B	1×10^{-6}	-	1×10^{-6}	1×10^{-6}	1×10^{-6}	1×10^{-6}	1×10^{-6}	1×10^{-6}
C	0.1	1×10^{-6}	-0.	0.1	1×10^{-6}	1	0.1	0.1
D	1×10^{-6}	1×10^{-6}	0.5	-	0.5	0.95	1	0.95
E	1×10^{-6}	1×10^{-6}	0.4	0.5	-	0.4	0.5	0.4
F	1×10^{-6}	1×10^{-6}	1	0.5	0.5	-	0.6	0.6
G	1×10^{-6}	1×10^{-6}	0.5	0.5	1×10^{-6}	0.6	-	0.95
H	1×10^{-6}	1×10^{-6}	0.1	0.3	1×10^{-6}	0.5	0.95	-

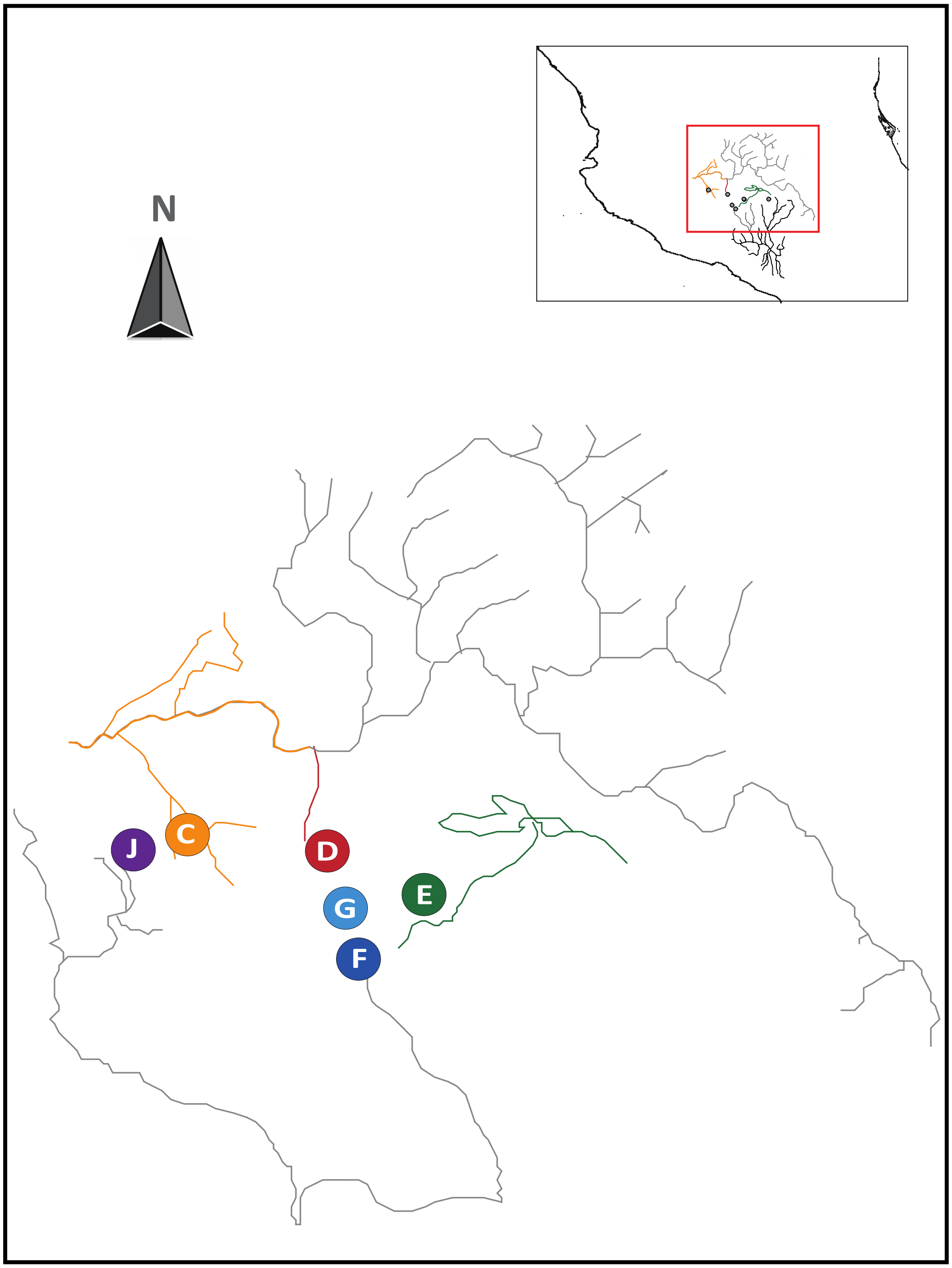
Time: 6.5 – 4 Ma.

	A	B	C	D	E	F	G	H
A	-	1×10^{-6}	1×10^{-6}	1×10^{-6}	1×10^{-6}	1×10^{-6}	1×10^{-6}	1×10^{-6}
B	1×10^{-6}	-	1×10^{-6}	1×10^{-6}	1×10^{-6}	1×10^{-6}	1×10^{-6}	1×10^{-6}
C	1×10^{-6}	1×10^{-6}	-	1×10^{-6}	1×10^{-6}	1	1×10^{-6}	1×10^{-6}
D	1×10^{-6}	1×10^{-6}	0.9	-	1	0.9	0.95	0.9
E	1×10^{-6}	1×10^{-6}	1×10^{-6}	0.5	-	1×10^{-6}	1×10^{-6}	1×10^{-6}
F	1×10^{-6}	1×10^{-6}	1	1×10^{-6}	1×10^{-6}	-	1×10^{-6}	1×10^{-6}
G	1×10^{-6}	1×10^{-6}	1×10^{-6}	0.3	1×10^{-6}	1×10^{-6}	-	1
H	1×10^{-6}	1×10^{-6}	1×10^{-6}	1×10^{-6}	1×10^{-6}	1×10^{-6}	1	-

Appendix S3A Table with codes employed for the Dispersal-Extinction-Cladogenesis (DEC; Model I: 0-1 Ma), analyses following Domínguez-Domínguez *et al*. (2006, 2010) hydrological systems (areas). Areas delimited based on the Mexican hydrological basins and sub-basins map produced by the Comisión Nacional para el Conocimiento y uso de la Biodiversidad (CONABIO, 1998; www.conabio.gob.mx). Appendix also shows locality and host taxa associated to each area where *Margotrema bravoae* Lineage III is distributed.

Area (subasin)	**Code area**	**Locality**	**Hosts**
Lower Lerma	C	La Luz spring, Jacona de Plancarte, Michoacán, México	*Zoogoneticus purhepechus*
Zacapu Lake	D	La Angostura, Zacapu Lake (balneario), Michoacán, México	*Allotoca zacapuensis,* *Zoogoneticus quitzeoensis*
Cuitzeo Lake	E	La Mintzita spring, Michoacán, México	*Alloophorus robustus,* *Zoogoneticus quitzeoensis*
Pátzcuaro Lake	F	Spring Chapultepec, Michoacán, México	*Allotoca diazi,* *Allotoca duguesi*
Zirahuén Lake	G	Opopeo Lake, Michoacán, México	*Allotoca meeki*
Cotija	J	Cotija, Michoacán, México	*Neoophorus regalis*

Appendix S3B Figure of the hydrological systems (areas) for *Margotrema bravoae* Lineage III from Central Mexico. The capital letters correspond to each water body of the locality where specimens of *Margotrema* spp. were collected from (for more details Appendix S3A).



Appendix S3C Matrix used in Lagrange dispersal constrains for different time slices (mirrow matrix) (Model II: 0-1 Ma).

Time: Plio-Pleistocen (> 1 Ma)

	C	D	E	F	G	J
C	-	1×10^{-6}	1×10^{-6}	1×10^{-6}	1×10^{-6}	1×10^{-6}
D		-	0.85	0.85	0.85	1×10^{-6}
E			-	0.85	0.85	1×10^{-6}
F				-	0.9	1×10^{-6}
G					-	1×10^{-6}
J						-

Time: 0 m.y.a

	C	D	E	F	G	J
C	-	1	1	1	1	1
D		-	1	1	1	1
E			-	1	1	1
F				-	1	1
G					-	1
J						-

Appendix S4A Table with codes employed for the Dispersal-Extinction-Cladogenesis (DEC) using the host species as areas and divergences timing, in million years ago (Ma) based on Domínguez- Domínguez *et al*., 2010, used in the dispersal matrix for cophylogenetical analysis Lagrange, of the most recent common ancestral (MRCA) of genus / species of hosts of *Margotrema* spp.

Host species (Areas)	**Code areas**	**Age of MRCA of genus / species**
Allotoca diazi	A	7.5 / < 1
Allotoca duguesi	B	7.5 / 3.9
Allotoca meeki	C	7.5 / < 1
Neoophorus regalis	D	6.9 / < 1
Alloophorus robustus	E	4 / 2.8
Allotoca zacapuensis	F	7.5 / 3.9
Allodontichthys zonistius	G	6.9 / 3.6
Codoma ornata	H	?
Characodon audax	I	15.5 / 1.8
Chapalichthys pardalis	J	4 / ?
Ilyodon furcidens	K	5.1 / ?
Ilyodon whitei	L	5.1 / ?
Xenotaenia resolanae	M	6.9 / 5.6
Zoogoneticus quitzeoensis	N	6.9 / 3.9
Zoogoneticus purhepechus	O	6.8 / 2.8

Appendix S4B Matrix used for Lagrange dispersal constrains for different time slices (mirrow matrix) (cophylogenetic model).

Time: 0 – 2 Ma.

	a	B	c	d	e	F	g	h	i	j	k	l	m	n	0
a	-	1	1	1	1	1	1×10^{-6}	1×10^{-6}	1×10^{-6}	1×10^{-6}	1×10^{-6}	1×10^{-6}	1×10^{-6}	1	1
b		-	1	1	1	1	1×10^{-6}	1×10^{-6}	1×10^{-6}	1×10^{-6}	1×10^{-6}	1×10^{-6}	1×10^{-6}	1	1
c			-	1	1	1	1×10^{-6}	1×10^{-6}	1×10^{-6}	1×10^{-6}	1×10^{-6}	1×10^{-6}	1×10^{-6}	1	1
d				-	1	1	1×10^{-6}	1×10^{-6}	1×10^{-6}	1×10^{-6}	1×10^{-6}	1×10^{-6}	1×10^{-6}	1	1
e					-	1	1×10^{-6}	1×10^{-6}	1×10^{-6}	1×10^{-6}	1×10^{-6}	1×10^{-6}	1×10^{-6}	1	1
f						-	1×10^{-6}	1×10^{-6}	1×10^{-6}	1×10^{-6}	1×10^{-6}	1×10^{-6}	1×10^{-6}	1	1
g							-	1×10^{-6}	1×10^{-6}	1	1	1	1×10^{-6}	1×10^{-6}	1×10^{-6}
h								-	1×10^{-6}	1×10^{-6}	1×10^{-6}	1×10^{-6}	1×10^{-6}	1×10^{-6}	1×10^{-6}
i									-	1×10^{-6}	1×10^{-6}	1×10^{-6}	1×10^{-6}	1×10^{-6}	1×10^{-6}
j										-	1	1	1×10^{-6}	1×10^{-6}	1×10^{-6}
k											-	1	1×10^{-6}	1×10^{-6}	1×10^{-6}
l												-	1×10^{-6}	1×10^{-6}	1×10^{-6}
m													-	1×10^{-6}	1×10^{-6}
n														-	1
o															-

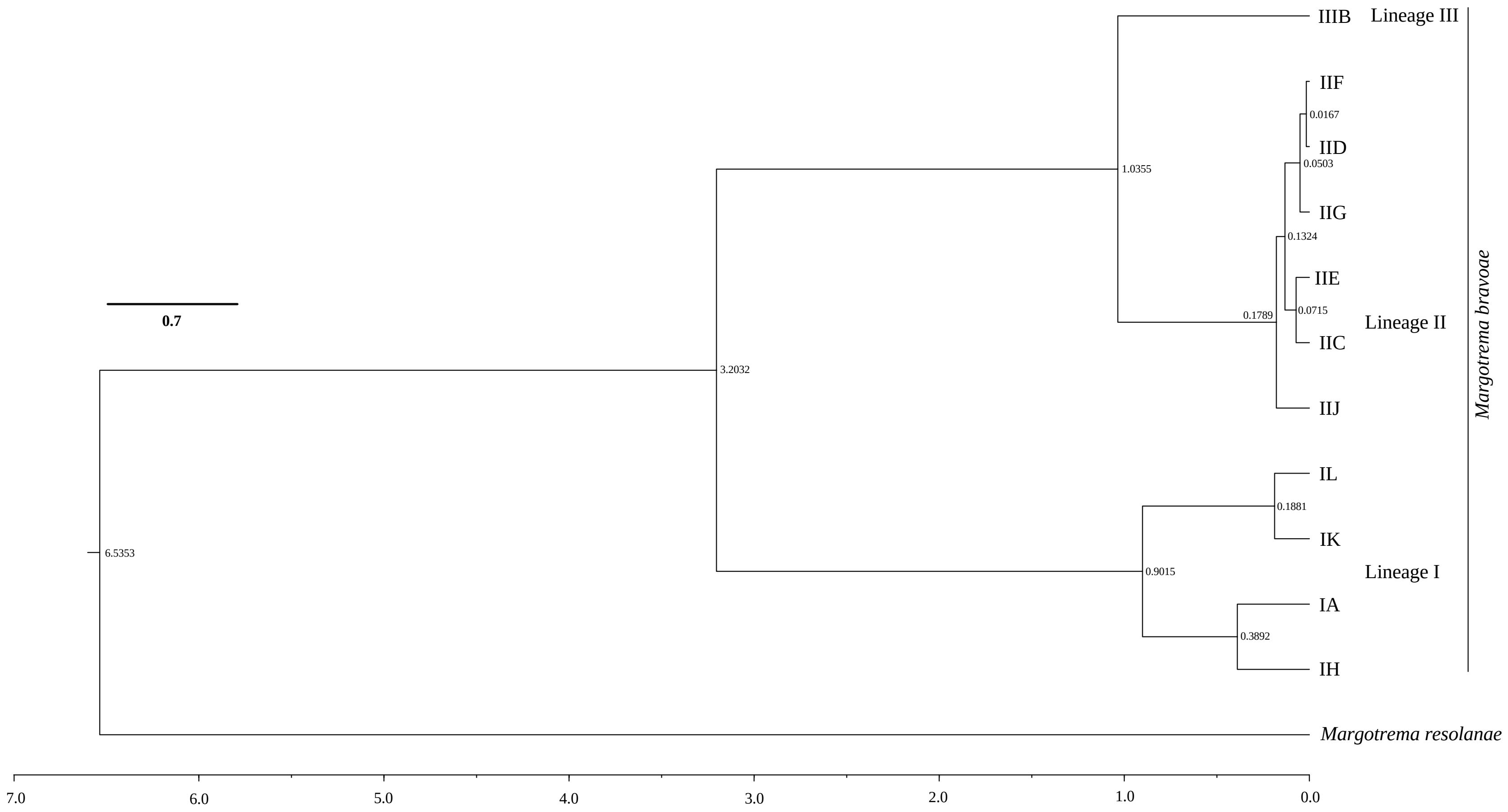
Time: 2 – 4 Ma.

	a	b	c	d	e	F	g	h	i	j	k	l	m	n	O
a	-	1×10^{-6}	1×10^{-6}	1×10^{-6}	1×10^{-6}	1×10^{-6}	1×10^{-6}	1×10^{-6}	1×10^{-6}	1×10^{-6}	1×10^{-6}	1×10^{-6}	1×10^{-6}	1×10^{-6}	1×10^{-6}
b		-	1×10^{-6}	0.5	1	1	1	1×10^{-6}	1×10^{-6}	0.5	1×10^{-6}	1×10^{-6}	1×10^{-6}	1	1
c			-	1×10^{-6}	1×10^{-6}	1×10^{-6}	1×10^{-6}	1×10^{-6}	1×10^{-6}	1×10^{-6}	1×10^{-6}	1×10^{-6}	1×10^{-6}	1×10^{-6}	1×10^{-6}
d				-	0.5	0.5	0.5	1×10^{-6}	1×10^{-6}	0.25	1×10^{-6}	1×10^{-6}	1×10^{-6}	0.5	0.5
e					-	1	1	1×10^{-6}	1×10^{-6}	0.5	1×10^{-6}	1×10^{-6}	1×10^{-6}	1	1
f						-	1	1×10^{-6}	1×10^{-6}	0.5	1×10^{-6}	1×10^{-6}	1×10^{-6}	1	1
g							-	1×10^{-6}	1×10^{-6}	0.5	1	1	0.1	1	1
h								-	0.1	1×10^{-6}	1×10^{-6}	1×10^{-6}	1×10^{-6}	1×10^{-6}	1×10^{-6}
i									-	1×10^{-6}	1×10^{-6}	1×10^{-6}	1×10^{-6}	1×10^{-6}	1×10^{-6}
j										-	1×10^{-6}	1×10^{-6}	1×10^{-6}	0.5	0.5
k											-	1	1×10^{-6}	1×10^{-6}	1×10^{-6}
l												-	1×10^{-6}	1×10^{-6}	1×10^{-6}
m													-	1×10^{-6}	1×10^{-6}
n														-	1
o															-

Time: 6.6 – 4 Ma.

	a	B	c	d	e	F	g	h	i	j	k	l	m	n	O
a	-	1×10^{-6}	1×10^{-6}	1×10^{-6}	1×10^{-6}	1×10^{-6}	1×10^{-6}	1×10^{-6}	1×10^{-6}	1×10^{-6}	1×10^{-6}	1×10^{-6}	1×10^{-6}	1×10^{-6}	1×10^{-6}
b		-	1×10^{-6}	1×10^{-6}	1×10^{-6}	1×10^{-6}	1×10^{-6}	1×10^{-6}	1×10^{-6}	1×10^{-6}	1×10^{-6}	1×10^{-6}	1×10^{-6}	1×10^{-6}	1×10^{-6}
c			-	1×10^{-6}	1×10^{-6}	1×10^{-6}	1×10^{-6}	1×10^{-6}	1×10^{-6}	1×10^{-6}	1×10^{-6}	1×10^{-6}	1×10^{-6}	1×10^{-6}	1×10^{-6}
d				-	1×10^{-6}	1×10^{-6}	1×10^{-6}	1×10^{-6}	1×10^{-6}	1×10^{-6}	1×10^{-6}	1×10^{-6}	1×10^{-6}	1×10^{-6}	1×10^{-6}
e					-	1×10^{-6}	1×10^{-6}	1×10^{-6}	1×10^{-6}	1×10^{-6}	1×10^{-6}	1×10^{-6}	1×10^{-6}	1×10^{-6}	1×10^{-6}
f						-	1×10^{-6}	1×10^{-6}	1×10^{-6}	1×10^{-6}	1×10^{-6}	1×10^{-6}	1×10^{-6}	1×10^{-6}	1×10^{-6}
g							-	1×10^{-6}	1×10^{-6}	1×10^{-6}	1×10^{-6}	1×10^{-6}	1	1×10^{-6}	1×10^{-6}
h								-	1×10^{-6}	1×10^{-6}	1×10^{-6}	1×10^{-6}	1×10^{-6}	1×10^{-6}	1×10^{-6}
i									-	1×10^{-6}	1×10^{-6}	1×10^{-6}	1×10^{-6}	1×10^{-6}	1×10^{-6}
j										-	1×10^{-6}	1×10^{-6}	1×10^{-6}	1×10^{-6}	1×10^{-6}
k											-	1×10^{-6}	1×10^{-6}	1×10^{-6}	1×10^{-6}
l												-	1×10^{-6}	1×10^{-6}	1×10^{-6}
m													-	1×10^{-6}	1×10^{-6}
n														-	1×10^{-6}
o															-

Appendix S5 Ultrametric tree resulting from the dating analysis of *Margotrema* spp. Mean ages are indicated in each node, the terminal codes are (localities) as in Appendix 1.



Appendix S6 Additional records of *Margotrema* in freshwater fish species from Mexico. The locality number (LN) corresponds in the Figure 4. For more details see text in the manuscript. * = Unpublished data.

LN	**Host species**	**Locality**	**Georreferences**
1	*Cyprinella lutrensis*	Buenaventura, Chihuahua	29° 50' 16.94" N; 107° 28' 24.86" W
2*	*Cyprinidae*	Conchos River, Chihuahua	Unpublished data
3*	*Cyprinidae*	Pagigochic River, Chihuahua	Unpublished data
4	*Cyprinodon nazas*	Río Guatimape in the Sofía town, Durango	24° 54’ 41.1’’N; 104° 32’ 7.4’’W
5	*Codoma ornata*	Río Piaxtla, Municipio de San Dimas, Durango	24°21'59" N; 105°31’7.8’’ W
6	*Allodontichthys hubbsi*	El Tule, Jalisco	19° 19' 34.2" N; 103° 22' 15" W
7	*Allodontichthys tamazulae*	Río Tamazula, Jalisco	19° 43' 22.7" N; 103° 12' 08.5" W
8	*Goodea atripinnis*	Verde River, Jalisco	21º 49" 12.0" N; 101º 46' 21.3" W
9	*Allotoca maculata*	Río San Marcos, Jalisco	20° 46' 35.7" N; 104° 09' 52.6" W
10	*Ilyodon furcidens*	Río Potrero Grande, Jalisco	19° 43' 22.7" N; 103° 12' 08.5" W
11	*Neoophorus regalis*	Los Reyes, Michoacán	19° 33' 43.5" N; 102° 27' 39" W
12	*Goodea atripinnis*	Ignacio Allende, Guanajuato	20° 55' N; 100° 50' W
13	*Ilyodon cortesae*	Manantial Cutzaróndiro, Michoacán	19° 10' 59" N; 101° 30' 13" W
14	*Girardinichthys multiradiatus*	Canal el Porvenir, Michoacán	19° 40' 29" N; 100° 38' 25" W
15	*Girardinichthys multiradiatus*	Villa Victoria, Estado de México	19° 27' 30" N; 99° 59' 39" W
16	*Girardinichthys multiradiatus*	Ciénega La Lagunilla, Estado de México	19° 08' 30" N; 99° 30' 12" W

DISCUSIÓN GENERAL

La asociación *Goodeinae-Margotrema*, representa un modelo biológico potencial para poner a prueba hipótesis acerca de la evolución de los helmintos parasitarios de peces dulceacuícolas de México, debido a que se distribuye sobre áreas geográficas restringidas y no puede dispersarse por fenómenos naturales a través de sistemas hidrológicos epicontinentales del centro de México, lo que impide el flujo génico entre huéspedes o sistemas hidrológicos (Martínez-Aquino *et al*., 2013). Por tanto, es posible recuperar una estructura genealógica de los organismos parásitos capaz de reflejarse tanto sobre el escenario geográfico en el que se distribuyen como con la historia evolutiva de sus huéspedes. En este contexto, el presente estudio tuvo como objetivo general detectar los patrones biogeográficos y cofilogenéticos que configuraron la asociación *Goodeinae-Margotrema*, grupos endémicos y con historia evolutiva estrechamente relacionada y ocurrida en sistemas hidrológicos del centro de México.

Implicaciones taxonómicas y patrones filogenéticos

Con base en los distintos análisis filogenéticos se detectó que la especie *Margotrema guillerminae* es sinónima de *M. bravoae* (Martínez-Aquino *et al*., 2013). Por tanto, *M. bravoae* representa una especie polimórfica en términos de ciertos rasgos morfológicos, tales como la distribución de los ciegos intestinales en relación a la posición de los testículos. A su vez, *M. bravoae* incluye tres linajes con evolución independiente, aparentemente asociados a sistemas hidrológicos y grupos particulares de huéspedes: a) *Margotrema bravoae* Linaje I, distribuido en los sistemas hidrológicos de los ríos Ayuquila, Balsas y Conchos, y asociado primordialmente a huéspedes de las tribus Ilyodontini. b) *Margotrema bravoae* Linaje II, distribuído en sistemas hidrológicos del río Lerma y asociado a huéspedes de las tribus Girardinichthyini y Chapalichthyini. c) *Margotrema bravoae* Linaje III, distribuido en sistemas hidrológicos del río Mezquital y asociado a un único huésped de la tribu Characodontini.

Con base en caracteres morfológicos y moleculares, y como parte de los análisis de la reconstrucción filogenética de *Margotrema*, se determinó y describió una especie nueva para la ciencia, *Margotrema resolanae* Pérez-Ponce de León, Martínez-Aquino & Mendoza-Garfías, 2013, asociada exclusivamente para *Xenotaenia resolanae* y microendémica para el río Cuzalapa, Jalisco (Pérez-Ponce de León *et al*., en prensa). Este reordenamiento taxonómico sin implicar un incremento en el numero de especies descritas para el género *Margotrema*, apoya la hipótesis de que la helmintofauna de peces dulceacuícolas de México esta cerca de ser completada (Pérez-Ponce de León & Choudhury, 2010). Sin embargo, es importante resaltar que la delimitación de los linajes intraespecíficos de *M. bravoae* nos acerca a entender cómo ocurren los procesos de diversificación parasitaria en helmintos parásitos de peces dulceacuícolas de México.

Patrones biogeográficos

La siguiente hipótesis biogeográfica se puso a prueba: "si existe congruencia biogeográfica entre la historia genealógica de *Margotrema* y la historia hidrogeomorfológica del centro de México, entonces se puede reflejar con la historia biogeográfica de sus huéspedes". De manera general, se observó que los patrones de distribución geográfica de *Margotrema* están asociados con las barreras biogeográficas que fragmentaron los sistemas hidrológicos del centro de México y que, a su vez, influenciaron también en el patrón de distribución de sus huéspedes a través de eventos de vicarianza. Por otra parte, el proceso de dispersión causado por sus huéspedes goodeinos juega un papel relevante en la estructura biogeográfica de *Margotrema*, debido a la vagilidad que presentaron históricamente los MRCA de cada una de las tribus de Goodeinae, mismos que expandieron el área de distribución de *Margotrema* sobre los sistemas hidrológicos del centro de México.

Con base en los resultados del análisis de DEC, se observó que el área ancestral de *Margotrema* estuvo ubicada en sistemas hidrológicos del Norte de México y que presentó una conexión relativamente ancestral (6.6 millones de años -ma-) con los sistemas hidrológicos del centro de México. Este resultado es apoyado con base en el patrón de distribución ancestral inferido para otros peces dulceacuícolas (Barbour, 1973; Chernoff & Miller, 1986; Domínguez-Domínguez *et al*., 2010). Al parecer, la expansión del área de distribución del MRCA de *Margotrema*, ocurrió debido a un evento de transmisión vertical (Rannala Michalakis, 2003), de tipo *Goodeinae-Margotrema.* Esto indica que esta asociación ancestral ocurrió entre un huésped basal de la tribu Ilydontini, y su posterior expansión geográfica causada por eventos de vicarianza-dispersión, seguida de asociaciones cofilogenéticas sobre el centro de México. En este contexto, los patrones de diversificación de *M. resolanae* y cada uno de los tres linajes de *M. bravoae* pueden reflejarse en primera instancia en los procesos de fragmentación de los sistemas hidrológicos del Centro de México y, como resultado de ello, pueden reflejarse en los patrones de distribución y diversificación de sus huéspedes Goodeinos.

Los patrones de distribución geográfica de *M. bravoae* Linaje II, datados en < 1 ma, muestran una relación espacio-temporal con los grandes paleolagos que existieron en el centro de México durante el pleistoceno. Futuros estudios con análisis de tipo *Isolation with migration model* (Nielsen & Wakeley, 2001; Hey & Nielsen, 2007; Hey, 2010), usando distintas localidades de distribución de *M. bravoae* Linaje II, podrán poner a prueba posibles rutas de expansión poblacional contrastadas con los de otros taxa dulceacuícolas en la región en donde ocurrieron. En este escenario, podrá detectarse como los taxa dulceacuícolas respondieron ante los eventos pleistocénicos ocurridos en el centro de México.

Patrones cofilogenéticos

Las siguientes hipótesis cofilogenéticas se pusieron a prueba: a) Hay congruencia filogenética entre Goodeinae (a nivel de especies y tribus) reflejada en la historia genealógica de *Margotrema* (a nivel de linajes y especies). b) Los tiempos de divergencia entre los principales clados de Goodeinae (Tribus) corresponden con los de cada especie / linaje de *Margotrema*. Con base en la congruencia biogeográfica detectada con las asociaciones especificas de huéspedes de Goodeinae y apoyada con los resultados de los análisis cofilogenéticos y con los tiempos de divergencia de los principales linajes para *Margotrema*, se identificaron tres niveles de asociaciones históricas distintas donde se apoyan dichas hipótesis cofilogenéticas. A continuación se menciona de manera general cada uno de estos tres niveles de asociación histórica.

a) *Especie-Especie*. Esta asociación representa un proceso de coespeciación (≈ coevolución), ocurrido entre *Xenotaenia resolanae*-*Margotrema resolanae*. El MRCA de *Margotrema* asociado a este evento fue datado hace 6.5 ma y es apoyado estadísticamente por los análisis cofilogenéticos de TreeMap. Por otra parte, esta hipótesis se sustenta por la edad de divergencia del MRCA del goodeino *A. zonistius* (6.9 ma), y el posterior evento de diversificación de *X. resolanae*, exclusivo para la región hidrológica del Río Purificación-Mascota (Domínguez-Domínguez *et al*., 2010), lo que corresponde con la edad de divergencia del MRCA de *Margotrema*.

b) *Especie-Linaje.* Esta asociación implica un proceso de especiación de huésped y una codiferenciación de linajes parasitarios que corresponde tanto con un evento de separación y aislamiento (Falla del Salto sobre el Río Mezquital, Durango, datado hace 1.8 ma), como para el tiempo de divergencia de *M. bravoae* Linaje III (1.0355 ma). Este evento causó la especiación alopátrica vicariante de los caracodontinos; *Ch. audax* y *Ch. lateralis* (Domínguez- Domínguez *et al*., 2006, 2010). *Characodon audax* actualmente está registrado como huésped exclusivo de *M. bravoae* Linaje III. Con base en esta evidencia biogeográfica y cofilogenética, se infiere que ocurrieron procesos de codiferenciación mediados por la vicarianza del Río Mezquital (entre el Río Mezquital Alto-Medio y el Río Mezquital Bajo), lo que promovió la codiferenciación entre el MRCA de *Ch. audax* y el MRCA de sus parásitos (*M. bravoae* Linaje III). Por otra parte, es posible que la asociación ancestral *Characodon-M. bravoae* Linaje III se perdiera en *Ch. lateralis* quizá por extinción natural de las poblaciones de *M. bravoae* Linaje III sobre el Río Mezquital Bajo; o bien, debida a una diferenciación ecológica mediada por los hábitos alimentarios herbívoros que presenta actualmente *Ch. lateralis*, lo que pudo causar la pérdida de la asociación con *M. bravoae* Linaje III. Futuros estudios podrán esclarecer ambos procesos de diversificación.

c) *Tribu-Linaje*. Esta asociación histórica representa patrones de codivergencia a nivel de tribu, históricamente asociados con una codiferenciación de parásitos a nivel de linajes. Este patrón fue observado empíricamente por Martínez-Aquino *et al*., (2013), donde se infirió que las asociaciones entre las tribus de Goodeiane y los tres linajes de *M. bravoae* están estrechamente relacionados tanto por la historia hidrogeomorfológica del centro de México como por los procesos de diversificación filogenética de Goodeinae. En el Capítulo III, se puso a prueba esta hipótesis y se descubrió que *M. bravoae* Linaje I esta estrechamente relacionado con la historia de los sistemas hidrológicos de los ríos Ayuquila y Balsas que a su vez moldearon la diversificación de la tribu Ilyodontini. Del mismo modo se detectó una estrecha relación entre la la diversificación de *M. bravoae* Linaje II y el Rio Lerma, en donde también existió una diversificación de Girardinichthyini y Chapalichthyini.

Con base en estos resultados se infiere que la divergencia de los linajes de *Margotrema*, en primera instancia, se debe a eventos vicariantes seguidos de los procesos de diversificación de los sus huéspedes goodeinos (i.e. Tribus). En este contexto, nuestros resultados se suman a otros trabajos donde se propone que el escenario geográfico rige, en primer orden, los procesos de diversificación de los organismos parásitos y, en segundo, las asociaciones con sus huéspedes (Nieberding *et al*., 2008, Mizukoshi *et al*., 2012). Los resultados de los análisis cofilogenéticos obtenidos en el presente trabajo nos permiten detectar que los procesos de codivergencia pueden actuar en tres niveles distintos, representado claramente que la evolución de los helmintos parásitos puede ocurrir de manera independiente, aún cuando están estrechamente relacionados filogenéticamente. En este contexto, es posible postular que la historia evolutiva del género *Margotrema* ocurrió sobre un *mosaico geográfico*, en el cual las poblaciones de parásitos difieren en sus características y especializaciones con respecto a las especies de huéspedes con las que están asociadas (Thompson, 2005).

El área de distribución observada para *M. bravoae* y *M. resolanae*, así como la datación de la divergencia molecular, sus relaciones filogenéticas, patrones biogeográficos y cofilogenéticos, en contraste con la datación de los eventos hidrogeomorfológicos sobre el centro de México, apoyan las hipótesis que se generaron en este trabajo. Estos resultados sugieren que al aplicar distintos medios de análisis filogenéticos, biogeográficos y cofilogenéticos, con información detallada tanto del área de distribución de los taxa a estudiar, como de las edades geológicas del escenario geográfico en cuestión, permiten definir con mayor objetividad la historia evolutiva de los helmintos parásitos de peces dulceacuícolas de México.

Implicaciones evolutivas

La historia evolutiva de *Margotrema* propuesta en este estudio es congruente con *la regla de Manter*, la cual infiere que los parásitos evolucionan más lentamente que sus huéspedes (Brooks & McLennan, 1993). La tasa de diversificación observada para Goodeinae refleja una radiación biológica relativamente alta ya que cuenta con 42 taxa taxonómicamente válidos (Domínguez-Domínguez *et al*., 2010). Actualmente se han registrado 55 taxa de helmintos parásitos para peces goodeinos (Capítulo I), sin embargo, solo 10 de estos pueden definirse como especialistas de Goodeinae: *Margotrema bravoae, M. resolanae, Phyllodistomum* sp., *Saccocoeloides* sp., *Gyrodactylus lamothei, G. mexicanus, Gyrodactylus* sp. 1, *Gyrodactylus* sp. 2, *Rhabdochona ahuehuellensis y R. lichtenfelsi.* La baja riqueza de especies del género *Margotrema* en goodeinos sugiere una tasa de especiación baja. Algo similar ha sido registrado en las especies de monogéneos de cíclidos de México, donde se ha sugerido que las tasas de diversificación de sus huéspedes han sido más rápidas que las de sus parásitos, por lo que el patrón de distribución huésped / parásito es desigual, es decir, pocas especies de monogéneos en muchas especies de huéspedes (Vidal-Martínez *et al*., 2001).

El área de distribución ancestral de *Margotrema* coincide con la hipótesis de que Goodeinae divergió en sistemas hidrológicos del norte de México (Parenti, 1981). Con base en ello, en este trabajo se infiere la hipótesis de un área ancestral de *Margotrema* más amplia sobre sistemas hidrológicos del norte del país, lo que puede ser coherente con los patrones de distribución de los fósiles de Empetrichthyinae, grupo hermano de la subfamilia Goodeinae (Parenti, 1981; Minckley *et al*., 1986). Futuros estudios, con base en análisis filogenéticos moleculares de *Margotrema* distribuidos en sistemas hidrológicos del norte del país, podrán apoyar o contrastar esta posible asociación histórica entre *Margotrema* y peces empetríctinos.

Los patrones biogegráficos y cofilogeneticos descubiertos en este trabajo para *Margotrema* dan pauta para generar nuevas preguntas. Por ejemplo, si *mapeamos* como caracteres los hábitos alimentarios de los peces goodeinos sobre su filogenia, se puede observar que aquellos peces que presentan carnivoría presentan están parasitados por *Margotrem*a. Con base en esta observación, tiene sentido formular cuestionamientos como: ¿existe un efecto ecológico-evolutivo en el hecho de presentar un hábito alimentario de tipo carnívoro, capaz de reflejarse en un patrón de transmisión parasitaria horizontal o vertical? ¿la transmisión parasitaria es regida por el tipo de hábito alimentario del huésped definitivo y esta asociado a sus relaciones filogenéticas? Los patrones cofilogenéticos detectados entre *Goodeinae-Margotrema* fueron interpretados sobre el marco teórico del *mosaíco geográfico* (Thompson, 2004). Sin embargo, es posible apoyar nuestras hipótesis con evidencia coadaptativa si exploramos estudios metagenómicos respondiendo preguntas como: ¿el complejo mayor de histocompatibilidad (MHC, por sus siglas en ingles *major histocompatibility complex*) de Goodeinae y el de *Margotrema*, puede reflejar una asociación coadaptativa en parte de su genoma? En este proyecto se cuantificó la variación genética de *Margotrema* sobre los huéspedes definitivos. Algunos autores han descubierto que el origen de la variación genética en digéneos de la familia Allocreadiidae ocurre en los huéspedes definitivos debido a que es ahí donde ocurren las fases meióticas de los digéneos adultos (Murty, 1975; Canning & Madhavi, 1977; Madhavi, 1978; Ramanjaneyulu & Madhavi, 1984; Willis, 2001). Para este estudio en *Margotrema*, resulta interesante preguntarnos ¿que porcentaje de la variación observada en los sitios nulceotídicos polimórficos, tanto a nivel inter e intrapoblacionales, se originó en cada uno de los huéspedes involucrados en su ciclo de vida? Modelar estudios de selección natural dependiente de la frecuencia para organismos parásitos (Poulin, 2007), permitirá observar este tipo de información por descubrir. El comportamiento identificado para las asociaciones históricas intrínsecas (gen-organismo, huésped-parásito y área-organismo) del modelo biológico *Goodeinae-Margotrema* permitió comprender los procesos de diversificación de los organismos parásitos de México. Este trabajo representa la arquitectura central a considerar para otros modelos biológicos (v. gr. helmintofaunas principales de México), en el sentido de expandir nuevas pautas de conocimiento sobre la vida parasitaria.

CONCLUSIONES GENERALES

< La subfamilia Goodeinae (Osteichthys: Cyprinodontiformes: Goodeidae), incluye un total de 55 taxones de helmintos parásitos para 36 especies de goodeinos de las 42 que existen, de los cuales 10 son categorizados como especies especialistas (principales, desde el punto de vista biogeográfico) a esta subfamilia.

< El género *Margotrema* representa un grupo monofilético, en el cual se incluyen dos especies: *M. bravoae* Lamothe-Argumeto, 1970 y *M. resolanae* Pérez-Ponce de León, Martínez-Aquino & Mendoza-Garfías, 2013.

< *Margotrema bravoae* presenta una estructura genealógica que comprende tres linajes con evolución independiente asociados estrechamente a sistemas hidrológicos y a grupos particulares de huéspedes: a) *Margotrema bravoae* Linaje I, distribuido sobre los sistemas hidrológicos de los ríos Ayuquila, Balsas y Conchos y estando asociados a huéspedes de las tribus Ilyodontini, Chapalichthyini y a una especie de ciprínido (*Codoma ornata*). b) *Margotrema bravoae* Linaje II, distribuído sobre sistemas hidrológicos del río Lerma y asociado a huéspedes de la tribu Girardinichthyini y Chapalichthyini. c) *Margotrema bravoae* Linaje III, distribuído sobre sistemas hidrológicos del río Mezquital y asociado a un único huésped de la tribu Characodontini (*Characodon audax*).

< Los patrones de distribución geográfica de *Margotrema* están asociados con los eventos que fragmentaron a los sistemas hidrológicos del centro de México y que, a su vez, influenciaron también el patrón de distribución de sus huéspedes goodeinos. Por tanto, existe congruencia biogeográfica entre la historia genealógica de las poblaciones de *Margotrema* y la historia hidrogeomorfológica del centro de México, así como con la historia biogeográfica de Goodeinae.

< El proceso de dispersión causado por los huéspedes goodeinos jugó un papel relevante en el patrón de distribución actual de *Margotrema*, debido a la vagilidad que presentó históricamente el ancestro común más reciente de cada una de las tribus de Goodeinae, mismos que expandieron el área de distribución de *Margotrema* sobre los sistemas hidrológicos del centro de México.

< Existe congruencia entra las relaciones filogenéticas de Goodeinae (a nivel de especies y tribus) y la historia genealógica de *Margotrema* (a nivel de linajes y especies). A su vez, este patrón cofilogenético se observa en los tiempos de divergencia estimados para Goodeinae (a nivel de especies y tribus), y para especies y linajes de *Margotrema*.

< La relación *Goodeinae-Margotrema* representa tres niveles de asociaciones históricas distintas que reflejan que los procesos de codivergencia de los taxones de *Margotrema*, en primera instancia, son debidos a eventos vicariantes seguidos de los procesos de diversificación de los grupos monofiléticos de goodeinos: a) *Especie-Especie*. Representada por la asociación histórica *Xenotaenia resolanae-Margotrema resolanae,* exclusivo del Río Cuzalapa. b) *Especie-Linaje.* (Codivergencia Tipo I). Representada por la asociación histórica *Characodon audax-Margotrema bravoae* Linaje III, exclusivo del Río Mezquital Medio-Alto. c) *Tribu-Linaje*. (Codivergencia Tipo II). Representado, a su vez, por dos tipos de asociaciones históricas distintas: 1) Ilyodontini-*Margotrema bravoae* Linaje I, distribuido en los sistemas hidrológicos de los Ríos Ayuquila y Balsas. 2) Girardinichthyini / Chapalichthyini -*Margotrema bravoae* Linaje III, distribuido en el Río Lerma.

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APÉNDICE

HELMINTH PARASITES OF FRESHWATER FISHES FROM CUATRO CIÉNEGAS, COAHUILA, IN THE CHIHUAHUA DESERT OF MEXICO: INVENTORY AND BIOGEOGRAPHICAL IMPLICATIONS

Durante el transcurso del presente proyecto doctoral (financiado por el Consejo Nacional de Ciencia y Tecnología a través de una beca para estudios de doctorado dentro del Programa de Posgrado en Ciencias Biológicas, de la Universidad Nacional Autónoma de México), de manera paralela, se genero un artículo en extenso referente a la biodiversidad de helmintos parásitos de peces dulceacuícolas del norte de México el cuál se muestra a modo de apéndice en este trabajo. A continuación se presenta la galera de este articulo en extenso aceptado para su publicación, intitulado:

> **Helminth parasites of freshwater fishes from Cuatro Ciénegas, Coahuila, in the Chihuahua, desert of Mexico: Inventory and biogeographical implications** (Aguilar-Aguilar *et al*., 2013).

Helminth parasites of freshwater fishes from Cuatro Ciénegas, Coahuila, in the Chihuahuan desert of Mexico: Inventory and biogeographical implications

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ABSTRACT. As a part of an ongoing inventory of the helminth parasites of freshwater fishes in Mexico, 570 individual fish were collected between April 2008 and October 2011 in 26 localities along the Cuatro Ciénegas region in Coahuila State, northern Mexico. Seventeen species of hosts, mostly corresponding to Nearctic freshwater elements, were studied. A total of 8324 individual worms were collected during this survey, representing 25 species of helminths of which 9 were digeneans, 3 monogeneans, 1 cestode, 3 acanthocephalans, and 9 nematodes. Most of the records in this checklist represent new host or locality records. The information provided in this checklist may be helpful for our understanding of the biodiversity and historical biogeography of this host-parasite system, since in the Cuatro Ciénegas region occur a Nearctic freshwater fish fauna, along with Neotropical and endemic elements and from a biogeographical point of view, may represent a transitional area.

KEY WORDS. Monogenea, Digenea, Cestoda, Acanthocephala, Nematoda,

Freshwater fishes, Cuatro Ciénegas, Mexico.

INTRODUCTION

Cuatro Ciénegas is a small intermontane valley edge of the Sierra Madre Oriental in Coahuila, northern Mexico. The approximately 1000 square km desert valley of Cuatro Ciénegas possess the greatest number of endemic species of any place in North America (Stein *et al.* 2000), holding a large biodiversity and more than 70 endemic species. In this valley, diverse aquatic and semi-aquatic habitats such as springs, marshes, rivers and lakes are found (Marsh 1984), which support an unusually diverse fish fauna for a North American desert region, conformed by at least 16 native fish species, 8 of which are endemic (Minckley 1984). The freshwater fish helminth parasite fauna of Cuatro Ciénegas has been scarcely studied (Contreras 2004). Published records include the digeneans *Genarchella isabellae* (Lamothe-Argumedo, 1977), *Culuwiya cichlidorum* Aguirre-Macedo & Scholz, 2005, *Crassicutis cichlasomae* Manter, 1936, and *Maritrema* sp. (species originally described as *Quadripaludis luistoddi, Saccocoelioides* sp., *Crassicutis bravoae,* and *Maritreminoides* sp., respectively, see Pérez-Ponce de León *et al.* 2007), all of them parasitizing *Cichlasoma* spp. (Jiménez *et al.* 1981; Guajardo-Martinez 1984). Other records correspond to monogeneans of the family Dactylogyridae as parasites of *Cichlasoma* spp. and *Etheostoma* sp. (Guajardo-Martinez 1984), the acanthocephalans *Paulisentis* sp. in *Gambusia marshi* (Meffe 1985), and other unidentified species of digeneans and nematodes from *Etheostoma* sp., *Cyprinodon bifasciatus* Miller, 1968 and *Gambusia marshi* Minckley & Craddock, 1962 (Guajardo-Martinez 1984; Meffe 1985). Additional records of helminth parasites of native freshwater fishes of Coahuila in nearby localities of Cuatro Ciénegas include the monogenean *Diplostamenides spinicirrus* MacCallum, 1918 (= *Microcotyle* *spinicirrus*) on the gills of *Aplodinotus grunniens* Rafinesque, 1819 (Bravo-Hollis & Jiménez 1982), the digeneans *Diplostomulum* sp. and *Alloglossidium corti* (Lamont, 1921) infecting *Ictalurus punctatus* (Rafinesque, 1818) (Caballero-Padilla, 1988 in Pérez-Ponce de León *et al.* 2007), and the metacercariae of *Centrocestus formosanus* (Nishigori, 1824) as a parasite of *Cyprinella lutrensis* (Baird & Girard, 1853) (Aguilar-Aguilar *et al.* 2009).

Cuatro Ciénegas basin possesses a wide variety of freshwater fishes with a biogeographical affinity mostly to the Nearctic region since it is located in northern Mexico, close to the border with the U.S.A., but there are some Neotropical elements (Minckley 1984) that reach their most northern distribution limits in the zone, providing an interesting model to study potential parasite faunal exchange. For this reason, the present study brings together previously published information and new data we have gathered during recent survey in that particular area, in the context of a more comprehensive work we have conducted in northern Mexico (see Martínez-Aquino *et al.* 2007; Martínez-Aquino & Aguilar-Aguilar 2008; Mendoza-Palmero & Aguilar-Aguilar 2008; Aguilar-Aguilar *et al.* 2009, 2010; Pérez-Ponce de León *et al.* 2009, 2010). The aims of this paper are to record the helminth parasite fauna of several fish species from Cuatro Ciénegas in order to contribute to the inventory of metazoan parasites in freshwater fishes from northern Mexico, and to briefly discuss the biogeographical implications of our findings.

MATERIALS AND METHODS

A total of 570 fishes from 26 localities in Cuatro Ciénegas hydrological system and neighboring zones were collected between April, 2008 and October, 2011 (Fig. 1, Table 1). Seventeen taxa of fish were examined: Catostomidae: *Catostomus* sp. ($n = 3$);

Centrarchidae: *Lepomis megalotis* (Rafinesque, 1820) (*n* = 7), *Micropterus salmoides* (Lacepède, 1802) (*n* = 8); Characidae: *Astyanax mexicanus* (De Filippi, 1853) (*n* = 55); Cichlidae: *Hemichromis bimaculatus* Gill, 1862 (n = 21), *Herichthys cyanoguttatus* (Baird & Girard, 1854) (*n* = 3), *H. minckleyi* (Kornfield & Taylor, 1983) (*n* = 58); Cyprinidae: *Cyprinella lutrensis* (Baird & Girard, 1853) (*n* = 31), *C. xanthicara* (Minckley & Lytle, 1969) (*n* = 24), *Dionda episcopa* (Girard, 1856) (*n* = 20), *Notropis* sp. (*n* = 3); Cyprinodontidae: *Cyprinodon atrorus* Miller, 1968 (*n* = 108); *C. atrorus x bifasciatus* (n = 6), *C. bifaciatus* Miller, 1968 (n = 28), *Lucania interioris* Hubbs & Miller, 1965 (*n* = 2); Ictaluridae: *Ictalurus lupus* (Girard, 1858) (*n* = 4); and Poeciliidae: *Gambusia marshii* Minckley & Craddock, 1962 (*n* = 189). Fish were collected using minnow traps and seine nets and were kept alive and studied for helminths no more than 4 hrs after capture. Fish were sacrificed and immediately studied for helminths. All internal organs were placed separately in 0.65% saline and examined under the stereoscope. The gills from each host were dissected, placed in Petri dishes with tap water and examined for monogeneans, which were fixed in glycerin ammonium-picrate (GAP) in order to study their sclerotized structures. After the morphological evaluation, specimens fixed with GAP were remounted in Canada balsam following Ergens (1969); some specimens were fixed in hot (steaming) formalin 4%, stained with Gomori's trichrome and mounted in Canada balsam to study their internal organs. Endoparasites were counted *in situ* and removed to a Petri dish with 0.65% saline prior fixation. Platyhelminths were fixed with hot (steaming) 4% formalin. Acanthocephalans were maintained at 4°C for 12 h in distilled water, and then fixed in cold 70% ethanol. Nematodes were fixed with hot (steaming) 4% formalin or hot (steaming) 70% ethanol. All helminths were processed following standard procedures (Lamothe-Argumedo

1997). Identification was completed using specialized literature, and voucher specimens of most helminth taxa were deposited in the Colección Nacional de Helmintos (CNHE), Instituto de Biología, Universidad Nacional Autónoma de México, Mexico City, Mexico. Fish specimens were deposited at the Colección Nacional de Peces (CNPE), Instituto de Biología, Universidad Nacional Autónoma de México, Mexico City, Mexico. The use of prevalence (% infected), mean intensity (number of parasites of the same species per infected fish) and abundance (number of parasites of the same species per examined fish) follows Bush *et al*. (1997).

RESULTS

In this survey, 570 individual fish from Cuatro Ciénegas basin and neighboring areas, corresponding to 17 taxa were sampled, with a sampling size per species varying from 2 to 189 individuals. Fish from 26 localities were sampled; these sites were isolated water bodies such as springs and ponds, but also rivers and man-made channels (Table 1, Fig. 1). The host species with the largest sample size was *Gambusia marshi*, which seems to be the most common and abundant fish species in water bodies of the Cuatro Ciénegas region. The examined species of hosts (excepting *Catostomus* sp. and *Cyprinodon bifasciatus*, which were negative for helminth parasites) harbored between 1 and 10 helminth species, with the robust gambusia *G. marshi* being the host species harboring the largest number of helminth taxa. A total of 8324 individual worms were collected during this 4-yr survey, representing 25 species of helminths of which 9 were digeneans, 3 monogeneans, 1 cestode, 3 acanthocephalans, and 9 nematodes.

The results of this survey are presented in two tables. The first one is the parasite-host list (Table 2), in which parasite data is organized by helminth group, and species within each group are listed alphabetically. The scientific name and authority are presented, followed by the abbreviation indicating the habitat(s) where the helminth taxon was found within the fish. Next, the host species in which the helminth was found is listed, followed by the locality and the values of prevalence, mean abundance and mean intensity of infection. When more than one host species harbored the same helminth taxa, those are listed alphabetically, and host species for which more than one locality was recorded are listed together. The host-parasite list (Table 3) is organized alphabetically by fish family. Within each family, species are also listed in alphabetical order. Each fish species (excluding those not parasitized), is followed by the list of its parasites, which includes developmental stage and an abbreviation of the helminth group to which that parasite belongs. The parasites previously recorded in the same geographical region are identified with an asterisk with the proper reference in parentheses.

DISCUSSION

This inventory present herein represents the continuation of our efforts to describe the biodiversity of helminth parasites of freshwater fishes in Mexico, in this case following a geographical region approach (see Pérez-Ponce de León and Choudhury 2010), considering several localities in the desert valley of Cuatro Ciénegas. In this survey we recorded 25 species of helminths, bringing the total number of helminth species in Cuatro Ciénegas freshwater fishes to 30. Most of the records we establish here are new locality or host records. With the exception of some helminth taxa (e.g. Allocreadiidae gen. sp., Ancyrocephalinae gen. sp., and *Procamallanus* sp.), which require the collection of more specimens and further taxonomic work to establish their taxonomic identity, and the species *Characithecium costaricensis*, which possesses a wide distribution in the Neotropical region, from southern Mexico to South America

(Mendoza-Franco *et al.* 2009), all remaining taxa have been previously recorded in freshwater fishes in North America (Hoffman 1999; Vidal-Martínez *et al.* 2001, Caspeta-Mandujano 2010; Pérez-Ponce de León *et al.* 2007; García-Prieto *et al.* 2010).

Species of hosts that were analyzed are included in 12 genera and 7 families. A large proportion of the fish fauna we sampled are typically Nearctic elements, belonging to groups usually found in North America such as carps and minnows (*Cyprinella lutrensis*, *C. xanthicara*, *Dionda episcopa* and *Notropis* sp.), North American catfishes (*Ictalurus lupus*), pupfishes and killifishes (*Cyprinodon atrorus*, *C. bifasciatus*, *C. atrorus x bifasciatus* and *Lucania interioris*), and suckers and sunfishes (*Lepomis megalotis* and *Micropterus salmoides*). However, some Neotropical components of the freshwater fish fauna of Cuatro Ciénegas were also sampled, such as livebearers (*Gambusia marshi*), characins (*Astyanax mexicanus*), and cichlids (*Herichthys cyanoguttatus* and *H. minckleyi*) (Minckley 1984). One single exception is represented by the cichlid *Hemichromis bimaculatus* which represents an introduced species as a result of anthropogenic activities (Miller *et al.* 2005). The presence of freshwater fishes with Nearctic and Neotropical affinities sets Cuatro Ciénegas as an interesting transitional zone from a biogeographical point of view, and certainly, the Neotropical elements almost represent the most northern distribution range for these species. The list of helminth species reported here, in conjunction with previous records supports the prediction made by Pérez-Ponce de León and Choudhury (2005) that the parasite fauna is largely circumscribed by higher levels of monophyletic host taxa, especially to the level of fish family, corroborating the fact that the historical biogeography of the parasites is closely related to that of their hosts (Pérez-Ponce de León & Choudhury 2002, 2005; Aguilar-Aguilar *et al.* 2003, 2008). In this study 2 species of *Crassicutis* as well as one species of *Homalometron* were recognized based on a combination of molecular and morphological data. In this respect, molecular date were instrumental and necessary to establish more robust species delimitation criteria for these helminth species. In the case of the digeneans *Crassicutis* spp., we recognized lineage I from Razo-Mendivil *et al.* (2010) (an undescribed cryptic species) as a parasite of *H. cyanoguttatus*, and *C. cichlasomae* (*sensu lato*) as a parasite of *H. minckleyi*. In *Homalometron*, sequences of the ITS and 28S nuclear rDNA were used to recognize that our specimens actually correspond with *H. pallidum*, a species originally described from fundulids in several parts of North America (Hoffman, 1999). The record of *H. pallidum* in an endemic fish species in Cuatro Ciénegas (*Gambusia marshi*) poses an interesting question that deserves further investigation to actually demonstrate they are conspecific, or they may represent a cryptic species, but other molecular markers such as COI are needed to have conclusive data.

Our study reveals that some typical Neotropical helminth species, which are part of the biogeographical core fauna of characins and cichlids were found, e.g., the digeneans *Crassicutis cichlasomae* and *Crassicutis* sp. (lineage I) and *Rhabdochona kidderi* as parasites of cichlids; while *Creptotrematina aguirrepequenoi*, *Characithecium costaricensis*, and *Procamallanus neocaballeroi* in characins. Likewise, some typical Nearctic helminth species were found. Helminth species with Nearctic affinities recorded here are the monogeneans of the genus *Salsuginus*, the digeneans *H. pallidum* and *Microphallus* cf. *opacus* and the acanthocephalans *Leptorhynchoides thecatus* and *Pomphorhynchus bulbocolli*. Monogeneans of the genus *Salsuginus* have been recorded in the Nearctic portion of Mexico in goodeids and pupfishes (Martínez-Aquino *et al.* 2004, 2007; Mendoza-Palmero 2007; Martínez-well as one species of *Homalometron* were recognized based on a combination of molecular and morphological data. In this respect, molecular date were instrumental and necessary to establish more robust species delimitation criteria for these helminth species. In the case of the digeneans *Crassicutis* spp., we recognized lineage I from Razo-Mendivil *et al.* (2010) (an undescribed cryptic species) as a parasite of *H. cyanoguttatus*, and *C. cichlasomae* (*sensu lato*) as a parasite of *H. minckleyi*. In *Homalometron*, sequences of the ITS and 28S nuclear rDNA were used to recognize that our specimens actually correspond with *H. pallidum*, a species originally described from fundulids in several parts of North America (Hoffman, 1999). The record of *H. pallidum* in an endemic fish species in Cuatro Ciénegas (*Gambusia marshi*) poses an interesting question that deserves further investigation to actually demonstrate they are conspecific, or they may represent a cryptic species, but other molecular markers such as COI are needed to have conclusive data.

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Aquino & Aguilar-Aguilar 2008), while *P. bulbocolli* is likely to be a parasite of suckers, with some records in other fish groups (Hoffman 1999; Pérez-Ponce de León *et al.* 2009). The record of *H. pallidum* and *Microphallus* cf. *opacus* represents a Nearctic influence since both species are found in some species of freshwater fishes from North America (see Hoffman, 1999). Interestingly, specimens of *Microphallus* cf. *opacus* were immature worms and no eggs were found in the uterus, although symmetrical testes and ovary located in dextral and pretesticular position were observed; still, the pear-body shape, spined body surface, and testes position allowed us to tentatively determine that species of digenean. New samplings in the locality looking for mature worms are necessary to corroborate this taxonomic identification or to establish this as a new species since the extension of the ceca does not correspond with the diagnosis of the genus. Likewise, records of *L. thecatus* presented here include 6 host species, suggesting a low specificity, however, only gravid females of *L. thecatus* were found among the specimens obtained from the intestine of *Micropterus salmoides*, which strongly fit with findings made by Lincicome and VanCleave (1949), who observed that although *L. thecatus* was reported from a long list of hosts, the females do not produce eggs in the vast majority of the fishes that are not centrarchids. Our observations of the specimens we collected corroborate that observation, supporting the suggestions of some authors who have found a close association of this acanthocephalan with sunfishes (Steinauer *et al.* 2007). The strongest case of host specificity is exhibited by the acanthocephalan *Atactorhynchus duranguensis*, which had been recorded previously in pupfishes of the genus *Cyprinodon* (García-Prieto *et al.* 2010), and by *Characithecium costaricensis*, which appears to be only associated with characid fishes of the genus *Astyanax* (Mendoza-Franco *et al.* 2009). For that reason, both helminth species can be considered as a part of the biogeographical core fauna of cyprinodontids and characids, respectively.

Data presented here corroborate that Cuatro Ciénegas basin is a complex region, with a mixture of Nearctic and Neotropical fish components, which is reflected in its helminth fauna. However, the level of endemism of the fish helminth fauna in this region is lower than that of their fish hosts. Up to date, no endemic species of helminth have been recorded in this area, however, genetic information may show cases of cryptic species (morphologically similar but genetically distinct) (see Pérez-Ponce de León & Choudhury 2010). Likewise, further analyses are required in order to establish the taxonomic identity of the monogeneans identified as Ancyrocephalinae gen. sp. from *Herichthys minckleyi* and *H. cyanoguttatus* (which probably belong to an undescribed genus, Mendoza-Palmero pers. comm.), and the nematode *Procamallanus* sp. from the intestine of *Ictalurus lupus*.

Despite the fact that the Cuatro Ciénegas region is as a center of endemism for diverse taxa, no novel helminth taxa infecting freshwater fish were found in this work, which corroborates the prediction made by Pérez-Ponce de León and Choudhury (2010) who, based on the extent of the inventory of the freshwater fish helminths of Mexico generated up to 2010, argued that, excepting for monogeneans, the inventory is nearing completion for most groups, and for this reason, few additional novel taxa could be expected to be found. These authors also suggested a strategic survey work combining the need to target missing components of the host spectrum with the choice of appropriate drainages based on biogeographic, faunistic, and hydrologic data in order to enhance the biodiversity inventory. In this context, this work provides further data on the helminth fauna of freshwater fishes in a very important region of the country that has been scarcely studied for helminth parasites.

Introduction of exotic species of freshwater taxa to hydrological systems has occurred frequently in Mexico. The Cuatro Ciénegas basin in not an exception and currently several introduced species of fishes as well as diverse aquatic invertebrates can be found (Contreras-Arquieta 1998; March & Martínez 2007). This introduction has promoted the establishment of some exotic helminth species, as the digenean *Centrocestus formosanus* and the cestode *Bothriocephalus acheilognathi*, which are now found in freshwater fishes of Cuatro Ciénegas and neighboring zones, representing a potential risk for the suitable conservation of these fish species. Both helminth taxa were introduced to hydrological systems of Mexico within their hosts, and because their low host specificity and great capability to adapt to different environmental conditions that allow them to thrive and increase their host and distributional range, they actually are recorded parasitizing numerous freshwater fish species in Mexico (Scholz & Salgado-Maldonado 2000; Salgado-Maldonado & Pineda-López 2003; Gutiérrez-Cabrera *et al*. 2005; Pérez-Ponce de León *et al*. 2007, 2009, 2010; Rojas-Sánchez & Garcia-Prieto 2008; Aguilar-Aguilar *et al*. 2009, 2010a, 2010b; Ortega *et al*. 2009; Mendez *et al*. 2010).

The data that we present in this survey represent a preliminary source of information on the helminth parasites of Cuatro Ciénegas. Clearly, more studies are needed to complete the inventory, however, our findings contribute to the accurate description of the species richness patterns of the freshwater fish helminth parasite fauna in Mexico, particularly from the northern region, and in addition, this information is very useful to further contribute with the description of the evolutionary and biogeographical processes that determine the host-parasite association.

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Figure 1.

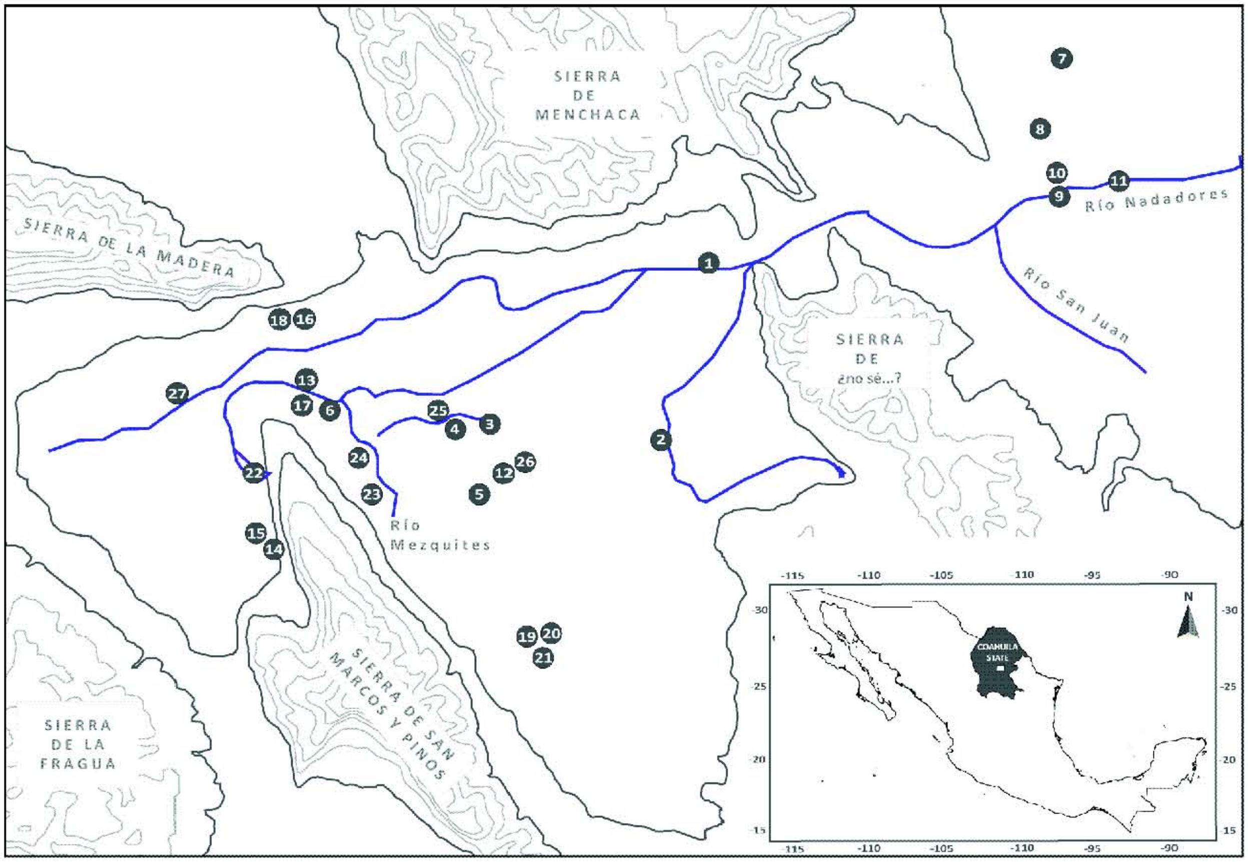
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TABLE 1. Localities in the Cuatro Ciénegas region where fish were collected for the helminth parasite survey. The numbers shown for each locality are used in the Fig. 1.

Code (Fig. 1)	Locality	Geographic reference
1	Canal San Juan Boquillas	26.9994 N, 101.8921 W
2	Canal entre la Vega y el Venado	26.9006 N, 101.9192 W
3	Poza Playitas	26.9095 N, 102.0139 W
4	Charcos Prietos	26.9099 N, 102.0360 W
5	Los Hundidos	26.8703 N, 102.0196 W
6	Río Mezquites	26.9172 N, 102.1031 W
7	Nadadores 1	27.1138 N, 101.6960 W
8	Nadadores 2 (Huizachal)	27.0738 N, 101.7078 W
9	Río en Celemania (Cortina)	27.0412 N, 101.6993 W
10	Río en Celemania	27.0446 N, 101.6985 W
11	Puente San José de las Águilas	27.0439 N, 101.6642 W
12	Poza temporal camino a Playitas	26.8823 N, 102.0062 W
13	Arroyo junto a Estación	29.9305 N, 102.1191 W
14	Manantial en Churince	26.8403 N, 102.1341 W
15	Laguna Intermedia	26.8485 N, 102.1434 W
16	Anteojo San Juan	26.9694 N, 102.1205 W
17	El Mojarral	26.9230 N, 102.1177 W
18	Antiojo	26.9694 N, 102.1275 W
19	Camino hacia La Tecla	26.7900 N, 102.0000 W
20	Santa Tecla	26.7908 N, 101.9932 W
21	La Teclita	26.7792 N, 101.9846 W
22	Poza La Becerra	26.8780 N, 102.1380 W
23	Poza Tío Cándido	26.8704 N, 102.0784 W
24	Camino hacia Playitas	26.9140 N, 102.0396 W
25	Poza Los Gatos	26.8881 N, 101.9980 W
26	El garabatal	26.9207 N, 102.1894 W

TABLE 2. Parasite-Host-Locality list with prevalence / mean abundance / mean intensity values. Sample sizes per locality are shown in parenthesis following the locality.

Digenea		
Allocreadiidae gen sp. (I)		
Gambusia marshi	Los Hundidos (8)	12.5 / 0.13 / 1
***Ascocotyle* sp.** (IW, H)		
Gambusia marshi	Canal entre La Vega y el Venado (3)	66.67 / 0.67 / 1
	La Teclita (3)	66.67 / 6.33 / 9.5
	Camino hacia la Tecla (6)	16.67 / 0.33 / 2
	Santa Tecla (3)	66.67 / 1.33 / 2
Centrocestus formosanus (Nishigori, 1924) **(G)**		
Astyanax mexicanus	Canal entre La Vega y el Venado (7)	28.57 / 1.71 / 6
	Rio en Celemania (19)	5.26 / 0.32 / 6
	Anteojo San Juan (7)	14.29 / 8.43 / 59
	Poza La Becerrra (4)	25 / 1 / 4
Cyprinella lutrensis	Puente San José de las Águilas (31)	
Remarks: This record was described separately by Aguilar-Aguilar *et al*. (2009)		
Cyprinella xanthicara	Canal entre La Vega y el Venado (1)	100 / 8 / 8
Cyprinodon atrorus x bifasciatus	Laguna intermedia (5)	20 / 17.6 / 88
Gambusia marshi	Puente San José de las Águilas (1)	100 / 2 / 2
	Poza La Becerra (7)	28.57 / 44 / 154
Lepomis megalotis	Rio en Celemania (2)	100 / 80 / 80
Micropterus salmoides	Puente San José de las Águilas (3)	100 / 14 / 14
Notropis sp.	Rio en Celemania (3)	66.67 / 263 / 394.5
Crassicutis cichlasomae Manter, 1936 (I)		
Herichthys minckleyi	Charcos Prietos (6)	83.33 / 23.83 / 28.6
	Laguna intermedia (3)	33.33 / 11.67 / 35
	Rio Mezquites (21)	42.86 / 1.43 / 3.33
	Poza La Becerra (9)	11.11 / 0.56 / 5
***Crassicutis* sp. (lineage I)** (I)		
Herichthys cyanoguttatus	Puente San José de las Águilas (1)	100 / 48 / 48
Creptotrematina aguirrepequenoi (Jiménez-Guzmán, 1973) (I)		
Astyanax mexicanus	Rio en Celemania (19)	5.26 / 0.11 / 2
Homalometron pallidum Stafford, 1904 (I)		
Gambusia marshi	Los Hundidos (8)	62.5 / 0.75 / 1.2
	Charcos Prietos (4)	25 / 0.75 / 3
	Poza Temporal hacia Playitas (7)	57.14 / 0.86 / 1.5
	Arroyo en La Estación (5)	20 / 0.2 / 1
	Manantial de Churince (49)	2.04 / 0.02 / 1
Microphallus* cf. *opacus (Ward, 1894)		
Cyprinodon atrorus	Poza Los Gatos (23)	4.35 / 0.13 / 3
	El Garabatal (6)	16.67 / 0.17 / 1
Posthodiplostomum minimum (MacCallum, 1921) (M)		
Cyprinodon atrorus	Poza temporal hacia playitas (13)	7.69 / 0.08 / 1
	Poza Los Gatos (23)	21.74 / 2.52 / 11.6

Gambusia marshi	Charcos prietos (4)	75 / 6.25 / 8.33
	Rio Mezquites (10)	10 / 0.1 / 1
Micropterus salmoides	Camino hacia Playitas (3)	33.33 / 1.67 / 5
	Camino hacia Playitas (1)	100 / 1 / 1
Monogenea		
Ancyrocephalinae gen. sp. (G)		
Herichthys cyanoguttatus	Rio en Celemania (2)	100 / 2.5 / 2.5
Herichthys minckleyi	Charcos Prietos (6)	16.67 / 0.33 / 2
	Poza La Becerra (9)	22.22 / 3.56 / 16
	Poza Tio Cándidio (1)	100 / 23 / 23
Characithecium costaricensis (Price & Bussing, 1967) (G)		
Astyanax mexicanus	Canal entre La Vega y el Venado (7)	71.43 / 27.71 / 38.8
	Anteojo San Juan (7)	57.14 / 4.71 / 8.25
	Poza La Becerra (4)	50 / 1.75 / 3.5
***Salsuginus* sp.** (G)		
Cyprinodon atrorus	Poza Playitas (44)	4.55 / 0.07 / 1.5
	Manantial de Churince (18)	22.22 / 0.5 / 2.25
Cyprinodon atrorus x bifasciatus	Laguna Intermedia (5)	20 / 0.2 / 1
Cestoda		
Bothriocephalus acheilognathi Yamaguti, 1934 (I)		
Cyprinella xanthicara	Rio Mezquites (2)	100 / 2.5 / 2.5
Gambusia marshi	Canal San Juan Boquillas (17)	11.76 / 0.12 / 1
Nematoda		
***Contracaecum* sp.** (BC)		
Herichthys minckleyi	Santa Tecla (6)	16.67 / 0.17 / 1
	Rio Mezquites (21)	4.76 / 0.05 / 1
	Poza Tio Cándido (1)	100 / 1 / 1
***Eustrongylides* sp.** (BC)		
Gambusia marshi	Nadadores 1 (21)	4.76 / 0.05 / 1
	Nadadores at Huizachal (7)	14.29 / 0.14 / 1
Herichthys minckleyi	Poza La Becerra (9)	11.11 / 0.56 / 5
Lepomis megalotis	Rio Mezquites (1)	100 / 1 / 1
Lucania interioris	Camino hacia Playitas (2)	50 / 0.5 / 1
***Procamallanus* sp.** (I)		
Ictalurus lupus	Charcos prietos (2)	50 / 1 / 2
Procamallanus neocaballeroi (Caballero-Deloya, 1977) (I)		
Astyanax mexicanus	Rio en Celemania (19)	68.42 / 1.11 / 1.62
***Rhabdochona* sp.** (I)		
Cyprinella xanthicara	Rio Mezquites (2)	50 / 0.5 / 1
	Laguna intermedia (17)	5.88 / 0.18 / 3
Ictalurus lupus	El Antiojo (2)	100 / 3 / 3
Lucania interioris	Camino hacia Playitas (2)	100 / 4.5 / 4.5
Rhabdochona kidderi (Pearse, 1936) (I)		
Gambusia marshi	Canal entre La Vega y el Venado (3)	33.33 / 0.67 / 2
	Charcos prietos (4)	50 / 1 / 2
Herichthys cyanoguttatus	Rio en Celemania (2)	100 / 7 / 7
Herichthys minckleyi	Canal entre La Vega y el Venado (1)	100 / 20 / 20

	Charcos prietos (6)	16.67 / 0.17 / 1
	Rio Mezquites (21)	42.86 / 2 / 4.67
	Poza La Becerra (9)	11.11 / 0.33 / 3
Serpinema trispinosum (Leidy, 1852) (I)		
Lepomis megalotis	Rio Mezquites (1)	100 / 1 / 1
	Manantial en Churince (4)	25 / 0.25 /1
Micropterus salmoides	Charcos prietos (1)	100 / 3 / 3
***Spinitectus* sp.** (I)		
Micropterus salmoides	Rio Mezquites (1)	100 / 10 / 10
***Spiroxys* sp.** (M)		
Astyanax mexicanus	Rio en Celemania (19)	42.11 / 1.68 / 4
	Camino hacia La Tecla (2)	50 / 0.5 / 1
	Poza La Becerra (4)	25 / 4.5 / 18
	Poza Tio Cándido (16)	100 / 11.13 / 11.13
Cyprinella xanthicara	Laguna Intermedia (17)	5.88 / 0.06 / 1
	Santa Tecla (3)	66.67 / 0.67 / 1
Cyprinodon atrorus	Poza Playitas (44)	6.82 / 0.07 / 1
	Poza temporal hacia playitas (13)	30.77 / 0.46 / 1.5
Dionda episcopa	Los Hundidos (20)	5 / 0.05 / 1
Gambusia marshi	Canal entre La Vega y el Venado (3)	33.33 / 0.33 / 1
	Poza Playitas (13)	7.69 / 0.08 / 1
	Poza temporal hacia playitas (7)	14.29 / 0.29 / 2
	La Teclita (3)	66.67 / 6.33 / 2
Hemichromis bimaculatus	Manantial en Churince (1)	100 / 4 / 4
	Laguna Intermedia (20)	25 / 0.35 / 1.4
Herichthys minckleyi	Charcos prietos (6)	50 / 5 / 10
	Rio Mezquites (21)	9.52 / 2.33 / 24.5
	Santa Tecla (6)	16.67 / 0.33 / 2
	Poza La Becerra (9)	11.11 / 0.22 / 2
	Camino hacia Playitas (1)	100 / 1 / 1
Lepomis megalotis	Rio en Celemania (2)	100 / 1.5 / 1.5
	Rio Mezquites (1)	100 / 1 / 1
Micropterus salmoides	Camino hacia Playitas (3)	66.67 / 0.67 / 1
Notropis sp.	Rio en Celemania (3)	33.33 / 0.67 / 2
Acanthocephala		
Atactorhynchus duranguensis Salgado-Maldonado, Aguilar-Aguilar & Cabañas-Carranza, 2005 (I)		
Cyprinodon atrorus	Poza Playitas (44)	40.91 / 0.64 / 1.56
	Poza temporal hacia playitas (13)	30.77 / 0.77 / 2.5
	Manantial en Churince (18)	38.89 / 0.61 / 1.57
	Laguna intermedia (3)	66.67 / 0.67 / 1
	Poza Los Gatos (23)	52.17 / 1.22 / 2.33
Cyprinodon atrorus x *bifasciatus*	Laguna intermedia (5)	80 / 1.4 / 1.75
Leptorhynchoides thecatus Linton, 1891 (I)		
Cyprinodon atrorus	Poza Playitas (44)	15.91 / 0.18 / 1.14
Gambusia marshi.	Poza Playitas (13)	7.69 / 0.08 / 1
	Charcos prietos (4)	25 / 0.25 / 1
	Poza temporal hacia playitas (7)	14.29 / 0.57 / 4
Herichthys minckleyi	Charcos prietos (6)	83.33 / 3 / 3.6

Ictalurus lupus	Charcos prietos (2)	100 / 15 / 15
Lepomis megalotis	Rio en Celemania (2)	50 / 0.5 / 1
	Manantial en Churince (4)	75 / 0.75 / 1
Micropterus salmoides	Charcos prietos (1)	100 / 6 / 6
	Camino hacia Playitas (3)	33.33 / 0.67 / 2
Pomphorhynchus bulbocolli (Van Cleave, 1916) (I)		
Cyprinella lutrensis	Puente San José de las Águilas (31)	12.9 / 0.32 / 2.5

BC = Body Cavity, G = Gills, GB = Gall bladder, H = Heart, I = Intestine, IC = Intestinal caeca, IW= Intestinal wall, L = Liver, M = Mesentery, UB = Urinary bladder.

TABLE 3. Host-Parasite list. Parasites previously recorded in the same host species in the region are marked with an asterisk, followed with the bibliographical reference in parenthesis.

Centrarchidae

Lepomis megalotis
- *Centrocestus formosanus* (Metacercariae, D)
- *Eustrongylides* sp. (Larvae, N)
- *Leptorhynchoides thecatus* (Adult, A)
- *Serpinema trispinosum* (Larvae, N)
- *Spiroxys* sp. (Larvae, N)

Micropterus salmoides
- *Centrocestus formosanus* (Metacercariae, D)
- *Leptorhynchoides thecatus* (Adult, A)
- *Posthodiplostomum minimum* (Metacercariae, D)
- *Serpinema trispinosum* (Larvae, N)
- *Spinitectus* sp. (Adult, N)
- *Spiroxys* sp. (Larvae, N)

Characidae

Astyanax mexicanus
- *Centrocestus formosanus* (Metacercariae, D)
- *Characithecium costaricensis* (Adult, M)
- *Creptotrema* aguirrepequenoi (Adult, N)
- *Procamallanus neocaballeroi* (Adult, N)
- *Spiroxys* sp. (Larvae, N)

Cichlidae

Hemichromis bimaculatus
- *Spiroxys sp.* (Larvae, N)

Herichthys cyanoguttatus
- Ancyrocephalinae gen. sp. (Adult, M)
- *Crassicutis cichlasomae* (Adult, D) * (as *C. bravoae* by Guajardo-Martinez 1984)
- *Rhabdochona kidderi* (Adult, N)

Herichthys minckleyi
- Ancyrocephalinae gen. sp. (Adult, M)
- *Contracaecum* sp. (Larvae, N)

  Crassicutis cichlasomae (Adult, D)
  Eustrongylides sp. (Larvae, N)
  Leptorhynchoides thecatus (Adult, A)
  Rhabdochona kidderi (Adult, N)
  Spiroxys sp. (Larvae, N)

Cyprinidae

Cyprinella lutrensis
  Centrocestus formosanus (Metacercariae, D) *(Aguilar-Aguilar *et al.* 2009)
  Pomphorhynchus bulbocolli (Adult?, A)

Cyprinella xanthicara
  Bothriocephalus acheilognathi (Adult, C)
  Centrocestus formosanus (Metacercariae, D)
  Rhabdochona sp. (Larvae, N)
  Spiroxys sp. (Larvae, N)

Dionda episcopa
  Spiroxys sp. (Larvae, N)

Notropis sp.
  Centrocestus formosanus (Metacercariae, D)
  Spiroxys sp. (Larvae, N)

Cyprinodontidae

Cyprinodon atrorus
  Atactorhynchus duranguensis (Adult, A)
  Gyrodactylus sp. (Adult, M)
  Leptorhynchoides thecatus (Adult, A)
  Microphallus cf. *opacus* (Adult, A)
  Posthodiplostomum minimum (Metacercariae, D)
  Salsuginus sp. (Adult, M)
  Spiroxys sp. (Larvae, N)

Cyprinodon atrorus x bifasciatus
  Atactorhynchus duranguensis (Adult, A)
  Centrocestus formosanus (Metacercariae, D)
  Salsuginus sp. (Adult, M)

Lucania interioris
  Eustrongylides sp. (Larvae, N)

- *Rhabdochona* sp. (Larvae, N)

Ictaluridae

Ictalurus lupus

- *Leptorhynchoides thecatus* (Adult, A)
- *Procamallanus* sp. (Adult, N)
- *Rhabdochona* sp. (Larvae, N)

Poeciliidae

Gambusia marshi

- Allocreadiidae gen sp. (Adult, D)
- *Ascocotyle* sp. (Metacercariae, D)
- *Bothriocephalus acheilognathi* (Adult, C)
- *Centrocestus formosanus* (Metacercariae, D)
- *Eustrongylides* sp. (Larvae, N)
- *Homalometron pallidum* (Adult, D)
- *Leptorhynchoides thecatus* (Adult, A)
- *Posthodiplostomum minimum* (Metacercariae, D)
- *Rhabdochona kidderi* (Adult, N)
- *Spiroxys* sp. (Larvae, N)

A = Acanthocephalan, C = Cestoda, D = Digenean, M = Monogenean, N = Nematoda.