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HISTORIA BIOGEOGRÁFICA Y SISTEMÁTICA DE LA HELMINTOFAUNA DE GOODEIDAE EN LA MESA CENTRAL DE MÉXICO

T E S I S

QUE PARA OBTENER EL GRADO ACADÉMICO DE

DOCTOR EN CIENCIAS

P R E S E N T A

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Por medio de la presente me permito informar a usted que en la reunión ordinaria del Comité Académico del Posgrado en Ciencias Biológicas, celebrada el día 27 de junio del 2005, se acordó poner a su consideración el siguiente jurado para el examen de DOCTOR EN CIENCIAS del alumno **MEJIA MADRID HUGO HARLAN** con número de cuenta **82539384** y número de expediente **71660**, con la tesis titulada: **"Historia Biogeográfica y Sistemática de la Helmintofauna de Goodeidae en la mesa central de México"**, bajo la dirección del **Dr. Gerardo Pérez Ponce de León.**

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Sin otro particular, quedo de usted.

A t e n t a m e n t e
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TABLA DE CONTENIDOS

RESUMEN. Los peces de agua dulce del centro de México, y en particular aquéllos que se distribuyen en la región conocida como Mesa Central, han tenido una diversificación muy grande por lo menos desde el Mioceno, en comparación con otras cuencas de Norteamérica. Por lo tanto su helmintofauna debe seguir un patrón de diversificiación semejante.

Se realizó el estudio de la bioeografía y sistemática de la helmintofauna de la subfamilia Goodeinae en la Mesa Central de México. El objetivo general de esta investigación fue inferir la historia biogeográfica de las cuencas hidrológicas de la Mesa Central de México con base en los helmintos en estado adulto de una subfamilia de peces de agua dulce diversificada en esta región, la subfamilia Goodeinae. Para esto se utilizaron 3 modelos de estudio: los helmintos de los peces de la subfamilia Goodeinae, la filogenia del nemátodo parásito de peces de agua dulce *Rhabdochona* Railliet, 1916 y la filogeografía de una especie del género de nematodos estudiado *Rhabdochona lichtenfelsi* Sánchez-Álvarez, García-Prieto et Pérez-Ponce de León, 1998.

El primer objetivo particular de dicha investigación, el faunístico, nos da como resultado un total de 10 especies de helmintos distribuidos en 32 especies de goodéinos. Estos registros incluyeron a 4 digéneos, 2 céstodos y 4 nemátodos. Entre los digéneos se registró a *Allocreadium lobatum* (primer registro para México), *A. mexicanum*, *Margotrema bravoae* y M. *guillerminae*. Entre los céstodos se registró a *Proteocephalus longicollis* (Cestoda) y a *Bothriocephalus acheilognathi*. Entre los nematodos, se describió una nueva especie, endémica de goodéinos, *Rhabdochona ahuehuellensis* y se registraron otras 3 especies pertenecientes al género *Rhabdochona*: *R. lichtenfelsi*, *R. mexicana*, y *R. xiphophori*. Se obtuvieron 29 nuevos registros de hospedero y 48 nuevos registros de localidad. Entre estas especies solamente 4 pueden ser consideradas especies núcleo, es

decir, que predeciblemente se les puede encontrar solamente en goodéinos por su enorme distribución y abundancia.

En el segundo objetivo de esta investigación, el filogenético, se encontró que el helminto más diverso y abundante de estas comunidades parasitarias, *R. lichtenfelsi*, no es la especie hermana de otras especies del mismo género que se encuentran en la Mesa Central u otras cuencas relacionadas. Solamente parece ser que *R. fortunatowi*, especie europea, es su especie hermana, sin embargo, *R. ahuehuellensis* y *R. xiphophori* se encuentran en clados distintos, lo cual indica que las especies de *Rhabdochona* de la Mesa Central no son monofiléticas y por lo tanto no indican relación alguna entre las cuencas de la Mesa Central.

El tercer objetivo de esta investigación, el filogeográfico, arroja mejores datos y conclusiones en cuanto a la historia biogeográfica de la Mesa Central, y concretamente, de las cuencas occidentales de la misma. Los haplotipos de *R. lichtenfelsi* ofrecen información sobre: a) la distribución actual de *R. lichtenfelsi* corresponde a aislamiento por distancia, lo cual indica que los hospederos de dichos nematodos llevaron a sus helmintos a cuencas que se separaron por lo menos en los últimos 9 millones de años, y rara vez se reconectaron; b) las cuencas del la región occidental de la Mesa Central estuvieron interconectadas entre sí, por lo menos desde hace 9.8 millones de años; c) los patrones observados en la distribución espacio-temporal de dichos haplotipos corresponde en algunos casos a la historia geológica y de otros grupos de animales de agua dulce, concretamente, al de los goodéinos.

La conclusión general de este trabajo de investigación es que los helmintos de peces de agua dulce de la Mesa Central, concretamente, la helmintofauna de la subfamilia Goodeindae, son un buen modelo a nivel poblacional debido a que no han respondido a eventos de especiación como lo han hecho sus hospederos, pero sí a procesos de

diversificación genética que corresponden a los eventos geológicos más importantes que han conformado la distribución actual de las cuencas de la región occidental de la Mesa Central. Adicionalmente, la presente investigación indica que las tasas de mutación entre estos helmintos y sus hospederos son distintas, probablemente siendo más lenta la de *R. lichtenfelsi* si se le compara con la de sus hospederos.

ABSTRACT. Freshwater fish from Central México and particularly those that are distributed in the region known as Mesa Central have had a widespread diversification dating from at least the Miocene if compared to other basins of North America. Therefore, its helminthfauna must follow a similar diversification pattern.

A biogeographic and systematic study of the helminthfauna of the subfamily Goodeinae of Mesa Central of México was undertaken. The main aim of this research was to infer the historical biogeography of the hydrologic basins of Mesa Central of Mexico based on adult endohelminths of the most diversified subfamily of freshwater fishes of this region, namely, the Goodeinae. To undertake this research 3 models were developed: the detailed study of the helminthfauna of Goodeinae, the phylogeny of the nematode parasite of freshwater fishes *Rhabdochona* Railliet, 1916 and the phylogeography of one species of the nematode genus studied *Rhabdochona lichtenfelsi* Sánchez-Álvarez, García-Prieto et Pérez-Ponce de León, 1998.

The first particular aim of this research, the faunistic one, resulted in a report totalling 10 species of helminths distributed among 32 species of goodeines. This report included 4 digeneans, 2 cestodes and 4 nematodes. Among the digeneans *Allocreadium lobatum* (first report in Mexico), *A. mexicanum*, *Margotrema bravoae* and *M. guillerminae* are reported. The cestodes, *Proteocephalus longicollis* (Cestoda) and *Bothriocephalus acheilognathi* are reported. A new species of nematode of goodeines was described,

Rhabdochona ahuehuellensis. Nematodes belonging to other 3 species of *Rhabdochona* are reported: *R. lichtenfelsi*, *R. mexicana*, and *R. xiphophori*. New host records totalled 29 and 48 new localities are reported for all helminths. Only 4 species are considered as the core species of goodeines, this means that only these species can be predicted to occur in goodeines as their distribution and abundance shows.

In the second aim of this research, the phylogenetic one, it was found that the most diverse and abundant helminth of these parasite communities, *R. lichtenfelsi*, is not sister species to other ones that belong to the same genus and are found in Mesa Central or other related basins. It seems that only *R. fortunatowi*, an European species, is its sister species. *Rhabdochona ahuehuellensis* and *R. xiphophori* belong to the same clade but in distinct positions. Therefore the species of *Rhabdochona* from Mesa Central are not monophyletic and show no relationship between the basins of Mesa Central.

The third aim, phylogeography, shows better results and conclusions. The haplotypes of *R. lichtenfelsi* show that: a) the modern distribution of *R. lichtenfelsi* corresponds to isolation by distance. This indicates that the hosts of this nematode dispersed their helminths to basins that afterwards separated at least during the last 9 million years and rarely reconnected again; b) the basins of the occidental region of Mesa Central were interconnected at least after 9.8 m.a.; c) the pattern observed in space-time of such haplotypes corresponds to the geologic history and distribution of other freshwater animals, specifically, the goodeines.

INTRODUCCIÓN.

Los helmintos son organismos que al explotar hábitats más restringidos espacialmente y más compartamentalizados que los de sus hospederos (Price, 1980) son indicadores finos de diversos procesos biológicos y geológicos a distintas escalas: poblacional, comunitaria, ecosistémica y biogeográfica. En sus ciclos de vida utilizan varios hospederos, lo cual agrega información mucho más detallada a la información evolutiva y ambiental proveniente de organismos de mayor talla, debido a que los cambios en sus condiciones tanto bióticas como abióticas nos darían una extensa información adicional con respecto a todo tipo de cambio ambiental (Brooks et al., 2001). Esto equivaldría a estudiar un organismo de vida libre que en cada fase de su ontogenia habitara un lugar distinto, cada uno localizado a varias decenas o cientos de kilómetros. Un cambio en cada uno de los distintos hábitats (varios hospederos y fases de vida libre o latentes) que los helmintos explotan durante todo su desarrollo nos daría información única y en plazos más cortos sobre los efectos en sus ciclos de vida a distintas escalas espacio-temporales.

De lo anterior se desprende que el origen de nuevas especies de parásitos tendrán lugar si los cambios en distintas escalas de tiempo geológico sumandos a grandes cambios ambientales afectan los ciclos de vida de los parásitos, ya sea por cambios de hospedero o cambio en el habitat de los hospederos conjuntamente con sus parásitos. Esto es lo que estudia la biogeografía histórica.

Prácticamente todos los continentes de la Tierra han sido investigados en cuanto a la fauna parasitaria de animales y plantas silvestres. El acopio de esta información ha permitido generar una de las bases de datos sobre biodiversidad mundial más importantes de la actualidad. Las bases de datos actuales son de una considerable calidad, sin embargo,

en cuanto a cantidad, hace falta más información detallada de distintos grupos de hospederos y varias regiones biogeográficas (Brooks & McLennan, 1993).

En la actualidad se cuenta con información empírica para poder sugerir que los helmintos parásitos de vertebrados acuáticos son modelos biológicos que arrojan información crítica sobre patrones y procesos evolutivos. Las herramientas de la biología que nos permiten estudiar estos patrones y procesos son: la sistemática filogenética, para dilucidar en primera instancia las relaciones filogenéticas de los helmintos; la biogeografía filogenética, para reconocer patrones de distribución y especiación; y por último, la filogeografía, para descubrir los patrones y los procesos de distribución espacial a través de la genética de poblaciones a niveles específico e infraespecífico y de esta forma afinar las relaciones biogeográficas a través del parentesco no solamente entre especies, sino entre individuos de una misma especie. Por lo tanto, la información que puede obtenerse del estudio de los helmintos parásitos de peces de aguas continentales es potencialmente muy diversa.

Los descubrimientos sistemáticos de la especiación en parásitos y, particularmente de los helmintos, se cuentan entre las primeras evidencias biológicas de la deriva continental (para un recuento histórico ver Brooks & McLennan, 1993). En las primeras investigaciones al respecto (fines del siglo XIX y principios del XX) se generó la idea que los parásitos evolucionaban por el fenómeno de la ortogénesis, es decir, que la evolución de aquéllos dependía de la de sus hospederos. De aquí se desprendió otra idea: que cada hospedero tiene un conjunto exclusivo de parásitos que lo infectan (especificidad hospedatoria). Sin embargo, existían datos documentados empíricamente que indicaban que en algunos casos ésto no sucede, especialmente cuando una especie de parásito infecta a distintas especies o grupos supraespecíficos de hospederos (distribución amplia).

Fue solamente hasta finales de la década de los años setenta (Brooks, 1977, 1979) que se produjeron los primeros trabajos sobre la evolución de los helmintos parásitos en un contexto moderno, al incorporar las nuevas ideas de la sistemática filogenética a la parasitología (Hennig 1966; Wiley, 1981). Finalmente, se descubrió que los parásitos y sus hospederos en ocasiones presentan patrones de coevolución (Brooks & McLennan, 1993), lo cual implica una dependencia biológica entre 2 linajes, el del parásito y el del hospedero; o bien, tienen filogenias distintas, es decir, historias evolutivas independientes, lo cual implica que no existe una dependencia biológica biunívoca, entre un linaje de parásitos y uno de hospederos.

La primera generación de parasitólogos que estudió el fenómeno de especiación en los parásitos entendió que éstos podrían ayudar a dilucidar las relaciones filogenéticas de sus hospederos y por lo tanto, las conexiones antiguas entre distintas áreas geográficas (Brooks & McLennan, 1993) debido a que son organismos que explotan hábitats más restringidos espacialmente que los de sus hospederos.

Esto quiere decir que los helmintos parásitos han dejado "rastros" de la misma evolución, no solo de sus hospederos sino también de las áreas que habitan alrededor del globo terrestre. El modo de especiación que predomina en los helmintos parásitos es, al igual que en otros grupos, el alopátrico y específicamente el de aislados periféricos también conocido como modelo del fundador, modelo IB, modelo II alopátrico, peripatría (Lynch, 1989). Se ha demostrado en repetidas ocasiones, que los parásitos, incluidos los helmintos, tienen distintas tasas de especiación con respecto a sus hospederos (Price, 1980). En algunos casos se ha descubierto coevolución de helmintos y sus hospederos (en nemátodos parásitos de monos catarrinos: Brooks & Glen, 1982; Brooks et al., 2002; Brooks, 1992, en mantarrayas y platelmintos parásitos: Brooks & McLennan, 2003). Estos trabajos ponen de

manifiesto que la especiación de los helmintos (y de los parásitos en general) y sus hospederos puede ser un evento interdependiente en algunos casos (coevolución) (Brooks & McLennan, 1993) o bien, independiente en otros, lo cual se ha observado al encontrar filogenias concordantes (Brooks & Glen, 1982; Brooks et al., 2002; Brooks, 1992, Brooks & McLennan, 2003) y discordantes (Hoberg, Gardner, 2004), respectivamente. Esto es fuente de información adicional para los estudios no solamente de los linajes de los mismos helmintos (sistemática), sino también para el estudio de la biogeografía, la biodiversidad y la ecología. A esta forma de abordar los problemas evolutivos por medio de la evolución de los parásitos se conoce con el nombre *parascript*, es decir, el estudio evolutivo de los parásitos que revela la historia evolutiva de sus hospederos y de las áreas que habitan (Brooks & McLennan, 1993).

Dentro de los helmintos más intensamente estudiados en México se encuentran aquellos que parasitan a vertebrados acuáticos (Pérez-Ponce de León et al., 1996; Pérez-Ponce de León & García-Prieto, 2001). Éstos son una fuente importante de información con relación a la biodiversidad global (Brooks et al., 2000 y referencias incluidas; Choudhury & Dick, 2000), a la sistemática filogenética (Brooks et al., 2000 y referencias incluidas) y a la biogeografía (Pérez-Ponce de León & Choudhury, 2002, 2005) debido a que se encuentran confinados a cuencas, en algunos casos endorréicas. Esto quiere decir que los helmintos parásitos de vertebrados acuáticos son modelos adecuados para describir fenómenos de vicarianza y/o dispersión, principalmente los que habitan en las cuencas mayores de México, infectando a los principales grupos de peces dulceacuícolas, donde se ha recogido una gran parte de la información que actualmente existe en las bases de datos de los helmintos de México (Pérez-Ponce de León et al., 1996; Lamothe et al., 1997; Pérez-Ponce de León & García-Prieto, 2001).

El hecho empírico más sobresaliente de la distribución actual de los helmintos parásitos que completan su ciclo de vida en vertebrados acuáticos, es que se encuentran confinados, no solo a hospederos que completan su ciclo de vida en el agua, sino a cuencas o subcuencas de aguas continentales. Además, los helmintos de estas zonas representan la prueba crítica más importante de la relación histórica entre las distintas cuencas, ya que se ha demostrado que éstos no son buenos dispersores y por ende tampoco colonizadores (Kennedy, 1976), siempre y cuando completen su ciclo de vida dentro de sus hospederos acuáticos. Esto quiere decir en una frase, *que los helmintos parásitos de vertebrados acuáticos han acumulado evidencia evolutiva de la historia de la biota de las aguas continentales.*

De ésto se desprende que los helmintos a estudiar deben de ser parásitos de vertebrados que se encuentren restringidos a las aguas dulces, como es el caso de los peces primarios, aunque también los peces secundarios y vicarios son buenos modelos, debido a que aunque tienen orígenes marinos o son tolerantes a la salinidad, actualmente se encuentran confinados en las aguas dulces. Los estudios existentes de la diversidad de los helmintos parásitos de peces de agua dulce en México han dado el primer paso para el estudio de sus relaciones filogenéticas y de su distribución geográfica.

La filogeografía es una disciplina nueva enmarcada dentro de la biogeografía y la genética de poblaciones (Avise, 1998, 2000). Sus objetivos son recuperar las filogenias de genes, principalmente a través de líneas matrilineales, los tiempos de coalescencia de esas líneas, su relación con la demografía de los grupos estudiados y correlacionar dichos resultados con la distribución geográfica de las genealogías. Su metodología comúnmente se basa en la construcción de genealogías de genes utilizando como fuente de información principalmente al DNA mitocondrial (mtDNA), debido a sus características de ser un

genoma corto, sin espaciadores intergénicos, haploide en la mayoría de los casos (contiene solamente herencia matrilineal), lo cual permite construir filogenias a niveles infraespecíficos, es decir no confunden las relaciones tocogenéticas (*sensu* Hennig, 1966) con las relaciones filogenéticas, que contiene una alta tasa de mutación neutral en animales y son pocos los casos en que se observan cambios de longitud en la molécula. Adicionalmente, el mtDNA parece indicar que existen fenómenos de eliminación interna de haplotipos (sweepstakes) y esto permite que uno solo de éstos domine por individuo, lo cual hace más fácil la delimitación de haplotipos ampliamente distribuidos con respecto a otros que aparecen con menor frecuencia (Avise, 2000). Esto hace que metodológicamente se simplifique el muestreo de haplotipos en las poblaciones a estudiar.

La reconstrucción de las filogenias de genes de esta forma implica que se pueden rastrear los tiempos de divergencia de los organismos atendiendo a la probabilidad de parentesco entre descendientes y ancestros (los tiempos en los cuales se pueden rastrear al menos 1 madre para sus descendientes hijas), a su tasa de mutación, al flujo génico y a sus parámetros poblaciones (Avise, 2000). Para esto, comúnmente se utilizan distribuciones teóricas de las familias de hembras (más comúnmente la distribución de Poisson). Esto ha permitido ratrear los orígenes de la especie humana a una hembra de 200, 000 años.

Algunos de los trabajos más recientes de esta joven rama de la biogeografía comienzan a explorar las relaciones genéticas de invertebrados y vertebrados a nivel geográfico en aguas continentales (Strecker et al., 1996, 2003; Consuegra et al., 2002; Koskinen et al., 2002; Kontula et al., 2003; Janzen et al., 2002; Veith et al., 2003; Witt et al., 2003; Hidding et al., 2003) relacionados con cambios climáticos recientes, especialmente pleistocénicos, que han afectado a peces dulceacuícolas, anfibios y microcrustáceos. Adicionalmente, se han realizado trabajos detallados con peces

dulceacuícolas en regiones donde existen fenómenos de captura de ríos, aislamiento geográfico de cuencas fluviales, desaparición de cabeceras fluviales y desvío de cauces (Hurwood & Hughes, 1998).

Los parasitólogos han incursionado recientemente en el campo de la filogeografía. El primer trabajo que se realizó de la filogenia de helmintos utilizando este nombre versa sobre el parásito zoonótico *Paragonimus westernmani* (Blair et al., 1997). Asimismo, se han realizado trabajos sobre la filogeografía de otro parásito de importancia médica, *Schistosoma* spp. (Attwood et al., 2002). Se podrían ennumerar una cantidad creciente de trabajos que se han realizado con helmintos de animales silvestres (Sinnappah, 1998; Brant & Ortí, 2003; Mes, 2003; Nieberding et al., 2004).

Tomando como ejemplo algunos trabajos (Nieberding et al., 2004) es importante reconocer que esta metodología ayuda a estudiar taxones de animales, con escasa o nula vagilidad como lo son los peces dulceacuícolas que habitan la Mesa Central de México. Mateos et al. (2002) y Mateos (2005) son un punto de partida teórico importante, que agrega a lo anterior el que los organismos o taxones estudiados se encuentren a ambos lados de una barrera geográfica, como lo es el Cinturón Volcánico Trans Mexicano (CVTM) que es el límite sureño de la Mesa Central. En términos de la filogeografía, como lo encontraron los autores señalados, las genealogías de genes serían concordantes, es decir, habría un componente norteño y uno sureño y el patrón que describe mejor estos fenómenos es el vicariante, con dispersión limitada y relacionada con el cierre final de las cuencas de la Mesa Central.

Sin embargo, todos los trabajos anteriores se han realizado entre regiones, pero ninguno todavía ha estudiado una región fragmentada en lo particular. La Mesa Central de México es una región que podría aportar información filogeográfica interesante, debido a que se

halla formada por una serie de cuencas que estuvieron relacionadas históricamente. Existen algunos grupos de peces de la Mesa Central que podrían aportar esta información, como son los Goodéidos (Webb, 1998; Webb et al., 2004) que se distribuyen en la cuenca del Río Lerma, dentro de la Mesa Central y al norte del CVTM, la cuenca del Río Pánuco, al noreste de la misma y la cuenca del Río Balsas, al sur del CVTM. Se reconoce actualmente (Webb et al., 2004; Doadrio & Domínguez, 2003) que los Goodéidos han especiado alopátricamente en estas cuencas. Sin embargo, por sus antecedentes de baja diversidad sus helmintos no parecen haber respondido a este evento de separación geográfica. Se pueden plantear un par de interrogantes al respecto: 1) debido a que es probable que los helmintos parásitos tengan una tasa de especiación más lenta que la de sus hospederos, no hubo especiación; 2) o bien, las poblaciones de sus helmintos pueden estar representando especies crípticas (Nekrutenko et al., 1999)

Las pruebas que pueda aportar la filogeografía están en función del hallazgo de haplotipos de helmintos diferenciados en varias cuencas de la Mesa Central de México es decir, hallar un modelo que ayude a dilucidar un patrón de diferenciación haplotípica vicariante (Templeton, 1998, 2004) o bien, de dispersión. Para ésto se parte de la idea de que los lagos de la Mesa Central (Chapala, Pátzcuaro, Zirahuén, Cuitzeo, Zacapu) estuvieron unidos en un tiempo. Esto nos llevaría a proponer que las cuencas de la Mesa Central y adyacentes, estuvieron conectadas entre sí, porque este fenómeno ha dejado huella en los peces primarios y secundarios principalmente (Barbour, 1973; Bradbury, 2000; Webb, 1998, 2004; Doadrio & Domínguez, 2003).

Álvarez del Villar (1972) fue el primer ictiólogo que propuso la existencia de un gran lago en donde ahora se encuentran los lagos de Chapala, Sayula, Magdalena, Pátzcuaro, Cuitzeo, Zirahuén, Cuitzeo y Zacapu, que se dividió sucesivamente o

simultáneamente hasta conformar las grandes cuencas de Jalisco y Michoacán, debido a los movimientos con dirección NNW-SSE del llamado bloque de Jalisco (Ferrari et al., 1999). Esto lleva a sugerir la idea de los helmintos parásitos de estos peces deberían reflejar en sus haplotipos la influencia que han ejercido estas barreras sobre sus poblaciones. Entonces, en alguna de estas cuencas, o en varias, encontraríamos un haplotipo más comúnmente distribuido que otros. Este haplotipo más comúnmente distribuido sería hipotéticamente el más antiguo. Los haplotipos más raros y que además presenten menor variabilidad genética, serían los periféricos y deberán de encontrarse en cuencas periféricas a la zona "central" de este gran lago antiguo, que puede postularse sería Chapala o áreas periféricas o cercanas (Lago de Pátzcuaro). Si muestreamos poblaciones de la misma especie en distintas regiones, encontraríamos "mayor" variabilidad genética relativa en aquel lugar donde se distribuía originalmente la especie, y baja variabilidad en aquellos lugares donde se postulen invasiones recientes o bien contracciones (cuellos de botella).

Sin embargo, como ya se mencionó, este patrón podría sufrir disrupción por dispersión, lo cual consecuentemente invalidaría el modelo puramente vicariancista. Esto se debe a que algunos de los hospederos que se estudian en el presente trabajo muestran claramente dispersión (Doadrio & Domínguez, 2004) y sus helmintos parásitos podrían reflejar dichos movimientos, por lo que se tendría que postular que existieron eventos sucesivos de separación, reconexión o captura de cuencas, análogo a la frecuencia temporal en que se fueron formando o separando los lagos de la Mesa Central. Además, se podrían encontrar períodos de contracción evidenciados en algunas cuencas tectónicas de la Mesa Central (Bradbury, 2000; Caballero et al., 2002) que se reflejarían en una baja diversidad haplotípica.

ANTECEDENTES.

Evolución lacustre del centro de México

La región central de México ha sido el escenario de una gran actividad geotectónica y biológica que se acentuó principalmente en el periodo Mioceno de la Era Terciaria al dividirse la placa de Farallón en el Pacífico oriental y dando origen a las placas de Cocos y a la placa de Rivera. Esto produjo una amplia zona de subducción de la placa de Cocos (localizada en el Pacífico sur mexicano y centroamericano) debajo de la placa de Norteamérica. Este fenómeno geotectónico dio origen al Eje Neovolcánico Transversal o Cinturón Volcánico Transversal Mexicano (CVTM) (Ferrari et al., 1999, 2000; Ruíz-Martínez et al., 2000; Soler-Arechalde y Urrutia-Fucugauchi, 2000) y posteriormente a la elevación del centro de México hace 9.8 millones de años (m.a.) que hoy se conoce como Mesa Central. La Mesa Central de México está limitada al sur por el Eje Neovolcánico Transversal, al oeste por la Sierra Madre Occidental y al este por la Sierra Madre Oriental, con un límite norteño no muy claro y colindante con la Mesa del Norte, pero con una cota altitudinal mínima de 1800 m.s.n.m. Debido a que la Mesa Central de México no es reconocida como una provincia biótica (Ferrusquía-Villafranca, 1990; Morrone, 2002, Morrone & Márquez, 2001), la mayoría de los estudios que involucran zonas dentro de la misma se refieren a la provincia Neovolcanense *sensu* Ferrusquía-Villafranca (1990) o al Trans Mexican Volcanic Belt (TMVB por sus siglas en inglés o CVTM en español) (Mateos et al., 2002).

Los estudios que explícitamente reconocen a la Mesa Central son de carácter biológico y la definen como una región donde existen varios grupos taxonómicos (Barbour, 1973, basado en West, 1958; Miller, 1986; Miller & Smith, 1986). Estos grupos, habitantes de las aguas dulces del centro de México, al parecer han especiado principalmente dentro

de esa región, bajo el concepto un tanto cuanto cuestionado de "centro de origen" (Croizat et al., 1974). Varios de esos grupos, especialmente los peces de agua dulce, se han originado fuera de la región, en cuencas aledañas a la Mesa Central, o cuando existía contigüidad entre las mismas (Barbour, 1973).

Los grupos taxonómicos de peces endémicos a la Mesa Central son principalmente tres: la subfamilia Goodeinae, el género *Algansea*, y el género *Chirostoma*. La subfamilia Goodeinae se originó probablemente en la cuenca del Río Mezquital en el Noroeste de México (Doadrio & Domínguez, 2004); del ciprínido del género *Algansea* se desconocen sus afinidades inmediatas claramente, sin embargo parece ser un género hermano de *Gila*, habitante de varias cuencas del norte de México y E.U.A (Coburn & Cavender, 1992). Además, se reconoce que el área de distribución del género mencionado rebasa a la Mesa Central y al CVTM (Miller, 1986; Miller & Smith, 1986; Espinosa-Pérez et al., 1993). Con el género *Chirostoma* ocurre una situación semejante con la diferencia de que este grupo se originó al parecer de ancestros marinos (Barbour, 1973; Barriga-Sosa & Arredondo-Figueroa, 2001; Echelle & Echelle, 1999). Debido a ésto, es más conveniente referirse a cada una de las grandes cuencas hidrológicas que se encuentran delimitadas al sur por el CVTM y al norte por la Mesa del Norte (*sensu* Ferrusquía-Villafranca, 1990) por separado y adicionalmente se reconoce en la actualidad que el mismo eje volcánico es tanto una zona de endemismos (Marshall & Liebherr, 2000), como una barrera geográfica para la dispersión de peces de agua dulce (Mateos et al., 2002, 2005).

Los estudios que han tratado de dilucidar las relaciones biogeográficas del CVTM con otras provincias bióticas han sido numerosos (Espinosa-Organista et al., 2000; Marshall & Liebherr, 2000; Morrone et al., 2002; Morrone et al., 2002, Morrone & Márquez, 2001; Brooks, 2005). Sin embargo, son menos los estudios que se refieren a las relaciones

biogeográficas *dentro* de la Mesa Central (Doadrio & Domínguez, 2004; Pérez-Ponce de León, 2003). Existen inclusive evidencias biológicas que han aportado datos importantes al estudio geológico de la región (Barbour, 1973; Doadrio & Domínguez, 2004). Estos estudios reflejan que la historia geológica del CVTM y su zona de influencia principalmente en la Mesa Central ha producido una región con marcados endemismos (Pérez-Ponce de León, 2003). Dentro de estos endemismos se cuenta con la subfamilia Goodeinae, con 39 especies aproximadamente; el género *Algansea*, con 7 especies nominales y el género *Chirostoma*, con 17 especies.

Los peces han sido objeto de diversos estudios biogeográficos en la Mesa Central: *Chirostoma* (Barbour, 1973; Barriga-Sosa & Arredondo-Figueroa, 2001); *Algansea* (Jensen & Barbour, 1981) y la familia Goodeidae (Doadrio & Domínguez, 2004). Otros taxones, de claras afinidades específicas a los cuerpos de agua dulce y con ciclos de vida más complejos que los de los vertebrados mencionados, han sido escasamente estudiados desde la perspectiva de la biogeografía histórica (Pérez-Ponce de León, 2003).

Se ha mencionado anteriormente que México se encuentra ligado fuertemente con eventos geológicos que se han dado en la región desde principios del período Terciario. Estos estudios han establecido patrones geomorfotectónicos para dicha zona, en especial el origen y evolución del denominado CVTM (Ferrari et al., 1999, 2000; Ruíz-Martínez et al., 2000; Soler-Arechalde y Urrutia-Fucugauchi, 2000). Este arco volcánico ha representado una barrera geográfica entre las regiones Neártica y Neotropical (Contreras et al., 1996; Mateos et al., 2002). Debido a que las cuencas lacustres del centro de México, en especial aquéllas que se encuentran en el CVTM (Lerma-Santiago, cuencas del Pacífico y afluentes norteños del Balsas) han sido materia de la mayor cantidad de estudios helmintológicos hasta nuestros días (Lamothe et al., 1997; Pérez-Ponce de León et al., 1996) y se

encuentran íntimamente relacionadas con el origen y evolución del CVTM, es muy importante describir su evolución lacustre.

El CVTM es una provincia volcánica de considerable altitud que atraviesa el centro de México desde el Océano Pacífico hasta el Golfo de México (~ 1000 km). Se le asocia actualmente con la subducción de las placas de Cocos y Rivera a lo largo de la fosa Mesoamericana.

El CVTM casi tenía la orientación actual desde mediados del Mioceno (Ferrari et al., 1999) y un arco con características del CVTM ya existía en el Mioceno Tardío, lo cual es interesante desde el punto de vista biológico, pues se ha hipotetizado que el origen y diversificación de la ictiofauna de la zona central de México, en particular en la Mesa Central data de ese tiempo (Miller & Smith, 1986). Sin embargo, a pesar de una buena cantidad de estudios en la última década sobre la evolución Miocénica del CVTM la evolución tectónica de México central durante el Mioceno no está completamente entendida (Ferrari et al., 2000).

Tectónicamente el CVTM se encuentra dividido en tres segmentos (Ruíz-Martínez et al., 2000). Un fallamiento Post-Pliocénico que resultó en los *rifts* de Tepic-Chapala (o Tepic-Zacoalco) y Chapala-Colima que forman una unión triple que caracteriza a este segmento occidental. El segmento central se caracteriza por fallas normales y "grabens" pequeños con dirección este-oeste (Cuitzeo, Acambay) que han estado activos en tiempos recientes. El tercer segmento se encuentra al este del lineamiento Querétaro-Taxco donde existen cuencas pequeñas con dirección noroeste-sureste y cordilleras que delinean una zona con vulcanismo extendido pero sin lineamientos este-oeste claros y grandes. Asimismo, a estos segmentos se les agrega la provincia alcalina del este de edad

oligocénica, que también representa vulcanismo reciente en el este de México hasta el Cuaternario.

Es claro que la historia hidrológica de los cuerpos de agua de la Mesa Central está íntimamente ligada a la evolución del CVTM como atestiguan algunos estudios recientes (Soler-Arechalde & Urrutia-Fucugauchi, 2000) en los cuales se comenta que la corriente del río Lerma sigue el cambio de orientación de los fallamientos del "graben" de Acambay (de Este-Oeste a Nornoroeste-Sursureste). Sin embargo, son muy limitados los casos en los cuales se da cuenta explícita de esta historia y todavía hoy la mayoría de las hipótesis biogeográficas sustentan parte de esta historia hidrológica (Barbour, 1973; Bradbury, 2000; Doadrio & Domínguez, 2004; Mateos et al., 2002, 2005; Mulcahy & Mendelson, 2000).

Aunque la historia hidrológica de la Mesa Central es compleja, dado que todavía existe materia de controversia en la geología de la región, Doadrio & Domínguez (2004) han esbozado una sucesión de eventos en la evolución lacustre del occidente y centro de la Mesa Central. El este de México sigue siendo materia aún de mayor controversia (Álvarez, 1972; Ruíz-Martínez, 2000).

Diversidad helmintológica en la Mesa Central de México

México se encuentra entre los 7 países con mayor diversidad en el mundo (Ramamoorthy et al., 1998). Se ha argumentado desde hace tiempo, que la diversidad de este país se debe a que se encuentra en una zona en la cual confluyen las biotas Neártica y Neotropical. Además, a este hecho se une otro, el que México es una región geomorfológicamente compleja, ya que, como se ha visto, en ésta confluyen varias placas tectónicas: Norteamericana, Caribeña, Cocos y Pacífica. Esto ha dado como resultado un relieve muy irregular que como consecuencia originó una enorme diversidad de hábitats. Una hipótesis biogeográfica más reciente, basada en la filogenia de los goodéidos, indica

que la evolución biológica dentro del centro de México se debe a diversos procesos alternados de vicarianza y dispersión (Domínguez-Domínguez et al., 2005). Por lo tanto, la importancia que reviste esta región tiene tres vertientes: la biótica, la biogeográfica y la geológica.

La diversidad de helmintos parásitos en vertebrados de aguas continentales de México ha sido inventariada con cierto detalle y analizada sistemática y biogeográficamente (Pérez-Ponce de León, 2001, Pérez-Ponce de León et al., 2000, Pérez-Ponce de León et al., 2001; Pérez-Ponce de León & Choudhury, 2005). La mayor base de datos existente en México (Colección Nacional de Helmintos, Instituto de Biología, UNAM, CNHE en adelante) contiene en su mayor parte, los registros de los helmintos que parasitan a vertebrados acuáticos, pero especialmente, la base de datos de helmintos de peces de aguas dulces es mayor en comparación con sus contrapartes en otros vertebrados (Pérez-Ponce de León et al., 1996; Pérez-Ponce de León & García-Prieto, 2001; Pérez-Ponce de León & Choudhury, 2005, ver Tabla 1).

Tabla 1. Número de especies de helmintos adultos registradas para México por familia de peces de agua dulce (existen especies de helmintos que se repiten, datos tomados de Pérez-Ponce de León & Choudhury, 2005).

	Acanthocephala	Cestoda	Digenea	Monogenea	Nematoda	TOTAL
Anguillidae					1	1
Atherinidae		1	1		4	6
Belonidae	1				1	2
Bythitidae					1	1
Catostomidae		1				1
Centrarchidae		1	1	2		4
Cichlidae	3	2	20	12	14	51
Clupeidae	1		1	3		5
Cyprinidae		2	2	2	3	9
Cyprinodontidae					2	2
Characidae		1	8	6	4	19
Goodeidae*		2	3	1	5	11
Hemiramphidae	1					1
Ictaluridae	1	4	6	3	4	18
Lepisosteidae		1	1			2
Mugilidae			1		3	4
Pimelodidae		2	5	1	7	15
Poeciliidae	1	1	4	1	5	12
Sciaenidae			1	1		2
Synbranchidae		1			3	4

* registros anteriores a 2004

Aun cuando las investigaciones sobre la helmintofauna de peces dulceacuícolas, así como de anfibios y reptiles, data de la década de 1930 (Lamothe et al., 1997), los inventarios de toda esta riqueza específica se han elaborado en los últimos 30 años (Pérez-Ponce de León et al., 1996; Pérez-Ponce de León & García-Prieto, 2001; Vidal-Martínez et al., 2001). Se ha realizado un enorme número de muestreos a lo largo de México, en algunas ocasiones concentrándose en el inventario exhaustivo de localidades particulares (Pérez-Ponce de León et al., 2000, Lago de Pátzcuaro; Pineda-López et al., 1985, en varios ríos y lagos de Tabasco y Chiapas;

Tabla 2. Número de especies de helmintos adultos registradas para México por cuenca (datos tomado de Pérez-Ponce de León & Choudhury, 2005).

	Peninsula de Yucatán	Grijalva-Usumacinta	Papaloapan, Pánuco	Balsas	Lerma-Santiago	Bravo
Anguillidae	1					1
Atherinidae				1	5	6
Belonidae		2				
Bythitidae	1					
Catostomidae			1			
Centrarchidae			1		2	2
Cichlidae	17	36	10	4	9	4
Clupeidae		1				4
Cyprinidae		1		2	7	2
Cyprinodontidae				2		
Characidae	10	5	7	1	3	4
Goodeidae*				2	9	
Hemiramphidae		1				
Ictaluridae		4	9	3	4	6
Lepisosteidae		2				
Mugilidae			2		3	
Pimelodidae	12	4	6			
Poeciliidae	5	5	5	3	4	
Sciaenidae						2
Synbranchidae			4			
TOTALES	46	61	45	18	46	31

* registros anteriores a 2004

Pérez-Ponce de León et al., 1992, Lago de Catemaco en Veracruz y Vidal-Martínez, 2001, Península de Yucatán) (ver Tabla 2).

De esta ictiofauna se han registrado helmintos parásitos de 127 especies de peces es decir el 31% del total, que pertenecen a 51 géneros y 23 familias (Pérez-Ponce de León & Choudhury, 2005). Esto representa alrededor de un tercio de la totalidad de la diversidad íctica de aguas dulces de México.

En la cuencas más extensas e intensamente estudiadas en México, se han estudiado 120 especies de helmintos adultos (Pérez-Ponce de León & Choudhury, 2005). Entre éstas, 61 se han registrado para las cuencas que delimitan la zona centro de México (Lerma-Santiago, Balsas, Pánuco, Papaloapan, y cuencas distribuidas entre éstas) dentro de un

conjunto de 111 especies, 61 de las cuales son únicas para las especies de peces y 50 son compartidas entre las mismas. Estas especies están repartidas en 13 familias de peces, 19 especies de peces del Papaloapan y Pánuco, 12 del Balsas, 47 Lerma-Santiago sumando un total de 78 especies estudiadas.

De las familias de nemátodos que parasitan peces de agua dulce en México, las familias Capillariidae Neveu-Lemaire, 1936 y Rhabdochonidae (Travassos, Artigas & Almeida, 1928) son las que cuentan con un mayor número de especies. Rhabdochonidae es la familia mejor estudiada de nemátodos de peces de agua dulce en México (Sánchez-Álvarez et al., 1998; Caspeta-Mandujano et al., 2000, 2001, 2002; Moravec, 1998; Mejía-Madrid & Pérez-Ponce de León, 2003). Estos descubrimientos parecen indicar que su diversificación es muy antigua y no se encuentra ligada con eventos geotectónicos en la Mesa Central de México.

El interés que esto ha tenido para los helmintológos de México y otras partes del mundo es el de describir patrones biogeográficos semejantes a los encontrados en organismos de vida libre. Esto se debe a que en la teoría biogeográfica moderna se investigan los patrones de distribución, es decir, una regularidad o repetición en la distribución de los organismos que permite establecer comparaciones y ensayar predicciones, aun cuando esa regularidad no sea perfecta (Morrone & Ruggiero, 2000). Esto es el objetivo principal de la panbiogeografía (Craw et al., 1999) y la biogeografía cladista (Humphries & Parenti, 1999), que son parte de la biogeografía histórica. Sin embargo, no se debe descartar en principio, que la información obtenida puede arrojar casos aislados no congruentes con el patrón vicariante pero que generan un patrón de dispersión asociado al aumento en la distribución de forma periódica (Wiley, 1988; Brooks and McLennan, 2001, Brooks & Van Veller, 2002).

El presente trabajo de tesis aborda el estudio de los helmintos parásitos de una subfamilia de peces de agua dulce endémicos de la la Mesa Central de México, los peces de la subfamilia Goodeinae en la Mesa Central de México en relación al estudio de la evolución y diversificación lacustre en el centro de México. La hipótesis de trabajo empleada es que si los peces de agua dulce de la Mesa Central de México han diversificado significativamente como consecuencia de la compleja historia geológica de la misma, los helmintos deben de mostrar patrones de diversificación semejantes.

OBJETIVOS.

El objetivo general del presente trabajo es reconstruir la historia biogeográfica de las cuencas hidrológicas de la Mesa Central de México utilizando dos modelos de estudio: los helmintos de los peces de la familia Goodeidae (subfamilia Goodeinae) y la filogenia de alguno de los helmintos más abundantes o diversificados, en este caso se escogío a las especies del nemátodo parásito de peces de agua dulce *Rhabdochona* Railliet, 1916, debido a que anteriormente se ha registrado en la Mesa Central y cuencas adyacentes.

Particularmente: a) se describirá la fauna helmintológica de los peces de la familia Goodeidae atendiendo principalmente al componente de especies autogénicas y en su caso, a las especies nuevas de helmintos; b) se comparará la composición de las comunidades de helmintos en Goodeidae en diferentes localidades representativas de las cuencas hidrológicas comprendidas dentro de la Mesa Central de México y otras cuencas relacionadas para que de esta forma encontremos si los patrones de diversificación de los helmintos han sido consecuencia de la fragmentación de las cuencas en las cuales habitan; c) se propondrá una hipótesis de las relaciones filogenéticas entre las especies mexicanas del género *Rhabdochona* utilizando únicamente caracteres morfológicos; de esta forma se pretende reconocer un patrón biogeográfico asociado a la historia geológica de la Mesa

Central y finalmente d) se dilucidará el patrón filogeográfico de *Rhabdochona lichtefelsi* para encontrar las relaciones históricas de las cuencas de la Mesa Central con base en la distribución geográfica de sus haplotipos.

Los resultados del presente trabajo de tesis se presentan como una serie de 4 artículos distribuidos en 3 capítulos distintos. Dos de los artículos incluídos ya han sido publicados y 2 más se encuentran en revisión. En los mismos se encontrarán las metodologías empleadas en cada caso, los resultados particulares para cada uno y la discusión y conclusione particulares. Al final del presente trabajo de tesis se encontrará la discusión general y las conclusiones de toda la investigación realizada.

El primer capítulo incluye 2 artículos: la descripción de una nueva especie de *Rhabdochona* Railliet, 1916 y la descripción de la fauna helmintológica de la subfamilia Goodeinae. En este último se incluyen los primeros registros de la helmintofauna de estos peces así como los nuevos registros obtenidos a partir de la presente investigación. En la discusión se podrán leer algunas de las conclusiones biogeográficas a las que se pudo llegar con el registro de esta helmintofauna.

El segundo capítulo incluye el manuscrito en revisión de la filogenia de las especies Americanas de *Rhabdochona* Railliet, 1916. La hipótesis de las relaciones filogenéticas de las especies de este género se discuten a la luz de la biogeografía de México en general y la Mesa Central en particular.

El tercer capítulo incluye la filogeografía de *Rhabdochona lichtenfelsi* Sánchez-Álvarez, García-Prieto & Pérez-Ponce de León, 1998. En este manuscrito se muestran los resultados que es factible obtener con la genealogía genética de una sola especie de helminto, ampliamente distribuido en la Mesa Central y su relación con los fenómenos

geológicos que han dado lugar a la diversificación de otros animales en esta región de México.

BIOLOGÍA DE LOS HOSPEDEROS.

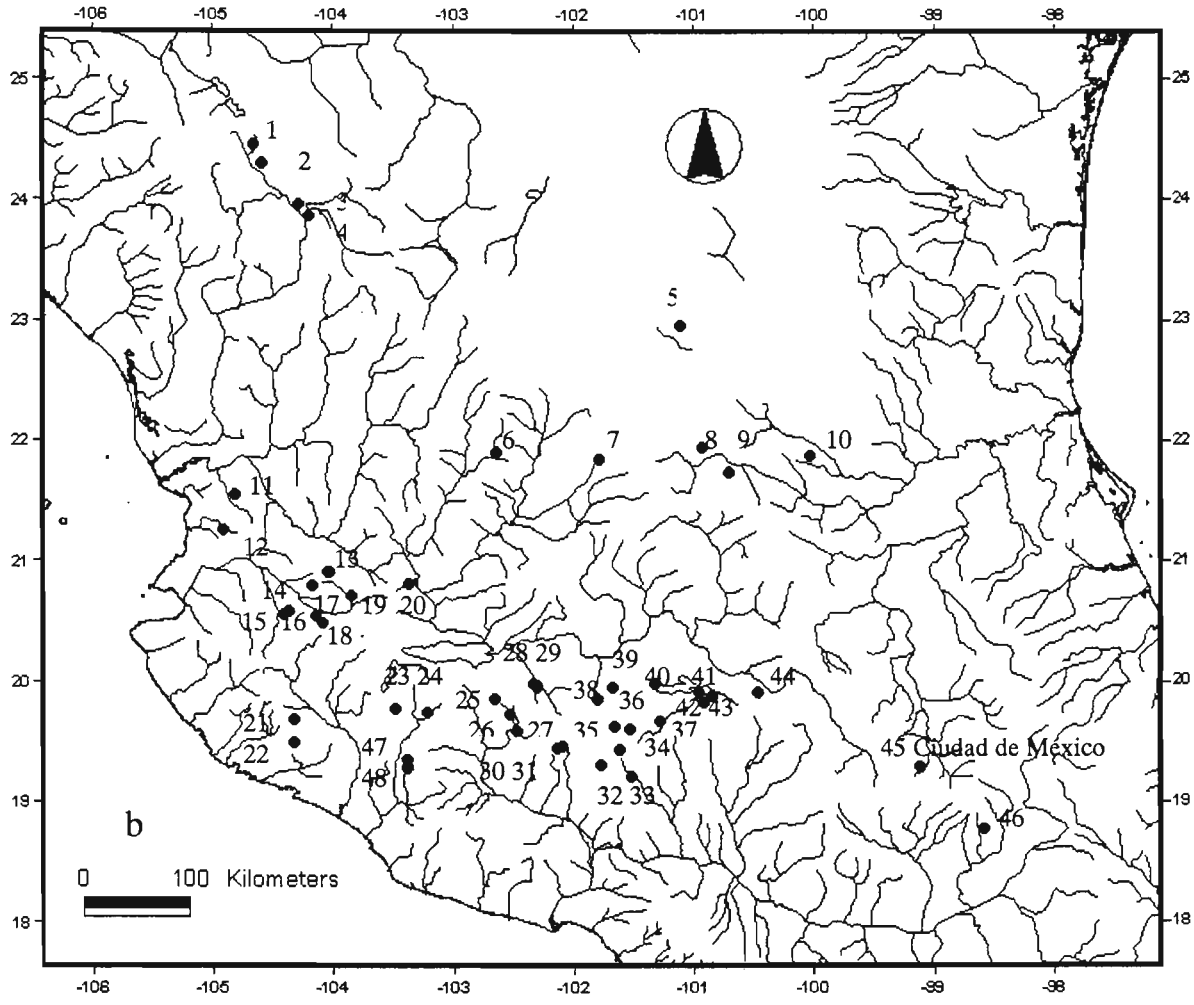
La biología de la subfamilia Goodeinae se detalla en la introducción del artículo correspondiente (Mejía-Madrid, H.H., O. Domínguez-Domínguez & G. Pérez-Ponce de León. 2005. Adult endohelminth parasites of Goodeinae (Cyprinodontiformes: Goodeidae) from México with Biogeographical Considerations. *Comparative Parasitology* 72:200-211).

ZONAS DE COLECTA.

Las 51 localidades muestreadas corresponden a 8 de las cuencas más grandes del centro de México, incluida la Mesa Central: Ameca, Armería, Balsas, Coahuayana, Cotija, Lerma-Santiago, Mezquital y Pánuco (Mapa 1). Adicionalmente, se muestrearon menos extensamente otras cuencas incluidas dentro del territorio de la Mesa Central: Magdalena y San Marcos. Las localidades y coordenadas se detallan en el capítulo I, en el artículo correspondiente (Mejía-Madrid, H.H., O. Domínguez-Domínguez & G. Pérez-Ponce de León. 2005. Adult endohelminth parasites of Goodeinae (Cyprinodontiformes: Goodeidae) from México with Biogeographical Considerations. *Comparative Parasitology* 72:200-211). Para mayores referencias ver Mapa 1.

Mapa 1. a) Mapa de las cuencas muestreadas para helmintos de goodéidos; b) Mapa de las localidades individuales. Nombres de las localidades en el Capítulo I (Mejía-Madrid et al., 2005).

a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CAPÍTULO I.

HELMINTOFAUNA DE LA SUBFAMILIA GOODEINAE (OSTEICHTHYES: CYPRINODONTIFORMES) EN LA MESA CENTRAL DE MÉXICO

De un total de 1, 294 ejemplares de peces de la subfamilia Goodeinae, que representaron 35 especies recolectadas en 51 localidades de México se obtuvieron 10 especies de helmintos entre septiembre de 2001 y diciembre de 2004. Estas 10 especies de helmintos comprenden a 4 especies de digéneos, 2 de céstodos, y 4 de nemátodos. Se registran por primera vez para México *Allocreadium lobatum* y *Proteocephalus longicollis*. La distribución geográfica de *Allocreadium mexicanum* se amplía e incluye a nuevas especies de hospederos dentro de los Goodeinae en la cuenca del Mezquital. *Margotrema bravoae* se encontró principalmente en la cuenca del río Lerma-Santiago y en menor cantidad en el río Balsas. *Margotrema guillerminae* solamente se encontró en las cuencas occidentales relacionadas con la Mesa Central.. *Proteocephalus longicollis* se encontró en 1 solo hospedero y en 1 sola localidad.

Rhabdochona lichtenfelsi fue el helminto más prevalente y abundante en las colectas de las cuencas del Lerma-Santiago y el Pánuco. Se le considera como la única especie de nemátodo endémica a goodéidos en ambas cuencas. *Rhabdochona ahuehuellensis* se encontró principalmente en el Balsas y en las cuencas occidentales que supuestamente estuvieron relacionadas anteriormente en tiempo geológico al Balsas. Asimismo se le encontró en el río Pánuco.

Margotrema bravoae, *M. guillerminae*, *R. ahuehuellensis* y *R. lichtenfelsi* se consideran la helmintofauna núcleo de las comunidades de helmintos de los Goodeinae. Se registran nuevos hospederos y localidades de 2 especies exóticas para México, *Botrhriocephalus acheilognathi* y *Pseudocapillaria tomentosa*. En total, se registran 29 nuevos hospederos y 48 nuevas localidades para los endohelmitnos adultos de peces goodéinos de México. La distribución de *Margotrema* spp. es congruente con un origen vicariante que refleja cercanamente la filogenia de Goodeidae, mientras que *Rhabdochona*

spp. no, lo cual indica que este último tiene orígenes múltiples proveniente de distintos hospedero y distintos orígenes geográficos en las Américas.

Dentro de este conjunto de especies, se describió a *R. ahuehuellensis* como nueva especie de *Ilyodon whitei* Meek. Los caracteres que la distinguen son las elevaciones cuticulares en medio del protomio, una terminación tricúspide de la espícula izquierda combinada con una forma de cuchara; la superficie de los huevos tiene pocos filamentos subpolares cortos. Los caracteres sitúan a esta especie lejanamente a otras especies descritas para Norteamérica, excepto *R. lichtenfelsi*. Con esta descripción aumenta el número de especies de *Rhabdochona* de la Mesa Central de México a cuatro, lo cual sugiere que éste género ha especiado en respuesta a la fragmentación de las cuencas de la Mesa Central de México.

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RHABDOCHONA AHUEHUELLENSIS N. SP. (NEMATODA: RHABDOCHONIDAE) FROM THE BALSAS GOODEID, *ILYODON WHITEI* (OSTEICHTHYES: GOODEIDAE), IN MEXICO

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ABSTRACT: *Rhabdochona ahuehuellensis* n. sp. is described from the Balsas goodeid, *Ilyodon whitei*. The distinctive characters are cuticular elevations in the middle of the prostom, tricuspid ending of the distal part of the left spicule combined with a scoop shape, and an egg surface with limited, short subpolar filaments. The characters place the present species farther apart from species described from North America, except *R. lichtenfelsi*. This description brings the total number of species of *Rhabdochona* in the Mesa Central of Mexico to 4, suggesting that this genus has undergone speciation in response to basin fragmentation within the Mesa Central of Mexico.

Species of *Rhabdochona* Railliet, 1916, infect the intestine of freshwater fishes all over the world except Australia (Moravec, 1975). Members of this genus are mainly found in cyprinids in Eurasia and also occur in members of other fish families in North America (Catostomidae, Cyprinidae, Ictaluridae, Sciaenidae, Cottidae, and to a lesser extent Salmonidae) and South America (Pimelodidae, Cichlidae, Characidae, and Poeciliidae) (Moravec, 1975, 1994, 1998; Hoffman, 1999). Catostomids, cyprinids, and salmonids seem to be the most important hosts for species in this genus because each fish family harbors 2 or 3 distinct species (up to 3). Despite this fact, *Rhabdochona* species have undergone extensive speciation in other North American freshwater fish species (Moravec and Arai, 1971; Moravec and Coy-Otero, 1987; Byrne, 1992; Maggenti et al., 1992; Sánchez-Alvarez et al., 1998).

Three new species of *Rhabdochona* in freshwater fishes from basins of the Mesa Central of Mexico and bordering areas have been added recently (plus the discovery of a similar nematode species in *Cichlasoma beani* Jordan): *R. lichtenfelsi* Sánchez-Alvarez, García-Prieto, and Pérez-Ponce de León, 1998, from the goodeid fishes *Goodea atripinnis* Jordan and *Alloophorus robustus* (Bean); *R. mexicana* Caspeta-Mandujano, Moravec, and Salgado-Maldonado, 2000, from the characid fishes *Astyanax mexicanus* (Filippi) and *A. fasciatus* (Cuvier); and *R. xiphophori* Caspeta-Mandujano, Moravec, and Salgado-Maldonado, 2001, from an unidentified species of *Xiphophorus* Heckel (Poeciliidae). Only *R. lichtenfelsi* has been discovered from the Mesa Central proper, from lakes Pátzcuaro and Cuitzeo, 2 lakes in the center of the aforementioned region (Sánchez-Alvarez et al., 1998). *Rhabdochona mexicana* was recovered from 2 species of characids from the Amacuzac River, a tributary of the Balsas Basin in the southern limit of the Mesa Central. *Rhabdochona xiphophori* was described mainly from immature specimens from a river in the northeastern limit of the Mesa, west of the Sierra Madre Oriental (Caspeta-Mandujano et al., 2000, 2001). In the present study a new species of *Rhabdochona* is described from *Ilyodon whitei* Meek, the Balsas goodeid.

MATERIALS AND METHODS

Specimens from which the new species is described were recovered from 40 individuals of the Balsas goodeid, *I. whitei*, caught in the Ahuehuello River (18°45'19.0"N, 98°34'20.4"W), the most western tributary of the Nexapa River in Puebla State, between November 2000 and August 2001. Fish were caught using a 4-ft × 30-in. × 0.25-in. (1.22 × 9.15 × 0.6 cm) seine.

Fish were taken alive to the laboratory and killed by pithing. Worms were recovered live from the intestine. After recovery, worms were killed in 4% formalin and preserved in 70% alcohol until they were studied. Helminths were cleared with lactophenol for 2 hr and later returned to the 70% alcohol. Drawings were made with the aid of a drawing tube. Some specimens were processed for scanning electron microscopy (SEM) as follows: the specimens were dehydrated in an ascending series of ethanol, critical point dried, and sputter coated with gold before being examined using a Hitachi S-2460N SEM unit. All measurements are given in micrometers unless otherwise indicated. Mean and standard deviation are given in brackets. For the purpose of comparison, additional specimens of the following species of *Rhabdochona* were borrowed from the Colección Nacional de Helmintos (CNHE), Instituto de Biología, Universidad Nacional Autónoma de México (UNAM), México City, Mexico: *R. paxmani* Maggenti, Abdel-Rahman, and Cid del Prado, 1992, CNHE 30705; *R. salmonis* Maggenti, Abdel-Rahman, and Cid del Prado, 1992, CNHE 30706; *R. lichtenfelsi* CNHE 3212, 3213, 3012, 3013, 3214, and 3215; *R. xiphophori* CNHE 3940, 3941, and 3942; and *R. kidderi* Pearse, 1936, CNHE 3286, 2698, and 2699.

DESCRIPTION

Rhabdochona ahuehuellensis n. sp.

(Figs. 1–13)

General: Body narrow, elongate, with broad cephalic end and tapering, rounded caudal end. Cuticle smooth, finely striated. Mouth oval with rudimentary pseudolabia, bordered with 4 submedian papillae, large amphids, and inner circlet of papillae. Prostom thick walled, funnel shaped; anterior half more widened than posterior half. Anterior half of prostom armed with 10 large conical teeth protruding from longitudinal thickenings, the 4 lateral (2 + 2) more massive than the 3 dorsoventral; middle part of prostom possesses minute cuticular elevations; basal part of prostom with 4 basal teeth, 1 dorsal, 1 ventral, and 2 bilateral. Vestibule (stoma) long, thick walled, straight, sometimes sinuous in gravid specimens. Deirids small, deeply bifurcated, situated in first half of cervical region (anterior half of vestibule).

Male (n = 15; holotype in parentheses): Body 3.16–6.36 mm [4.96 ± 0.80 mm] (3.16 mm) long, 0.06–0.11 mm [0.08 ± 0.01 mm] (0.06 mm) wide; prostom 12–18 [15 ± 1] (15) long, 7–11 [9 ± 1] (7) wide; vestibule including prostom 69–120 [98 ± 16] (91) long. Deirids 16–52 [38 ± 10] (52), excretory pore 126–257 [202 ± 42] (170); nerve ring 99–180 [136 ± 20] (115) from anterior end. Esophagus 27.8–35.1% [31 ± 2.5%] (35%) of body length. Muscular esophagus 102–210 [166 ± 28] (155) long; glandular esophagus 0.95–1.74 mm [1.33 ± 0.21 mm] (0.95 mm) long, representing one third of body length; ratio length of glandular to muscular esophagus 1:5.6–17.1 [1:8.3 ± 2.9] (1:5.9). Tail conical, tapered, rounded, 80–219 [151 ± 35] (104) long. Male tail with 8–11 pairs of subventral preanal papillae in combinations of 10 + 11 (holotype), 9 + 8, 10 + 9, 8 + 8, 9 + 9 (number of papillae on right and left sides, respectively). One additional pair lateral, lying at level of third pair of subventral papillae (counted anteriorly from cloaca); postanal papillae represented by 5 subventral pairs, 1 additional lateral pair at level of first pair (counted posteriorly

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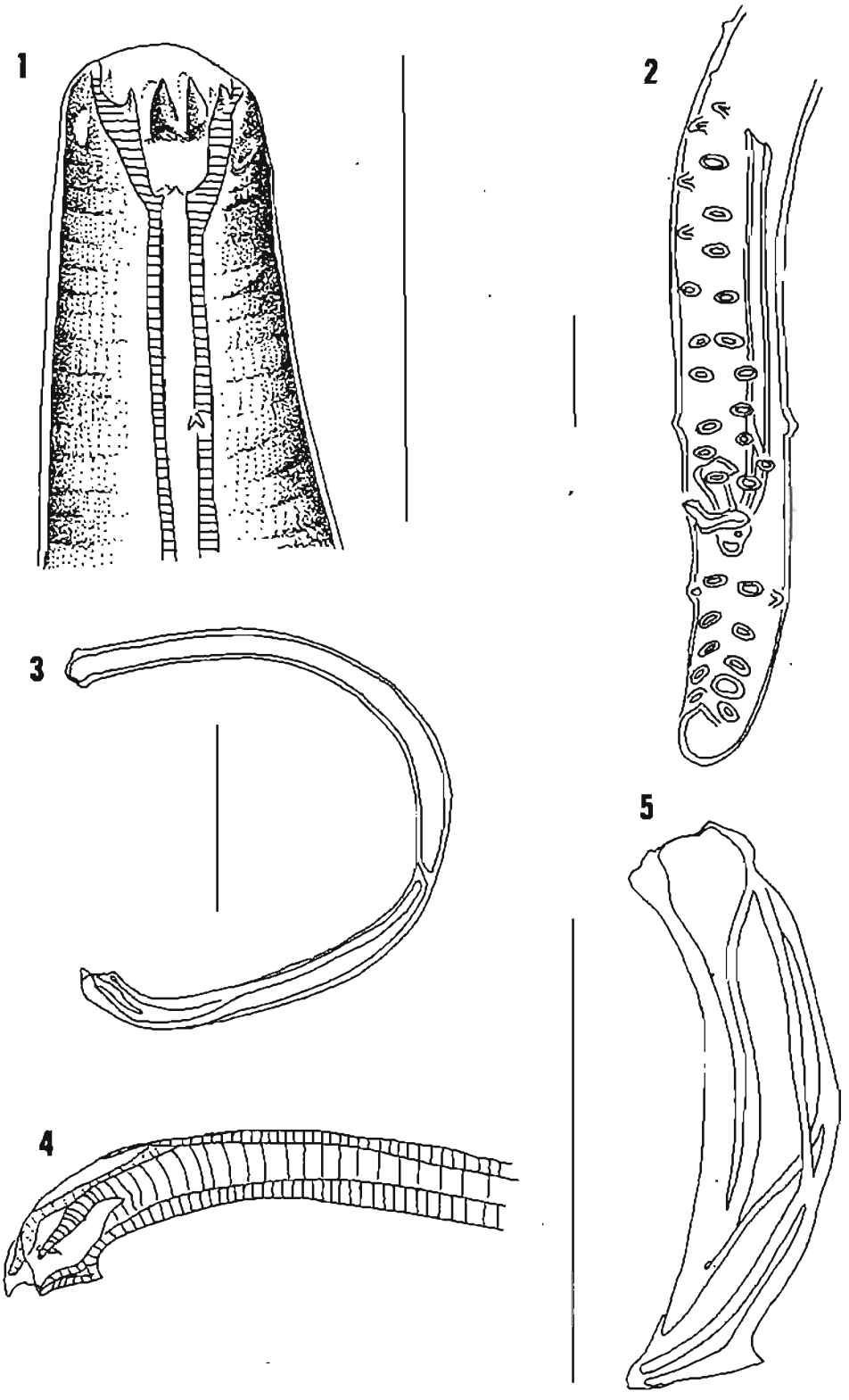
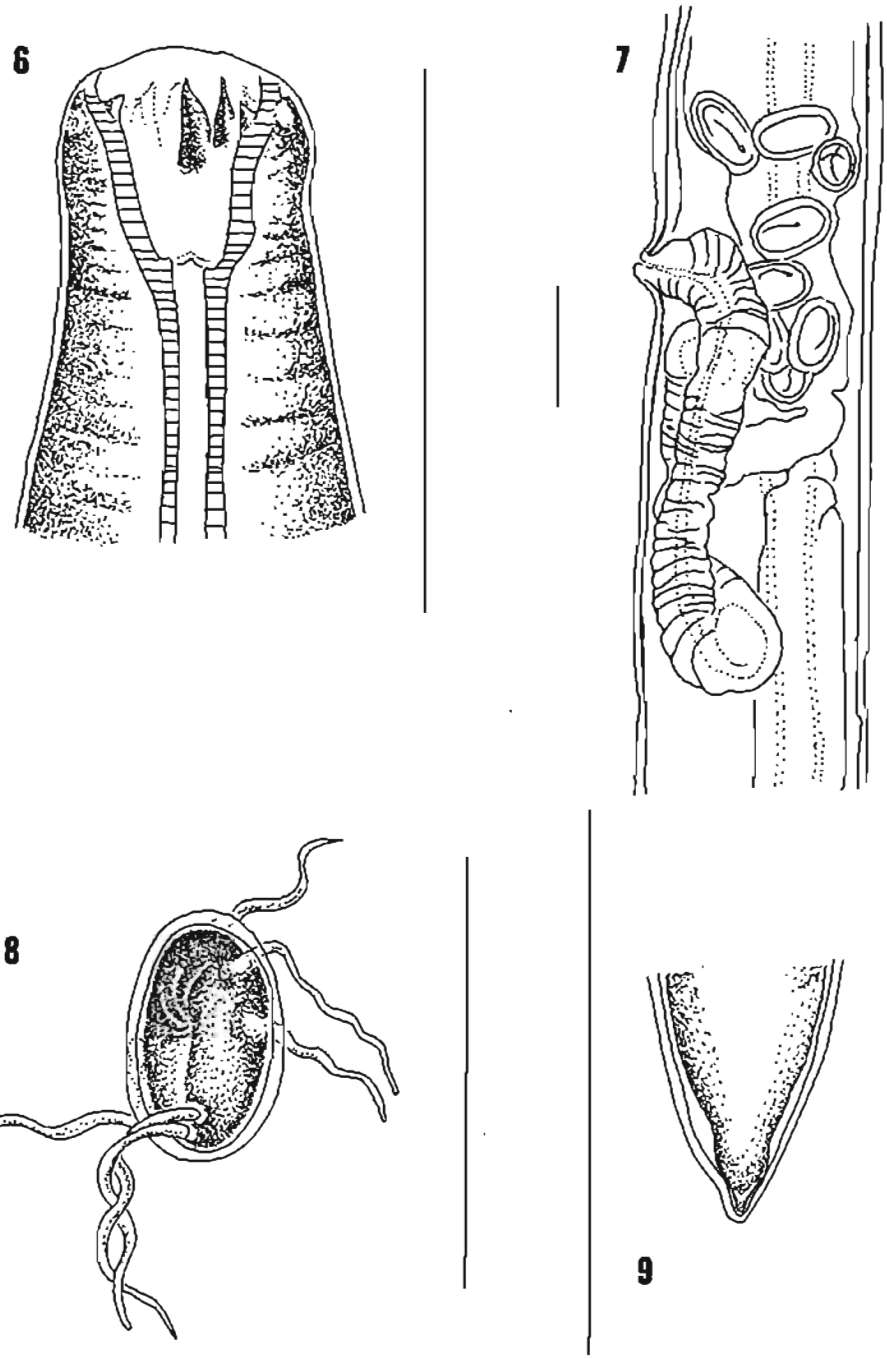


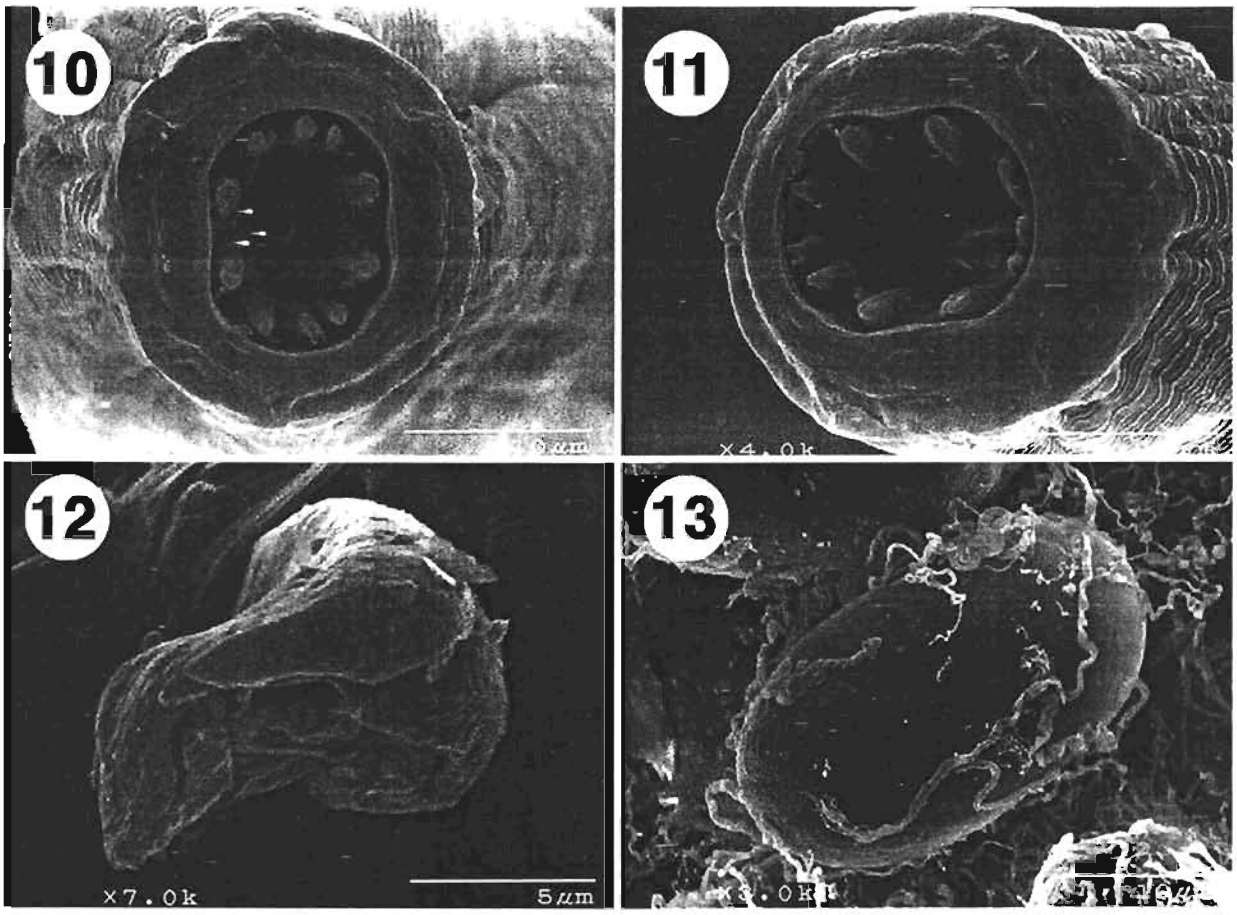
FIGURES 1–5. *Rhabdochona ahuehuellensis* n. sp. 1. Anterior end, male. Paratype. 2. Male terminal end. Holotype. 3. Left spicule. Paratype. 4. Left spicule, distal end. Paratype. 5. Right spicule, lateral. Paratype. Bars = 50 μm.



FIGURES 6–9. *Rhabdochona ahuehuellensis* n. sp. 6. Anterior end, female. Paratype. 7. Vagina. Paratype. 8. Egg. Allotype. 9. Tail, female. Paratype. Bars = 50 μm.

from cloaca). Area rugosa absent. Spicules unequal; left spicule larger, well sclerotized, curved outwardly to right, strongly bifurcated, with dorsal branch less sclerotized than ventral branch, which is bifurcated, making spicule trifurcate. This ventral branch possesses ventral curved barb directed posteriorly. Left spicule 127–248 [214 ± 29] (196) long, 6–9 [7 ± 1] (8) wide. Shaft 61–116 [92 ± 15] (61) long, representing 30.9–66.9% [43.3 ± 7.7%] (31%) of spicule length. Right spicule bifurcated, 41–73 [60 ± 8] (41) long, with membrane; straight dorsal barb (occasionally reflected backwards) in distal region, with spike in proximal region. Ratio of left to right spicule length 1:1.9–4.7 [1:3.6 ± 0.75] (1:4.7).

Female (n = 15; allotype in parentheses): Body 2.75–8.92 mm [7.02 ± 1.5 mm] (6.07 mm) long, 0.05–0.12 mm [0.09 ± 0.02 mm] (0.12 mm) maximum width. Prostom 11–20 [18 ± 2] (19) long, 7–15 [11 ± 3] (15) wide. Vestibule, including prostom, 20–188 [99 ± 34] (109) long. Deirids 25–86 [44 ± 16] (35), excretory pore 70–306 [186 ± 67] (160), highly ganglionated nerve ring 67–213 [138 ± 32] (136) from anterior end. Esophagus 13.6–49.9% [21.8 ± 8.2%] (21.4%) of body length. Muscular esophagus 74–307 [195 ± 60] (140) long. Glandular esophagus 0.86–1.57 [1.25 ± 0.19] (1.16) long, representing one third of body length. Glandular–muscular esophagus length ratio 1: 3.6–11.7 (1:6.9 ± 2) (1:8.3). Amphidelphic; anterior ovary reaching posterior



FIGURES 10–13. Scanning electron micrographs of *Rhabdochona ahuehuellensis* n. sp. **10.** En face, female. **11.** Cephalic end, lateral, female. **12.** Left spicule. Lateral–frontal view. **13.** Eggs near vulva.

end of muscular esophagus, posterior ovary reaching anal level. Vulva bordered by protruding posterior lip, 1.32–4.08 mm [3.31 ± 0.69 mm] (2.9 mm) from posterior end; ovijector present; vagina directed posteriorly. Ovarial ends folding once. Eggs ovoid, 32–61 [36 ± 7] (33) long and 18–30 [20 ± 3] (21) wide, with smooth surfaces, with 4–6 short, thick subpolar filaments originating from hyaline points. Tail conical, 96–278 [186 ± 61] (161) long and tapered to rounded tip.

Taxonomic summary

Type host: Ilyodon whitei Meek, the Balsas goodeid or mexcalpique of the Balsas.

Site of infection: Anterior and middle intestine.

Type locality: Ahuehuello River (Río Ahuehuello) at 18°45′19.0″N, 98°34′20.4″W; altitude 1,536 m; Municipality of Huaquechula, State of Puebla, Mexico.

Prevalence: 58.3%.

Intensity: 1.0–4.4 worms per infected host.

Specimens deposited: Holotype, CNHE 4417; allotype, CNHE 4418; paratypes, CNHE 4419 and 4420, USNPC 92333, 92334, 92335, and 92336.

Etymology: This species is named after its type locality, the Ahuehuello River.

Remarks

Only 5 of the 21 species of *Rhabdochona* described from the Americas possess 10 teeth in the upper prostom and bifurcate deirids: *R. kisutchi* Margolis, Moravec, and McDonald, 1975, *R. paxmoni*, *R. salmonis*, *R. lichtenfelsi*, and *R. mexicana*, all from North America. *Rhabdochona ahuehuellensis* n. sp. is the sixth species with this combination of characters.

Rhabdochona ahuehuellensis n. sp. has several outstanding features that set it apart sharply from these species, as well as from the rest of the species described from the Americas (see Moravec and Arai, 1971; Kayton et al., 1979; Moravec and Coy-Otero, 1987; Sánchez-Alvarez et al., 1998; Caspeta-Mandujano et al., 2000, 2001) and from other parts of the world (see Moravec, 1972, 1975, 1994, 1998). One of these is the presence of a prostom lined internally with cuticular elevations in its middle part and the presence of large dorsal, ventral, and lateral teeth at the base of the prostom. Scanning electron microscopy studies on the internal lining of the prostom are still lacking for most species of *Rhabdochona* (see Byrne, 1992). Another distinct character is the trifurcate nature of the left spicule, which is unique among the species of this genus. The limited number of filaments on eggs, not directly over the polar region, also sets it apart from other related rhabdochonids that possess filamentous eggs.

The new species differs from *R. mexicana* in having eggs with filaments on their surface. Eggs of *R. paxmoni*, *R. kisutchi*, and *R. salmonis* possess floats. *Rhabdochona lichtenfelsi*, *R. milleri*, *R. canadensis*, *R. ovifilamenta*, and *R. canestomi* also have filamented eggs. Yet the position and length of the filaments in these species is quite different. Although *R. lichtenfelsi*, *R. milleri*, *R. canadensis*, and *R. catostomi* possess long polar filaments, *R. ahuehuellensis* possesses short filaments on the surface near the polar ends but not originating directly from them. It does not possess numerous filaments as in *R. ovifilamenta*.

Rhabdochona ahuehuellensis n. sp. most closely resembles *R. lichtenfelsi*, a parasite of 2 other goodeid fishes, *G. atripinnis* Jordan and

A. robustus (Bean), found in Lake Cuitzeo and Lake Pátzcuaro. Both species possess a prostom with 10 teeth and bifurcated deirids. However, males of *R. ahuehuellensis* n. sp. and *R. lichtenfelsi* possess different spicular structures, the former with a trifurcate left spicule with a ventral bifurcated barb, which can be seen at ×400 magnification (Fig. 4), and the latter a bifurcate spicule with a simple ventral projection. In addition, both differ in the structure of the right spicule: *R. ahuehuellensis* n. sp. possesses a dorsal gorgeret lacking in *R. lichtenfelsi*; the proximal end of the right spicule of *R. ahuehuellensis* n. sp. possesses a cuticular spine similar to that of *R. lichtenfelsi*.

In both sexes tail tips are very different. Whereas *R. lichtenfelsi* possesses a pointed end, the new species has a tapered, rounded end. On the whole, *R. ahuehuellinsis* n. sp. can be set apart from the rest of the North American species of *Rhabdochona* by the trifurcate structure of the left spicule and the thick nonpolar filaments of the eggs.

DISCUSSION

Rhabdochona ahuehuellensis is unusual among species of its genus in the Americas because of its highly restricted distribution. It has been found only in the headwaters of the Balsas River and not in other localities along its main drainage where *I. whitei* is found. Besides, the only host in which it was found, in its type locality, is the Balsas goodeid, *I. whitei* Meek, and it was not found in other hosts examined simultaneously, a poeciliid, *Poecilia sphenops* Valenciennes, and a characid, *Astyanax mexicanus* (Filippi). Another species of *Rhabdochona*, namely, *R. kidderi* Pearse, 1936, has been found in the same river, parasitizing *I. whitei* in the Amacuzac River, some 200 km west of the present locality, on the other side of the volcanic Sierra near Mount Popocatépetl. That record might be considered a consequence of a recent introduction or transfaunation because it appears that *R. kidderi* is more typical of pimelodids and cichlids (see Pérez-Ponce de León et al., 1996), which are mainly found in southeastern Mexico. Additionally, 4 other species of *Rhabdochona* were reported recently from localities of the Balsas River basin: *R. canadensis* Moravec and Arai, 1971 in *Hybopsis boucardi* (Günther); *R. kidderi* in *C. istlanum* (Jordan and Snyder) and *C. nigrofasciatum*; *R. lichtenfelsi* in *G. atripinnis*; and *R. mexicana* in *Astyanax fasciatus* (Caspeta-Mandujano et al., 2000; Salgado-Maldonado et al., 2001). However, the taxonomic identity of that material could not be confirmed because specimens reported as having been deposited at CNHE (Salgado-Maldonado et al., 2001) were in fact never deposited.

Species of *Rhabdochona* have flourished in the Americas in species of Cyprinidae and Catostomidae and may have had speciation events through host switching to salmonids, cottids, etc. The species of this genus seems particularly species rich in cyprinids, and the Cyprinidae may comprise its original host family. If this hypothesis is corroborated by a phylogenetic analysis, then a route of invasion toward South America can be postulated, one that involves cyprinids and other families of freshwater fish. However, the biogeographical history of these fish families indicates that their distributional range extends only southward to central Mexico and, rarely (Ictaluridae, Catostomidae), to some parts of southeastern Mexico into the Neotropical region (Miller and Smith, 1986). The occurrence of a few species of *Rhabdochona* in freshwater fishes in South America, infecting typical components of the fish fauna of the Neotropics (pimelodids, cichlids, and characids), poses another interesting question, i.e., whether or not the species of *Rhabdochona* in the Americas constitute a monophyletic assemblage. The geographical position of Mexico, where the Neotropical and Nearctic regions converge, will constitute the key element in the understanding of the historical biogeography of *Rhabdochona* in the Americas once a phylogenetic hypothesis is proposed for this group of nematodes. Empirical evidence available at the moment makes it difficult to disentangle host and geographical specificity among members of *Rhabdochona* inhabiting freshwater fishes of central Mexico. It is hoped that parascript studies (see Brooks and McLennan, 1993), involving *Rhabdochona* spp. and their hosts, along with studies on other taxa of helminths, e.g., *Spinitectus* spp., corallobothrine cestodes (Choudhury and Pérez-Ponce de León, 2001; Pérez-Ponce de León and Choudhury, 2002), will provide useful information for understanding the historical biogeography of host–parasite associations of this region.

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Adult Endohelminth Parasites of Goodeinae (Cyprinodontiformes: Goodeidae) from México with Biogeographical Considerations

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ABSTRACT: A total of 1,294 goodeinid fish representing 35 species collected from 51 localities in Mexico was examined for ?1 adult intestinal helminths. Sampling was conducted between September 2001 and December 2004. Ten helminth species were collected (4 digeneans, 2 cestodes, and 4 nematodes). *Allocreadium lobatum* and *Proteocephalus longicollis* are reported for the first time in Mexico. The geographic and host range of *Allocreadium mexicanum* is extended to include new host species within Goodeinae in the Mezquital basin. *Margotrema bravoae* was found mainly in the Lerma-Santiago River basin and sparsely in the Balsas River, and *Margotrema guillerminae* was only found in the Western basins related to the Mesa Central. *Proteocephalus longicollis* was found in 1 host and 1 location. *Rhabdochona lichtenfelsi* was the most prevalent and abundant helminth in collections from the freshwater basins of Lerma-Santiago and Pánuco river systems. It is considered as the only ?2 nematode species of goodeids endemic to both basins. *Rhabdochona ahuehuellensis* was found mainly in the Balsas, in the Western Basins supposed to have been formerly related in geological time to the Balsas, and in the Panuco basin. *Margotrema bravoae*, *M. guillerminae*, *R. ahuehuellensis*, and *R. lichtenfelsi* are considered to be the core species of the helminth communities of Goodeinae. Two exotic helminths, *Bothriocephalus acheilognathi* and *Pseudocapillaria tomentosa*, are reported from new hosts and locations. Overall, 29 new host and 48 new locality records are reported for endohelminths of goodeinid fishes in Mexico. Distribution of *Margotrema* spp. is congruent with a vicariant origin that closely mirrors the phylogeny of the Goodeidae whereas *Rhabdochona* spp. does not, indicating it has multiple host and biogeographical origins within the Americas.

KEY WORDS: Endohelminths, exotic, native, Goodeinae, Mexico, *Allocreadium*, *Margotrema*, *Bothriocephalus*, *Proteocephalus*, *Pseudocapillaria*, *Rhabdochona*.

Endemic freshwater fishes of North America that belong to the family Goodeidae (sensu Parenti, 1981) have been studied for more than a century (see Doadrio and Domínguez, 2004; Webb et al., 2004 and references therein). These freshwater fishes constitute 42 species representing 2 subfamilies, Empetrichthynae and Goodeinae. The distribution of the former is limited to some springs in the southwestern United States; the latter is endemic to central Mexico (Miller, 1986; Miller and Smith, 1986; Doadrio and Domínguez, 2004; Webb et al., 2004). Subfamily Goodeinae includes 39 species representing 16 genera and is distributed mainly (11 of 16 genera, Uyeno et al., 1983) in the freshwater basins of the Mesa Central of México (Lerma, Balsas, Pánuco, and several smaller basins and springs). In addition, the Mesa Central is a biogeographical zone that has been tectonically subject to intermittent periods of geological activity ever since its uplift in the Upper Miocene. These processes have had profound influence on the biogeographic history of its freshwater fauna (Álvarez, 1972; Barbour, 1973; Mateos et al., 2002; Doadrio and Domínguez, 2004). The helminth fauna of the goodeine fishes has been explored irregularly ever since 1987 (Mejía-Madrid, 1987; unpublished thesis, Facultad de Ciencias, UNAM, México D.F., Mexico; Peresbarbosa et al., 1994; Martínez-Aquino et al., 2004; Sánchez-Nava et al., 2004), and no previous records of this fish subfamily as a whole exist. We ?3 present results of the first extensive survey of the adult endohelminths of goodeines, reporting endohelminths from 32 host species representing 15 genera collected from 51 localities in 10 freshwater basins of central México: Ameca, Lerma-Santiago, and Mexico City basins, and the headwaters of neighboring basins, Balsas, Mezquital, San Marcos, Magdalena, Panuco, Armeria, and Coahuayana basins. We incorporate previous faunal and distributional records to reveal major biogeographical patterns.

MATERIALS AND METHODS

Goodeinid fishes from 51 localities representing 10 distinct freshwater basins in central Mexico were collected between September 2001 and December 2004. Geographic coordinates and sample size for each host species collected for each

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locality are as follows. Mexico—Aguascalientes: Río Calvillo (21°52'48.5"N; 102°37'54.8"W), *Goodea atripinnis* (15); Río Juchipila (21°49'30.4"N; 101°46'04.9"W), *G. atripinnis* (5); Durango: Amado Nervo (23°50'32.0"N; 104°11'13.7"W), *Characodon lateralis* (15); El Toboso (24°16'32.5"N; 104°34'56.4"W), *Characodon audax* (10); Guadalupe Aguilera (24°26'04.9"N; 104°38'45.8"W), *C. audax* (10); Los Berros (23°56'18.2"N, 104°16'26.4"W), *C. audax* (13); Jalisco: Ahuacapán (19°39'44.7"N; 104°19'13.7"W), *Allodontichthys zonistius* (10), *Ilyodon furcidens* (3); El Tule (19°19'34.2"; 103°22'15.0"W), *Allodontichthys hubbsi* (6), *I. furcidens* (10); Guachinango (20°32'0.5"N; 104°24'8.7"W), *I. furcidens* (12); La Coronilla (20°28'9.4"N; 104°04'10.6"W), *G. atripinnis* (15); Laguna de Zapotlán (19°45'03.4"N; 103°28'00.9"W), *Xenotoca melanosoma* (1); Pihuamo (19°15'23.5"N; 103°22'37.3"W), *A. hubbsi* (5), *I. furcidens* (8); Potrero Grande (20°31'14.9"N; 104°07'36.8"W), *I. furcidens* (4), *Allotoca goslinei* (9); Presa Tacotán (20°33' 21.2"N; 104°21'44.1"W), *I. furcidens* (6); Río Cuzalapa (19°27'40.1"N; 104°19'12.3"W), *Xenotaenia resolanae* (15); Río Magdalena (20°53'29.4"N; 104°01'55.2"W), *G. atripinnis* (1), *Allotoca maculata* (12); Río San Marcos (20°46'35.7"N; 104°09'52.6"W), *X. melanosoma* (11); *A. maculata* (15), *G. atripinnis* (3); Río San Marcos (20°53'28.7"N; 104°01'17.2"W), *Xenotoca eiseni* (5); Río Tamazula (19°43'22.7"N; 103°12'08.5"W), *I. furcidens* (11), *Allodontichthys tamazulae* (7), *X. eiseni* (3); Río Tecolote (19°27'40.1"N; 104°19'12.3"W), *X resolanae* (14), *I. furcidens* (10); Río Verde (21°49'12.0"N; 101°46'21.3"W), *G. atripinnis* (25); San Isidro (20°47'10.3"N; 103°21' 49.3"W), *G. atripinnis* (6); Teuchitlán (20°41'20.6"N; 103°50'29.9"W), *Ameca splendens* (17), *G. atripinnis* (7); Mexico City: Xochimilco (19°15'58"N; 99°06'31"W), *G. atripinnis* (20); Michoacán: Queréndaro (19°48'02.7"N; 100°54'25.2"W), *Allotoca dugesii* (5); La Luz (19°56' 08.1"N, 102°18'00.2"W), *G. atripinnis* (19), *Alloophorus robustus* (10), *Chapalichthys encaustus* (17), *Skiffia multipunctata* (22); La Minzita (19°38'40.3"N; 101°16'28.5"W), *A. robustus* (4), *G. atripinnis* (5), (25), *Xenotoca variata* (10), *Zoogoneticus quitzeoensis* (17); Lago de Orandino (19°57'21.8"N; 102°19'29.7"W), *A. robustus* (13), *G. atripinnis* (17), *X. variata* (26), *S. multipunctata* (1); Lago de Pátzcuaro (19°36'5"N; 101°39'13"W), *Allotoca diazi* (10), *A. robustus* (19), *G. atripinnis* (18); Lago de Zacapu (19°49'35"N; 101°47'10"W), *Hubbsina turneri* (14), *Z. quitzeoensis* (15), *Allotoca zacapuensis* (17); Laguna de Opopeo (19°24'16.7"N; 101°36'09.1"W), *Allotoca meeki* (19), *G. atripinnis* (7); Los Reyes (19°33'43.5"N; 102°27'39"W), *Allotoca regalis* (7); Manantial Cutzaróndiro (19°10'59.0"N; 101°30'13.0"W), *Ilyodon cortesae* (30); Manantial Chapultepec (19°34'20"N; 101°31'18.7"W), *A. diazi* (34), *A. dugesii* (6), *Skiffia lermae* (51); Maravatío (19°52'56.1"N; 100°26'51.9"W), *Girardinichthys multiradiatus* (4), *G. atripinnis* (5); Naranja de Tapia (19°16'58.2"N, 101°45'50.3"W), *G atripinnis* (15), *X. variata* (12); Presa Arsteo Mercado (19°55'34.6"N; 101°39'38"W), *G. atripinnis* (15); Presa Caliztonzin (19°25'14.8"N; 102°07'05.8"W), *Allotoca catarinae* (16), *Ilyodon whitei* (10), Presa Cupatitzio (19°25.9'00"N; 102°4'59.5"W), *A. catarinae* (7); Puente Río Queréndaro (19°53'09.6"N; 100°57'06.9"W), *G. atripinnis* (15), *Skiffia bilineata* (15); Queréndaro (19°48'02.7"N; 100°54'25.2"W), *A. dugesii* (5); Río San Marcos (20°53' 28.7"N; 104°01'17.2"W), *G. atripinnis* (3); San Cristóbal (19°57'41.6"N; 101°18'57.3"W), *X. variata* (21), *A. robustus* (1), *G. atripinnis* (15); San Juanico (19°50'13.3"N; 102°38'35.4"W), *Chapalichthys peraticus* (10); Tocumbo (19°42'07"N; 102°30'58.4"W), *Chapalichthys pardalis* (11); Tocumbo (19°42'07"N; 102°30'58.4"W), *G atripinnis* (9); Zinapécuaro (19°48'08.7"N; 100°54'03.2"W) and (19°51' 49.7"N; 100°50'24.4"W), *A. dugesii* (4), *G atripinnis* (3), *X. variata* (9); Nayarit: Colonia 6 de enero (21°31'31.7"N; 104°48'14.8"W), *X. eiseni* (14); Río Compostela (21°14' 24.3"N; 104°54'38.7"W), *X. eiseni* (15); Puebla: Río Ahuehuello (18°45'19"N; 98°34'20.4"W), *I. whitei* (180); San Luis Potosí. Lago de la Media Luna (21°51'18.6"N; 100°01'22.3"W), *Ataeniobius toweri* (19); Jesús María (21°55'31.0"N; 100°54'38.3"W), *Goodea gracilis* (14); Tierra Quemada (21°42'39.1"N, 100°41'32.6"W), *G. gracilis* (15); El Venado (22°56'02.9"N; 101°06'25.2"W), *Xenoophorus captivus* (20); Tierra Quemada (21°42'39.1"N; 100°41' 32.6"W), *X. captivus* (11), *X. variata* (6). Fish were taken alive to the laboratory, pithed, and examined individually for intestinal helminths. Other organs (liver, gall bladder, spleen, and swim bladder) were examined under a stereomicroscope in separate petri dishes with 6.5% saline. Digeneans and cestodes were fixed with 4% hot (steaming) formalin and nematodes with 70% boiling ethanol or 4% hot formalin. Platyhelminths were stained with Harris hematoxylin, Gomori trichrome, or chlorhidric carmine. Nematodes were cleared in Amman lactophenol. Voucher specimens are deposited in the Colección Nacional de Helmintos (CNHE), Instituto de Biología, Universidad Nacional Autónoma de México (UNAM), México D.F., Mexico. Abbreviations associated with specimen accession numbers presented in this study refer to the following museums and collections: CNHE, Harold W. Manter Laboratory (HWML), and United States National Parasite Collection, Beltsville, Maryland (USNPC). Where no identifying records are listed, no specimen was deposited. Prevalence and mean abundance were calculated and used as defined in Margolis et al. (1982) and as modified by Bush et al. (1997).

Digenea

Allocreadium lobatum Wallin, 1909

Hosts, localities, prevalence, and mean abundance: *Allotoca zacapuensis*, Lago de Zacapu, 1 of 17 hosts examined (5.8%, 0.1 ± 0.5); *Z. quitzeoensis*, Lago de Zacapu, 2 of 15 (13.3%, 0.4 ± 1.3).

Site of infection: Intestine.

Type host: Semotilus bullaris.

Reported hosts in Mexico: None.

Other locality records in Mexico: This is the first report from Mexico.

Specimens deposited: CNHE 5133–5134.

Remarks: Allocreadium lobatum has been extensively reported in cyprinids and to a lesser extent in salmonids and catostomids from freshwater fishes in North America (Hoffman, 1999). This is the first record in Mexico of this species and extends its

geographic range down to the Lerma-Santiago basin, the southernmost locality where *A. lobatum* is found. In addition, they may also represent a species with a Nearctic origin. The ecological host-extension (Brooks and McLennan, 1993) of this digenean is hard to explain in the absence of more information from the hosts related to the families mentioned above. *Allocreadium lobatum* is readily distinguished from *Allocreadium mexicanum* Osorio-Sarabia, Pérez-Ponce de León and Salgado-Maldonado, 1986, in the relative size of the oral and ventral suckers, the extension of the vitelline follicles, the form, size, and position of the testes, the position of the ovary, and the extension of the excretory vesicle.

Allocreadium mexicanum Osorio-Sarabia, Pérez-Ponce de León and Salgado-Maldonado, 1986

Hosts, localities, prevalence, and mean abundance: Characodon audax, El Toboso, 2 of 10 (20%; 0.8 ± 1.7); *C. lateralis*, Amado Nervo, 7 of 15 (46.7%; 0.8 ± 1.3).

Site of infection: Intestine.

Type host: Chirostoma estor.

Reported hosts in Mexico: Chirostoma estor (Osorio-Sarabia et al., 1986; Pérez-Ponce de León et al., 2000); *Chirostoma attenuatum* (Pérez-Ponce de León et al., 1994); *Atherinella crystallina* (Salgado-Maldonado, Cabañas-Carranza, Soto-Galera, et al., 2001); *Chirostoma riojai* (Salgado-Maldonado, Cabañas-Carranza, Soto-Galera, et al., 2001).

Other locality records in Mexico: Estado de México—Santiago Tilapa, Laguna de Guadalupe Victoria (Salgado-Maldonado, Cabañas-Carranza, Soto-Galera, et al., 2001); Michoacán—Lago de Pátzcuaro (Osorio-Sarabia et al., 1986; Pérez-Ponce de León et al., 2000); Lago de Zirahuén (Pérez-Ponce de León et al., 1994); Nayarit—Río Santiago (Aguamilpa) (Salgado-Maldonado, Cabañas-Carranza, Soto-Galera, et al., 2001).

Specimens deposited: CNHE 5135–5136.

Other specimens from Mexico: ex. *Chirostoma estor*, CNHE 697–700, 709; ex. *C. attenuatum*, CNHE 1351.

Remarks: Allocreadium mexicanum has been reported previously only from Atherinidae and in diverse water bodies that belong to the Lerma-Santiago basin. This report extends its host and geographical range to include Goodeids distributed further northward to the Mezquital basin in Durango.

Margotrema bravoae Lamothe-Argumedo, 1970

Hosts, localities, prevalence, and mean abundance: Alloophorus robustus, La Luz 1 of 10 (10%, 1.6); *A. diazi*, Lago de Pátzcuaro 1 of 10 (10%, 0.4 ± 1.3), Manantial Chapultepec 5 of 34 (14.7%, 0.3 ± 0.7); *A. dugesii*, Manantial Chapultepec 6 of 6 (100%, 3.7 ± 1.7); *A. maculata*, Río San Marcos 10 of 15 (66.8%, 2.1 ± 2.8); *A. meeki*, Laguna de Opopeo 15 of 19 (79%, 5 ± 9.1); *A. regalis*, Los Reyes 1 of 7 (14.3%, 0.1 ± 0.4); *A. zacapuensis*, Lago de Zacapu 6 of 17 (35.3%, 0.6 ± 1); *G. airipinnis*, Río Verde 3 of 25 (12%; 0.2 ± 0.5).

Site of infection: Intestine.

Type host: Girardinichthys multiradiatus.

Reported hosts in Mexico: Girardinichthys multiradiatus (Lamothe-Argumedo, 1970; Salgado-Maldonado, Cabañas-Carranza, Soto-Galera, et al., 2001; Sánchez-Nava et al., 2004); *A. robustus* (Pérez-Ponce de León, 2001); *A. diazi* (Pérez-Ponce de León, 2001); *A. zonistius* (Salgado-Maldonado, Mercado-Silva, et al., 2004).

Other locality records in Mexico: Estado de México—La Lagunilla (Lamothe-Argumedo, 1970); Villa Victoria (Salgado-Maldonado, Cabañas-Carranza, Soto-Galera, et al., 2001; Sánchez-Nava et al., 2004); Michoacán—Canal el Porvenir, Ciudad Hidalgo (Sánchez-Nava et al., 2004); Lago de Pátzcuaro (Pérez-Ponce de León, 2001); Jalisco—Río Ayuquila, Sierra de Manantlán Biosphere Reserve (Salgado-Maldonado, Mercado-Silva, et al., 2004).

Specimens deposited: CNHE 5137–5145.

Other specimens from Mexico: ex. *Girardinichthys multiradiatus*, CNHE 869, 4759; ex. *A. diazi*, CNHE 4211–4212; ex. *A. zonistius* CNHE 4808.

Remarks: Margotrema bravoae has been recorded in the Lerma basin ever since Lamothe-Argumedo (1970) erected the genus *Margotrema*. Pérez-Ponce de León (2001) extended its geographic range to other sites in Michoacán state. This is the first record in goodeids of the upper Santiago basin in Jalisco, Mexico, extending its geographic range and pointing to a hydrological relationship between the Lerma and Santiago basins based on their helminth faunas. *Margotrema bravoae* is primarily found in *Allotoca* spp. Records of this species in *A. zonistius* from Río Ayuquila by

Salgado-Maldonado, Mercado-Silva, et al. (2004) is uncertain because the single specimen deposited at CNHE is in a poor condition. The extension of both ceca and vitellaria cannot be observed, and they are required to establish species differentiation.

Margotrema guillerminae Pérez-Ponce de León, 2001

Hosts, localities, prevalence, and mean abundance: Allodontichthys hubbsi, El Tule 2 of 6 (33.3%; 0.5 ± 0.8); *A. tamazulae*, Río Tamazula 3 of 7 (42.9%, 1 ± 1.4); *A. zonistius*, Ahuacapán 7 of 10 (70%; 3 ± 2.8); *C. pardalis*, Tocumbo 2 of 11 (18.2%, 0.4 ± 0.9); *C. audax*, El Toboso 1 of 10 (10%; 0.3 ± 0.9); *I. cortesae*, Manantial Cutzaróndiro 2 of 30 (6.7%, 0.1 ± 0.4); *I. furcidens*, Potrero Grande 1 of 4 (25%, 0.25 ± 0.5), Río Tamazula 1 of 11 (9.1%, 0.1 ± 0.3); *I. whitei*, Río Ahuchuellp 130 of 180 (72.2%, 2.7 ± 4.6); *X. melanosoma*, Río San Marcos 6 of 11 (54.5%, 1.8 ± 2.6).

Site of infection: Intestine.

Type host: Hybopsis calientis.

Reported hosts in Mexico: Alloophorus robustus (Pérez-Ponce de León, 2001). CNHE 3965, 3966, 28 4210; HWML 16380, 16381).

Other locality records in Mexico: Michoacán—Lago de Zacapu (Pérez-Ponce de León, 2001).

Specimens deposited: CNHE 5146–5155.

Other specimens from Mexico: ex. *Allophoorus robustus* CHNE 3966, 4210; ex. *H. calientis* CHNE 3965.

Remarks: Margotrema guillerminae had only been reported previously from Lago de Zacapu, Michoacan. This report extends its range down to the Pacific basins west of Mesa Central, where it is mainly found, although the type locality lies within the central basins of Michoacan. A total of 757 individuals belonging to the 2 recognized species of *Margotrema* were collected. A total of 186 *M. bravoae* and 558 *M. guillerminae* were collected, making *M. guillerminae* the most abundant digenean in goodeinids. Of the 35 species of goodeinids examined, 18 were infected with *Margotrema* spp.; 10 were infected with *M. bravoae*, and 8 were infected with *M. guillerminae*. According to the phylogenetic analysis of the Goodeidae based on mitochondrial DNA (Doadrio and Domínguez, 2004), *M. guillerminae* is predominantly associated with the basal portions of the host clade (Illyodontini and Characontini) and *M bravoae* is associated with the derived host clades, predominantly Girardinichthyni.

Cestoda

Bothriocephalus achellognathi Yamaguti, 1934

Hosts, localities, prevalence, and mean abundance: Ailotoca zacapuensis, Lago de Zacapu 2 of 17 (11.8%, 0.3 ± 0.1); *C. audax*, Los Berros 1 of 13 (7.7%, 0.3 ± 1.1); *G. multiradiatus*, Maravatío 2 of 4 (50%, 0.5 ± 0.6); *I. cortesae*, Manantial Cutzaróndiro 2 of 30 (6.7%, 0.2 ± 0.9); *S. bilineata*, Puente Río Querendaro 1 of 15 (6.6, 0.1 ± 0.3).

Site of infection: Intestine.

Type host: Cyprinus carpio. 29

Reported hosts in Mexico: Cyprinidae—*Algansea lacustris* (Mendoza-Garfias et al., 1996); *Algansea rubescens* (García-Prieto and Osorio-Sarabia, 1991); *Algansea tincella* (Salgado-Maldonado, Cabañas-Carranza, Soto-Galera, et al., 2001); *Carassius auratus* (Sanabria-Espinosa and Sánchez-Santana, 1989); *Carassius carassius* (Alarcón-González, 1988; Alarcón-González and Castro-Aguirre, 1988); *Ctenopharyngodon idella* (López-Jiménez, 1981, 1982; Guillén-Hernández et al., 1991); *C. carpio* (Salgado-Maldonado et al., 1986; García-Prieto and Osorio-Sarabia, 1991; León-Règagnon, 1992; Salgado-Maldonado, Cabañas-Carranza, Soto-Galera, et al., 2001); *Dionda ipni* (Salgado-Maldonado and Pineda-López, 2003); *Hybopsis boucardi* (Salgado-Maldonado, Cabañas-Carranza, Caspeta-Mandujano, et al., 2001); *Megalobrema amblycephala* (García-Prieto and Osorio-Sarabia, 1991); *Notropis celayensis* (Salgado-Maldonado and Pineda-López, 2003); *Notropis sallei* (León-Règagnon, 1992; Salgado-Maldonado, Cabañas-Carranza, Soto-Galera, et al., 2001; Salgado-Maldonado and Pineda-López, 2003); *Yuriria alta* (Salgado-Maldonado and Pineda-López, 2003; Salgado-Maldonado, Cabañas-Carranza, Soto-Galera, et al., 2001); Characidae—*Astyanax fasciatus* (Salgado-Maldonado, Cabañas-Carranza, Soto-Galera, et al., 2001); Goodeidae—*A. robustus* (Peresbarbosa et al., 1994); *A. diazi* (Peresbarbosa et al., 1994); *G. multiradiatus* (León-Règagnon, 1992; Astudillo-Ramos and Soto-Galera, 1997; Salgado-Maldonado, Cabañas-Carranza, Soto-Galera, et al., 2001; Sánchez-Nava et al., 2004); *G. atripinnis* (García-Prieto and Osorio-Sarabia, 1991; Pineda-López and González-Enríquez, 1997; Salgado-Maldonado and Pineda-López, 2003); *X. variata* (Pineda-López and González-Enríquez, 1997;

Salgado-Maldonado, Cabañas-Carranza, Soto-Galera, et al., 2001; Salgado-Maldonado and Pineda-López, 2003); Poeciliidae—*Gambusia yucatana* (Scholz, 1997); *Gambusia vittata* (Salgado-Maldonado and Pineda-López, 2003); *Heterandria bimaculata* (Salgado-Maldonado, Cabañas-Carranza, Caspeta-Mandujano, et al., 2001; Salgado-Maldonado and Pineda-López, 2003); *Poecilia butleri* (Salgado-Maldonado and Pineda-López, 2003); *Poecilia mexicana* (Salgado-Maldonado and Pineda-López, 2003); *Poecilia sphenops* (Salgado-Maldonado, Cabañas-Carranza, Caspeta-Mandujano, et al., 2001); *Poecilia reticulata* (Salgado-Maldonado, Cabañas-Carranza, Caspeta-Mandujano, et al., 2001); *Poeciliopsis baenschi* (Salgado-Maldonado and Pineda-López, 2003); *Poeciliopsis gracilis* (Salgado-Maldonado, Cabañas-Carranza, Caspeta-Mandujano, et al., 2001); *Poeciliopsis* sp. (Salgado-Maldonado and Pineda-López, 2003); Atherinidae—*A. crystallina* (Salgado-Maldonado, Cabañas-Carranza, Soto-Galera, et al., 2001); *Chirostoma arge* (Salgado-Maldonado and Pineda-López, 2003); *C. attenuatum* (García-Prieto and Osorio-Sarabia, 1991); *C. estor* (Osorio-Sarabia et al., 1986; Salgado-Maldonado et al., 1986; Guillén-Hernández et al., 1991; Pérez-Ponce de León et al., 1994); *Chirostoma grandocule* (García-Prieto and Osorio-Sarabia, 1991); *Chirostoma humboldtianum* (Astudillo-Ramos and Soto-Galera, 1997); *Chirostoma jordani* (Salgado-Maldonado, Cabañas-Carranza, Soto-Galera, et al., 2001); *Chirostoma labarcae* (Salgado-Maldonado, Cabañas-Carranza, Soto-Galera, et al., 2001); *Chirostoma scotilanae* (García-Prieto and Osorio-Sarabia, 1991); *C. riojai* (Salgado-Maldonado, Cabañas-Carranza, Soto-Galera, et al., 2001); *Chirostoma* sp. (Pineda-López and González-Enríquez, 1997); *Melaniris balsanus* (García-Prieto and Osorio-Sarabia, 1991); Centrarchidae—*Micropterus salmoides* (Salgado-Maldonado et al., 1986); Cichlidae—*Cichlasoma cyanoguttatum* (Salgado-Maldonado and Pineda-López, 2003); *Cichlasoma istlanum* (Salgado-Maldonado, Cabañas-Carranza, Caspeta-Mandujano, et al., 2001; Salgado-Maldonado and Pineda-López, 2003); *Nandopsis istlanum* (Salgado-Maldonado, Mercado-Silva, et al., 2004); *Cichlasoma labridens* (Salgado-Maldonado and Pineda-López, 2003); *Cichlasoma meeki* (Vidal-Martínez et al., 2001); *Cichlasoma urophthalmus* (Salgado-Maldonado et al., 1997); *Cichlasoma nigrofasciatum* (Salgado-Maldonado, Cabañas-Carranza, Caspeta-Mandujano, et al., 2001); *Oreochromis niloticus* (Pineda-López and González-Enríquez, 1997).

Other locality records in Mexico: Río Acamulco (no state reported) (Salgado-Maldonado and Pineda-López, 2003), Campeche—Lago Nuevo Becal (Vidal-Martínez et al., 2001); Estado de México—Atlacomulco (Sánchez-Nava, 2004), Ciénega de Lerma (León-Règagnon, 1992), el CIMMYT (Sánchez-Nava et al., 2004), La Lagunilla, Lago de Chicnahuapan, Parque Sierra Morelos, Presa Ignacio Ramírez (Salgado-Maldonado, Cabañas-Carranza, Soto-Galera, et al., 2001; Sánchez-Nava et al., 2004), Lago Santiago Tilapa (Salgado-Maldonado, Cabañas-Carranza, Soto-Galera, et al., 2001), Rancho La venta, Acambay, Salazar, San Juanico, San Pedro del Rosal (Sánchez-Nava et al., 2004), Presa La Goleta (Sanabria-Espinosa and Sánchez-Santana, 1989), Presa Trinidad Fabela (Astudillo-Ramos and Soto-Galera, 1997), Río San Gerónimo, Ixtapan de la Sal (Salgado-Maldonado, Cabañas-Carranza, Caspeta-Mandujano, et al., 2001); Guanajuato—Manantial El Realito (Salgado-Maldonado and Pineda-López, 2003), Presa Ignacio Allende, Presa La Biznaga, Presa Trinidad Fabela (Salgado-Maldonado, Cabañas-Carranza, Soto-Galera, et al., 2001), Río Los Galvanes, San Miguel de Allende (Salgado-Maldonado and Pineda-López, 2003); Guerrero—Presa Tepecoacuilco, Río Acatlán, Río Petatlán (Salgado-Maldonado, Cabañas-Carranza, Caspeta-Mandujano, et al., 2001); Hidalgo—Río Amajac (Salgado-Maldonado and Pineda-López, 2003), Río Atlapexco; Hidalgo—Río Talol, Río Venados (Salgado-Maldonado and Pineda-López, 2003); Hidalgo—Tezontepec (López-Jiménez, 1981; García-Prieto and Osorio-Sarabia, 1991); Jalisco—Lago de Chapala (García-Prieto and Osorio-Sarabia, 1991), Río Ayuquila (Salgado-Maldonado et al., 2004), El Chacalito, El Grullo, Manantlán, Achacales, Palo Blanco (Salgado-Maldonado and Pineda-López, 2003); Michoacán—Lago de Pátzcuaro (Osorio-Sarabia et al., 1986; Salgado-Maldonado et al., 1986; García-Prieto and Osorio-Sarabia, 1991; Guillén-Hernández et al., 1991; Peresbarbosa et al., 1994; Mendoza-Garfias et al., 1996), Lago de Zirahuén (Pérez-Ponce de León et al., 1994), Presa Cointzio (Astudillo-Ramos and Soto-Galera, 1997), Presa de Infiernillo (López-Jiménez, 1981), Presa Infiernillo (García-Prieto and Osorio-Sarabia, 1991); Morelos—Río Amacuzac, Contlalco, Río, Huajintlán (Salgado-Maldonado, Cabañas-Carranza, Caspeta-Mandujano, et al., 2001); Nayarit—Presa Aguamilpa, Río Santiago (Salgado-Maldonado, Cabañas-Carranza, Soto-Galera, et al., 2001); Oaxaca—Río Cuyotepeji, Río Michapa, Río Petlalcingo, Río Huajuapan de León (Salgado-Maldonado, Cabañas-Carranza, Caspeta-Mandujano, et al., 2001); Querétaro—Manantial Los Váquez (Salgado-Maldonado

210

and Pineda-López, 2003), Presa Constitución 1917, Presa El Batán, Presa El Batán (Pineda-López and González-Enríquez, 1997), Presa El Carmen (Salgado-Maldonado and Pineda-López, 2003), Presa Rayas (Pineda-López and González-Enríquez, 1997), Quemada (Salgado-Maldonado and Pineda-López, 2003), Río Concá (Pineda-López and González-Enríquez, 1997), Río Grande (Salgado-Maldonado and Pineda-López, 2003), Río Jalpan (Pineda-López and González-Enríquez, 1997), Río Las Zúñigas, Río Quiotillos, Río San Pedro, Río Xote (Salgado-Maldonado and Pineda-López, 2003); Tabasco—Río Jonuta (Salgado-Maldonado et al., 1997); Tlaxcala—Atlangatepec (Alarcón-González, 1988; Alarcón-González and Castro-Aguirre, 1988; Guillén-Hernández et al., 1991); Yucatán—Cenote Homún (Scholz, 1997).

?11 Laguna de Celestún (Salgado-Maldonado et al., 1997).

?12 *Specimens deposited:* CNHE 5156–5160.

Other specimens from Mexico: ex. *Algansea lacustris*, CNHE 1326; ex. *A. rubescens*, CNHE 440; ex. *C. idella*, CNHE 446; ex. *C. carpio*, CNHE 438; ex. *D. ipni*, CNHE 4935; ex. *N. sallei*, CNHE 437, 4849; ex. *A. robustus*, CNHE 1317; ex. *A. diazi*, CNHE 1323; ex. *G. multiradiatus*, CNHE 439; ex. *P. hnileri*, CNHE 4809; ex. *P. mexicana*, CNHE 4937; ex. *P. baenschi*, CNHE 4792; ex. *C. attenuatum*, CNHE 1318; ex. *C. estor*, CNHE 1811; ex. *C. ocotlanae*, CNHE 2497; ex. *M. balsanus*, CNHE 445; ex. *N. istlanum*, CNHE 4791.

Remarks: This is 1 of the most widely distributed helminth species in Mexico. At least 50 species of hosts and 58 localities in this country have been recorded. García-Prieto and Osorio-Sarabia (1991), Pérez-Ponce de León et al. (1996), and Salgado-Maldonado and Pineda-López (2003) include comprehensive reviews of the distribution and host records of this tapeworm in Mexico.

Proteocephalus longicollis (Zeder, 1800)

Hosts, localities, prevalence, and mean abundance: Skiffia lermae, La Minzita 6 of 25 (24%, 0.4 ± 1).

Site of infection: Intestine.

Type host: Salmo trutta.

Reported hosts in Mexico: Goodea atripinnis (Mejía-Madrid, 1987, unpublished thesis).

Other locality records in México: Michoacán—Lago de Pátzcuaro (Mejía-Madrid, 1987, unpublished thesis).

Specimens deposited: CNHE 5161.

Other specimens from Mexico: None.

Remarks: Previously, a proteocephalid tapeworm identified as *Proteocephalus pusillus* Ward, 1910, was recorded infecting *G. atripinnis* in Lago de Pátzcuaro, Michoacán (Mejía-Madrid, 1987, unpublished thesis). This new record closely resembles the specimens from Lago de Pátzcuaro. *Proteocephalus pusillus* is a junior synonym of *P. longicollis* sensu Scholz and Hanselová (1998); it is here established that the former material belongs to *P. longicollis*. *Proteocephalus exiguus* La Rue, 1911, is also a junior synonym of *P. longicollis* according to Scholz and Hanselová (1998) but is primarily reported from salmonid fishes in North America (Hoffman, 1999). More detailed studies of the proteocephalids reported in goodeids will enable to establish new morphological variations that will add to those already recognized in the recent literature.

Nematoda
Pseudocapillaria tomentosa (Dujardin, 1843)

Hosts, localities, prevalence, and mean abundance: Skiffia lermae, Manantial Chapultepec 3 of 51 (5.8%, 0.2 ± 0.8); *G. atripinnis*, Lago de Pátzcuaro 2 of 18 (11.1%, 0.11 ± 0.3), Maravatío 1 of 5 (20%, 0.2 ± 0.4); *G. gracilis*, Jesús María, 1 of 14 (7.1%, 0.1 ± 0.3).

Site of infection: Intestine.

Type host: Catostomus commersoni.

Reported hosts in Mexico: Chirostoma estor (Osorio-Sarabia et al., 1986; Espinosa-Huerta et al., 1996; Pérez-Ponce de León et al., 2000); *C. attenuatum* (Pérez-Ponce de León et al., 1994, 2000); *G. atripinnis* (Mejía-Madrid, 1987, unpublished thesis; Pérez-Ponce de León et al., 2000); *A. lacustris* (Mendoza-Garfias et al., 1996; Pérez-Ponce de León et al., 2000); *C. carpio* (see Pérez-Ponce de León et al., 1996, 2000); *A. robustus* (Pérez-Ponce de León et al., 2000); *N. sallei* (Salgado-Maldonado, Cabañas-Carranza, Soto-Galera, et al., 2001).

Other locality records in Mexico: Estado de México—Presa Ignacio Ramírez (Salgado-Maldonado, Cabañas-Carranza, Soto-Galera, et al., 2001); Michoacán—Lago de Pátzcuaro (Osorio-Sarabia et al., 1986; Mejía-Madrid, 1987, unpublished thesis; Pérez-Ponce de León et al., 1994, 1996, 2000;

Espinosa-Huerta et al., 1996; Mendoza-Garfias et al., 1996).

Specimens deposited: CNHE 5162–5165.

Other specimens from Mexico: ex. *Chirostoma estor*, CNHE 2255; ex. *G. atripinnis*, CNHE 2256; ex. *A. robustus*, CNHE 4079.

Remarks: This species was originally described as *Capillaria patzcuarensis* Osorio-Sarabia, Pérez-Ponce de León and Salgado-Maldonado, 1986. Moravec et al. (2000) pointed out that the type material corresponded to *Ornithocapillaria appendiculata* Teixeira de Freitas, 1933, therefore *C. patzcuarensis* should be redescribed on the basis of new material. Later, Moravec et al. (2001) studied new material from Lago de Pátzcuaro and proposed the synonymy of this species with *P. tomentosa*. Therefore, records of *C. patzcuarensis* in Osorio-Sarabia et al. (1986), Mendoza-Garfias et al. (1986), Espinosa-Huerta et al. (1996), and Pérez-Ponce de León et al. (1994, 1996, 2000) reflect reports of *P. tomentosa*.

Rhabdochona ahuehuellensis Mejía-Madrid and Pérez-Ponce de León, 2003

Hosts, localities, prevalence, and mean abundance: Allodontichthys hubbsi, Pihuamo 3 of 5 (60%; 1 ± 1.1); *A. tamazulae*, Río Tamazula 3 of 7 (42.9%, 0.6 ± 0.8); *A. toweri*, Lago de la Media Luna 16 of 19 (84.2%, 4.8 ± 3.6); *I. furcidens*, Guachinango 1 of 12 (8.3%, 0.08 ± 0.3), Pihuamo 5 of 8 (62.5%, 1.7 ± 1.7), Rio Tamazula 4 of 11 (36.4%, 0.5 ± 0.7); *I. whitei*, Río Ahuehuello 115 of 180 (63.9%, 1.14 ± 2.4); *X. resolanae*, Río Tecolote 4 of 14 (28.6%, 0.4 ± 0.6).

Site of infection: Intestine.

Type host: Ilyodon whitei.

Reported hosts in Mexico: Ilyodon whitei (Mejía
?13 Madrid and Pérez-Ponce de León, 2003).

Other locality records in Mexico: Puebla—Río Ahuehuello (Mejía Madrid and Pérez-Ponce de León, 2003).

Specimens deposited: CNHE 5166–5173.

Other specimens from Mexico: ex. *Ilyodon whitei*, CNHE 4417–4420; USNPC 92333–92336.

Remarks: Rhabdochona ahuehuellensis is extensively associated among the basal groups of Goodeidae, mainly in the Ilyodontini (Doadrio and Dominguez, 2004). The present records extend its geographical range within the Balsas basin. Sampling sites along the Pánuco basin are new locality records for this recently described rhabdochonid. *Rhabdochona ahuehuellensis* has been found only in the aforementioned basins, which points to an ancient relationship between both; a hypothesis put forward by Chernoff and Miller (1981) for the modern distribution of *N. sallei*.

Rhabdochona lichtenfelsi Sánchez-Álvarez, García-Prieto & Pérez-Ponce de León, 1998

Hosts, localities, prevalence, and mean abundance: Alloophorus robustus, La Luz 8 of 10 (80%, 1.9 ± 1.2), La Minzita 2 of 4 (50%, 1.5 ± 2.4), Lago de Orandino 1 of 13 (7.7%, 0.1 ± 0.3), Lago de Pátzcuaro 12 of 19 (63.2%, 5.4 ± 8), San Cristóbal 1 of 1 (100%, 2); *A. diazi*, Lago de Pátzcuaro 4 of 10 (40%, 6.1 ± 12.2); *A. zacapuensis*, Lago de Zacapu 13 of 17 (76.5%, 9 ± 13.8); *A. splendens*, Teuchitlan 4 of 17 (23.5, 0.2 ± 0.4); *C. encaustus*, La Luz 10 of 17 (58.8%, 1 ± 1.2); *C. pardalis*, Tocumbo 4 of 11 (36.4%, 0.9 ± 2.1); *C. audax*, Los Berros 5 of 13 (38.5%, 0.7 ± 1.4); *G. atripinnis*, La Luz 15 of 19 (78.9%, 12 ± 15.7), La Minzita 4 of 5 (80%, 13 ± 12), Lago de Orandino 2 of 17 (11.8%, 0.8 ± 2.7), Lago de Pátzcuaro 17 of 18 (94.4%, 9 ± 7.7), Naranja de Tapia 14 of 15 (93.3%, 24.3 ± 21.7), Presa Aristeo Mercado 8 of 15 (53.3%, 2.4 ± 3.5), Puente Río Queréndaro 1 of 15 (6.7%, 0.1 ± 0.2), San Cristóbal 14 of 15 (93.3%, 5.7 ± 3.1), Tocumbo 6 of 9 (66.7%, 7.2 ± 8.4), La Coronilla 12 of 15 (80%, 2.3 ± 2.1), Río Verde 15 of 25 (60%, 3.8 ± 6.0), Teuchitlán 7 of 7 (100%, 9.6 ± 6.2); *G. gracilis*, Tierra Quemada 22 of 24 (92%, 4.0 ± 3.4); *H. turneri* Lago de Zacapu 13 of 14 (92.9%, 13.9 ± 12.4); *S. bilineata* Puente Río Queréndaro 1 of 15 (6.7%, 0.1 ± 0.3); *S. lermae* La Minzita 22 of 25 (88%, 9.6 ± 8.4); *S. multipunctata* La Luz 19 of 22 hosts (86.4%, 5.3 ± 6.1); *X. variata* Lago de Orandino 9 of 26 (34.6%, 1.2 ± 2.1), Naranja de Tapia 8 of 12 (66.75, 3 ± 3.4), San Cristóbal 9 of 21 (42.9%, 1.5 ± 2.6); *Z. quitzeoensis*, Lago de Zacapu 7 of 15 (46.7%, 1.5 ± 3.1).

Site of infection: Intestine.

Type host: Goodea atripinnis.

Reported hosts in Mexico: Alloophorus robustus (Peresbarbosa et al., 1994; Pérez-Ponce de León et al., 1996; Sánchez-Álvarez et al., 1998; Pérez-Ponce de León et al., 2000); *A. diazi* (Peresbarbosa et al., 1994; Pérez-Ponce de León et al., 1996,

2000); *C. encaustus* (Martínez-Aquino et al., 2004); *G. atripinnis* (Peresbarbosa et al., 1994; Pérez-Ponce de León et al., 1996, 2000; Sánchez-Álvarez et al., 1998; Salgado-Maldonado, Cabañas-Carranza, Caspeta-Mandujano, et al., 2001, Salgado-Maldonado, Cabañas-Carranza, et al. 2004); *I. furcidens* (Salgado-Maldonado, Mercado-Silva, et al., 2004); *P. mexicana* (Salgado-Maldonado, Cabañas-Carranza, et al., 2004).

Other locality records in Mexico: Hidalgo—Río Estórax (Salgado-Maldonado, Cabañas-Carranza, et al., 2004); Michoacán—Lago de Cuitzeo (Sánchez-Álvarez et al., 1998); Lago de Pátzcuaro (Mejía-Madrid, 1987, unpublished thesis; Peresbarbosa et al., 1994; Pérez-Ponce de León et al., 1996, 2000; Sánchez-Álvarez et al., 1998), Presa San Juanico (Salgado-Maldonado, Cabañas-Carranza, Caspeta-Mandujano, et al., 2001); Jalisco—Ayuquila River (Salgado-Maldonado, Mercado-Silva, et al., 2004), Lago de Chapala (Martínez-Aquino et al., 2004).

Specimens deposited: CNHE 5174–5205.

Other specimens from Mexico: ex. *Alloophorus robustus*, CNHE 1998, 2279, 3012, 3013, 3212–3215, USNPC 87766; ex. *A. diazi*, CNHE 2281; ex. *G. atripinnis*, CNHE 2280, 3212.

Remarks: This nematode is the most widely distributed nematode parasitizing the Goodeidae but has never been reported from other fish families (except for *P. mexicana*, where it seems to be an accidental infection). It ranges from the Mezquital and Santiago rivers from Western Central Mexico, both being new records, to the Lerma and Pánuco basins, Central and Eastern Mexico, as reported in this study. Mejía-Madrid (1987, unpublished thesis), Peresbarbosa et al. (1994), and Pérez-Ponce de León et al. (1996) recorded this species erroneously as *Rhabdochona milleri* Choquette, 1951. For correct specimen designations of *R. lichtenfelsi* refer to Sánchez-Álvarez et al. (1998). This nematode is absent in the Ayuquila basin, contrary to some probable misidentifications of specimens found in *I. furcidens* (Salgado-Maldonado, Mercado-Silva, et al., 2004). The species of *Rhabdochona* present in all *Ilyodon* species is *R. ahuehuellensis*.

Rhabdochona xiphophori Caspeta-Mandujano, Moravec and Salgado-Maldonado, 2001

Hosts, localities, prevalence, and mean abundance: Alloloca catarinae, Presa Caltzontzin 3 of 16 (18.8%, 2.8 ± 6.4); *X. eiseni*, Colonia 6 de enero 5 of 14 (35.7%, 0.6 ± 1.2).

Site of infection: Intestine.

Type host: Xiphophorus sp.

Reported hosts in Mexico: Xiphophorus sp. (Caspeta-Mandujano et al., 2001, CNHE 3940, 3941).

Other locality records in Mexico: Hidalgo—Río Tenango (Caspeta-Mandujano et al., 2001).

Specimens deposited: CNHE 5206–5207

Other specimens from Mexico: ex. *Xiphophorus* sp., CNHE 3940, 3941.

Remarks: This nematode was first reported from some unknown species of *Xiphophorus* (Caspeta-Mandujano et al., 2001) in the Pánuco basin (Salgado-Maldonado, Cabañas-Carranza, et al., 2004). It has been found in *Xiphophorus helleri* in the río Armería near Colima City, in Mexico (Mejía-Madrid and Pérez-Ponce de León, unpublished data). In addition, it is here reported in 2 distinct species of goodeids in the Western basins of the Mesa Central, Balsas basin, and Compostela basin (the latter belongs to the Santiago basin). Rhabdochonid nematodes totaling 2,751 individuals comprising 327 specimens of *R. ahuehuellensis*, 2,369 specimens of *R. lichtenfelsi*, and 55 specimens of *R. xiphophori*. *Rhabdochona lichtenfelsi* is the most abundant nematode of the assemblage, followed by *R. ahuehuellensis*. The former species is present in 15 of the 22 species where *Rhabdochona* spp. were found, whereas the latter was only present in 5 species. *Rhabdochona xiphophori* is a more typical parasite of poeciliids (*X. helleri*) and has probably passed into at least 2 species of goodeids (*A. catarinae* and *X. eiseni*) through ecological host-extension.

DISCUSSION

Of the 1,294 goodeinid fishes representing 35 species and 16 genera that were collected from 51 localities, 679 individuals representing 32 species and 15 genera were infected with at least 1 species of adult endohelminth. *Allotoca goslinei* (1 site), *C. peraticus* (1 site), and *X. captivus* (2 sites) were the only fish species sampled that were not infected with adult helminths. Before this study, the only species of Goodeids studied were *A. diazi*, *A. robustus*, *C. encaustus*, *G. atripinnis*, *I. whitei*, and *G. multiradiatus*. Of the 32 species of goodeid hosts examined,

29 represent new records of adult endohelminths, including 12 new host records for *R. lichtenfelsi*, 9 for *M. guillerminae*, 5 for *M. bravoae*, 5 for *R. ahuehuellensis*, 3 for *B. acheilognathi*, 2 for *A. mexicanum*, 2 for *P. tomentosa*, 2 for *R. xiphophori*, 2 for *A. lobatum*, and 1 for *P. longicollis*. Adult helminths were collected from 34 of 51 localities, yielding 15 new locality records for *R. lichtenfelsi*, 10 for *M. guillerminae*, 7 for *M. bravoae*, 6 for *R. ahuehuellensis*, 5 for *B. acheilognathi*, 3 for *P. tomentosa*, 2 for *A. mexicanum*, 2 for *R. xiphophori*, 1 for *A. lobatum*, and 1 for *P. longicollis*.

The core adult endohelminth fauna of the Goodeinae (sensu Pérez-Ponce de León and Choudhury, 2002, 2005) is represented by *M. bravoae*, *M. guillerminae*, *R. ahuehuellensis*, and *R. lichtenfelsi*. By any measure, these are the dominant intestinal endohelminths of goodeinid fishes in Mesa Central, Mexico: number of individuals, number of species of hosts infected, prevalence, mean abun- ?14 dance, and geographical range. Nevertheless, species of *Margotrema* and *Rhabdochona* co-occurred in only 9 localities. The basins where these species pairs occurred were distributed as follows: in 5 species of goodeinids collected in the Lerma basin, *A. robustus*, *A. diazi*, *A. zacapuensis*, *C. pardalis*, and *Z. quitzeoensis*; 2 in the Tamazula basin, *A. tamazulae*, *I. furcidens*; and 1 in Balsas basin, *I. whitei*, and 1 in the Santiago basin, *G. atripinnis*.

Our results support the hypothesis that the parasite fauna in freshwater fishes is largely circumscribed by higher levels of monophyletic host taxa (Pérez-Ponce de León and Choudhury, 2005). The 4 biogeographical core endohelminths of goodeinids are associated with members of both basal and derived goodeinid clades, but no case of host species specificity was observed. The other species of helminths found in goodeids of the Mesa Central represent either instances of ecological host-extension (e.g., *A. mexicanum*, a parasite of atherinids or *R. xiphophori*, a parasite of poeciliids) or cases of a wide host use by an introduced exotic (e.g., *B. acheilognathi* and *P. tomentosa*). As predicted by Pérez-Ponce de León and Choudhury (2005), instances of ecological host-extension are limited.

The modern distribution of helminth parasites that complete their life cycles in obligate aquatic vertebrates are confined not only to their hosts but also to basins or tributaries of continental waters. As such, they can provide evidence of the historical basin relationships when testing a vicariance null hypothesis. The modern distribution of the goodeinid core helminth fauna reflects the separation of the northern Pacific basins (i.e., Mezquital basin from the Lerma-Santiago and Ameca) and the southern Mesa Central (Armería and Coahuayana basins) from the Balsas. This hypothesis was proposed when the phylogeny of the Goodeidae (Doadrio and Domínguez, 2004) correlated with available geological evidence. The only endemic helminth sister species that track this event are *M. guillerminae* and *M. bravoae*. *Margotrema guillerminae* is present almost exclusively in the Characontini and Ilyodontini (sensu Doadrio and Domínguez, 2004), which are distributed in the northern Mezquital rivers and in the Balsas, Armería, and Coahuayana basins. In contrast, *M. bravoae* is associated with the most derived Chapalichthyini, the Goodini, and Girardinichthyni. The basal Chaplichthyni, which are present in some basins within the Ilyodontini distribution area might have acquired *M. guillerminae* through ecological host-extension or phylogenetic retention. ?15

An emerging picture of the biogeographical relationships of the rivers and streams of the Mesa Central and neighboring areas is disclosed by the modern distribution of *Margotrema* spp., one in which *M. guillerminae* is distributed outside the immediate boundaries of the Mesa Central and the other, *M. bravoae*, is distributed within the basins of the Mesa Central proper. The distribution is probably a consequence of the split between the Pacific and Mesa Central goodeids, a hypothesis strongly supported by phylogenetic analysis (see Doadrio and Domínguez, 2004). Apparently, the occurrence of the sister group of the Goodeinae in southern California and Nevada (subfamily Empethrichthynae) implies a continuous ancestral distribution in southern North America. Pérez-Ponce de León and Choudhury (2005) discussed how the 2 species of *Margotrema* may provide a potentially different line of evidence. Both species are placed along with species of *Wallinia* Pearse, *Magnivitellinum* Floss, and *Creptotrematina* Yamaguti in the Walliniinae, a group of allocreadid or macroderoidid digeneans commonly found in characids in Neotropical freshwater fishes (see Choudhury et al., 2002). Proper phylogenetic analysis will pro- ?16 vide evidence to determine whether the presence of *Margotrema* spp. in endemic goodeids from the Mesa Central of Mexico is a result of a host-switching event from either Nearctic or Neotropical species of freshwater fishes (Pérez-Ponce de León and Choudhury, 2005), the latter case being supported by the Gondwanalandic origin of the Cyprinodontiformes (Parenti, 1981).

No conclusions can be drawn from the same database assembled here for *Rhabdochona* spp. It is quite

clear that the present distribution of *Rhabdochona* species in North American freshwaters is not the result of vicariant events that took place, at least recently, in any of those modern basins, for this group of nematodes is not monophyletic in the Americas and even less in the Mesa Central of Mexico. However, those data point to the fact that species of *Rhabdochona* are more closely related to the Nearctic species than to the Neotropical ones. The rest of the adult endohelminths reflects mainly the relationships of the Mesa Central of Mexico and surrounding basins to the Nearctic freshwater fish fauna, as exemplified by *A. lobatum* and *P. longicollis* establishing the so-called Nearctic connection (Pérez-Ponce de León and Choudhury, 2005).

The results of this study lead to a more accurate framework for future studies based on the helminth faunas of specific freshwater fish families, which has proved to be a better research strategy (Vidal-Martínez et al., 2001; Pérez-Ponce de León and Choudhury, 2002; Salgado-Maldonado, Moravec, et al., 2004) rather than the accumulation of information from extensive freshwater basins, where the ?17 majority of helminths found are larval forms. Adult helminths are more indicative of modern and past distributions not only of helminth taxa themselves but also of their fish hosts as well. This will enable helminthologists, ichthyologists, and biogeographers to draw a better and more clear picture of the past and present relationships of continental waters beyond the local spatiotemporal scale.

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CAPÍTULO II.

FILOGENIA Y BIOGEOGRAFÍA DEL GÉNERO *Rhabdochona* Railliet, 1916 (NEMATODA: RHABDOCHONIDAE) EN AMÉRICA

En este capítulo se desarrolló un análisis filogenético de 44 especies del género *Rhabdochona* Railliet, 1916 en el cual se incluyeron de manera especial las especies que parasitan a peces de agua dulce de las Américas. El análisis resultó en 19 árboles igualmente parsimoniosos con la búsqueda island hopper/ratchet y 1733 árboles, también parsimoniosos, con búsqueda heurística al azar, los cuales indican que el género *Rhabdochona* es monofilético. La topología de los árboles de consenso estricto obtenidos es casi idéntica. Las ambigüedades presentes se originan de la poca resolución de los caracteres que no han sido descritos adecuadamente para *Rhabdochona*. El único caracter que demostró ser consistente en ambos análisis fue la forma de la espícula izquierda de los machos. Solamente en un clado el número de dientes fue consistente y contiene a un grupo importante de especies Neárticas y Neotropicales. Las especies de las Américas no son monofiléticas y se encuentran ampliamente distribuidas en al menos 5 clados distintos. Las especies de *Rhabdochona* que se encuentran asociadas a ciertos grupos de hospederos tales como salmónidos, catostómidos y goodéidos, no siempre forman grupos monofiléticos, así como las especies asociadas a áreas pequeñas, con respecto a las Américas, tales como la Mesa Central de México. Esto indica que es más común que la evolución de *Rhabdochona* en las Américas se haya llevado al cabo por un amplio cambio de hospederos más que por coespeciación. Los patrones filogenéticos rebelan un origen antiguo para este género que antecede en el tiempo a la configuración actual de los continentes.

Phylogeny and biogeography of *Rhabdochona* Railliet, 1916 (Nematoda : Rhabdochonidae) species from the Americas

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Abstract

A phylogenetic systematic analysis based on morphological characters was carried out on 44 species of the genus *Rhabdochona* Railliet, 1916 including 23 that parasitize freshwater fish of the Americas. The analyses resulted in 19 to 1733 equally most parsimonious trees generated from different searches, island hopper/ratchet and heuristic, respectively. Both revealed that *Rhabdochona* is arguably monophyletic. Both searches identified at least 5 clades that include species from the Americas and species from other continents. Species from the Americas are therefore not monophyletic. The sinapomorhphies identified from all 5 clades stem from left spicule form of males. Teeth number was consistent only in 1 clade. This means that most characters that are recognized as valuable for taxonomic description and species identification, perform poorly in phylogenetic analyses. Species of *Rhabdochona* associated with certain host groups such as salmonids, catostomids and goodeids do not always form monophyletic assemblages, nor do species associated with smaller discrete areas, such as the Mesa Central of Mexico. This indicates widespread host switching rather than co-speciation as the main phenomenon in the evolution of this group

in the Americas. Phylogenetic patterns reveal an ancient origin for the group that probably pre-dates current continental configurations.

Introduction

The genus *Rhabdochona* Railliet, 1916, comprises 104 species that are distributed worldwide in freshwater fishes, except in Australia (Moravec, 1975): 41 species in the Oriental, 30 in the Palaearctic, 17 in the Nearctic, 10 in the African and 6 in the Neotropical bigeographic regions. This genus belongs to the subfamily Rhabdochoninae Travassos, Artigas & Pereira, 1928 (Moravec, 1975), family Rhabdochonidae Skrjabin, 1946, which in turn is classified within the Superfamily Thelazioidea Sobolev, 1949 (Anderson, 2000). The subfamily is defined (Moravec, 1975) by rudimentary pseudolabia or lack thereof, a rounded or hexagonal mouth, the common (but not universal) presence of 'teeth' in the anterior end of the vestibule, the general lack of caudal alae, and sessile caudal papillae. Of the 23 species in the Americas, 21 belong solely to North America, where 17 are Nearctic and 6 are Neotropical. Only 2, or most probably 1, actually belong to the South American helminth fauna (Cremonte et al., 2002). The 21 North American species are primarily parasites of the Cyprinidae (minnows), Catostomidae (suckers) and Salmonidae (trouts), and to a lesser extent, Characidae (characins), Cottidae (sculpins), Cyprinodontidae (killifishes), Goodeidae (splitfins), Ictaluridae (nearctic catfishes) and Percidae (perches). Of these, at least 5 inhabit the most northern tropical plateau, the Mesa Central of Mexico, and its neighboring freshwater basins.

Species of *Rhabdochona* apparently exhibit high levels of host specificity (Moravec, 1975). Several species of *Rhabdochona* also parasitize hosts belonging to monophyletic lineages (e.g., 43 species in cyprinids, 5 in salmonids, 3 in catosotomids, 2 in goodeids, etc.). Such lineage restriction raises the possibility of coevolution but the wide distribution

and diverse host-associations of *Rhabdochona* spp. may also suggest an evolutionary history of extensive ecological host extensions and host switching. The considerable diversification of the genus *Rhabdochona* in the Americas provides us with the raw material to examine these ideas in a phylogenetic context, which in turn will provide insights into the biogeographical history of this speciose group of nematodes.

The specific aims of the present study are: to propose a phylogenetic hypothesis of the species of *Rhabdochona* in the Americas, to determine if the group conforms a monophyletic group in that continent, by including in this analysis some widely distributed and well described species from Eurasia and Africa, to infer to what extent the species of the genus has co-evolved with its hosts or host switched between fish hosts of different families, and finally to discover the highlights of its biogeographical history in this region.

Materials and methods

Information on the morphology of *Rhabdochona* spp. and outgroups were obtained from three sources: specimens from field collections, museum depositions, and literature on those species not readily available for study.

Field collections

Canada- *Cystidicola farionis* and *R. milleri* collected from salmonids and catostomids in Manitoba, Canada, during 1992.

México- *R. ahuehuellensis* – Río Balsas, Ayuquila, and Pánuco Basins, hosts: *Ilyodon whitei*, *Allodontichthys hubbsi*, *A. zonistius*, and *Ataeniobius toweri*; 2000, 2001, and 2003; *R. guerreroensis* – Río Ayuquila Basin, host *Sicydium multipunctatum*, 2005; *R. kidderi* – Río Santiago Basin, host *Haplochromis niloticus;* 2001; *R. lichtenfelsi* – Río Lerma and Pánuco Basins, various goodeid hosts, 2002-2003; *R. mexicana*-Río Mezquital Basin, host

Characodon audax, *R. xiphophori* – Armería, Balsas, and Santiago Basins, hosts: *Xiphophorus helleri*, *Allotoca catarinae*, and *Xenotoca eiseni*, 2001 and 2003.

Museum Collections

Colección Nacional de Helmintos (CNHE): *Beaninema nayaritense* 3937 (paratypes), *Heptochona* sp. 3604; *R. ahuehuellensis* 4417 (holotype), 4418 (allotype), 4419 (paratypes); *R. californiensis* 3074 (voucher), *R. kidderi* 2698, 2699, 3286 (voucher), *R. lichtenfelsi* 3212 (holotype), 3213 (allotype), 3012, 3013, 3214, 3215 (paratypes), *R. mexicana* 4031 (holotype), 4032 (allotype), 4033 (paratypes), *R. paxmani* 3075 (voucher), *R. salgadoi* 3886 (holotype), 3887 (allotype), 3888 (paratypes), *R. salmonis* 3076, *R. xiphophori* 3940 (holotype), 3941 (allotype), 3942 (paratypes).

United States National Parasite Collection (USNPC): *R. canadensis* 71792, 78919, 79044 (paratypes), *R. canadenisis bifilamentosa* 80005 (paratypes), *R. catostomi* 74896, 74899, 74900 (slides 1268-23, 1268-24) (paratypes), *R. congolensis* 749290 (1225-10, 1225-11, voucher), *R. cotti* 36991, 78739, 83640 (voucher), *R. decaturensis* 36992, 84546 (voucher), *R. kidderi texensis* 80006 (paratypes), *R. longleyi* 80004 (paratypes), *R. ovifilamenta* 77981 (paratype), *R. penangensis* (syn. of *R. hospeti*) (voucher) 60306, *R. rotundicaudadum* 81937 (paratypes).

Harold W. Manter Laboratory of Parasitology UNL (HWML): *R. cascadilla* 37587 (voucher).

Species considered

An analysis was carried out on 23 species of the Americas. These include: *R. acuminata* (Molin, 1860), *R. ahuehuellensis* Mejía-Madrid & Pérez-Ponce de León, 2003, *R. californiensis* Maggenti, Abdel-Rahman & Cid del Prado, 1992, *R. canadensis* Moravec & Arai, 1971, *R. cascadilla* Wigdor, 1918 *R. catostomi* Kayton, Kritsky & Tobias, 1979, *R.*

cotti Gustafson, 1949, *R. cubensis* Moravec & Coy-Otero, 1987, *R. decaturensis* Gustafson, 1949, *R. guerreroensis* Caspeta-Mandujano, Moravec & Salgado, 2002; *R. kidderi* Pearse, 1936, *R. kisutchi* Margolis, Moravec & McDonald, 1975, *R. lichtenfelsi* Sánchez-Álvarez, García-Prieto & Pérez-Ponce de León, 1998, *R. longleyi* Moravec & Huffman, 1988, *R. mexicana* Caspeta-Mandujano, Moravec & Salgado-Maldonado, 2000, *R. milleri* Choquette, 1951, *R. ovifilamenta* Weller, 1938, *R. paxmani* Maggenti, Abdel-Rahman & Cid del Prado, 1992, *R. rotundicaudatum* Byrne, 1992, *R. salgadoi* Caspeta-Mandujano & Moravec, 2001, *R. salmonis* Maggenti, Abdel-Rahman & Cid del Prado, 1992, *R. uruyeni* Díaz-Ungría, 1968, and *R. xiphophori* Caspeta-Mandujano, Moravec & Salgado-Maldonado, 2001. The subspecies, *R. kidderi kidderi* Pearse, 1936, *R. kidderi texensis* Moravec & Huffman, 1988 and *R. canadensis bifilamentosa* Moravec & Huffman, 1988, were not considered.

Three African species were included with the foregoing: *R. congolensis* Campana-Rouget, 1961, *R. gambiana* Gendre, 1922, and *R. paski* Baylis, 1928. *Rhabdochona congolensis* and *R. paski* may be conspecific, but adult characters were only considered (Puylaert, 1969). The 16 Eurasian species considered in the analysis were: *R. anguillae* Spaul, 1927, *R. coronacauda* Belouss, 1965, *R. denudata* (Dujardin, 1945), *R. ergensi* Moravec, 1968, *R. fortunatowi* Dinnik, 1933, *R. gnedini* Skrjabin, 1946, *R. hellichi* (Šrámek, 1901), *R. hospeti* Thapar, 1950, *R. humili* Roytman & Trofimenko, 1964, *R. japonica* Moravec, 1975, *R. jiangxiensis* Wang, Zhao, Wang & Zhang, 1979, *R. oncorhynchi* (Fujita, 1921), *R. phoxini* Moravec, 1968, *R. squalobarbi* Moravec & Sey, 1988, *R. vietnamensis* Moravec & Sey, 1988, and *R. zacconis* Yamaguti, 1935.

Cystidicola farionis Fischer, 1798 (Cystidicolidae) was used as the outgroup as it is a representative, in some measure, of the spirurids outside the superfamily Thelazioidea.

Beaninema nayaritense Caspeta-Mandujano, Moravec & Salgado-Maldonado, 2001, *Pancreatonema torriense* Gibson & McVicar, 1988 and, *Vasorhabdochona cablei* Martin & Zam, 1967, (Rhabdochoninae *sensu* Moravec, 1975), were also included without specifying their phylogenetic status, in order to explore outgroup and ingroup relationships simultaneously.

Phylogenetic analysis

Fifty one characters were considered for analysis. These included one external somatic character, 11 cephalic and oral characters, two cervical characters, 33 caudal characters, and four reproductive characters. Characters of the 44 taxa used in the analysis were coded in a matrix using MacClade 4 (Maddison & Maddison, 2000) as binary (28 characters) or multistate (23 characters) summing a total 78 apomorphic states. Characters and character states were coded according to their designation in the literature (Rasheed, 1965; Moravec, 1972a, 1975 and references therein). For further information, refer to character argumentation (below). Unknown characters were coded as '?', inapplicable characters were coded '-'. Analyses were performed with NONA V. 2.0 (Goloboff, 1993), trees drawn with WinClada V. 1.00.08 (Nixon, 1999) and PAUP*V4.0b10 (Swofford, 2000). Characters were equally weighted and unordered. Optimization criterion used was ACCTRAN/Fast Optimization. Since more than 25 species were analyzed (Kitching et al., 1998), a heuristic search was undertaken with PAUP, TBR algorithm, random addition of taxa with 100 replicates and an island hopper/ratchet was undertaken with NONA with 200 iterations with 100 replicates per iteration, TBR algorithm. Two analyses are presented: an initial with PAUP and a second analysis with NONA with the assumptions already outlined. A fast boostrap analysis with 100 replicates, step-wise addition with random addtion sequence was performed with PAUP*V4.0b10 (Swofforrd, 2000) and a bootstrap

with 100 replicates was perfomed with NONA in order to assess branch support in both analyses.

Character argumentation

(Figures 1 & 2)

External somatic character

1. *Body lateral alae.* Two states: absent - 0, present – 1. This is a character that has only been found in *R. coronacauda* and *R. squalobarbi* (Belouss, 1965 In Moravec, et al. 1981; Moravec, 1975; Moravec & Sey, 1988).

Cephalic and oral characters

2. *Anterior region.* Two states: wide – 0, tapered - 1. This character refers to the narrowed condition of the cephalic and cervical regions exhibited by *Pancreatonema torriense* as stated by Gibson & McVicar (1988). *Beaninema nayaritense* and *Rhabdochona* spp. possess the wide condition.
3. *Pseudolabia.* Two states: Present - 0, absent - 1. We follow Chabaud (1971) in that *Rhabdochona* spp. lack this character. Moravec (1972a, 1975), but not mentioned by Rasheed (1965), states that this character is present. Nevertheless, SEM observations of various species of *Rhabdochona* as compared to *C. farionis* do not lend support to those observations.
4. *Base of vestibule.* Two states: annulated - 0; straight - 1. This character has not been described in all those groups classified in the Rhabdochoninae considered here, yet it is depicted in drawings included in those descriptions plus personal obsevations, of *B. nayaritense* and *Heptochona* sp. (see Fig. 2), plus drawings depicted in *P. torriense* (Gibson & McVicar, 1988).

5. *Prostom dimensions.* Three states: narrow - 0, wide – 1, expanded – 2. Our coding follows the description of Moravec (1975) in his brief introductory account of Rhabdochoninae. According to Rasheed (1965) the expanded condition is apparently congruent with the absence (0) or presence (1) of basal teeth (character 16), in her revision of the genus *Rhabdochona* (page 408). The narrow condition is only present in *C. farionis*, where no basal teeth are found; in all other cases the prevailing condition in *Rhabdochona* is the expanded state where the prostom is separated from the mesostom by basal teeth in some, but not all of the species (see Appendix I).
6. *Prostom funnel shaped.* Two states: absent – 0, present - 1.
7. *Vestibule.* Two states: short - 0, long - 1. The coding was done after a meristic (not reported here) discontinuity was found between short and long vestibules that apparently discriminates non-*Rhabdochona* species from *Rhabdochona* spp., respectively.
8. *Teeth.* Two states: absent – 0, present – 1. This undeniably is a character that is symplesiomorphic for the whole of *Rhabdochona* as a genus. The homology of the so called longitudinal thickenings with other species outside this family, e.g., *Cystidicola farionis*, makes this character so important that the latter was chosen as an outgroup.
9. *Number of teeth in anterior prostom.* Seven states: Absent - 0, 6 - 1, 8 - 2, 10 - 3, 14 - 4, 16 – 5, 12-6. It is quite apparent that some species may exhibit some combinations of both teeth numbers, i.e., 14 and 16 teeth (*R. ovifilamenta*, Moravec & Arai, 1971; 6 and 8 (*R. coronacauda*, see Moravec & Sey, 1978), 14 or more (*R. japonica*, see Moravec, 1998). Nervertheless, when one of two states was found in one single species, no polymorphisms were considered and only the apomorphic condition was coded. Seemingly, in the Americas there are no 8 teeth species. It has been demonstrated

through careful developmental studies, that the third-stage larvae of several species of *Rhabdochona* have only 2 lateral teeth (called cystidicoline stage), that become 6 in the fourth-stage larva. The dorsal and ventral teeth appear in L4 as single teeth, that presumably increase in number (duplicates or triplicates) according to species. This has been observed in *R. acuminata*, *R. ergensi*, *R. kidderi*, *R. oncorhynchi*, and *R. phoxini*, and in *R. lichtenfelsi* (personal observations). Therefore this character could follow ontogeny, according to Moravec & Huffman (2001), and Moravec, 1972b). But as *a priori* considerations of recapitulatory phenomena in the developmental biology of *Rhabdochona* were not entertained here, this character is treated as unordered.

10. *Prostom basal teeth*. Two states: Absent - 0, present – 1. *Beaninema nayaritense* is described with basal teeth in the original description (Caspeta et al., 2001), but Moravec et al., (2001) re-named these as "middle teeth". The original designation is coded for the analysis.
11. *Dorso-ventral external teeth*. Two states: absent – 0, present – 1.
12. *Lateral external teeth*. Two states: absent – 0, present – 1.

Several of the aforementioned characters, and some of following ones that only appear in *C. farionis*, are coded as plesiomorphic.

Cervical characters

13. *Deirid*s. Three states: absent – 0, simple –1, bifurcate –2.
14. *Deirid position*. Four states: absent – 0; anterior - 1, middle - 2, posterior - 3. The position of deirids is designated in relation to the vestibule (Moravec, 1972a), i.e., close to prostom (near), middle of the vestibule or near its posterior end (Moravec, 1972a).

Caudal characters

15. *Area rugosa*. Two states: absent - 0, present - 1. New observations by the authors reveal that *R. xiphophori* possesses an area rugosa in adult forms (Fig. 1). Apparently, Caspeta-Mandujano et al. (2001) described immature specimens of this nematode, because they described eggs found in females as immature. Other character codifications distinct from the original description of this nematode will be found in what folllows.
16. *Caudal alae.* Two states: absent - 0, present - 1.
17. *Cloacal deep flap*. Two states: absent - 0, present - 1.
18. *Circumcloacal papillae*. Two states: absent – 0; present – 1.
19. *Pedunculate papillae*. Two states: absent – 0, present – 1.
20. *Papillae position*. Two states: ventral - 0, subventral and lateral - 1. Another character that is symplesiomorphic to *Rhabdochona* relative to the outgroup and the other species.
21. *Postanal papillae number*. Two states: 2-5 pairs - 0, 6-7 pairs - 1. Six to 7 pairs is exclusive of *Rhabdochona,* although some species in the latter genus present 5 pairs (*R. equispiculata* and *R. gambiana*).
22. *Preanal papillae number*. Two states: 1-2 pairs - 0, more than 2 pairs - 1. Supernumerary preanal papillae is exclusive in its apomorphic state to *Rhabdochona* relative to the outgroup and the other species.
23. *Single adcloacal papillae.* Two states: absent - 0, present - 1.
24. *Gubernaculum.* Two states: present - 0, absent - 1.
25. *Left spicule distal.* Six states: pointed - 0, pronged - 1, lanceolate thin - 2, lanceolate wide - 3, bifurcate – 4, blunt - 5. (Fig. 1). Probably there is no other character so

remarkably complex and species specific within *Rhabdochona* than the distal end of the left spicule of males (Rasheed, 1965). Unfortunately, this structure cannot be well seen if not observed outside the nematode body (Moravec, 1972a). Detailed examination of this structure plus SEM photographs have revealed it is a complex structure sclerotized into 3 branches, 2 dorsal and 1 ventral, this latter futher divided in some species (Figs.1 & 2). The structure was found to be so complex that further coding of each of its character transformations into a multistate TS would have gone beyond the limit number of multistate character coding. Therefore, each structure had to be coded as binary or multistate as to render all of the variations of each distinct spicule type. For all structures coded from 25 to 41 please refer to Figs. 1 -2.

26. *Lanceolate thin membrane size.* Three states: absent – 0, membrane short or indistinct – 1, membrane wide-2.
27. *Lanceolate thin membrane extension.* Three states: absent – 0, dorsal membrane extended –1, dorsal membrane not extended –2.
28. *Lanceolate thin extent of bifurcation.* Three states: absent- 0, bifurcation of membrane and blade slight – 1, bifurcation of membrane and blade deep – 2.
29. *Lanceolate wide keel.* Two states: lateral keel absent – 0, lateral keel present –1. This structure was first named by Moravec & Amin (1978) when describing *R. denudata dzhalilovi* form cyprinids of Afghanistan.
30. *Lanceolate wide keel fusion.* Three states: lateral keel absent 0, lateral keel fused – 1, lateral keel free – 2.
31. *Lanceolate wide keel position.* Three states: lateral keel absent 0, lateral keel shallow – 1, lateral keel deep – 2.

32. *Lanceolate wide keel size.* Three states: lateral keel absent – 0, lateral keel long – 1, lateral keel short – 2.

33. *Lanceolate wide branch size.* Three states: absent – 0, dorsal branch short – 1, dorsal branch long - 2

34. *Lanceolate wide dorsal branch.* Three states: absent – 0, dorsal branch directed upwards –1, dorsal branch hooked – 2.

35. *Bifurcate furcae symmetry.* Three states: furcae absent – 0, furcae same size – 1, furcae distinct size –2.

36. *Bifurcate furcae size.* Three states: absent – 0, furcae long – 1, furcae short – 2.

37. *Bifurcate furcae/membrane.* Four states: absent – 0, furcae without membrane – 1, furcae outside membrane – 2, furcae inside membrane – 3.

38. *Blunt branches.* Three states: absent –0, simple distended distal tip – 1, dorsal and ventral ends duplicated –2.

39. *Blunt keel.* Three states: absent – 0, no ventral keel – 1, ventral keel 2.

40. *Blunt bifurcation.* Three states: absent – 0, blade not bifurcated –1, blade bifurcated - 2.

41. *Prong with cone.* Two states: absent – 0, present – 1.

42. *Left spicule, proximal.* Two states: simple - 0, broad - 1. Broad refers to distention or bifurcation of this internal region. This character might lead to multistates once better descriptions within *Rhabdochona* are reported.

43. *Right spicule distal-dorsal branch.* Two states: smooth - 0, barbed – 1.

44. *Right spicule proximal.* Two states: simple - 0, bulbous - 1.

45. *Right spicule shape.* Two states: straight (parallell sides) - 0, pyramidal form or "boat shaped" - 1.

46. *Spicule ratio.* Four states: 0-1:2.0 - 0, 1:1.2.1-1:4.0 -1, 1:4.1-1:6.0 – 2, 1:6.1-1:14.0 - 3.

47. *Tail.* Five states: conical pointed - 0, rounded or blunt - 1, sharp cuticular spike - 2, small cuticular processes - 3, terminus conoid with spicate mucro – 4. Tails with terminal small cuticular processes present in some African, American and Asian species will deserve closer attention. While the number of processes is variable, (not considered here), this character is sexually polymorphic, except in *R. equispiculata* and *R. salgadoi* from Laos and Mexico, respectively, where both males and females present this character. Such polymorphism was not included in the present analysis and only the apomorphic state was coded.

Reproductive characters

48. *Egg protuberances.* Four states: smooth - 0, floats - 1, filaments at or near poles - 2, flock-like structures – 3. These different covers are not exclusive of the eggs of *Rhabdochona,* as other groups taxonomically related to this genus possess them, i.e., *Cystidicola farionis.*

49. *Vagina direction.* Two states: posteriorly - 0, anteriorly - 1.

50. *Vulva position.* Two states: anterior - 0, middle – 1. This character was coded according to original descriptions where genera are distinguished by a distinct position of the vulva, as compared, i.e., to *Trichospirura* species (Moravec, 1975).

51. *Vulvar lips.* Three states: symmetrical - 0, asymmetrical upper lip (larger of the 2) - 1, asymmetrical lower lip (larger of the 2) – 2 (Fig. 1).

Results

A strict consensus tree of the 19 equally parsimonious trees (not shown) with 175 steps long, with a consistency index (CI) of 0.49, and a retention index (RI) of 0.69 resulted from the analysis with NONA (Figure 3). A slightly different topolgy was obtained when the matrix was analysed with PAUP. The strict consensus tree represented by 1733 trees

obtained from that analysis is shown (Figure 4). All trees recovered with PAUP had identical length, consistency, and retention indices to those of NONA.

The overall results show a topology of 2 ingroups: one monophyletic group formed by (*B. nayaritense* (*V. cablei* (*P. torriense*))) and the other formed by species of *Rhabdochona*. Both groups showed a bootstrap value of 100%. This means that both ingroups are closely related (Moravec, 1975). *Rhabdochona* is a monophyletic group (boostrap value 81%). The differences between both analyses stem from the level of resolution between some species of *Rhabdochona*, namely, *R. gambiana*, *R. cotti*, *R. anguillae*, associated to the basal clades and *R. kidderi*, *R. decaturensis* and *R. vietnamensis* within the group defined by bifurcate left spicules (character state 25-4). The rest of the clades are constant in all other resulting trees. They all show the same degree of polytomy among the 5 clades of *Rhabdochona* common to both analyses. Nevertheless, they are always grouped by the same sinapomorphies especially those related to left spicule of males.

The only characters that showed a high CI (1.00 or near it) are those that are related to left spicule structure of males (characters 25 to 41). Only in 1 case was teeth number a sinapomorphic character (9-1, 6 teeth) and groups the 7 species that include 5 American and 2 Asian species (Fig. 3 & 4). The high level of homoplasy observed in *Rhabdochona* spp. stems from the lack of resolution of most of the characters analysed as there appeared 35 false sinapomorphies that are really homoplasic characters against 25 true sinapomorphies distributed in the strict consensus tree as stated above (Fig. 3).

Mapping of hosts of the *Rhabdochona* spp. onto the strict consensus tree (Figure 5) indicates that Cyprinidae is the main host group but do not host any major clade of *Rhabdochona*. A crown clade involving Salmonidae appears to be the only consistent pattern of a clade of *Rhabdochona* spp. associated with any particular host group.

Mapping of geographical areas onto the strict consensus tree (Figure 6) indicates no consistent pattern of inter-continental relationships. A single clade of eight species (Fig. 3 & 4, bottom clade) can be identified as being associated with the Nearctic region. This clade is as well associated with three species from central and eastern Asia.

Discussion

The hypothesis herein presented sums up our present knowledge of the phylogenetic and biogeographic relationships in the genus *Rhabdochona*. While phylogenetic hypotheses of nematodes based on morphology have been problematic and show that such characters exhibit a high degree of homoplasy which calls for an immediate and complementary input of molecular data (Blaxter, 1996 In: Kennedy & Harnett, 2001), morphological hypotheses still represent important starting points in understanding the historical processes of diversification in these organisms.

The grouping of *B. nayaritense*, *V. cablei* and *P. torriense* into one clade and species of *Rhabdochona* into another supports the grouping of the genera by Moravec (1975) under one family Rhabdochonidae (Moravec et al., 2001). According to our analyses, there are 4 sinapomorphies that define family Rhabdochonidae: pseudolabia absent (character state 3-1), prostom funnel present (6-1), middle position of deirids (14-2), and bulbous form of the proximal end of right spicule (44-1). Distribution of character state 3-1 tells us that *P. torriense* actually lacks pseudolabia if compared to *C. farionis*.

Most characters are homoplasic. Characters that would appear to be consistent (*sensu* Kitching et al., 2000) are actually not informative characters for deducing phylogenetic relationships, e.g., teeth number, deirid form, egg protuberances, and tail form. Tail cuticular processes are present in a number of African and Asian species of *Rhabdochona*. *Rhabdochona salgadoi* is the only known species from the Americas that possesses that

character. Nevertheless, *R. gambiana*, that shares this character with other African species, is situated at the base of the present tree, while the others that possess this character are mostly grouped in 1 clade formed by (*salgadoi* (*congolensis* (*paski*))). Another character to which much attention has been paid in previous taxonomic work is number of anterior prostomial teeth. As our analyses show, 14 teeth appears as the original plesiomorphic state of *Rhabdochona*. Six teeth appear as a sinapomorphy for (*kisitchi acuminata cubensis xiphophori longleyi* (*coronacauda zacconis*)). Nevertheless, a reversal to 16 teeth occurs twice for *R. ovifilamenta* in 1 clade and *R. salgadoi* in another. From here on, 8 teeth defines another clade containing (*congolensis paski*) and appears again only in *coronacauda*.

There is no strong support from the results that the species of *Rhabdochona* from the Americas with 10 teeth are monophyletic, except for those that are seemingly associated with salmonid fishes, like *R. kisutchi*, , *R. paxmani*, *R. salmonis*, and *R. oncorhynchi* from Europe. Other species related to the latter possess 10 teeth: *R. ahuehuellensis* and *R. mexicana*. Egg filaments, which were thought three decades ago could bring together distinct species of *Rhabdochona*, do not support any monophyletic groups. The presence of filaments is plesiomorphic, due to their presence in the outgroup. The presence of basal teeth as a character appears only in *Rhabdochona* but is shared outside the genus with *B. nayaritense*. It seems that this character is not homologous if considered in *B. nayaritense* and *Rhabdochona* spp.

Analyses of character distributions in the cladogram shows that homoplasy concentrates on the cephalic and caudal regions, where more characters were sampled. Nevertheless, the greater number of homoplasies in reproductive traits is due to the nature of egg protuberances (filaments, flock-like coverings, polar caps or smooth covers).

Several published revisions of the genus *Rhabdochona* have already addressed the phylogenetic relationships within the broader scope of the family Rhabdochonidae (see Skrjabin et al., 1971 for a brief account). The account of Moravec, 1972b describes some general trends in the evolution of *Rhabdochona* with the support of ontological evidence. He states that primitive teeth number is 6. This observation is based solely on the developmental stages of these nematodes, where 2 teeth precede 6 teeth in the 3rd and 4th larval stages, respectively. Despite the above statement, present analysis indicates that 14 teeth is the plesiomorphic state (derived from a more numerous teeth number character state, probably 16 as shown), and 6 teeth most likely arose as a paedomorphic character that probably originated but only once. This is not new in the extensive literature of the genus, for Puylaert (1973) had already pointed to the probable paedomorphic origin of several characters in some of the African species of *Rhabdochona*.

In describing the distal tip of left spicules, there seems a clear departure from a basic lanceolate type found in other species not described with such a term. We have observed in *R. salgadoi* that despite their possessing prongs in this part of the spicule, there is a basical lanceolate framework that originates the so called "coned" structure seen (Moravec, 1972c).

Our premise for codification was that such a complex structure, especially in some species, would be difficult to be acquired more than once. So, very limited modifications might ensue from any of these 5 categories, especially more in *Rhabdochona* than in its putative sister genera simply because the latter exhibit a more generalized type of spicule. Other characters vary in these latter species, but left spicule form remains quite constant.

In assigning character codes we have followed closely the terminology of Moravec (1968, 1972a,b,c, Moravec and Arai, 1971, for South American, Asian, African and North

American species, respectively) and Rasheed (1965). We have re-defined terms from other authors (Byrne, 1992; Maggenti et al., 1992; Kayton et al., 1992) that are clearly equivalent to the original terms used in Moravec's descriptions, i.e., rounded ventral, or ventral, conical rounded protuberance = extended ventral process formed by membrane or ventral tooth-like process formed by membrane. In some cases, i.e., *R. ahuehuellensis* and *R. guerreroensis*, we have imported the use of "barb" (a term originally used for the "ventral barb" of *R. catostomi*, a structure equivalent to the "wide cuticular membrane forming a ventral process" of *R. oncorhynchi*, see Figure 2 and Kayton et al., 1992) for a structure that is actually sclerotized and not only formed by a ventral membranous process or tooth-like process, as in many *Rhabdochona* spp. from Asia and North America, i.e., *R. denudata*, *R. oncorhynchi*, *R. squalobarbi*, *R. zacconis*, *R. californiensis*, *R. catostomi*, *R. cubensis*, *R. kisutchi*, *R. paxmani*, etc.

We have only sought carefully those outstanding characters that could group species by spicule character states. In a few instances we had to infer if, i.e., "wide" stood for "lanceolate", by personal observations or if material not available, through drawings or photograph inspection. The same happened with the term used in a few species as "lanceolate broad", which in 1 case actually stood for bifurcate, as drawing interpretation showed.

From the phylogenetic analysis of *Rhabdochona* spp. it seems that species could be better grouped by spicule form than by any other character, mainly because all other characters are so constant as to render them of very little help in phylogenetic analyses. Yet, those characters are valuable for taxonomic purposes. As we could code at least 4 spicule types within genus *Rhabdochona*, we have re-classified this structure in order to include all species herein analysed, to relate some species directly to American species and

to eliminate any ambiguities in future taxonomic descriptions or redescriptions of species of *Rhabdochona*.

Chabaud (1975) considered *Heptochona*, *Johnstonmawsonia*, *Trichospirura*, and *Hepatinema* as the most derived Rhabdochonidae. In the present hypothesis it appears that the increase in vestibule length (mainly in the mesostom part) (character state 7-0) is not a derived character within the subfamily, but a plesiomorphic one, and that the shortening of the vestibule in Rhabdochonidae is not congruent with the appearance of prostomial teeth (character state 8-1) and with the widening of the prostom itself (character 5) as Rasheed (1965) had claimed.

If *Cystidicola farionis* actually contains the characters ancestral to the Rhabdochonidae, this family evolved via a simplification of the base of the vestibule from annulated to simple; widening of the prostom, the appearance of deirids and the presence of lateral papillae in the male tail, additionally to the ventral ones present in the outgroups. This contradicts the statement that a prosotm provided at its base with basal teeth and lower number of anterior teeth is more primitive than that lacking these basal teeth and having a higher number of anterior teeth (Moravec, 1972b).

Taxonomically, this phylogenetic hypothesis of *Rhabdochona* spp. uncovers the form of erection of new species within this genus: new species are erected on the basis of new combinations of characters. Most species entertain autapomorphic characters which are homoplasic, so the question here is to what extent what has been described are really geographic variations and nothing more. In the foregoing phylogenetic analysis, the so called American subspecies of *Rhabdochona* were not included, due to the fact that they represent only geographic variants of the original species and do not provide any apomorphic characters. The criterion used to state this is that left spicule structure has

unperceivable variation among these alleged subspecies. Undoubtedly more species within the Americas, especially in those regions where there is a singular concentration of species (for example, Mexico), will be erected on this basis, further complicating the taxonomy of this group. Molecular analysis within (intraspecific variation, represented by phylogeographic analyses) and between species (interspecific variation, represented by phylogeny) could shed light on the evolutionary history of *Rhabdochona*.

The biogeography of *Rhabdochona*

A closer examination of the results also reveals several salient aspects of the biogeography and host associations of *Rhabdochona*. As it seems that the origin of *Rhabdochona* is most probably African and Asian the pattern recovered quite clearly points to an ancient southern Laurasian and northern Gondwanian distribution, probably related to basins of Tethys Sea. Some more recent exchanges might have given origin to new sepcies of *Rhabdochona*, as the resolved clades from our analyses show.

Species of *Rhabdochona* in the Americas do not represent a monophyletic group. This indicates that the species of *Rhabdochona* of the Americas do not have a single origin, be it by dispersion or vicariance. What is constant in all resolved clades where species of the Americas are present is that mostly every pair of sister species seems to be represented by an American species and another one from a distinct continental area. This was something that was anticipated by Moravec & Arai (1971).

Rhabdochona has been reported from all of the biogeographic zones of the world, except the Australasian region. The foregoing analysis includes species from every region of the world, and from all their hosts reported up to date (Figures 5 and 6). Figure 5 shows only few patterns as to potential parasite-host coevolutionary relationships. In 1 clade of *Rhabdochona* parastizing the Pacific salmonids (*Oncorhynchus* spp.) and goodeids, the

basal members are all from the western region of the continent and eastern Asia with what seems to be instances of host-extensions from cyprinids into salmonids and goodeids. What is most striking is that Cyprinidae seem to be the main host family in all of the biogeographic zones where *Rhabdochona* spp. have been found. Salmonidae and Catostomidae, the 2nd and 3rd families where most of the species of *Rhabdochona* are found, only reflect the modern distribution of these helminths, but shows no clue as to their ancestral area relationships due to the antiquity of such, reflected in the present phylogenetic hypothesis. The wealth of host fishes where several species of *Rhabdochona* have been found, do not conform to its alleged host specificity (Moravec 1975).

Thus, the biogeographical pattern within *Rhabdochona* represents a very old one: it seems likely that *Rhabdochona* originated in southern Laurasia and northern Gondwana, and diversified relatively fast into distinct freshwater basins, especially in the northern basins of the Tethys Sea, where today we find a great deal, but not the majority, of species in the Aral and Caspian basins. Such species are widely distributed and are shared with basins in the Oriental and Palaearctic regions. Its diversification probably does not reflect the diversification of their modern definitive hosts, but most probably the diversification of their ancient, and yet unknown, hosts. Nevertheless, the ancestry of the species of *Rhabdochona* that parasitize freshwater fishes in the Americas shows Trans-Pacific geographical relationships (that cannot be explained by simple dispersal of their fish hosts) which in the future might be explained by alternative hypothesis of earth crust evolution (Brooks and McLennan, 1993; Shields, 1979), but in the meantime, can only be a matter of speculation.

Rhabdochona has as well traversed the Nearctic-Neotropical realm and has invaded South America probably only after the emergence of the Panamanian isthmus. A similar

biogeographic hypothesis has been recently proposed for the species of Proteocephalidea (de Chambrier et al., 2004). It is noteworthy that the principal host group of *Rhabdochona*, the Cypriniformes (e.g., Cyprinidae and Catostomidae) are absent from the Neotropical realm. This could explain the sparsity of species in that subcontinent and the absence of phylogenetic relationships between the African and South American species.

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FIGURE LEGENDS

Figure 1. Cephalic ends of males of Rhabdochonidae: A. *B. nayaritense*; B. *R. lichtenfelsi*; C. *R. decaturensis*; D. *R. cotti*.

Figure 2. Left spicules of males of *Rhabdochona* spp.: A. *R. salgadoi* (pronged); B. *R. decaturensis* (bifurcate); C. *R. catostomi* (lanceolate broad); D. *R. milleri* (blunt).

Figure 3. Strict consensus of 19 cladograms of *Rhabdochona* spp. Only unambiguous changes are shown. One asterisk* indicates American species. Two asterisks** indicate species present in both Asia and America. Number above branches is character number and below its corresponding state. Upper case numbers below branches are bootstrap values > 50%.

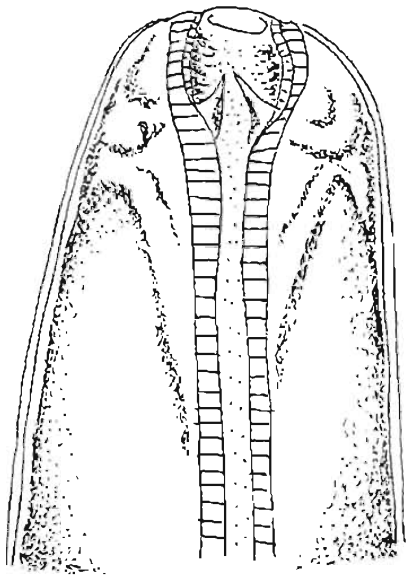
Figure 4. Strict consensus of 1733 cladograms of *Rhabdochona* spp. Character distribution is similar to cladogram of Figure 2. One asterisk* indicates American species. Two asterisks** indicate species present in both Asia and America. Number above branches is character number and below its corresponding state.

Figure 5 Host families superimposed on *Rhabdochona* species strict consensus of 19 cladograms. * indicates hosts associated with American species of *Rhabdochona*.

Figure 6. Area cladogram of species analyzed and outgroup species of *Rhabdochona* superimposed on strict consensus of 19 cladograms. * indicates biogeographic regions associated with American species of *Rhabdochona*.

Appendix I. Character matrix of *Rhabdochona*.

1



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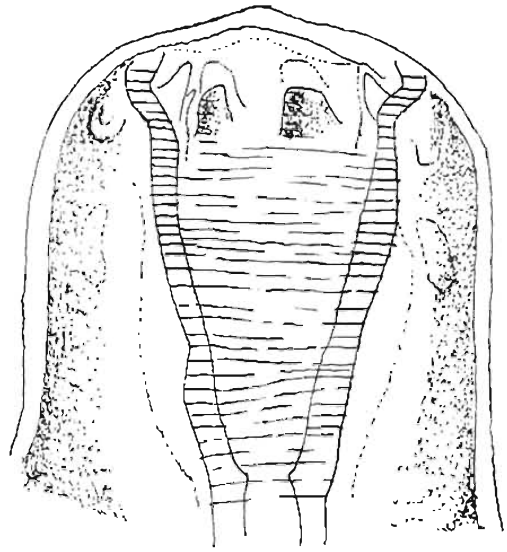

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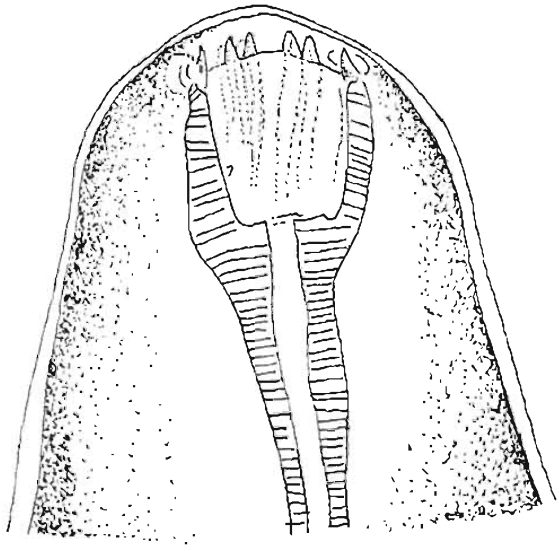

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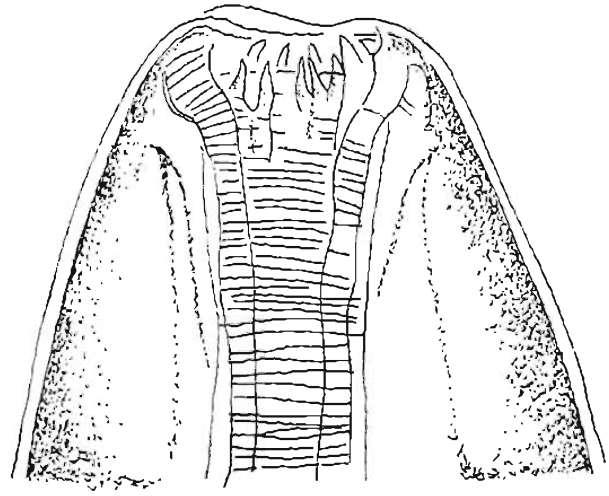

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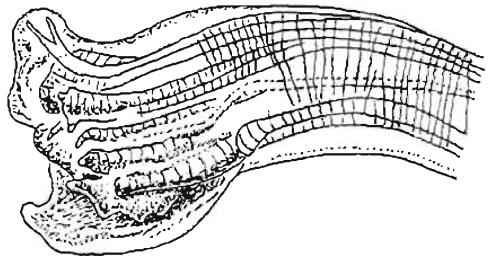

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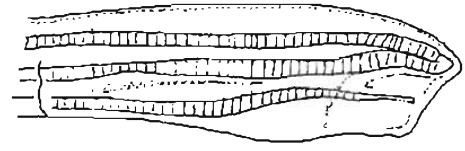

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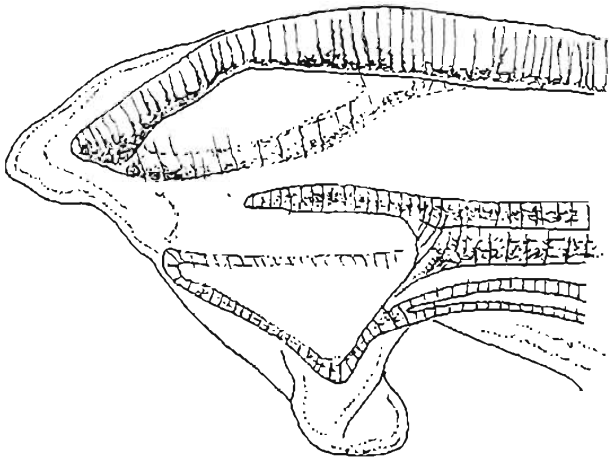

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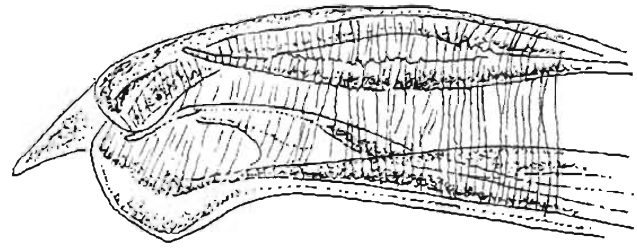

D

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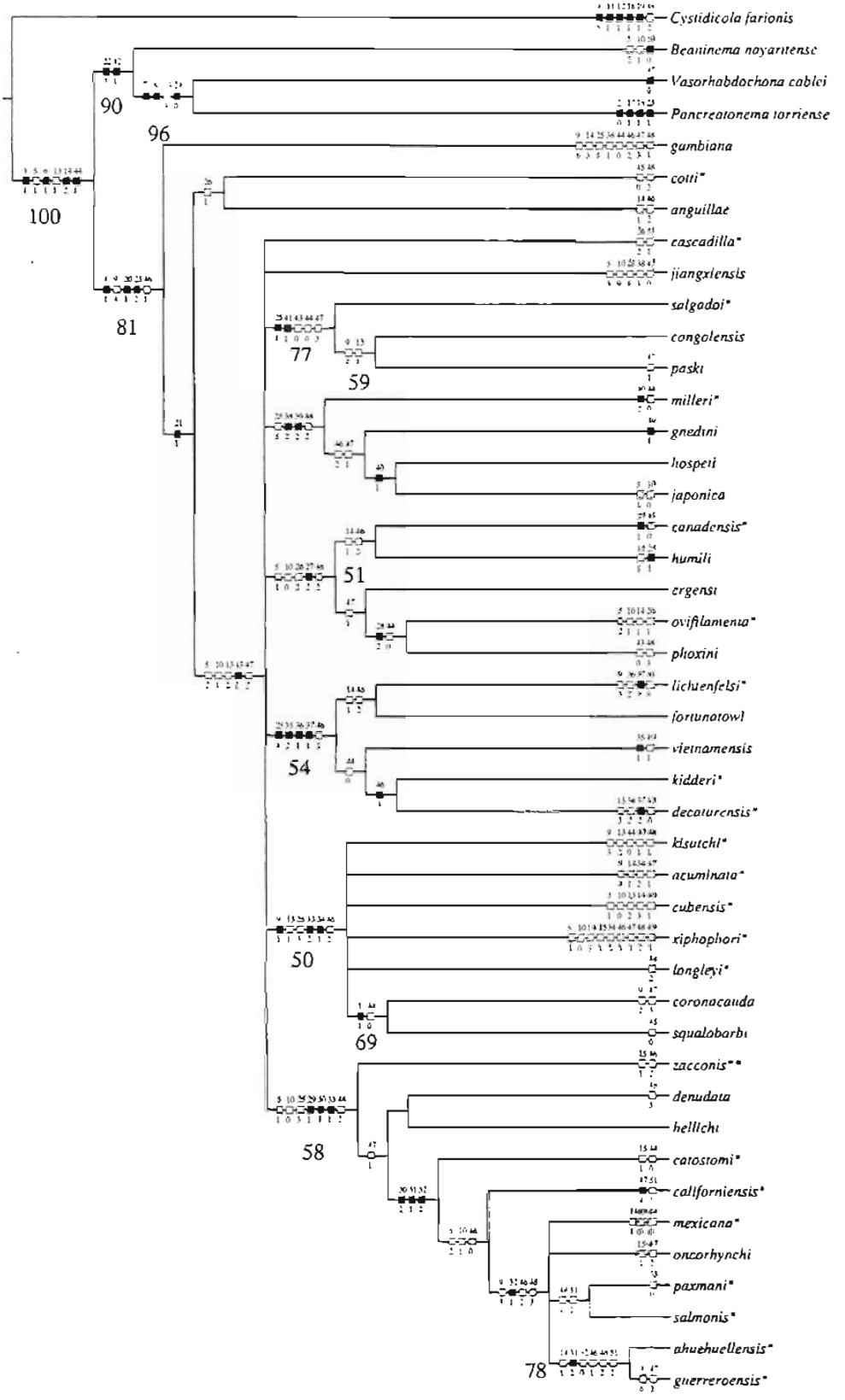
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Vasorhabdochona cablei
Pancreatonema torriense
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cotti*
anguillae
cascadilla*
jiangxiensis
salgadoi*
congolensis
paski
milleri*
gnedini
hospeti
japonica
canadensis*
humili
ergensi
ovifilamenta*
phoxini
lichuenfelsi*
fortunatowi
vietnamensis
kidderi*
decaturensis*
klsutchi*
acuminata*
cubensis*
xiphophori*
longleyi*
coronacauda
squalobarbi
zacconis**
denudata
helllchi
catostomi*
californiensis*
mexicana*
oncorhynchi
paxmani*
salmonis*
ahuehuellensis*
guerreroensis*
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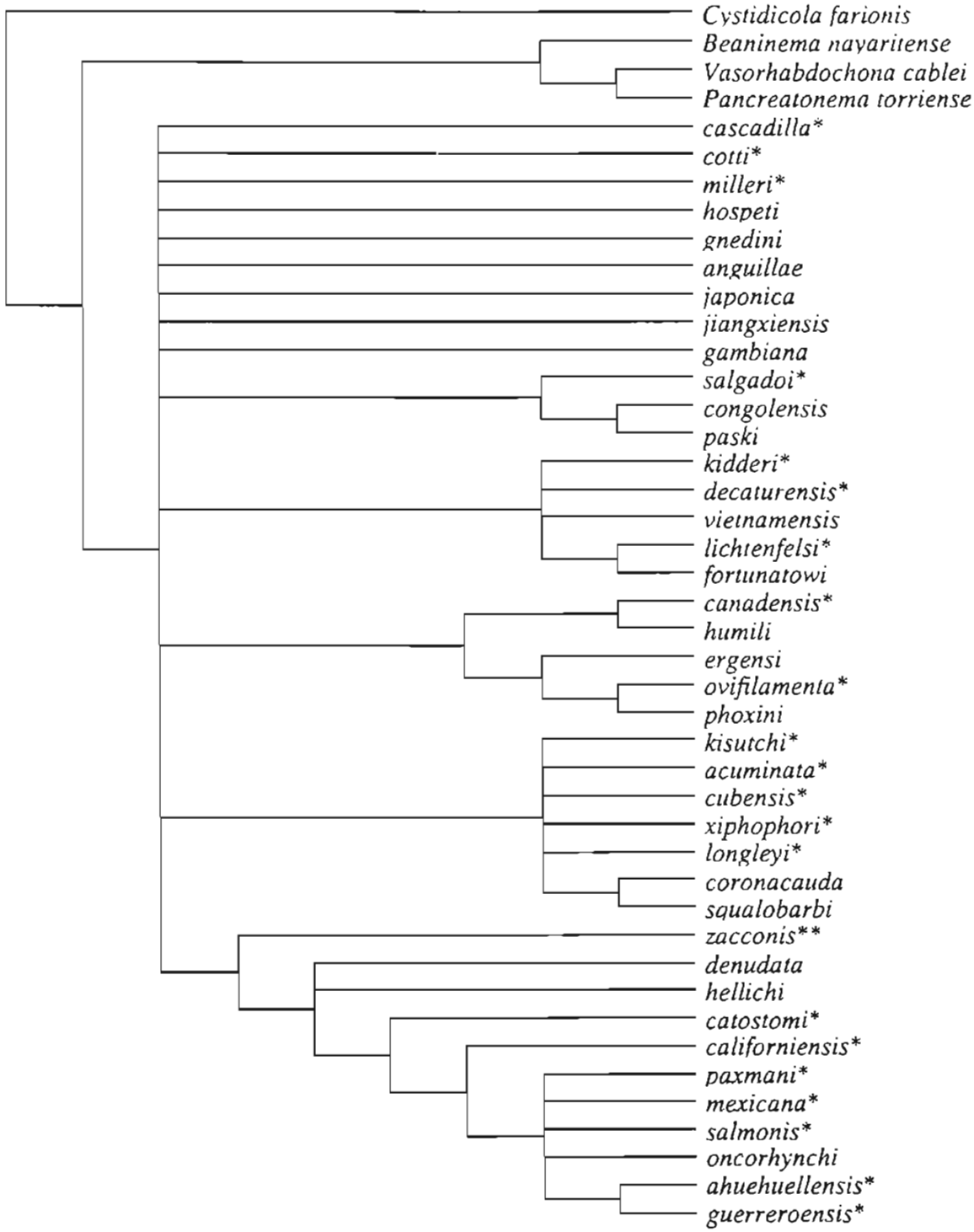
90
96
81
77
59
51
54
50
69
58
78

4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cotti*
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anguillae
japonica
jiangxiensis
gambiana
salgadoi*
congolensis
paski
kidderi*
decaturensis*
vietnamensis
lichtenfelsi*
fortunatowi
canadensis*
humili
ergensi
ovifilamenta*
phoxini
kisutchi*
acuminata*
cubensis*
xiphophori*
longleyi*
coronacauda
squalobarbi
zacconis**
denudata
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mexicana*
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oncorhynchi
ahuehuellensis*
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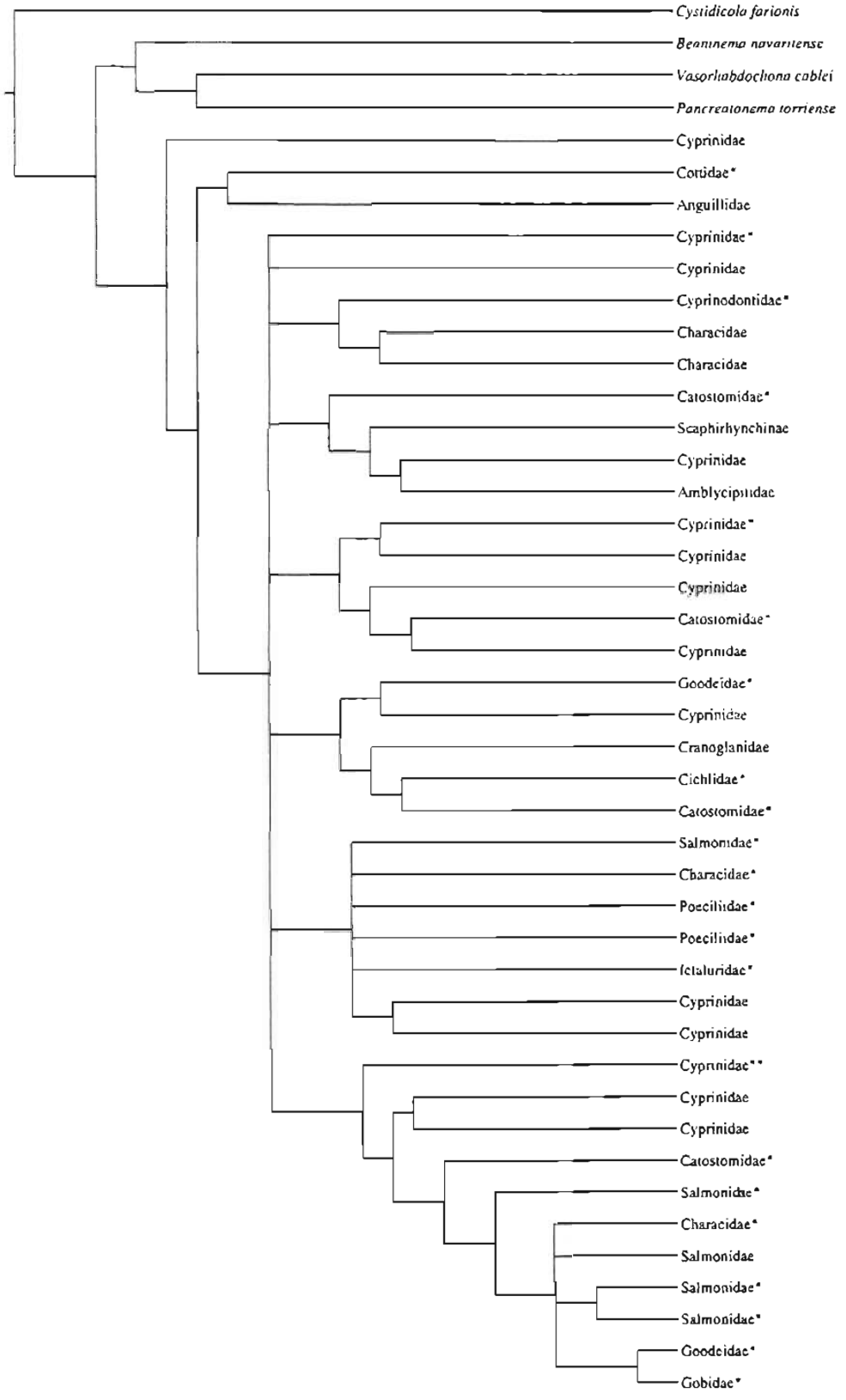
5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Cottidae*
Anguillidae
Cyprinidae*
Cyprinidae
Cyprinodontidae*
Characidae
Characidae
Catostomidae*
Scaphirhynchinae
Cyprinidae
Amblycipitidae
Cyprinidae*
Cyprinidae
Cyprinidae
Catostomidae*
Cyprinidae
Goodeidae*
Cyprinidae
Cranoglanidae
Cichlidae*
Catostomidae*
Salmonidae*
Characidae*
Poecilidae*
Poecilidae*
Ictaluridae*
Cyprinidae
Cyprinidae
Cyprinidae**
Cyprinidae
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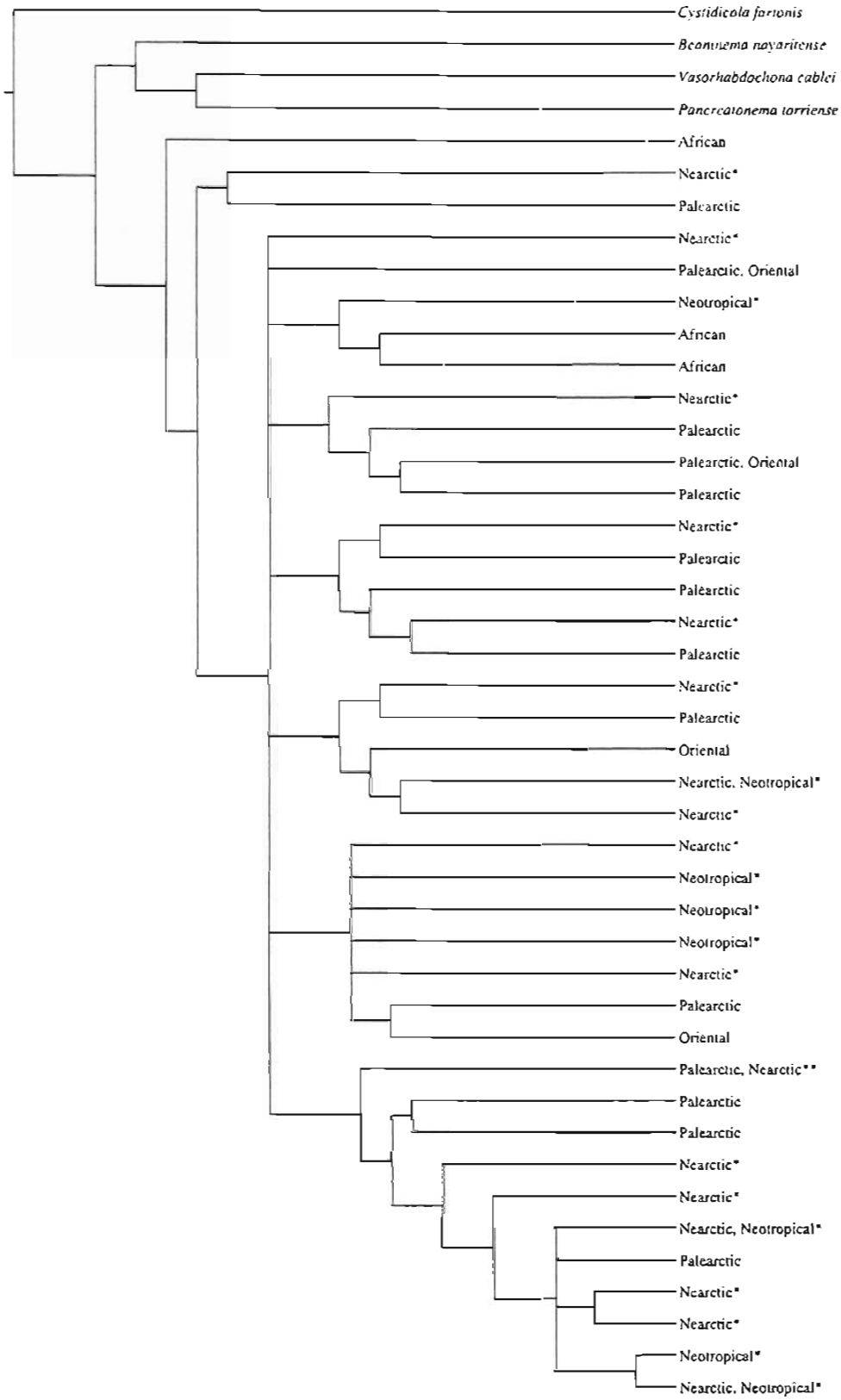
Salmonidae*
Salmonidae*
Goodeidae*
Gobidae*

6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Nearctic*
Palearctic
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Neotropical*
African
African
Nearctic*
Palearctic
Palearctic, Oriental
Palearctic
Nearctic*
Palearctic
Palearctic
Nearctic*
Palearctic
Nearctic*
Palearctic
Oriental
Nearctic, Neotropical*
Nearctic*
Nearctic*
Neotropical*
Neotropical*
Neotropical*
Nearctic*
Palearctic
Oriental
Palearctic, Nearctic**
Palearctic
Palearctic
Nearctic*
Nearctic*
Nearctic, Neotropical*
Palearctic
Nearctic*
Nearctic*
Neotropical*
Nearctic, Neotropical*

Apendix I. Data matrix for *Rhabdochona* spp. Characters are numbered and coded as in text.

	CHARACTER				
	10	20	30	40	50
TAXA	.	.	.	.	.
Vasorhabdochona cablei	0110110000001300000000000000000000000000001011000010
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Cystidicola farionis	0100001150110001001001010000000000000000000001012010
kisutchi	0111211131002200000111013000000021000000000101211010
paxmani	0111211131002200000111013000121100000000000111211012
lichtenfelsi	0111211131002100000111014000000000223000000011222010
mexicana	0111211131002100000111013000121110000000000001213010
californiensis	0111211141002200000111013000121210000000000111140011
salmonis	0111211131002200000111013000121110000000000111211012
cascadilla	0111211141002200000111012200000000000000000111120011
kidderi	0111211141002200000111014000000000021100000101320010
decaturensis	0111211141001200000111014000000000222000000001320010
canadensis	0111111140002100000111012210000000000000000110222010
cotti	0111111140001200000111012100000000000000000010112010
milleri	0111211141002200000111015000000000000022200101122010
acuminata	0111211141001100000111013000000022000000000111210010
cubensis	0111111110002300000111013000000021000000000111220110
xiphophori	0111111110001110000111013000000022000000000111112110
ovifilamenta	0111211141002100000111012122000000000000000101112010
salgadoi	0111211141002200000111011000000000000000010001130010
ahuehuellensis	0111211131002100000111013000122010000000000111112012
guerreroensis	0111211161002100000111013000122010000000000111122012
longleyi	0111211111001200000111013000000021000000000111222010
catostomi	0111111140002210000111013000121210000000000101112010
denudata	0111111140002200000111013000110010000000000111113010
oncorhynchi	0111211131002210000111013000121110000000000111223010
zacconis	0111111140002210000111013000110010000000000111222010
phoxini	0111111140002200000111012222000000000000000001113010
ergensi	0111111140002200000111012220000000000000000111112012
hellichi	0111111140002200000111013000110010000000000111112012
humili	0111111140002110000111012221000000000 0000111?2201?
hospeti	0111211141002200000111015000000000000221001111?1201?
gnedini	0111211141002200000111015000000000000210001112120010
fortunatowi	0111211141002100000111012 400000000021100000111222010
anguillae	0111111140001100000111012100000000000000000011210010
coronacauda	1111211121001200000111013000000021000000000101230010
japonica	0111111140002220000011101500000000000002210011121201 0
jiangxiensis	0111111140002200000111015000000000000010000011120010
vietnamensis	0111211141002200000111014000000000111000001012201 10
squalobarbi	1111211111001200000111013000000021000000000100220010
congolensis	0111211121001200000111011000000000000000010001130010
paski	0111211121001200000111011000000000000000010001110010
gambiana	0111111160001300000101015000000000000010000001231010

CAPÍTULO III. FILOGEOGRAFÍA DE *Rhabdochona lichtenfelsi* EN EL CENTRO DE MÉXICO

Rhabdochona lichenfelsi es un nemátodo parásito específico de goodéidos con una distribución que abarca el río Lerma-Santiago y el río Pánuco. En el siguiente estudio, un fragmento de 456 pb de CO1 se utilizó para estimar la variación genética de 38 secuencias distribuidas en 10 poblaciones de *R. lichtenfelsi* dentro de la cuenca del Lerma. Se encontró que 6 de 10 poblaciones tienen haplotipos únicos en cada localidad muestreada a nivel de cuenca. La variación genética total de las 10 poblaciones es alta. Estas poblaciones exhibieron diferencias significativas alejadas de panmixia. El proceso que se identificó como responsable de la estructura de las poblaciones fue flujo génico restringido con aislamiento por distancia, tal y como se obtuvo a través del análisis de parsimonia de clados anidados. Las implicaciones biogeográficas de estos resultados es que antiguamente existieron en la Mesa Central de México cuencas hidrográficas y conexiones entre las mismas donde hoy solamente quedan cuerpos de agua dulce aislados.

Phylogeography of *Rhabdochona lichtenfelsi* (Nematoda) parasite of freshwater fish in Central Mexico as revealed by a fragment of mitochondrial gene CO1

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Abstract

Rhabdochona lichtenfelsi is a nematode parasite specific to Goodeids with a distribution that extends the whole range of the Lerma-Santiago river basin in Central Mexico and into the Panuco basin. In the foregoing study, a fragment of 456 bp of COI was used to estimate genetic variation on 38 sequences distributed in 10 populations of *R.lichtenfelsi* within the the Lerma Basin. It was found that 6 out of 10 populations possesed unique haplotypes in each locality sampled at basin level. The overall genetic variation of the 10 populations is high. These populations exhibited a highly significant departure from panmixis. The process identified as responsible for population structure is restricted gene flow with isolation by distance, as recovered by nested clade parsimony analysis. The biogeographical implications of these results is that there were ancient basins and connections where today stand isolated freshwater bodies in the Mesa Central of Mexico.

Keywords: COI; phylogeography; gene flow. nematodes; *Rhabdochona lichtenfelsi*; Goodeinae

1. Introduction. Patterns of genetic variability of the Earth's biota in terms of gene genealogies and their geographical distribution have been extensively studied during the last 3 decades within the discipline of phylogeography (Avise, 1998, 2000; Emerson et al., 2001). Phylogeography has a two-fold aim: 1) to explain demographic issues related to genetic variability through the comparison of haploid gene frequencies (intraspecific and interspecific) within and between gene populations and 2) to explain the genealogy of that variabitlity in terms of time, through coalescent theory, and through the correlation of gene distances vs. geographic distance, which is a biogeographical issue in its own right. Phylogeography has chosen mithocondrial DNA as its model genetic molecular marker mainly because it is not affected by recombination and is inherited maternally (Avise, 2000). Therefore, mtDNA can reveal genealogic patterns below the species level that can hopefuly link microevolution with macroevolution. This avoids that any genetic recombination would actually assess tocogenetic relationships (Hennig, 1966) instead of genealogical or phylogenetic relationships.

Model organisms in phylogeography have centered in the Animal Kingdom. The range of model organisms is now wide and includes terrestrial invertebrates, vertebrates and freshwater as well as sea organisms (Avise, 2000). Among the latter, freshwater invertebrates (including Mollusca and Arthropoda mainly) have been used to investigate riverine histories of ancient watershed courses subject to historic periods of fragmentation and separation.

More interestingly, patterns of basin fragmentation and reconnection can be studied with a certain degree of confidence, if the geological history of such water systems is well known. Phylogeography can disclose biological patterns that probably broad geological phenomena studies have been unable to recognize in detail. Colonization, vicariance and

geographic expansions within and between freshwater basins are all phenomena that can be studied with parasites as models within the context of phylogeography, as will be seen.

Patterns of genetic variability of nematode parasites of vertebrates are important in terms of biogeographical history, especially those of areas thought to have been formerly related in geological time (Nieberding et al., 2004). Their study has enabled to assess closely their patterns of vicariance, colonization and migration. Most of the studies involving these issues have centered on the role gene flow has played in those nematodes of economic importance. Other studies have revealed cryptic phylogeographic patterns associated with the withdrawal of glaciers during the last million years (Nieberding et al., 2004). Needless to say, nematodes as parasites have a set of features that make them suitable models for phylogeographic sudies. Parasites are phylogenetically or ecologically restricted to 1 or several hosts during a part of their life histories. Some of their definitive hosts have more vagility than others (amphibians and reptiles vs. fishes). Additionally, if parasite life cycles are restricted in space, i.e., to freshwater habitats, the environment in which they can develop is naturally limited. Therefore, phenomena like vicariance and dispersion could be hypothetically assessed through changes in watercourses, connection and reconnection due to geological and climatic changes.

One of a group of geological phenomena that has changed signifcicantly the watercourses of a broad region in the Americas and that bas received much attention by systemtatists and biogeographers recently is the Trans-Mexican Volcanic Belt (TMBV). In broad terms this volcanic arc has been considered a geographical barrier to the range expansion of freshwater species from South America to North America and viceversa (Marshal and Liebherr, 2000; Mateos et al., 2002; Mateos, 2005). Nevertheless, there is evidence that it has been an area where dispersal has taken place in and out of it (Halas et

al., 2005). Studies on the speciation patterns leading to morphologically new species within the TMBV and its surroundings has dealt with freshwater fishes (Álvarez, 1972a). Álvarez (1972a, 1972b) established the basic hypotheses for explaining the surprising freshwater fish diversity within the Mesa Central. Barbour (1973) followed Álvarez in much of his account on the biogeography of the silverside *Chirostoma*. This diversity includes species of the subfamily Goodeinae, species of the genus *Chirostoma* (Family Atherinidae) and *Algansea* (Family Cyprinidae), which have undergone dramatic events of speciation since the Miocene. Yet, the genetic differentiation processes leading sometimes to speciation of freshwater organisms *within* the TMBV has begun to be recently explored, as revealed by the study of Doadrio and Domínguez (2004) on the Family Goodeidae, subfamily Goodeinae that inhabit exclusively Central Mexico.

Within the TMBV, the goodeinae have undergone a series of speciation events that seem to have no parallel with other freshwater vertebrate taxa in Central Mexico and are highly related to the geological history of this region (Doadrio and Domínguez, 2004). This history implies that extant freshwater basins in Mesa Central are the result of a series of vicariant events which have led to the fragmentation of those basins. In the course of their evolution these basins have remained separate, have reconnected through erosion or faulting, such as the Colima Graben, which is the result of an erosive process which once bridged Mesa del Norte with the southwestern Pacific drainages of Mexico (Mateos et al., 2000).

Parasites of goodeine fishes, are considered in this paper as models that can further substantiate the hypothesis of Doadrio and Domínguez (2004) on the geological evolution of a part of Central Mexico. The main reason is that at least a suite of 4 species of helminth parasites in Central Mexico including 2 species of digeneans (*Margotrema bravoae* and *M.*

guillerminae) and 2 species of nematodes (*Rhabdochona ahuehuellensis* and *R. lichtenfelsi*), are goodeine specialists (Mejía-Madrid et al., 2005; Pérez-Ponce de León, 2003).

Present interest in using freshwater fish helminths stems from the fact that such parasites are restricted geographically at different scales. In space, helminths are restricted by their host ranges (intermediate and definitive), i.e., freshwater basins. In time, they are restricted by their life cycles and therefore the opportunities for speciation offered either by vicariance or dispersion of their ancestors depends solely in the vagility of their hosts.

The species of *Rhabdochona* Railliet, 1916 could be a model for testing the structuring of populations in a rather changing environment. This genus comprises more than 100 species present in freshwater fishes from all continents except Australia (Moravec, 1975). There exist up to 8 distinct nominal species in Mexico. Among these, *Rhabdochona ahuehuellensis*, *R. canadensis*, *R. guerreroensis*, *R. mexicana*, *R. lichtenfelsi*, *R. salgadoi* and *R. xiphophori*, are seemingly endemic to Central Mexico. *Rhabdochona canadensis* and *R. kidderi* are the only widely distributed species in Mexico as the former has been reported from North America parasitizing only cyprinids and the latter from Central America to southern Texas parasitizing mainly cichlid fishes.

One of the most common and dominant species of these nematodes parasitizing freshwater fish hosts of the endemic subfamily Goodeinae is *Rhabdochona lichtenfelsi*. *Rhabdochona lichtenfelsi* parasitizes those goodeines inhabiting only the Mesa Central basins, Lerma-Santiago and Ameca, and outside Mesa Central, the Panuco basin (Mejía-Madrid et al., 2005). Its complete definitive host range has been recently reported (Mejía-Madrid et al., 2005) and the conclusion is that it is specific to the most derived clades (*sensu* Doadrio and Domínguez, 2004) of Goodeinae.

The aforementioned species can be distinguished from other members of its genus because it possesses 10 prostomial teeth equal in size, polar filamented eggs and a distinctive pair of spicules in the males of which the morphology of the left one is species specific (Sánchez-Álvarez et al., 1998). A related species appears to be *R. mexicana*, which posseses a suite of characters closely similar to *R. lichtenfelsi*, except for the left spicule which relates the former to other *Rhabdochona* spp. present in Northwestern U.S. Although *R. mexicana* is found mainly in characid fishes of the genus *Astyanax* in northeastern, central and southern Mexican basins, it has been found in one species of the most basal clade of Goodeinae, *Characodon audax* (Mejía-Madrid et al., 2005) in the Mezquital basin in northwestern Mexico. This nematode has never been found within Mesa Central freshwater basins.

Although its complete life cycle has not been worked out, there is direct evidence that its intermediate host is the naiad of a mayfly species (personal observations). This means that *R. lichtenfelsi* completes its life cycle nearly in the same way as other conspecifics in other parts of the world (Moravec, 1972, 1976;. Moravec and Huffman, 2001; Shimazu, 1996).

There exists no evidence that after the uplift of Mesa Central, some 9.8 million years ago, another species of *Rhabdochona* originated within the basins limited by it. Where other taxa have produced clear patterns of vicariance and dispersal in the Mesa Central i.e., the Goodeinae, *Rhabdochona* as a genus, has not. Therefore, *R. lichtenfelsi* remains as the only freshwater fish nematode that belongs to this genus that inhabits the Mesa Central. It has been geologically documented that this area has been subject not only to watershed fragmentation, but to periods of erosion and river reconnection and further fragmentation added to river piracy that has produced new species of freshwater fishes

(Doadrio and Domínguez, 2004; Mateos et al., 2002, 2005) but no cophylogeny has co-ocurred with its helminth fauna.

In the light of these facts, *Rhabdochona lichtenfelsi* could reveal the same history of the basins of Western Mesa Central through the study of its demographic and genetic structure or disclose new patterns of the history of those basins in which it parasitizes goodeines, without the taxonomic status problems that other freshwater species usually present (i.e., *Chirostoma*).

The aim of the present paper is to determine the genetic structure of the population of *Rhabdochona lichtenfelsi* from 7 different basins from Western Central Mexico whose headwaters originate within Mesa Central or represent endorreic basins once connected to a main watercourse (Ameca, Cotija, Cuitzeo, Lerma, Pátzcuaro, río Verde, and Zacapu) utilizing a fragment of the COI mitochondrial gene of this nematode and subject these data to a basic phylogeographic analysis in order to assess if 1) the present genetic structure of *Rhabdochona lichtenfelsi* can account for, to some extent, the historical basin fragmentation that has undergone Western Mesa Central, i.e., the separation of Ameca, Cotija and Lerma basins and the relatedness of several of the Michoacan lakes, i.e., Cuitzeo and Pátzcuaro, and if 2) that genetic structure can account for basin connections or reconnections in the past.

2. Materials and methods

Rhabdochona lichtenfelsi was sampled from *Goodea atripinnis* in most cases (Table 1) from 7 major basins of eastern Central Mesa Central that represent 10 different localities (Figure 1); exceptions are listed where no *G. atripinnis* were found at the period of year sampled. It is understood that as *R. lichtenfelsi* moves freely within Lerma and Ameca goodeinae, there exists no preference of this nematode for any particular species of these

fish. Additionally, Mezquital basin was sampled for *Characodon audax* at Los Berros (coordinates given) for *R. mexicana*. The general criterion for the election of these basins was that *R. lichtenfelsi* was previously reported from a restricted group of basins centered in Lerma-Santiago and adyacent basins plus others explored during the course of faunistic research on the helminth fauna of Goodeinae (Mejía-Madrid et al., 2005).

Individuals of *Rhabdochona lichtenfelsi* were collected from Ameca (hereafter Teuchitlan and La Coronilla), Cotija (hereafter Tocumbo), Cuitzeo (hereafter San Cristobal), Lerma (hereafter La Luz, Minzita Naranja de Tapia, and Pátzcuaro), río Verde (hereafter Santiago), and Zacapu basins (Table 1). A total of 5-10 individuals (exclusively females) per locality were used for DNA extraction. The nematodes were collected from pithed specimens of *Goodea atripinnis* within the next 48 hours of collection, except for Zacapu, where nematodes were collected from *Hubbsina turneri* and *Allotoca zacapuensis*, and Pátzcuaro, where nematodes were collected simultaneously from *Alloophorus robustus* and *G. atripinnis* (Table 1). *Rhabdochona mexicana* specimens were recovered from *Characodon audax* from Mezquital basin. Voucher specimens were deposited in the Colección Nacional de Helmintos, Mexico City (CNHE 5174-5205).

Complete female (larger sex) individuals were fixed in absolute alcohol and frozen at –20°C. DNA extraction protocol used was phenol-chloroform basic extraction method, as described in Hillis et al (1996; pages 342-343). Final products of crude extract amounted to aliquots of approximately 20-50 µl with a concentration of total DNA of $\leq$ 100 ng/50 µl.

Primers synthesized for amplifiying COI fragments of *Rhabdochona mexicana* and *R. lichtenfelsi* were those used previously for assessing the phylogenetic relationships of filarial nematodes (Casiraghi et al., 2001). Forward (TGATTGGTGTTTTGGTAA) and

backward (ATAAGTACGAGTATCAATATC) primers (Invitrogen Technologies) were used to amplify a fragment of the COI gene using the thermal profile detailed in Casiraghi et al (2001): denaturing temperature 94°C° 45 sec, annealing temperature 52°C 45 sec and extension at 72°C 90 sec for 40 cycles with an Eppendorf Mastercycler thermocycler. Sequence reaction was perfomed with Big Dye Terminator v.3.1 solution (Invitrogen Technologies): re-amplicfication of COI purified products was undertaken with the program ABIX2552, e.g., a general thermal cycle temperature profile programmed with the annealing temperature employed in the initial amplification step. The product was then purfied with a 10% Sephadex column centrifuged at 2, 800 r.p.m. and finally taken to a concentration of 10ng of product in 20µl. Product was finally dried with a vacuum centrifuge at 30°C. Final product was sequenced with an ABIPrism technology sequencer. Sequences were deposited in GenBank (accession numbers XXXXX-XXXXX).

Alignment was performed by visual inspection (BioEdit Sequence Alignment Editor) simultaneously with homologous fragments of the most closely allied nematodes found in a NCBI nucleotide/nucleotide blast: *Onchocerca volvulus*, *Dirofilaria repens*, *Thelazia lacrymalis*, *T. callipaeda* and *T. gulosa*. This was done because no previous records of any species of *Rhabdochona* or putative closely related species had been sequenced before. Special care was taken to group bases by homologous codons. Only those sequences that were homologous were kept for further analyses. The alignment was finally compared to a single sequence of a female of *Rhabdochona mexicana*. Aligned sequences were then compiled in a text matrix with Nexus format without interleaving for the following analyses.

Analyses of MP and NJ were performed with PAUP V4.0b10 (Swofford, 2000) for recovering the pattern of relationships between sequences. For MP analysis, characters were equally weighted and unordered. Analysis was carried out with a heuristic branch pattern analysis (because 25 > taxa were obtained) and Tree Bisection Reconection (TBR) branch-swapping algorithm. Taxa were added with random addition with 100 replicates, with retention of 100 trees at each step, with an initial seed of 0. Optimization algorithm used was ACCTRAN. Bootstrap analysis was performed on the sequence matrix after clearing all trees from memory with 100 replicates utilizing the same heuristic analysis as discussed above.

NJ distance analysis was performed on the same sequence matrix with an uncorrected "p". If more than one tree was obtained, only the one shown on the screen by PAUP (Swofford, 1998) was reported. A distance matrix was obtained where genetic distances between the 2 species and within *R. lichtenfelsi* could be compared.

The alignment matrix was subjected to DnaSP v.3 (Rozas and Rozas, 1999) for calculation of haplotypes, polymorphism and demographic analyses. These analyses included: the determination of the genetic code used (mt DNA flatworm), number of segregating sites, number of mutations, haplotype/nucleotide diversity and neutrality tests (Tajima's D, Fu and Li's D test, Fu and Li's F test, Fu's F). All these were calculated with the total number of mutations. After grouping sequences into their corresponding 10 populations, gene flow and genetic differentiation and the estimates of the latter (chi-square values were tested at a 0.001% level to make decisions as to any significative departure from a panmictic population) were determined. This step lead us to perform a nested clade parsimony analysis (NCPA, see below) test if chi-square values departed significantly from

panmixis. Population growth was calculated with a mismatch pairwise frequency distribution between each pair of sequences.

TCS v 1.18 was implemented for obtaining a minimum spanning haplotype network (probability was set at 95% and 99%). Nested clades were determined following the procedures of Templeton (1987, 1998). Those clades recovered and exhibiting different haplotypes found in different localities, were used for performing permutation analyses with the program GeoDis v. 1.0 (Posada et al., 2000) based on 1000 resamples and considered correlation between genetic and geographic distances at the 5% level. Statistically significant permutations between haplotype tree and geography (localities in this case) were inferred from the most recent key published by Templeton (2004).

Finally, the pairwise genetic distances as measured by Gst were plotted against the pairwise geographical distance in order to assess a direct correlation coefficient between both distances.

3. Results

A fragment of 456 bp was recovered from 38 individuals of *R. lichtenfelsi* plus 1 individual of *R. mexicana* belonging to 7 basins in the Western Mesa Central. The fragment corresponds to bases 2653 to 3108 of the complete mithocondrial genome of *O. volvulus* that is homologous wth a fragment of the COI gene of the same (Keddie et al., 1998).

Analyses of MP and NJ, the latter with uncorrected "p", were performed to recover the genetic distance between the haplotypes (Figures 2 and 3). Both analyses were performed using *Thelazia lacrymalis* (a putative relative of *Rhabdochona* spp. classified in the Superfamily Thelazioidea (Anderson 2000) (Figures 2 and 3) and *Rhabdochona mexicana* (Figure 4) as outgroups. The MP tree is represented by a strict consensus tree recovered from 68 trees (Fig. 2). There were 27 parsimony informative characters (sites),

324 constant characters and 105 parsimony-uninformative characters. All trees recovered from 1 island had a length of 163 steps, CI 0.877, RI 0.896 and RC 0.786. The relationships recovered by MP indicate that *R. lichtenfelsi* sequences group within one single clade, hereafter ingroup. The sequences form a polytomy, which indicates that molecular synapomorphies are not clear. Nevertheless, 6 different clades group together within the ingroup according to localities sampled. Of 38 sequences analyzed, 28 group within these 6 clades and 10 do not group at all. Those sequences that group together are: Teuchitlan (2 sequences), Santiago (2 sequences), La Coronilla (3 sequences), Zacapu (5 sequences), Patzcuaro together with Naranja de Tapia (8 sequences), and Patzcuaro with La Minzita, San Cristobal and La Luz (8 sequences). Only the clade next to the last mentioned contains a subIcade that further groups 2 sequences from Naranja de Tapia. Those sequences within the ingroup that do not conform any "locality" clade are: Teuchitlan (2 sequences), La Coronilla (1 sequence), La Luz (1 sequence), Tocumbo (4 sequences = total number of individuals sampled), and Santiago (2 sequences). At least 4 out of 5 sequences collected from the latter appear represented in the resolved clades. This topology was expected due to the close relationships among the sequences studied. A boostrap analysis with parameters indicated above recovered the same tree topology of the MP analysis (Figure 2).

The NJ trees (Figure 3 - 4) show that the distance between *R. mexicana* and *R. lichtenfelsi* has a range of 3.7-6.0% and the maximum distance between the sequences of *R. lichtenfelsi* is no less than 4.0-5% between Naranja de Tapia, La Luz, Teuchitlan and Tocumbo and the minimum distance is 0% between Patzcuaro, Minzita, Naranja de Tapia, San Cristobal; between the samples of Santiago and Ameca and the samples from Tocumbo. This colaterally would mean that species distinction within closely allied *Rhabdochona* spp., as is the case, is within the order of 4-6% or probably more, not being

able to establish a more precise divergence percentage between species for the time being. Additionally, there is overlap between these figures when some localities are compared (Figure 4), i.e,. Narranja de Tapia sequences L30 and L32, La Luz sequences L27 and L28.. This could mean that the retention of genetic polymorphisms from related sequences, namely M1, *R. mexicana*, might be responsible for these results. Moreover, this is a lower genetic distance value if compared to other nematode species' genetic divergence for mithocondrial, i.e., *Haemonchus* spp. (especially ND4) or nuclear markers, i.e., Ascaridida (18s and 28s) (Blouin et al., 1999; Nadler and Hudspeth, 1998, respectively) where divergence between closelly allied species is equal or well over 10%.

The NJ trees already show from the outset that certain sequences have 0 or less distance (NJ was allowed to freely calculate negative numbers) indicating the presence of at least 25 haplotypes. DNAsp showed that actually this data set contains information that groups the 38 sequences analyzed into 23 haplotypes.

Estimates of haplotypic diversity (Hd) and nucleotide diversity (p) for *R. lichtenfelsi* are presented in Table 2. Number of segregating sites obtained was 10.9% of the total number of sites analyzed (50/456, see Apendix I). Haplotype diversity was high in 5 populations, where it ranged from 0.8 in Rio Verde to 1.0 in Teuchitlan, San Cristobal, La Luz and Naranja de Tapia, being the first and the last 3 samples from Lerma. Haplotype diverstiy ranged from moderate to low in Coronilla, Patzcuaro, Tocumbo and Zacapu. Minzita exhibited 0 diversity. Total sample had a high haplotype diversity, nearing 1.0. Nucleotide diversity is low within populations, due to small sample sizes, and slightly higher in the total sample. Chi-square (table) for total sample (456 sites) was significant ($P = 0.00$, d.f. = 198 with a $P < 0.001$). Gene flow estimates from haplotypes amounted to Gst = 0.30 and Nm = 1.2 (Nei, 1973). Nei, 1982, Linch and Crease, 1990, and Hudson, Slatkin

and Maddison estimates from gene flow were Nm = 0.22, 0.33 and 0.33, respectively. This means that gene flow is or was highly limited. These results show that the population of *R. lichtenfelsi* is therefore structured and subdivided into smaller populations, probably matching those localities sampled.

Generally, haplotypes were highly particular for each basin involved (Table 2). Among localities, 6 out of 10 populations possesed unique haplotypes in each locality sampled at basin level. Only 3 haplotypes were present in more than 1 basin, namely, A4 which includes 1 sequence from Teuchitlan and 1 from Rio Verde, indicating the former relationship between Ameca and Lerma, respectively; L8 was present in Patzcuaro, Minzita, and San Cristobal showing that these water bodies were once connected in an ancient Lerma mainstream and L4 in Patzcuaro and Naranja de Tapia, now close freshwater bodies. Naranja de Tapia was expected to be more related to Zacapu, but it was a surprise that they did not share any haplotypes as today they are geographically close and even belong to the same basin.

Overall, Gst values (Table 3) show that among the most differentiated populations there appears to be no relationship with their geographic distance (Figures 1 and 8), i.e., Minzita from Zacapu (0.6). At the same time some far ranging localities had higher distance values, too, i. e., Coronilla and Minzita (0.56) and Minzita and Cotija (0.56).

The values of θ have been used as a relative estimate of population size (Cuenca et al., 2003). It was found that a θ value of 3.58 for the total sample of *Rhabdochona lichtenfelsi* approximates the mean value observed in the field. Nevertheless, caution must be taken when applying this value to metapopulation (several hosts of same species)

samples, as the value of θ obtained only estimates the value at the infrapopulation (1 host) regardless of parasite distribution, which tends to be aggregate (Crofton, 1971). Probably θ estimates a value equivalent to mean abundance (number of parasites/host sampled).

Some haplotypes of *Rhabdochona lichtenfelsi* seem to have differentiated geographically. In order to find evidence of this, two strategies were undertaken. The first one consisted of simply plotting the Gst values from Table 2 against the pairwise distance differences amongst populations. This resulted into a plot with a correlation coefficient of 0.04 (Figure 8).

The second strategy consisted in subjecting the sequence matrix data to TCS in order to recover a minimum spanning network of haplotypes. The initial result, at a default 95% significance limit, was a large net (not shown) with a considerable number of hypothetical haplotypes because the 9 step limit was reached between Patzcuaro, Naranja de Tapia, San Cristobal and the rest of the haplotypes. These haplotype groups separated when a 4 step limit was reached when the matrix was re-run at a 99% significance limit, and 3 distinct networks were recovered (Figure 6 – 7, only 2 networks shown), 2 with Patzcuaro, Minzita and San Cristobal haplotypes as an outgroup and a single group of 11 haplotypes with haplotype A4 as outgroup. As sampling bias might be generating these results, only the latter network was analyzed. Nevertheless, it is worth considering that both of the 2 networks left out involve Patzcuaro, plus the other haplotypes previously mentioned as outgroups (probability = 1.00000). This could mean that Patzcuaro has not only been an endorrheic basin along its history, as shown by haplotypes L8 and L17, represented by 5 sequences: Patzcuaro (L8) + Minzita (L9, L10, L11) + San Cristobal (L17,

L18) but was probably part of a lotic system. Another group of 5 sequences represented by L4, L7 (Patzcuaro), L31, L32, and L33 (Naranja de Tapia) indicate that it was a lentic system as well. The rest of the 11 haplotypes occur in Ameca/Santiago as an outgroup (sequences A4 and S3). It must be kept in mind, from the outset, that Santiago here is represented by 4 sequences from samples from rio Verde. Rio Verde was formerly connected to Lerma basin, through the Turbio-Silao basin in the state of Guanajuato (Barbour, 1973) as its fish fauna is identical to that in Lerma and is not related to the Santiago fish fauna. The haplotype network (Fig. 6) shows that A4 is closely related to other haplotypes in the same river basin (A6 and A1) although they belong to 2 distant localities, Coronilla and Teuchitlan (Table 1). It is closely related as well to (this means 1 step) Rio Verde and secondarily to Tocumbo, La Luz and Zacapu. Zacapu therefore nests with Rio Verde indicating that both basins were once connected via the Rio Turbio-Silao waterway, now cancelled but nevertheless not far apart from Rio Verde at present (less than 20 km, Domínguez-Domínguez, pers. comm.).

Nested clade analysis resulted in 1 clade (2-2) being statistically significant (see Table 4). Following Templeton's inference key (Templeton, 2004) it was found that they correspond to restricted gene flow with isolation by distance (sequence chain 1-NO-2-YES-3-NO-4-NO) which corresponds to Santiago/Zacapu/La Luz/Tocumbo basins. This involves 5 populations out of 10.

Pairwise mismatch distribution is clearly unimodal and corresponds to the number of differences among individuals within lineages (Figure 9). *Rhabdochona lichtenfelsi* has undergone at least 2 waves of expansion (increase in frequency of differences among individual sequences) under a constant population growth model. Yet, it shows a recent and sustained decrease with 2 slight waves of growth, but not comparable to the peaking section

of the graph. This means that the genetic diversity of *R. lichtenfelsi* has been steadily decreasing in the recent past.

4. Discussion

4.1. Genetic variation

The area covered by the present study corresponds to the Western basins of Mesa Central in Mexico. The haplotypes recovered represent a small sample of the nematode parasite of goodeines, *R. lichtenfelsi*. Nevertheless, some of the data discussed below indicate that this nematode in its present state is structued into distinct populations, as the populations of haplotypes found have very limited gene flow and the values of χ^2 between populations show that there are significative differences between them. Genetic differentiation into distinct haplotypes in different geographical regions (basins) would evidence that *R. lichtenfelsi* has differentiated along with basin fragmentation of this part of the Mesa Central of Mexico.

4.2. Genetic differentiation among populations

As fragments of COI are recognized to have considerable variation over time in nematodes (Blouin et al., 1992) it is considered that variation found in the present study is well within those limits. Intraspecific variation is considerably small if compared to interspecific variation in nematodes (Blouin et al., 1992). Nevertheless, the present work reports retricted genetic variation within the limits of the geographical realm inhabited by this nematode. There is only a genetic distance of 4 to 6% between the 2 species analyzed here, *R. lichtenfelsi* and *R. mexicana*. Intraspecific differences are well below that value, and overlap with distances (4 -5%) between some samples of *R. lichtenfelsi*, which could indicate that we are dealing with relatively recent episodes of genetic differentiation.

4.3. Geographic distribution of haplotypes and nested clade analysis

Nested clade analysis has been previously applied to complex river topographies for different taxa, i.e., fishes (Hurwood and Hughes, 1998), crayfish (Fetzner, Jr. and Crandall, 2003), and ephemeropterans (Hughes et al., 2003a; Hughes et al., 2003b). The common result found by all these works is that genetic differentiation within a sampled watershed is smaller, in some cases by an order of magnitude, than that among different watersheds. In the case of helminths, namely *R. lichtenfelsi*, we have found similar results, where haplotypes were unique to a particular drainage or a group of neighboring drainages, indicating very little movement of individuals among drainages.

The outcome that haplotypes of *R. lichtenfelsi* only differentiate at long distances, i.e., very large areas, means that the basins of Mesa Central have undergone fragmentation. Only Goodeines show any evidence up to this day and remains the only study that correlates sequences of freshwater species with the evolution of Mesa Central landscape (Doadrio and Domínguez, 2004; Moncayo-Estrada et al., 2001). Our evidence, although limited, reinforces a former connection of some of these freshwater basins, mainly Ameca and Lerma. The connection found between Santiago, Ameca and Cotija seems to reinforce previous information on the ancient distribution of different species of goodeines in this area (Doadrio and Dominguez, 2004). Yet, the connection with the Zacapu basin implies that further sampling between this basin and Río Verde is needed. Nevertheless, limited small drainages are found between those larger basins, i.e., Turbio-Silao river, so it is probable that an ancient relationship between both freshwater bodies has left some tracks that can be discovered up to this day even with limited information.

4.4. Demographic history

The populations of *R. lichtenfelsi* present a steady state increase during most of its history followed by stasis and a final decrease in recent generations. Still, nothing can be said about the probable mutational rates, generation times and possible ancestry of *R. lichtenfelsi* up to this date. Pairwise differences argument strongly in behalf of an recent origin for *R. lichtenfelsi*, well within the aftermath of the uplift of Mesa Central. Several peaks in the distribution of the differences could be evidence of population fragmentation in the past.

4.5. Biogeography

The present distribution of the species of Goodeinae have reinforced some of the historical geomorphological data of the evolution of the Mesa Central (Doadrio and Domínguez, 2004). The present work done on a nematode parasite endemic of Goodeinae, *R. lichtenfelsi*, reinforces to a certain extent some of the discoveries done by the goodeine investigations.

Some of the expected relationships between the basins of Western Mesa Central have been fulfilled. Other, new ones, were found through the study of the haplotypes of *R. lichtenfelsi*. For example, Zacapu basin had been thought to have been an isolated freshwater body throught its history, only connected to Cuitzeo and probably forming at times a part of it. Present evidence indicates that it had connections to those tributaries of the Santiago (Río Verde) that apparently formerly flowed directly into the Lerma river system through the Turbio-Silao river. Ancient faulting in the north of Lake Chapala that resulted in the formation of the Tepic-Chapala rift and river piracy of Western Mesa Central are probably responsible for this.

Lake Patzcuaro seems to have had a long history of isolation (Bradbury, 2000). In the absence of other data, it seems that Patzcuaro has existed as a basin since 3 Ma. (Israde and Domínguez, personal communication). This could readily explain the unique star phylogeny found for the Patzcuaro and Naranja de Tapia haplotypes, which seems so recent that it defies any further analysis.

Overall, population structuring of *R. lichtenfelsi* seems to be detectable even at low sample sizes, and well differentiated haplotypes seem to conform its present distribution. The present work seems to reinforce the idea of a recent Patzcuaro/Cuitzeo/Rio Grande de Morelia (represented here as Minzita) connection where there still exists no genetic differentiation between populations now geographically separated but genetically not structured.

The fact that freshwater vertebrate populations like goodeines are genetically and geographically structured at the species level, and their helminths do not, can owe to the lower rate of base substitutions of COI in the nematode at specific sites when compared to their definitive hosts. *Rhabdochona lichtenfelsi* is an example of a helminth with a limited distribution that infects a myriad of goodeine species in the Ameca, Lerma and Cotija basins, and could further be considered as a model where to look for evidence of relatively recent basin fragmentation and the ancient relationships of the lakes and rivers of the Western Mesa Central of Mexico.

Other evidences will demonstrate or reject what we propose herein. Information from other parasites and even fish hosts will determine the possibility to integrate a comparative study from which general phylogeographic patterns of the Mesa Central of Mexico will be established.

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Legend of figures

Figure 1. Localities of Western Mesa Central of Mexico sampled for *Rhabdochona lichtenfelsi* found mainly in *Goodea atripinnis*; sample of nematode found in *Alloophorus robustus* belongs to Lago de Patzcuaro, and samples from Zacapu are from *Allotoca zacapuensis* and *Hubbsina turneri*.

Figure 2. MP strict consensus cladogram, heuristic search with random addition, 100 replicates of sequences of *Rhabdochona lichtenfelsi* from Western Mesa Central basins. Bootstrap values with > 50% indicated over branches.

Figure 3. Neighbor joining tree with, uncorrected p. *Thelazia lacrymalis* as outgroup.

Figure 4. Neighbor joining tree with uncorrected p. *Rhabdochona mexicana* (M1) as outgroup to show explicitly distances between sequences of *R. lichtenfelsi*.

Figure 5. Haplotype network superimposed on localities sampled for *Rhabdochona lichtenfelsi*.

Figure 6. Nested clades for analysis of haplotypes of *Rhabdochona lichtenfelsi* found in Ameca, Santiago, La Luz, Tocumbo and Zacapu localities. Dotted lines represent 2 step clades.

Figure 7. Nested clade (not analyzed) of Patzcuaro and Naranja de Tapia localities. Dotted lines represent 2 step clades.

Figure 8. Paired genetic distances between populations plotted against paired geographic distances of localities sampled for *Rhabdochona lichtenfelsi*. Correlation coefficient is 0.04. Lines correspond to least squares fit.

Figure 9. Pairwise mismatch distribution of sequences of *Rhabdochona lichtenfelsi* from Mesa Central.

Table 1. Localities and hosts sampled for COI fragments of *Rhabdochona lichtenfelsi* in Western Mexico.

Table 2. Genetic diversity of sequences of 38 sequences sampled of *Rhabdochona lichtenfelsi*

Table 3. Gst (above 0 diagonal) and Da (below 0 diagonal) values for 10 populations of COI (38 sequences) of *Rhabdochona lichtenfelsi* from Western Mesa Central.

Table 4. Permutational contingency test of clade 2-2 of *Rhabdochona lichtenfelsi*

Apendixes. Appendix 1. Variable sites within haplotypes of *R. lichtenfelsi*.

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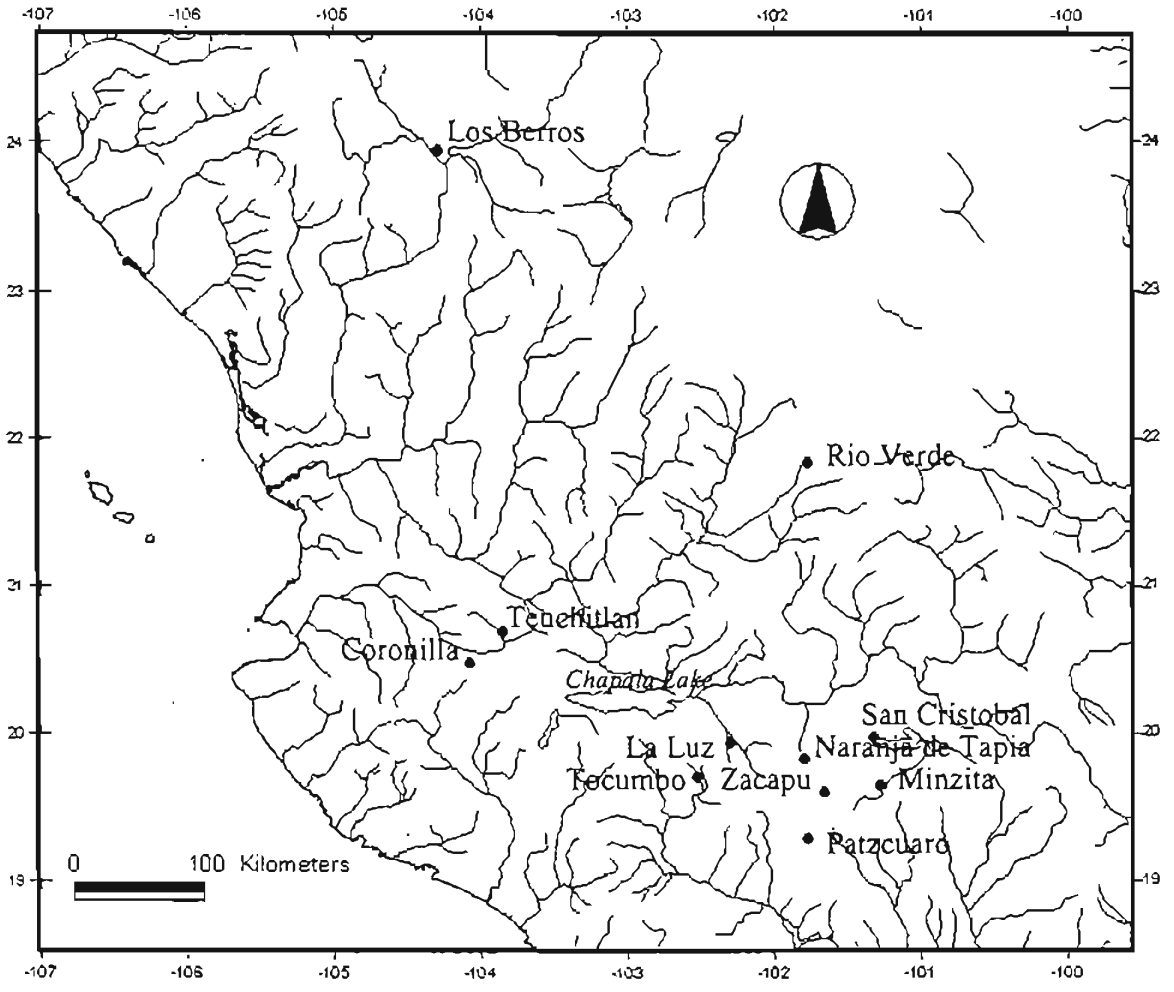
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Teuchitlan
Coronilla
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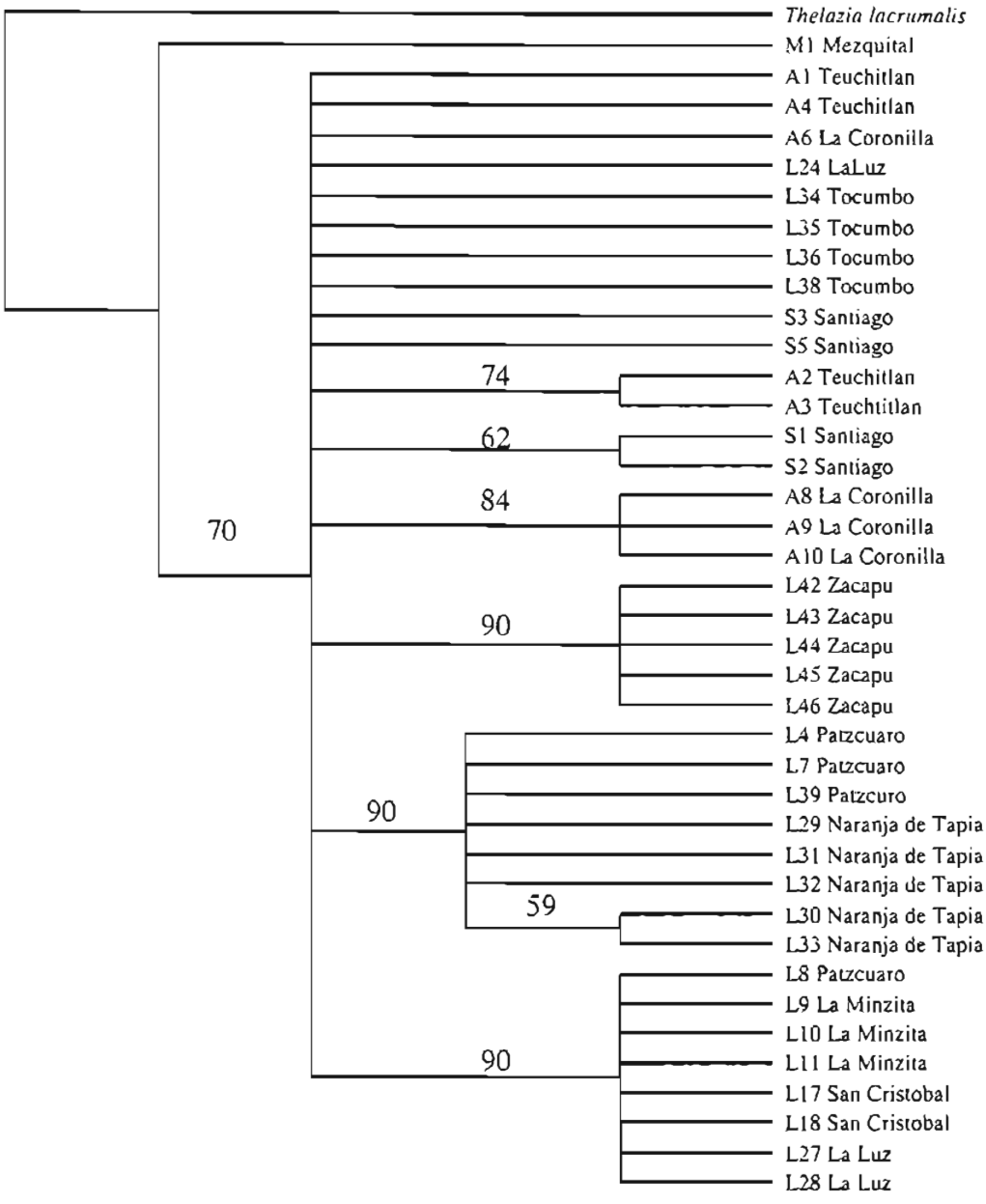
Zacapu
Minzita
Patzcuaro
0
100 Kilometers

2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A4 Teuchitlan
A6 La Coronilla
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L34 Tocumbo
L35 Tocumbo
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L38 Tocumbo
S3 Santiago
S5 Santiago
74
A2 Teuchitlan
A3 Teuchtitlan
62
S1 Santiago
S2 Santiago
84
A8 La Coronilla
70
A9 La Coronilla
A10 La Coronilla
L42 Zacapu
90
L43 Zacapu
L44 Zacapu
L45 Zacapu
L46 Zacapu
L4 Patzcuaro
L7 Patzcuaro
L39 Patzcuro
90
L29 Naranja de Tapia
L31 Naranja de Tapia
L32 Naranja de Tapia
59
L30 Naranja de Tapia
L33 Naranja de Tapia
L8 Patzcuaro
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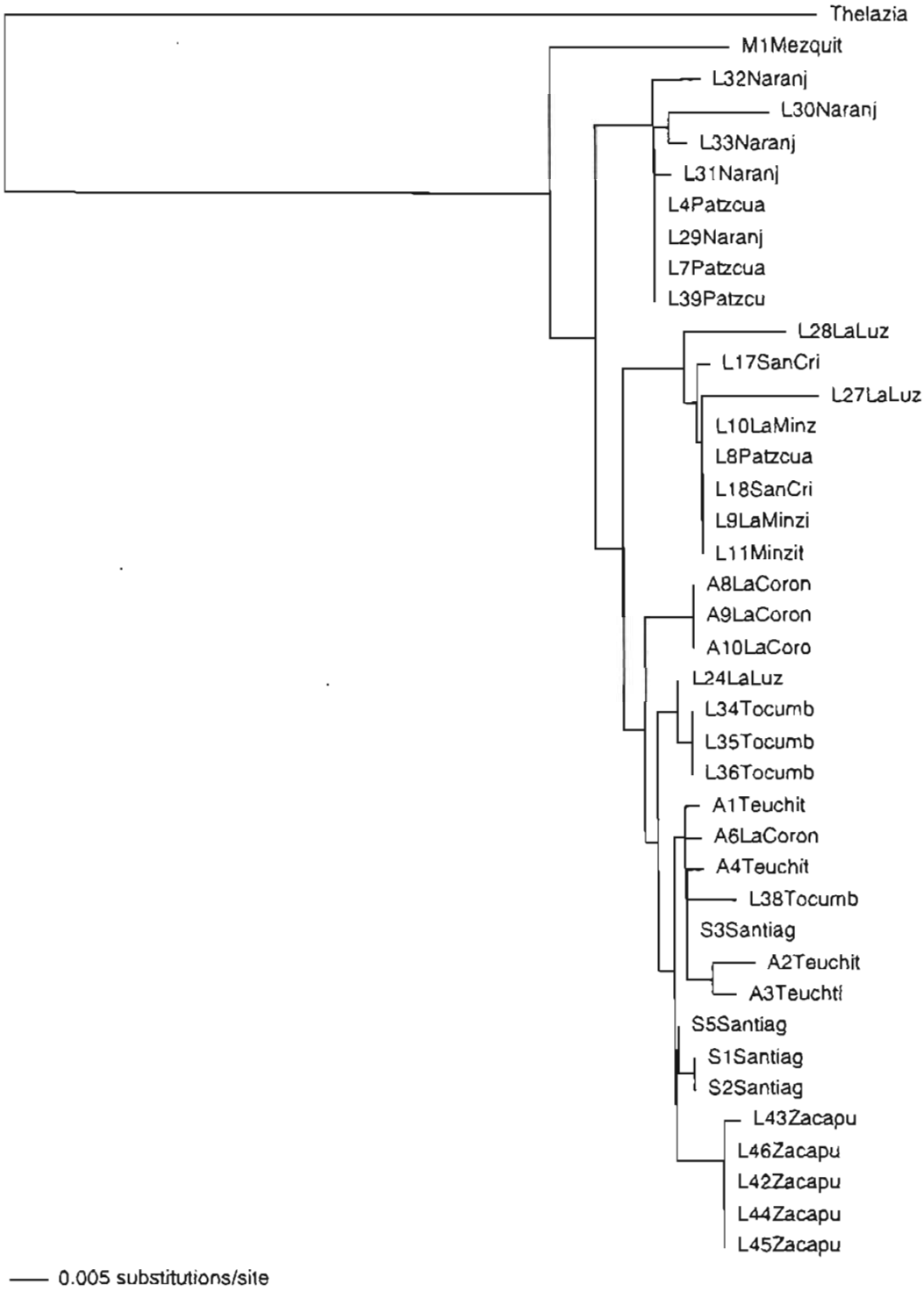
L11 La Minzita
L17 San Cristobal
L18 San Cristobal
L27 La Luz
L28 La Luz

3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L27LaLuz
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L18SanCri
L9LaMinzi
L11Minzit
A8LaCoron
A9LaCoron
A10LaCoro
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L35Tocumb
L36Tocumb
A1Teuchit
A6LaCoron
A4Teuchit
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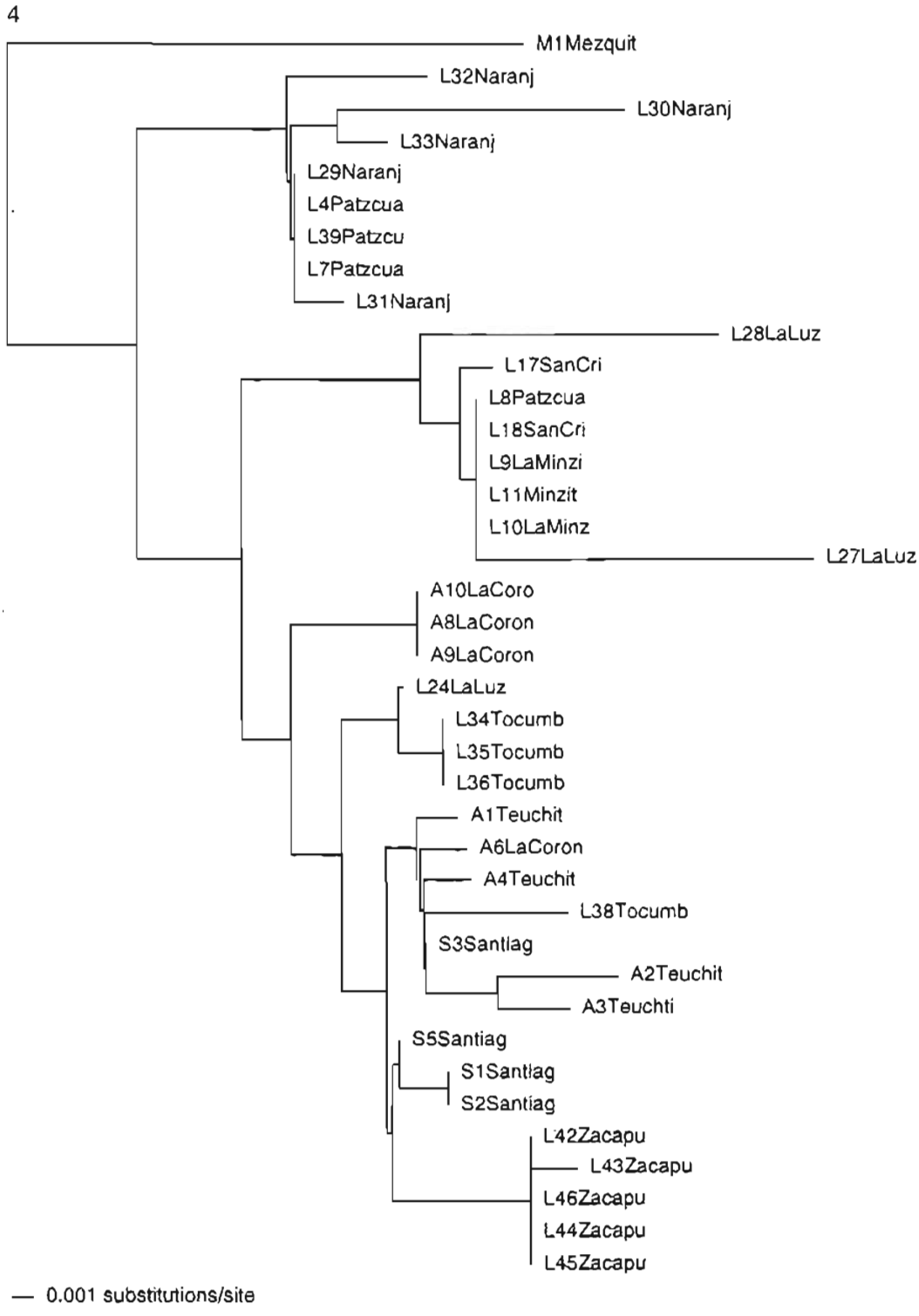
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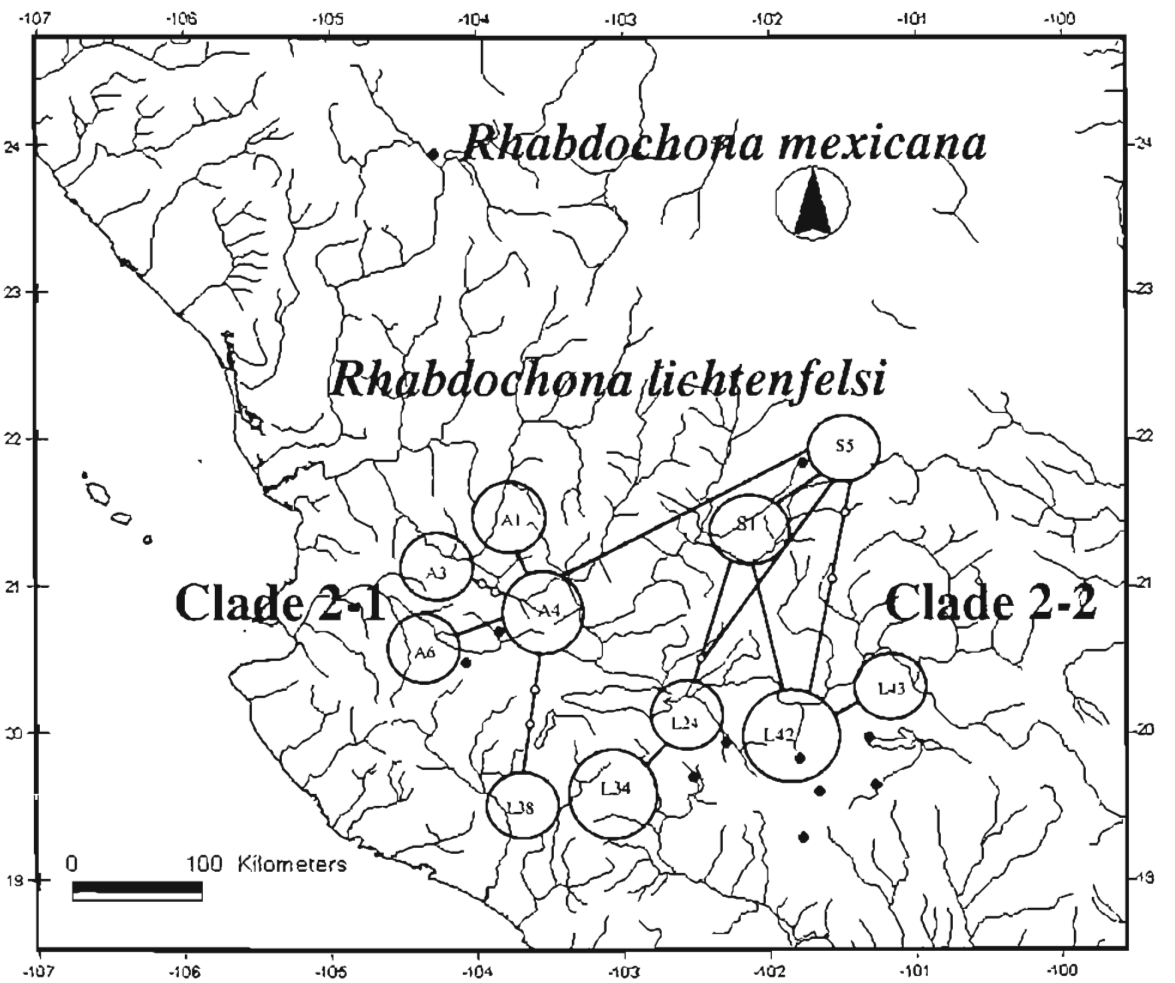
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Rhabdochona lichtenfelsi
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Clade 2-2
S5
S1
A1
A3
A4
A6
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L34
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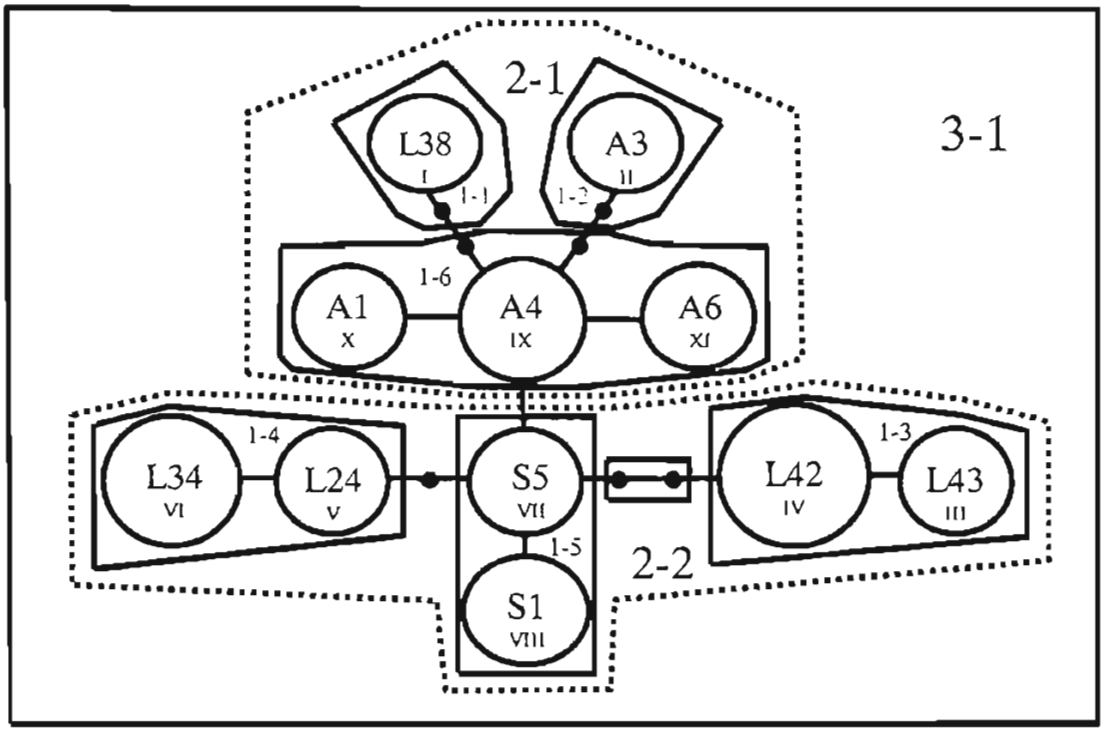
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IV
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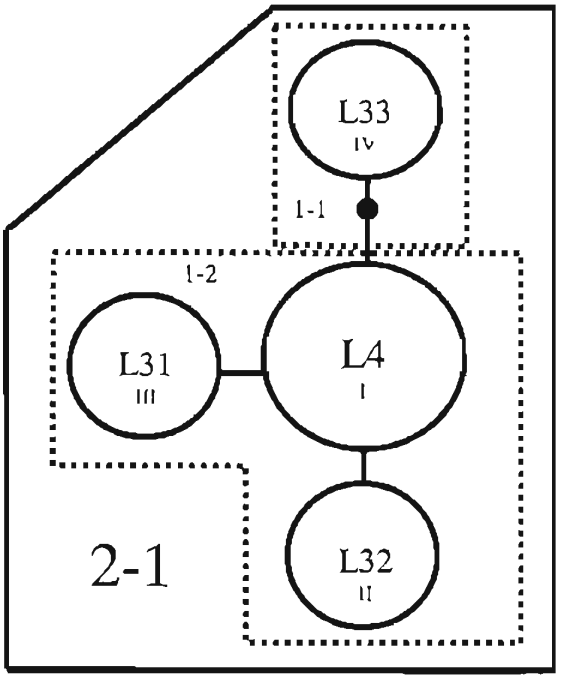
III

7



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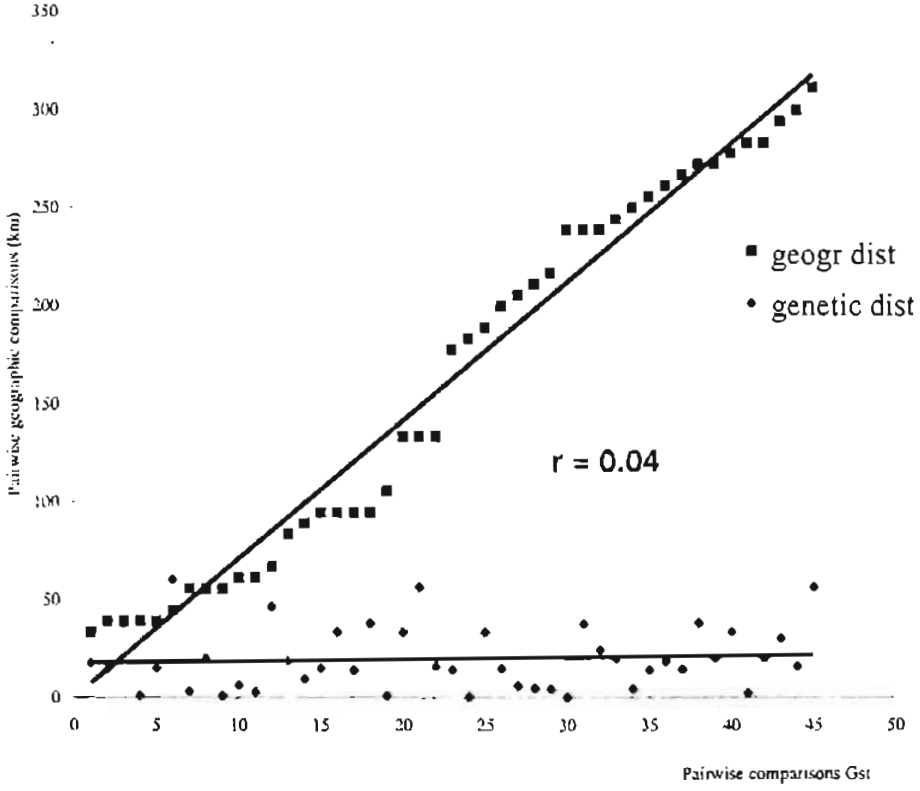
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Pairwise geographic comparisons (km)
Pairwise comparisons Gst
geogr dist
genetic dist
r = 0.04

9

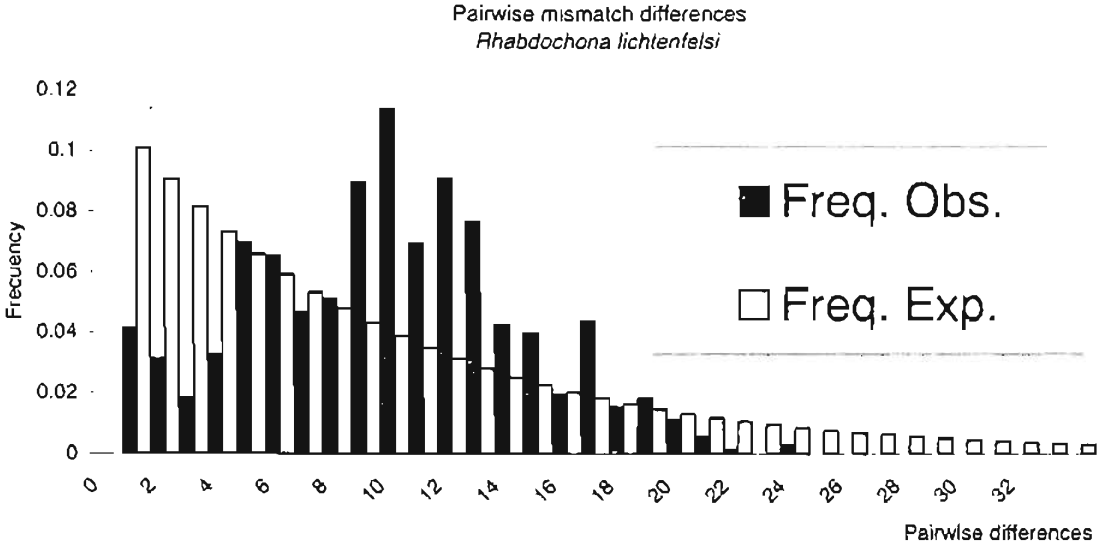
Pairwise mismatch differences
Rhabdochona lichtenfelsi
Frecuency
0.12
0.1
0.08
0.06
0.04
0.02
0
Freq. Obs.
Freq. Exp.
0
2
4
6
8
10
12
14
16
18
20
22
24
26
28
30
32
Pairwise differences

Table 1. Localities and hosts sampled for COI fragments of *Rhabdochona lichtenfelsi* in Western Mexico.

Locality (number, name)	River basin	Host	Sequences (code, number sampled)		Latitude (North)			Longitude (West)		
					Deg	Min	Sec	Deg	Min	Sec
1 Los Berros	Mezquital	*Characodon audax**	M1	1	23	56	18.2	104	16	26.4
2 La Coronilla	Ameca	*Goodea atripinnis*	A6, A8, A9, A10	4	20	28	9.4	104	4	10.6
3 Río Verde	Santiago	*Goodea atripinnis*	S1, S2, S3, S5	4	21	49	12	101	46	21.3
4 Teuchitlan	Ameca	*Goodea atripinnis*	A1-A4	4	20	41	20.6	103	50	29.9
5 La Luz	Former Lerma	*Goodea atripinnis*	L24, L27, L28	3	19	56	8.1	102	18	0.2
6 La Minzita	Middle Lerma	*Goodea atripinnis*	L9, L10, L11	3	19	38	40.3	101	16	28.5
7 Lago de Patzcuaro	Middle Lerma	*Goodea atripinnis* and *Alloophorus robustus*	L4, L7, L8, L39	4	19	41	0.0	100	32	0.0
8 Lago de Zacapu	Middle Lerma	*Allotoca zacapuensis* and *Hubbsina turneri*	L42-L46	5	19	49	0.0	101	47	0.0
9 Naranja de Tapia	Middle Lerma	*Goodea atripinnis*	L29-L33	5	19	16	58.2	101	45	50.3
11San Cristobal	Cuitzeo	*Goodea atripinnis*	L17, L18	2	19	57	41.6	101	18	57.3
12Tocumbo	Cotija	*Goodea atripinnis*	L34, L35, L36, L38	4	19	42	7	102	30	58.4

* *Rhabdochona mexicana*.

Table 2. Genetic diversity of sequences of 38 sequences sampled of *Rhabdochona lichtenfelsi*.

Haplotype code	Basin	Population name throughout this study	Individuals/ sequences	Haplotypes	S	Hd	*p*
A1, A2, A3, A4	Ameca	1: Teuchitlan	4	4	7	1.00000	0.00877
A6, A8, A9, A10	Ameca	2: Coronilla	4	2	5	0.50000	0.00548
L4, L7, L8, L39	Lerma	3: Patzcuaro	4	2	9	0.50000	0.00987
L9, L10, L11	Lerma	4: Minzita	3	1	0	0.00000	0.00000
L17, L18	Lerma	5: San_Cristobal	2	2	1	1.00000	0.00219
L24, L27, L28	Lerma	6: La_Luz	3	3	23	1.00000	0.03363
L29, L30, L31, L32, L33	Lerma	7: Naranja_de_Tapia	5	5	12	1.00000	0.01096
L34, L35, L36, L38	Cotija	8: Tocumbo	4	2	7	0.50000	0.00768
L42, L43, L44, L45, L46	Lerma	9: Zacapu	5	2	1	0.40000	0.00088
S1, S2, S3, S5	Santiago	10: Río Verde	4	3	2	0.83333	0.00256
Total sample			38	23	50	0.95875	0.01955

S = number of segregating sites
Hd = haplotype diversity
π = nucleotide diversity

Table 3. Gst (above 0 diagonal) and Da (below 0 diagonal) values for 10 populations of COI (38 sequences) of *Rhabdochona lichtenfelsi* from Western Mesa Central.

	Population name and number									
	1	2	3	4	5	6	7	8	9	10
1 Teuchitlan	0	0.1429	0.1429	0.3015	0.0204	0.003	0.0014	0.1429	0.1828	0.0435
2 Coronilla	0.0074	0	0.3333	0.5631	0.1579	0.1485	0.138	0.3333	0.3798	0.2
3 Patzcuaro	0.0159	0.0115	0	0.4624	0.0943	0.1485	0.0609	0.3333	0.3798	0.2
4 Mintzita	0.0192	0.0165	0.0099	0	0.0083	0.3333	0.2771	0.5631	0.6013	0.3762
5 San Cristobal	0.017	0.0159	0.0099	0	0	0.0083	0.0311	0.1579	0.1977	0.0588
6 Luz	0.0073	0.0066	0.0051	0.0007	0.0007	0	0.0083	0.1485	0.1892	0.0459
7 Naranja	0.0214	0.0163	0.0002	0.02	0.02	0.0132	0	0.138	0.1765	0.0438
8 Tocumbo	0.0049	0.0047	0.0126	0.0208	0.0203	0.0062	0.0167	0	0.3798	0.2
9 Zacapu	0.0088	0.0132	0.0208	0.0241	0.0241	0.0102	0.0261	0.0093	0	0.2429
10 Rio Verde	0.002	0.0067	0.0146	0.0179	0.0174	0.0055	0.0203	0.0034	0.0069	0

Table 4. Permutational contingency test of clade 2-2 of *Rhabdochona lichtenfelsi*

Clade	χ^2	p	major basins involved
2-2	24.0	0.00*	Coronilla/Luz/Teuchitlan/Tocumbo/Santiago/Zacapu/

* significative at less than 5% level

DISCUSIÓN.

La Mesa Central de México ha sido un área intensamente estudiada en relación con la evolución de sus peces de agua dulce desde las décadas de los 60 y 70, con los trabajos de especiación y biogeografía de José Álvarez del Villar (1972, 1978). Esta región, que se puede considerar el límite norteño de la provincia ictiofaunística de la Zona Central de México, tiene una serie de especies tanto de afinidad Neotropical como Neártica (Álvarez, 1972, 1978; Miller, 1986; Miller & Smith, 1986). Sin embargo, el estudio de la evolución de los helmintos que parasitan a tan diversa ictiofauna se encuentra todavía en sus etapas iniciales. A pesar de ésto, se cuenta con un inventario que puede ser utilizado para generar información filogenética y biogeográfica. Se han comenzado a generar investigaciones relacionadas principalmente con la helmintofauna de las familias más diversas de peces de la Mesa Central, encontrándose la presente investigación entre uno de esos primeros trabajos.

Este estudio sobre la historia biogeográfica de la Mesa Central de México, realizado con los helmintos adultos parásitos de peces de la Subfamilia Goodeinae, describió una helmintofauna que reúne las características ecológicas y geográficas indicadas por Pérez-Ponce de León & Choudhury (2002) y Pérez-Ponce de León (2003): se trata de una helmintofauna núcleo asociada a peces endémicos de esta región y tiene afinidades neárticas y/o neotropicales. Adicionalmente, cuenta con 4 especies de helmintos que son endémicos de esta subfamilia: *Margotrema bravoae*, *M. guillerminae* (Platyhelminthes: Allocreadiidae); *Rhabdochona ahuehuellensis* y *R. lichtenfelsi* (Nematoda: Rhabdochonidae). Se las considera especies núcleo debido a que dichos helmintos son los que se encuentran con mayor prevalencia e intensidad en los peces estudiados además de tener una distribución amplia dentro de la Mesa Central y otras cuencas ya mencionadas.

Los hospederos pertenecientes a un determinado taxón generalmente tienden a conservar su helmintofauna original en su área de distribución. Sin embargo, en la periferia de su distribución estos hospederos tienden a perder su helmintofauna original, la cual no es sustituida por la helmintofauna de otros peces se distribuyen en la misma región y comparten los mismos hábitats (espacialmente), al menos recientemente (Choudhury & Dick, 2000). Es decir, es limitada la compartición de hospederos (host-sharing), o el cambio de hospederos (host-switching) (Choudhury & Dick, 2000; Pérez-Ponce de León & Choudhury, 2002). Este último caso, el cambio de hospederos (host-switching) en algunos casos parece haber dado origen a nuevas especies de helmintos como resultado precisamente de especiación alopátrica vicariante por aislados periféricos (Brooks & McLennan, 1993).

En helmintos de peces dulceacuícolas de Norteamérica, el nivel taxonómico de los hospederos en el que se observa este fenómeno es el de familia (Pérez-Ponce de León & Choudhury, 2005). Estos autores han postulado que la helmintofauna se mantiene a nivel de familia de peces, pero hay pérdidas en la periferia de la distribución de los hospederos, como ocurre con la helmintofauna de la familia Ictaluridae (Pérez-Ponce de León & Choudhury, 2002). Además no hay substitución de helmintofaunas (las originales por las nativas). Esto explica el hecho que especies de Goodeinae como *Xenotoca variata* prácticamente estén desprovistas de helmintos intestinales en los límites de su área de distribución (cuenca del Pánuco).

Las especies introducidas, helmintos como *B. acheilognathi* y *P. tomentosa*, no se encuentran tan ampliamente distribuidas en goodéidos, pese a que la primera especie mencionada se ha distribuido rápidamente en distintas especies de peces tanto exóticos como nativos (Pineda-López & Salgado-Maldonado, 2003). Esto puede deberse a que no

todos los goodéidos han sido susceptibles de infección por *B. acheilognathi* y probablemente a que aquellos no se encuentran emparentados cercanamente con los ciprínidos, la familia de peces que preferentemente infecta este céstodo. Sin embargo, se debe mencionar que *B. acheilognathi* ha llegado a infectar a especies del género *Chirostoma* (Pineda-López & Salgado-Maldonado, 2003).

La composición de las comunidades de helmintos estudiadas se reduce a la presencia de las 4 especies mencionadas. Mejía-Madrid et al. (2005) mencionan que difícilmente las 4 especies de helmintos encontradas coinciden en los mismos hospederos en la misma localidad. Por lo tanto, no existe registrado hasta este momento ninguna interacción potencial entre estas especies dentro de un mismo hospedero o individuo de la misma especie de goodéido, ya que parece ser que estos helmintos se encuentran en distintos individuos de la misma especie de goodéidos o en distintas especies. Por ende, es raro encontrar 2 especies distintas de helmintos en el intestino de una especie de pez. En aquellas ocasiones en las cuales se recolectaron helmintos de distintas especies ya sea, en el mismo individuo o en la misma especie de goodéidos, generalmente se trataba de una de las dos especies de *Margotrema* y una especie de *Rhabdochona*, pero nunca 2 especies pertenecientes al mismo género. Además, estas especies generalmente se distribuían en distintas regiones del intestino, donde *Rhabdochona* ocupaba preferencialmente la mayor parte del intestino medio y *Margotrema* se limitaba al recto. Aún en ausencia de una u otra especie, el caso generalmente encontrado, dichos helmintos limitaban su distribución a las regiones intestinales mencionadas.

La distribución geográfica de la helmintofauna estudiada se encuentra dentro de las cuencas hidrográficas de la Mesa Central y otras, como las cuencas de los ríos Pánuco, Santiago, Mezquital, Armería, Coahuayana y Balsas. La distribución de la helmintofauna

núcleo corresponde cercanamente a la distribución de sus hospederos, ya que hay localidades donde no se encontró a ninguna de las especies mencionadas.

La distribución geográfica de dichos helmintos, adicionalmente, se encuentra en cuencas determinadas. *Rhabdochona lichtenfelsi* es un nemátodo que parasita a goodéidos del Lerma y algunos afluentes orientales del Santiago, pero nunca se le halló en el Santiago Bajo, es decir, entre el Lago Chapala y su desembocadura. En el presente trabajo se muestreó el río Santiago cerca de la presa de Aguamilpa, donde se halló a *R. kidderi* parasitando a *Cichlasoma beani*. En los afluentes del Santiago en la región del río Compostela se encontró a *R. xiphophori* parasitando a *Xenotoca eiseni*. Por otra parte, *R. lichtenfelsi* parasita a *Goodea gracilis* en la región estudiada del alto Pánuco. En esta región se reconoció que *R. ahuehuellensis* se encuentra parasitando a otro goodéido, *Ataeniobius toweri* en el Lago de la Media Luna (Mejía-Madrid et al., 2005). Cabe agregar que *R. lichtenfelsi* también fue hallado en *Chapalichthys pardalis* (Mejía-Madrid et al., 2005) en Tocumbo, cuenca del río Cotija, un afluente que antes perteneció al Lerma pero que fue desviado en tiempos históricos hacia el río Balsas. *Rhabdochona lichtenfelsi* revela, por lo tanto, la relación anterior de la cuenca del Cotija con el Lerma y no con el Balsas, donde se encuentra *R. ahuehuellensis* parasitando a goodéidos.

Rhabdochona ahuehuellensis se encuentra ampliamente distribuida en el río Balsas. Además se la encontró parasitando goodéidos en la parte alta de las cuencas del río Armería (Ayuquila) y Coahuayana (Tamazula y otros afluentes menores).

Margotrema bravoae sigue un patrón de distribución semejante al encontrado para *R. lichtenfelsi*, excepto que no se la encontró en el río Pánuco. Parece ser que *M. bravoae* se limita a infectar goodéidos en la cuenca del río Lerma y en los afluentes del alto Santiago (río Verde). Por otra parte, se encontró *M. bravoae* en *Allotoca regalis* en la cuenca del río

Cotija. Esto puede deberse a lo que se mencionó para el caso de la distribución de *R. lichtenfelsi*.

De todo esto se desprende que la Mesa Central de México al menos tiene 2 helmintos que se distribuyen exclusivamente en sus aguas: *Margotrema bravoae* y *Rhabdochona lichtenfelsi*. Este último helminto además extiende su distribución al río Pánuco.

Es evidente además, que estos helmintos demuestran una diversidad muy baja, si solamente se los compara con la diversidad, a nivel de especie, de sus hospederos. La Mesa Central muestra una serie de cuencas en las cuales se ha llevado al cabo la especiación de peces que, como los goodéidos, cuentan hasta con 39 especies. A partir del análisis tan solo faunístico es claro que los helmintos no han diversificado conjuntamente con sus hospederos. Por lo tanto, la helmintofauna intestinal de Goodeinae, así como de otros grupos de peces de la Mesa Central como las especies de los géneros *Chirostoma* y *Algansea*, es pobre (*sensu* Choudhury & Dick, 2000), si se la compara con la helmintofauna de otros peces de México, p. ej., Cichlidae (Vidal-Martínez et al., 2001) e incluso de peces de otras partes del mundo (Choudhury and Dick, 2000). Los factores responsables de una helmintofauna poco diversa han sido discutidos desde el punto de vista ecológico en términos generales por Choudhury & Dick (2000). Existen otros ejemplos de baja diversidad en helmintofaunas de peces mexicanos (Espinosa-Huerta, et al., 1996).

A partir de esta evidencia, no fue posible detallar la historia biogeográfica de las cuencas de la Mesa Central. Sin embargo, estos datos representan el primer paso para caracterizar a una helmintofauna regional con límites geográficos claros.

Se ha reconocido recientemente que los helmintos que parasitan peces (y otros vertebrados acuáticos) de la región central de México, incluyendo a la Mesa Central, tienen

afinidades no solo faunísticas, sino filogenéticas en ambas regiones biogeográficas, aunque trabajos previos han encontrado que tienen relación principalmente con la región Neártica (Pérez-Ponce de León et al., 1996, Pérez-Ponce de León, 2003; Pérez-Ponce de León & Choudhury, 2005) y otras son endémicas de la región (Pérez-Ponce de León, 2002). Solamente en contados casos se cuenta con helmintos de claras afinidades neotropicales (Pérez-Ponce de León, 2003).

En el presente trabajo se ha encontrado que las especies que se encuentran en la Mesa Central (Lerma-Santiago) y cuencas adyacentes (cuencas del Pacífico, Balsas y Pánuco) son probablemente de origen neártico, como lo indica la evidencia obtenida de la filogenia de *Rhabdochona* Railliet, 1916. Asimismo, dicha filogenia indica que las especies americanas de *Rhabdochona* no conforman un grupo monofilético, lo cual traducido a términos biogeográficos es evidencia de que su invasión a cuencas americanas no fue resultado de un solo evento histórico. Los clados que componen dicha filogenia tienen componentes asiáticos, africanos y europeos y las especies americanas se encuentran formando parte de todos estos clados. Esto indica por una parte que el género *Rhabdochona* ha ingresado a cuencas americanas en distintas épocas, no estando en posibilidad de fechar aún sus distintos orígenes. Esto será el objetivo de una filogenia molecular basada en varios genes y con fechamientos aproximados que utilicen el reloj molecular.

Las especies que se encuentran en el centro de México, y en particular en la Mesa Central, tienen claras afinidades neárticas. *Rhabdochona lichtenfelsi* está cercanamente relacionada con un clado al cual pertenecen especies europeas como *R. fortunatowi* o asiáticas como *R. vietnamensis*. Además, esta especie, claramente del Lerma-Santiago, es la especie hermana de *R. decaturensis*, un nemátodo parásito de las familias Sciaenidae,

Ictaluridae, Catostomidae e Hiodontidae en Norteamérica, que no ha sido encontrado parasitando a estas familias de peces en México.

Otras filogenias parecen señalar un patrón biogeográfico semejante, como lo indica la filogenia del género de céstodos de vertebrados dulceacuícolas, *Proteocephalus* (de Chambrier et al., 2004), es decir, tienen un origen eurasiático y a partir de esta amplia región, se diseminaron por todo el mundo o simplemente especiarion vicariantemente, como se indicó anteriormente (Brooks & McLennan, 1993).

Ya que no se encontraron evidencias de especiación asociadas a las distintas cuencas de la Mesa Central o cuencas relacionadas, fue lógico pensar que el modelo a estudiar debería de ser filogeográfico, debido a que de esta forma se podría estudiar una especie a nivel genético y por lo tanto infraespecífico en distintos cuerpos de agua dentro de esta zona. Como se encontró que *Rhabdochona lichtenfelsi* es el helminto de goodéidos más prevalente y ampliamente distribuído en la mayoría de los cuerpos de agua estudiados, se reconoció como un modelo que podría dilucidar las relaciones históricas entre las cuencas de la Mesa Central, al menos donde se le encontró. Cabe recalcar que este es el primer trabajo de su tipo que se realiza en México con helmintos de peces de aguas dulces.

Los resultados encontrados indican que: a) la diferenciación genética de *Rhabdochona lichtenfelsi* en distintos cuerpos de agua del Occidente de la Mesa Central es alta: el o los haplotipos encontrados en cada cuerpo de agua son únicos e indican estructuración geográfica debido a que el flujo génico entre distintas poblaciones o localidades de *R. lichtenfelsi* ha sido históricamente escaso o nulo ($m < 2$); b) esto indica que el aislamiento geográfico de distintos haplotipos de *R. lichtenfelsi* ha dado como resultado aislamiento genético en distintos cuerpos de agua desde hace probablemente unos 9 millones de años que fue cuando se elevó la Mesa Central, pero seguramente a una tasa de substitución más

baja que la de sus hospederos, lo cual explica porqué no ha habido especiación en *Rhabdochona* en la Mesa Central, a pesar de que sus hospederos han especiado; c) la diferenciación genética detectable en *R. lichtenfelsi* corresponde a la separación de 4 cuerpos de agua principalmente: La Luz (Lerma) del Ameca, Tocumbo (Cotija) del Lerma y Santiago (río Verde) de Zacapu. Este último caso en realidad es una separación del Lerma provocada por el cambio de dirección en el flujo de las aguas del Lerma Medio hacia el Alto Santiago después del Plioceno y que corresponde al surgimiento del *rift* de Tepic-Chapala.

La filogeografía abre una serie de posibilidades para la investigación de las helmintofaunas de distintas familias de peces en México, es decir, una ampliación de estos estudios que pasarían del nivel específico al infraespecífico.

En los escritos que resumen la biogeografía del Gran Intercamabio Americano (Jackson, et al., 1996) se encuentra ausente un capítulo sobre la biogeografía histórica de los vertebrados de agua dulce. Los estudios recientes realizados sobre el intercambio de iciofaunas se han comenzado a multiplicar sobretodo desde la persepectiva biogeográfica (Bermingham & Martin, 1998). La Mesa Central de México ha sido postulada como una zona de transición por algunos autores (Marshall & Liebherr, 2000), sin embargo, para otros no (Morrone & Márquez, 2001). Desde la perspectiva de los helmintos de peces de agua dulce, la Mesa Central no es una zona de transición ya que la mayoría de los helmintos que parasitan a vertebrados acuáticos de esta región tienen afinidades Neárticas (Pérez-Ponce de León, 2003). En el presente trabajo hemos corroborado las aseveraciones de Pérez-Ponce de León (2003). La filogenia del nemátodo parásito más abundante y mejor distribuido de goodéidos de la Mesa Central, es *Rhabdochona lichtenfelsi* lo que indica que es de origen Neártico, al igual que otros helmintos parásitos de goodéidos (Pérez-Ponce de

León com. pers.). Por lo tanto, se afirma que la Mesa Central de México no es una zona de transición.

Por otra parte, las relaciones biogeográficas dentro de la Mesa Central indican, como lo venían anunciando otros autores (Álvarez, 1972, 1978; Barbour, 1973; Pérez-Ponce de León, 2003) que las cuencas hidrográficas que se encuentran dentro de sus límites, así como el nacimiento de varios otros ríos (Pánuco y Balsas), han formado grandes cuerpos de agua anteriormente y que han estado sujetas a periodos intermitentes de desecación y recuperación (Álvarez. 1972). Los haplotipos de *R. lichtenfelsi*, tal como se estudiaron en el tercer capítulo de esta investigación indican la relación antigua del Río Lerma con el Río Ameca, así como con otras cuencas de la región occidental de la Mesa Central, donde se encuentra la región de los grandes lagos Michoacanos y Chapala. Los haplotipos de *R. lichtenfelsi* asimismo indican que la dinámica de la historia hidrográfica y geológica de la región no solamente muestran eventos de vicarianza, es decir, separación entre distintas cuencas, sino también dispersión. La primera se encuentra representada por la diversidad de haplotipos encontrados y privativos de cada una de las cuencas analizadas, pero al mismo tiempo indica una mezcla de haplotipos, muy limitada, pero que significa que estos cuerpos de agua una vez separados se comunicaron de nuevo.

El futuro de la investigación de la historia biogeográfica de los helmintos de la Mesa Central implica que se deberán de abordar estudios cada vez más detallados sobre la composición genética de la helmintofauna tan poco diversa de la región y asimismo, deberá ampliarse a helmintos de otras familias de peces que se encuentran en la región, así como a helmintos de otras familias de vertebrados. Solamente así se podrá completar la reconstrucción paleohistórica de la biogeografía de la Mesa Central de México.

CONCLUSIONES.

1) La helmintofauna intestinal de la subfamilia Goodeinae Parenti, 1981 está compuesta de 11 especies de helmintos adultos que pertenecen a 2 phyla: Platyhelminthes y Nematoda.

   a) El phylum Platyhelminthes se encuentra representado por 6 especies, pertenecientes a las clases Digenea (4 especies) y Cestoda (2 especies).

   b) El phylum Nematoda se encuentra representado por 5 especies, pertenecientes a las clases Adenophorea (1 especie) y Secernentea (4 especies).

   c) Las especies pertenecientes a la clase Digenea son: *Allocreadium lobatum*, *A. mexicanum*, *Margotrema bravoae* y *M. guillerminae.*

   d) Las especies pertenecientes a la clase Eucestoda son: *Bothriocephalus acheilognathi* y *Proteccephalus longicollis.*

   e) La clase Adenophorea está representada por una sola especie, *Psedocapillaria tomentosa*;

   f) La clase Secernentea está representada por 4 especies pertenecientes al orden Spirurata, Superfamilia Thelazioidea, familia Rhabdochonidae: *Rhabdochona ahuehuellensis*, *R. lichtenfelsi*, *R. mexicana* y *R. xiphophori.*

2) Se consideran 29 nuevos registros de hospederos y 48 nuevos registros de localidad, lo cual hace este el registro más completo de los helmintos de la subfamilia Goodeinae.

3) Se encontró una nueva especie de Rhabdochonidae, *R. ahuehuellensis* Mejía-Madrid & Pérez-Ponce de León, 2003 en el goodéido *Ilyodon whitei* Meek en la cuenca del Alto Balsas en el Estado de Puebla, México.

4) La helmintofauna intestinal de Goodeinae es pobre si se la compara con la helmintofauna de otros peces de México, p. ej., Cichlidae, sin embargo es semejante en cuanto a pobreza con la helmintofauna de Atherinidae y de Cyprinidae nativos.

5) La helmintofauna núcleo se encuentra compuesta por 4 especies: *Margotrema bravoae*, *M. guillerminae* (Platyhelminthes: Allocreadiidae); *Rhabdochona ahuehuellensis* y *R. lichtenfelsi* (Nematoda: Rhabdochonidae).

6) Las especies introducidas, como *B. acheilognathi* y *P. tomentosa*, no se encuentran tan ampliamente distribuidas en goodéidos.

7) La distribución de la helmintofauna estudiada se encuentra dentro de las cuencas hidrográficas de la Mesa Central y otras, como la cuenca del río Pánuco, Santiago, Mezquital, Armería, Coahuayana y Balsas.

9) *Rhabdochona lichtenfelsi* es un nemátodo que parasita a goodéidos del Lerma y algunos afluentes orientales del Santiago, pero nunca se le halló en el Santiago Bajo, es decir, entre el Lago Chapala y su desembocadura.

10) *Rhabdochona ahuehuellensis* se encuentra ampliamente distribuido en el río Balsas. Además se le encontró parasitando goodéidos en la parte alta de las cuencas del río Armería (Ayuquila) y Coahuayana (Tamazula y otros afluentes menores).

11) *Margotrema bravoae* sigue un patrón de distribución semejante al encontrado para *R. lichtenfelsi*, excepto que no se le encontró en el río Pánuco.

12) En la filogenia morfológica del género *Rhabdochona* se encontró que las especies americanas que lo componen no representan un grupo monofilético.

13) Las 2 especies principales, *R. ahuehuellensis* y *R. lichtenfelsi* no pertenecen a un grupo monofilético.

14) La filogeografía de *R lichtenfelsi* indicó que sus poblaciones están estructuradas genética y geográficamente aunque la única correlación entre la distancia genética y la distancia geográfica se obtuvo a partir de las poblaciones de algunas de las cuencas occidentales de la Mesa Central y áreas circunvecinas, es decir, en Ameca, Cotija, Lerma-Santiago y Zacapu.

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