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Taxonomía integrativa de algunas especies del género
***Saccocoelioides* (Digenea: Haploporidae)**

TESIS

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PRESENTA:

Biól. LEOPOLDO ANDRADE GÓMEZ

TUTOR PRINCIPAL DE TESIS: DR. JOSÉ MARTÍN GARCÍA VARELA, Instituto de Biología, UNAM
COMITÉ TUTOR: DR. ROGELIO AGUILAR AGUILAR, Facultad de Ciencias, UNAM
DR. GERARDO PÉREZ PONCE DE LEÓN, Instituto de Biología, UNAM

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I. RESUMEN

En el presente trabajo de tesis se estudiaron algunas de las especies del género *Saccocoelioides*, parásitos de peces dulceacuícolas y estuarinos en diferentes localidades de México y Centro América. Como resultado de la tesis fueron sometidos dos manuscritos publicados. En el primero corresponde con la descripción de una nueva especie, *Saccocoelioides olmecae* n. sp., parásito del intestino del pez dormilón *Dormitator maculatus* (Bloch) (Perciformes: Eleotridae) colectados en seis localidades a lo largo del Golfo de México. Esta especie de parásito se caracteriza por tener un cuerpo alargado, ciegos en forma de saco, un útero que se extiende al primer tercio del cuerpo y folículos vitelinos longitudinalmente alargados alcanzando la parte posterior del cuerpo. Se generaron secuencias de la subunidad mayor (LSU por sus siglas en inglés) del ADN ribosomal (dominios D1–D3) y del espaciador transcrito interno (ITS2), los cuales fueron utilizados independientemente y combinado de ambos (LSU + ITS2) con el objetivo de contrastar la reconstrucción filogenética con su diferenciación morfológica entre *S. olmecae* n. sp., *S. chauhani* y *S. lamothei*, los últimos dos parásitos de peces dulceacuícolas y salobres distribuidos en Norteamérica. La divergencia genética estimada utilizando las distancias “p” sin corregir entre las tres especies de *Saccocoelioides* es baja: 1% para LSU y de 1 a 4% para ITS2. Los análisis filogenéticos inferidos con los métodos de Máxima Verosimilitud (ML) e Inferencia Bayesiana (BI) para cada base de datos y el combinado (LSU + ITS2) reveló que *S. olmecae* n. sp. representa un clado independiente con apoyo de bootstrap y probabilidades posteriores moderado. En un segundo manuscrito se analizó la posición sistemática de *Culuwiya cichlidorum* Aguirre-Macedo y Scholz, 2005 debido que en un inicio había sido identificada como *Saccocoelioides* y posteriormente transferida al género *Culuwiya*. Se utilizaron datos morfológicos y secuencias parciales de dos genes nucleares (LSU e ITS2). En total, se analizaron 62 especímenes correspondientes con 4 especies de *Saccocoelioides* (41 de *Saccocoelioides sogandaresi*, tres de *Saccocoelioides chauhani*, seis de *Saccocoelioides lamothei*, y 12 de *Saccocoelioides olmecae*), más 12 especímenes de *Culuwiya cichlidorum* de cuatro localidades en Nicaragua y Costa Rica. Los análisis filogenéticos inferidos con ML y BI mostraron que cuatro especies de *Saccocoelioides* formaron 4 sub-clados que se corresponden con las especies descritas previamente. Además, los siete ejemplares identificados como *Culuwiya cichlidorum*

secuenciados de la localidad y huésped tipo no se recuperaron como grupo monofilético, incluyéndose en dos sub-clados, el primero con especímenes de *C. cichlidorum* de Costa Rica y el segundo con especímenes de *S. sogandaresi* de México, Honduras, Nicaragua y Costa Rica. Con base en nuestros resultados, se propone que la especie *C. cichlidorum* sea reasignada dentro del género *Saccocoelioides*, nombrada *Saccocoelioides cichlidorum* (Aguirre-Macedo y Scholz, 2005) n. comb., cuya distribución se restringe a Nicaragua y Costa Rica; asociada con peces de la familia Cichlidae. Por otra parte, los especímenes identificados como *Culuwiya cichlidorum* de las costas del Golfo de México por Aguirre-Macedo y Scholz, (2005) pertenecen a *S. sogandaresi*, el cual tiene una amplia distribución y está asociado con peces de las familias Cichlidae y Poeciliidae. Nuevos datos morfológicos del tegumento del cuerpo mostraron un patrón de espinación característico que puede ser útil para distinguir entre *S. cichlidorum* n. comb. y *S. sogandaresi*.

II. ABSTRACT

In the present Master's thesis, we studied some species of genera *Saccocoelioides* parasites of freshwater and estuarine fishes in different localities from Mexico and Middle America. Two manuscripts were published as a result of the study. In the first paper, *Saccocoelioides olmecae* n. sp. is described from specimens recovered from the intestine of the fat sleeper *Dormitator maculatus* (Bloch) (Perciformes: Eleotridae) collected in six localities along the coast of the Gulf of Mexico. The new species is distinguished by having an elongated body, a sac-like caecum, an uterus that extends to the first third of body and vitelline follicles, longitudinally elongated reaching the posterior end of the body. Sequences of the large subunit (LSU) of the ribosomal DNA, including the domain D1–D3, and the internal transcribed spacer (ITS2) were used independently and in a concatenated analysis to corroborate the morphological distinction among *S. olmecae* n. sp., *S. chauhani* and *S. lamothei* as parasites of freshwater and brackish-water fish across North-America. The genetic divergence estimated using uncorrected “p” distances among the three species of *Saccocoelioides* was very low: 1% for LSU and from 1 to 4% for ITS2. Maximum likelihood and Bayesian inference analyses for each dataset and both datasets combined revealed that *S. olmecae* n. sp. represents an independent clade with moderate bootstrap support and posterior probabilities. In the second paper, we investigated the systematic position of *Culuwiya cichlidorum* Aguirre-Macedo and Scholz, 2005 due to initially was identified as *Saccocoelioides* and later transfer as *Culuwiya*. New morphological data and partial sequences of two nuclear genes (LSU and ITS2) were used in the analyses. In total, 62 specimens representing four species of *Saccocoelioides* (41 of *Saccocoelioides sogandaresi*, three of *Saccocoelioides chauhani*, six of *Saccocoelioides lamothei*, and 12 of *Saccocoelioides olmecae*), plus 12 specimens of *Culuwiya cichlidorum* in four localities of Nicaragua and Costa Rica were analyzed. The phylogenetic analyses inferred with ML and BI showed that the four species of *Saccocoelioides* formed a clade divided into 4 sub clades representing each species. In addition, seven specimens sequenced from the type host and locality of *Culuwiya cichlidorum* did not recover as monophyletic, and they were include in two clades, the first clade with specimens of *C. cichlidorum* from Costa Rica and the second clade with specimens of *S. sogandaresi* from Mexico, Honduras, Nicaragua and Costa Rica. According to our data, *C. cichlidorum*, should be reallocated into

Saccocoelioides. *Saccocoelioides cichlidorum* (Aguirre-Macedo and Scholz, 2005) n. comb., is restricted to Nicaragua and Costa Rica and is associated with cichlid fishes. In contrast, the specimens identified previously as *Culuwiya cichlidorum* from the coast of Gulf of Mexico by Aguirre-Macedo and Scholz, (2005) belong to *S. sogandaresi*, which has a wide distribution and is associated with poeciliid and cichlid fishes. New morphological data of the body surface show a characteristic pattern of spination that is useful to distinguish between of *S. cichlidorum* n. comb. and *S. sogandaresi*.

III. INTRODUCCIÓN

III. I. Taxonomía integrativa

La taxonomía es una disciplina de la biología que clasifica, identifica, describe y nombra las especies vivas y extintas (Padial et al., 2010). La especie es considerada como la unidad básica en los estudios de biodiversidad y conservación; y es definida como una entidad dinámica (Valdecasas et al., 2013; Sukumaran y Gopalakrishnan, 2015). Miles de especies han sido nombradas desde que Linneo (1758) propuso el sistema binominal de nomenclatura actual. No obstante, se han propuesto diferentes conceptos sobre la definición de especie, lo que ha generado controversias sobre la delimitación de las mismas (Sites y Marshall, 2004).

La delimitación de especies en la actualidad requiere de una combinación de diferentes caracteres como: ecológicos, moleculares, biogeográficos, evolutivos entre otros, así como de los caracteres morfológicos. El empleo de estos criterios de información en la delimitación de especies es conocido como taxonomía integrativa (Schlick-Steiner et al., 2010).

En el caso particular de los helmintos (parásitos vermiformes), la clasificación de las especies está sustentada en su mayoría por la taxonomía alfa. Sin embargo, la incertidumbre sobre la validez de los caracteres morfológicos ha generado cuestionamientos sobre éstos, dificultando su identificación a nivel de especie. En años recientes se han descrito especies de helmintos empleando nuevas herramientas por ejemplo, datos moleculares (Blasco-Costa et al., 2009a, Pinacho-Pinacho et al., 2012; García-Varela et al., 2016, Tkach et al., 2016), siendo fundamentales y complementarios para llevar a cabo la descripción y delimitación de especies, es decir, empleando taxonomía integrativa.

III. II. Marcadores moleculares en digeneos

Los genes ribosomales del ADN están formados por las regiones codificantes (18S, 5.8S, y 28S), regiones no codificantes (ITS1 e ITS2) y una región no transcrita (NTS). En conjunto a estos genes se les conoce como unidad de transcripción las cuales se encuentran en repeticiones tándem a lo largo de todo el genoma y evolucionan concertadamente. Esto se debe a eventos de recombinación que homogeniza las copias (Fig. 1) (Wei et al., 2006;

Eickbush y Eickbush, 2007). Las regiones codificantes al ser conservadas pueden ser usadas para inferir relaciones filogenéticas a nivel supraespecífico, mientras que las regiones no codificantes son más variables y pueden ser útiles para establecer relaciones entre géneros, e incluso especies (Nolan y Cribb, 2005).

Particularmente, los genes del 28S e ITS2 del ADN ribosomal han sido utilizados dentro de los digeneos para delimitar y describir especies (Anderson y Barker, 1998; Bell et al., 2001). Se ha demostrado que en la familia Haploporidae Nicoll, 1914 (Digenea) estos dos marcadores moleculares tienen variación suficiente para delimitar a las especies. A partir de estos marcadores, las relaciones filogenéticas dentro de la familia se conocen, además han sido utilizados para delimitar las subfamilias así como erigir nuevos géneros, y han permitido la descripción de nuevas especies (Blasco-Costa et al., 2009b; Pulis et al., 2013; Andres et al., 2015; Atopkin et al., 2015).

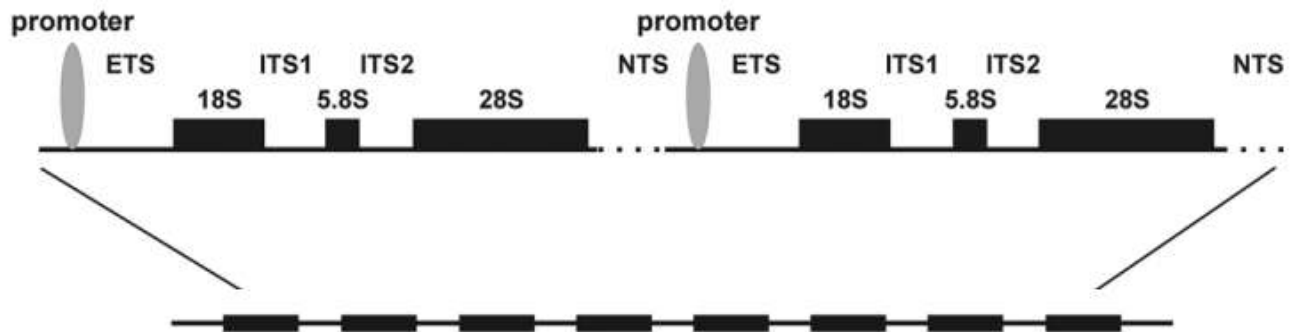


Fig. 1 Unidad de transcripción del ADN ribosomal en Eucarionotes. Designaciones: 18S, 5.8S, y 28S, genes del ADN ribosomal; NTS, espaciador no transcrito; ETS, espaciador transcrito externo; ITS1 e ITS2, espaciadores transcritos internos; ovalo gris, señala el promotor de la ARN polimerasa I. (Modificado de Mukha et al., 2011).

III. III. Características de *Saccocoelioides* Szidat, 1954 (Haploporidae: Digenea)

Saccocoelioides Szidat, 1954 es un género de endoparásitos que consta de 17 especies, los cuales parasitan el intestino de peces dulceacuícolas y en ocasiones peces estuarinos. Las especies del género se distribuyen únicamente en el Continente Americano. Taxonómicamente, el género *Saccocoelioides* se encuentra dentro de la subfamilia Chalcinotrematinae Overstreet y Curran, 2005 que a su vez está ubicada en la familia Haploporidae Nicoll, 1914. Esta familia se caracteriza morfológicamente por poseer un saco hermafrodita y un solo testículo (Overstreet y Curran, 2005).

Las especies del género *Saccocoelioides* son digeneos pequeños que miden entre 300 µm a 1200 µm; éstos se caracterizan principalmente por tener un cuerpo alargado a fusiforme, glándulas vitelógenas que rodean al testículo, con ciegos cortos y la presencia de manchas oculares (Fig. 2). En la revisión taxonómica más reciente de la familia, Overstreet y Curran (2005) erigieron un nuevo género: *Culuwiya* Overstreet y Curran, 2005 para acomodar cuatro especies previamente descritas como *Saccocoelioides* de peces principalmente del género *Mugil* y *Agonostomus* (mugílidos) y de *Sicydium plumieri* Bloch 1786 (góbido). Además de una quinta especie que había sido descrita previamente como *Carassotrema tilapiae* Nasir y Gómez, 1976 de peces de la familia Cichlidae. En ese mismo año, Aguirre-Macedo y Scholz (2005), describieron una nueva especie de *Culuwiya*: *C. cichlidorum* de peces dulceacuícolas de la familia Cichlidae de Nicaragua y México la cual previamente había sido reportada como un *Saccocoelioides* sp. (Aguirre-Macedo et al., 2001). Con un total de 6 especies dentro del género *Culuwiya*, éste fue clasificado dentro de la subfamilia Waretrematinae Svrstava, 1937 la cual parasita principalmente peces marinos y estuarinos.

El único carácter morfológico diagnóstico que diferencia al género *Saccocoelioides* del género *Culuwiya* es la distribución del útero (Overstreet y Curran, 2005). Por ejemplo, *Saccocoelioides* presenta un útero que se distribuye desde el nivel del esófago a la zona posterior del testículo. Mientras que el género *Culuwiya*, se extiende solamente entre el saco hermafrodita y llega a nivel testicular (Overstreet y Curran, 2005). En este sentido, el carácter antes mencionado debería ponerse a prueba con otras fuentes de evidencia para constatar la validez de ambos géneros.

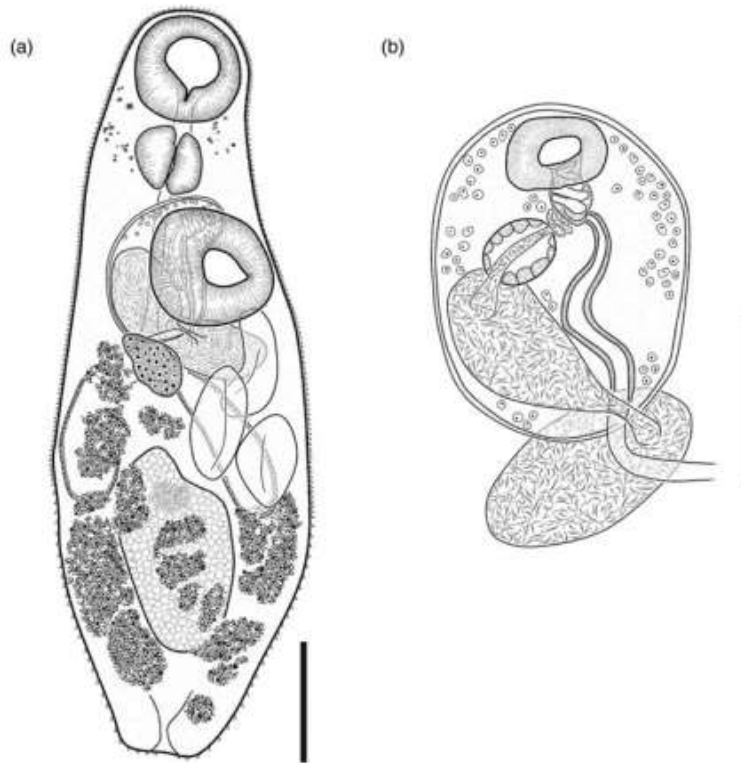


Fig. 2 a) Esquema de *Saccocoelioides olmecae* de *Dormitator maculatus* escala= 100 μ b) Saco hermafrodita (Tomado de Andrade-Gómez et al., 2016) escala= 50 μ .

III. IV. Ciclo de vida de *Saccocoelioides tarpazensis* Díaz y González, 1990

El ciclo de vida de *Saccocoelioides tarpazensis* se obtuvo experimentalmente por Díaz y González, 1990. Los parásitos adultos maduran en el intestino de peces donde se lleva a cabo la reproducción. Una vez que se forman los huevos, son liberados a través de las heces de los peces al medio acuático. Los miracidios eclosionan del huevo y son probablemente ingeridos por el huésped intermediario (gasterópodo acuático de la familia Physidae). Dentro de la glándula digestiva del caracol, se desarrolla la redia y por medio de la reproducción asexual darán origen entre 7-17 cercarias de diferentes etapas de desarrollo. Cuando las cercarias gimnocéfalas están desarrolladas, son liberadas al medio acuático en donde se enquistan (metacercaria) preferentemente en la superficie del agua. El ciclo de vida es completado cuando las metacercarias son ingeridas por el huésped definitivo donde llega al intestino y tarda aproximadamente 20 días en desarrollar la forma adulta (Díaz y González, 1990).

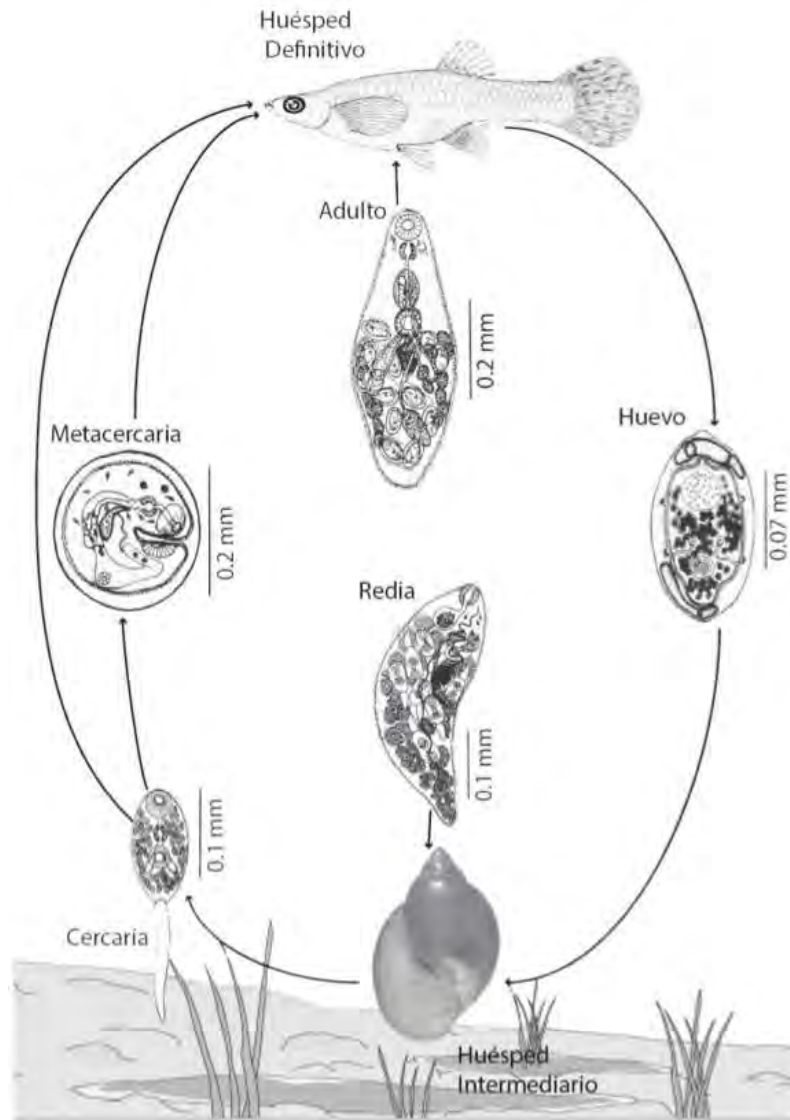


Fig. 3 Ciclo de vida de *Saccocoelioides tarpazensis* (Modificado de Díaz y González, 1990.)

III. V. Registros de *Saccocoelioides* en Norte América

Hasta el 2008, se habían registrado 17 especies del género *Saccocoelioides*, 14 de ellas reportadas en América del Sur y tres especies en Centro América y Norte América. Dos de estas tres especies fueron descritas en México como parásitos de peces dulceacuícolas y estuarinos: *Saccocoelioides chauhani* Lamothe-Argumedo, 1974 del carácido *Astyanax aeneus* (Günther, 1860) y *Saccocoelioides lamothei* Aguirre-Macedo y Violante-González, 2008 del eleótrido *Dormitator latifrons* (Richardson, 1844). La tercera especie, *Saccocoelioides sogandaresi* Lumsden, 1963 fue descrita en Estados Unidos del poeciliido *Poecilia latipinna* (Lesueur, 1821), sin embargo ha sido reportada ampliamente en México en distintas familias de peces (Poeciliidae, Gobiidae, Goodeidae, Mugilidae) (Scholz et al., 1995; Salgado-Maldonado et al., 2005; Pérez-Ponce de León et al., 2007; Martínez-Aquino et al., 2014).

La compleja morfología del género ha derivado en determinaciones taxonómicas dudosas o confusas, por ejemplo algunos registros de *S. sogandaresi* han sido reportados como *Saccocoelioides* c. f. *sogandaresi* (Salgado-Maldonado et al., 2004; Pérez-Ponce de León et al., 2013), y esto se debe al tamaño que poseen ya que oscila entre 300-500 µm, dificultando la observación de los caracteres diagnósticos de la especie, y generando identificaciones inciertas. Por otro lado, la descripción de *C. cichlidorum* (Aguirre-Macedo y Scholz, 2005) del cíclido *Vieja maculicauda* (Regan, 1905) de Nicaragua es otro caso de confusión dentro de la familia Haploporidae, debido a la morfología parecida a la de un *Saccocoelioides*. Tal es la similitud que en un inicio esta especie había sido reportada como *Saccocoelioides* sp. (Aguirre-Macedo et al., 2001) y posteriormente descrita como *Culuwiya*, en el mismo año que el género *Culuwiya* fue erigido. En sí, el género *Culuwiya* posee caracteres que se asemejan a los del género *Saccocoelioides* siendo un solo carácter morfológico lo que diferencia ambos géneros, el cual es la distribución del útero. En este sentido, la identidad de la especie *C. cichlidorum*, así como algunos registros de *S. sogandaresi* deben ponerse a prueba empleando taxonomía integrativa para corroborar su validez taxonómica.

IV. OBJETIVOS

Objetivo General.

- Determinar molecular y morfológicamente a las especies del género *Saccocoelioides* que se distribuyen en México y Centro América asociadas a diferentes familias de peces.

Objetivos Particulares.

- Proponer una hipótesis filogenética de las especies de *Saccocoelioides* distribuidas en México y Centro América utilizando marcadores moleculares nucleares.
- Realizar la descripción y comparación morfológica de las especies del género *Saccocoelioides* asociados a distintas familias peces.
- Estimar la diversidad de especies de *Saccocoelioides* que existen en México y Centro América.
- Probar la posición taxonómica de la especie *Culuwiya cichlidorum* asociada a peces cíclidos de Centro América y México.

V. RESULTADOS

Los resultados de este estudio se presentan en forma de dos artículos publicados. El primero de ellos en la revista *Journal of Helminthology* y el segundo en el *Journal of Parasitology*.

V. I. PUBLICACIÓN 1

Primer artículo publicado en el *Journal of Helminthology*.

RH: L. Andrade-Gómez et al. A new species of *Saccocoelioides* from the Gulf of Mexico

Morphological and molecular analyses of a new species of *Saccocoelioides* Szidat, 1954 (Haploporidae Nicoll, 1914) in the fat sleeper *Dormitator maculatus* (Bloch) (Perciformes: Eleotridae) from the Gulf of Mexico

Leopoldo Andrade-Gómez¹, Carlos Daniel Pinacho-Pinacho¹, Jesús Servando Hernández-Orts², Ana Lucía Sereno-Uribe³ and Martín García Varela^{3*}.

¹Posgrado en Ciencias Biológicas, Instituto de Biología, Universidad Nacional Autónoma de México, A. P. 70-153, C.P. 04510, Ciudad de México, México: ²Instituto de Biología Marina y Pesquera Almirante Storni, Universidad Nacional del Comahue, Güemes 1030, 8520 San Antonio Oeste, Río Negro, Argentina: ³Departamento de Zoología, Instituto de Biología, Universidad Nacional Autónoma de México, A. P. 70-153, C.P. 04510, Ciudad de México.

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Corresponding author:

Martín García Varela Departamento de Zoología, Instituto de Biología, UNAM, 04510. México D.F., México. Email: garciaiv@ibiologia.unam.mx Phone:(525)56229130
Fax: (525) 5550 0164

Morphological and molecular analyses of a new species of *Saccocoelioides* Szidat, 1954 (Haploporidae Nicoll, 1914) in the fat sleeper *Dormitator maculatus* (Bloch) (Perciformes: Eleotridae) from the Gulf of Mexico

L. Andrade-Gómez¹, C.D. Pinacho-Pinacho¹, J.S. Hernández-Orts²,
A.L. Sereno-Uribe³ and M. García-Varela^{3*}

¹Posgrado en Ciencias Biológicas, Instituto de Biología, Universidad Nacional Autónoma de México, A. P. 70-153, C.P. 04510, Ciudad de México; ²Instituto de Biología Marina y Pesquera Almirante Storni, Universidad Nacional del Comahue, Güemes 1030, 8520 San Antonio Oeste, Río Negro, Argentina; ³Departamento de Zoología, Instituto de Biología, Universidad Nacional Autónoma de México, A. P. 70-153, C.P. 04510, Ciudad de México

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Abstract

Saccocoelioides olmecae n. sp. is described from specimens recovered from the intestine of the fat sleeper *Dormitator maculatus* (Bloch) (Perciformes: Eleotridae) collected in six localities along the coast of the Gulf of Mexico. The new species is mainly distinguished from the other three described species of *Saccocoelioides* Szidat, 1954 from North and Middle America (i.e. *S. sogandaresi* Lumsden, 1963, *S. chauhani* Lamothe-Argumedo, 1974 and *S. lamothei* Aguirre-Macedo & Violante-González, 2008) by having an elongated body, a sac-like caecum, a uterus that extends to the first third of body and by having vitelline follicles longitudinally elongated reaching the posterior end of the body. Sequences of the large subunit (LSU) of the ribosomal DNA, including the domain D1–D3, and the internal transcribed spacer 2 (ITS2) were used independently and concatenated to corroborate the morphological distinction among *S. olmecae* n. sp., *S. chauhani* and *S. lamothei* from freshwater and brackish-water fish from Middle America. The genetic divergence estimated among the three species of *Saccocoelioides* was very low: 1% for LSU and from 1 to 4% for ITS2. Maximum likelihood and Bayesian inference analyses for each dataset and both datasets combined revealed that *S. olmecae* n. sp. represents an independent clade with moderate bootstrap support and posterior probabilities. This is the third species of *Saccocoelioides* described in Mexico, and the 17th species from the Americas.

*E-mail: garciav@ib.unam.mx

Introduction

Haploporidae Nicoll, 1914 is a family of trematode endoparasites of fish distributed worldwide. This family is characterized morphologically by the presence of a hermaphroditic sac and a single testis. In the recent taxonomic revision of the family, Overstreet & Curran (2005) erected the subfamily Chalcinotrematinae Overstreet & Curran, 2005 to accommodate the genera *Chalcinotrema* Freitas, 1947, *Paralecithobotrys* Freitas, 1948, *Saccocoelioides* Szidat, 1954, *Megacoelium* Szidat, 1954 and *Unicoelium* Thatcher & Dossman, 1975.

Species of the genus *Saccocoelioides* are parasites of freshwater and brackish-water fishes from the Americas (Lumsden, 1963; Travassos *et al.*, 1969; Szidat, 1970, 1973; Lamothe-Argumedo, 1974). The taxonomic history, as well as the species composition of this genus, have been controversial (see Yamaguti, 1958). In the last review of Haploporidae, Overstreet & Curran (2005) validated the genus *Saccocoelioides*, and transferred it to the subfamily Chalcinotrematinae. In North and Middle America three species of *Saccocoelioides* have been found: *S. sogandaresi* Lumsden, 1963 was described from *Poecilia latipinna* Lesueur from Texas, USA (Lumsden, 1963). In Mexico, *S. sogandaresi* has been recorded in poeciliids, characids, cichlids, gobiids, eleotrids and goodeids (Scholz *et al.*, 1995; Salgado-Maldonado *et al.*, 2005; Pérez-Ponce de León *et al.*, 2007; Martínez-Aquino *et al.*, 2014). *Saccocoelioides chauhani* Lamothe-Argumedo, 1974 was described from the characid fish *Astyanax aeneus* Günther and it has also been recorded in poeciliids from Catemaco Lake, Veracruz, Mexico (Jiménez-García, 1996; Pérez-Ponce de León *et al.*, 2007). Finally, *S. lamothei* Aguirre-Macedo & Violante-González, 2008 was described from the eleotrid fish *Dormitator latifrons* Richardson from Tres Palos Lagoon, Guerrero, Mexico (Aguirre-Macedo & Violante-González, 2008), and it has been recorded in other fishes such as gobiids, poeciliids and profundulids (Andrade-Gómez, 2015; Pinacho-Pinacho *et al.*, 2015).

In the present study, we collected haploporids from the fat sleeper *Dormitator maculatus* (Bloch) (Perciformes: Eleotridae) in six localities along the coast of the Gulf of Mexico. Additionally, adults of *S. chauhani* and *S. lamothei* were also collected from their type host and type locality. The morphological examination of the specimens from *D. maculatus* in combination with information from two nuclear molecular markers suggests that these trematodes represent a new species of *Saccocoelioides*. The new species is described herein. In addition, new descriptions of *S. chauhani* and *S. lamothei* are also provided.

Materials and methods

Sample collection

Haploporids were collected from the intestines of four fish species from nine localities in Mexico (see fig. 1): *Dormitator latifrons* from (1) Tres Palos Lagoon, Guerrero, type host and type locality of *S. lamothei* (16°48'0"N, 99°47'0"W), (2) Chacahua Lagoon, Oaxaca (15°58'5"N, 97°40'55"W); *Astyanax aeneus* from (3) Catemaco Lake, Veracruz, type host and type locality of *S. chauhani*

(18°25'0"N, 95°7'0"W); *Dormitator maculatus* from (4) El Milagro Lagoon, Campeche (18°52'25"N, 91°24'39"W), (5) Boca del Río, Veracruz (19°6'29"N, 96°6'46"W), (6) Tamiahua Lagoon, Veracruz (21°15'49"N, 97°27'41"W), (7) Tlacotalpan, Veracruz (18°36'0"N, 95°39'0"W), (8) La Palma River, Veracruz (18°33'21"N, 95°2'59"W) and (9) El Corchito, Progreso, Yucatan (21°16'40"N, 89°38'38"W) where *Gambusia yucatanana* was also collected. Fish were captured with seine nets or electrofished, transported alive to the laboratory and identified following Miller *et al.* (2005). Individual fish were euthanized by pithing and immediately examined for helminths. Trematodes were fixed in hot (steaming) 4% formalin or 100% ethanol for DNA analyses.

Morphological description

Unflattened digeneans preserved in 4% formalin were stained with Mayer's paracarmine or iron acetocarmine, dehydrated in an ethanol series, cleared with methyl salicylate and mounted in Canada balsam. All the specimens were examined using a bright-field Leica DM 1000 LED microscope (Leica, Wetzlar, Germany). Morphometric measurements were taken using the Leica Application Suite microscope software. Drawings were made with the aid of a drawing tube. All measurements are in micrometres.

Specimens collected in the present study were compared with the original description and with the holotype and paratypes of *S. lamothei* deposited in the Colección Nacional de Helminths (CNHE), Instituto de Biología, Universidad Nacional Autónoma de México, Ciudad de México, México (holotype: CNHE 5920; paratypes: CNHE 5921a, 5921b). Type material of *S. chauhani* could not be examined since they were not available in the CNHE. Voucher specimens from the present study were deposited in the CNHE: *S. lamothei* from *D. latifrons*, 4 specimens, CNHE 9373; *S. chauhani* from *A. aeneus*, 5 specimens, CNHE 9852.

DNA extraction, sequencing and phylogenetic analyses

For DNA extraction, specimens were placed individually in tubes and digested overnight at 56°C in a solution containing 10 mM Tris-HCl (pH 7.6), 20 mM NaCl, 100 mM disodium EDTA (pH 8.0), 1% Sarkosyl and 0.1 mg/ml proteinase K. Following digestion, DNA was extracted from the supernatant using the DNAzol reagent (Molecular Research Center, Cincinnati, Ohio, USA) following the DNA protocol established by García-Varela & Nadler (2005). Internal transcribed spacer 2 (ITS2) and the D1-D3 domains of the large subunit (LSU) from the ribosomal DNA (rDNA) were amplified using the forward primer BD3, 5'-GAA CAT CGA CAT CTT GAA CG-3' (Hernández-Mena *et al.*, 2014), and the reverse primer 536, 5'-CAG CTA TCC TGA GGG AAAC-3' (García-Varela & Nadler, 2005). Polymerase chain reactions (PCR) (25 µl) consisted of 1 µl of 10 µM of each primer, 2.5 µl of 10 × PCR Rxn buffer, 1.5 µl 2 mM MgCl₂, 0.5 µl of deoxynucleoside triphosphates (dNTPs) 10 mM, 16.375 µl of water, 2 µl of genomic DNA and 0.125 µl (1 U) of *Taq* DNA polymerase (Platinum *Taq*, Invitrogen Corporation, São Paulo, Brazil). PCR cycling parameters for rDNA amplifications included denaturation at 94°C for 1 min; followed by 35 cycles of 94°C for 1 min, annealing at 50°C for 1 min and

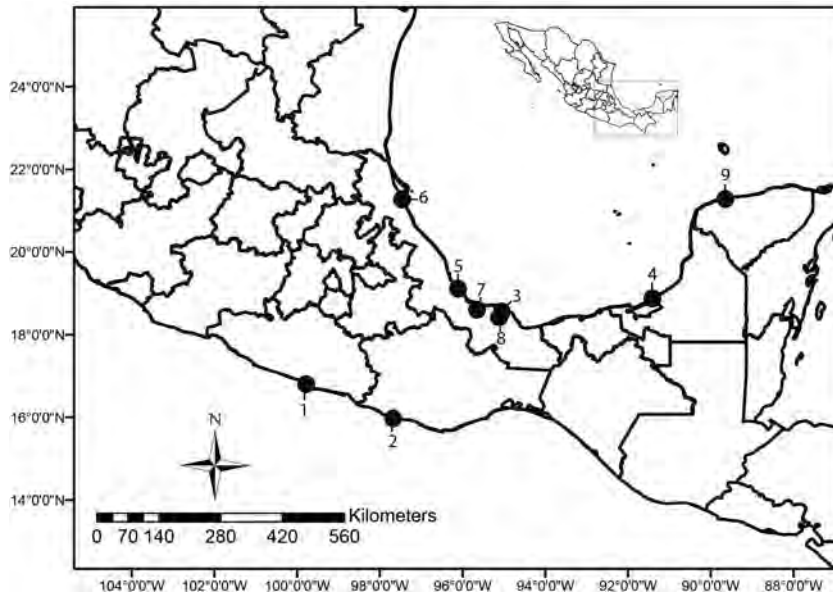


Fig. 1. Sampling sites in Mexico for specimens of *Saccocoelioides* spp. Legend for localities: 1, Tres Palos Lagoon, Guerrero; 2, Chachagua Lagoon, Oaxaca; 3, Catemaco Lake, Veracruz; 4, El Milagro Lagoon, Campeche; 5, Boca del Río, Veracruz; 6, Tamiagua Lagoon, Veracruz; 7, Tlacotalpan, Veracruz; 8, La Palma River, Veracruz; 9, El Corchito Progreso, Yucatan.

extension at 72°C for 1 min; followed by a post-amplification incubation at 72°C for 10 min. Sequencing reactions were performed using the forward and reverse primers mentioned above and four internal primers: 504 (5'-CGT CTT GAA ACA CGG ACT AAG G-3'), 502 (5'-CAA GTA CCG TGA GGG AAA GTT GC-3') (García-Varela & Nadler, 2005), 503 (5'-CCT TGG TCC GTG TTT CAA GAC G-3') (Stock *et al.*, 2001) and BD2 (5'-TAT GCT TAA ATT CAG CGG GT-3') (Luton *et al.*, 1992), with ABI Big Dye (Applied Biosystems, Boston, Massachusetts, USA) terminator sequencing chemistry, and reaction products were separated and detected using an ABI 3730 capillary DNA sequencer. The electropherograms of each sequence were assembled conforming a consensus sequence, and base-calling differences were resolved using Codoncode Aligner version 5.0.2 (Codoncode Corporation, Dedham, Massachusetts, USA).

Sequences obtained in the current research from LSU rDNA and ITS2 were aligned separately with data from another 32 haploporid species downloaded from the GenBank dataset, including a sequence of *Saccocoelioides* sp. from Nicaragua (see Curran *et al.*, 2006), plus other species from three families that were used as the outgroup (see supplementary table S1). The alignment consisted of 58 sequences with 1275 nucleotides for the LSU rDNA and 43 sequences with 417 nucleotides for the ITS2. The concatenated alignment contained 43 sequences with 1687 nucleotides. Alignments were constructed using the software Clustal W (Thompson *et al.*, 1997) with default parameters and adjusted manually with the Mesquite program (Maddison & Maddison, 2011). The best model of nucleotide substitution for each dataset was identified with the Akaike Information Criterion (AIC) implemented in jModelTest v0.1.1 (Posada, 2008). For ML analyses, the program RAxML v.7.0.4 (Stamatakis, 2006)

was used with the option GTRGAMMAI and with 10,000 bootstrap replicates. The best model for each dataset was TVM+I+G for ITS2 and GTR+I+G for LSU, which were used with the Bayesian analyses (Huelsenbeck & Ronquist, 2001). Settings were two simultaneous runs of the Markov chain (MCMC) for 10 million generations, sampling every 1000 generations, a heating parameter value of 0.2 and a 'burn-in' of 25%. Trees were drawn using FigTree version 1.3.1 (Rambaut, 2006). The genetic divergence among taxa was estimated using uncorrected *p* distances with the program MEGA version 6 (Tamura *et al.*, 2013).

Results

Family: Haploporidae Nicoll, 1914.

Subfamily: Chalcinotrematinae Overstreet & Curran, 2005.

Genus: *Saccocoelioides* Szidat, 1954.

Saccocoelioides lamothei

Description

The specimens identified as *S. lamothei* collected from *D. latifrons* on Chachagua Lagoon (see fig. 1, locality 2; table 1) are similar to the type material of *S. lamothei* by having a pyriform to slightly elongated body with numerous eggs (10–11), the tegument with fine spines. Eye-spot remnants dispersed from oral opening to level of anterior margin of hermaphroditic sac. Oral sucker subterminal spherical. Ventral sucker spherical pre-equatorial. An absent prepharynx, pharynx oval, strongly muscular. Caecum sac-like, thick-walled, ending at mid-level of testis in unflattened specimens. Testis single, oval to spherical,

Table 1. Comparative morphometric data for species of *Saccocoelioides* from North and Middle America. Data of the holotype of *S. lamothei* in parenthesis (CNHE 5920).

Species	<i>S. olmecae</i> n. sp.	<i>S. sogandaresi</i> Lumsden, 1963	<i>S. chauhani</i> Lamothe- Argumedo, 1974	<i>S. chauhani</i> This study	<i>S. lamothei</i> Aguirre- Macedo & Violante- González, 2008	<i>S. lamothei</i> This study
No. specimens examined	14	10	2	5	12	4
Body length	340–527	341–512	536–966	574–672	420–850 (670)	560–850
Body width	150–250	165–307	289–418	198–224	240–510 (328)	280–460
Oral sucker length	50–80	55–103	108–112	70–77	62–155 (66)	65–95
Oral sucker width	55–84	60–87	108–112	82–93	77–127 (80)	88–102
Ventral sucker length	55–90	62–105	101–105	74–81	71–112 (97)	110
Ventral sucker width	60–94	75–103	86–108	81–87	82–107 (75)	100–102
Ventral sucker position	Pre-equatorial	Pre-equatorial	Pre-equatorial	Pre-equatorial	Pre-equatorial	Pre-equatorial
Prepharynx length	13–35	1–12	18–26	17–29	8–12 (12)	Not observed
Pharynx length	40–53	45–73	48–60	42–56	52–112 (62)	34–100
Pharynx width	49–73	37–70	45–52	45–56	55–97 (75)	85–120
Distance from anterior end to caecum bifurcation	221–242	172 ^a	218–405	246–297	157–270 (270)	201–287
Maximum extension of caecum	First third of testis	Posterior third of body	Pre-testicular	Pre-testicular or first third of testis	Pre-testicular	Middle level of testis
Genital pore position	Anterior to ventral sucker	Between pharynx and ventral sucker	Anterior to ventral sucker	Anterior to ventral sucker	Between pharynx and ventral sucker	Anterior to ventral sucker
Hermaphroditic sac length	81–117	45–98	112–128	77–105	100–215 (100)	90–223
Hermaphroditic sac width	57–85	50–84	75–82	52–74	60–130 (100)	72–125
Hermaphroditic sac position	Dorsal to ventral sucker	Between pharynx and ventral sucker	Between pharynx and ventral sucker	Between midlevel of oesophagus and ventral sucker	Between pharynx and ventral sucker	Between pharynx and ventral sucker
External seminal vesicle length	30–75	38–83 ^a	48	35–47	40–70 (not observed)	55–162
External seminal vesicle width	32–75	28–48 ^a	33–37	28–51	40–55 (not observed)	31–117
Testis length	72–140	95 ^a	112–120	93–116	90–152 (109)	85–132
Testis width	70–97	75–112	75–180	58–94	67–137 (80)	77–137
Ovary length	22–40	41–62	45–82	52–66	42–62 (62)	62
Ovary width	20–35	41–62	52–75	32–40	25–42 (38)	52
Ovary position	Post-equatorial or equatorial	Middle level of body	Post-equatorial	Post-equatorial	Post-equatorial	Middle level of body
Seminal receptacle length	64–224	–	–	47–101	30–45 (37)	90–121
Seminal receptacle width	21–62	–	–	23–31	37–52 (50)	29–58
Uterus	Between ventral sucker and first third of testis	Mostly in hind-body	Between ventral sucker and testis	Between ventral sucker and testis level or ovary level	Occupying ventral sucker to testicular level	Occupying ventral sucker level to post-testicular level
Egg length	60–89	78–87	101–105	70–97	80–135 (115)	100–132
Egg width	28–47	37–55	56–63	50–68	50–78 (62)	45–75

Table 1. (Cont.)

Species	<i>S. olmeca</i> n. sp.	<i>S. sogandaresi</i> Lumsden, 1963	<i>S. chauhanii</i> Lamothe- Argumedo, 1974	<i>S. chauhanii</i> This study	<i>S. lamothei</i> Aguirre- Macedo & Violante- González, 2008	<i>S. lamothei</i> This study
Vitelline follicles position	From ovary to post-testicular region	From middle level of ventral sucker to post-testicular region	From posterior end of ventral sucker to posterior end of body	From ovary level to posterior end of testis or at testis level	From ovary to post-testicular region	From ovary to post-testicular region
Sucker length ratio	1:0.87–1.3	1.5 ^a	1:0.93	1:0.98–1.08	1:0.84–1.23 (1.4)	1:1.15
Sucker width ratio	1:0.94–1.28	1:1.2	1:0.96	1:0.87–1	1:0.88–1.075* (0.88)	1:0.88–1

^a Measured from the published figure. *Measured from holotype and paratype.

terminal (flattened specimens) or subterminal (unflattened). Hermaphroditic sac oval. Internal seminal vesicle oval, filling posterior third of hermaphroditic sac. Ejaculatory duct short, prostatic cells in peripheral region of hermaphroditic sac. Genital pore pre-equatorial. Ovary oval to cylindrical, post-equatorial between ventral sucker and the testis. Uterus extends posterior to the testis; Vitelline follicles irregular in size and shape, can reach terminal or subterminal part of the body (fig. 2a, b).

Remarks

The specimens of *S. lamothei* show morphological plasticity; for example, metrical data of newly collected material provided lower limits for pharynx length (34–100 this study vs. 52–112 original description) and external seminal vesicle width (31–117 vs. 40–55). Likewise, newly collected material provided higher limits for pharynx width (85–120 vs. 55–97), external seminal vesicle length (55–162 vs. 40–70) and seminal receptacle length (90–121 vs. 30–45) (see table 1).

Saccocoelioides chauhanii

Description

The haploporids identified as *S. chauhanii* collected from the intestine of *Astyanax aeneus* in Catemaco Lake (see fig. 1, locality 3; table 1) are similar to the type material of *S. chauhanii*, by having an elongated body, with spines. Eye-spot remnants dispersed from prepharynx level to hermaphroditic sac level, not represented in original description. Oral sucker subterminal, spherical. Ventral sucker spherical, pre-equatorial. Prepharynx present. Pharynx oval, muscular. Caecum sac-like, ending at level pretesticular or at first third of testis in some specimens. Testis spherical, subterminal, located in the hind body. External seminal vesicle small and spherical. Hermaphroditic sac oval, located at ventral sucker level. Internal seminal vesicle oval, prostatic cells in peripheral region of hermaphroditic sac. Genital pore pre-equatorial. Ovary cylindrical, post-equatorial half between ventral sucker and testis. Uterus between ventral sucker level and testis level or ovary level. Excretory vesicle Y-shaped, bifurcating at anterior level of testis. Miracidium not developed (fig. 2c, d). Vitelline follicles reaching posterior end of body in flattened organisms (original description), and subterminal in unflattened organisms at testis level (fig. 2c, d).

Remarks

The specimens of *S. chauhanii* from type locality and type host show morphological plasticity; for example, metrical data obtained for the newly collected material provided lower limits for body maximum width (198–224 this study vs. 289–418 original description), oral sucker length (70–77 vs. 108–112) and width (82–93 vs. 108–112), ventral sucker length (74–81 vs. 101–105), hermaphroditic sac length (77–105 vs. 112–128), testis length (93–116 vs. 112–120), ovary width (32–40 vs. 52–75) and egg length (70–97 vs. 101–105) (see table 1). Additionally, we also found higher limits on the range for the external seminal vesicle width (28–51 vs. 33–37) (table 1).

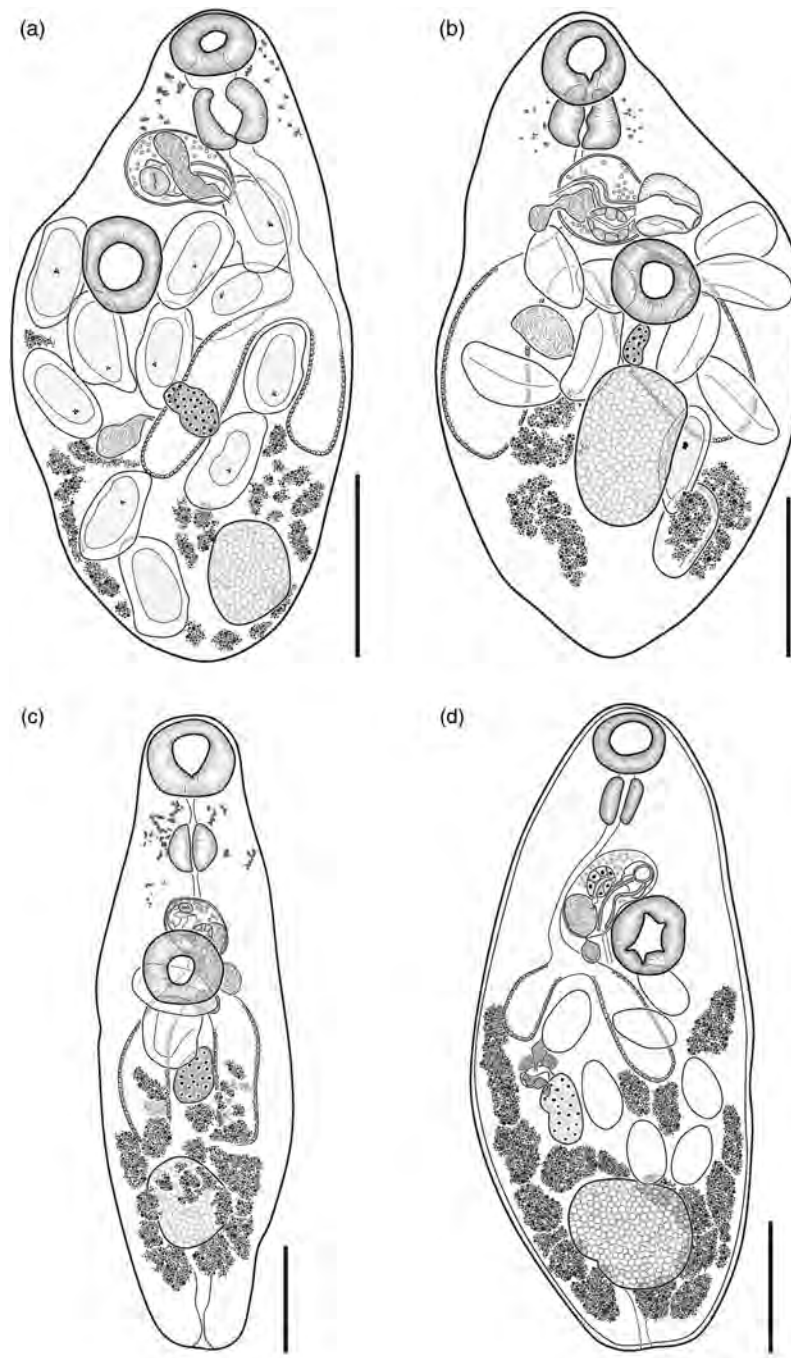


Fig. 2. *Saccocoelioides lamothei*: (a) holotype (CNHE 5920) from *Dormitator latifrons*, (b) specimen (CNHE 9373) from *D. latifrons*. *Saccocoelioides chauhani*: (c) specimen (CNHE 9852) from *Astyanax aeneus*, (d) specimen from *A. aeneus* (from Lamothe-Argumedo, 1974). Scale bars: (a, b, d) 200 μ m; (c) 100 μ m.

Saccocoelioides olmecae n. sp.

Description

Based on 14 specimens from the type locality (see table 1). Measurements from holotype. Body elongated

(fig. 3a), with maximum width at second third of body 527×200 . Tegument thin, armed with fine spines. Eyespot remnants dispersed from oral opening to level of anterior margin of hermaphroditic sac. Oral sucker spherical, subterminal 75×73 . Ventral sucker spherical, larger

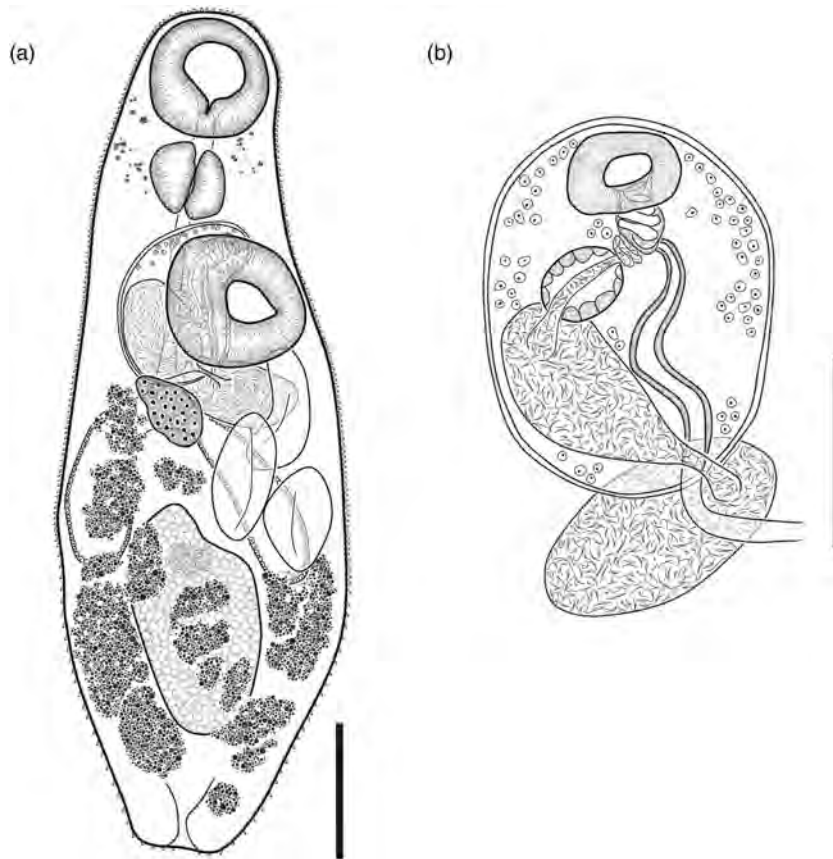


Fig. 3. *Saccocoelioides olmecae* n. sp. from *Dormitator maculatus*: (a) whole worm, holotype, ventral view; (b) hermaphroditic sac, paratype, ventral view. Scale bars: (a) 100 μ m; (b) 50 μ m.

than oral sucker, pre-equatorial, 90×94 ; sucker length ratio 1:1.2; sucker width ratio 1:1.28. Prepharynx short, 14. Pharynx oval, large, strongly muscular, 42×50 , shorter than oral sucker length. Oesophagus longer than pharynx 103, extending posterior to level of ventral sucker. Intestinal bifurcation at level of the ovary. Caecum sac-like, thick-walled, ending at level first third of testis. Testis single, oval to spherical, subterminal, located in the posterior third of the body. External seminal vesicle spherical to oval, dorsal to ventral sucker, larger than internal seminal vesicle. Hermaphroditic sac oval to ellipsoidal (fig. 3b), obliquely arched around ventral sucker dorsally, 117×82 . Internal seminal vesicle elongated-oval, filling posterior third to half of hermaphroditic sac length. Pars prostatic swollen, contiguous to the internal seminal vesicle. Ejaculatory duct and hermaphroditic duct are short and of similar length. Prostatic cells in peripheral region of hermaphroditic sac. Genital pore pre-equatorial, prominent, thick walled. Ovary elongated-oval, equatorial or post-equatorial 40×34 , contiguous or separated from testis. Uterus occupying from level of ventral sucker opening to hermaphroditic sac, extending to first third of testis, metraterm thick walled. Numerous vitelline follicles (16) usually longitudinally elongate, distributed in two lateral fields, from ovary level, confluent

to post-testicular level, reaching posterior end of the body. Eggs $72\text{--}89 \times 40\text{--}41$, miracidia not developed; at maximum 3 eggs in uterus from holotype. Excretory vesicle Y-shaped, excretory pore terminal.

Taxonomic summary

Type host. *Dormitator maculatus* (Bloch) (Perciformes: Eleotridae).

Accidental host. *Gambusia yucatana* Regan (Cyprinodontiformes: Poeciliidae) (see Discussion).

Site. Intestine.

Type locality. Boca del Río, Veracruz, Mexico ($19^{\circ}6'29''\text{N}$, $96^{\circ}6'46''\text{W}$).

Other localities in Mexico. Tamiahua Lagoon, Veracruz ($21^{\circ}15'49''\text{N}$, $97^{\circ}27'41''\text{W}$); Tlacotalpan, Veracruz ($18^{\circ}36'0''\text{N}$, $95^{\circ}39'0''\text{W}$); La Palma River, Veracruz ($18^{\circ}33'21''\text{N}$, $95^{\circ}2'59''\text{W}$); El Corchito Progreso, Yucatan ($21^{\circ}16'40''\text{N}$, $89^{\circ}38'38''\text{W}$); El Milagro lagoon, Campeche ($18^{\circ}52'25''\text{N}$, $91^{\circ}24'39''\text{W}$).

Type material. Holotype CNHE: 9846; paratypes CNHE: 9847. Voucher material CNHE: 9848–9851.

Representative sequences. *Saccocoelioides olmecae* n. sp. *D. maculatus* [GenBank KU061125–KU061133; KU061135–KU061136 (LSU rDNA gene); GenBank KU061106–KU061113; KU061115–KU061116 (ITS2 rDNA gene)]; *S. olmecae* n. sp. *G. yucatanana* [GenBank KU061134 (LSU rDNA gene); GenBank KU061114 (ITS2 rDNA gene)].

Etymology. The specific epithet refers to Olmeca, a Mesoamerican civilization that lived along the Gulf of Mexico between 1400 and 1200 BCE.

Remarks

The new species possesses the main morphological characters of the genus *Saccocoelioides* (Overstreet & Curran, 2005). Of the 16 species of genus *Saccocoelioides* reported from the Americas, 13 species are described from South America (Overstreet & Curran, 2005). *Saccocoelioides olmecae* n. sp. can be distinguished morphologically from 10 South American species (*S. magnus* Szidat, 1954; *S. elongatus* Szidat, 1954; *S. szidati* Travassos, Freitas & Kohn, 1969; *S. octavus* Szidat, 1970; *S. quintus* Thatcher, 1978; *S. saccodontis* Thatcher, 1978; *S. magnorchis* Thatcher, 1978; *S. antonioi* Lunaschi, 1984; *S. carolae* Lunaschi, 1984 and *S. godoyi* Kohn & Miranda-Froes, 1986) by having the smallest body size (340–527 × 150–250) (see Szidat, 1954, 1970; Travassos *et al.*, 1969; Thatcher, 1978; Lunaschi, 1984; Kohn & Miranda-Froes, 1986). *Saccocoelioides olmecae* n. sp. can be distinguished from *S. magniovatus* Szidat, 1954 as the latter has a plump body and vitellarium at pre-testicular level vs. body elongated and vitellarium at testicular level in *S. olmecae* n. sp. *Saccocoelioides tarpazensis* Díaz & González, 1990 differs from the new species by having vitelline follicles that are not confluent at post-testicular level vs. vitelline follicles confluent at post-testicular level in *S. olmecae* n. sp., a wider oral sucker (80–130 for *S. tarpazensis* vs. 55–84 for *S. olmecae* n. sp.) and by the size of eggs (110–130 × 50–125 for *S. tarpazensis* vs. 60–89 × 28–47 for *S. olmecae* n. sp.). Finally, *S. olmecae* n. sp. differs from *S. nanii* Szidat, 1954 in the distribution of the uterus – in the latter, from ventral sucker level to pre-testicular level vs. from ventral sucker level to testicular level in *S. olmecae* n. sp. – and by distribution of the vitellarium – in the latter, not confluent at post-testicular level vs. confluent at post-testicular level in *S. olmecae* n. sp. (Szidat, 1954; Díaz & González, 1990).

Three of the 16 species of *Saccocoelioides* (*S. lamothei*, *S. chauhani* and *S. sogandaresi*) have been recorded in Mexico. *Saccocoelioides olmecae* n. sp. can be differentiated from *S. lamothei* by the following characters: a pyriform-elongated body, ovary length and by having vitelline follicles usually longitudinally elongated (see table 1). *Saccocoelioides olmecae* n. sp. differs from *S. sogandaresi* by having an elongated body (vs. pyriform, see Fig. 1 in Lumsden, 1963), a sac-like caecum, vitelline follicles reaching posterior end of body, pre-pharynx length and on the ovary size (see table 1). Finally, *S. olmecae* n. sp. can be differentiated from *S. chauhani* on the following characters: body length, ovary size and by having a uterus that extends to the first third of the body (see table 1).

Molecular characterization and phylogenetic analyses

In this study, sequences of the LSU obtained from 12 individuals of *S. olmecae* n. sp. from six localities along the Gulf of Mexico (see supplementary table S1, fig. 1) were aligned with a LSU dataset containing new sequences of five specimens of *S. lamothei* and three specimens of *S. chauhani* from type locality plus *Saccocoelioides* sp. from Nicaragua (Curran *et al.*, 2006), in addition to sequences of other haploporids (i.e. *Capitimitta* Pulis & Overstreet, 1982; *Dicrogaster* Looss, 1902; *Forticulcita*, Overstreet, 1982; *Platydidymus* Overstreet & Curran, 2005; *Hapladena* Linton, 1910; *Haploporus*, Looss, 1902; *Intrromugil*, Overstreet & Curran, 2005; *Lecithobotrys*, Looss, 1902; *Litosaccus*, Pulis, Cribb & Overstreet, 2014; *Parasaccocoelium*, Zhukov, 1971; *Ragaia* Blasco-Costa, Montero, Gibson, Balbuena & Kostadinova, 2009; *Saccocoelium*, Looss, 1902; *Spiritestis*, Nagaty, 1948; *Skrjabinolecithum*, Belous, 1954; *Xiha*, Andres, Curran, Fayton, Pulis & Overstreet, 2015), and *Atractotrema*, Goto & Ozaki, 1929, *Pseudomegasolena*, Machida & Komiya, 1976, *Preptetos*, Pritchard, 1960, *Cadenatella*, Dollfus, 1946 and *Paragonimus*, Braun, 1899 from another four families were used as the outgroup. The LSU dataset was conformed by 1277 characters. The phylogenetic tree inferred with this dataset suggested that Haploporidae and 15 other genera of haploporids are monophyletic. However, this tree suggests that the taxonomy of *Hapladena* should be examined using morphological and molecular data because this genus is nested among outgroup taxa (fig. 4). The genus *Intrromugil*, which includes trematodes infecting mullet fish, is sister to *Saccocoelioides*, which is divided into three clades corresponding *S. lamothei*, *S. chauhani* and *S. olmecae* n. sp. (fig. 4). The genetic divergence among the three species of *Saccocoelioides* was 1%, whereas isolates of *S. olmecae* n. sp. from *D. maculatus* and one isolate from *G. yucatanana* were identical among the six localities sampled. The three sequences of *S. chauhani* were also identical. The five isolates of *S. lamothei* had a genetic divergence of 1%, including the sequences generated by Curran *et al.* (2006) from an unidentified poeciliid from Nicaragua.

The ITS2 sequences of *S. olmecae* n. sp. (11 adults) were aligned with three isolates of *S. lamothei*, four isolates of *S. chauhani* and other genera of haploporids, forming a dataset of 419 characters. The genetic divergence among *S. lamothei*, *S. chauhani* and *S. olmecae* n. sp. ranged from 1 to 4%. The genetic divergence among the 11 isolates of the new species was very low, ranging from 0 to 1%. The phylogenetic tree inferred from this dataset did not support the monophyly of Haploporidae, and some genera, such as *Saccocoelium* and *Dicrogaster*, were paraphyletic with low support of bootstrap and posterior probabilities (fig. 5). This tree also showed that *Intrromugil* is not a sister to *Saccocoelioides* (fig. 5).

The concatenated alignment (LSU + ITS2) contained 11 specimens of *S. olmecae* n. sp., four isolates of *S. lamothei*, three isolates of *S. chauhani*, plus the same taxa used on the alignments with LSU and ITS2. Maximum likelihood (ML) analysis inferred with LSU, ITS2 and the combined dataset of both (LSU + ITS2) each yielded a single tree that was very similar in topology to the Bayesian inference (BI) consensus tree (figs 4–6). The ML and Bayesian

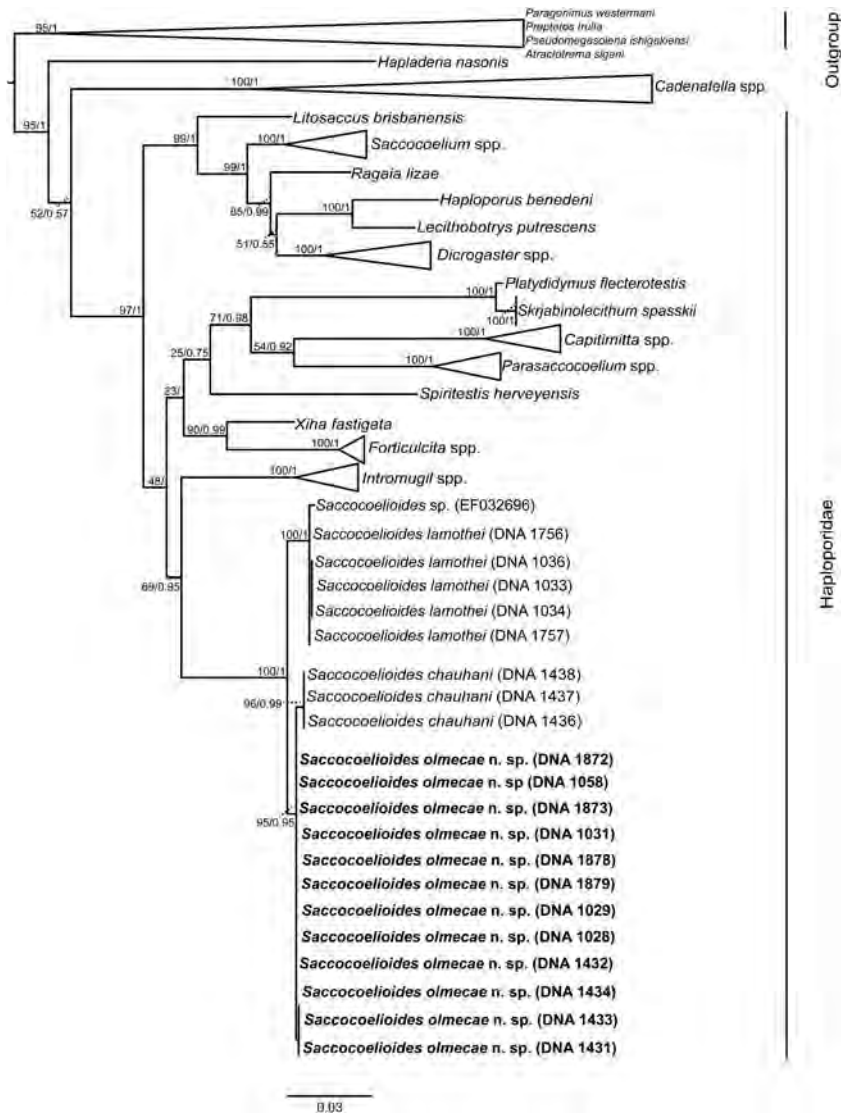


Fig. 4. Maximum likelihood tree and consensus Bayesian inference trees inferred from the LSU dataset. Numbers near internal nodes show ML bootstrap clade frequencies and posterior probabilities (BI).

consensus trees inferred with LSU (fig. 4) and that concatenated from both genes (fig. 6), showed that *Intromugil* is sister to *Saccocoelioides*, which is formed by *S. lamothei*, *S. chauhani* and *S. olmecae* n. sp. In addition, all the sequences of *S. olmecae* n. sp. are nested together within a monophyletic clade, with moderate bootstrap support and Bayesian posterior probability values (95/0.95 with LSU, 63/0.80 with ITS2 and 80/0.57 with the concatenated dataset) (figs 4–6).

Discussion

The species analysed in this study belong to *Saccocoelioides* according to the distribution of vitelline follicles in lateral fields of the body and by parasitizing freshwater fish from the Americas (see Lumsden, 1963;

Lamothe-Argumedo, 1974; Overstreet & Curran, 2005; Aguirre-Macedo & Violante-González, 2008). However, other morphological characters diagnostic of the genus *Saccocoelioides*, such as the distribution of the uterus, the presence of a pre-pharynx, caecum sac-like and position of the testis are similar to those of *Culuwiya*, and therefore a taxonomic debate has emerged regarding *Saccocoelioides* and *Culuwiya*. The inclusion of molecular data is necessary to resolve the validity of some species of *Saccocoelioides* and those of the genus *Culuwiya*, as some species, such as *Culuwiya beauforti* Hunter & Thomas, 1961; *C. overstreeti* Bargiela, 1988; *C. papernai* Bargiela, 1988; *C. agonostomus* Dyer, Bunkley-Williams & Williams, 1999, originally identified as *Saccocoelioides*, were transferred to the genus *Culuwiya* by Overstreet & Curran (2005).

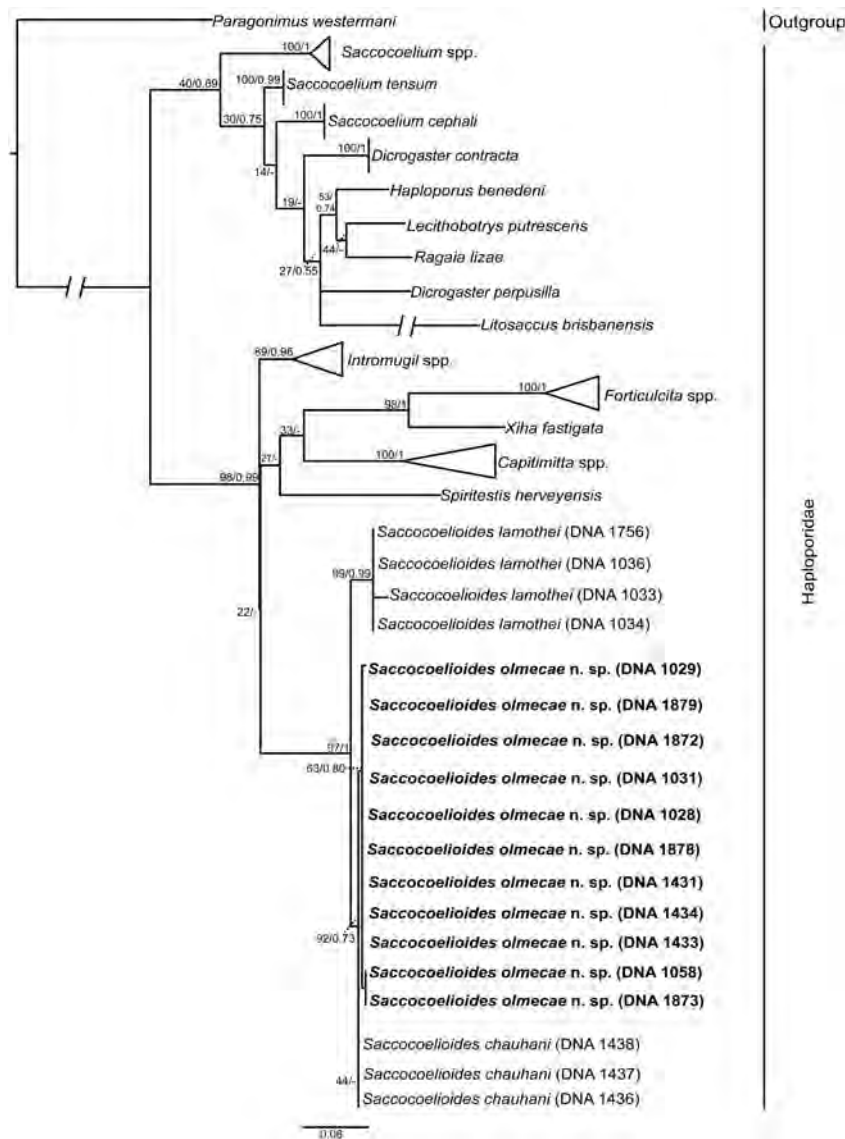


Fig. 5. Maximum likelihood tree and consensus Bayesian inference trees inferred from the ITS2 data set. Numbers near internal nodes show ML bootstrap clade frequencies and posterior probabilities (BI).

Our phylogenetic trees, obtained with the concatenated and separate datasets, confirmed that *Saccocoelioides* is monophyletic, with three sub-clades, each one represented by the three species analysed herein (*S. lamothei*, *S. chauhani* and *S. olmecae* n. sp.), with moderate support of bootstrap and posterior probability values. The phylogenetic analyses inferred with LSU and concatenated datasets showed that *Saccocoelioides* is sister to *Intromugil*, which contains four species associated with fish of the genus *Mugil* distributed along the Atlantic coast (Pulis *et al.*, 2013). The genetic divergence estimated with the LSU dataset among species of *Saccocoelioides* was 1.0%, and it ranged from 1.0 to 4% with ITS2. These values of genetic divergence among species are similar those of other haploporids. For instance, the genetic divergence found among four species of

Saccocoelium (*S. cephal* Blasco-Costa, Montero, Gibson, Blabuena, Raga & Kostadinova, 2009; *S. tensum* Looss, 1902; *S. obesum* Looss, 1902 and *S. brayi* Blasco-Costa, Balbuena, Raga, Kostadinova & Olson, 2010) ranged from 0.9 to 4.8% with LSU and from 2.1 to 10.9% with ITS2; between two species of *Dicrogaster* (*D. perpusilla* Looss, 1902 and *D. contracta* Looss, 1902) the genetic divergence was 4.6% and 8.7% with LSU and ITS2, respectively; and among three species of the genus *Capitimita* (*Capitimita* sp., *C. darwinensis* Pulis & Overstreet, 2013 and *C. costata* Pulis & Overstreet, 2013), it ranged from 2.7 to 2.8% with LSU and from 7.3 to 11% with ITS2 (see Blasco-Costa *et al.*, 2009, 2010; Pulis & Overstreet, 2013).

Currently, *Saccocoelioides* contains 17 described species distributed in the Americas. In Middle America, *S. olmecae*

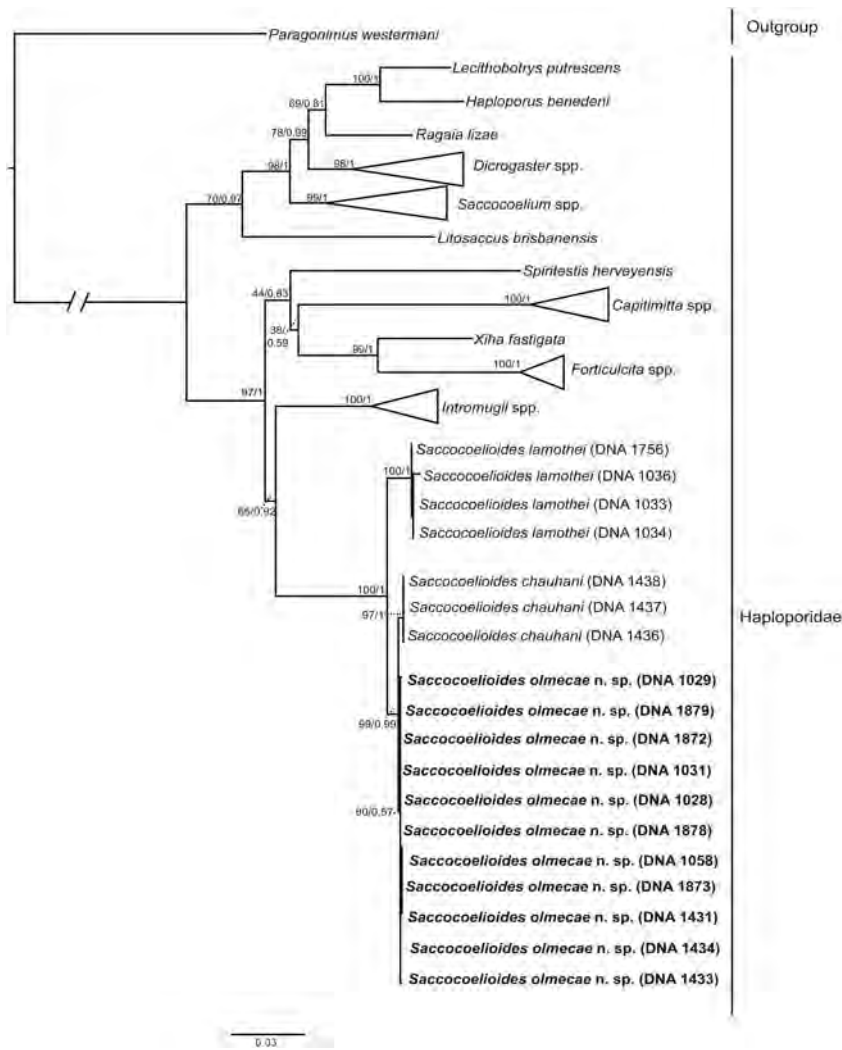


Fig. 6. Maximum likelihood tree and consensus Bayesian inference trees inferred with the concatenated (LSU + ITS2) dataset. Numbers near internal nodes show ML bootstrap clade frequencies and posterior probabilities (BI).

n. sp. is the third species of the genus infecting eleotrid fish of the genus *Dormitator* Gill and it shows some level of host specificity, due to the fact that *S. olmecae* n. sp. was found in the fat sleeper (*D. maculatus*) along the Gulf of Mexico. However, a single, poorly developed specimen was collected from the intestine of *G. yucatanana*, suggesting that this fish can act as an accidental host. *Saccocoelioides lamothei* has been reported from the intestine of *D. latifrons* from the Pacific coast of Mexico and from another four unrelated families of fish (Eleotridae, Gobiidae, Poeciliidae and Profundulidae) (Aguirre-Macedo & Violante-González, 2008; Andrade-Gómez, 2015; Pinacho-Pinacho *et al.*, 2015). *Saccocoelioides sogandaresi* has been found in fish of the orders Cyprinodontiformes (Goodeidae, Poeciliidae), Mugiliformes (Mugilidae) and Perciformes (Cichlidae, Gobiidae) from central and south-eastern Mexico (Scholz *et al.*, 1995; Pérez-Ponce de León *et al.*, 2007; Martínez-Aquino *et al.*,

2014). Finally, *S. chauhani* has only been found mainly in characid and poeciliid fish from Catemaco Lake in Veracruz, Mexico (Pérez-Ponce de León *et al.*, 2007). In addition, our molecular analyses suggested that the specimen of *Saccocoelioides* sp. from Nicaragua, found in an unidentified poeciliid, belonged to *S. lamothei* (see Curran *et al.*, 2006).

In this study, we combined morphological and molecular data with the aim of describing a new species of *Saccocoelioides* from along the coast of the Gulf of Mexico, and we also generated new morphological and molecular data of *S. chauhani* and *S. lamothei* that could represent the starting point to the study of this group of parasites. The inclusion of other species of *Saccocoelioides* from South America and North America, such as *S. sogandaresi*, is fundamental to a better understanding of the phylogenetic history of this complex and fascinating group of parasites.

Supplementary material

To view supplementary material for this article, please visit <http://dx.doi.org/10.1017/S0022149X1600047X>

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Conflict of interest

None.

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V. II. PUBLICACIÓN 2

Segundo artículo publicado en el *Journal of Parasitology*.

RH: L. Andrade-Gómez et al. Reallocation of *Culuwiya cichlidorum*

Molecular, morphological and ecological data of *Saccocoelioides* Szidat, 1954 (Digenea: Haploporidae) from Middle America supported the reallocation from *Culuwiya cichlidorum* to *Saccocoelioides*

Leopoldo Andrade-Gómez, Carlos Daniel Pinacho-Pinacho*, and Martín García-Varela†

Posgrado en Ciencias Biológicas, Instituto de Biología, Universidad Nacional Autónoma de México, A. P. 70-153, C. P. 04510, México D. F., México. *Universidad de la Sierra Sur, División de Estudios de Postgrado, Guillermo Rojas Mijangos S/N, C. P. 70800, Ciudad Universitaria, Miahuatlán de Porfirio Díaz, Oaxaca, México. †Departamento de Zoología, Instituto de Biología, Universidad Nacional Autónoma de México, Avenida Universidad 3000; Ciudad Universitaria; C.P. 04510; Ciudad de México.

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Correspondence should be sent to

Martín García-Varela at: garciav@ib.unam.mx

Molecular, Morphological, and Ecological Data of *Saccocoelioides* Szidat, 1954 (Digenea: Haploporidae) from Middle America Supported the Reallocation from *Culuwiya cichlidorum* to *Saccocoelioides*

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MOLECULAR, MORPHOLOGICAL, AND ECOLOGICAL DATA OF *SACCOCOELIOIDES* SZIDAT, 1954 (DIGENEA: HAPLOPORIDAE) FROM MIDDLE AMERICA SUPPORTED THE REALLOCATION FROM *CULUWIYA CICHLIDORUM* TO *SACCOCOELIOIDES*

Leopoldo Andrade-Gómez, Carlos Daniel Pinacho-Pinacho*, and Martín García-Varela†

Posgrado en Ciencias Biológicas, Instituto de Biología, Universidad Nacional Autónoma de México, A. P. 70-153, C. P. 04510, Mexico D.F., Mexico. Correspondence should be sent to Martín García-Varela at: garcia@ib.unam.mx

ABSTRACT: This study investigates the systematic position of some species of the genus *Saccocoeloides* Szidat, 1954, and the species *Culuwiya cichlidorum* Aguirre-Macedo and Scholz, 2005, from North and Middle America using new morphological data and partial sequences of 2 nuclear genes, the large subunit and the internal transcribed spacer 2 from ribosomal DNA. In total 74 specimens representing 4 species of *Saccocoeloides* (41 of *Saccocoeloides sogandaresi*, 3 of *Saccocoeloides chauhani*, 6 of *Saccocoeloides lamothei*, and 12 of *Saccocoeloides olmecae*) plus 12 specimens of *Culuwiya cichlidorum* were analyzed. The phylogenetic analyses inferred with maximum likelihood method and Bayesian inference showed that the 4 species of *Saccocoeloides* formed a clade divided into 4 subclades representing each species. In addition, 7 specimens sequenced from the type host and locality of *Culuwiya cichlidorum* were nested in 2 clades: the first clade with specimens of *C. cichlidorum* from Costa Rica, and the second clade with specimens of *S. sogandaresi* from Mexico, Honduras, Nicaragua, and Costa Rica. Our analyses suggest that *C. cichlidorum* should be reallocated into *Saccocoeloides cichlidorum* n. comb., is restricted to Nicaragua and Costa Rica and associated with cichlid fishes. In contrast, the specimens identified previously as *Culuwiya cichlidorum* from the coast of Gulf of Mexico by Aguirre-Macedo and Scholz belong to *S. sogandaresi*, which has a wide distribution that extends from Galveston Bay (U.S.A.) in Gulf of Mexico to Atlantic and Pacific slopes associated with poeciliid and cichlid fishes. New morphological data derived from scanning electron microscopy photographs of the body surface show a characteristic pattern of spination that is useful to distinguish between *S. cichlidorum* n. comb. and *S. sogandaresi*.

Digeneans of the genus *Saccocoeloides* Szidat, 1954 (Haploporidae) are intestinal parasites of freshwater and brackish water fishes distributed in the Americas (Overstreet and Curran, 2005). Currently the genus contains 18 nominal species (Overstreet and Curran, 2005; Aguirre-Macedo and Violante-González, 2008; Andrade-Gómez et al., 2016) of which 14 are distributed in South America: 9 in Argentina (*Saccocoeloides nanii* [type-species] Szidat, 1954; *Saccocoeloides elongatus* Szidat, 1954; *Saccocoeloides magniovatus* Szidat, 1954; *Saccocoeloides magnus* Szidat, 1954; *Saccocoeloides quintus* Thatcher, 1978; *Saccocoeloides szidati* Travassos, Freitas and Kohn, 1969; *Saccocoeloides octavus* Szidat, 1970; *Saccocoeloides carolae* Lunaschi, 1984; and *Saccocoeloides antonioi* Lunaschi, 1984), 2 in Colombia (*Saccocoeloides saccodontis* Thatcher, 1978, and *Saccocoeloides magnorchis* Thatcher, 1978), 2 in Brazil (*Saccocoeloides godoyi* Kohn and Miranda-Froes, 1986, and *Saccocoeloides rotundus* Thatcher and Jégu, 1996), and a single species in Venezuela (*Saccocoeloides tarpazensis* Díaz and González, 1990). Four species of *Saccocoeloides* have been described from North and Middle America (*Saccocoeloides sogandaresi* Lumsden, 1963, in the United States and reported in Mexico), and the other 3 species have been described in Mexico (*Saccocoeloides chauhani* Lamothe-Argumedo, 1974; *Saccocoeloides lamothei* Aguirre-Macedo and Violante-

González, 2008; and *Saccocoeloides olmecae* Andrade-Gómez, Pinacho-Pinacho, Hernández-Orts, Sereno-Urbe and García-Varela, 2016).

From a survey of helminth parasites from Nicaragua, Aguirre-Macedo et al. (2001), reported 2 undescribed species of *Saccocoeloides*, one associated with the blackbelt cichlid, *Vieja maculicauda* Regan, and the other with the characid *Astyanax fasciatus* Cuvier. Aguirre-Macedo and Scholz (2005) described one of these species as *Culuwiya cichlidorum* Aguirre-Macedo and Scholz, 2005, from *V. maculicauda*. In the same study, those authors reviewed the specimens previously identified as *Saccocoeloides* sp., from cichlid fishes from southeastern Mexico (see Vidal-Martínez et al., 2001) and identified them as *C. cichlidorum*, extending its distribution in Middle America from southeastern Mexico to Nicaragua (Aguirre-Macedo and Scholz, 2005). However, the morphological characters diagnostic of the genera *Saccocoeloides* and *Culuwiya*, such as the distribution of the uterus (Overstreet and Curran, 2005), presence of prepharynx, caeca sac-like, and position of testis, are overlapping (Aguirre-Macedo and Scholz, 2005). Therefore the taxonomy of some species of these genera is uncertain, and it is essential to evaluate their taxonomy with new evidence based on morphological and molecular data.

The taxonomic status of some species of *Saccocoeloides* such as *Saccocoeloides beauforti* Hunter and Thomas 1961, *Saccocoeloides overstreeti* Bargiela, 1988, *Saccocoeloides papernai* Bargiela, 1988, *Saccocoeloides agonostomus* Dyer, and Bunkley-Williams and Williams, 1999, were controversial, and in the most recent review of the genus, Overstreet and Curran (2005) transferred those 4 species to a new genus named *Culuwiya*. Currently the genus *Culuwiya* includes 6 species (*Culuwiya tilapiae* Nasir and Gómez, 1976; *C. cichlidorum*) that are parasites mainly of mullets, but they have also been found in gobiid and cichlid

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* Universidad de la Sierra Sur, División de Estudios de Postgrado, Guillermo Rojas Mijangos S/N, C. P. 70800, Ciudad Universitaria, Miahuatlán de Porfirio Díaz, Oaxaca, Mexico.

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

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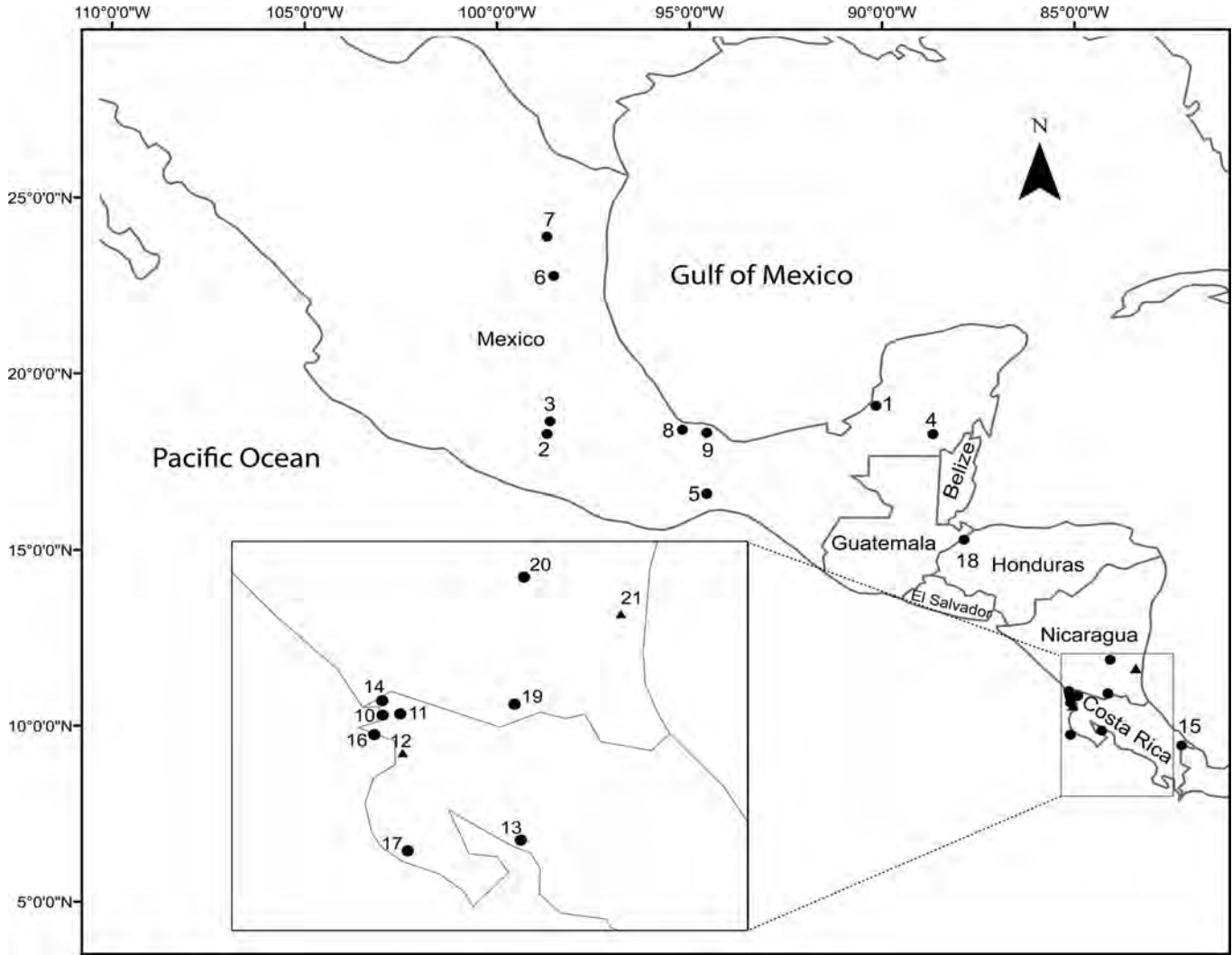


FIGURE 1. Sampling sites (see Table I) of fish hosts with *Saccocoelioides sogandaresi* and *Culawiya cichlidorum* from Mexico, Honduras, Nicaragua, and Costa Rica. Triangle marks are where both species inhabit.

fishes in the Americas (Aguirre-Macedo and Scholz, 2005; Overstreet and Curran, 2005).

In the current research, we sampled adult digeneans of cichlid and poeciliid fishes in 4 countries from Middle America: Mexico, Nicaragua (including from the type host and type locality of *C. cichlidorum*), Honduras, and Costa Rica. The aims of this study were to 1) redescribe morphologically the species *Saccocoelioides sogandaresi* and *Culawiya cichlidorum*, and 2) explore the systematic position of both species within Haploporidae using DNA sequence of large subunit sequence (LSU) including the domains D1–D3 and the internal transcribed spacer 2 (ITS2) of ribosomal DNA.

MATERIALS AND METHODS

Specimen collection

Adult digeneans were collected from December 2011 through April 2016 from the intestine of poeciliid and cichlid fishes in 21 localities from Middle America, 9 in Mexico, 8 in Costa Rica, 3 in Nicaragua, and 1 in Honduras (see Table I; Fig. 1). Fishes were captured with seine nets and electrofishing and maintained alive

and transported to the laboratory. Individual fish was euthanized and immediately examined for digeneans. Digeneans collected from the intestine were fixed by immersion in hot (steaming) 4% formalin without flattening and stored in 4% formalin for morphological comparisons; other specimens were preserved in 100% ethanol for molecular analysis. Fishes were identified following the keys of Miller et al. (2005) and Bussing (2002).

Morphological analyses

Unflattened specimens preserved in formalin were stained with Mayer’s paracarmine (Merck, Darmstadt, Germany), dehydrated in a graded ethanol series, cleared with methyl salicylate, and mounted on microscope slides using Canada balsam. Mounted specimens were examined under a bright field Leica DM 1000 LED microscope (Leica, Wetzlar, Germany), and drawings were made using a drawing tube attachment to the microscope. Measurements were taken using the Leica Application Suite microscope software (Leica) and are given in micrometers (µm).

Voucher specimens were deposited in the Colección Nacional de Helmintos (CNHE), Instituto de Biología, Universidad

TABLE I. Digeneans collected in this study, including collection sites (CS), localities, host species, geographical coordinates, and GenBank accession number. The collection site number (CS) for each locality corresponds with the number in Figure 1. Species marked with * are transferred to *Saccocoelioides sogandaresi* in this study.

Locality	CS	Host	Coordinates		GenBank accession numbers		Species identified as
			N	W	28S	ITS2	
Mexico							
Campeche							
Ulmal	1	<i>Paratheraps bifasciatus</i> <i>Torchithys helleri</i>	19°16'43"	90°37'26"	KY489602 KY489603 KY489601	KY489551 KY489552 KY489550	<i>Culuwiya cichlidorum</i> * <i>Culuwiya cichlidorum</i> *
Morelos							
Huixtla	2	<i>Poeciliopsis balsas</i>	18°28'50"	99°9'56"	KY489625	KY489572	<i>Saccocoelioides sogandaresi</i>
Yautepec	3	<i>Pseudoxiphophorus</i> sp.	18°50'7"	99°5'26"	KY489606 KY489607	KY489555 KY489556	<i>Saccocoelioides sogandaresi</i>
Quintana Roo							
Los Milagros	4	<i>Poecilia</i> sp.	18°28'26"	89°8'39"	KY489599 KY489600	KY489548 KY489549	<i>Saccocoelioides sogandaresi</i>
Oaxaca							
Matías Romero	5	<i>Poecilia mexicana</i>	16°47'30"	95°0'59"	KY489612	KY489559	<i>Saccocoelioides sogandaresi</i>
Tamaulipas							
Río Frio	6	<i>Poecilia mexicana</i>	22°58'11"	98°59'36"	KY489623	KY489570	<i>Saccocoelioides sogandaresi</i>
Río Purificación	7	<i>Poecilia formosa</i> <i>Herichthys cyanoguttatus</i>	24°5'21"	99°9'54"	KY489618– KY489620 KY489621 KY489622 KY489624	KY489565– KY489567 KY489568 KY489569 KY489571	<i>Saccocoelioides sogandaresi</i> <i>Culuwiya cichlidorum</i> *
Veracruz							
Tlacotalpan	8	<i>Poecilia sphenops</i>	18°36'0"	95°39'0"	KY489593	KY489542	<i>Saccocoelioides sogandaresi</i>
Laguna de Sontecomapa	9	<i>Xiphophorus helleri</i>	18°30'50"	95°1'6"	KY489594–95	KY489543–44	<i>Saccocoelioides sogandaresi</i>
Costa Rica							
Río Las Animas	10	<i>Archocentrus nigrofasciatus</i> <i>Astatheros alfari</i> <i>Archocentrus siquia</i>	11°02'58"	85°35'12"	KY489638 KY489639 KY489641 KY489640	KY489585 KY489586 KY489588 KY489587	<i>Culuwiya cichlidorum</i> <i>Culuwiya cichlidorum</i> <i>Culuwiya cichlidorum</i>
Río Orosí	11	<i>Hypsophrys nematopus</i>	11°02'50"	85°22'48"	KY489634 KY489635	KY489581 KY489582	<i>Culuwiya cichlidorum</i>
Río Irigaray	12	<i>Archocentrus siquia</i> <i>Poecilia gillii</i>	10°43'21"	85°30'38"	KY489636 KY489637 KY489614 KY489615	KY489583 KY489584 KY489561 KY489562	<i>Culuwiya cichlidorum</i> <i>Saccocoelioides sogandaresi</i>
Río Ciruelas	13	<i>Poecilia gillii</i>	10°3'38"	84°45'31"	KY489596 KY489608– KY489610	KY489545 KY489557 KY489558	<i>Saccocoelioides sogandaresi</i>
Río Las Vueltas	14	<i>Poecilia gillii</i>	11°10'56"	85°36'58"	KY489616 KY489617	KY489563 KY489564	<i>Saccocoelioides sogandaresi</i>
Playa Grande (Puerto viejo)	15	<i>Poecilia mexicana</i>	9°38'15"	82°40'56"	KY489597	KY489546	<i>Saccocoelioides sogandaresi</i>
Quebrada Puercos	16	<i>Poecilia gillii</i>	10°51'32"	85°34'15"	KY489598 KY489604 KY489605	KY489547 KY489553 KY489554	<i>Saccocoelioides sogandaresi</i>
Centeno	17	<i>Poecilia gillii</i>	9°56'28"	85°34'15"	KY489611		<i>Saccocoelioides sogandaresi</i>
Honduras							
Río Chamelecón	18	<i>Poecilia</i> sp.	15°29'7"	88°20'13"	KY489613	KY489560	<i>Saccocoelioides sogandaresi</i>
Nicaragua							
Palo de Arquito	19	<i>Poecilia gillii</i>	11°7'12"	84°36'5"	KY489629 KY489630	KY489576 KY489577	<i>Saccocoelioides sogandaresi</i>
Río Mico	20	<i>Poecilia gillii</i>	12°4'21"	84°32'13"	KY489628	KY489575	<i>Saccocoelioides sogandaresi</i>
Río Torsuani	21	<i>Vieja maculicauda</i> <i>Brychon</i> sp.	11°47'06"	83°52'38"	KY489644–45 KY489626–27 KY489631– KY489633 KY489642 KY489643	KY489591–92 KY489573–74 KY489578– KY489580 KY489589 KY489590	<i>Culuwiya cichlidorum</i> <i>Saccocoelioides</i> sp.

Nacional Autónoma de México, Mexico City. The species identification was conducted following the key of Overstreet and Curran (2005) and original descriptions (see Lumsden, 1963; Aguirre-Macedo and Scholz, 2005). Type material of *C. cichlidorum* could not be examined because they were not available in the CNHE. However, paratypes of *C. cichlidorum* (No. cat. D511) deposited in the Helminthological Collection of the Institute of Parasitology, Biology Centre ASCR, České Budějovice, Czech Republic (IPCAS), were examined.

For scanning electron microscopy (SEM), 2 specimens of *C. cichlidorum* fixed in formalin from *V. maculicauda* from Río Torsuani, Nicaragua, 1 from *Herichthys cyanoguttatus* from Río Purificación Tamaulipas, Mexico, and 1 from *Hypsophrys nematopus* from Río Orosí, Costa Rica, plus 1 specimen of *S. sogandaresi* from *Poecilia formosa* from Río Purificación, Mexico, were dehydrated through an ethanol series, critical point dried, sputter-coated with gold, and examined with a Hitachi Stereoscan Model S-2469N scanning electron microscope, operating at 15 kV, from the Instituto de Biología, Universidad Nacional Autónoma de México (UNAM).

Amplification and sequencing of DNA

A total of 53 specimens were placed individually in tubes and digested overnight at 56 C in a solution containing 10 mM Tris-HCl (pH 7.6), 20 mM NaCl, 100 mM Na₂ EDTA (pH 8.0), 1% Sarkosyl, and 10 mg/ml of proteinase K (see Table I). Following digestion, DNA was extracted from the supernatant using the DNAzol reagent (Molecular Research Center, Cincinnati, Ohio) according to the manufacturer's instructions.

Internal transcribed spacer 2 (ITS2) and the D1-D3 domains of the large subunit (LSU) from ribosomal DNA (rDNA) were amplified using the forward primer BD3 5'-GAACATCGA-CATCTTGAACG-3' (Hernández-Mena et al., 2014) and the reverse primer 536 5'-CAGCTATCCTGAGGGAAAC-3' (García-Varela and Nadler, 2005). PCR reactions (25 µl) consisted of 1 µl 10 µM of each primer, 2.5 µl of 10× PCR Rxn buffer, 1.5 µl 2 mM MgCl₂, 0.5 µl of dNTP 10 mM, 16.375 µl of water, 2 µl of genomic DNA, and 1 unit of Taq DNA polymerase (Platinum Taq, Invitrogen Corporation, São Paulo, Brazil). PCR cycling parameters for rDNA amplifications included denaturation at 94 C for 1 min, followed by 35 cycles of 94 C for 1 min, annealing at 50 C for 1 min, and extension at 72 C for 1 min, followed by a post-amplification incubation at 72 C for 10 min. Sequencing reactions were performed using the primers forward and reverse mentioned above and 4 internal primers: 504 (5'-CGTCTTGAAACACGGACTAAGG-3'), 502 (5'-CAAG-TACCGTGAGGGAAAGTTGC-3') (García-Varela and Nadler, 2005), 503 (5'-CCTTGGTCCGTGTTTCAAGACG-3') (Stock et al., 2001), and BD2 (5'-TATGCTTAAATTCAGCGGGT-3') (Luton et al., 1992), with ABI Big Dye (Applied Biosystems, Boston, Massachusetts) terminator sequencing chemistry, and reaction products were separated and detected using an ABI 3730 capillary DNA sequencer. Contigs were assembled and base-calling differences resolved using Codoncode Aligner version 5.0.2 (Codoncode Corporation, Dedham, Massachusetts).

Alignments and phylogenetic analyses

Sequences obtained in the current research from ITS2 ranged from 203 to 380 base pair (bp) and from LSU ranged from 952 to

1,339 bp long. Those markers were aligned separately using the software Clustal W (Thompson et al., 1997) and adjusted manually with Mesquite (Maddison and Maddison, 2011). The final alignment consisted of 91 sequences with 1,354 nucleotides for the LSU and 82 sequences with 414 nucleotides for the ITS2. Phylogenetic trees were constructed by maximum-likelihood method (ML) and Bayesian inference (BI). For ML analyses, the program RAXML v7.0.4 (Stamatakis, 2006) was used. A GTRGAMMAI substitution model was selected for ML analyses, with 10,000 bootstrap replicates. The best nucleotide substitution models were selected for both data set with jModelTest v0.1.1 using the Akaike information criterion (Posada, 2008). The best model for both data set was GTR + G + I with different parameters and was used with the Bayesian analyses (Ronquist et al., 2012). Settings were 2 simultaneous runs of the Markov chain (MCMC) for 10 million generations, sampling every 1,000 generations, a heating parameter value of 0.2, and a "burn-in" of 25%. Trees were drawn using FigTree version 1.3.1 (Rambaut, 2006). The genetic divergence among species of *Saccocoelioides* and among genera of Haploporidae was estimated using uncorrected "p" distances with the program MEGA version 6 (Tamura et al., 2013).

RESULTS

Phylogenetic analyses

The LSU data set consisted of 1,354 characters. Maximum likelihood analysis of this data set yielded a tree with $-\ln$ of 7,273.73. The 4 species of *Saccocoelioides* (*S. sogandaresi*, *S. chauhani*, *S. lamothei*, and *S. olmecae*) formed a clade with strong bootstrap support (100%) and Bayesian posterior probabilities (1.0). The ML and Bayesian trees show that specimens of *Culuwiya cichlidorum* nested inside those *Saccocoelioides* species. The specimens analyzed from the type host and locality of *C. cichlidorum* formed 2 clades, the first with specimens of *C. cichlidorum* from Costa Rica and the second with specimens identified as *S. sogandaresi* from Mexico, Honduras, Nicaragua, and Costa Rica, suggesting that *C. cichlidorum* and *S. sogandaresi* occur sympatrically in 2 localities (Figs. 1, 2). The ML tree inferred with the ITS2 data set (with $-\ln$ of 2,701.15) had similar topology to the ML and Bayesian trees inferred with LSU data set, including the sister relationship of *Culuwiya cichlidorum* with *S. lamothei* (Fig. 3). The genetic divergence among the 4 species of *Saccocoelioides* (*S. sogandaresi*, *S. chauhani*, *S. lamothei*, and *S. olmecae*) was low in both molecular markers and ranged from 1.1% to 1.6% for LSU and from 0.7% to 3.4% for ITS2. The genetic divergence between species of *Saccocoelioides* and *Culuwiya cichlidorum* ranged from 0.7% to 1.6% (5–18 nt) and from 0.7% to 2% (3–14 nt) with LSU and ITS2, respectively. Both phylogenetic analyses support the reallocation of *Culuwiya cichlidorum* to *Saccocoelioides cichlidorum* n. comb. (Aguirre-Macedo and Scholz, 2005).

DESCRIPTIONS

Saccocoelioides cichlidorum (Aguirre-Macedo and Scholz, 2005) n. comb.

Syn. *Culuwiya cichlidorum* Aguirre-Macedo and Scholz, 2005

Description based on 16 individuals collected from 3 species of cichlid fishes: *Vieja maculicauda* (type-host of *C. cichlidorum*),

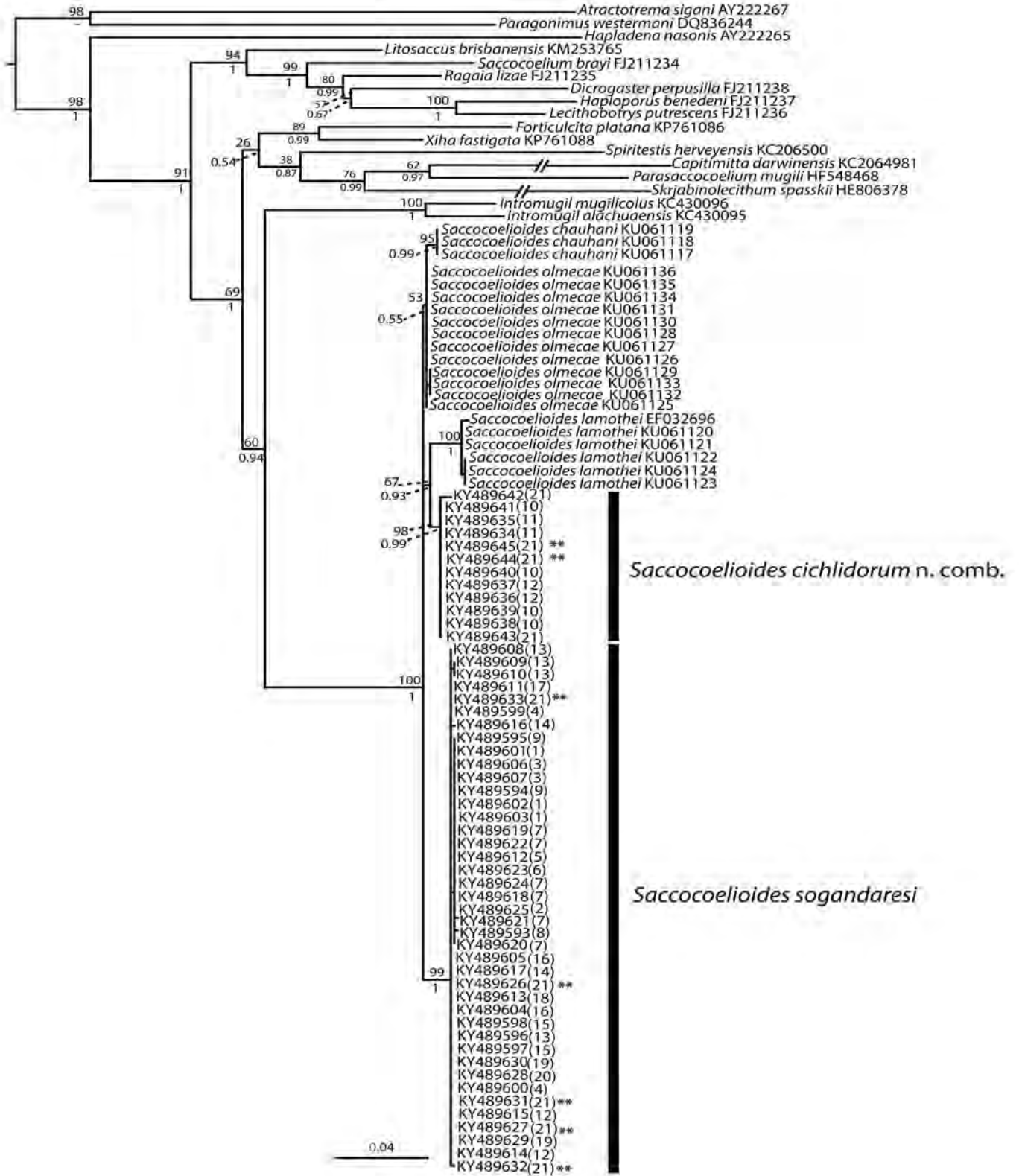


FIGURE 2. Maximum likelihood tree and consensus Bayesian inference trees inferred from the LSU dataset. Numbers near internal nodes show ML bootstrap clade frequencies and posterior probabilities (BI). DNA numbers are shown in Table I. ** Specimens from type-locality and type-hosts from *Saccocoelioides cichlidorum* n. comb.

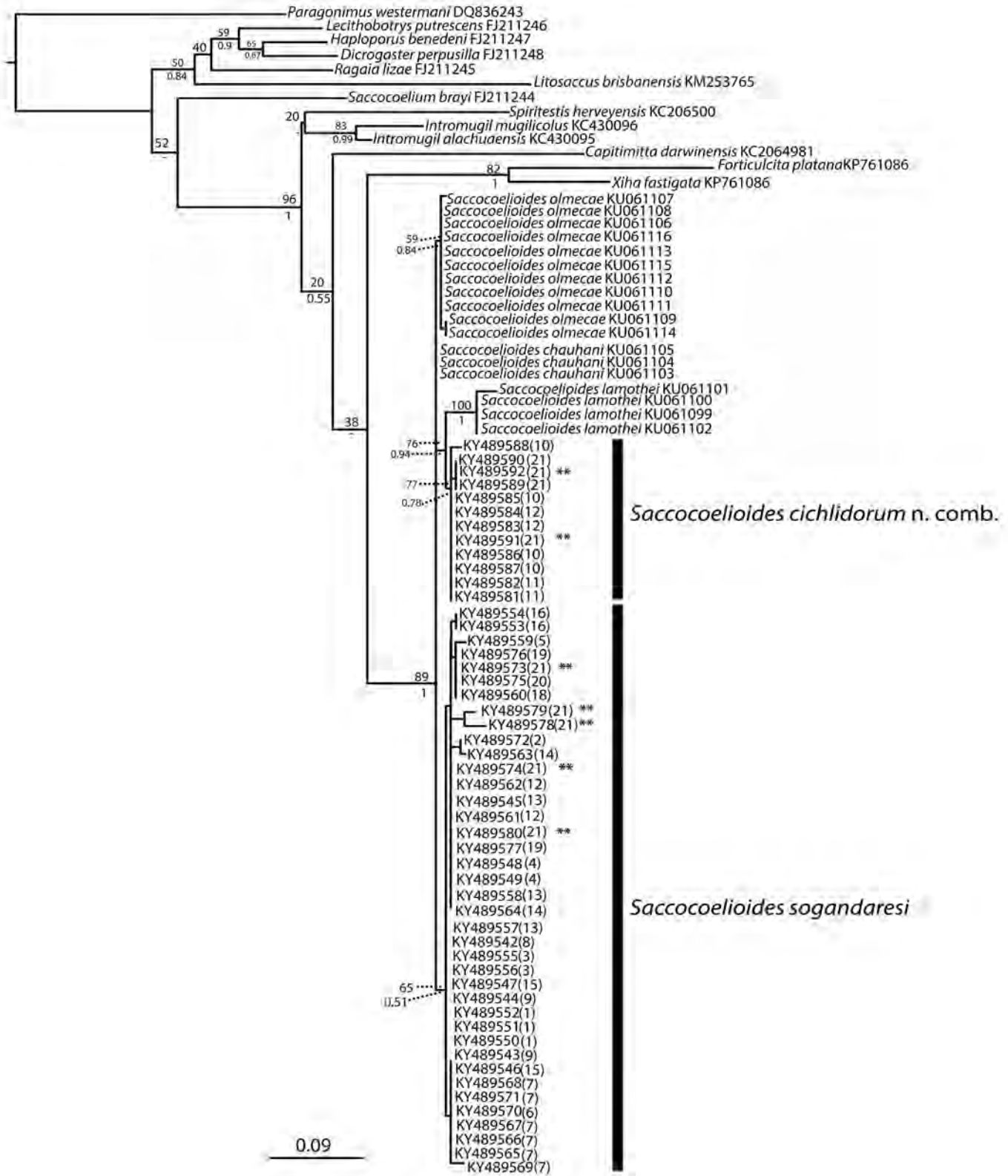


FIGURE 3. Maximum likelihood tree and consensus Bayesian inference trees inferred from the ITS2 dataset. Numbers near internal nodes show ML bootstrap clade frequencies and posterior probabilities (BI). DNA numbers are shown in Table I. ** Specimens from type-locality and type-hosts from *Saccocoelioides cichlidorum* n. comb.

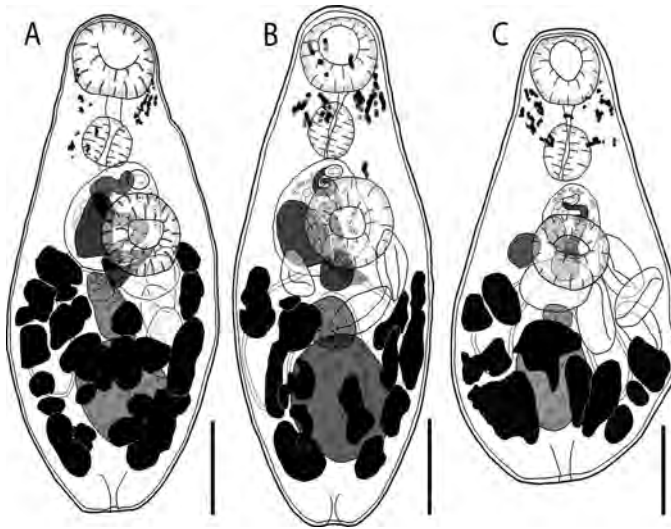


FIGURE 4. (A) *Saccocoelioides cichlidorum* n. comb., from *Hypsophrys nematopus* from Río Orosí, Costa Rica. (B) *Saccocoelioides sogandaresi* from *Paratheraps bifasciatus* from Ulumal, Campeche, Mexico. (C) *Saccocoelioides sogandaresi* from *Poecilia gillii* from Centeno, Costa Rica. Scale bars = 100 μ m.

Hypsophrys nematopus, and *Amatitlania nigrofasciatus* from Río Torsuani in Nicaragua (type-locality of *C. cichlidorum*) and Costa Rica: Body pyriform (Fig. 4A) with maximum width between second and third part of body. Tegument entirely spined (Fig. 5A, B). Eye-spot remnants dispersed from oral sucker to pharyngeal level. Oral sucker, subterminal spherical. Ventral sucker spherical pre-equatorial, very similar in size to oral sucker. Prepharynx present; pharynx oval to spherical, strongly muscular. Oesophagus long, extending to posterior half of ventral sucker. Intestinal bifurcation at posterior level of hermaphroditic sac. Caeca sac-like, thick-walled, terminating at one-half or two-thirds of testis length. Testis, spherical, subterminal, located in posterior third of body. External seminal vesicle bipartite with posterior part swollen when full. Hermaphroditic sac oval to spherical, obliquely arched around ventral sucker. Internal seminal vesicle oval to elongate, filling one-third of hermaphroditic sac, hermaphroditic duct long; prostatic cells in peripheral region of hermaphroditic sac. Genital pore immediately anterior to ventral sucker. Ovary spherical to elongate, in middle of body, just pretesticular. Seminal receptacle small and not observed in most specimens. Laurer's canal not observed. Uterus occupying space between hermaphroditic sac to first third of testis; metraterm thick walled, opening to hermaphroditic duct. Vitelline follicles numerous large, usually longitudinally elongate, distributed from posterior margin of ventral sucker level to posterior to testis, and confluent in posterior of body but not reaching terminal body level. Eggs operculate; maximum 4 eggs in uterus. Miracidium not observed. Excretory vesicle Y shaped, bifurcating at anterior level of testis; excretory pore terminal (see Table II).

Taxonomic summary

Type host: *Vieja maculicauda* Regan (Cichlidae).
 Other definitive hosts: Cichlidae: *Amatitlania nigrofasciatus* Gunter, *Amatitlania siquia* Schmitter-Soto, *Astatheros alfari*

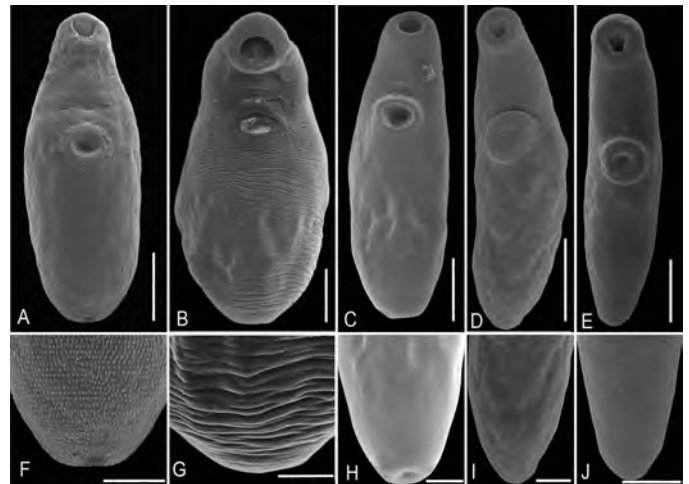


FIGURE 5. Scanning electron micrographs of whole specimens of *Saccocoelioides cichlidorum* n. comb. (A, B) and *Saccocoelioides sogandaresi* (C–E) and posterior end of body (F–J). Specimen from *Hypsophrys nematopus* from Río Orosí, Costa Rica (A, F). Specimens from *Vieja maculicauda* from Río Torsuani, Nicaragua (B, C, G, H). Specimen from *Poecilia formosa* from Río Purificación, Mexico (D, I). Specimen from *Herichthys cyanoguttatus* from Río Purificación, Mexico (E, J). Scale bar (A–E) = 100 μ m. Scale bar (F–J) = 50 μ m.

Meek, *Hypsophrys nematopus* Gunter, *Hypsophrys nicaraguensis* Gunter.

- Accidental host: *Brychon* sp. (Characidae) (see Remarks).
- Infection site: Intestine.
- Geographical distribution: Nicaragua and Costa Rica.
- Type locality: Nicaragua: Río Torsuani.
- Other localities: Costa Rica: Río Orosí, Río Animas, Río Irigaray.
- Previous records: Costa Rica: Guanacaste.
- Site in definitive hosts: Intestine.
- Newly collected material: Nicaragua: 5 specimens from *V. maculicauda* Río Torsuani (CNHE No. cat. 10267); Costa Rica: 11 specimens from *H. nematopus* Río Orosí (CNHE No. cat. 10268), and 5 specimens from *A. nigrofasciatus* Río Las Animas (CNHE No. cat. 10269).
- Specimens deposited: GenBank accession number: KY489634-45 by LSU and KY489581-92 by ITS2.

Remarks

The specimens found in cichlid fishes from Nicaragua and Costa Rica were identified as *S. cichlidorum* n. comb., on the basis of metrical data and morphological description. Our specimens are very similar to those of the original description (Aguirre-Macedo and Scholz, 2005) (see Table II). Our SEM study also confirmed that the tegument is entirely spined (see Fig. 5A, B, F, G). However, our specimens differ morphometrically from the original description of the prepharynx length (36–72 vs. 13–21, in this study provides lower limits) and number of eggs (15 vs. 4, in this study). Two specimens of *S. cichlidorum* n. comb., found in a freshwater fish, *Brychon* sp. (Characidae), from Río Torsuani, Nicaragua, had poor development, suggesting that the infection is accidental. Based on the current study, the specimens previously identified as *Saccocoelioides* sp. 3 and *Saccocoelioides* sp. 4, from

TABLE II. Comparative morphometric data of *Saccocoelioides sogandaresi* and *Saccocoelioides cichlidorum* n. comb.

Species	<i>S. sogandaresi</i> Lumsden, 1963	<i>S. sogandaresi</i> This study	<i>S. sogandaresi</i> This study	<i>S. sogandaresi</i> “ <i>C. cichlidorum</i> ” Aguirre-Macedo & Scholz, 2005	<i>C. cichlidorum</i> Aguirre-Macedo & Scholz, 2005	<i>S. cichlidorum</i> n. comb. This study
Host	<i>Poecilia latipinna</i>	<i>Poecilia gillii</i> <i>P. formosa</i>	<i>Vieja bifasciata</i>	<i>Paraneetroplus</i> <i>synspilus</i>	<i>Vieja maculicauda</i>	<i>Hypsophrys nematopus</i>
Distribution	Galveston Bay, Texas, U.S.A.	Centeno, Costa Rica Río Purificación, Mexico	Ulumal Campeche	Tabasco, Mexico	Nicaragua and Mexico	Río Orosi and Animas, Costa Rica
Specimens examined	10	9	14	—	15	12
Body shape	Pyriiform	Pyriiform	Pyriiform	Pyriiform	Pyriiform	Pyriiform
Body length	341–512	500–620	400–690	400–690	470–680	448–641
Body width	165–307	260–270	120–260	157–243	150–335	227–283
Oral sucker length	55–103	72–80	52–100	58–81	67–105	77–98
Oral sucker width	60–87	80–95	67–110	70–98	75–125	90–106
Ventral sucker length	62–105	85–95	67–100	64–104	57–120	76–96
Ventral sucker width	75–103	90–100	60–110	70–104	50–120	90–106
Sucker ratio length	1:1.2	1:1.2	1:0.93–1.28	Ventral sucker slightly larger	1:1–1.2	1:0.97–1.1
Prepharynx length	1–12	15–22	8–25	Short	36–72	13–21
Pharynx length	45–73	60–72	49–75	43–74	50–82	46–64
Pharynx width	37–70	57–65	45–85	49–80	47–75	61–75
Maximum extension of caeca	Posterior third of body	At middle or posterior of testis	At half or two-thirds of testis	Anterior margin of testis	At half or two-thirds of testis	At half or two-thirds of testis
Genital pore position	Between pharynx and ventral sucker	Anterior to ventral sucker	Anterior to ventral sucker	—	Anterior to ventral sucker	Anterior to ventral sucker
Hermaphroditic sac length	45–98	75–90	90–167	86–150	77–162	99–147
Hermaphroditic sac width	50–84	30–60	52–110	75–93	62–135	99–120
External seminal vesicle length	38–83*	35–55	30–100	—	—	128–177
External seminal vesicle width	28–48*	35–45	35–75	—	—	23–33
External seminal vesicle shape	Saccular	Spherical	Oval	Spherical to club shaped	Bipartite	Bipartite
Testis length	95*	80–112	82–147	78–142	72–175	110–126
Testis width	75–112	87–99	50–135	59–114	58–145	83–125
Ovary length	41–62	26–40	35–70	30–76	37–87	66–82
Ovary width	41–62	25–39	42–66	29–60	35–85	43–61
Ovary shape	Round	Spherical	Spherical	Spherical	Spherical	Spherical to elongate
Uterus	Mostly in hind-body	Between margin posterior of hermaphroditic sac to the first third of testis	Between hermaphroditic sac to anterior or slightly overlapping the testis	Pretesticular	Pretesticular	Between hermaphroditic sac to first to first third of testis
Eggs (n)	1*	(5–12)	(1–4)	—	(4–15)	(1–4)
Egg length	78–87	72–97	37–87	68–89	67–81	57–84
Egg width	37–55	40–57	25–80	42–53	36–47	34–90
Vitelline follicles position	From middle level of ventral sucker to post-testicular region	From posterior margin of ventral sucker level to posterior to testis, converging in testis zone	From posterior margin of ventral sucker to posterior of testis	Post-testicular level	Post-testicular level	From posterior ventral sucker level to posterior of testis, can confluent or not in posterior of body
Excretory vesicle	Tubular	Y-shaped	Y-shaped	Y-shaped	Y-shaped	Y-shaped

* Measured from the published figure.

cichlid fishes from Guanacaste, Costa Rica (Sandlund et al., 2010) can be assigned to *S. cichlidorum* n. comb.

***Saccocoelioides sogandaresi* Lumsden, 1963**

Description based on 9 individuals collected from poeciliid fishes (Poecilia formosa and Poecilia gillii) from 2 localities in 2 countries: Body pyriform, with maximum width in third part of body. Tegumental spines cover three-fourths of body (Fig. 4C). Eye-spot remnants dispersed from posterior margin of oral sucker to pharyngeal level. Oral sucker, subterminal, spherical. Ventral sucker spherical, slightly pre-equatorial, smaller or larger than oral sucker. Prepharynx present long; pharynx oval to spherical, strongly muscular. Oesophagus long, extending to half or posterior half of ventral sucker. Intestinal bifurcation posterior to hermaphroditic sac. Caeca sac-like, thick-walled, terminating at two-thirds of testis length. Testis, oval, subterminal, located in posterior third of body. External seminal vesicle spherical, small, dorsal to hermaphroditic sac, similar in size to internal seminal vesicle. Hermaphroditic sac small, oval to spherical, slightly smaller than and dorsal to ventral sucker. Internal seminal vesicle oval to elongate, filling one-third of hermaphroditic sac; hermaphroditic duct long; prostatic cells in peripheral region of hermaphroditic sac. Genital pore immediately anterior to ventral sucker. Ovary spherical, in middle of body, slightly overlapping testis. Seminal receptacle present, contiguous with hermaphroditic sac and connecting to ovary. Laurer's canal not observed. Uterus occupying space between margin posterior of hermaphroditic sac to first third of testis; metraterm thick walled, opening to hermaphroditic duct. Vitelline follicles numerous, large, usually irregular in shape, distributed from posterior margin of ventral sucker level to posterior to testis, converging in testis zone, not reaching posterior extremity. Eggs operculate; maximum 4 eggs in uterus. Miracidium not observed. Excretory vesicle Y shaped, bifurcating anterior at level of testis; excretory pore terminal (see Table II).

Taxonomic summary

Type host: *Poecilia latipinna* Lesueur (Poeciliidae).

Other definitive hosts: Poeciliidae: *Poecilia formosa* Girard, *Poecilia gillii* Kner, *Poecilia latipunctata* Meek, *Poecilia mexicana* Steindachner, *Poecilia sphenops* Valenciennes, *Poecilia velifera* Regan, *Poecilia* sp., *Poeciliopsis balsas* Hubbs, *Poeciliopsis gracilis* Heckel, *Pseudoxiphophorus bimaculata* Heckel, *Pseudoxiphophorus* sp., and *Xiphophorus helleri* Heckel. Cichlidae: *Herichthys cyanoguttatus* Baird and Girard, *Mayaheros urophthalmus* Gunther, *Paraneotroplus synspilus* Hubbs, *Paratheraps bifasciatus* Steindachner, *Vieja maculicauda* Regan, and *Thorichthys* sp.

Geographical distribution: Gulf of Mexico, Atlantic and Pacific Ocean slopes.

Type locality: United States, Galveston, Texas.

Other localities: Mexico: Ulumal, Campeche; Huixtla and Yauatepec, Morelos; Los Milagros, Quintana Roo; Río Frío and Río Purificación, Tamaulipas; Sontecomapan and Tlacotalpan, Veracruz; Costa Rica: Río Irigaray, Río Ciruelas, Río Las Vueltas, Playa Grande, Quebrada Puercos, Centeno; Honduras: Río Chamelecón; Nicaragua: Río Torsuani, Río Mico, and Palo de Arquito.

Previous records on poeciliid fishes: Mexico: Río Tecoloco, Río Amajac, Río San Pedro, and Arroyo Atenango, Hidalgo. El

Rodeo, La Planta, Río Verde, and Huichihuaya, San Luis Potosí. Río Puyacatengo, Tabasco. Los Tuxtlas, Río Palma, Arroyo Balzapote, Río Maquinas, Laguna Escondida, and Río Papaloapan, Veracruz. Cenote Azul and Río Hondo, Quintana Roo. Cenote Chen-Ha in Yucatán. Río Amacuzac, Morelos. Nicaragua: Caiio Marañón and Lago Nicaragua. Costa Rica: Guanacaste (see Watson, 1976; Scholz et al., 1995; Scholz and Vargas-Vázquez, 1998; Aguirre-Macedo et al., 2001; Salgado-Maldonado et al., 2001, 2004, 2005a, 2005b; Pineda-López et al., 2005; Sandlund et al., 2010; Pérez-Ponce de León et al., 2013).

Previous records on cichlid fishes: Mexico: Río San Pedro, Laguna El Guanal, Laguna Santa Anita, Laguna de las Ilusiones, Laguna El Espino, Estanque Tucta, and Río Camellones, Tabasco. Cenote Azul, Quintana Roo. Río La Antigua, Veracruz (see Salgado-Maldonado et al., 1997, 2004; Vidal-Martínez et al., 2001; Salgado-Maldonado, 2006).

Site in definitive hosts: Intestine.

Newly collected material: Mexico: 9 specimens from *P. formosa*, Río Purificación (CNHE No. cat. 10270); 1 specimen from *P. sphenops*, Tlacotalpan (CNHE No. cat. 10271), 2 from *Pseudoxiphophorus* sp., Yauatepec (CNHE No. cat. 10272); 15 specimens from *H. cyanoguttatus*, Río Purificación (CNHE No. cat. 10273); 14 specimens from *P. bifasciatus* (CNHE No. cat. 10274); and 13 from *Thorichthys* sp. Ulumal (CNHE No. 10275); Costa Rica: 6 specimens from *P. gillii*, Río Las Vueltas (CNHE No. cat. 10276), 3 specimens from *P. gillii*, Río Centeno (CNHE No. cat. 10277); Nicaragua: 7 specimens from *V. maculicauda*, Río Torsuani (CNHE No. cat. 10278).

Specimens deposited: GenBank accession number: KY489593-KY489633 by LSU and KY489542-80 by ITS2.

Remarks

The specimens analyzed in the current study from poeciliid fishes are morphologically similar to those of the original description of *S. sogandaresi* (see Lumsden, 1963). However, this study provides new metrical data for length of body, prepharynx, testis, ovary, eggs, and new metrical data for width of hermaphroditic sac, testis, and ovary (see Table II). We provided data to the external seminal vesicle and number of eggs that were not reported in the original description (see Table II). Specimens of *S. sogandaresi* have also been found in cichlid fishes from southeastern Mexico, the Pacific, and the Atlantic, including from the type and host locality of *S. cichlidorum* n. comb. We found that *S. sogandaresi* shows a wide morphological variation along its distribution area (see Table II). When *S. sogandaresi* infects cichlid fishes they resemble *S. cichlidorum* n. comb. in the metrical data and morphology (see Table II; Fig. 4B) suggesting that their morphology is affected by the host, but with the help of the molecular data, we confirm that those specimens represent 2 species. Our SEM analysis also shows a characteristic pattern of spination in the tegument that is useful to distinguish *S. sogandaresi* (tegument spined extended three-fourths of the body) from *S. cichlidorum* n. comb. (tegument entirely spined) (Fig. 5C–E, H–J). Our study suggests that the specimens previously identified as *Culuwiya cichlidorum* from cichlid fishes in Mexico (Salgado-Maldonado et al., 1997, 2004; Vidal-Martínez et al., 2001) and from a single poeciliid fish (Salgado-Maldonado, 2006) belong to *S. sogandaresi*. In addition, the other records of *S. sogandaresi* from other fish families need to be re-evaluated using

new molecular and morphometrical data to explore their taxonomic identities (Salgado-Maldonado et al., 2005a; Martínez-Aquino et al., 2014).

DISCUSSION

The ML and Bayesian trees inferred from the LSU and ITS2 data set in the current study consistently show that the species *Culuwiya cichlidorum* should be reallocated into *Saccocoelioides* as *S. cichlidorum* n. comb. and that it is closely related to *S. lamothei*. Our analysis also revealed that *S. cichlidorum* n. comb. and *S. sogandaresi* occur in sympatry in Nicaragua and Costa Rica (localities 12 and 21, see Fig. 1). In addition, *S. cichlidorum* n. comb. is distributed in 4 localities from 2 countries (Nicaragua and Costa Rica) associated with cichlid fishes. A second result of the phylogenetic analyses is that the specimens associated with poeciliid and cichlid fishes from Mexico, Honduras, Costa Rica, and Nicaragua belong to *S. sogandaresi*. The low genetic divergence found with the 2 molecular markers among the species of *Saccocoelioides* also supports the transfer of *S. cichlidorum* n. comb.; i.e., the genetic divergence among 5 species of *Saccocoelioides* (including *S. cichlidorum* n. comb.) with LSU ranged from 1% to 1.6% and with ITS2 ranged from 0.07% to 3.4%. These values of genetic divergence among species are similar to those between other haploporids. For instance, the genetic divergence found among 4 species of *Saccocoelium* (*Saccocoelium cephalis* Blasco-Costa, Montero, Gibson, Blabuena, Raga and Kostadinova, 2009, *Saccocoelium tensum* Looss, 1902, *Saccocoelium obesum* Looss, 1902, and *Saccocoelium brayi* Blasco-Costa, Balbuena, Raga, Kostadinova and Olson, 2010) ranged from 0.9% to 4.8% for LSU and from 2.1% to 10.9% for ITS2, between 2 species of *Dicrogaster* (*Dicrogaster perpusilla* Looss, 1902, and *Dicrogaster contracta* Looss, 1902) the genetic divergence was 4.6% and 8.7% for LSU and ITS2 respectively, and among 3 species of the genus *Capitimita* (*Capitimita* sp., *Capitimita darwinensis* Pulis and Overstreet, 2013, and *Capitimita costata* Pulis and Overstreet, 2013), ranged from 2.7% to 2.8% for LSU and from 7.3% to 11% for ITS2 (Blasco-Costa et al., 2009; Pulis and Overstreet, 2013).

The use of 2 molecular markers in the current study was fundamental to the phylogenetic analyses, which allowed us to reallocate *S. cichlidorum* n. comb. and better understand the systematic of *Saccocoelioides*, their ecological associations, and the distribution pattern of the 5 species of *Saccocoelioides* in Middle and North America. The ultrastructural studies provided extra comparative data. Thus we observed that *S. cichlidorum* n. comb. has the tegument entirely spined, whereas *S. sogandaresi* has tegumental spines covering only three-fourths of body (see Fig. 5).

With the reallocation of *S. cichlidorum*, the genus *Saccocoelioides* now contains 19 nominal species, with 5 distributed in Middle and North America (*S. sogandaresi*, *S. chauhani*, *S. lamothei*, *S. olmecae*, and *S. cichlidorum*) associated with freshwater and brackish water fishes, whereas the genus *Culuwiya* contains 5 species (*C. beauforti*, *C. overstreeti*, *C. papernai*, *C. tilapiae*, and *C. agonostomus*), parasites mainly of mullets and occasionally of freshwater fishes such as gobiids and cichlids (Overstreet and Curran, 2005). However, the morphological characters diagnostic that distinguished *Saccocoelioides* and *Culuwiya* overlaps and requires further taxonomic revision using

phylogenetic systematic and a re-examination of morphological and ecological characters.

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V. I. DISCUSIÓN GENERAL

En la familia Haploporidae (Digenea), la taxonomía alfa es la base actual de la clasificación (Overstreet y Curran, 2005), sin embargo, por la plasticidad fenotípica de los caracteres diagnósticos que presentan algunas especies (Blasco-Costa et al., 2009c) ha generado confusión en la delimitación de las mismas sobre todo con un grupo de organismos pequeños y en donde las estructuras internas diagnósticas son difíciles de caracterizar. A priori a este trabajo, los principales caracteres diagnósticos utilizados para diferenciar las especies de *Saccocoelioides* de Centro y Norte América eran: 1) Distribución del útero, 2) Posición del testículo, 3) Presencia de prefaringe, 4) Forma del cuerpo. En el presente trabajo se observó que algunos de estos caracteres están influenciados por la fijación y/o el proceso de montaje. Por ejemplo, la distribución del útero está ligado al aplanamiento de los trematodos ya que entre más aplanado se encuentre el ejemplar, la distribución del útero es más amplia. Por otro lado, encontramos que la forma del cuerpo, funciona como buen carácter para discernir entre especies, debido a que no es afectada por el aplanamiento como en otros caracteres. Asimismo, otro carácter que se propone en el presente estudio para diferenciar entre especies es la forma de los ciegos, ya que estos pueden ser delgados en algunas especies o en forma de saco en otras. En este sentido, los caracteres morfológicos diagnósticos deben ser tomados con precaución al momento de hacer la diagnosis de las especies. Por lo anterior, es indispensable emplear otros caracteres además de los morfológicos para delimitar a las especies correctamente y proponer un esquema filogenético que nos permita entender la evolución de los grupos así como sus asociaciones ecológicas con sus huéspedes definitivos.

Este es el primer estudio que aborda aspectos taxonómicos y de sistemática del género *Saccocoelioides*. El principal objetivo del trabajo fue determinar las especies del género *Saccocoelioides* que se distribuyen en México y Centro América. Con base en los datos generados se confirma la presencia de cuatro especies del género *Saccocoelioides* en México: *S. lamothei*, *S. chauhani*, *S. olmecae* y *S. sogandaresi*; y de una quinta especie que se distribuye en Centro América, *S. cichlidorum*. Además se logró dilucidar las relaciones filogenéticas entre estas especies.

Con base en nuevos ejemplares colectados en este trabajo, fue posible ampliar la

diagnosis de *S. lamothei* y *S. chauhani* así como describir de una nueva especie de *Saccocoelioides*. Esta especie fue denominada como *Saccocoelioides olmecae* con una distribución en las vertientes del Golfo de México, y que posee una estrecha relación con su huésped *Dormitator maculatus*.

Por otro lado, nuestros resultados sugieren que *S. sogandaresi* está asociada primordialmente a poeciliidos de Norte y Centro América; y cíclidos principalmente de México. El rango de distribución de esta especie abarca desde Texas (E.U.A.) hasta Costa Rica, siendo la especie de *Saccocoelioides* con el más amplio intervalo de distribución. Por lo anterior, los registros de la especie *S. sogandaresi* en distintas familias de peces como Characidae, Eleotridae, Mugilidae y Goodeidae (Salgado-Maldonado et al., 2005; Pérez-Ponce de León et al., 2007; Martínez-Aquino et al., 2014) deben ser evaluadas con otras fuentes de evidencia para conocer si en realidad pertenecen a esta especie. En el presente trabajo de tesis, se realizó una exhaustiva colecta de distintos huéspedes de diferentes familias de peces como Characidae, Goodeidae, Eleotridae, Gobiidae, Mugilidae, Poeciliidae y Cichlidae, sin embargo la especie *S. sogandaresi* solamente fue encontrada en las dos familias antes mencionadas (Poeciliidae y Cichlidae). En cambio, la especie *Saccocoelioides lamothei* se encontró parasitando algunas de las familias como Goodeidae, Eleotridae, Gobiidae, Poeciliidae y Mugilidae en ríos principalmente con vertientes al Océano Pacífico, siendo la especie con el menor grado de especificidad hospedatoria. Actualmente, un tercer manuscrito sobre la distribución de la especie *S. lamothei* así como un listado de huéspedes a los que está asociado fue recientemente sometido para su publicación a la revista *Zootaxa*.

Los resultados encontrados en el presente trabajo de tesis muestran que el género *Saccocoelioides* es versátil en su capacidad hospedatoria así como en su rango de distribución. Por ejemplo, encontramos que *S. chauhani* posee una especificidad hospedatoria muy alta, parasitando solamente una especie de hospedero en una localidad; mientras que la especie *S. lamothei* es una especie generalista que puede parasitar al menos 5 familias de peces en vertientes del Pacífico. Las otras tres especies (*S. sogandaresi*, *S. olmecae* y *S. cichlidorum*) tienen una especificidad hospedatoria alta parasitando una o dos familias de peces, pero con un amplio rango de distribución como es el caso más extremo de la especie *S. sogandaresi* que va desde Texas, Estados Unidos de América a Costa Rica

sobre todo con vertientes hacia el Golfo de México y Océano Atlántico. Con respecto a su distribución, el género *Saccocoelioides* se distribuye en la región Neotropical extendiéndose desde Argentina hasta Estados Unidos. Los huéspedes reportados en esta tesis son de origen neotropical, sin embargo se han distribuido a la región Neártica como es el caso de poeciliidos en Estados Unidos. La única familia de peces de origen Neártico que encontramos en este estudio pertenece a la familia Goodeidae que están parasitados por la especie *S. lamothei*, que es la especie generalista. Existen reportes en la Meseta Central de México donde otros parásitos han sido introducidos de los poeciliidos a los goodeidos debido a la introducción de diferentes especies de poeciliidos en la Meseta Central (García-Vásquez et al. 2017; Contreras-MacBeath et al. 2014). Con base en lo anterior, se propone que la especie de *S. lamothei* fue transfaunada también por los poeciliidos.

Por otra parte, uno de los objetivos planteados del presente trabajo fue probar mediante una taxonomía integrativa, si la especie previamente identificada como *C. cichlidorum* era representante del género *Culuwiya* o del género *Saccocoelioides*. Con base en la nueva evidencia generada en el actual estudio, se confirmó que *C. cichlidorum* pertenece al género *Saccocoelioides* como había sido propuesto inicialmente por Aguirre-Macedo et al., (2001). Por lo tanto, en este trabajo se hizo la transferencia de género, ahora denominada *Saccocoelioides cichlidorum*. Además se reconoció un patrón de espinación característico para la especie, el cual cubre todo el cuerpo. Asimismo, la evidencia ecológica y biogeográfica apoyaron la transferencia de *S. cichlidorum*. Esta especie solamente parasita peces de la familia Cichlidae en dos países de Centro América (Costa Rica y Nicaragua).

Con base en lo antes mencionado, el género *Culuwiya* propuesto por Overstreet y Curran (2005) para acomodar especies que habían sido descritas en su mayoría como *Saccocoelioides* de peces mugílidos debe revisarse empleando taxonomía integrativa. Únicamente un carácter morfológico diagnóstico (distribución del útero) es utilizado para distinguir ambos géneros, y ello crea confusión entre éstos, ya que la fijación de los especímenes puede modificar la distribución del útero. En cambio, el carácter ecológico referido como la asociación hospedatoria (parásito de peces estuarinos, en su mayoría lisas) puede ser la diferencia diagnóstica entre los dos géneros (*Culuwiya* y *Saccocoelioides*). Por ello, es trascendental coleccionar las especies de *Culuwiya* asociadas a peces del género *Mugil*

(Mugilidae) y analizarlas con nuevas herramientas complementarias a la taxonomía alfa. Con esto se podrá constatar la validez del género, y más aún se podrá conocer cómo son sus relaciones filogenéticas.

Finalmente, el presente trabajo permitió conocer las relaciones filogenéticas entre las especies de *Saccocoelioides* distribuidas en Centro y Norte América. No obstante, es importante coleccionar las especies de *Saccocoelioides* que se encuentran en América del Sur y analizarlas empleando taxonomía integrativa y con base en la nueva evidencia, aclarar la historia evolutiva de este grupo de parásitos y entender los patrones biogeográficos de las especies.

VII. CONCLUSIONES

1. Con base en caracteres morfológicos, ecológicos y moleculares, se reconoce una nueva especie de *Saccocoelioides* denominada como *Saccocoelioides olmecae* en seis localidades de las costas del Golfo de México.
2. Cuatro especies del género *Saccocoelioides* se encuentran distribuidas en México: *S. lamothei*, *S. chauhani*, *S. sogandaresi* y *S. olmecae*.
3. La especie *Saccocoelioides lamothei* se encuentra asociada al pez eleótrido *Dormitator latifrons* en el Océano Pacífico. Actualmente un tercer manuscrito sobre la distribución y las otras familias de peces que parasita fue enviado a la revista de *Zootaxa*.
4. La especie *Saccocoelioides chauhani* tiene una distribución restringida en el Lago de Catemaco, Veracruz como parásito del carácido *Astyanax aeneus*.
5. La especie *Saccocoelioides sogandaresi* tiene el más amplio rango de distribución en el Golfo de México (Estado Unidos de América y México) y en vertientes principalmente del Océano Atlántico en Centro América (Honduras, Nicaragua y Costa Rica). Esta especie está asociada a peces de las familias Poeciliidae y Cichlidae.
6. Los análisis filogenéticos derivados de genes nucleares, en combinación con datos morfológicos, apoyaron la transferencia de *Culuwiya cichlidorum* a *Saccocoelioides cichlidorum*, la cual se encuentra distribuida en dos países de Centro América: Costa Rica y Nicaragua, asociada a cíclidos.
7. Con la nueva composición taxonómica propuesta en este trabajo, el género *Saccocoelioides* contiene 19 especies distribuidas en el Continente Americano en la región Neotropical.

IX. REFERENCIAS

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