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TESIS DOCTORAL

Caracterización morfológica y molecular de *Protomicrocotyle* Johnston & Tiegs, 1922 (Monogenea: Protomicrocotylidae) de *Caranx* spp. (Carangiformes: Carangidae) de algunas localidades del Golfo de México

Para obtener el grado de

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Presenta

M. en C. Edgar Salvador Ramírez Cruz

Directora de tesis

Dra. Griselda Pulido Flores

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Instituto de Ciencias Básicas e Ingeniería
School of Engineering and Basic Sciences
Área Académica de Biología
Department of Biology

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Suplente

JFO/CIAF

Ciudad del Conocimiento, Carretera Pachuca-Tulancingo Km. 4.5 Colonia Carboneras, Mineral de la Reforma, Hidalgo, México. C.P. 42184
Teléfono: 52 (771) 71 720 00 Ext. 40063, 40064 y 40065
aab_icbi@uaeh.edu.mx, maritzal@uaeh.edu.mx

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Resumen general

Los monogéneos del género *Protomicrocotyle* son parásitos que se encuentran en los arcos branquiales (entre los filamentos del arco) de peces marinos de la familia Carangidae. Se caracterizan por tener un haptor asimétrico con cuatro clamps en fila longitudinal en un solo lado del haptor, los clamps son del tipo gastrocotilido por presentar una esclerita accesoria. El apéndice larvario es elongado en posición transversal y se encuentra armado con dos o tres pares de ganchos, los laterales son mayor tamaño. El órgano copulador masculino puede formar un bulbo muscular provisto de una corona de espinas. El poro genital está en posición ventral al esófago. El ovario se localiza en la mitad anterior o posterior del cuerpo. Los huevos tienen un filamento polar en cada polo, y puede presentarse de uno a tres en el útero. El vestíbulo vaginal abre en la región ventral, hacia la parte posterior derecha o izquierda del poro genital, se puede encontrar armada con numerosas espinas. Actualmente, el género Protomicrocotyle está representado por 10 especies a nivel global. En México, se han registrado cuatro especies, *Protomicrocotyle manteri* y *P. nayaritensis* en el océano Pacífico, y *P. mirabilis* y *P. veracruzensis* en el Golfo de México. Sin embargo, la distribución y presencia de *P. manteri* en el Golfo de México podría ser un error de identificación taxonómica, mientras que la amplia distribución de *P. mirabilis* podría estar sujeta a discusión. Con base en la distribución potencial de sus hospederos definitivos y en las diferentes condiciones ambientales presentes en su área de distribución, las provincias biogeográficas y ecorregiones marinas, así como el comportamiento de los hospederos se podría esperar, que los ejemplares de *P. mirabilis* registrados en la costa del Golfo de México y Mar Caribe correspondan a un conjunto de especies aún no descritas taxonómicamente. Por esto, el objetivo del presente trabajo fue evaluar la variación morfológica y molecular de las especies de Protomicrocotyle en diferentes localidades costeras del Golfo de México. Como parte de este trabajo, se analizaron especímenes de la Colección Nacional de Helmintos (CNHE) del Instituto de Biología de la Universidad Nacional Autónoma de México, de la Colección de Helmintos (CHE) del Centro de Investigaciones Biológicas de la Universidad Autónoma del Estado de Hidalgo, de Harold W. Manter Laboratory of Parasitology Collection (HWML) University of Nebraska–Lincoln, y especímenes recolectados en las localidades de Casitas, Veracruz; Ciudad del Carmen, Campeche; Puerto de Veracruz, Veracruz; Tampico, Tamaulipas; Tecolutla y Tuxpan, Veracruz. Los especímenes recolectados en campo se guardaron en alcohol al 70% y absoluto para estudios morfológicos y moleculares, respectivamente. Los especímenes que se utilizaron para estudios

morfológicos se tiñeron y montaron en preparaciones permanentes. Para los análisis morfológicos, los especímenes se midieron con un ocular micrométrico calibrado y adaptado a un microscopio óptico. Las medidas se capturaron en una matriz de datos y se analizaron con estadísticos multivariados. Para los estudios moleculares, de forma individual, cada espécimen fue procesado para realizar el procedimiento de digestión, extracción, amplificación y secuenciación del material genético. Todas las secuencias obtenidas con CO1, 28S y 18S se adjuntaron en una base de datos de cada gen con secuencias de otras especies de la familia Protomicrocotylidae y se analizó de forma independiente para la obtención de la matriz de divergencias genéticas y fenogramas de Neighbor-Joining. Para el análisis filogenético, se generaron matrices con los datos morfológicos, moleculares y combinados que se analizaron con el método de inferencia de Máxima Parsimonia. En total, 187 especímenes se analizaron con los análisis morfométricos y 91 variables morfológicas. Se muestran cinco nuevos morfotipos de *Protomicrocotyle* en los hospederos *Caranx hippos, C. latus* y *Caranx* sp., con distribución en el Golfo de México y Mar Caribe. En el análisis filogenético de evidencia total, se muestra a las especies de *Protomicrocotyle* de México como un grupo monofilético y a *Neomicrocotyle pacifica* como grupo hermano. La topología de la cladograma de evidencia total demuestra que Protomicrocotyle de México se divide en tres clados. Con el presente estudio, se contribuye al conocimiento de la diversidad de especies de este género de monogéneos y representa el primer estudio morfológico y filogenético de *Protomicrocotyle* en México.

Abstract

Monogeneans of the genus *Protomicrocotyle* are parasites that are found in the gill arches (between the filaments of the arch) of marine fish belonging to the Carangidae family. These parasites are distinguished by an asymmetrical haptor, comprising four clamps in a longitudinal row on one side. The clamps are of the gastrocotylid type, exhibiting an accessory sclerite. The haptoral lappet is elongated in a transverse position and is equipped with two or three pairs of hooks, with the lateral ones being larger in size. The male copulatory organ is capable of developing into a muscular bulb, which is furnished with a crown of spines. The genital pore is situated in a ventral position relative to the esophagus. The ovary is situated in either the anterior or posterior half of the body. The eggs are characterized by the presence of a polar filament at each pole, with the number of such filaments varying between one and three. The vaginal vestibule opens in the ventral region, in the vicinity of the right or left posterior aspect of the genital pore, and is equipped with a multitude of spines. At present, the genus *Protomicrocotyle* is represented by 10 species worldwide. In Mexico, four species have been documented: *Protomicrocotyle manteri* and *P. nayaritensis* in the Pacific Ocean, and *P. mirabilis* and *P. veracruzensis* in the Gulf of Mexico. However, the distribution and presence of *P. manteri* in the Gulf of Mexico may warrant further taxonomic scrutiny, while the wide distribution of *P. mirabilis* may be open to further discussion. Based on the potential distribution of its definitive hosts and the different environmental conditions present in its distribution area, as well as the behavior of the hosts, it can be postulated that the specimens of *P. mirabilis* recorded on the coast of the Gulf of Mexico and the Caribbean Sea correspond to a set of species not yet taxonomically described. In order to complete this work, specimens were obtained from several sources, including the National Helminth Collection (CNHE) of the Institute of Biology of the National Autonomous University of Mexico, the Helminth Collection (CHE) of the Center for Biological Research of the Autonomous University of the State of Hidalgo, the Harold W. The Maintain Laboratory of Parasitology Collection (HWML) at the University of Nebraska-Lincoln, along with specimens gathered from the localities of Casitas, Veracruz; Ciudad del Carmen, Campeche; Puerto de Veracruz, Veracruz; Tampico, Tamaulipas; Tecolutla and Tuxpan, Veracruz, were subjected to analysis. The specimens collected in the field were stored in 70% ethanol and absolute alcohol, respectively, for morphological and molecular studies. The specimens utilized for morphological studies were subjected to staining and mounting in permanent preparations. For the

purpose of conducting morphological analyses, the specimens were measured with the aid of a calibrated micrometer eyepiece, which was adapted to an optical microscope. The data matrix was utilized to record and subsequently analyze the measurements via multivariate statistical techniques. For the purpose of molecular study, each specimen was subjected to a series of individualized processes, including digestion, extraction, amplification and sequencing of the genetic material. All sequences obtained with CO1, 28S, and 18S were incorporated into a database comprising sequences from other species within the Protomicrocotylidae family. These sequences were then analyzed independently to generate a matrix of genetic divergences and Neighbor-Joining phenograms. For phylogenetic analysis, matrices were generated with the morphological, molecular, and combined data, which were then analyzed with the Maximum Parsimony inference method. In total, 187 specimens were subjected to morphometric analysis, with 91 morphological variables. Five novel morphotypes of *Protomicrocotyle* are illustrated on the hosts *Caranx hippos*, *C. latus*, and *Caranx* sp., with a distribution in the Gulf of Mexico and the Caribbean Sea. The total evidence phylogenetic analysis indicates that the *Protomicrocotyle* species from Mexico constitute a monophyletic group, with *Neomicrocotyle pacifica* identified as sister group. The total evidence cladogram reveals that the species of *Protomicrocotyle* from Mexico are distributed across three distinct clades. The present study contributes to the knowledge of species diversity within this monogenean genus and represents the first morphological and phylogenetic study of *Protomicrocotyle* in Mexico.

CAPÍTULO I

Historia taxonómica del género *Protomicrocotyle* Johnston & Tiegs, 1922

Taxonomic history of *Protomicrocotyle* Johnston & Tiegs, 1922

1. Introducción

El phylum Platyhelminthes es un grupo monofilético que incluye a gusanos planos, parásitos de fauna silvestre y en México es uno de los grupos más diversos de helmintos. Este taxón está conformado por las clases Trematoda, Cestoda y Monogenea (Zamparo et al., 2001; García-Prieto et al., 2014). En la clase Monogenea se incluyen especies de ectoparásitos de las branquias, narinas, línea lateral y piel de peces marinos y de aguas continentales (García-Prieto et al., 2014; Mendoza-Garfias et al., 2017), aunque también se han registrado en la cloaca y vejiga urinaria de anfibios y quelonios (Paredes-León et al., 2008; Cohen et al., 2013; Mendoza-Garfias et al., 2017).

Los integrantes de la clase Monogenea presentan un cuerpo generalmente bilateral, y en el caso de las especies con asimetría parcial se debe principalmente por la morfología del haptor. El cuerpo está dividido en las regiones cefálica, tronco y haptor (Figura 1.1). En la región cefálica o prohaptor se encuentran algunas estructuras de fijación, como las glándulas cefálicas, surcos adhesivos y/o ventosas, también se encuentra la apertura oral, faringe, esófago y ganglio nervioso. En la región del tronco se ubican los órganos reproductores masculino y femenino. El ciclo de vida de los monogéneos es monoxeno, la mayoría de las especies son ovíparas, aunque también existen especies vivíparas como las especies pertenecientes al género *Macrogyrodactylus* Malmberg, 1957 (Barson et al., 2010) y *Gyrodactylus* Nordmann, 1832, en las cuales, un individuo puede presentar hasta tres generaciones: como muñeca rusa, un gusano puede contener en su interior a su hija y esta, a su vez, a su nieta (Bakke et al., 2007).

En el caso de los monogéneos ovíparos se distinguen tres fases; el huevo, el oncomiracidio y el adulto. Los huevos son expulsados al medio acuático, la morfología del huevo varía considerablemente entre especies, géneros y familias, pero en general, tienen dos filamentos polares, los cuales pueden ser largos o cortos, con los que se fijan al hospedero (Kearn, 1986). Al eclosionar, la larva que se libera se denomina oncomiracidio, en la mayoría de las especies es un organismo ciliado, sin embargo, existen larvas no ciliadas como en la especie *Acanthocotyle greeni* MacDonald y Llewellyn, 1980 (MacDonald & Llewellyn, 1980). Esta larva tiene un promedio de 24 horas de vida, dependiendo de la temperatura del agua, al encontrar un hospedero se fija en él y comienza la madurez sexual (Buchmann & Bresciani, 2006a; Roberts & Janovy, 2009).

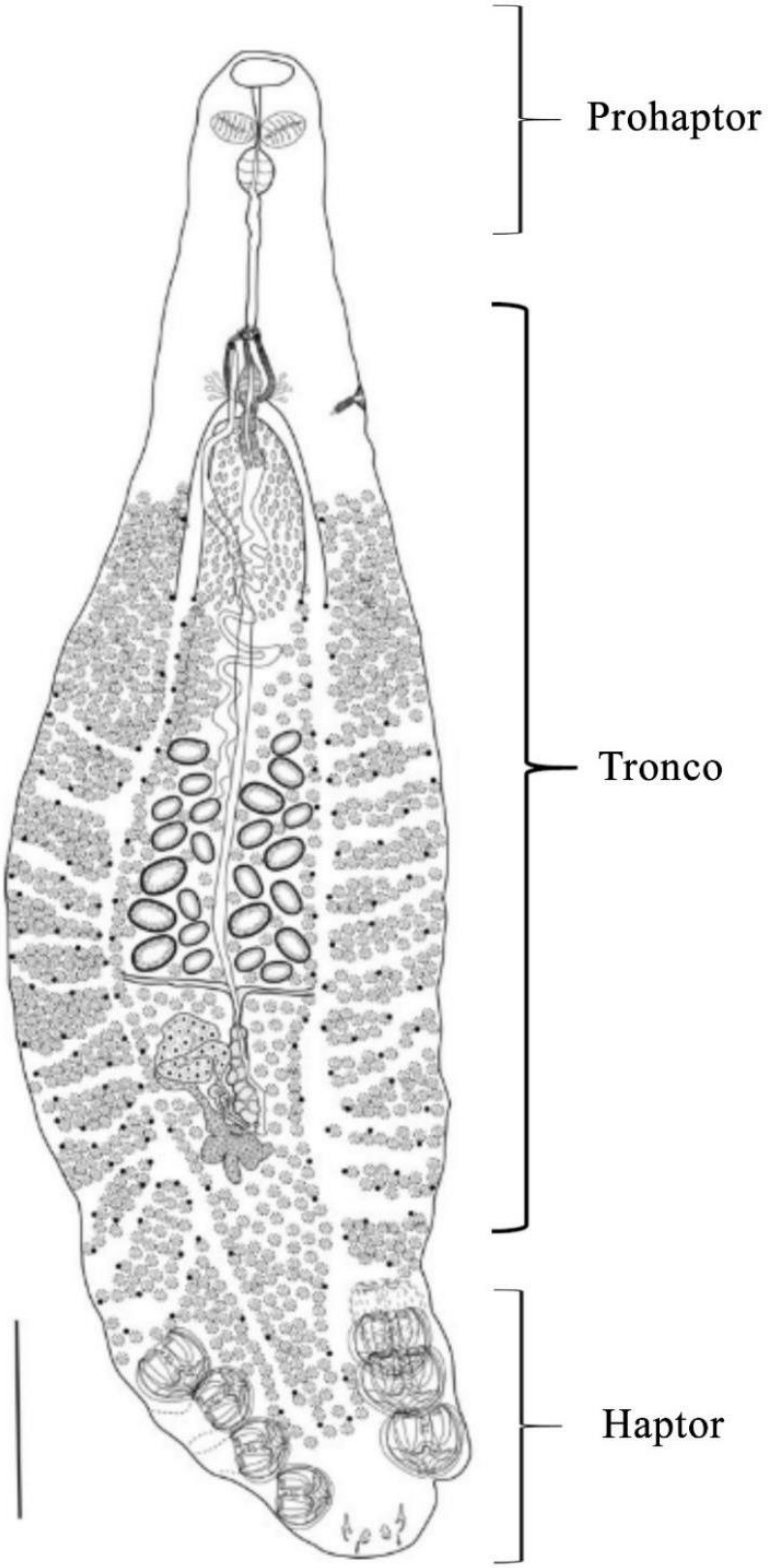

Figura 1. 1. Morfología general de un monogéneo. Representación de *Pseudomazocraes sulamericana* Camargo y Santos, 2019, barra de escala = 100 µm (tomado y editado de Camargo & Santos (2020)).

El haptor, es el órgano de fijación, se compone por diversas estructuras de adhesión, como anchors o hamulis (ganchos grandes), hooklets (ganchos marginales), clamps (abrazaderas o pinzas esclerosadas), ventosas (Figura 2). Estas estructuras de fijación son variables en los distintos géneros y especies de monogéneos (Roberts & Janovy, 2009; Justine et al., 2013; García-Prieto et al., 2014).

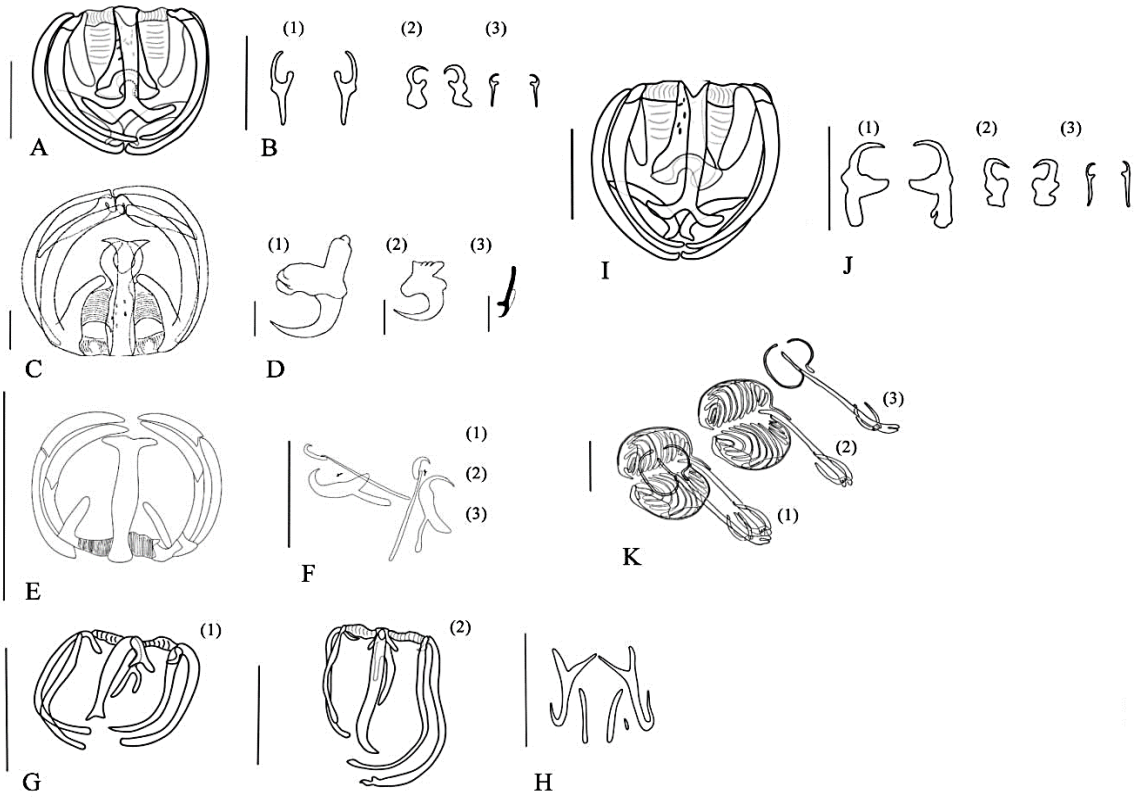

Figura 1. 2. Diversidad de estructuras asociadas al haptor. (A) clamps de *Pseudomazocraes sulamericana*, escala= 50 µm; (B) ganchos, (1) gancho larval más grande; (2) gancho mediano; (3) gancho pequeño, escala= 50 µm (Camargo & Santos, 2020). (C) clamp de *Protomicrocotyle mirabilis* (MacCallum, 1918) Johnston y Tiegs, 1922, escala= 10 µm; (D) ganchos (1) gancho lateral; (2) gancho; (3) gancho medio, escala= 10 µm (Kritsky et al., 2011). (E) clamp cerrado de *Axinoides euzeti* Châari, Derbel y Neifar, 2016, escala= 50 µm. (F) Ganchos, (1) hamuli mediano; (2) uncinuli postero-lateral; (3) hamuli lateral, escala= 50 µm (Châari et al., 2016). (G) clamps de *Cemocotyle carangis* (MacCallum, 1913) Sproston, 1946. (1) clamp grande; (2) clamp chico, escala= 50 µm (H) larval hooks, barra de escala= 50 µm. (I) Clamps de *Pseudomazocraes selene* Hargis, 1957, barra de escala= 50 µm. (J) ganchos (1) ancla más grande; (2) ancla mediana; (3) anzuelos larvales más pequeños, escala= 50 µm (Camargo y Santos, 2019). (K) clamps de *Heteromicrocotyloides megaspinosus* Barton, Beaufrère, Justine y Whittington, 2009, escala= 50 µm. (1) clamp con pedúnculo completo compuesto por la válvula grande y chica; (2) válvula grande; (3) válvula chica (Barton et al., 2009).

Se estima que existen 13,570 especies de monogéneos. Sin embargo, menos de 2,000 especies se han descrito alrededor del mundo (Hugot et al., 2001; García-Prieto et al., 2014). En México, la diversidad de esta clase asciende a 313 especies nominales que han sido registradas en peces dulceacuícolas, marinos, de agua salobre, en anuros y tortugas (Mendoza-Garfias et al., 2017). En *Caranx caballus* Günther, 1868, *C. hippos* (Linnaeus, 1766), *C. crysos* (Mitchill, 1815), y *C. latus* Agassiz, 1831 (Cuadro 1.1) pertenecientes a la familia Carangidae Rafinesque, 1815, se han reportado especies de monogeneos de las familias Allopyragraphoridae Yamaguti, 1953, Chauhaneidae Euzet & Trilles, 1960, Heteraxinidae Unnithan, 1957, Pyragraphoridae Yamaguti, 1963 y Protomicrocotylidae Johnston & Tiegs, 1922 (Cuadro 1.1) (Mendoza-Garfias et al., 2017).

La familia Protomicrocotylidae Johnston y Tiegs, 1922 está integrada por los géneros: *Abortipedia* Unnithan, 1962; *Bilaterocotyle* Chauhan, 1945; *Bilaterocotyloides* Ramalingam, 1961; *Chauhanocotyle* Khoche y Dad, 1975; *Lethacotyle* Manter y Price, 1953; *Neomicrocotyle* Ramalingam, 1960; *Protomicrocotyle* Johnston y Tiegs, 1922; *Vallisiopsis* Subhapradha, 1951 y *Youngiopsis* Lebedev, 1972 (Lebedev, 1986; WoRMS, 2024). Las características morfológicas diagnósticas para Protomicrocotylidae son un cuerpo musculoso aplanado y de forma ovalada irregular y asimétrica. El haptor, que es la estructura de fijación que utilizan para adherirse a los arcos branquiales de los peces, se forma de dos partes oponibles de diferente origen morfológico (Yamaguti, 1963). En la primera sección del haptor se encuentran los clamps (=abrazaderas) y se hallan en diferente número como son cuatro en *Protomicrocotyle*, *Neomicrocotyle* y *Abortipedia* (Ramalingam, 1960; Unnithan, 1962; Bravo-Hollis, 1966; Bravo-Hollis, 1979; Kritsky et al., 2011), seis y arregladas en dos filas como en *Bilaterocotyle chirocentrosus* Chauhan, 1945 (Chauhan, 1945), y *Bilaterocotyloides carangis* Ramalingam, 1961 (Yamaguti, 1963), ocho y arregladas en dos filas como en *Vallisiopsis contorta* Subhapradha, 1951 (Subhapradha, 1951; Al-Zubaidy, 2013), siete y arreglados en dos filas como en *Youngiopsis australis* (Young, 1968) Lebedev, 1972 (Young, 1968) o ausentes como en *Lethacotyle vera* Justine, Rahmouni, Gey, Schoelinck y Hoberg, 2013 y *L. fijiensis* Manter y Price, 1953 (Manter & Prince, 1953; Justine et al., 2013) (Figura 3). La segunda parte del haptor se forma por el apéndice larvario muscular y transversalmente alargado, y en él se encuentran tres pares de ganchos de diferente tamaño (Figura 1.3) (Chauhan, 1945; Yamaguti, 1963; Kritsky et al., 2011; Justine et al., 2013).

Algunas características importantes del aparato reproductor es que el ovario se localiza en la región posterior a los testículos, el vestíbulo vaginal y poro genital se ubican en la región anterior del cuerpo en posición anterior o posterior a la bifurcación cecal, estos están armados con un número variable de espinas de diferente tamaño. Los testículos se encuentran en la parte central del cuerpo, presentan variación en cuanto a su forma y número. Los adultos llegan a presentar de uno a tres huevos operculados y con filamentos polares (Chauhan, 1945; Yamaguti, 1963; Kritsky et al., 2011; Justine et al., 2013). Actualmente, se cuenta con un registro de 42 especies de la Protomicrocotylidae (WoRMS, 2024).

El género *Protomicrocotyle* Johnston & Tiegs, 1922 fue descrito originalmente como *Acanthodiscus* MacCallum, 1918 para integrar a la especie tipo *Acanthodiscus mirabile* MacCallum (1918) (MacCallum, 1918). Posteriormente, el género fue revisado y renombrado como *Protomicrocotyle* y la especie tipo se cambió a *Protomicrocotyle mirabilis* (MacCallum, 1918) Johnston & Tiegs, 1922 parásito de *Caranx hippos* obtenido del acuario de Nueva York (Johnston & Tiegs, 1922; Yamaguti, 1963). En la actualidad, este género se integra por diez especies; *P. mirabilis* en el Oceano Atlántico, y se ha reportado en Alligator, Harbour Florida (Hargis, 1957), Everglades National Park, Florida (Kritsky et al., 2011), y en Port Aransas, Texas (Koratha, 1955b) en Estados Unidos de América. En México, se han realizado reportes en diferentes localidades del Estado de Campeche, Quintana Roo, Tamaulipas y Veracruz (Caballero y Caballero & Bravo-Hollis, 1967; Bravo-Hollis, 1989; Montoya-Mendoza et al., 2017; 2021), y también existen registros en Puerto Rico, Venezuela y Brasil (Bunkley-Williams & Williams, 1994; 1995; Williams & Bunkley-Williams. 1996; Luque et al., 2000; Luque & Ramos Alves, 2001; Boada et al., 2012); *P. celebensis* Yamaguti, 1953 se registró en la isla Célebes, Indonesia en *Caranx* sp. (Yamaguti, 1953); *P. madrasensis* Ramalingam, 1960 en *C. affinis* Rüppell, 1836, *P. mannarensis* Ramalingam, 1960 y *P. minutum* en *C. sexfaciatus* Quoy & Gaimard, 1825 de la India (Ramalingam, 1960); *P. manteri* se registró en La Paz, Baja California, México como parásito de *Trachinotus paitensis* Cuvier, 1832 (Bravo-Hollis, 1966); *P. ivoriensis* Wahl, 1972 en la Laguna Ebrié cerca de Abidjan en *C. hippos* (Wahl, 1972); *P. carangis* Sivasankar Pillai & Krishna Pillai, 1978 de Hawaii en el pez *C. lugubris* Poey, 1860 (WoRMS, 2024) y *P. nayaritensis* en Isla Isabel, Nayarit, Mexico en *C. hippos caninus* (= *C. caninus* Günther, 1867) (Bravo-Hollis, 1979). Recientemente se describió la especie *Protomicrocotyle veracruzensis* Ramírez-Cruz,

Monks, Manríquez-Morán, Violante-González, & Pulido-Flores, 2023 en *C. latus* de las localidades de Casitas y Puerto de Veracruz, Veracruz (Ramírez-Cruz et al., 2023).

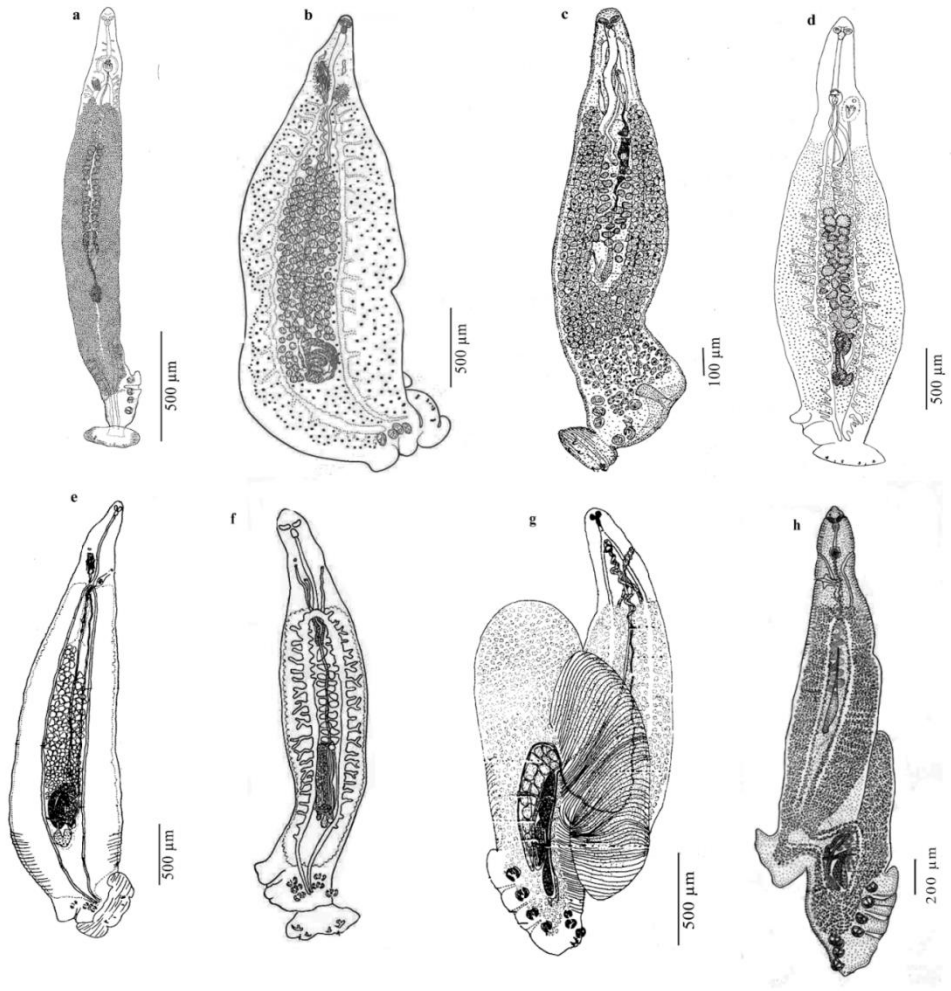

Figura 1. 3. Algunas especies de la familia Protomicrocotylidae. (a) *Protomicrocotyle mirabilis* (Kritsky et al., 2011); (b) *Abortipedia indica* Unnithan, 1962 (Unnithan, 1962); (c) *Bilaterocotyle chirocentrosus* (Chauhan, 1945); (d) *Lethacotyle fijiensis* (Manter y Price, 1953); (e) *Neomicrocotyle indica* Ramalingam, 1960 (Ramalingam, 1960); (f) *Bilaterocotyloides carangis* (Yamaguti, 1963); (g) *Youngiopsis australis* (Young, 1968) y (h) *Vallisiopsis contorta* (Subhapradha, 1951).

Las características morfológicas de *Protomicrocotyle* son la presencia de un haptor asimétrico con cuatro *clamps* en fila longitudinal en un solo lado del haptor, los clamps son del tipo gastrocotilido por presentar una esclerita accesoria (Figura 4) (Yamaguti, 1963; Justine et al., 2013). El apéndice larvario es elongado en posición transversal, o en forma de campana, y se encuentra armado con dos o tres pares de ganchos, los laterales son mayor tamaño. El esófago

presenta divertículos y los ciegos intestinales son ramificados. Las glándulas vitelógenas pueden o no extenderse hasta el extremo posterior del cuerpo. Los testículos se ubican en posición anterior al ovario, en la tercera porción del cuerpo, estos pueden ser ovales o elongados, y de número variable. El órgano copulador masculino puede formar un bulbo muscular provisto de una corona de espinas. El poro genital está en posición ventral al esófago. El ovario puede estar enrollado, plegado o compacto, se localiza en la mitad anterior o posterior del cuerpo. Los huevos tienen un filamento polar en cada polo, y puede presentarse de uno a tres en el útero. El vestíbulo vaginal abre en la región ventral, hacia la parte posterior derecha o izquierda del poro genital, se puede encontrar armada con numerosas espinas. Son parásitos principalmente de peces de la familia Carangidae (Yamaguti, 1963).

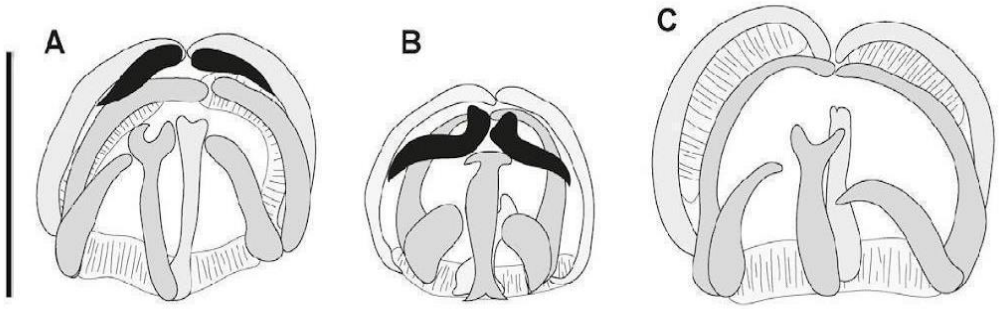

Figura 1. 4. Ejemplos de clamps (=abrazaderas) en diferentes géneros de la familia Protomicrocotylidae. **A.** *Bilaterocotyloides*. **B.** *Protomicrocotyle*. **C.** *Neomicrocotyle*, escala= 50 µm. Esclerita accesoria característica del clamp tipo gastrocotilido en negro (Justine et al., 2013).

Cuadro 1. 1. Monogéneos registrados en *Caranx crysos*, *C. hippos* y *C. latus* en el océano Pacífico y Golfo de México.

Monogéneo	Hospedero	Localidad	Océano	Referencia
Ahpua piscicola	*Caranx caballus*	Salina Cruz, Oaxaca	Pacífico	Lamothe-Argumedo et al., 1997
	C. hippos	Ciudad del Carmen, Campeche	Golfo de México	
	C. latus♦	Salina Cruz, Oaxaca	Pacífico	
Allopyragraphorus incomparabilis	*C. crysos*	Isla Mujeres, Quintana Roo		Mendoza-Garfias et al., 2017
Allopyragraphorus caballeroi	*C. caballus*	Zihuatanejo, Guerrero		
	C. hippos♦	Manzanillo, Colima		
		Bahía de Chamela, Jalisco		
		Salina Cruz, Oaxaca		
Allopyragraphorus hippos	*C. hippos*	Alvarado, Veracruz	Golfo de México	Montoya-Mendoza et al., 2008
Allopyragraphorus winteri	*C. hippos*	Ciudad del Carmen, Campeche		Mendoza-Garfias et al., 2017
		Sontecomapan, Veracruz		Bravo-Hollis, 1989
	C. latus	Laguna Madre, Tamaulipas		Iruegas-Buentello, 1999
Axine sp.	*C. hippos*	Tuxpan, Veracruz		Caballero y Caballero & Bravo-Hollis, 1965b
Cemocotyle carangis	*Caranx crysos*	Alvarado, Veracruz		Montoya-Mendoza et al., 2008
	C. hippos			

Cuadro 1.1. *Continuación*.

Cemocotyle noveboracensis	*C. crysos*	Alvarado, Veracruz	Golfo de México	Montoya-Mendoza et al., 2008
	C. hippos	Alvarado, Veracruz		
		Campeche, Campeche		
		Ciudad del Carmen, Campeche		
		Arrecife el Cabezo, Veracruz		
		El Saladero, Veracruz		
		Sontecomapan, Veracruz		
	C. latus	Laguna Madre, Tamaulipas		Iruegas-Buentello, 1999
Cemocotylella elongata	*C. hippos*♦	Bahía de Chamela, Jalisco	Pacífico	Mendoza-Garfias et al., 2017
		Arrecife el Cabezo, Veracruz	Golfo de México	
	C. latus	Bahía de Chetumal, Quintana Roo		Montoya-Mendoza et al., 2008
Neomicrocotyle carangis	*Caranx* sp.	Bahía de Chamela, Jalisco	Pacífico	Lamothe-Argumedo et al., 1997
Neomicrocotyle pacifica	*C. crysos*	Bahía de la Paz, Baja California Sur		
	C. caballus	Bahía de Chamela, Jalisco		Pérez-Ponce de León et al., 1999
	C. hippos♦			
	C. caballus	Puerto Ángel, Oaxaca		Lamothe-Argumedo et al., 1997

Cuadro 1.1. *Continuación*.

	C. hippos♦		Pacífico	Bravo-Hollis & Salgado-Maldonado, 1985
Protomicrocotyle manteri	*C. crysos*	Bahía de la Paz, BCS		Lamothe-Argumedo et al., 1997
	C. hippos♦			
	C. caballus	Bahía de Chamela, Jalisco.		Pérez-Ponce de León et al., 1999
	C. hippos♦			
	Caranx sp.			
	C. hippos♦	San Blás, Nayarit		
		Puerto Escondido, Oaxaca.		Lamothe-Argumedo, 1970
	Caranx sp.			Mendoza-Garfias et al., 2017
Protomicrocotyle mirabilis	*C. hippos*	Campeche, Campeche.	Golfo de México	Caballero y Caballero & Bravo-Hollis, 1967
	C. latus	Bahía de Chetumal, Quintana Roo.	Mar Caribe	Bravo-Hollis, 1989
	C. crysos	Isla Cozumel, Quintana Roo.		
	Caranx sp.	Isla Mujeres, Quintana Roo		

Cuadro 1.1. *Continuación*.

	C. latus	Laguna Madre, Tamaulipas	Golfo de México	Iruegas-Buentello, 1999
	C. crysos	Arrecife el Cabezo, Veracruz		Montoya-Mendoza et al., 2008
	C. hippos	Las Barrancas, Veracruz		
		El Saladero, Tamiahua, Veracruz		Porraz-Álvarez, 2006
	C. hippos	Laguna de Sontecomapan, Veracruz		
	C. latus	Tuxpan, Veracruz		Caballero y Caballero & Bravo-Hollis, 1965a
Protomicrocotyle veracruzensis	*C. latus*	Casitas, Veracruz		Ramírez-Cruz et al., 2023
		Puerto de Veracruz, Veracruz		
Protomicrocotyle nayaritensis	*C. hippos*	Isla Isabel, Nayarit.	Pacífico	(Bravo-Hollis, 1979)
		San Blas, Nayarit.		Lamothe-Argumedo et al., 1997
Pseudomazocraes selene	*C. caballus*			
	C. crysos	Arrecife el Cabezo, Veracruz.	Golfo de México	Montoya-Mendoza et al., 2008
		Jicacal, Veracruz		Bravo-Hollis, 1989

Cuadro 1.1. *Continuación*.

		Alvarado, Veracruz	Golfo de México	Montoya-Mendoza et al., 2008
	C. hippos♦	Bahía de Chamela, Jalisco	Pacífico	
		Jicacal, Veracruz	Golfo de México	Bravo-Hollis, 1989
		Alvarado, Veracruz		Montoya-Mendoza et al., 2008
	C. latus	Tuxpan, Veracruz		Caballero y Caballero & Bravo-Hollis, 1965a
Pseudomazocraes monsivaisae	*C. hippos*♦	Salina Cruz, Oaxaca	Pacífico	Mendoza-Garfias et al., 2017
		Zihuatanejo, Guerrero		
Pseudomazocraes riojai		Salina Cruz, Oaxaca		Caballero y Caballero & Bravo-Hollis, 1963
Salinacotyle mexicana		Salina Cruz, Oaxaca		
Zeuxapta seriolae		Zihuatanejo, Guerrero		Mendoza-Garfias et al., 2017

♦Estas especies no se distribuyen en el Océano Pacífico, por lo que los registros realizados pertenecen a otras especies del género *Caranx*.

2. Justificación

En México, se ha realizado la descripción de tres especies de *Protomicrocotyle*: *P. manteri*, y *P. nayaritensis* en el Océano Pacífico (Bravo-Hollis, 1966; 1979), y *P. veracruzensis* en el Golfo de México (Ramírez-Cruz et al., 2023), respectivamente. Así como registros de *P. mirabilis* en diferentes localidades de las costas del Golfo de México y Mar Caribe (Bravo-Hollis, 1989; Ramírez-Cruz et al., 2023), y *P. manteri* (Lamothe-Argumedo, 1976; Bravo-Hollis, 1989). Sin embargo, la distribución y presencia de *P. manteri* en el Golfo de México podría ser un error en la identificación taxonómica, mientras que la amplia distribución de *P. mirabilis* podría estar sujeta a discusión. Con base en la distribución potencial de sus hospederos definitivos (peces de la familia Carangidae) y a las diferentes condiciones ambientales a las que se someten, considerando las provincias biogeográficas y ecorregiones marinas, así como el comportamiento del hospedero se podría esperar, que los ejemplares de *Protomicrocotyle mirabilis* que se han registrado en la costa del Golfo de México y Mar Caribe correspondan a un conjunto de especies que aún no se han identificado. Por lo cual, se requiere de la evaluación morfométrica de los ejemplares almacenados en las diferentes colecciones biológicas de helmintos y obtener material genético para complementar la información morfológica. Es por esta razón, que en el presente trabajo se utilizarán las herramientas de la taxonomía integrativa para abordar la delimitación y caracterización de los monogéneos del género *Protomicrocotyle* del Golfo de México con el objetivo de realizar la delimitación de las especies de este género.

3. Hipótesis

Hipótesis nula

La especie de *P. mirabilis* descrita para el Golfo de México es una única especie con variación intraespecífica en las diferentes poblaciones.

Hipótesis alternativa

La especie de *Protomicrocotyle mirabilis* descrita en el Golfo de México en peces de la familia Carangidae no representan una única especie, por lo que podría ser un conjunto de especies aún no descritas para la ciencia.

4. Objetivos

Objetivo general

Evaluar la variación morfológica y molecular de las especies de *Protomicrocotyle* en diferentes localidades del Golfo de México.

Objetivos específicos

Contribuir al conocimiento de la diversidad de especies de *Protomicrocotyle* en el Golfo de México.

Realizar la caracterización morfométrica de las especies de *Protomicrocotyle* en el Golfo de México.

Inferir con estadísticos multivariables y marcadores moleculares la diferenciación de las especies de *Protomicrocotyle*.

Proponer una hipótesis de relaciones filogenéticas de *Protomicrocotyle* y su posición filogenética con respecto a otros miembros de Protomicrocotylidae con base a información morfológica y molecular.

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CAPÍTULO II

A new morphotypes of *Protomicrocotyle* Johnston & Tiegs, 1922 (Monogenea: Protomicrocotylidae) in fish of the genus *Caranx* Lacepède, 1801 (Carangiformes: Carangidae) from the Gulf of Mexico and the Caribbean Sea

Nuevos morfotipos de *Protomicrocotyle* Johnston & Tiegs, 1922 (Monogenea: Protomicrocotylidae) en peces del género *Caranx* Lacepède, 1801 (Carangiformes: Carangidae) del Golfo de México y Mar Caribe

Without taxonomy to give shape to the bricks, and systematics to tell us how to put them together, the house of biological science is a meaningless jumble.

Lord Robert M. May (1990)

1. Introduction

The biological collections are a systematized collection of biological material where cured specimens are protected, preserved, and studied. They are of great importance in the development of biological sciences, since they are the reservoirs and the reference of the knowledge of biological diversity available for a region of a municipality, state, and/or country (Navarro et al., 2003; Luna Plascencia et al., 2011). Mexico is one of the main world megadiverse countries. With around 200,000 different species, Mexico is home to the 10 to 12 percent of the world's Biodiversity (Martínez-Meyer et al., 2014), and therefore different national and state biological collections are responsible for preserving biological material.

The Colección Nacional de Helmintos (CNHE) of the Laboratorio of Helmintología of the Instituto de Biología, of the Universidad Nacional Autónoma de Mexico, founded in 1932 by Dr. Eduardo Caballero y Caballero, is considered the most important of its kind in this country and was created to safeguard the different species of helminths that parasitize the vertebrates of Mexico, as well as specimens that were donated by researchers from other countries (Lamothe-Argumedo et al., 1997). The helminths are a polyphyletic group of parasitic worms that include members of the phylum Platyhelminthes (digeneans [flukes], aspidogastreans, monogeneans, and cestodes [tapeworms]), Phylum Acanthocephala (spiny-headed worms), Phylum Nematoda (roundworms), and Phylum Annelida (only hirudineans) (Pérez-Ponce de León et al., 2011). This group is integrated by invertebrates characterized by elongated, flat or round bodies, different sizes, ranging from very small, less than a millimeter, to several meters in length, and with specialized anatomical structures to adhere to the host organism they parasitize. The life cycles of the helminths can be divided into two types: monoxenous (direct life cycle): intermediate host is not required, and heteroxenous (indirect life cycle): at least one or two intermediate hosts are required for biological development (Pérez-Ponce de León et al., 2011; Thaenkham et al., 2022c).

In Mexico, the diversity of the species of helminths amounts to a total of 1,508 nominal species (Martínez-Meyer et al., 2014). Platyhelminthes are the most species-rich group with 1,015 species (García-Prieto et al., 2014), followed by Nematoda with 402 (García-Prieto et al., 2014c), Acanthocephala with 60 (García-Prieto et al., 2014a), and finally Euhirudinea (leeches) with 31 recorded species (Oceguera-Figueroa & León-Règagnon, 2014). Monogeneans are a non-monophyletic group, common parasites of freshwater and marine fish, and mainly comprise

ectoparasitic flatworms with a monoxenous life cycle (Buchmann & Bresciani, 2006b; Mendoza-Garfias et al., 2017). This group of helminths includes an approximate global diversity of 5,000 monogenean species comprising 716 genera and 63 families have been described to date (Caira & Littlewood, 2013). In Mexico, this class is the second group with the highest number of recorded species, with approximately 313 nominal species and 54 undetermined taxa; altogether, the records correspond to 162 genera included in 29 families (Mendoza-Garfias et al., 2017).

The monogeneans members of the Protomicrocotylidae family are characterized by being parasites of the gill arches of marine fish, mainly carangid fish (Ramalingam, 1960; Yamaguti, 1963; Caballero y Caballero & Bravo-Hollis, 1965a; Caballero y Caballero & Bravo-Hollis, 1967; Bravo-Hollis, 1979; Kritsky et al., 2011). Currently, is represented by nine genera: *Abortipedia* Unnithan, 1962, *Bilaterocotyle* Chauhan, 1945, *Bilaterocotyloides* Ramalingam, 1961, *Chauhanocotyle* Khoche & Dad, 1975, *Lethacotyle* Manter & Price, 1953, *Neomicrocotyle* Ramalingam, 1960, *Protomicrocotyle* Johnston & Tiegs, 1922, *Vallisiopsis* Subhapradha, 1951, and *Youngiopsis* Lebedev, 1972, with approximately 43 species assigned to the family (WoRMS, 2024). Species of *Protomicrocotyle* are found as parasites of fish of the family Carangidae Nelson, 1984, mainly of the genera *Caranx* Lacepède, 1801 and *Trachinotus* Lacepède, 1801 (Yamaguti, 1953; Hargis, 1957; Ramalingam, 1960; Caballero y Caballero & Bravo-Hollis, 1965a; Bravo-Hollis, 1966; Wahl, 1972; Bravo-Hollis, 1979; Iruegas-Buentello, 1999; Luque & Ramos Alves, 2001; Kritsky et al., 2011; Boada et al., 2012; Montoya-Mendoza et al., 2017; Violante-González et al., 2019; Vianna et al., 2020).

Protomicrocotyle currently contains 10 species: *P. mirabilis* (type species) in the Atlantic Ocean in *C. hippos* from the New York Aquarium (MacCallum, 1918); *P. manteri* from La Paz, Baja California Sur, Mexico in *Trachinotus paitensis* (Bravo-Hollis, 1966); *P. nayaritensis* from the Isla Isabel, Nayarit, Mexico in *C. caninus* (described as *C. hippos caninus*) (Bravo-Hollis, 1979); *P. ivoriensis* Wahl, 1972 from Ebrié Lagoon, Ivory Coast, Western Africa in *C. hippos* (Wahl, 1972); *P. celebesensis* Yamaguti, 1953 from Celebes Island, Indonesia from *Caranx* sp. (Yamaguti, 1953); *P. madrasensis* in *C. affinis*, *P. mannarensis* and *P. minutum* in *C. sexfasciatus* from India (Ramalingam, 1960); *P. carangis* Pillai & Pillai, 1978 from Kerala coast, India in *C. ignobilis* (Forsskål, 1775) (= *C. sansun* [Fabricius, 1775]) (Pillai & Pillai, 1978); and

P. veracruzensis from Casitas and Puerto de Veracruz, Veracruz, Mexico in *C. latus* (Ramírez-Cruz et al., 2023).

The historical research on helminthology in Mexico has furnished us with the requisite information to ascertain the diversity of helminth species and to comprehend the ecological, biogeographical, and evolutionary processes of parasites, as well as significant data regarding the relationship between parasites and their hosts. Consequently, the endeavor to persist in documenting species diversity by procuring specimens housed in biological collections and specimens gathered in the field contributes to augmenting species diversity. Therefore, the objective of this study was to characterize five morphotypes of the genus *Protomicrocotyle* based on morphological data and morphometric analysis with multivariate statistics.

2. Material and methods

Sample area, material examined and specimen's deposit

During different sampling trips, 85 specimens of *Caranx hippos* and five of *C. latus* were collected from six localities along the Gulf of Mexico between 2019 and 2023 (Table 2.1). Sampled localities were: Casitas, Veracruz (20° 15' 31.5" N, 96° 47' 49.5" W), Ciudad del Carmen, Campeche (18° 38 ′ 18″ N, 91° 50′ 07″ W), Puerto de Veracruz, Veracruz (19° 13' 11.2" N, 96° 09' 24.4" W), Tampico, Tamaulipas (22° 15' 52.7" N, 97° 45' 20.5" W), Tecolutla (20° 28′ 39″ N, 97° 00′ 30″ W) and Tuxpan, Veracruz (20° 57′ 46″ N, 97° 24′ 01″ W) (Figure 1).

Table 2. 1. Sampled localities in the present study.

Locality	Species	Number of fish collected	Collection date	Coordinates
Casitas	*Caranx hippos*	22	14 Nov 2021	20° 15' 31.5" N, 96° 47' 49.5" W
		7	21 May 2022	
		2	10 Ago 2022	
		4	17 May 2023	
	C. latus	3	19 Aug 2019	

Table 2. 1. *Continuation*.

Ciudad del Carmen	*C. hippos*	10	10 Sep 2022	18° 38 ′ 18″ N, 91° 50′ 07″ W
Puerto de Veracruz	*C. hippos*	8	24 May 2022	19° 13' 11.2" N, 96° 09' 24.4" W
	C. latus	1	24 May 2022	
Tampico		6	21 Oct 2022 25 May 2023	22° 15’ 52.7” N, 97° 45’ 20.5” W
	C. latus	1	20 Oct 2022 26 May 2023	
Tecolutla	*C. hippos*	14	12 Nov 2021	20° 29' 00.8" N, 97° 00' 07.2" W
Tuxpan		12	19 Aug 2019	20° 58' 09.2" N, 97° 19' 43.4 "W

Some specimens of the Colección Nacional de Helmintos (CNHE) of the Instituto de Biología of the Universidad Nacional Autónoma de Mexico (UNAM), those from the Colección de Helmintos (CHE), of the Centro de Investigaciones Biológicas (CIB) of the Universidad Autónoma del Estado de Hidalgo (UAEH), and specimens collected in the field as part of this study were analyzed. In total, 147 specimens were included in the morphological and morphometric analyses. Specimens from the following localities were studied: Campeche (CNHE-82) (18° 38′ 18″ N, 91° 50′ 07″ W), Chetumal, Quintana Roo (CNHE-160; 165) (18° 30′ 13″ N, 88°18′19″ W; n = 4), Cozumel, Quintana Roo (CNHE-167) (20° 30′ 36″ N, 86° 56′ 56″ W; n = 10), Jicacal, Veracruz (CNHE-168) (18°12′ 56″ N, -94° 36′ 48″ W; n = 10) , Sontecomapan, Veracruz (CNHE-161;162) (18° 30' 13" N, 95° 2' 6" W; n = 6) and Tuxpan, Veracruz (CNHE-111). From the Pacific Ocean were the following localities: Chamela, Jalisco (CNHE-3114; 3115) (19° 31′ 38″ N, 105° 04′ 24″ W; n = 6), La Paz, Baja California Sur (CNHE-346; 347; 348) (22° 52' 19" N, 115° 04' 56" W; n = 5), Isla Isabel, Nayarit (CNHE-158; 159; 351) (21º 52' 30 " N, 105º

54' 54" W; n = 9) and Puerto Escondido, Oaxaca (CNHE-134) (15° 52' 10" N -97° 4' 22" W; n = 9), as well as specimens of *Neomicrocotyle pacifica* (Meserve, 1938) Yamaguti, 1968 from Chamela, Jalisco (CNHE-71; 371; 372; 3116) (19° 31′ 36″ N, 105° 04′ 26″ W; n= 12). From the CHE, the specimens correspond to the localities: Acapulco, Guerrero (CHE-GRO-21-01) (16° 51′ 46″ N, 99° 53′ 13″ W; n =10),Boca del Río, Veracruz (CHE-VER-15-126) (19°06′03″N, 96°06′26″ W; n = 4), Casitas, Veracruz (CHE-VER-05-105) (20° 15′ 33.64″ N, 96° 48′ 01.30″ W, n = 13), and Tuxpan, Veracruz (CHE- VER-19-348) (20° 57′ 46″ N, 97° 24′ 01″ W; n = 10). As part of this study, field trips were carried out to collect biological material in the localities of Tuxpan, Veracruz, Tecolutla, Veracruz (20° 28′ 39″ N, 97° 00′ 30″ W; n = 19), Ciudad del Carmen, Campeche (18° 38′ 18″ N, 91° 50′ 07″ W), Tampico, Tamaulipas (22° 15' 52.7" N, 97° 45' 20.5" W, n = 9), and Puerto de Veracruz, Veracruz (19° 13' 11.2" N, 96° 09' 24.4" W; n = 11) (Figure 2.1, Table 2.1).

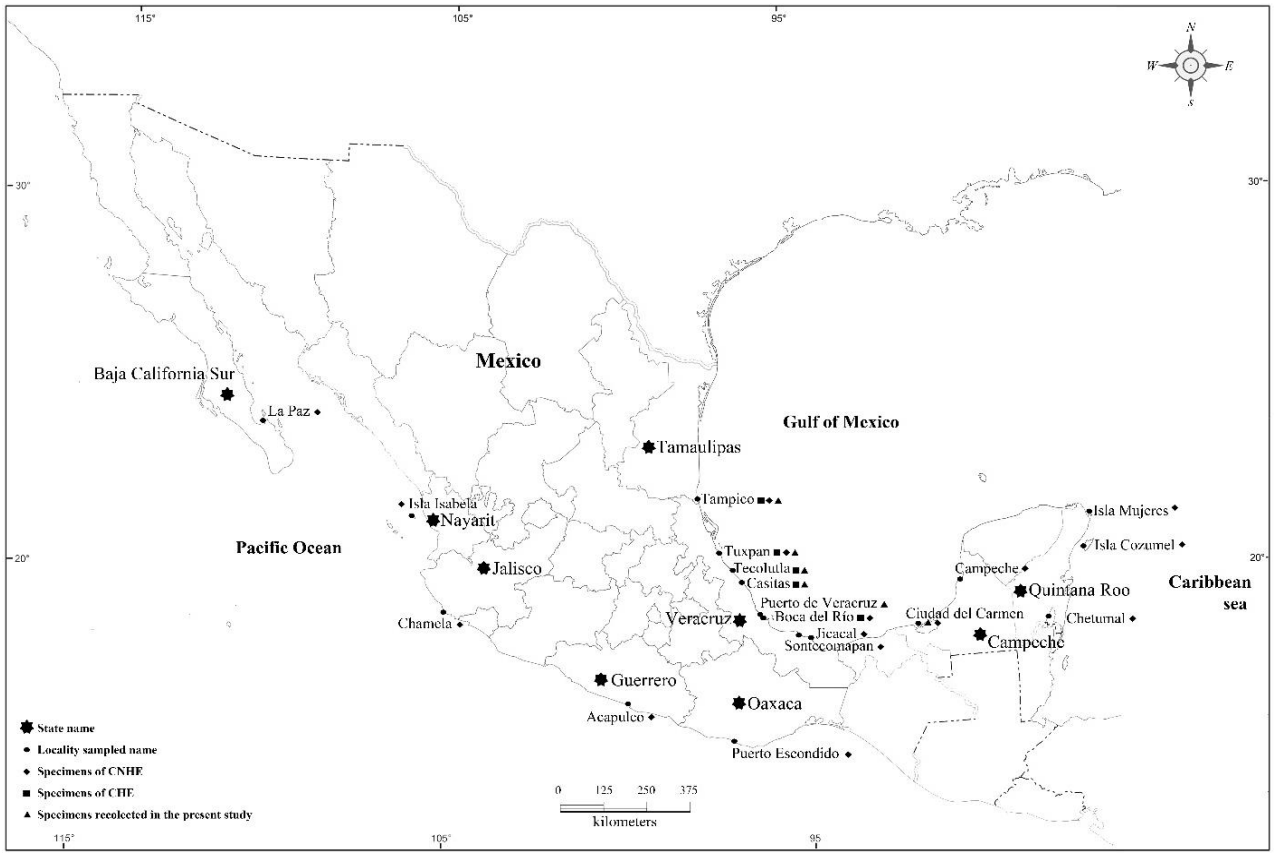

Figure 2. 1. Localities at Mexico where *Caranx hippos* and *C. latus* were sampled from the present study. Additionally, the localities that were sampled in previous studies are included.

Some specimens were previously identified as *Protomicrocotyle mirabilis* from Sontecomapan, Veracruz (CNHE-161; 162) collected in *Caranx hippos* (Bravo-Hollis, 1989), as well as the specimens identified as *P. manteri* from the localities of Chetumal Quintana Roo (CNHE-160), Cozumel, Quintana Roo (CNHE-167), and Jicacal, Veracruz (CNHE-168) (Bravo-Hollis, 1989). These specimens were collected in the 1970s by Biol. Jorge Caballero Deloya and M. en C. Margarita Bravo-Hollis and processed by personnel from the Laboratorio of Helmintología. There is no information on the coordinates of each of the localities mentioned in the original publication, although in this study the information available in the CNHE catalog was used (Lamothe-Argumedo et al., 1997). The staining technique by which they were stained is not described. The specimens are preserved in a permanent preparation with Canada balsam (Bravo-Hollis, 1989).

Figure 2. 2. Host collection and field work. (a) *Caranx hippos* from Tecolutla, Veracruz. (b) Gills of *C. hippos*. (c) Search of monogeneans in the gills of *C. hippos*.

In each of the localities that were sampled as part of this study (Figure 2.1, Table 2.1), the fish were obtained from local fishermen. The fish specimens were sexed, measured and photographed to carry out the taxonomic identification according to the specialized literature (Nelson, 2006). The helminthological review was carried out in the field, the gill arches were extracted from the gill cavity of the fish, kept in a bag with its respective label, and placed on ice for later review. Using a Leica Zoom 2000 stereoscopic microscope, each of the branchial arches was reviewed with the aid of dissecting needles, looking for monogeneans in each of the gill filaments (Figure 2.2). All the monogeneans found were relaxed with hot water and stored in AFA for morphological analysis (Pritchard & Kruse, 1982; Lamothe-Argumedo, 1997), and other specimens were stored at Alcohol 96% (OH96%) for molecular analyses.

Morphological study

The morphological characters were chosen based on the available literature for *Protomicrocotyle* (Yamaguti, 1953; Ramalingam, 1960; Caballero y Caballero & Bravo-Hollis, 1965a; Wahl, 1972; Bravo-Hollis, 1979; Bravo-Hollis, 1989; Kritsky et al., 2011), as well as the characters that were used in *P. veracruzensis* (Ramírez-Cruz et al., 2023). The morphological measurements were taken using a compound optical microscope equipped with differential interference contrast (DIC) optics, and given in μm; by the minimum and maximum range, in parentheses is the average, standard deviation and the number of individual or anatomical structures that were measured, represented for *n*.

For morphological study, specimens of *Protomicrocotyle* were stained using Gomori's trichrome, Mayer's carmalum, or Delafield's hematoxylin, dehydrated in an ethanol series, cleared in methyl salicylate, and mounted individually as whole-mounts on slides in Canada balsam. The identification of specimens was made based on morphological keys and specialized literature from the genus and the family (Ramalingam, 1960; Yamaguti, 1963; Bravo-Hollis, 1966; Bravo-Hollis, 1979; Kritsky et al., 2011). A measurement protocol was established for the morphological characteristics of the specimens of *Protomicrocotyle*, and the lengths of the haptoral lappet, the anchors and hooks were measured as indicated in (Ramírez-Cruz et al., 2023). Specimens were examined using a Leica CME compound optical binocular microscope equipped with differential interference contrast optics, and measurements were taken with a calibrated micrometric ocular microscope and are presented in micrometers (μm), with the following format: minimum,

maximum, and in parentheses, mean, standard deviation, and n, which represents the number of individuals or anatomical structures that were measured. The shape of the anatomical structures is based on the taxonomic proposal of Clopton (2004). The line drawings were made with an optical tube adapted to a clear camera in an optical microscope and processed in Adobe® Illustrator® CS6 2012 and Adobe Photoshop® CS6 2012 software (Adobe® Systems Inc., San Jose, California). The photographs were taken with a Digital Microscope VHX-7000 series belonging to the Unidad Central de Laboratorios de la Universidad Autonóma del Estado de Hidalgo.

Morphometry analyses

The information obtained from each of the measured and coding specimens was stored in an Excel database with data on the specimen number, locality, marine ecoregion, host, and the respective number of the collection to which it corresponds. The multivariate analyses used in this study were: Principal Component Analysis (PCA), Principal Coordinate Analysis (PCoA), Discriminant Analysis (DA), and cluster analysis with the Ward´s method. The PCA is an algebraic-statistical method that tries to synthesize and give a structure to the information contained in a data matrix. The procedure consists of homologating said matrix to a vector space and the objective is to reduce the number of variables introduced (Labrín & Urdinez, 2020; Palacio et al., 2020; Perelman & Puhl, 2023). The PCA analyzes a data matrix representing observations described by several dependent variables, which are, in general, inter-correlated, and represents the pattern of similarity of the observations and the variables by displaying them as points in plots (Labrín & Urdinez, 2020; Palacio et al., 2020).

The DA is a statistical method through which one seeks to know which variables (measured in objects or in anatomical structures of specimens) best explain the attribution of the difference of the groups to which the study units. In the DA the study units are already assigned to each of the groups, and the method determines to what degree the variables analyzed discriminate said groupings. This technique works as a predictive tool since it allows classifying the study units (Palacio et al., 2020). The DA is explicitly a multiple-group procedure and assumes that the groups are known correctly before analysis based on extrinsic criteria and that all individuals are members of one of the known groups (Strauss, 2010; Torrado-Fonseca & Berlanga-Silvente, 2013; Palacio et al., 2020). PCoA is a method developed by Gower (1966), in which a data set of the study units

is represented in a Euclidean space whose relationships are quantified by any similarity method, so continuous and discrete variables can be used.

The cluster analyses comprise a set of techniques that form groups of the study units that are associated by their degree of similarity, these techniques result in a dendrogram which is the graphical representation in the form of a tree that shows the hierarchical relationships between the units of study according to their similarity values. To know the degree to which the dendrogram represents the values of the similarity matrix, the cophenetic correlation coefficient (CCC) technique is used, which consists of building a new similarity matrix from the values of the dendrogram, this new matrix is named cophenetic matrix and the Pearson correlation coefficient is calculated between the similarity matrix that resulted in the dendrogram and the cophenetic matrix, resulting in a cophenetic correlation value. Values above 0.75 are a sign that there is little distortion (Palacio et al., 2020).

For data analysis, the quantitative variables (continuous and meristic) were transformed into Natural Logarithms (*Ln*). Two PCAs were carried out, both only with continuous and meristic variables, the first one was carried out with the objective of reducing the dimensionality and identifying the variables that contribute the most to the variance, and the second one only with 27 variables. The AD was performed with the total of the continuous variables standardized to *Ln*. The PCoA was performed with the combination of the 27 quantitative variables and 20 discrete variables, based on the Gower similarity coefficient. For the clustering analysis, the unweighted pair group method with arithmetic mean (UPGMA) technique was used, using all quantitative and discrete variables using the Gower similarity coefficient (Palacio et al., 2020). These multivariate techniques were used in order to know the behavior of morphological variables and their importance in the grouping of *Protomicrocotyle* specimens. All analyzes were performed in PAST v.4.12b (Hammer et al., 2009).

3. Results

A total of 147 specimens of *Protomicrocotyle* were examined, of which 81 correspond to specimens deposited in the Colección Nacional de Helmintos and 66 to specimens deposited in the Colección de Helmintos (CIB-UAEH) and specimens collected in the field as part of this project, as well as 12 specimens of *Neomicrocotyle pacifica* from the CNHE. The results are presented in three sections: The first section presents a list of the anatomical structures that were measured, and

counted, the second section presents the morphological characterization of the *Protomicrocotyle* specimens, and the third section shows the morphometric analyses.

List of morphological characters

Continuous characters

1. Body length (LT).

The measurement was taken from the anterior end of the body to the posterior end, including the larval appendage.

2. Body width (AT).

The maximum width was measured in the anterior part of the ovary. In the area of intersection between the testicles and the oviduct.

3. Length of oral opening (LAO).

It was measured from the anterior end to the posterior end of the oral suction cup.

4. Oral opening width (AAO).

It was measured from the right lateral end to the left lateral end of the oral opening.

5. Pharyngeal length (LF).

It was measured from the anterior end to the posterior end of the pharynx.

6. Pharyngeal width (AF).

It was measured from the right lateral end to the left lateral end of the pharynx.

7. Length of esophagus (LEGO).

It was measured from the posterior end of the pharynx to the beginning of the cecal bifurcation.

8. Width of esophagus (AEGO).

It was measured from the right lateral end to the left lateral end in the middle of the esophagus.

9. Right pseudo-sucker length (LPD).

It was measured from the anterior end to the posterior end of the prohaptor pseudosucker.

10. Width of right pseudo-sucker (APD).

It was measured from the right lateral end to the left lateral end of the prohaptor pseudo-sucker.

11. Length of left pseudo-sucker cup (LPI).

It was measured from the anterior end to the posterior end of the prohaptor pseudo-sucker.

12. Width of left pseudo-sucker (API).

It was measured from the right lateral end to the left lateral end of the prohaptor pseudo-sucker.

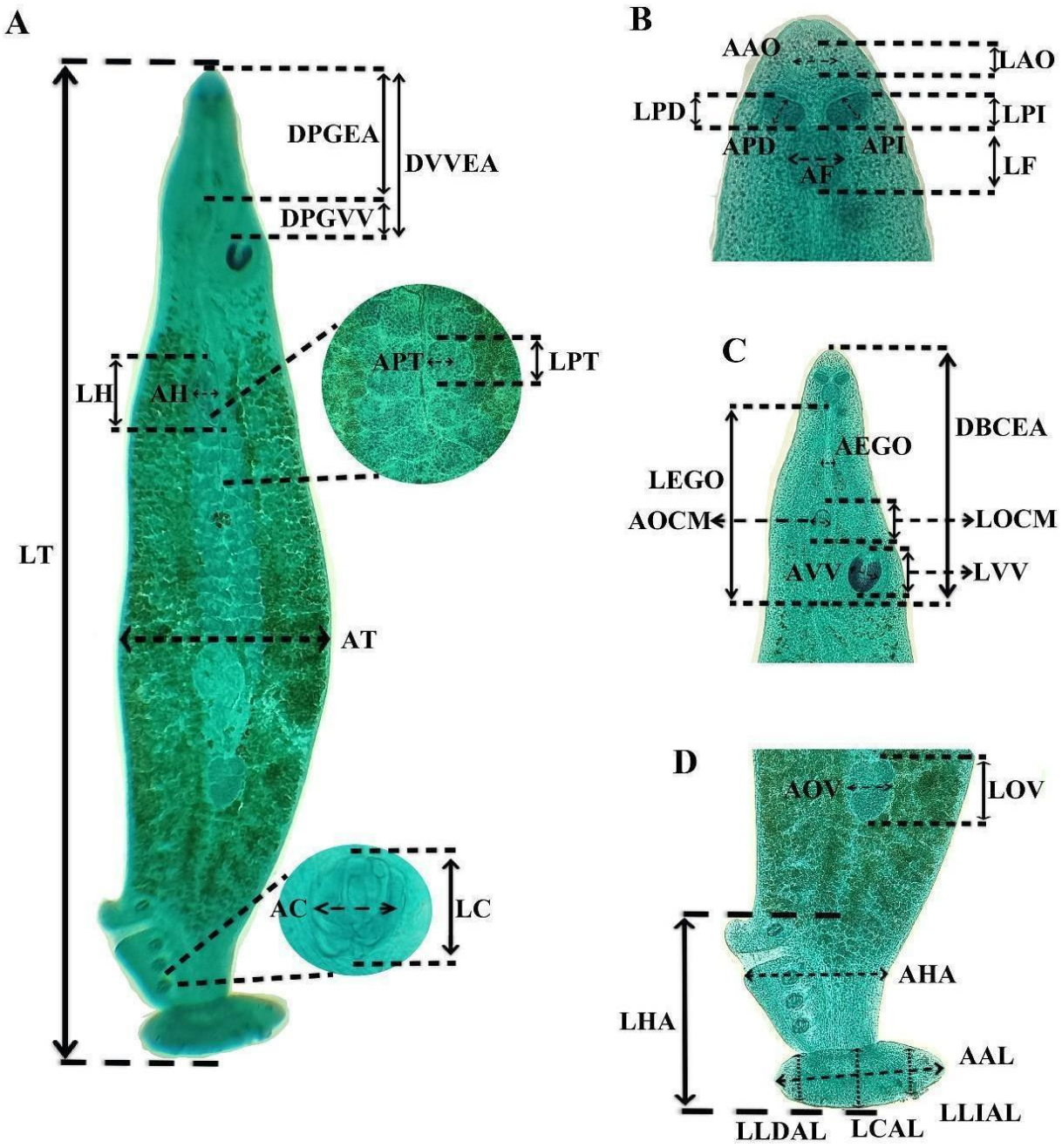

Figure 2. 3. Examples of some characters evaluated in the morphometric analysis of *Protomicrocotyle* are shown. (A) Full body; (B) prohaptor end; (C) prohaptor and anterior region of the trunk; and (D) posterior region of the trunk and haptor. Abbreviations, (A). T: Total length, AT: Total width, DPGEA: Distance from genital pore to anterior end, DPGVV: Distance from genital pore to vaginal vestibule, DVVEA: Distance from vaginal vestibule to anterior end, LH: Egg length, AH: Width of egg, LPT: Average length of the testicles, APT: Average width of the testicles, AC: Width of the clamp. (B). LAO: Length of oral opening, AAO: Width of oral opening, LPD: Length of right pseudo-sucker, APD: Width of right pseudo-sucker, LPI: Length of left pseudo-sucker, API: Width of left pseudo-sucker, LF: Pharynx length, AF: Wide pharynx. (C). DBCEA: Distance from cecal bifurcation to anterior end, LOCM: Length of male copulatory

organ, AOCM: Width of male copulatory organ, LVV: Length of vaginal vestibule, AVV: Width of vaginal vestibule, LEGO: Length of esophagus, AEGO: Width of esophagus. (D). LOV: Length of ovary, AOV: Width of ovary, LHA: Length of haptor, AHA: Width of haptor, LLDAL: Right lateral length of haptoral lappet, LCAL: Central length of haptoral lappet, LLIAL: Left lateral length of haptoral lappet, AAL: Width of the haptoral lappet.

13. Haptor length (LHA).

It was measured from the anterior part of the first clamp (from anterior to posterior) to the posterior end of the haptoral lappet.

14. Haptor width (AHA).

It was measured in the middle region between the second and third clamp.

15. Long right lateral haptoral lappet (LLDAL).

It was measured from the anterior end to the posterior end of the larval appendage on the right side.

16. Long left lateral haptoral lappet (LLIAL).

It was measured from the anterior end to the posterior end of the haptoral lappet on the left side.

17. Long central haptoral lappet (LCAL).

It was measured from anterior end to posterior end in the central part of the larval appendage.

18. Larval haptoral lappet (AAL).

It was measured from the right lateral end to the left lateral end in the central part of the haptoral lappet.

19. Length of first clamp (LPP).

It was measured from the anterior end to the posterior end

20. Width of first clamp (APP).

It was measured from the right lateral extreme to the left lateral extreme.

21. Length of second clamp (LSP).

It was measured from the anterior end to the posterior end.

22. Width of second clamp (ASP).

It was measured from the right lateral extreme to the left lateral extreme.

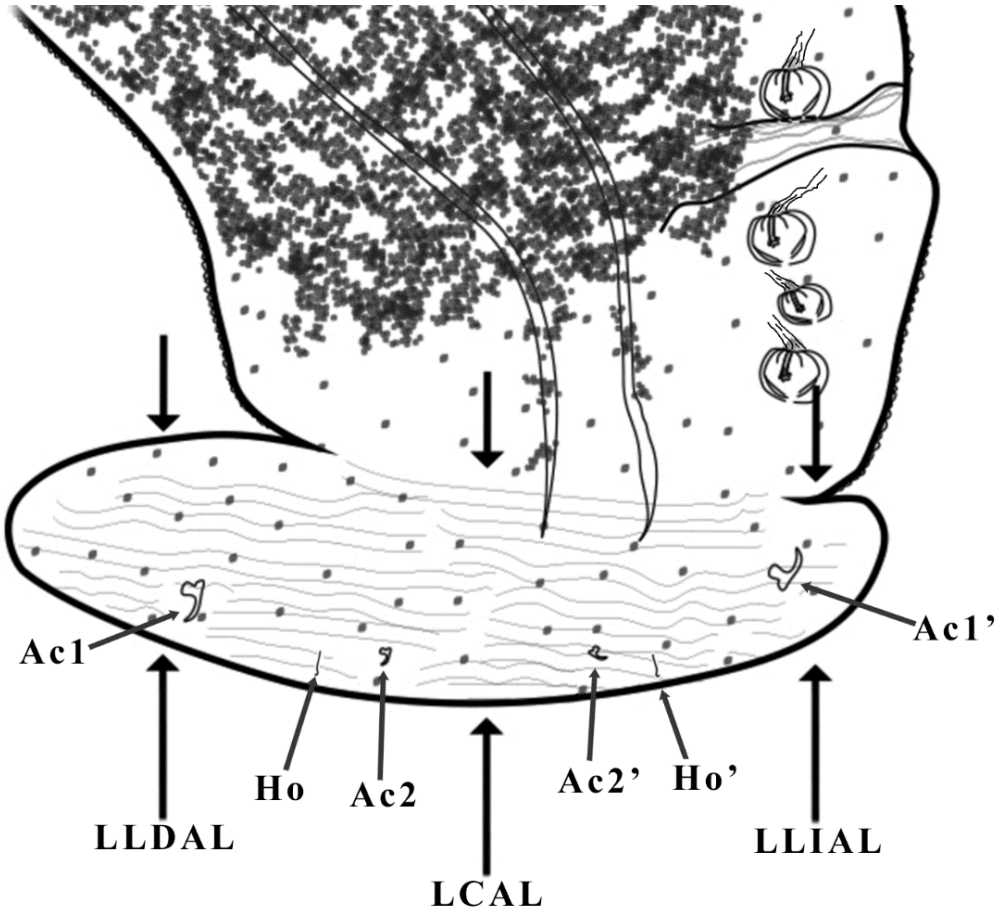

Figure 2. 4. Posterior end of the haptor, showing how the haptoral lappet was measured: Right lateral length of the larval appendage (LLDAL), left lateral length of the larval appendage (LLIAL) and central length of the larval appendage (LCAL) and the name of the hooks present on this anatomical structure: right lateral hook (Ac1), left lateral hook (Ac1'), right median hook (Ac2), left median hook (Ac2'), right hook (Ho) and left hook (Ho') (see Ramírez-Cruz, et al. (2023)).

23. Length of third clamp (LTP).

It was measured from the anterior end to the posterior end.

24. Width of third clamp (ATP).

It was measured from the right lateral extreme to the left lateral extreme.

25. Length of fourth clamp (LCP).

It was measured from the anterior end to the posterior end.

26. Width of fourth clamp (ACP).

It was measured from the right lateral extreme to the left lateral extreme.

27. Distance from the cecal bifurcation to the anterior end (DBCEA).

It was measured from the beginning of the cecal bifurcation to the anterior end of the body.

28. Distance from genital pore to anterior end (DPGEA).

It was measured from the genital pore to the anterior end of the body.

29. Distance from vaginal vestibule to anterior end (DVVEA).

It was measured from the anterior end of the vaginal vestibule to the anterior end of the body.

30. Distance from the genital pore to the vaginal vestibule (DPGVV).

It was measured from the genital pore to the vaginal vestibule.

31. Right side hook length (Anchor) (LGLD).

It was measured from the anterior end of the root to the recurved point and from there to the end of the tip.

32. Right side hook width (AGLD).

It was measured from the widest part of the hook.

33. Right side hook opening length (LAGLD).

It was measured from the tip of the hook to the posterior lateral end of the external root.

34. Internal root length of right lateral hook (LRIGLD).

It was measured from the anterior end of the internal root to the beginning of the hook.

35. Width of internal root of the right lateral hook (ARIGLD).

The lateral ends of the internal root were measured.

36. Right side hook handle length (LMGLD).

It was measured from the posterior region of the external root to the recurved point and from there to the end of the tip.

37. External root length of the right lateral hook (LREGLD).

It was measured from the anterior end to the posterior end of the external root.

38. Right side hook main part length (LPPGLD).

It was measured from the tip of the hook to the recurved point until the union of the external root with the beginning of the hook.

39. Right Lateral Hook Tip Length (LPGLD).

It was measured from the recurved point to the tip of the hook.

40. Left lateral hook length (anchor) (LGLI).

It was measured from the anterior end of the root to the recurved point and from there to the end of the tip.

41. Left lateral hook width (AGLI).

It was measured from the widest part of the hook.

42. Left side hook opening length (LAGLI).

It was measured from the tip of the hook to the posterior lateral end of the external root.

43. Internal root length of the left lateral hook (LRIGLI).

It was measured from the anterior end of the internal root to the beginning of the hook.

44. Width of internal root of the right lateral hook (ARIGLI).

The lateral ends of the internal root were measured.

45. Left Side Hook Handle Length (LMGLI).

It was measured from the posterior region of the external root to the recurved point and from there to the end of the tip.

46. External root length of the left lateral hook (LREGLI).

It was measured from the anterior end to the posterior end of the external root.

47. Length of main part of the left lateral hook (LPPGLI).

It was measured from the tip of the hook to the recurved point until the union of the external root with the beginning of the hook.

48. Left lateral hook tip length (LPGLI).

It was measured from the recurved point to the tip of the hook.

49. Right middle hook length (Anchor) (LGMD).

It was measured from the anterior end of the hook to the recurved point and from there to the tip of the hook.

50. Hook width middle right (AGMD).

The lateral ends of the hook were measured.

51. Right middle hook opening length (LAGMD).

It was measured from the tip of the hook to the posterior lateral end of the external root.

52. Internal root length of the right middle hook (LRIGMD).

It was measured from the anterior end of the hook to the junction between the internal root and the external root.

53. Width of internal root of the right middle hook (ARIGMD).

The lateral ends of the internal root were measured.

54. Right middle hook handle length (LMGMD).

It was measured from the posterior region of the external root to the recurved point and from there to the end of the tip.

55. External root length of the right middle hook (LREGMD).

It was measured from the anterior end to the posterior end of the external root.

56. Length of main part of right middle hook (LPPGMD).

It was measured from the tip of the hook to the recurved point until the union of the external root with the beginning of the hook.

57. Right middle hook tip length (LPGMD).

It was measured from the recurved point to the tip of the hook.

58. Left middle hook length (Anchor) (LGMI).

It was measured from the anterior end of the hook to the recurved point and from there to the tip of the hook.

59. Left middle hook width (AGMI).

The lateral ends of the hook were measured.

60. Left middle hook opening length (LAGMI).

It was measured from the tip of the hook to the posterior lateral end of the external root.

61. Internal root length of the left middle hook (LRIGMI).

It was measured from the anterior end of the hook to the junction between the internal root and the external root.

62. Width of the internal root of the left middle hook (ARIGMI).

The lateral ends of the internal root were measured.

63. Left middle hook handle length (LMGMD).

It was measured from the posterior region of the external root to the recurved point and from there to the end of the tip.

64. External root length of the left middle hook (LREGMI).

It was measured from the anterior end to the posterior end of the external root.

65. Length of main part of left middle hook (LPPGMD).

It was measured from the tip of the hook to the recurved point until the union of the external root with the beginning of the hook.

66. Left middle hook tip length (LPGMD)

It was measured from the recurved point to the tip of the hook.

67. Total length of right center hook (LTGCD).

It was measured from the anterior end to the posterior end of the hook.

68. Right center hook width (ATGCD).

It was measured at the widest part of the hook.

69. Total length of left center hook (LTGCI).

It was measured from the anterior end to the posterior end of the hook.

70. Left center hook width (ATGCI).

It was measured at the widest part of the hook.

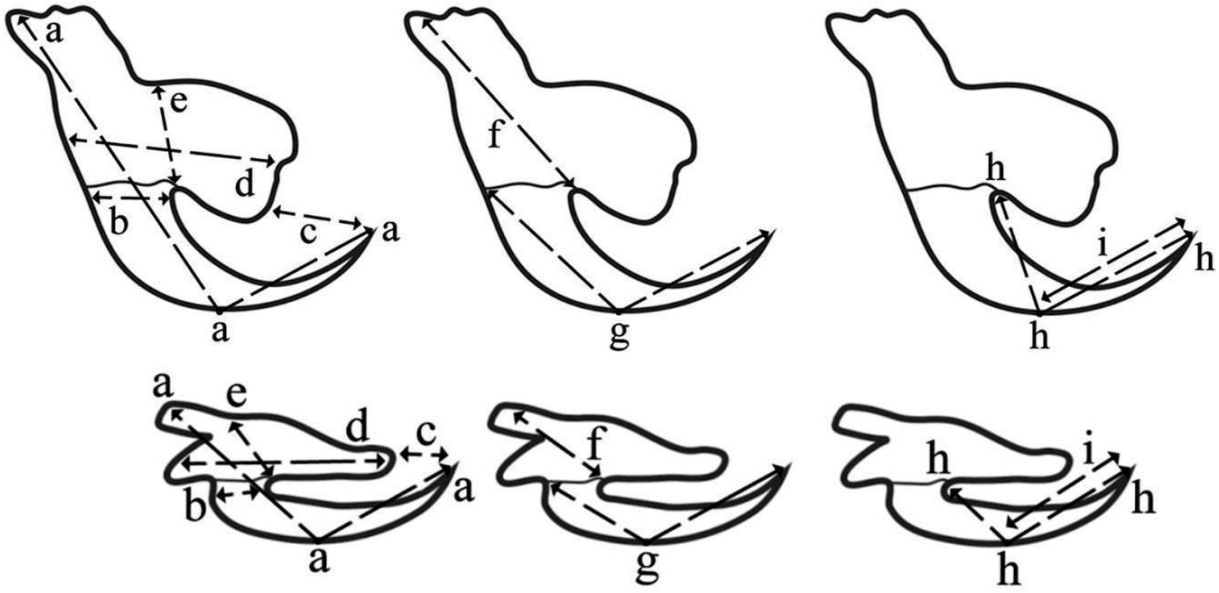

Figure 2. 5. Detail of the measurements taken on the hooks. (a) total hook length, (b) hook width, (c) hook opening, (d) internal root length, (e) internal root width, (f) external root length, (g) length of hook handle, (h) hook main part length, (i) hook tip length (see Ramírez-Cruz, et al. (2023)).

71. Average length of the testicles (LPT).

The distance between the anterior and posterior ends of some testicles in each specimen was measured.

72. Average width of the testicles (APT).

The lateral ends of some testicles in each specimen were measured.

73. Length of male copulatory organ (LOCM).

It was measured from the anterior end to the posterior end.

74. Width of male copulatory organ (AOCM).

The lateral ends were measured.

75. Length of spines of the male copulatory organ (LEOCM).

It was measured from the anterior end to the posterior end of the spine.

76. Width of spines of the male copulatory organ (AEOCM).

The lateral ends of the spine were measured.

77. Long of the ovary (LOV).

It was measured from the anterior end to the posterior end.

78. Ovary width (AOV).

The lateral ends were measured.

79. Length of vaginal vestibule (LVV).

It was measured from the anterior end to the posterior end of the vestibule.

80. Vaginal vestibule width (AVV).

The lateral ends of the vestibule were measured.

81. Length of spines of the vaginal vestibule (LEVV).

It was measured from the end to the anterior to the posterior end of the spine.

82. Width of spines of the vaginal vestibule (AEVV).

The lateral ends of the spine were measured.

83. Egg length (LH).

It was measured from the anterior end to the posterior end.

84. Egg width (AH).

The lateral ends of the egg were measured in the middle part.

85. Filament length (anterior region) (LFDHRA).

It was measured from the beginning of the filament in the anterior part of the egg to the end of the filament.

86. Filament width (anterior region) (AFDHRA).

The lateral ends of the filament were measured.

87. Filament length (posterior region) (LFDHRP).

It was measured from the beginning of the filament at the back of the egg to the end of the filament.

88. Filament width (posterior region) (AFDHRP).

The lateral ends of the filament were measured.

Meristic characteristics

89. Number of spines in the vaginal vestibule.

It represents the number of spines present in the vaginal vestibule of each individual.

90. Number of spines in the male copulatory organ.

It represents the number of spines on the male copulatory organ of each individual.

91. Number of testes.

Represents the number of testes present in each individual that was measured.

Morphological descriptions

Class Monogenea van Beneden, 1858

Subclass Polyopisthocotylea Odhner, 1912

Order Mazocraeidea Bychowsky, 1937

Family Protomicrocotylidae Johnston & Tiegs, 1922

Genus *Protomicrocotyle* Johnston & Tiegs, 1922

Protomicrocotyle **sp. 1**

Figures 2.6, 2.7 and 2.8.

This morphological description was based in the measurements and observation of six specimens of the CNHE (161; 162) from the locality of Sontecomapan, Veracruz, that was previously identified as *P. mirabilis*. Description: Body wide fusiform, the anterior region is narrower than the posterior region, the length of the body goes from 1379–1781 (1605 ± 69, n= 6) including the haptor. The wide goes from 134–171 (150 ± 7, n= 6) (Figure 6a). The tegument presents cuticular striations that give the margins of the body a serrated appearance. These appear mainly from the middle region of the body to the posterior region, and in some specimens, they are also observed in the region of the prohaptor (Figure 2.6b). In the anterior region, there are a pair of prohaptoral suckers that are septate, muscular, oblique, and very shallowly dolliform, the right prohaptoral sucker is 12–17 (15 ± 1, n= 6) long, 12–17 (14 ± 1, n= 6) wide, and the left prohaptoral sucker is 13–17 (16 ± 1, n= 6) long, 11–17 (16 ± 1, n= 6) wide (Figure 2.6b). The oral cavity is in the anterior end on the ventral side of the body, terminal, finely ovoid, surrounded by

small lobes similar to the lips, is 7–12 (9 ± 1, n= 6) long and 11–25 (16 ± 2, n= 6) wide. Pre-pharynx absent (Figures 2.6b, 2.8b). Glandomuscular organ present, showing like two lips (Figure 6b, 8b). The pharynx is broadly elliptoid, muscular, 13–26 (21 ± 2, n= 6) long and 13–24 (19 ± 1, n= 6) wide. The esophagus is a long tube and has diverticula. The cecal bifurcation is posterior to the male copulatory organ (MCO), and the intestinal caecum extends to the haptor region, reaching the larval appendix and presenting many diverticula extending to the body's lateral margins (Figures 2.6a, 2.8a).

The haptor is asymmetrical and is armed with a row of four gastrocotylid-type clamps with the presence of a small, short muscular peduncle and a haptoral groove that is located between the first and second clamps (the body of specimen is on the ventral side and considering that the first clamp is the one closest to the anterior region) (Figures 2.6a, 2.8a). The length of the haptor goes from 220–290 (245 ± 11, n= 6) to 80–160 (114 ± 11, n= 6) wide, at the level of the second clamp. The clamps have a similar size (Figure 7b); the first clamp goes from 19–24 (21 ± 1, n= 6) long to 16–20 (18 ± 1, n= 6) wide, the second clamp 20–24 (22 ± 1, n= 6) long, 11–20 (17 ± 1, n= 6) wide, the third clamp 17–23 (21 ± 1, n= 6) long, 11–22 (17 ± 2, n= 6) wide, and the fourth clamp 18–23 (21 ± 1, n= 6) long, 14–19 (18 ± 1, n= 6) wide. The haptoral lappet is transversely very shallowly doliform. Length of lappet measured in three regions; right side of haptoral lappet 48–65 (55 ± 2, n= 6) long, middle region of the haptoral lappet 43–58 (51 ± 2, n= 6) long, and left side of the haptoral lappet 47–56 (51 ± 2, n= 5) long. Haptoral lappet 125–180 (143 ± 8, n= 6) wide, armed with two pairs of anchors, and one pair of hooks (Figures 2.7c, 2.7d, 2.7e) (Table 2.3).

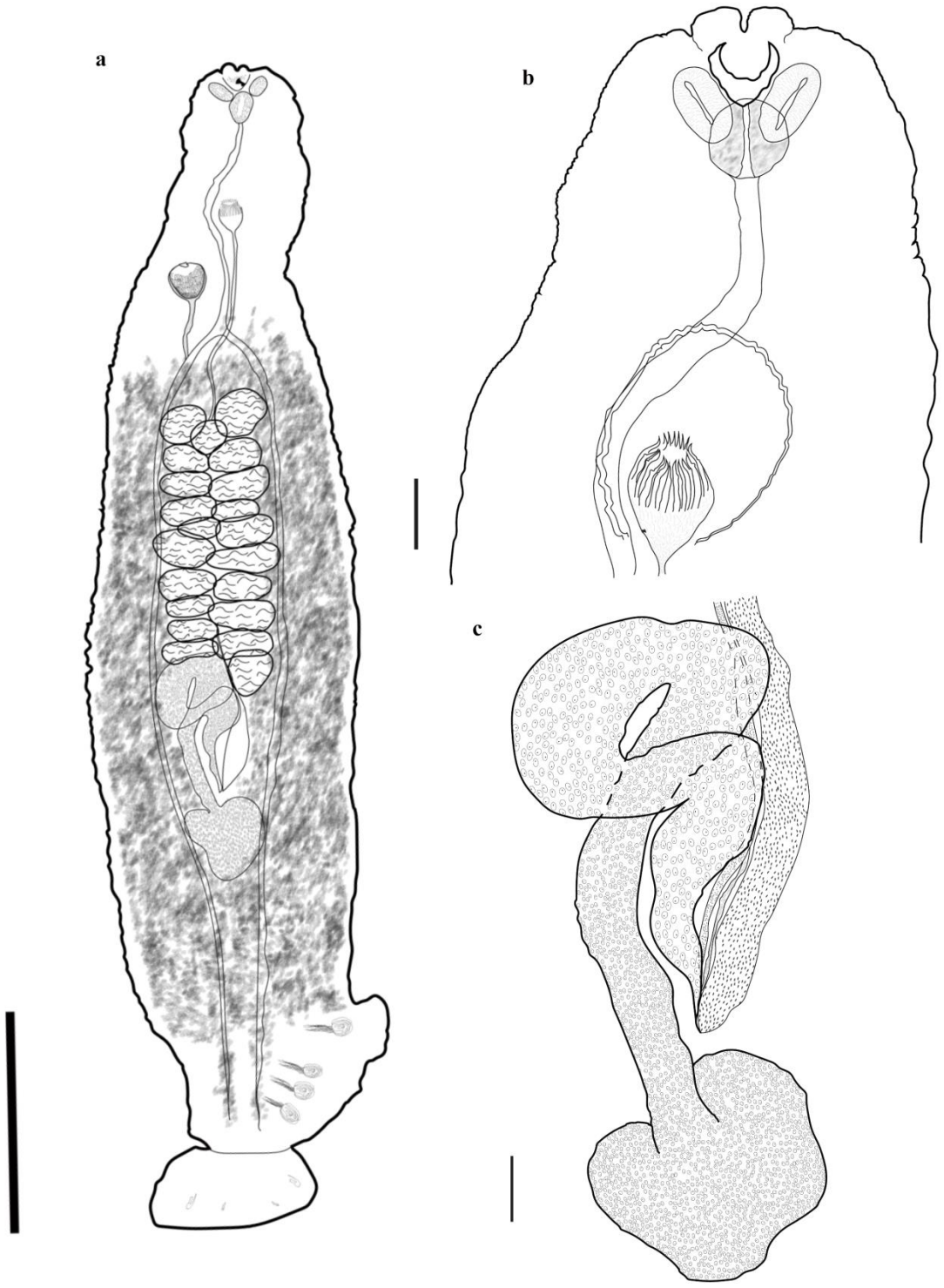

Figure 2. 6. Line drawings of *Protomicrocotyle* **sp. 1**. (a) Complete body of *Protomicrocotyle* **sp. 1** from the gills of *Caranx hippos*, ventral view, Bar 500 µm; (b) Anterior region of *Protomicrocotyle* **sp. 1**, the glandomuscular organ is shown in the apical region, Bar 100 µm; (c) Female reproductive organs, Bar 100 µm.

Testes very broadly doliform shape, irregular size, pre-ovarian and are arranged in two fields and in each field, there is a row. The number 26–31 (28 ± 2, n= 6) with an average length of 30–42 (38 ± 2, n= 6) and 29–42 (34 ± 2, n= 6) wide (Figures 2.6a, 2.8a, 2.8c). The ratio between the L: A = 1:0.9. The vas deferens go in the direction of the anterior region; in the part anterior to the testicular region, it expands and coils, then narrows and continues in the form of a straight tube until the MCO. The MCO is cup-shaped, muscular, with 31–38 (34 ± 1, n= 6) long and 17–23 (19 ± 1, n= 6) wide. Armed with 18–24 (20 ± 1, n= 6) spines. Spines of MCO hook-like shaped, with 16–18 (17, n= 6) long, 1–2 (2, n= 6) wide, arranged in a circle on anterior part of MCO (Figure 2.6b). Each spine with small knob in the anterior region just posterior to curved tip, knob and curved tip directed outwards. The genital atrium is located in the anterior region of the body, is subspherical, has muscular edges, and is without any type of sclerotized structure. The distance from the genital pore to the anterior end is 165–230 (199 ± 11, n= 6).

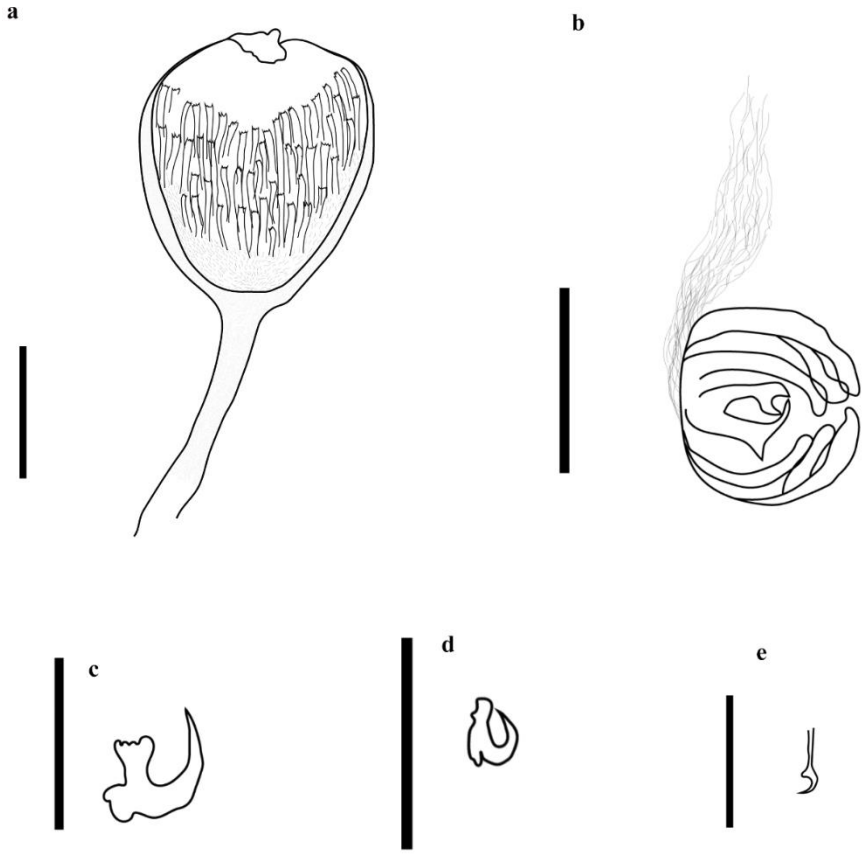

Figure 2. 7. Line drawings of *Protomicrocotyle* **sp. 1**. (a) Vaginal vestibule, Bar 100 µm; (b) Clamp with peduncle, Bar 100 µm; (c) Lateral anchor, Bar 100 µm; (d) Median anchor, Bar 100 µm; (e) Central hook, Bar 100 µm.

The ovary is located in the post-testicular region, is oval, and has lobed edges. The length of the ovary is 43–115 (73± 10, n= 6) with 29–46 (36 ± 2, n= 6) wide. The oviduct goes towards the anterior region, subsequently extends towards the posterior region, again towards the anterior region, and finally empties into the region of the oötype. From this region arises the uterus, which goes towards the anterior end in the middle region between the rows of the testicles until reaching the genital atrium (Figure 2.7c). The seminal vesicle is located in the region of the oötype, in the shape of a spindle. In the uterus, in some specimens, 1 to 2 eggs with long filaments at each polar end are observed; the egg is 84–124 (104 ± 20, n= 2) long and 34–40 (37± 3, n= 2) wide. The anterior polar end filament could not be measured, and the posterior polar end filament of the egg ranges from 151–263 (207 ± 52, n= 2) long and 5–8 (7 ± 2, n= 2) wide. The vaginal pore is located anterior to the vaginal vestibule, which is ovoid, is located on the opposite side to the clamps, and has a length of 29–46 (36 ± 2, n= 6) and 20–44 (32 ± 3, n = 6) wide and is armed with 53–75 (65 ± 3, n= 6) spines, with a flat shape and a variable size, with those found in the anterior part of the vestibule being larger than those found in the vestibule. They are found at the base; these spines have small extensions in the form of smaller spines in the distal region of each spine. The length of the spines ranges from 14–19 (16 ± 1, n= 6) and 2–2 (4, n= 6) wide. The vaginal opening is located at a distance of 225–305 (269 ± 12, n= 6), and the genital pore at 70–105 (84 ± 6, n= 6) of the anterior end. The vitelline glands are found covering most of the body, starting in the region anterior or posterior to the ceca bifurcation and extending to the haptor region.

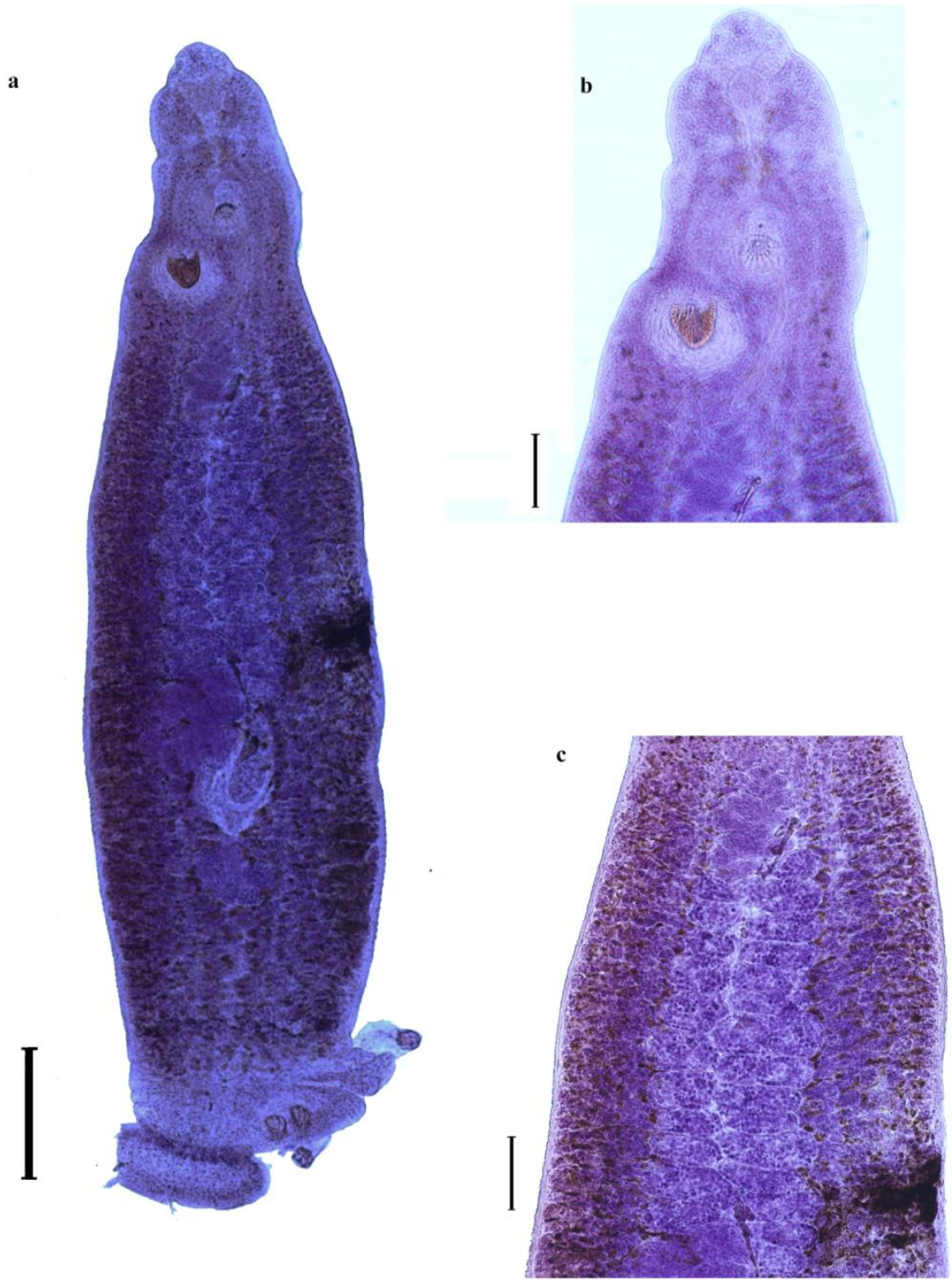

Figure 2. 8. Light micrographs of *Protomicrocotyle* **sp. 1**. (a) Complete body of *Protomicrocotyle* **sp. 1** from the gills of *Caranx hippos*, ventral view, Bar 250 µm; (b) Anterior region of *Protomicrocotyle* **sp. 1**, the glandomuscular organ is shown in the apical region, Bar 100 µm; (c) Arrangement of the testes, Bar 100 µm.

Taxonomic summary

Host: *Caranx hippos* (Linnaeus, 1766).

Common name: Crevalle jack.

Site of infection: Gills

Locality: Laguna de Sontecomapan, Veracruz, Mexico (Bravo-Hollis, 1989).

Geographic coordinates: 18° 25′ N, 95° 07′ W (Lamothe-Argumedo et al., 1997).

Remarks

The specimens described here were identified as *Protomicrocotyle mirabilis* by Bravo-Hollis (1989); however, with comparative morphological and morphometric analysis, it was determined that they correspond to a different morphotype of *Protomicrocotyle*. This morphotype differs from *P. mirabilis* in body length, since *P. mirabilis* has an average length of 2861 µm, and the morphotype has an average length of 1605 µm. *Protomicrocotyle mirabilis* has between 27 and 31 testes (Table 2.2), which are spherical and arranged in two rows (in most of the specimens, well defined). In contrast, the morphotype has, on average, 28 testes, they are elongated in shape and arranged in two rows, and in the upper part of the middle, there are some braids. The morphotype of *Protomicrocotyle* **sp. 1** differs from *P. veracruzensis* in that it is smaller, 1605 vs. 3858, and has a greater number of spines in the vaginal vestibule (65 vs. 45) (Table 2.2). *Protomicrocotyle* **sp. 1** differs from the morphotypes of *Protomicrocotyle* **sp. 2** and *Protomicrocotyle* **sp. 3**, mainly in size and shape of the body, since the morphotype of *Protomicrocotyle* **sp. 2** has a broad fusiform body and the morphotype of *Protomicrocotyle* **sp. 3** is fusiform, as well as by the number, shape, and arrangement of the testes (Table 2. 2). The distinguishing characteristics of *Protomicrocotyle* **sp. 1** and *Protomicrocotyle* **sp. 4** include the following: *Protomicrocotyle* **sp. 4** exhibits a longer body width, a larger haptor and haptoral lappet, as well as clamps, a wider testicular width, and a greater number of these (Table 2. 2). *Protomicrocotyle* **sp. 5** exhibits a more considerable body length, in addition to variations in the number, morphology, and arrangement of the testes (Table 2. 2).

Table 2. 2. Summary and comparison of the morphometric data for the morphotypes of *Protomicrocotyle* from the Gulf of Mexico and Caribbean Sea.

Species	*Protomicrocotyle* **sp. 1**	*Protomicrocotyle* **sp. 2**	*Protomicrocotyle* **sp. 3**	*Protomicrocotyle* **sp. 4**	*Protomicrocotyle* **sp. 5**	*P. veracruzensis*	*P. mirabilis*	*P. mirabilis*
Locality	Laguna de Sontecomapan, Veracruz	**Jicacal, Veracruz** and Isla Cozumel, Quintana Roo	Bahía de Chetumal, Quintana Roo	Tecolutla, Veracruz	Tecolutla, Veracruz	Casitas and Puerto de Veracruz, Veracruz, Mexico	Veracruz, Mexico	Florida Bay, USA
Host	*Caranx hippos*	***C. hippos*** and *Caranx* sp.	*C. latus*	*C. hippos*	*C. hippos*	*C. latus*	*C. hippos*	*C. hippos*
Total length	1605 (1379–1781)	2330 (1635–3489)	3312 (3245–3355)	1682 (1135–1879)	4117 (3721–4380)	3858 (3209–4709)	2861 (805–4673)	2240 (1470–3080)
Total Width	150 (134–171)	874 (561–1244)	656 (490–817)	474 (390–622)	537 (488–573)	804 (586–1098)	295 (134–537)	322 (214–449)
Haptor width	114 (80–160)	624 (435–880)	374 (270–425)	307 (275–350)	438 (410–470)	575 (415–980)	255 (70–425)	161
Right side of haptoral lappet length	55 (48–65)	131 (101–168)	150 (124–184)	100 (72–113)	174 (145–187)	173 (136–240)	117 (60–217)	64**
Left side of haptoral lappet length	51 (47–56)	124 (78–172)	138 (103–182)	105 (90–126)	169 (124–198)	194 (124–259)	120 (40–202)	72**

Table 2. 2. *Continuation.*

Central haptoral lappet length	51 (43–58)	128 (80–173)	146 (113–185)	114 (82–131)	196 (170–220)	206 (164–259)	125 (40–214)	88**
Haptoral lappet width	143 (125–180)	423 (360–490)	460 (375–520)	337 (295–375)	726 (645–820)	769 (580–920)	437 (105–785)	241
Clamps length	21 (21–22)*	39 (39–40)*	37 (32–45)*	37 (33–40)*	45 (41–48)*	64 (47–74)*	45 (14–65)*	57 (49–67)
Number of spines in MCO	20 (18–24)	21 (16–26)	21 (20–23)	24 (18–26)	21 (13–35)	23 (16–28)	22 (16–29)	19 (16–21)
Number of spines in vaginal vestibule	65 (53–75)	49 (41–59)	44 (37–48)	52 (43–58)	45 (35–61)	49 (33–59)	45 (31–65)	43
Number of testes	28 (26–31)	42 (38–48)	56 (53–59)	39 (31–48)	60 (53–67)	47 (36–69)	31 (21–38)	27 (23–33)
Testes wide	34 (29–42)	162 (101–217)	91 (84–98)	89 (66–109)	82 (65–102)	142 (96–176)	50 (14–86)	43 (28–54)
Genital pore to anterior end distance	199 (165–230)	327 (250–440)	380 (145–475)	257 (190–350)	552 (500–625)	506 (340–615)	370 (140–660)	250

Table 2. 2. *Continuation*.

Vaginal opening to anterior end distance	269 (225–305)	443 (320–655)	589 (575–625)	346 (270–445)	690 (635–755)	610 (440–730)	464 (175–740)	330
Reference	This study	This study	This study	This study	This study	Ramírez-Cruz et al., 2023	Ramírez-Cruz et al., 2023	Kritsky et al., 2011

*Data obtained from the average length of the four clamps; ** data obtained from the drawings of the article of each species.

The bold names correspond to the locality and host types.

Protomicrocotyle **sp. 2**

Figures 2.9 and 2.10.

This morphological description was based in the measurements and observation of 20 specimens of the CNHE 167; 168 from the localities of Isla Cozumel, Quintana Roo and Jicacal, Veracruz, that were previously identified as *P. manteri*. Description: Broad fusiform body, the anterior end is ovoid, body length ranges from 1635–3489 (2330 ± 568, n= 20) including the haptor, and width ranges from 561–1244 (874 ± 167, n= 20) (Figures 2.9a, 2.10a). The tegument presents cuticular striations mainly in the anterior region, giving a rough appearance to the lateral margins of the body. In the anterior region, there are a pair of pseudo-suckers, septate, muscular, oblique, and very shallowly dolliform; the right pseudo-sucker is 30–72 (57 ± 12, n= 20) long and 23–58 (38 ± 8, n= 20) wide, and the left pseudo-sucker ranges from 31–67 (56 ± 11, n= 20) long and 23–62 (38 ± 9, n= 20) wide. Retractile glandomuscular organ is present in the anterior region (Figures 2.9a, 2.10a, 2.10b). The oral opening is located in the ventral region of the body, terminal, finely ovoid, 11–30 (19 ± 5, n= 17) long, and 8–53 (34 ± 12, n= 17) wide. Pre-pharynx absent. The pharynx is broadly elliptoid, muscular, 48–62 (58 ± 5, n= 20) long, and 36–73 (56 ± 9, n= 20) wide. The esophagus is short, without diverticula, 430–515 (465 ± 29, n= 20) long and 15–40 (26 ± 8, n= 19) wide. The cecal bifurcation is located at the level of the MCO and 130–410 (192 ± 59, n= 20) distance from the anterior end. The intestinal ceca extend to the haptor region, reaching the haptoral lappet, and presents diverticula that extend to the lateral margins of the body.

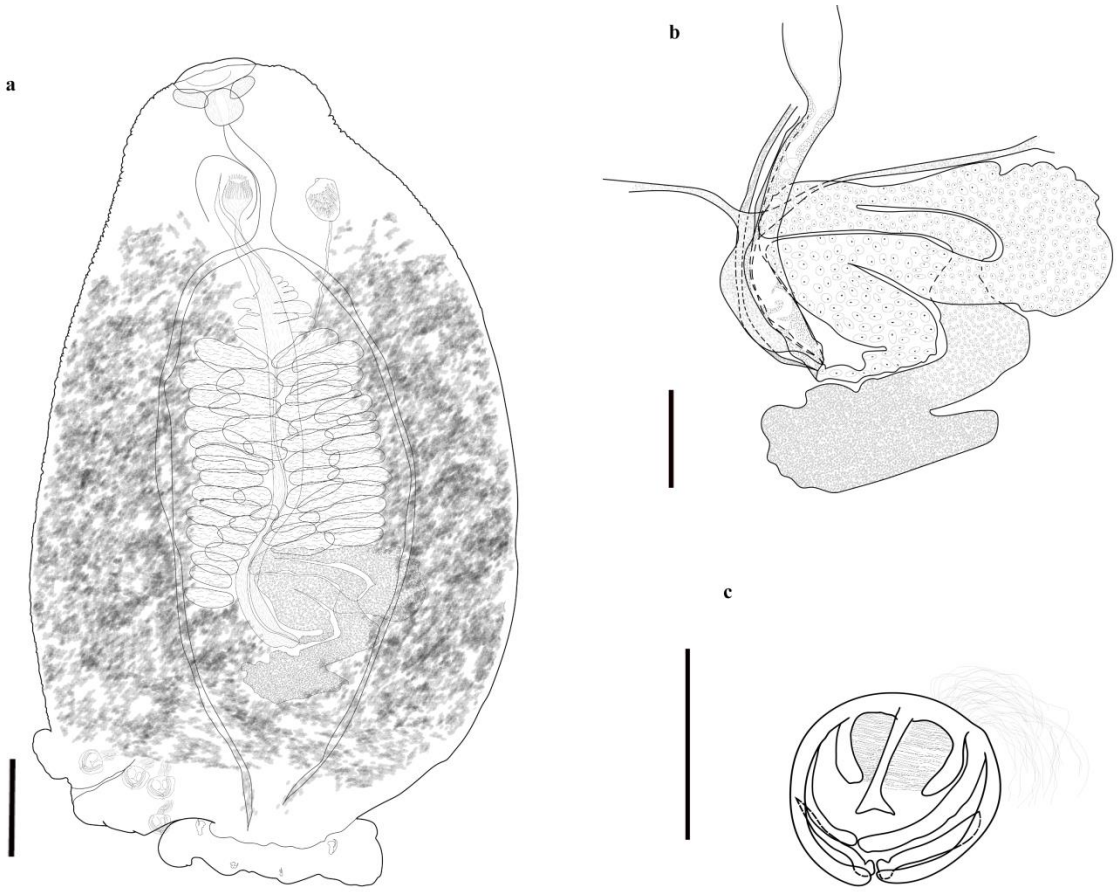

Figure 2. 9. Line drawings of *Protomicrocotyle* **sp. 2.** (a) Complete body of *Protomicrocotyle* **sp. 2** from the gills of *Caranx hippos*, ventral view, Bar 200 µm; (b) female reproductive organs, Bar 100 µm; (c) Gastrocotylid-type clamp with the peduncle, Bar 50 µm.

The haptor is asymmetrical and wide, and it is armed with a row of four gastrocotylid-type clamps with the presence of a small, short muscular peduncle and a haptoral groove that is located between the first and second clamps. The haptor ranges from 260–630 (448 ± 103, n= 19) long and 435–880 (624 ± 99, n= 19) wide at the level of the second clamp. The clamps have a similar size: the first clamp is 29–54 (40 ± 6, n= 20) long by 38–55 (50 ± 4, n = 20) wide; the second clamp is 26–50 (40 ± 40, n= 19) long by 46–61 (54 ± 4, n= 19) wide; the third clamp ranges from 26–52 (39 ± 6, n= 20) long by 41–60 (53 ± 4, n = 20) wide; and the fourth clamp ranges from 28–52 (40 ± 6, n= 20) long by 37–78 (52 ± 8, n= 20) wide. The haptoral lappet is transversely elongated and asymmetrical, with lobes on the lateral margins and at the posterior end (Figures 2.9a, 2.10c). It has a right lateral length of 101–168 (131 ± 15, n= 17), a left lateral length of 78–172 (124 ± 23, n= 17), and a central length of 80–173 (128 ± 25, n= 17) and a width of 360–490 (423 ± 35, n=

18) and is armed with three pairs of ventral hooks of the gastrocotylid type (one pair of hooks; two pairs of anchors) (Table 2.3).

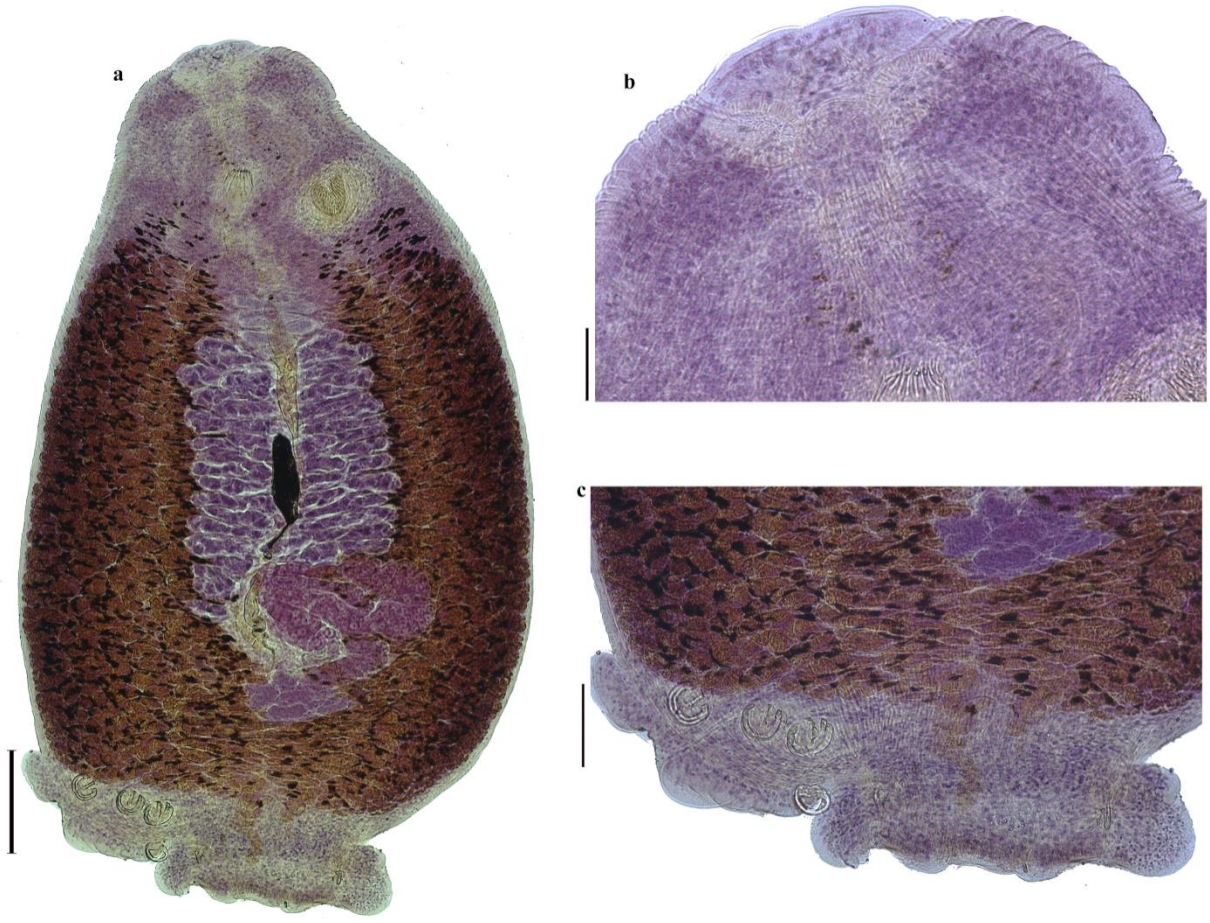

Figure 2. 10. Light micrographs of *Protomicrocotyle* **sp. 2.** (a) Complete body of *Protomicrocotyle* **sp. 2** from the gills of *Caranx hippos*, ventral view, Bar 200 µm; (b) Anterior region, Bar 50 µm; (c) Posterior region, Bar 100 µm.

The testes are depressed doliform, irregular in size, and pre-ovarian and arranged in one field. The number of testes ranges from 38–48 (42 ± 3, n= 20) with a length of 24–76 (43 ± 14, n= 20) and 101–217 (162 ± 30, n= 20) width (Figures 2.9a, 2.10a). The ratio between the L: A = 1: 3.47. The vas deferens go in the direction of the anterior region; in the part anterior to the testicular region, they expand and coil, then narrow and continue in the form of a straight tube until the MCO. The MCO is cup-shaped, muscular, and has a length of 46–100 (76 ± 13, n= 20) and 42–67 (56 ± 6, n= 20) wide. It is armed with 16–26 (25 ± 4, n= 20) spines that form a crown on the distal part of the MCO. The spines are hook-shaped, and the tips are directed outwards from the MCO. The length of the spines ranges from 31–43 (39 ± 3, n= 20) with a width of 4–5 (4 ± 1, n= 20). The

genital atrium is located in the anterior region of the body, subspherical, with muscular edges and without any type of sclerotized structure, and the distance from the genital pore to the anterior end is 250–440 (237 ± 57, n= 19).

The ovary is located in the post-testicular region, is oval, and has lobed edges, the length of the ovary ranges from 55–184 (118 ± 36, n= 20) to 113–238 (180 ± 30, n= 20) Wide. The oviduct goes towards the anterior region and subsequently extends towards the posterior region and again towards the anterior region and finally empties into the region of the oötype, from this region arises the uterus that goes towards the anterior end in the middle region between the rows of the testicles until reaching the genital atrium (Figure 2.9b). The seminal vesicle could not be measured. In the uterus, in some specimens, 2 eggs with filaments at each polar end are observed, the egg is 136–247 (207 ± 39, n= 8) long and 37–86 (59 ± 16, n= 8) wide. The anterior end filament ranges from 148–278 (216 ± 51, n= 5) long and 5–11 (7 ± 2, n= 5) wide and the polar filament from the posterior end of the egg ranges from 49–316 (181 ± 115, n= 5) long and 1–6 (4 ± 2, n= 5) wide. The vaginal pore is located anterior to the vaginal vestibule, it is ovoid, is located on the opposite side to the clamps, and has a length of 73–115 (97 ± 11, n= 19) and 64–118 (80 ± 13, n = 20) wide and is armed with 41–59 (49 ± 6, n= 20) spines, with a conical shape, of variable size, with those found in the anterior part of the vestibule being larger than those found in the vestibule. They are found at the base; these spines have small extensions in the form of smaller spines in the distal region of each spine. The length of the spines ranges from 31–43 (39 ± 3, n= 20) and 4–5 (4 ± 1, n= 20) wide. The vaginal vestibule is located at a distance of 55–655 (418 ± 126, n= 19) and the genital pore at 165–310 (202 ± 33, n= 19). The vitelline glands are found covering most of the body, starting in the region anterior or posterior to the ceca bifurcation and extending to the haptor region (Figures 2.9a, 2.10a).

Taxonomic summary

Host: *Caranx hippos*.

Common name: Crevalle Jack.

Site of infection: Gills.

Locality: Jicacal, Veracruz, Mexico (Bravo-Hollis, 1989).

Geographic coordinates: 20° 37′ N, 98° 12′ W (Lamothe-Argumedo et al., 1997).

Another host: *Caranx* sp.

Site of infection; Gills.

Other locality: Isla Cozumel, Quintana Roo, Mexico (Bravo-Hollis, 1989).

Geographic coordinates: 20° 31´N, 86° 57´W (Lamothe-Argumedo et al., 1997).

Remarks

These specimens were identified as *P. manteri* (CNHE 167: 168) parasites of *Caranx hippos* and *Caranx* sp. on the coasts of the Gulf of Mexico and Caribbean Sea, respectively, by (Bravo-Hollis, 1966). This morphotype differs from P*rotomicrocotyle mirabilis*, *P. veracruzensis*, *Protomicrocotyle* **sp. 1,** *Protomicrocotyle* **sp. 3**, *Protomicrocotyle* **sp. 4**, and *Protomicrocotyle* **sp. 5** in the shape of the body, since this new species has a broad fusiform body, as well as the presence of a retractile glandomuscular organ, the arrangement of the testes that is in a single field, the testes are wider than long and the haptoral lappet has lobes at the lateral ends (a little like to the haptoral lappet of *Neomicrocotyle*). *Protomicrocotyle ivoriensis* is another species reported in the Atlantic Ocean, and like this new species, in *P. ivoriensis* it is reported that the haptoral lappet has lobed lateral ends, giving it the shape of anchors, as in the genus *Neomicrocotyle*, although this new species differs from *P. ivoriensis* in the shape of the body, number, shape, and arrangement of the testes. Also, in *P. ivoriensis*, the vagina is tubular with sclerotized structures around it (Wahl, 1972).

Protomicrocotyle **sp. 3**

Figures 2.11, 2.12 and 2.13.

This morphological description is based on the measurement and observation of four specimens of CNHE-160 from the locality of Chetumal, Quintana Roo. Description: the body is fusiform; the anterior end is conical; the body length ranges from 3245–3355 (3312 ± 49, n= 4) including the haptor; and the width ranges from 490–817 (656 ± 143, n= 4) (Figures 2.11a, 2.13a). The integument presents cuticular striations mainly in the posterior region, giving a serrated appearance to the lateral margins of the body (Figures 2.11a, 2.13a). In the anterior region, there are a pair of pseudo-suckers, septate, muscular, oblique, and very shallowly dolliform; the right

pseudo-sucker is 36–42 (40 ± 3, n= 4) long and 24–35 (40 ± 3, n= 4) wide, and the left pseudo-sucker ranges from 34–42 (38 ± 3, n= 4) long and 30–35 (33 ± 2, n= 4). The oral opening is located in the ventral region of the body, subterminal, finely ovoid, with 11–26 (21 ± 7, n= 4) long, and 17–31 (24 ± 6, n= 4) wide. Pre-pharynx absent. The pharynx is broadly elliptoid, muscular, 46–54 (51 ± 4, n= 4) long, and 40–54 (48 ± 6, n= 4). The esophagus is long with diverticula, 610–640 (626 ± 12, n= 4) long and 20–25 (24 ± 3, n= 4) wide. The cecal bifurcation is located posterior to the MCO and 240–290 (265 ± 21, n= 4) distance from the anterior end. The intestinal ceca extend to the haptor region, reaching the haptoral lappet, and presents diverticula that extend to the margins of the body (Figures 11b, 13b).

The haptor is asymmetrical and is armed with a row of four gastrocotylid-type clamps with the presence of a small, short muscular peduncle and a haptoral groove that is located between the first and second clamps. The haptor ranges from 530–715 (599 ± 81, n= 4) long and 270–425 (374 ± 70, n= 4) wide at the level of the second clamp (Figures 11a, 13a, 13b). The clamps have a similar size: the first clamp is 29–37 (32 ± 4, n= 4) long by 49–53 (51± 2, n= 4) wide; the second clamp is 25–42 (34 ± 7, n= 4) long by 49–54 (53 ± 2, n= 4) wide; the third clamp ranges from 36–43 (38 ± 3, n= 4) long by 40–67 (52 ± 12, n= 4) wide; and the fourth clamp ranges from 42–53 (45 ± 5, n= 4) long by 38–53 (46 ± 6, n= 4) wide. The haptoral lappet is transversely elongated, asymmetrical, and at the posterior end. It has a right lateral length of 124–184 (149 ± 25, n= 4), a left lateral length of 103–182 (138 ± 4, n= 4), and a central length of 113–185 (146 ± 30) and width of 375–520 (460 ± 62, n= 4) and is armed with three pairs of ventral hooks of the gastrocotylid type (one pair of hooks and two pairs of anchors) (Figure 2.13c) (Table 2.3).

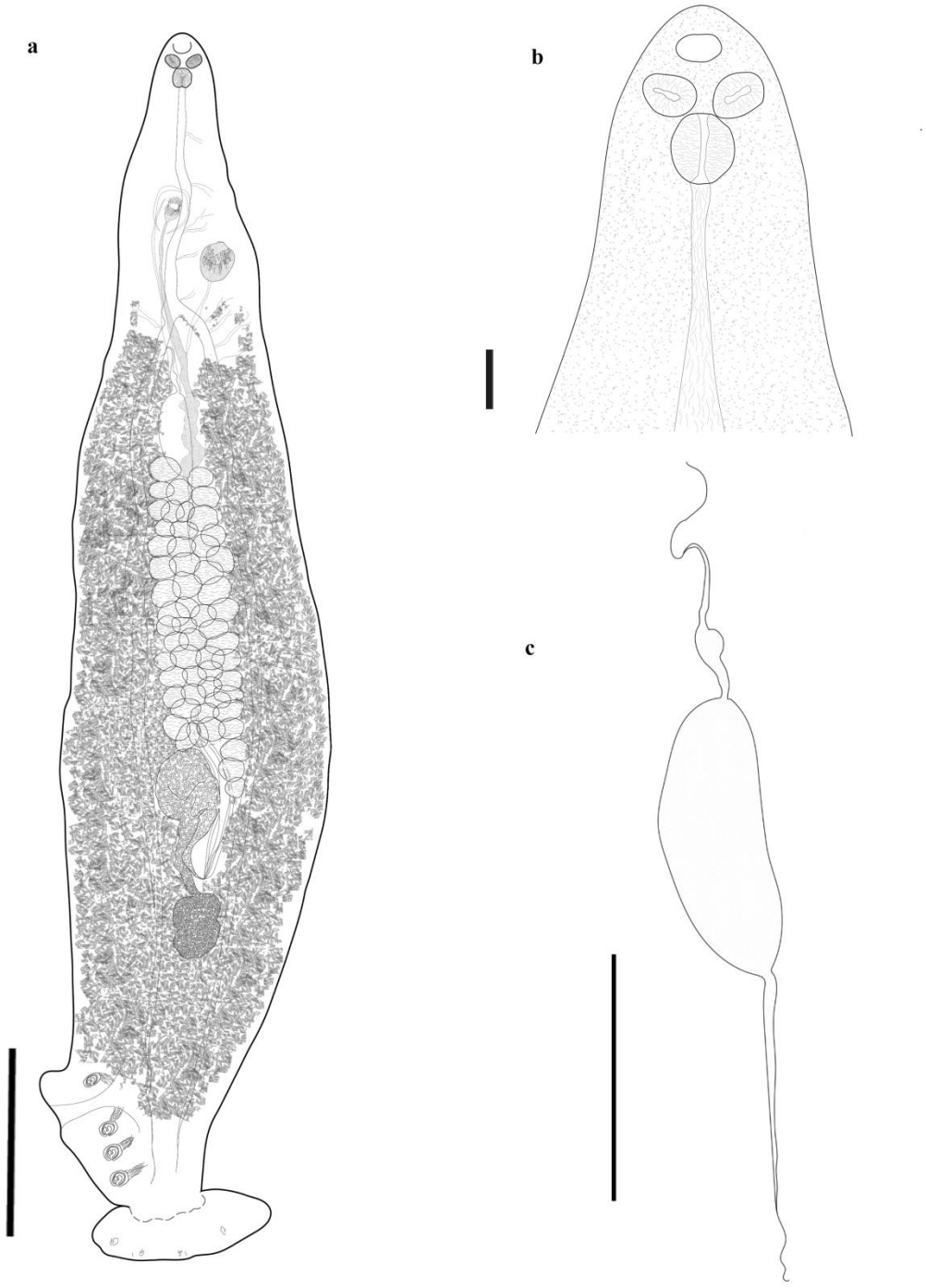

Figure 2. 11. Line drawings of *Protomicrocotyle* **sp. 3.** (a) Complete body of *Protomicrocotyle* **sp. 3** from the gills of *Caranx latus*, ventral view, Bar 200 µm; (b) Anterior region, Bar 100 µm; (c) Egg, Bar 200 µm.

The testes are shallowly doliform and subspherical, irregular in size, pre-ovarian, and arranged in a single field. The number of testes ranges from 53–59 (55 ± 3, n= 4) with a length of 55–75 (61 ± 7, n= 4) and 84–98 (91 ± 7, n= 4) width (Figures 2.11a, 2.13a). The ratio between the

L: A = 1: 2.09. The vas deferens go in the direction of the anterior region; in the part anterior to the testicular region, they expand and coil, then narrow and continue in the form of a straight tube until the MCO. The MCO is cup-shaped, muscular, and has a length of 70–95 (78 ± 11, n= 4) and 46–61 (52 ± 7, n= 4) width. It is armed with 20–23 (26 ± 6, n= 4) spines that form a crown on the distal part of the MCO. The spines are hook-shaped, and the tips are directed outwards from the MCO. The length of the spines ranges from 37–44 (41 ± 3, n= 4) with a width of 2–4 (3 ± 1, n= 4) (Figure 2.12b). The genital atrium is located in the anterior region of the body, subspherical, with muscular edges, and without any type of sclerotized structure. The distance from the genital pore to the anterior end is 145–475 (380 ± 157, n= 4).

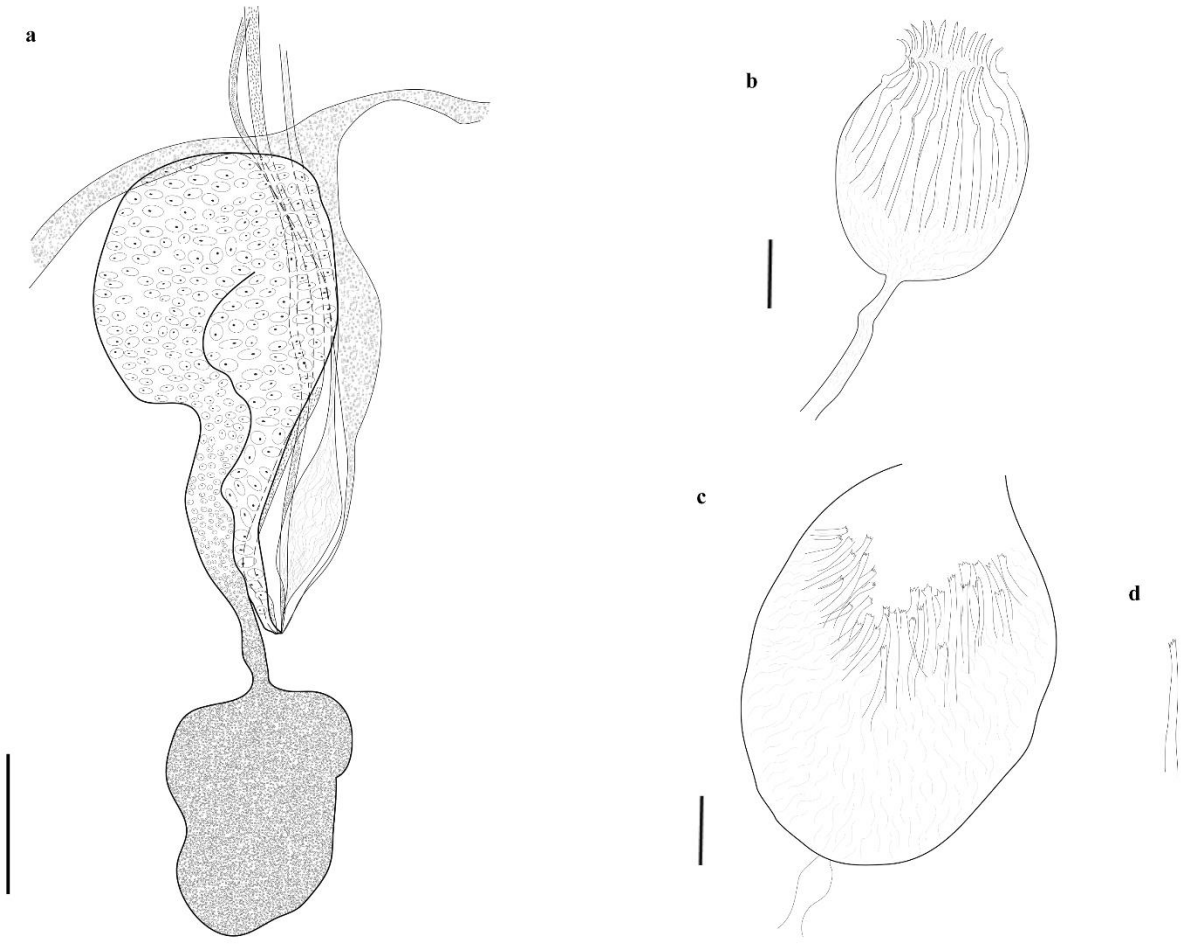

Figure 2. 12. Line drawings of the reproductive anatomical organs of *Protomicrocotyle* **sp. 3**. (a) Female reproductive organ, Bar 100 µm; (b) Male copulatory organ, Bar 20 µm;(c) Vaginal vestibule, Bar; 20 µm; (d) Spine of the vaginal vestibule, Bar 20 µm.

The ovary is located in the post-testicular region, oval and with lobed edges. The length of the ovary ranges from 150–175 (163 ± 10, n= 4) to 89–158 (131 ± 32, n= 4). The oviduct goes towards the anterior region, subsequently extends towards the posterior region, again towards the anterior region, and finally empties into the region of the oötype. From this region arises the uterus, which goes towards the anterior end in the middle region between the rows of the testicles until reaching the genital atrium. The seminal vesicle could not be measured (Figure 2.12a). In the uterus, in some specimens, an egg with filaments at each polar end can be observed. The egg is 162–223 (191 ± 25, n= 4) long and 66–84 (71 ± 9, n= 4). The anterior end filament ranges from 131–286 (193 ± 67, n= 4) long and 5–7 (6 ± 1, n= 4) wide, and the posterior end filament could not be measured (Figure 2.11c). The vaginal pore is located anterior to the vaginal vestibule; it is ovoid, is located on the opposite side to the clamps, has a length of 95–118 (103 ± 11, n= 4) and 83–109 (91 ± 12, n = 4) wide, and is armed with 37–48 (44 ± 5, n= 4) spines. The spines are conical and have a variable size, with those found in the anterior part of the vestibule being larger than those found in the vestibule. They are found at the base; these spines have small extensions in the form of smaller spines in the distal region of each spine. The length of the spines ranges from 32–41 (38 ± 4, n= 4) and 4–5 (4 ± 1, n= 4) wide. The vaginal vestibule is located at a distance of 575–625 (589 ± 24, n= 4) and the genital pore at a distance of 110–205 (171 ± 43, n= 4) (Figures 2.12c, 2.12d). The vitelline glands are found covering most of the body, starting in the region anterior or posterior to the ceca bifurcation and extending to the haptor region (Figures 2.11a, 2.13a).

Figure 2. 13. Light micrographs of *Protomicrocotyle* **sp. 3**. (a) Complete body of *Protomicrocotyle* **sp. 3** from the gills of *Caranx latus*, ventral view, Bar 500 µm; (b) Anterior region, Bar 200 µm; (c) Posterior region of the body, Bar 100 µm.

Taxonomic summary

Host: *Caranx latus* Agassiz, 1831

Site of infection: Gills

Locality: Bahía de Chetumal, Quintana Roo (Bravo-Hollis, 1989).

Geographic coordinates: 18° 30´N, 88° 18´W (Lamothe-Argumedo et al., 1997).

Remarks

Protomicrocotyle **sp. 3** differs from *P. mirabilis* in that this new species has a greater number of testes and a larger size; they are subspherical and are arranged in a single field. The difference between *Protomicrocotyle* **sp. 3** and *P. veracruzensis* is mainly in the shape and arrangement of the testes, although the testes of *P. veracruzensis* are considerably wider than they are long (Table 2.2), and the average length of the clamps of *Protomicrocotyle* **sp. 3** is smaller in size than those of *P. veracruzensis*. This morphotype differs from *Protomicrocotyle* **sp. 1** in the length of the body, as well as the number, shape, and arrangement of the testes, as in *Protomicrocotyle* **sp. 2**, although this species has a wider body, as well as the testes (Table 2.2). This morphotype differs from *P. ivoriensis* in the shape of the body, number, shape, and arrangement of the testes. Also, in *P. ivoriensis*, the vagina is tubular with sclerotized structures around it, while in this morphotype it is globular (Wahl, 1972). The differences between *Protomicrocotyle* **sp. 3** and *Protomicrocotyle* **sp. 4** are mainly in the length and shape of the body, and the shape and arrangement of the testes (Table 2.2). The differences between *Protomicrocotyle* **sp. 3** and *Protomicrocotyle* **sp. 5**, the latter is larger than *Protomicrocotyle* **sp. 3**, and in general terms of most of the variables that were measured and compared (Table 2.2), although the size of the testes and ovary is larger in *Protomicrocotyle* **sp. 3**.

Table 2. 3. The comparison of the anchors and hooks of *Protomicrocotyle* morphotypes with other morphologically similar species.

Morphotypes	*Protomicrocotyle* **sp. 1**	*Protomicrocotyle* **sp. 2**	*Protomicrocotyle* **sp. 3**	*Protomicrocotyle* **sp. 4**	*Protomicrocotyle* **sp. 5**	*P. veracruzensis*	*P. mirabilis*
Locality	Laguna de Sontecomapan, Veracruz	**Jicacal, Veracruz** and Isla Cozumel, Quintana Roo	Bahía de Chetumal, Quintana Roo	Tecolutla, Veracruz	Tecolutla, Veracruz	Casitas and Puerto de Veracruz, Veracruz, Mexico	Veracruz, Mexico
Host	*Caranx hippos*	***C hippos*** and *Caranx sp.*	*C. latus*	*C. hippos*	*C. hippos*	*C. latus*	*C. hippos*
Site	Gill filaments	Gill filaments	Gill filaments	Gill filaments	Gill filaments	Gill filaments	Gill filaments
Right side hook length (anchor)	24–29 (27 ± 1, n= 6)	41–61 (53 ± 5, n= 19)	49–54 (51 ± 2, n= 4)	43–50 (46 ± 3, n= 7)	50–60 (54 ± 3, n= 7)	32–65 (47 ± 9, n= 37)	38–70 (49 ± 8, n= 26)
Right side hook width	2–4 (2, n= 6)	4–7 (5 ± 1, n= 19)	4–6 (5 ± 1, n= 4)	4–5 (5 ± 0, n= 7)	5–6 (5 ± 0, n= 6)	4–6 (5 ± 1, n= 37)	5–7 (5 ± 1, n= 26)
Right side hook opening length	5–7 (6, n= 6)	7–16 (10 ± 2, n= 18)	11–13 (12 ± 1, n= 4)	7–10 (9 ± 1, n= 7)	7–7 (7 ± 0, n= 4)	5–14 (9 ± 3, n= 37)	7–13 (10 ± 2, n= 24)
Internal root length of right lateral hook	4–7 (6, n= 6)	8–16 (13 ± 2, n= 20)	8–13 (11 ± 2, n= 4)	11–14 (13 ± 1, n= 7)	13–18 (14 ± 2, n= 7)	4–19 (13 ± 4, n= 37)	11–20 (16 ± 3, n= 26)
Width of internal root of the right lateral hook	1–5 (3 ± 1, n= 6)	5–8 (6 ± 1, n= 20)	6–7 (7 ± 1, n= 4)	5–6 (6 ± 1, n= 7)	6–8 (7 ± 1, n= 5)	2–7 (6 ± 2, n= 36)	5–10 (7 ± 1, n= 27)

Table 2. 3. *Continuation.*

Right side hook handle length	7–13 (8 ± 1, n= 6)	20–43 (27 ± 6, n= 19)	22–25 (24 ± 2, n= 4)	11–24 (21 ± 4, n= 7)	23–28 (26 ± 2, n= 7)	17–26 (22 ± 2, n= 37)	18–29 (23 ± 3, n= 26)
External root length of the right lateral hook	5–7 (6, n= 6)	13–20 (16 ± 2, n= 20)	13–18 (15 ± 2, n= 4)	8–12 (11 ± 1, n= 7)	8–13 (12 ± 2, n= 5)	5–19 (12 ± 4, n= 37)	12–20 (15 ± 2, n= 27)
Length of main part of the right-side hook	11–14 (13 ± 1, n= 6)	1–37 (22 ± 7, n= 19)	17–22 (20 ± 2, n= 4)	17–22 (20 ± 2, n= 7)	17–23 (21 ± 2, n= 7)	14–23 (20 ± 2, n= 37)	10–25 (19 ± 3, n= 26)
Right side hook tip length	5–7 (6, n= 6)	8–31 (18 ± 5, n= 19)	11–17 (15 ± 3, n= 4)	10–14 (12 ± 1, n= 7)	11–20 (16 ± 3, n= 7)	11–18 (14 ± 2, n= 37)	5–19 (14 ± 3, n= 26)
Left side hook length (anchor)	23–30 (27 ± 1, n= 6)	7–64 (51 ± 11, n= 20)	48–55 (52 ± 3, n= 4)	46–56 (51 ± 3, n= 7)	52–58 (54 ± 2, n= 7)	37–68 (50 ± 8, n= 42)	40–64 (50 ± 8, n= 29)
Left side hook width	2–4 (3, n= 6)	4–7 (5 ± 1, n= 20)	5–6 (6 ± 1, n= 4)	4–6 (5 ± 1, n= 7)	5–6 (5 ± 0, n= 7)	2–7 (5 ± 1, n= 42)	5–7 (6 ± 1, n= 28)
Left side hook opening length	5–7 (6, n= 6)	4–17 (9 ± 3, n= 17)	7–11 (9 ± 2, n= 4)	8–12 (10 ± 2, n= 7)	4–10 (7 ± 2, n= 8)	5–14 (10 ± 2, n= 39)	7–16 (10 ± 2, n= 25)
Internal root length of left lateral hook	4–7 (6, n= 6)	11–16 (14 ± 1, n= 19)	12–14 (13 ± 1, n= 4)	12–14 (13 ± 1, n= 7)	13–18 (15 ± 2, n= 8)	11–19 (15 ± 3, n= 42)	11–20 (16 ± 2, n= 29)

Table 2. 3. *Continuation.*

Width of internal root of the left lateral hook	2–4 (3, n= 6)	5–18 (8 ± 3, n= 19)	5–6 (5 ± 1, n= 4)	5–7 (6 ± 1, n= 7)	6–8 (7 ± 1, n= 8)	4–8 (6 ± 1, n= 41)	5–8 (6 ± 1, n= 29)
Left side hook handle length	6–13 (9 ± 1, n= 6)	22–37 (27 ± 4, n= 20)	24–25 (25 ± 1, n= 4)	22–25 (23 ± 1, n= 7)	23–28 (25 ± 2, n= 8)	12–46 (24 ± 5, n= 42)	17–34 (24 ± 3, n= 29)
External root length of the left lateral hook	5–7 (6, n= 6)	12–18 (16 ± 2, n= 20)	13–16 (14 ± 1, n= 4)	10–13 (12 ± 1, n= 7)	8–14 (11 ± 2, n= 8)	7–20 (13 ± 3, n= 42)	10–19 (14 ± 3, n= 28)
Length of main part of the left side hook	11–16 (13 ± 1, n= 6)	18–31 (24 ± 4, n= 20)	19–22 (21 ± 2, n= 4)	17–20 (19 ± 1, n= 7)	19–22 (20 ± 1, n= 8)	14–26 (20 ± 3, n= 42)	13–31 (20 ± 3, n= 29)
Left side hook tip length	5–6 (6, n= 6)	13–28 (19 ± 4, n= 20)	13–18 (16 ± 2, n= 4)	12–14 (13 ± 1, n= 7)	10–17 (14 ± 2, n= 8)	10–19 (14 ± 2, n= 42)	8–24 (15 ± 3, n= 29)
Right half hook length (anchor)	13–16 (14 ± 1, n= 6)	24–31 (29 ± 2, n= 17)	23–29 (26 ± 3, n= 4)	24–28 (26 ± 1, n= 6)	25–31 (28 ± 2, n= 8)	23–38 (29 ± 4, n= 41)	1–31 (26 ± 5, n= 26)
Right half hook width	1–2 (2, n= 6)	4–5 (5 ± 1, n= 17)	4–5 (5 ± 1, n= 4)	2–4 (2 ± 1, n= 6)	2–4 (3 ± 1, n= 8)	2–5 (4 ± 1, n= 41)	2–5 (4 ± 1, n= 26)

Table 2. 3. *Continuation.*

Right middle hook opening length	2–5 (4, n= 6)	4–7 (6 ± 1, n= 14)	4–7 (6 ± 1, n= 4)	4–5 (4 ± 1, n= 6)	2–6 (4 ± 1, n= 6)	2–7 (5 ± 1, n= 41)	4–8 (5 ± 1, n= 24)
Internal root length of right middle hook	2–4 (3, n= 6)	5–8 (6 ± 1, n= 16)	5–7 (6 ± 1, n= 4)	4–6 (5 ± 1, n= 6)	4–7 (5 ± 1, n= 6)	4–8 (5 ± 1, n= 41)	4–11 (6 ± 2, n= 23)
Internal root width of the right middle hook	1–1 (1, n= 6)	2–7 (4 ± 2, n= 16)	2–4 (3 ± 1, n= 4)	2–2 (2, n= 6)	2–4 (3 ± 1, n= 5)	1–4 (3 ± 1, n= 40)	2–4 (3 ± 1, n= 23)
Middle right hook handle length	4–10 (7 ± 1, n= 6)	14–20 (18 ± 1, n= 16)	17–19 (18 ± 1, n= 4)	14–17 (16 ± 1, n= 6)	16–20 (18 ± 1, n= 6)	12–160 (21 ± 23, n= 41)	11–19 (16 ± 2, n= 24)
External root length of the right middle hook	2–6 (4 ± 1, n= 6)	5–8 (6 ± 1, n= 16)	6–7 (7 ± 1, n= 4)	4–7 (5 ± 1, n= 6)	4–8 (6 ± 1, n= 6)	4–8 (6 ± 1, n= 39)	4–13 (7 ± 2, n= 23)
Length of main part of right middle hook	7–12 (10 ± 1, n= 6)	12–18 (15 ± 2, n= 16)	14–17 (16 ± 1, n= 4)	11–13 (12 ± 1, n= 6)	12–18 (16 ± 2, n= 6)	8–23 (15 ± 3, n= 41)	8–17 (13 ± 2, n= 23)
Right middle hook tip length	4–6 (5, n= 6)	10–16 (12 ± 1, n= 16)	11–12 (12 ± 1, n= 4)	7–12 (9 ± 2, n= 6)	10–13 (12 ± 1, n= 6)	6–14 (11 ± 2, n= 41)	5–14 (10 ± 2, n= 26)

Table 2. 3. *Continuation.*

Left middle hook length (anchor)	12–14 (13, n= 6)	25–35 (29 ± 2, n= 17)	28–32 (30 ± 2, n= 4)	25–29 (26 ± 2, n= 6)	25–29 (27 ± 2, n= 6)	23–34 (28 ± 3, n= 41)	23–32 (28 ± 2, n= 26)
Middle left hook width	1–2 (2, n= 6)	4–6 (4 ± 1, n= 17)	4–5 (5 ± 1, n= 4)	2–4 (3 ± 1, n= 6)	2–4 (4 ± 1, n= 5)	2–5 (4 ± 1, n= 41)	2–4 (4 ± 0, n= 26)
Left middle hook opening length	4–5 (4, n= 6)	2–7 (5 ± 1, n= 16)	4–6 (5 ± 1, n= 4)	1–4 (3 ± 2, n= 6)	2–5 (4 ± 1, n= 4)	2–8 (5 ± 1, n= 40)	4–11 (5 ± 1, n= 24)
Internal root length of left middle hook	2–4 (3, n= 6)	4–8 (6 ± 1, n= 16)	4–8 (6 ± 2, n= 4)	2–4 (3 ± 1, n= 6)	2–6 (5 ± 2, n= 4)	4–8 (5 ± 1, n= 41)	2–12 (7 ± 3, n= 24)
Width of the internal root of the left middle hook	1–1 (1, n= 6)	2–5 (3 ± 1, n= 16)	2–4 (3 ± 1, n= 4)	2–2 (2, n= 6)	2–4 (3 ± 1, n= 4)	2–5 (3 ± 1, n= 41)	2–5 (3 ± 1, n= 23)
Left Middle Hook Handle Length	5–10 (6, n= 6)	8–23 (19 ± 4, n= 16)	17–22 (20 ± 2, n= 4)	14–17 (16 ± 1, n= 6)	17–19 (19 ± 1, n= 4)	13–29 (18 ± 4, n= 41)	12–20 (17 ± 2, n= 25)
External root length of the left middle hook	2–4 (3, n= 6)	6–8 (7 ± 1, n= 16)	6–10 (8 ± 2, n= 4)	5–6 (5 ± 1, n= 6)	4–5 (5 ± 1, n= 4)	2–8 (5 ± 1, n= 40)	4–11 (7 ± 2, n= 24)

Table 2. 3. *Continuation.*

Length of main part of left middle hook	7–11 (9 ± 1, n= 6)	14–20 (17 ± 2, n= 16)	16–18 (17 ± 1, n= 4)	12–14 (13 ± 1, n= 6)	14–17 (16 ± 1, n= 4)	11–25 (15 ± 4, n= 41)	8–20 (14 ± 3, n= 25)
Left middle hook tip length	4–6 (5, n= 6)	11–16 (13 ± 1, n= 16)	11–13 (12 ± 1, n= 4)	6–11 (9 ± 2, n= 6)	12–13 (13 ± 1, n= 4)	6–19 (11 ± 3, n= 41)	8–14 (11 ± 2, n= 26)
Total length of right center hook	6–8 (7, n= 6)	11–22 (17 ± 3, n= 14)	16–19 (17 ± 2, n= 3)	14–16 (14 ± 1, n= 8)	13–18 (16 ± 1, n= 11)	12–23 (17 ± 2, n= 41)	12–19 (17 ± 1, n= 30)
Right center hook width	1–1 (1, n= 6)	2–2 (2, n= 1|4)	2–2 (2, n= 3)	-	2–2 (2, n= 3)	1–4 (2 ± 1, n= 39)	1–2 (2, n= 30)
Total length of left center hook	7 (7–8, n= 6)	17 (13–19, n= 13)	17 (16–18, n= 4)	15 (13–17, n= 8)	17 (16–19, n= 9)	17 (13–19, n= 29)	17 (13–19, n= 41)
Left center hook width	1 (n= 6)	2 (n= 13)	2 (1–2, n= 4)	-	2 (n= 3)	2 (1–2, n= 29)	2 (1–2, n= 38)

Data presentation format: minimum, maximum, and in parentheses, mean, standard deviation, and n, which represents the number of individuals or anatomical structures that were measured.

Protomicrocotyle **sp. 4**

Figures 2.14 and 2.15.

This morphological characterization is based on the measurement and observation of 8 specimens from the CHE-CIB-UAEH from the locality of Tecolutla, Veracruz. Description: Body fusiform; the anterior end is conical; the body length ranges from 1135–1879 (1682 ± 244, n= 8) including the haptor; and the width ranges from 390–622 (474 ± 72, n= 8) (Figure 14a). The tegument has cuticular striations. In the anterior region, there are a pair of pseudo-suckers, septate, muscular, oblique, and very shallowly dolliform; the right pseudo-sucker is 36–50 (42 ± 6, n= 8) long and 23–30 (27 ± 2, n= 8) wide, and the left pseudo-sucker ranges from 37–53 (43 ± 5, n= 8) long and 24–31 (28 ± 3, n= 8). The oral opening is located in the ventral region of the body, subterminal, finely ovoid, 25–29 (26 ± 2, n= 6) long, and 20–40 (29 ± 8, n= 6) wide. Pre-pharynx absent. Pharynx broadly elliptoid, muscular, 30–55 (43 ± 7, n= 8) long, and 41–48 (44 ± 3, n= 8) (Figures 14a, 14b, 15a, 15c). The esophagus is long and, with diverticula, 320 (n= 1) long and 20 (n= 1) wide. The cecal bifurcation is located posterior to the MCO and 470 (n= 1) from the anterior end. The intestinal ceca extend to the haptor region, reaching the haptoral lappet and present diverticula that extend to the margins of the body.

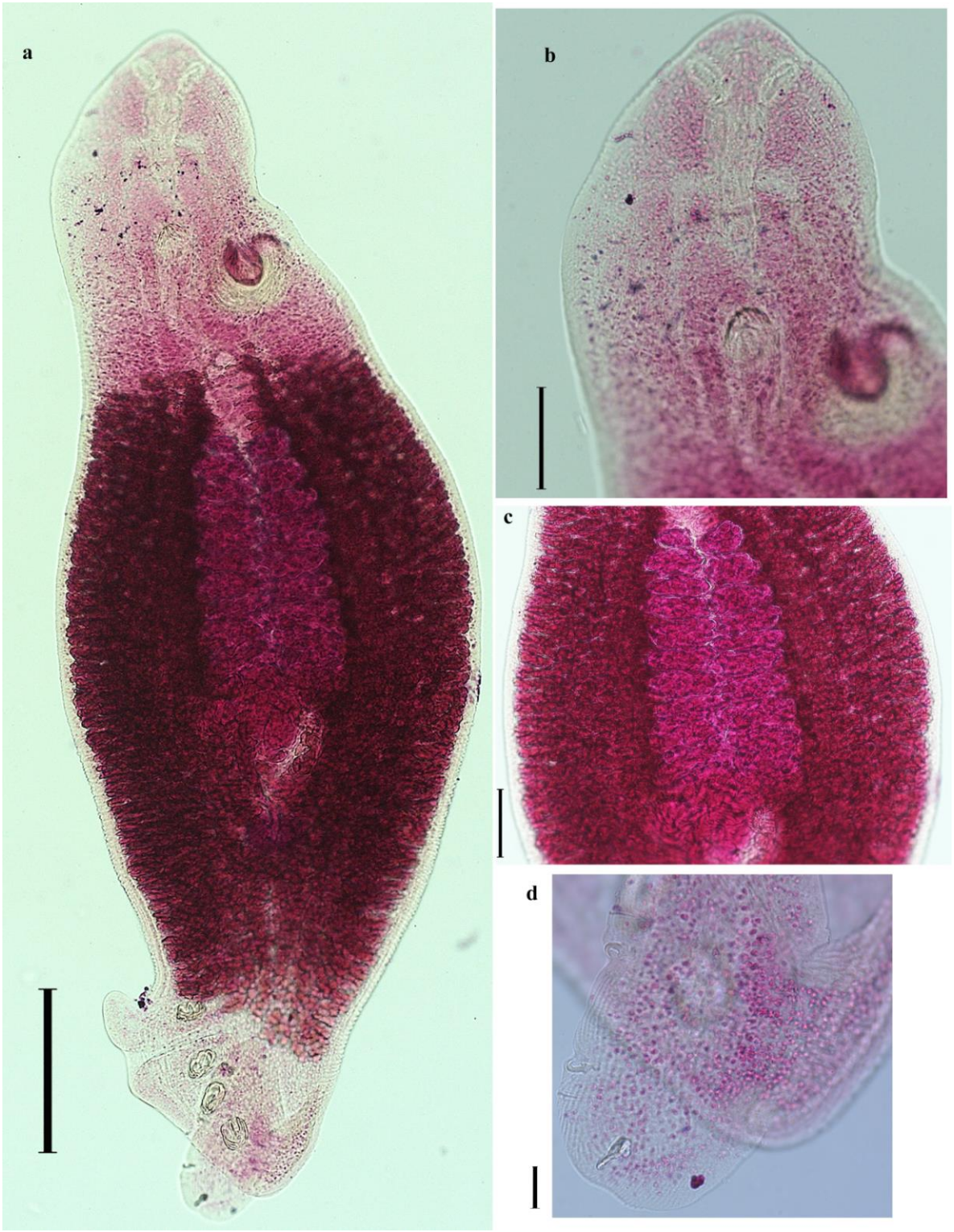

Figure 2. 14. Light micrographs of *Protomicrocotyle* **sp. 4**. (a) Complete body of *Protomicrocotyle* **sp. 4** from the gills of *Caranx hippos*, ventral view, Bar 250 µm; (b) anterior region, Bar 100 µm; (c) Arrangement of the testes, Bar 100 µm; (c) Haptoral lappet with the lateral and medium anchors and hooks, Bar 25 µm.

The haptor is asymmetrical and armed with a row of four gastrocotylid-type clamps with a small, short muscular peduncle and a haptoral groove between the first and second clamps. The haptor ranges from 245–430 (334 ± 55, n= 8) long and 275–350 (307 ± 30, n= 8) wide at the level of the second clamp (Figure 2.14d). The clamps have a similar size: the first clamp is 29–50 (39 ± 11, n= 6) long by 36–48 (42 ± 5, n= 6) wide; the second clamp is 28–44 (33 ± 6, n= 5) long by 41–48 (46 ± 3, n= 5) wide; the third clamp ranges from 28–49 (40 ± 9, n= 6) long by 37–53 (42 ± 6, n= 6) wide; and the fourth clamp ranges from 28–47 (39 ± 7, n= 7) long by 37–55 (45 ± 8, n= 7) wide (Figure 2.15d). The haptoral lappet is transversely elongate with the lateral ends lobed. It has a right lateral length of 72–113 (100 ± 16, n= 7), a left lateral length of 90–126 (105 ± 12, n= 7), and a central length of 82–131 (114 ± 18, n= 7), with a width of 295–375 (337 ± 24, n= 8) and is armed with three pairs of ventral hooks of the gastrocotylid type (one pair of hooks; two pairs of anchors) (Figure 2.15d) (Table 2.3).

The testes are very shallowly doliform (horizontally elongated), irregular in size, preovarian, and arranged in two fields, each with a single row. The number of testes ranges from 31–48 (39 ± 7, n= 8) with a length of 23–43 (35 ± 9, n= 80) and 66–109 (89 ± 14, n= 8) width (Figures 2.14a, 2.14c). The ratio between the L: A = 1: 1.8. The vas deferens goes in the direction of the anterior region; in the part anterior to the testicular region, it expands and coils, then narrows and continues in the form of a straight tube until the MCO. The MCO is cup-shaped and muscular and has a length of 37–52 (45 ± 6, n= 8) and 40–50 (44 ± 4, n= 8) width. It is armed with 18–26 (24 ± 2, n= 8) spines that form a crown on the distal part of the MCO; the spines are hook-shaped; and the tips are directed outwards from the MCO. The length of the spines ranges from 25–31 (28 ± 2, n= 8) with a width of 2–4 (3 ± 1, n= 8). The genital atrium is located in the anterior region of the body, subspherical, with muscular edges and without any sclerotized structure, and the distance from the genital pore to the anterior end is 190–350 (257 ± 48, n= 8) (Figures 2.15a, 2.15b).

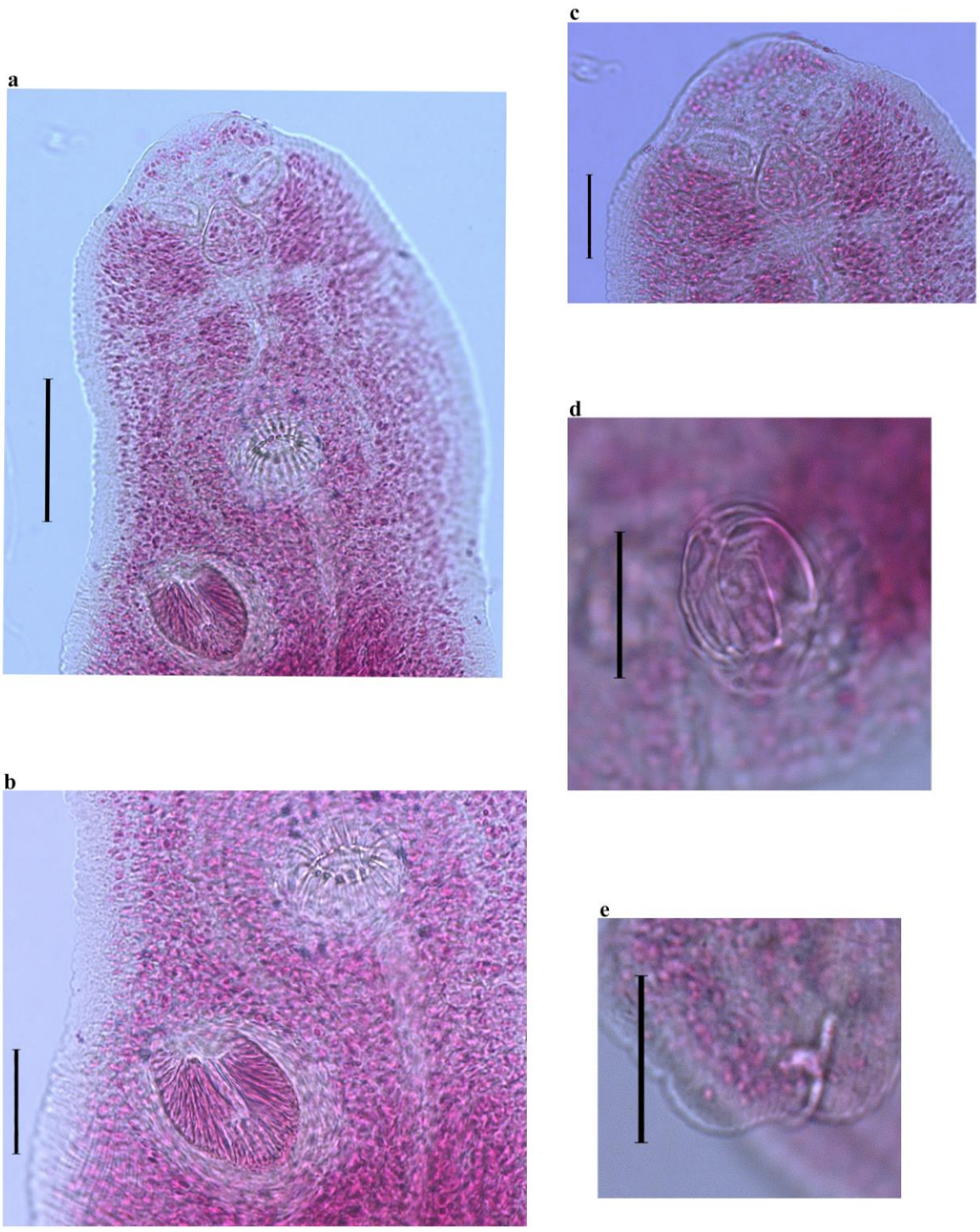

Figure 2. 15. Light micrographs of *Protomicrocotyle* **sp. 4**. (a) Anterior region, Bar 100 µm;(b) Male copulatory organ and vestibule vaginal, Bar 50 µm;(c) Anterior region of the prohaptor, the dome termination of the prohaptor is shown, Bar 50 µm;(d) Clamp, 50 µm;(e) Lateral anchor, Bar 50 µm.

The ovary is located in the post-testicular region, is oval, and has lobed edges. The length of the ovary ranges from 48–104 (74 ± 20, n= 6) to 60–103 (80 ± 15, n= 6). The

oviduct goes towards the anterior region, subsequently extends towards the posterior region, again towards the anterior region, and finally empties into the region of the oötype. From this region arises the uterus, which goes towards the anterior end in the middle region between the rows of the testicles until reaching the genital atrium. The seminal vesicle could not be observed. In the uterus, in some specimens, it is possible to observe an egg with filaments at each polar end; the egg is 136–166 (155 ± 13, n= 4) long and 42–53 (48 ± 5, n= 4) wide. The anterior end filament had a length of 209 (n= 1) long by 5 (n= 1) wide, and the posterior end filament ranged from 260 (n= 1) long by 6 (n= 1) wide.

The vaginal pore is located anterior to the vaginal vestibule; it is deltoid in shape; it is located on the opposite side to the clamps; it has a length of 54–88 (75 ± 10, n= 8) and 44–58 (51 ± 5, n = 8) wide; and it is armed with 43–58 (52 ± 7, n= 6) spines, with a flat shape and a variable size, with those found in the anterior part of the vestibule being larger than those found in the vestibule. They are found at the base; these spines have small extensions in the form of smaller spines in the distal region of each spine. The length of the spines ranges from 23–26 (25 ± 1, n= 8) and 2–4 (2, n= 14) wide. The vaginal vestibule is located at a distance of 270–445 (346 ± 56, n= 8) and the genital pore at 70–180 (120 ± 24, n= 8) (Figures 2.15a, 2.15b). The vitelline glands are found covering most of the body, starting in the region anterior or posterior to the ceca bifurcation and extending to the haptor region.

Taxonomic summary

Host: *Caranx hippos* (Linnaeus, 1766)

Site of infection: Gills

Locality: Tecolutla, Veracruz (20°28′39″N, 97°00′30″W).

Remarks

Protomicrocotyle **sp. 4** differs from *P. mirabilis* in that it is smaller in size, the testes are wider than long, unlike *P. mirabilis*, which are spherical or subspherical, and the haptoral lappet has lobed lateral ends. *Protomicrocotyle* sp. 4 is differentiated from *P. veracruzensis* in that it is smaller in size, has a smaller number of testes (Table 2.2), and these are arranged in two fields with a row in each field, whereas in *P. veracruzensis* they are arranged in two

fields with more than one row in each field. While the differences between *Protomicrocotyle* **sp. 1**, *Protomicrocotyle* **sp. 2**, *Protomicrocotyle* **sp. 3**, and *Protomicrocotyle* **sp. 5** are found in the length and shape of the body, in the arrangement, number, and shape of the testes, as well as the haptoral lappet.

Protomicrocotyle **sp. 5**

Figures 2.16 and 2.17.

This morphological characterization is based on the measurement and observation of 11 specimens from the CHE-CIB-UAEH from the locality of Tecolutla, Veracruz. Description: Body fusiform; the anterior end is conical; the body length ranges from 3721–4380 (4117 ± 246, n= 11) including the haptor; and the width ranges from 488–573 (537 ± 27, n= 11). The tegument has cuticular striations, mainly in the middle and posterior regions of the body. In the anterior region, there are a pair of pseudo-suckers, septate, muscular, oblique, and very shallowly dolliform; the right pseudo-sucker is 42–59 (52 ± 5, n= 11) long and 40–48 (43 ± 2, n= 11) wide, and the left pseudo-sucker ranges from 47–60 (54 ± 5, n= 11) long and 42–55 (44 ± 4, n= 11) wide. The oral opening is located in the ventral region of the body, subterminal, ovoid (finely ovoid), 31–35 (34 ± 2, n= 3) long, and 25–59 (42 ± 10, n= 8) wide. Pre-pharynx absent. Pharynx elliptical (broadly elliptoid), muscular, 30–55 (45 ± 6, n= 14) long, and 40–48 (43 ± 3, n= 14). The esophagus is long, with diverticula, 605–765 (663 ± 46, n= 10) long and 20–30 (24 ± 3, n= 10) wide. The cecal bifurcation is located posterior to the MCO and 775–920 (846 ± 51, n= 10) distance from the anterior end. The intestinal ceca extend to the haptor region, reaching the larval appendix and present diverticula that extend to the margins of the body.

The haptor is asymmetrical and armed with a row of four gastrocotylid-type clamps with a small, short muscular peduncle and a haptoral groove between the first and second clamps (Figure 2.18b). The haptor ranges from 655–835 (726 ± 52, n= 10) long and 410–470 (438 ± 19, n= 10) wide at the level of the second clamp. The clamps have a similar size: the first clamp is 36–47 (41 ± 3, n= 8) long by 49–58 (54 ± 3, n= 8) wide; the second clamp is 42–50 (46 ± 3, n= 8) long by 54–62 (41± 55, n= 10) wide; the third clamp ranges from 41–55 (46 ± 4, n= 10) long by 54–65 (59 ± 3, n= 10) wide; and the fourth clamp ranges from 40–

56 (48 ± 6, n= 9) long by 54–62 (59 ± 3, n= 8) wide (Figures 2.18b, 2.18c). The haptoral lappet is transversely elongated. It has a right lateral length of 145–187 (174 ± 14, n= 10), a left lateral length of 124–198 (169 ± 21, n= 10), a central length of 170–220 (196 ± 14, n= 10), a width of 645–820 (726 ± 48, n= 11) and is armed with three pairs of ventral hooks of the gastrocotylid type (one pair of hooks; two pairs of anchors) (Figure 2.18d) (Table 2.3).

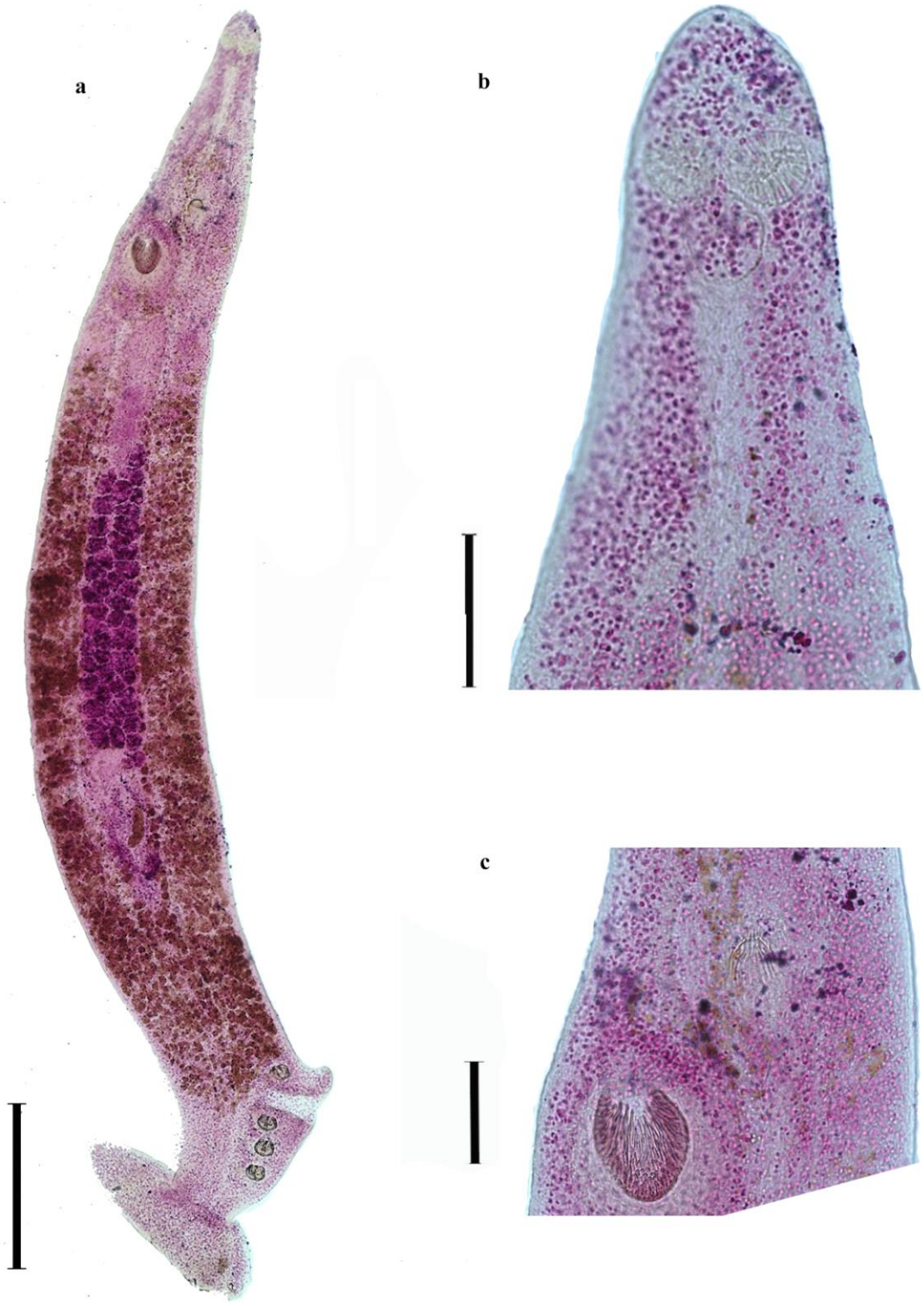

Figure 2. 16. Light micrographs of *Protomicrocotyle* **sp. 5**. (a) Complete body of *Protomicrocotyle* **sp. 5** from the gills of *Caranx hippos*, ventral view, Bar 500 µm; (b) Anterior region, Bar 100 µm;(c) Male copulatory organ and vestibule vaginal, Bar 100 µm.

The testes are broadly elliptoid (subspherical), irregular in size, pre-ovarian, and arranged in a single field in the intercecal region of the trunk. The number of testes ranges from 53–67 (60 ± 5, n= 11) with a length of 43–66 (53 ± 8, n= 11) and 65–102 (82 ± 14, n= 11) width (Figure 2.18a). The ratio between the L: A = 1: 1.5. The vas deferens goes in the direction of the anterior region; in the part anterior to the testicular region, it expands and coils, then narrows and continues in the form of a straight tube until the MCO. The MCO is cup-shaped, muscular, and has a length of 59–74 (64 ± 6, n= 10) and 43–55 (49 ± 3, n= 11) wide. It is armed with 13–35 (21 ± 7, n= 11) spines that form a crown on the distal part of the MCO. The spines are hook-shaped, and the tips are directed outwards from the MCO. The length of the spines ranges from 34–49 (42 ± 4, n= 11) with a width of 2–5 (4 ± 1, n= 11) (Figure 2.16c). The genital atrium is located in the anterior region of the body, subspherical, with muscular edges and without any sclerotized structure, and the distance from the genital pore to the anterior end is 500–625 (552 ± 40, n= 11).

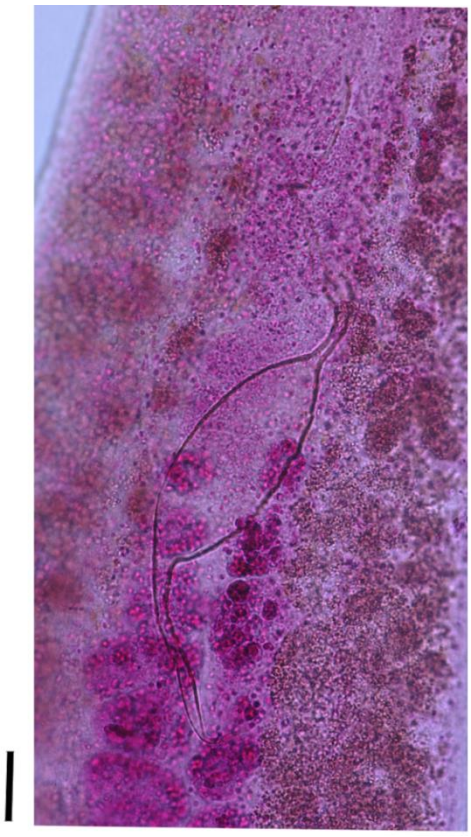

Figure 2. 17. Egg of *Protomicrocotyle* **sp. 5**, Bar 50 µm**.**

The ovary is located in the post-testicular region, is oval, and with lobed edges, the length of the ovary ranges from 124–193 (157 ± 21, n= 9) to 72–131 (102 ± 16, n= 9) wide. The oviduct goes towards the anterior region and subsequently extends towards the posterior region and again towards the anterior region and finally empties into the region of the oötype, from this region arises the uterus that goes towards the anterior end in the middle region between the rows of the testicles until reaching the genital atrium. The seminal vesicle could not be observed. In the uterus, in some specimens it is possible to observe an egg with filaments at each polar end, the egg is 164–280 (192 ± 21, n= 9) long and 72–131 (102 ± 16, n= 9) wide. The anterior end filament ranges 125–244 (193 ± 40, n= 7) long by 6–7 (7 ± 1, n= 7) wide and the posterior end filament ranges 268–325 (296 ± 41, n= 2) long by 5–7 (6 ± 2, n= 2) wide (Figure 2.17).

The vaginal pore is located anterior to the vaginal vestibule, it is deltoid in shape, is located on the opposite side to the clamps, it has a length of 101–133 (113 ± 9, n= 11) and 76–92 (84 ± 6, n = 11) wide and is armed with 35–61 (45 ± 7, n= 11) spines, with a flat shape and a variable size, with those found in the anterior part of the vestibule being larger than those found in the vestibule. They are found at the base; these spines have small extensions in the form of smaller spines in the distal region of each spine. The length of the spines ranges from 34–47 (41 ± 3, n= 11) and 4–6 (5 ± 1, n= 11) wide. The vaginal vestibule is located at a distance of 635–755 (690 ± 46, n= 11), and the genital pore at a distance of 150–210 (179 ± 17, n= 11) (Figure 2.16c). The vitelline glands are found covering most of the body, starting in the region anterior or posterior to the ceca bifurcation and extending to the haptor region.

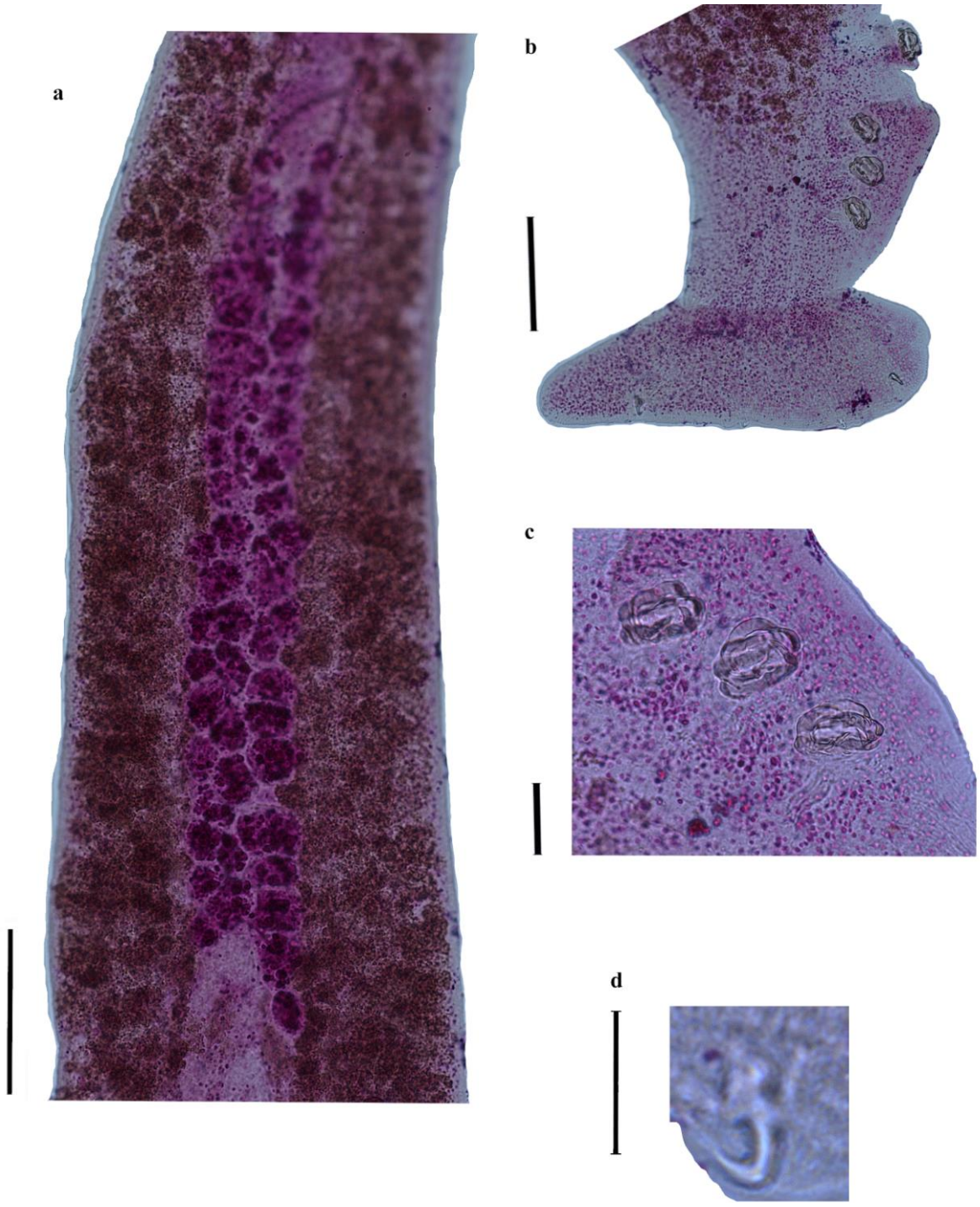

Figure 2. 18. Light micrographs of *Protomicrocotyle* **sp. 5**. (a) Median Region of the body, showing the arrangement and shape of the testes, Bar 200 µm; (b) Posterior region, Bar 200 µm; (c) Clamps, Bar 50 µm; (d) Median anchor, Bar 20 µm.

Taxonomic summary

Host: *Caranx hippos* (Linnaeus, 1766)

Site of infection: Gills

Locality: Tecolutla, Veracruz (20°28′39″N, 97°00′30″W).

Remarks

The specimens determined as *Protomicrocotyle* **sp. 5** are differentiated from *P. mirabilis*, *P. veracruzensis*, *Protomicrocotyle* **sp. 1**, *Protomicrocotyle* **sp. 2**, *Protomicrocotyle* **sp. 3**, and *Protomicrocotyle* **sp. 4** by having a larger body, being the largest of the species that have been recorded in the Gulf of Mexico. It is also differentiated by having the testes spherical in shape and arranged in a single field (Table 2.2). The clamps of *Protomicrocotyle* **sp. 5** are larger than those of *P. mirabilis*, *Protomicrocotyle* **sp. 1**, *Protomicrocotyle* **sp. 2**, *Protomicrocotyle* **sp. 3**, and *Protomicrocotyle* **sp. 4**, yet smaller than those of *P. veracruzensis*. The testes are spherical in shape and on average, there are 60 in number (Table 2.2), which differentiates it from the rest of the species and morphotypes of *Protomicrocotyle*.

Morphometric analyses

The PCA results show a cumulative variance of 57.12% (Table 2.4) for the first two principal components (Figure 2.19), eigenvalues and accumulated variance for the first two principal components are presented in Table 2.4. The variables that are considered the most informative (in order of importance) are, from the first component: the body width, the ovary width, the haptor width, the width of the haptoral lappet, large of the central haptoral lappet, the left lateral length of haptoral lappet, the right lateral length of haptoral lappet, the vaginal vestibule to genital pore distance and the average width of the testes. From the second component are: the number of spines in the male copulatory organ, and internal root width of right lateral hook.

Table 2. 4. Percentage of the variance explained for *Protomicrocotyle* used in the principal component's analysis.

Principal components	Eigenvalue	% Variance	% Accumulate variance
1	0.8395	44.13	44.13
2	0.24723	13.00	57.12

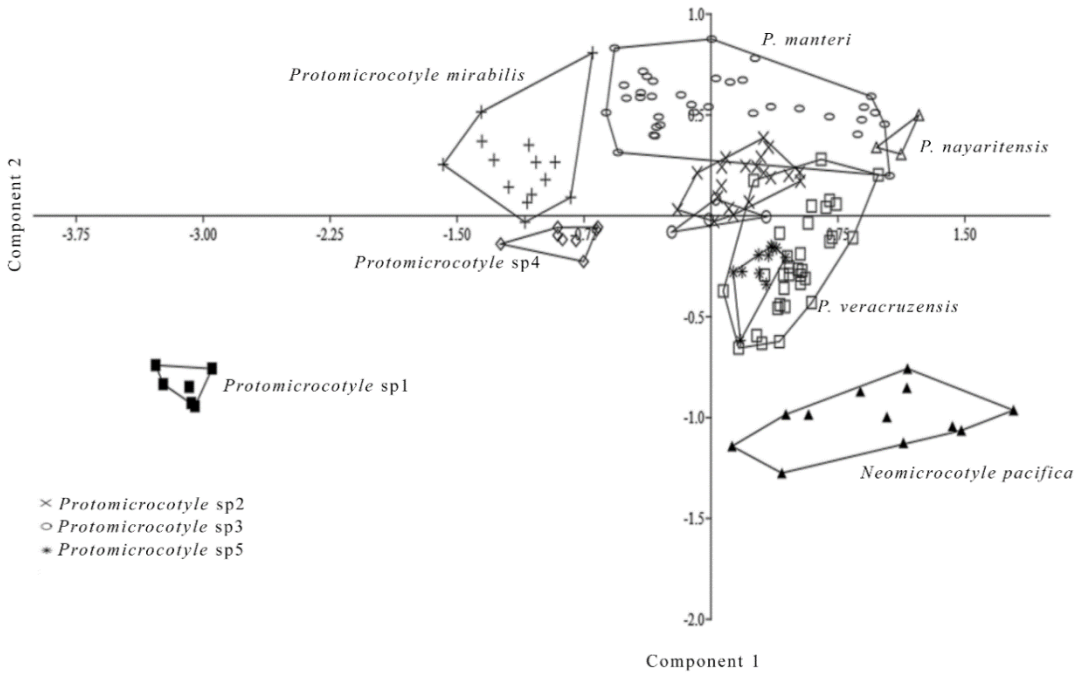

Figure 2. 19. Principal component analysis (PCA) of log-transformed morphological variables from *Protomicrocotyle* and *Neomicrocotyle*.

The DA, the first two factor axes explained 64.32% of the total variation. The first factor (eigenvalue 108.24, 35.25 of variation) alone was the main discriminant function, which arranged the species of *Protomicrocotyle* with the morphological variables, in order of importance: the number of the spines of the vestibule vaginal, number of spines of the MCO, width of the body, width of the haptoral lappet, and width of the haptor. The second factor (eigenvalue 89.105, 29.04 of variation) has a value almost equal to the first factor and

separated the species of *Protomicrocotyle* with the morphological variables, in order of importance: number of testes and, the width of the ovary.

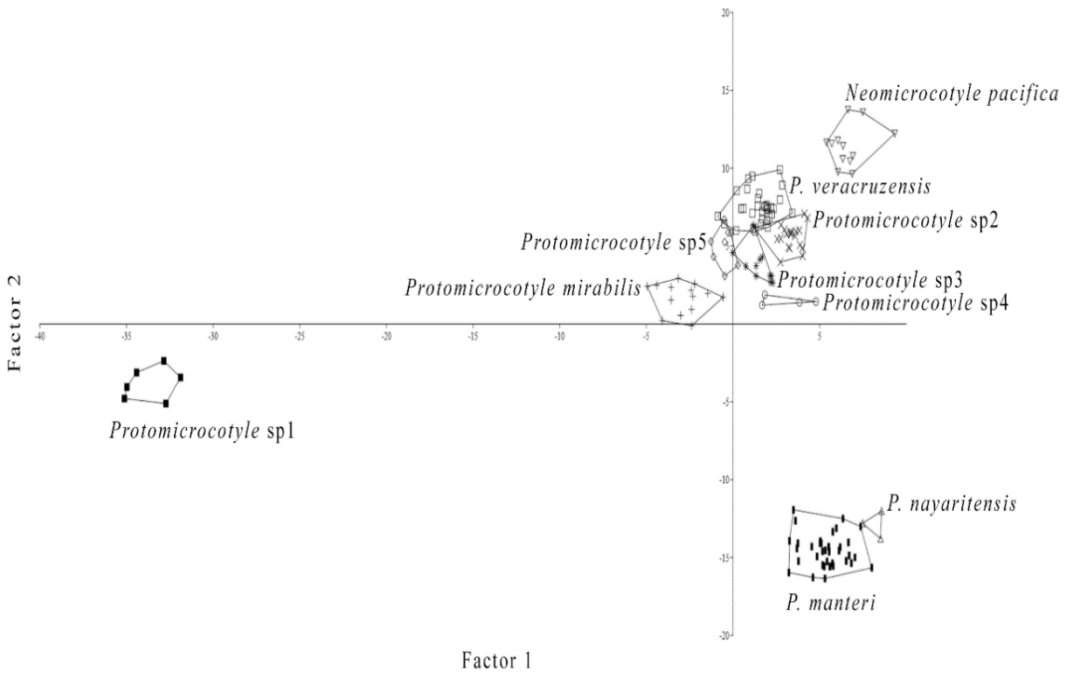

Figure 2. 20. Discriminant analyses (DA) of log-transformed morphological variables from *Protomicrocotyle* and *Neomicrocotyle*.

The PCoA analysis shows the dispersion of the *Protomicrocotyle* and *Neomicrocotyle* specimens along the two axes of the Cartesian plane (Figure 2. 21) and the first two axes explain 43.35% of the variance. It is evident that the specimens identified as *Protomicrocotyle* **sp. 1**, *Protomicrocotyle* **sp. 2**, *Protomicrocotyle* **sp. 3**, *Protomicrocotyle* **sp. 4**, and *Protomicrocotyle* **sp. 5** form independent groups. These results show that there is differentiation among *Protomicrocotyle* specimens from the Gulf of Mexico with respect to *P. manteri* and *P. nayaritensis*, species found in the Pacific Ocean (Figure 2.21).

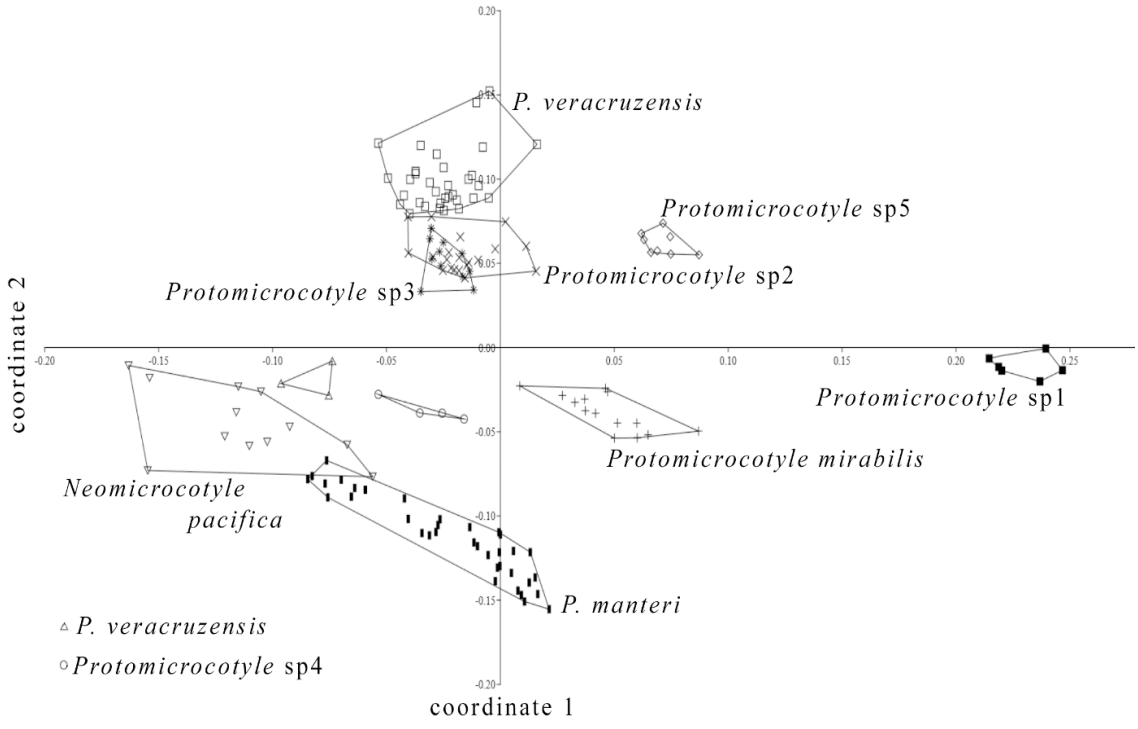

Figure 2. 21. Principal coordinate analysis (PCoA) with continuous and discrete variables showing the degree of dispersion of *Protomicrocotyle* and *Neomicrocotyle* specimens.

The UPGMA analysis showed a cophenetic correlation of 0.91 which indicates that there is little distortion in the matrix generated from the dendrogram data. The dendrogram shows the formation of different groups of *Protomicrocotyle*; The specimens that make up the *P. mirabilis* group are conformed of specimens from the localities of Boca del Rio, Casitas, Puerto de Veracruz, Tecolutla, and Tuxpan in the state of Veracruz, and from the localities of Campeche and Ciudad del Carmen in the state of Campeche, Chetumal, Quintana Roo, and Tampico, Tamaulipas. The *Protomicrocotyle veracruzensis* group is formed by specimens from the localities of Casitas and Puerto de Veracruz, Veracruz, and Tampico Tamaulipas. The *Protomicrocotyle* **sp. 1** group is formed by specimens from the locality of Sontecomapan, Veracruz. The *Protomicrocotyle* **sp. 2** group is formed by specimens from Jicacal, Veracruz, and Isla Cozumel, Quintana Roo. The *Protomicrocotyle* **sp. 3** group is formed by four specimens from a locality in Chetumal, Quintana Roo. The *Protomicrocotyle* **sp. 4** and *Protomicrocotyle* **sp. 5** groups are formed by specimens from Tecolutla, Veracruz (Figure 2.22).

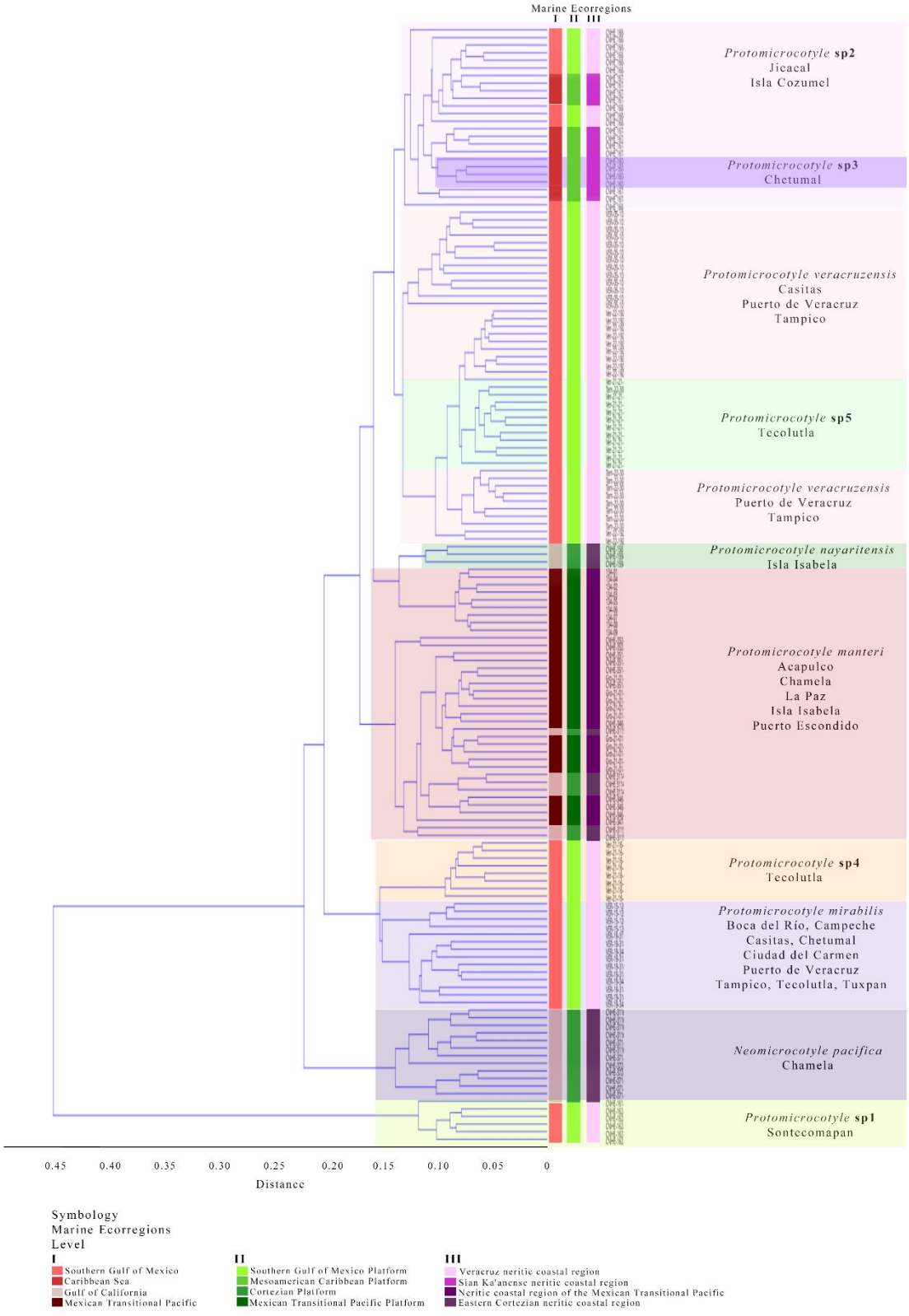

Figure 2. 22. Dendrogram of UPGMA analysis using Gower's similarity coefficient for *Protomicrocotyle* and *Neomicrocotyle* specimens from Mexico.

4. Discussion

Biological collections are important reservoirs of biodiversity, allowing for studies of taxonomy, physiology, and ecology, among others, which are essential to promoting environmental conservation strategies. Biological collections are essential to increase knowledge of biological processes and document the existing biodiversity on Earth, and the systematic study of the species that make them up reveals new taxonomic and diversity information (Rocha et al., 2014; Crisci & Katinas, 2017). Throughout taxonomic history, organisms have been described based mainly on morphology and with additional information about geographic location, and currently many kinds of data, including allozymes, scanning and transmission electron microscopy and DNA-sequence data were used to supporting the species distinction (Cook et al., 2010). However, when the conditions of the specimens under study do not permit the procedures required for the extraction, amplification, and sequencing of DNA to be carried out, it is necessary to conduct a thorough examination of the specimens and the use of multivariate analyses in order to identify the morphological characteristics that group them into a species that has already been described, or to separate them into new groups with morphological characteristics that are diagnostic, thus enabling the description of new species.

Reanalysis over time, using new instruments, methodologies and techniques, such as morphometric analyses, provides new data from specimens already studied (Swing et al., 2014). The morphometric analyses have been and still are a useful tool in the study of morphological variation in monogeneans, since, with their help, the delimitation of different species has been carried out (Shinn et al., 2000; Rubtsova et al., 2006; Dmitrieva et al., 2012; Soo & Lim, 2015; Ramírez-Cruz et al., 2023), as well as, based on morphometric analysis, the redescription of species has been carried out and has contributed to increasing the number of characters that allow individuals of a species they differ from others, which are morphologically similar (Sailaja et al., 2016).

The morphological characteristics of *Protomicrocotyle* are fusiform body with an asymmetric haptor with four gastrocotylid-type clamps (with an accessory oblique sclerite) in a longitudinal row on only one side of the haptor, haptoral lappet elongated with a transverse or bell-shaped shape armed with three pairs of hooks of different sizes, testes

anterior to the ovary with a variable shape and with an arrangement that shows a characteristic pattern between species, ovary posterior to the testes, male copulatory organ, and vaginal vestibule armed with a variable number of spines and eggs with two polar filaments (Ramalingam, 1960; Yamaguti, 1963; Kritsky et al., 2011; Ramírez-Cruz et al., 2023).

The description of these five morphotypes is done with the comparison of the anatomical organs. Since they were specimens that were subjected to fixation, staining, and mounting processes, the extraction of DNA is very difficult, so in future studies, it is advisable to carry out sampling in the type localities of these morphotypes to obtain new biological material and conserve it with the ideal conditions for obtaining genetic material that can be compared with what has been documented for the genus *Protomicrocotyle* (Ramírez-Cruz et al., 2023) and in general for the Protomicrocotylidae family (Tambireddy et al., 2016). This includes a greater amount of both morphological and molecular information for future phylogenetic studies of this group of helminths.

The results of the multivariate and clustering analyses allow us to differentiate five morphotypes of *Protomicrocotyle*. *Protomicrocotyle* **sp. 1** corresponds to specimens that were deposited in the CNHE with numbers 161 and 162 collected from *Caranx hippos* in the locality of Sontecomapan, Veracruz and that were identified as *P. mirabilis* (Bravo-Hollis, 1989). These specimens are differentiated from *P. mirabilis* by the length of the body, since they are smaller (2752 vs. 1605), by the number of spines in the vaginal vestibule (49 vs. 65), the number of testes (30 vs. 46) (Table 2.2), and in the shape and arrangement of these, since in *P. mirabilis* they appear as regular spherical structures, arranged in two fields and in *Protomicrocotyle* **sp. 1** they are horizontally elongated and arranged in two fields.

The specimens that were deposited in the CNHE that were identified as *Protomicrocotyle manteri* from the localities of Chetumal (CNHE-160), Isla Cozumel (CNHE-167), and Jicacal (CNHE-168) by (Bravo-Hollis, 1989) are specimens that, based on the description of *P. manteri* made by (Bravo-Hollis, 1966), do not correspond to the diagnostic characteristics of this species, so they are considered without a specific taxonomic identity and are nominated as morphotype *Protomicrocotyle* **sp. 2** for the specimens from Isla Cozumel and Jicacal and as *Protomicrocotyle* **sp. 3** for the specimens from Chetumal,

based on the observations, measurements, and results of the multivariate and cluster analyses carried out. The *Protomicrocotyle* **sp. 2** morphotype differs from *P. manteri* mainly by the number of spines in the vaginal vestibule; *P. manteri* has an average of 18 spines, while the *Protomicrocotyle* **sp. 2** morphotype has an average of 49, and by the number of spines on the copulatory organ, since *P. manteri* has an average of 44 spines and *Protomicrocotyle* **sp. 2** morphotype has an average of 21. Just as the geographical distribution of *P. manteri* corresponds to the Pacific Ocean region, whereas *Protomicrocotyle* **sp. 2** morphotype is found in the Gulf of Mexico and the Caribbean Sea.

Protomicrocotyle **sp. 2**, differs in body shape of *P. mirabilis*, this has a thin fusiform body with an average width of 294, while *Protomicrocotyle* **sp. 2** has a robust fusiform body with an average width of 874. In relation to the number of spines in the vaginal vestibule and male copulatory organ, there is not much difference, nor is there any difference in the number of testes; however, the shape of the testes and arrangement is elongated horizontally and come to form a row with the testes joined together, also differentiating from the morphotypes of *Protomicrocotyle* **sp. 1** and *Protomicrocotyle* **sp. 3**.

Protomicrocotyle **sp. 3** differs from *P. manteri* by the number of spines in the vaginal vestibule (44 vs. 18) and male copulatory organ (21 vs. 44), as well as by the average number of testes (55 vs. 31). From *P. mirabilis*, it is differentiated by the number of testes (55 vs. 30) and their arrangement, since *Protomicrocotyle* **sp. 3** presents subspherical testes arranged in a single field in the intercecal region of the body. This morphological characteristic also differentiates it from the remaining morphotypes. The morphotypes *Protomicrocotyle* **sp. 4** and *Protomicrocotyle* **sp. 5** correspond to specimens collected in *C. hippos* from the locality of Tecolutla, Veracruz. The main morphological characteristics that separate them are the length of the body, with *Protomicrocotyle* **sp. 4** being smaller (1682) than the morphotype *Protomicrocotyle* **sp. 5** (4117), as well as the number of testes (39 vs. 60), the shape (elongated horizontally vs. subspherical), and the arrangement of the testes (two fields, with a row in each field vs. a single field). These morphological characteristics, in conjunction with the other measured variables and the results of the statistical analyses, result in the separation of the specimens into different groups from the rest of the morphotypes studied.

In order to ascertain the current distribution of the *Protomicrocotyle* species present in the Atlantic Ocean (*P. mirabilis*, *P. ivoriensis*, *P. veracruzensis*, *Protomicrocotyle* **sp. 1**,

Protomicrocotyle **sp. 2**, *Protomicrocotyle* **sp. 3**, *Protomicrocotyle* **sp. 4**, and *Protomicrocotyle* **sp. 5**,) and in Pacific Ocean (*P. manteri* and *P. nayaritensis*), it is essential to investigate the biogeographic history of the fish of the genus *Caranx* that function as hosts for these parasite species and the environmental conditions in which the host is currently distributed. Based on currently available information, the family Carangidae is believed to have originated approximately 79 million years ago, at the time of the Cretaceous-Paleogene extinction event. The family is divided into four subfamilies (Trachinotinae, Scomberoidinae, Naucratinae, and Caranginae) (Nelson et al., 2016). Molecular information has been used to support this classification (Reed et al., 2002; Miya et al., 2013; Swart et al., 2015). The divergence of Trachinotinae into Naucratinae and Caranginae occurred approximately 66.69 million years ago, during the middle Paleocene. The divergence time of the subfamilies Naucratinae and Caranginae could be traced back to 65.91 million years ago (Li et al., 2020).

The environmental changes that occurred during the Cretaceous period, which resulted in the mass extinction of that period, led most species to differentiate during the Paleogene period (Miya et al., 2013; Li et al., 2020). At this time in history, the continental masses of North America and South America were not yet joined by the Isthmus of Panama, resulting in the Pacific and Atlantic oceans being in contact, and hence the marine fauna. The genus *Trachinotus* is estimated to have begun to differentiate approximately 66.69 million years ago, which would correspond to the Paleocene, based on the information provided by the complete mitogenome analysis (Li et al., 2020). With regard to *Caranx*, the available information is limited. Bannikov (1987) states that *Caranx* originated during the Eocene. However, if we consider the findings of Li et al. (2020), it can be postulated that the diversification of this genus commenced approximately between 14.54 and 19.74 million years ago, most likely at the end of the Miocene.

In light of the aforementioned information regarding fish, and considering that the first monogeneans began parasitizing placoderm fish approximately 400 million years ago during the Silurian period (Rohde, 1994), it can be posited that monogenean species, particularly *Protomicrocotyle* in *Caranx*, were already firmly established in these hosts by the time of the emergence of the Isthmus of Panama during the Pleistocene epoch. The

establishment of the Panama Bridge constituted the geographic barrier known as the New World land barrier (Okolodkov, 2010), thereby preventing the tropical marine fauna of the Pacific and Atlantic oceans from having contact. The formation of this barrier not only prevented the movement of marine fauna, but also altered the dynamics of marine ecosystems. Tropical currents in the Caribbean ceased flowing westward and instead shifted northward, causing changes in salinity and temperature conditions and strengthening the Gulf Stream. As the Pacific Ocean became isolated from the inflow of tropical ocean waters, it cooled and became nutrient-rich due to deep water cooling off the coast of Central America (Thacker, 2017). The occurrence of significant environmental alterations led to the extinction of numerous marine faunal groups in both oceans, while other lineages were initiated and subsequently diverged.

The Gulf of Mexico is divided into two major biogeographic provinces, the Carolina Province and the Caribbean Province (Briggs & Bowen, 2012). The Carolina Province is divided into two sections: The Northern Gulf of Mexico and the Atlantic Coast. In the corresponding Gulf region, the biota is distributed north of the tropical boundaries between Cabo Romano, Florida, and Cabo Rojo, Tamaulipas. The province is characterized by a warm-temperate climate, with the Gulf section exhibiting the greatest biodiversity. This is evidenced by the observation that fish and invertebrates exhibit approximately 10% of endemism. The Caribbean Province extends from Bermuda and Cape Canaveral, Florida, to the Amazon River. The Caribbean Province is home to a greater number of species than the Carolina Province (Briggs & Bowen, 2012; López-Herrera et al., 2021).

The provinces are divided into ecoregions on the basis of their distinctive geographical, floral, faunal, and ecosystem characteristics. Consequently, the division is made at three levels. The first level of classification considers the differences between marine ecosystems that occur at the scale of ocean basins. These include temperature and the circulation of large currents, and marine water masses. Of the 21 ecoregions defined for North America, eight are wholly or partially included within the Mexican territory in marine waters. With regard to the Gulf of Mexico, these include the Northern Gulf of Mexico, the Southern Gulf of Mexico, and the Caribbean Sea (Lara-Lara et al., 2008; Wilkinson, 2009).

Level II ecoregions reflect the distribution of benthic environments and include differences between benthic-neritic environments (from the continental shelf to a depth of approximately 200 m) and pelagic-oceanic environments (epipelagic). In addition, there are differences in the mesopelagic, bathypelagic, and abyssopelagic zones, as well as in large-scale morpho-structures such as continental slopes, abyssal plains, oceanic islands, trenches, and submarine mountain ranges. Of the total 28 ecoregions, nine are located in the Gulf of Mexico and Caribbean Sea. The delineation of Level III ecoregions is based on locally significant variations (water mass characteristics, seafloor formations, and type of biological communities) for each of the 24 regions. This level III is limited to the continental shelf, as only this area has sufficient information for a more precise scale delimitation (Lara-Lara et al., 2008; Wilkinson, 2009).

The biogeographical history of these oceanic regions, in conjunction with the influence of ocean currents, as well as the environmental conditions of each ecoregion and the biological, biogeographical, and ecological characteristics of the organisms that serve as definitive hosts of *Protomicrocotyle*, collectively influence the distribution patterns and morphological differentiation of this group of helminths. Previously, it was known that only *P. mirabilis* or *P. manteri* were found as a parasite of *Caranx* fish. Consequently, all specimens found were identified as such (Bravo-Hollis, 1989). The present study employed traditional morphometric tools (MacLeod, 2017; Luna, 2020), to identify a greater diversity of morphotypes within the genus *Protomicrocotyle*, as well as to discern morphological characters of taxonomic significance that facilitate the differentiation between specimens of this genus and enhance our understanding of this group of monogeneans. Morphometric analyses have been and are a useful tool in the study of morphological variation in monogeneans since, with their help, the delimitation of different species has been carried out (Shinn et al., 2000; Rubtsova et al., 2006; Dmitrieva et al., 2012; Soo & Lim, 2015), as well as, based on morphometric analysis, the redescription of species has been carried out and has contributed to increasing the number of characters that allow that individuals of a species differ from others, which are morphologically similar (Sailaja et al., 2016). For the genus *Protomicrocotyle*, this is the first study that includes additional information on measurements of anatomical structures such as the spines of the male copulatory organ, the spines of the vaginal vestibule, details of the measurements of the hooks, measurements of the length of

the haptoral lappet, and the distance from the cecal bifurcation, genital pore, and vaginal vestibule to the anterior end and from the vaginal vestibule to the genital pore.

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CAPÍTULO III

Total evidence study of Protomicrocotylidae Johnston & Tiegs, 1922 (Monogenea: Mazocraeidea), parasites of the gills of *Caranx* spp. (Carangiformes: Carangidae): emphasis on the species of *Protomicrocotyle* Johnston & Tiegs, 1922 from Mexico

Estudio de evidencia total de Protomicrocotylidae Johnston & Tiegs, 1922 (Monogenea: Mazocraeidea) parásitos de los arcos branquiales de *Caranx* spp. (Carangiformes: Carangidae): con énfasis en las especies de *Protomicrocotyle* Johnston & Tiegs, 1922de Mexico

1. Introduction

The family Carangidae, distributed worldwide, is comprised of fish commonly known as trevally, jacks, scads, mackerels, kingfish, queenfish, rainbow runners, darts, amberfish and pompanos organized into four subfamilies (Trachinotinae, Scomberoidinae, Naucratinae and Caranginae), with 32 genera and approximately 148 species. Members of the genus mostly inhabit marine tropical to subtropical waters, but they penetrate into brackish waters (Carpenter, 2002; Betancur-R et al., 2017; Damerau et al., 2018; Froese & Pauly, 2022). The species are ecologically and commercially important. They play significant roles in the trophic chain as top predators and are a major commercial fishing resources, both as a food source and as sport fish (Souza & Mafalda Júnior, 2008). Unfortunately, anthropocentric activities and intensive fishing have caused populations of all species of fish to decline on a global scale, highlighting the need of the implementation of new management and conservation strategies; for this, it is necessary to have increased knowledge of each species' natural history, behavior, habitat and ecology, as well as their helminth parasites (Pauly, 2008; Santos et al., 2010).

The genus *Caranx* is comprised of 18 species with worldwide distribution (Nelson et al., 2016; Froese & Pauly, 2022) that are characterized morphologically by having a moderately large to very large deep body, 20-31 gill rakers on the first gill arch, 7 dorsal spines, and 1-2 anal spines (Carpenter, 2002). In the Gulf of Mexico, the most common species of *Caranx* are *C. hippos*, the crevalle jack, *C. crysos*, the blue runner, *C. latus*, the Horse-eye jack, *C. ruber* (Bloch, 1793), the bar jack, and *C. bartholomaei* Cuvier, 1833, the yellow jack (López-Herrera et al., 2021; Froese & Pauly, 2022). Taxonomic and ecological studies of helminths in marine fish are numerous in Mexico (Violante-González et al., 2016; Montoya-Mendoza et al., 2017; Montoya-Mendoza et al., 2018; Santos-Bustos et al., 2018; Gallegos-Navarro et al., 2019; Montoya-Mendoza et al., 2021; Fajer-Ávila et al., 2022; Carpio-Hernandez et al., 2024; Carpio-Hernández et al., 2024), and different species of helminths have been reported and described (Pinacho-Pinacho et al., 2012; Quiterio-Rendon et al., 2018; Rodríguez-Ibarra et al., 2018; Zaragoza-Tapia et al., 2019; Zaragoza-Tapia et al., 2020; Andrade-Gómez et al., 2023; Ramírez-Cruz et al., 2023; Rodríguez-Ibarra et al., 2023). Several species of helminths have been reported from *C. hippos* and *C. latus* from the coastal waters of the Gulf of Mexico and Caribbean Sea (see Mendoza-Garfias et al. (2017);

and Montoya-Mendoza et al. (2017)); mainly monogeneans have been reported in these species.

Monogeneans principally are ectoparasites of brackish, freshwater and marine bony fish, sharks, rays; even chelonians, amphibians and crustaceans can be hosts of monogeneans (Buchmann & Bresciani, 2006b; Pulido-Flores, 2024). They are one of the most diverse groups of helminths in terms of the number of species, morphology, and ecology. In comparison with the other members of Platyhelminthes, the members of Monogenea have greater host specificity; there are species that infect only one or a few species or host or only hosts of families that are phylogenetically related (Whittington et al., 2000; Poulin, 2002; Mariniello et al., 2004). Being very host-specific organisms, monogeneans and their hosts have been widely used in coevolutionary studies (Sasal et al., 1998; Desdevises et al., 2002b; Šimková & Morand, 2008), studies of co-speciation (da Graca et al., 2018; Rahmouni et al., 2022), biogeography (Pariselle et al., 2011; Šimková et al., 2022), and they have been used for the identification of population stocks of marine fish (i.e., biological tags) (MacKenzie & Abaunza, 2014). In Mexico, monogeneans have been studied continuously for more of 80 years, although the knowledge of the diversity of species that have been studied in the different geographical areas and the variety of hosts that have been examined hosts remains fragmentary (Mendoza-Garfias et al., 2017).

Members of Protomicrocotylidae are characterized by being parasites of the gill arches of marine fish, mainly carangid fish (Yamaguti, 1963; Kritsky et al., 2011). Currently, the family is represented by nine genera: *Abortipedia* Unnithan, 1962; *Bilaterocotyle* Chauhan, 1945; *Bilaterocotyloides* Ramalingam, 1961; *Chauhanocotyle* Khoche & Dad, 1975; *Lethacotyle* Manter & Price, 1953; *Neomicrocotyle* Ramalingam, 1960; *Protomicrocotyle* Johnston & Tiegs, 1922; *Vallisiopsis* Subhapradha, 1951; and *Youngiopsis* Lebedev, 1972, with approximately 43 species assigned to the family (Lebedev, 1986; WoRMS, 2024). Currently, 10 species are assigned to the genus *Protomicrocotyle* (Ramírez-Cruz et al., 2023).

In a phylogenetic framework, some studies based on morphological characters have provided evidence in support of the monophyly of Protomicrocotylidae. However, the number of species included in these studies was relatively limited (Boeger & Kritsky, 1993;

Boeger & Kritsky, 2001). However, in those studies, the analysis was polarized based on characters coded for *Protomicrocotyle* and *Neomicrocotyle*, so a more comprehensive study is necessary. Olson & Littlewood (2002) evaluated phylogenetic relationships of 27 families of monogeneans, including the family Protomicrocotylidae, using morphological and molecular characters (in separate analyses), using the sequences of the large (D1-D2) and small ribosomal subunits (ssrDNA). They discussed the morphological characters of Boeger & Kritsky (2001), but they chose not to perform a combined analysis. For Protomicrocotylidae, *Neomicrocotyle pacifica* was the only representative of the family, in a clade with polytomy with the families Thoracocotylidae Price, 1936 (described as Neothoracocotylidae Lebedev, 1969), Gastrocotylidae Price, 1943 and Gotocotylidae Yamaguti, 1963 (Olson & Littlewood, 2002).

The aforementioned studies primarily have proposed phylogenetic hypotheses at the family level. As Olson & Littlewood (2002) noted, the combination of morphological and molecular data to obtain a total evidence solution has proven challenging to previous authors. This is largely because the matrix presented by Boeger & Kritsky (2001) is coded at the family level, rather than the species level; thus, not allowing evaluation of the monophyly of the families and included genera. The authors also highlighted that although possible incongruence between morphological and molecular hypotheses would be most effectively explored through a fully complementary approach (Total Evidence, *sensu* Kluge [1998]) that would include increased sampling of taxa for morphological and molecular data.

From a molecular perspective, Tambireddy et al. (2016) undertook a phylogenetic analysis of the families that comprise the order Mazocraeidea Bychowsky, 1937. In this study, the28S rDNA nuclear gene was employed to propose a phylogenetic hypothesis for the group. In the analysis, the following species were included: *Bilaterocotyloides madrasensis* Radha, 1966; *B. carangis*; *Neomicrocotyle pacifica*; *Neomicrocotyle* sp. and *Lethacotyle vera* (Protomicrocotylidae), as well as other members of Mazocraeidea. The phylogenetic hypothesis proposed for the members of Protomicrocotylidae position the species of *Bilaterocotyloides* basal to *Neomicrocotyle* and *Lethacotyle*, the latter being more derived (see Figure 5 of Tambireddy et al. [2016]). The morphological characteristic that is associated with the evolutionary changes that occur in the clamps: *Bilaterocotyloides* has the complex derived gastrocotylid-type clamps, while in *Neomicrocotyle* they are of the

microcotylid type (plesiomorphic) and clamps are absent (= lost; Justine et al. [2013]) in *Lethacotyle*. In the phylogenetic tree, the families Allodiscotylidae and Protomicrocotylidae form a monophyletic clade.

More recently, Kamio & Nitta (2022) studied a group of monogeneans study that included members of Mazocraeidae. This analysis employed sequences of cytochrome c oxidase 1 (CO1-mitochondrial) and 28S nuclear gene. In their results, representative species in Protomicrocotylidae were grouped with Chauhaneidae and Allodiscocotylidae, supporting the results of the morphological analyses of Boeger & Kritsky (1993; 001) and the genetic (28S and 18S) analyses of Camargo & Santos (2020). The general consensus of taxonomists is that Protomicrocotylidae is represented by the following genera: *Abortipedia*, *Bilaterocotyle*, *Bilaterocotyloides*, *Chauhanocotyle*, *Lethacotyle*, *Neomicrocotyle*, *Protomicrocotyle*, *Vallisiopsis* y *Youngiopsis* (Lebedev, 1986; WoRMS, 2024). However, only *Protomicrocotyle*, *Neomicrocotyle*), *Bilaterocotyle*, *Bilaterocotyloides*, and *Lethacotyle* have been integrated into phylogenetic analyzes (Boeger & Kritsky, 1993; 2001; Olson and Littlewood, 2002; Tambireddy et al., 2016; Kamio & Nitta, 2022).

The use of molecular tools is necessary to facilitate and improve species delimitation and identification. The use of different genes or sets of molecular markers has proven valuable in taxonomic studies, phylogeographic studies, and in establishing phylogenetic relationships between members of different taxonomic categories (Singh, 2012; Shaw et al., 2013; León-Règagnon & Topan, 2018; Moreira et al., 2019). Molecular markers (partial or complete genes) also aid in studies of evolution, ecology and diversity, and the different types of markers are distinguished by their ability to detect polymorphisms that allow the separation of groups, populations, species or larger taxonomic groups (Alcántara, 2007; Godoy, 2009). Analyses that combine morphological and molecular data (analyses of Total Evidence *sensu* Kluge [1998]) provide a more complete taxonomic view (Perkins et al., 2009; Hahn et al., 2011; Camargo & Santos, 2020). The objective of this study is to conduct a thorough examination of the evidence pertaining to the genus *Protomicrocotyle*, integrating morphological and genetic sequence data in order to derive a phylogenetic hypothesis for *Protomicrocotyle*.

2. Material and methods

Taxon selection

During different sampling trips, 85 specimens of *Caranx hippos* and five of *C. latus* were collected from six localities along the Gulf of Mexico between 2019 and 2023 (Table 2.1). Sampled localities were: Casitas, Veracruz (20° 15' 31.5" N, 96° 47' 49.5" W), Ciudad del Carmen, Campeche (18° 38 ′ 18″ N, 91° 50′ 07″ W), Puerto de Veracruz, Veracruz (19° 13' 11.2" N, 96° 09' 24.4" W), Tampico, Tamaulipas (22° 15’ 52.7” N, 97° 45’ 20.5” W), Tecolutla (20° 28′ 39″ N, 97° 00′ 30″ W) and Tuxpan, Veracruz (20° 57′ 46″ N, 97° 24′ 01″ W).

For the comparative, codification and analysis purposes, the following specimens of *Protomicrocotyle* and *Neomicrocotyle* were obtained from the Colección Nacional de Helmintos (CNHE), Instituto de Biología, Universidad Nacional Autónoma de Mexico, Mexico City, Mexico and the Colección de Helmintos (CHE), Centro de Investigaciones Biológicas, Universidad Autónoma del Estado de Hidalgo, Pachuca de Soto, Hidalgo, Mexico were examined: *Neomicrocotyle pacifica* (CNHE 071, 072, 3116), *Protomicrocotyle manteri* (CNHE 220, 134, 351, 3114, 3115; CHE-GRO-21-01), *P. nayaritensis* (CNHE 158, 159), *P. mirabilis* (CNHE 82, 111, 161, 165; CNHE 12822-12825; CHE-P 00147), *P. veracruzensis* (CNHE 12820-12821; CHE-P 00148).

Fish were obtained from local fishermen in each of the localities that were sampled. The fish were sexed, measured and photographed for taxonomic identification according to specialized literature (Nelson, 2006). The helminthological examination was carried out in the field; gill arches were extracted from the gill cavity of the fish, stored temporarily in a labeled bag, and placed on ice for later review. Using a Leica Zoom 2000 stereoscopic microscope, each of the branchial arches was examined for monogeneans in each of the gill filaments. All monogeneans were relaxed with hot water and stored in AFA for morphological analysis (Pritchard & Kruse, 1982; Lamothe-Argumedo, 1997), and other specimens were stored at Alcohol 96% (EtOH 96%) for molecular analyses.

Phylogenetic inference

Morphological variables and argumentation of characters

For morphological study, specimens of *Protomicrocotyle* were stained using Gomori's trichrome, Mayer's carmalum, or Delafield's hematoxylin (each stain enfasizes different structures, dehydrated in an ethanol series, cleared in methyl salicylate, and mounted individually as whole mounts on slides in Canada balsam. The morphological study was performed using a Leica DMLB2 compound optical microscope equipped with differential interference contrast (DIC) optics. The identification of specimens was made based on published keys and specialized literature (Ramalingam, 1960; Yamaguti, 1963; Bravo-Hollis, 1966; 1979; Kritsky et al., 2011).

A measurement protocol was established for the morphological features of the specimens as described in Chapter II; the lengths of the haptoral lappet, the anchors and hooks were measured as indicated in Ramírez-Cruz et al. (2023). Measurements were made using an ocular micrometer; all measurements are given in micrometers. Information for morphological characters was taken by direct examination of specimens corresponding to each of the collections previously mentioned, as well as from specimens that were collected in the different localities as part of the present study. Information from literature was used for species of Protomicrocotylidae not available for direct examination (Table 3.1).

Coding of the discrete characters was obtained based on the comparison of the shape of anatomical structures and the presence or absence of these. For the phylogenetic analysis, data were included from the ingroup of genera of Protomicrocotylidae for which there is information. For the outgroup, information from species of Allodiscocotylidae and Chauhaneidae was included; the outgroup was based on the previous studies of Monogenea (Boeger & Kritsky, 1993; 2001; Olson & Littlewood, 2002; Tambireddy et al., 2016; Kamio & Nitta, 2022).

A total of 140 morphological characters were coded: 91 continuous characters (88 measurements and 3 countable meristic characters) and 49 discrete characters (binary and multi-state transformations). Some continuous and discrete morphological characters were

conceptionalized and modified as unordered multi-state characters (non-additive) based on characters used by Boeger & Kritsky (1993); those authors used the additive coding.

Molecular study

Extraction and amplification of DNA were carried out using specimens collected in the field during 2019, 2021, and 2022, and identified as *Protomicrocotyle mirabilis*, *P. veracruzensis* or *Protomicrocotyle* sp. Genomic DNA was extracted from the samples using a DNeasy Blood and Tissue Kit (Qiagen, Hilden, Germany) in accordance with the manufacturer's instructions. Fragments of CO1 were amplified using the primers JB3-F (ASmit1) (5'-TTTTTTGGGCATCCTGAGGTTTAT-3') and JB4-R (ASmit2) (5'-TAAAGAAAGAACATAATGAAAATG-3') (Bowles et al., 1993; Tambireddy et al., 2016). The primers LSU5-F (5'-TAGGTCGACCCGCTGAAYTTAAGC-3') and EC-D2-R (5'-CCTTGGTCCGTGTTTCAAGACGGG-3') were used for amplification of 28S rDNA (Littlewood et al., 1997; Tkach et al., 2003), and for 18S, the primers F18 (5'-ACCTGGTTGATCCTGCCAGTAG-3') and IR5-R (5'-TACGGAAACCTTGTTACGAC-3') were used (Littlewood et al., 1997; Bentz et al., 2003).

The Polymerase Chain Reactions (PCR) were performed in a total of 25 µL, consisting of 4 µL of template DNA (primer), 4.85 µL of master solution of 0.15 µL of Taq DNA polymerase (5 µ/mL; BioTecMol), 1 µL dNTPs (2.5 mM; Promega), 0.2 µL of each primer (10 nM), 1.8 µL of 5x PCR buffer (BioTecMol), 1.5 µL of $MgCl_2$ (25 mM; BioTecMol), and 16.15 µL of distilled water. The cycling conditions included initial denaturation at 94°C for 5 min, followed by 38 cycles of 94°C for 30 s, 45°C for 30 s, and 72°C for 1:10 min, and a final extension of 7:00 min at 72°C. Products of the PCR were visualized by electrophoresis of an agarose gel and then purified using a polyethylene glycol (PEG) protocol. Genes were sequenced using BigDye Terminator v3.1 Cycle Sequencing Kit in 10 µL reactions and 3730xl DNA Analyzer-Thermo Fisher Scientific using the JB3-F and LSU5-F primers, respectively, performed at the Instituto de Biología, UNAM, Mexico. Sequence data and electropherograms were inspected and edited using Pregap4 and Gap4 modules in Staden software V.1.6 (Staden, 1996).

Table 3. 1. List of the species and the literature that was used to code the morphological characters of each of them for the phylogenetic analyses.

Species	Family	Host	Locality	State/Country	Reference
Allodiscocotyla chorinemi Yamaguti, 1953	Allodiscocotylidae	*Scomberoides moadetta* (Cuvier, 1832)	Celebes	Indonesia	Yamaguti, 1953
A. diacanthi Unnithan, 1962		*Chorinemus sanctipetri* Cuvier, 1832	Trivandrum	India	Unnithan, 1962
Metacamopia lebedevi Nguyen, Nguyen, Ha, Ngoc, Ngoc, Le, Tatonova & Greiman, 2020		*Acanthopagrus pacificus* Iwatsuki, Kume & Yoshino, 2010	Gulf of Tonkin	Quang Ninh Province, Vietnam	Nguyen et al., 2020
M. oligoplites Takemoto, Amato & Luque, 1996		*Oligoplites palometa* (Cuvier, 1832)	Itacuruçá	Brazil	Takemoto et al., 1996
Gemmaecaputia corrugata Tripathi, 1959		*Sphyraena forsteri* Cuvier, 1829	Off Yomitan Town	Okinawa-jima island, Japan	Kamio & Nitta, 2022
Protomicrocotyle mirabilis	Protomicrocotylidae	*Caranx hippos*	Campeche	Campeche	Ramírez-Cruz et al., 2023

Table 3. 1. *Continuation.*

			Casitas	Veracruz	
			Ciudad del Carmen	Campeche	
			Puerto de Veracruz	Veracruz	
			Tecolutla	Veracruz	
			Tuxpan	Veracruz	
			Tampico	Tamaulipas	
P. veracruzensis Ramírez-Cruz, Monks, Manríquez-Morán, Violante-González & Pulido-Flores, 2023	Protomicrocotylidae	*C. latus*	Casitas	Veracruz	Ramírez-Cruz et al., 2023
			Puerto de Veracruz	Veracruz	
			Tampico	Veracruz	
P. manteri Bravo-Hollis, 1966		*C. caballus* Günther, 1868	Chamela	Jalisco	CNHE
			La Paz	Baja Califronia Sur	

Table 3. 1. *Continuation.*

			Puerto Escondido	Oaxaca	
			Acapulco	Guerrero	CHE-UAEH
P. nayaritensis Bravo-Hollis, 1979	Protomicrocotylidae	*C. caballus*	Isla Isabel	Nayarit	Bravo-Hollis, 1979
P. mannaresensis Ramalingam 1960		*C. sexfasciatus* Quoy & Gaimard, 1825	Mandapam	Tamil Nadu, India	Ramalingam, 1960
P. miutum Ramalingam 1960					
P. madrasensis Ramalingam 1960		*C. affinis*			
P. carangis Sivasankar Pillai & Krishna Pillai, 1978		*C. sansun* (Forsskål, 1775)	Trivandrum	Kerala, India	Pillai & Pillai, 1978
P. celebesensis Yamaguti, 1953		*Caranx sp.*	Macasar	Celebes, Indonesia	Yamaguti, 1953
P. iviorensis Wahl, 1972		*C. hippos*	Ebrié Lagoon	Côte d'Ivoire, Western Africa	Wahl, 1972
Protomicrocotyle **sp. 1**		*C. hippos*	Sontecomapan	Veracruz	This study
Protomicrocotyle **sp. 2**			Jicacal	Veracruz	

Table 3. 1. *Continuation.*

		Caranx sp.	Isla Cozumel	Quintana Roo	
Protomicrocotyle **sp. 3**		*C. latus*	Chetumal	Quintana Roo	This study
Protomicrocotyle **sp. 4**		*C. hippos*	Tecolutla	Veracruz	
Protomicrocotyle **sp. 5**			Tecolutla	Veracruz	
Neomicrocotyle pacifica (Meserve, 1938) Yamaguti, 1968	Protomicrocotylidae	*C. hippos**	Puerto Ángel	Oaxaca	Bravo-Hollis & Salgado-Maldonado, 1985
N. elongata Sivasankar Pillai & Krishna Pillai, 1978		*Megalaspis cordyla* (Linnaeus, 1758)	Trivandrum	Kerala, India	Pillai & Pillai, 1978
N. indica Ramalingam, 1960		*C. sexfasciatus*	Mandapam	Tamil Nadu, India	Ramalingam 1960
Bilaterocotyle chirocentrosus Chauhan, 1945		*Sciaena belengeri* (Cuvier, 1830)	Bombay Port	Bombay, India	Chauhan, 1945
Bilaterocotyloides carangis Ramalingam, 1961		*Megalaspis cordyla*	Trivandrum	Kerala, India	Pillai & Pillai, 1978

Table 3. 1. *Continuation.*

Lethacotyle fijiensis Manter & Prince, 1953		Yellow Jack**	Suava	Fiji Islands, Japan	Manter & Prince, 1953
L. vera Justine, Rahmouni, Gey, Schoelinck & Hoberg, 2013		*C. papuensis* Alleyne & MacLeay, 1877	Off Nouméa	New Caledonia	Justine et al., 2013
Vallisiopsis contorta Subhapradha, 1951	Protomicrocotylidae	*Lactarius lactarius* (Bloch & Schneider, 1801)	Madras Regional Station	India	Subhapradha, 1951
Youngiopsis australis Young, 1968		*Sphyraena obtusata* Cuvier, 1829	Moreton Bay	Queensland, Australia	Young, 1968

*The taxonomic identity is consistent with that of another host, as *C. hippos* is not geographically distributed in the Pacific Ocean.

**The scientific name of the host was not provided in the original description.

Sequence alignment, p-distance analysis and Neighbor-Joining three

Partial sequences obtained in the present study (some published in (Ramírez-Cruz et al., 2023)), for CO1, 28S and 18S regions were aligned with sequences from other protomicrocotylids retrieved from GenBank. *Allodiscocotyla chorinemi*, *A. diacanthi*, *Metacamopia lebedevi* and *M. oligoplites* (Allodiscocotylidae), and *Gemmaecaputia corrugata* (Chauhaneidae) as outgroups, based on previous phylogenetic analyses of Monogenea (Table 3.2) (Boeger & Kritsky, 1993; 2001; Tambireddy et al., 2016). The alignment of each gene was conducted using the MEGA 11 software (Tamura et al., 2021) with Muscle (Edgar, 2004).

The *p-distance* is a statical measure that calculates the distance between two sequences based on the fraction of positions in which two sequences differ (Van de Peer and Salemi, 2009). Average *p-distance* between conspecific sequences from GenBank and collected samples (Table 3.2), the calculations were performed in MEGA 11 with uniform rates and default settings (Tamura et al., 2021). A distance matrix was used for the clustering analysis and the presentation of the tree topology. The Neighbor-Joining (NJ) method count the number of dissimilarities and use these distances to build a phenogram (Van de Peer & Salemi, 2009). The NJ analyses from CO1, 28S and 18S was performed in MEGA 11 with bootstrap analysis based on 1000 resampling of each data set (Tamura et al., 2021). The bootstrap and distance values are shown in the phenogram. The trees were edited in Adobe® Photoshop®.

Morphology, molecular and total evidence phylogenetic analyses

In this study, the intent was to perform a combined analysis that included both morphological and molecular data rather than perform analyses of each data type separately (Kluge, 1998; Kluge, 2004). The following taxa were used as outgroups: *Allodiscocotyla chorinemi*, *A. diacanthi*, *Metacamopia lebedevi* and *M. oligoplites* of Allodiscocotylidae, and *Gemmaecaputia corrugata* of Chauhaneidae (Table 3.2) based on previous phylogenetic analyses of Monogenea (Boeger & Kritsky, 1993; 2001; Tambireddy et al., 2016). Characters

(morphological and molecular) that were not applicable for a particular taxon or for which the character state is unknown were coded as unknown ("?").

The analyses were performed with the Maximum Parsimony (MP) inference method, which the trees are evaluated on the basis of a general metric, the minimum number of character state changes required to generate the data on a given tree, without assuming a specific distribution (Morrone, 2013). The analyses were performed in the program TNT (Tree Analysis using New Technology) (Goloboff et al., 2006). The program TNT (Tree Analysis using New Technology) was selected for the analyses because it allows continuous characters to be analyzed without prior transformation to discrete values. The use of continuous characters with traditional discrete characters provides a combination of all available data types that can provide the most robust hypotheses of phylogenetic relationships (Luna, 2020). Discrete multi-state characters were considered non-additive to provide an unbiased evaluation of previous hypothesized evolution in these taxa (Boeger & Kritsky, 1993; 2001).

The alignments exported in "*Fasta*" format were modified for TNT (Tree analysis using New Technology) in the Mesquite program version 3.81 (Maddison, 2008). The most parsimonious tree for each data matrix was searched using "New Technology Search" (heuristics) (Goloboff, 1999; Goloboff et al., 2003b; Goloboff et al., 2006), using the TNT program version 1.6 (Goloboff and Morales, 2023). The ratchet (Nixon, 1999), and drift (Goloboff, 1999) procedures were added. All characters were equally weighted and gaps were treated as missing data. The strictly consensus tree was calculated. Clade robustness was assessed using a Bootstrap analysis with 10,000 replicates. These values are derived from resampling the character matrix with replacement and analyzing the recovered topology with each iteration. Bootstrap support values are presented here as Group-present/Group-contradicted (GC) frequencies (Goloboff et al., 2003a). Descriptive tree statistics as tree length, the consistency indices (CI) and the retention indices (RI) were calculated with the TNT script "stats.run" and the resulting cladogram was edited in Adobe Photoshop®.

Morphology

Continuous characters were coded based on the proposal of Goloboff et al. (2006), in which intervals obtained from the natural logarithm + 1 of the average value of each

anatomical structure measured and the natural logarithm + 1 of the standard deviation were used. The interval of the first value is the result of the subtraction of the natural logarithm + 1 of the average value and the interval of the second value is the result of the sum of the natural logarithm + 1 of the standard deviation. Discrete characters were treated as unordered, and all characters were weighted equally.

Molecular

Sequences were generated for 35 specimens of *Protomicrocotyle* that were collected as part of this study (some published in Ramírez-Cruz et al. (2023)). These sequences were augmented with sequences of related taxa available in GenBank (Table 3.2). The sequences for CO1, 28S and 18S are shown in Table 3. 2.

Total evidence

The combined matrix is composed of five blocks: The first block was the continuous data; the second block, the discrete data, and the third, fourth and fifth blocks correspond to the molecular data of CO1, 28S and 18S respectively. The data matrix included 48 OTU (6 from the outgroup and 42 from the ingroup) and a total of 2118 characters (141 morphological and 1977 molecular base pairs) (Anexo B: Supplementary material A).

Table 3. 2. List of monogenean species used in this study with CO1, 28S and 18S GenBank accession numbers.

Species of monogeneans	GenBank accession number			References
	CO1	***28S***	***18S***	
Allodiscocotyla diacanthi	KF804045	KF804040	–	Tambireddy et al., 2016
	KF804046	–	–	
Metacamopia oligoplites	–	AF382038	AJ287538	Littlewood & Bray, 2000; Olson & Littlewood, 2002
Gemmaecaputia corrugata	LC623880	LC623879	–	Kamio & Nitta, 2022
Bilaterocotyloides carangis	KF804043	KF804032	KT267184*	Tambireddy et al., 2016
Bilaterocotyle madrasensis	KF804041	KF804029	–	
Lethacotyle vera	–	KF378588	–	
Neomicrocotyle pacifica	–	AF382043	AJ228787	Littlewood & Bray, 2000; Olson & Littlewood, 2002
Protomicrocotyle mirabilis	OR282821	OR282885	ES03*****	Ramírez-Cruz et al., 2023
	OR282822	OR282886	ES04*****	This study
	OR282823	OR282887	ES06*****	
	OR282824	OR282888	ES15*****	
	OR282825	OR282889		
	OR282826	OR282890		
	OR282827	OR282891		
	OR282828	OR282892	ES17*****	
	OR282829	OR282893	ES49*****	

Table 3. 2. *Continuation*.

	OR282830	OR282894	ES52*****	Ramírez-Cruz et al., 2023
	OR282831	ES65*	ES53*****	This study
	OR282832	ES66*		
Protomicrocotyle veracruzensis	OR282833	OR282895	ES45*****	
	OR282836	OR282896	ES48*****	
	OR282837	OR282897	–	
	OR282838	OR282898	–	
Protomicrocotyle **sp. 4**	ES81*****	ES81*****	–	
	ES82*****	ES82*****	–	
	ES83*****	ES87*****	–	
	ES87*	ES88*****	–	
Protomicrocotyle **sp. 5**	ES19*****	ES19*****	–	
	ES20*****	ES20*****	–	
	ES21*****	ES21*****	–	
		ES22*****		

*****Unpublished data

4. Results

Morphological information was taken from the examination of 147 specimens of *Protomicrocotyle* sp. that were examined, of which 81 correspond to specimens that had been deposited previously in the Colección Nacional de Helmintos, 66 specimens that had been deposited in the Colección de Helmintos (CIB-UAEH), and additional specimens collected in the field as part of the current study. An additional 12 specimens of *Neomicrocotyle pacifica* from the CNHE were examined.

Sequence alignment, p-distance analysis and Neighbor-Joining three

The data matrices for CO1, 28S and 18S were aligned individually using Muscle in the MEGA software. The CO1 matrix comprised a total of 30 sequences, of which 23 were generated in the present study (Table 3.2). The length of the final alignment was 429 bp. The CO1 p-distance data matrix indicates an intraspecific variation was 0% to 2.01% from *P. mirabilis*; *P. veracruzensis* was 0.25% to 0.75%; *Protomicrocotyle* **sp. 4** present 0% to 0.48%; and *Protomicrocotyle* **sp. 5** ranges from 0% to 0.25% (Table 3.3). Furthermore, interspecific variation between *P. mirabilis* and *P. veracruzensis* was 8.48% to 10.53%; *P. mirabilis* and *Protomicrocotyle* **sp. 4** was 1.75% to 3.26%; and *P. mirabilis* and *Protomicrocotyle* **sp. 5** 7.73% to 9.02%. The interspecific variation between *P. veracruzensis* and *Protomicrocotyle* **sp. 4** was 7.44% to 8.19%; and *P. veracruzensis* and *Protomicrocotyle* **sp. 5** was 4.47% to 5.21%. The interspecific variation between *Protomicrocotyle* **sp. 4** and *Protomicrocotyle* **sp. 5** was 7.43% to 8.17% (Table 3.3). The NJ tree, the sequences of *Protomicrocotyle* **sp. 4** cluster with those of *P. mirabilis* and those of *Protomicrocotyle* **sp. 5** cluster with *P. veracruzensis*, indicating the similarity between specimens of the already-described species and undescribed specimens (Figure 3.1).

Table 3. 3. Intraspecific and interspecific genetic distances estimated for CO1 of species of *Protomicrocotyle*. Pairwise uncorrected p-distances are expressed as percentages (%).

Species, GenBank accession number and identifier of the sequence	*Allodiscocotyla diacanthi* KF804045	*A. diacanthi* KF804046	*Gemmaecaputia corrugata* LC623880	*Bilaterocotyloides carangis* KU872042	*B. carangis* KF804043	*Bilaterocotyle madrasensis* KF804041	*B. madrasensis* KJ201023	*Protomicrocotyle mirabilis* OR282821	*P. mirabilis* OR282822
Allodiscocotyla diacanthi KF804045									
A. diacanthi KF804046	3.55%								
Gemmaecaputia corrugata LC623880	25.18%	28.37%							
Bilaterocotyloides carangis KU872042	19.50%	21.63%	27.79%						
B. carangis KF804043	18.09%	20.21%	24.11%	2.48%					
*Bilaterocotyle madrasensis*KF804041	19.86%	22.34%	23.05%	14.54%	12.41%				
B. madrasensis KJ201023	19.86%	22.34%	23.01%	14.84%	12.41%	0.00%			
Protomicrocotyle mirabilis OR282821	20.21%	23.05%	21.14%	17.96%	15.25%	16.67%	15.18%		
P. mirabilis OR282822	20.21%	23.05%	21.39%	17.66%	14.89%	16.31%	15.18%	1.00%	
P. mirabilis OR282823	20.21%	23.05%	21.64%	18.16%	14.89%	16.31%	15.48%	1.24%	0.74%
P. mirabilis OR282824	20.92%	23.76%	20.97%	17.95%	14.89%	16.31%	14.88%	0.77%	1.28%
P. mirabilis OR282825	20.21%	23.05%	21.14%	17.66%	14.89%	16.31%	14.88%	0.50%	0.50%
P. mirabilis OR282826	20.21%	23.05%	21.30%	17.84%	15.25%	16.31%	15.18%	0.75%	0.75%

Table 3. 3. *Continuation.*

P. mirabilis OR282827	20.21%	23.05%	21.20%	17.50%	14.89%	16.31%	14.93%	0.50%	0.25%
P. mirabilis OR282828	20.21%	23.05%	21.64%	17.91%	15.25%	16.31%	15.48%	1.00%	0.99%
P. mirabilis OR282829	20.57%	23.40%	21.89%	17.66%	15.25%	16.67%	15.77%	1.00%	0.99%
P. mirabilis OR282830	20.21%	23.05%	21.64%	17.66%	14.89%	16.31%	15.48%	0.75%	0.74%
P. mirabilis OR282831	19.86%	22.70%	21.30%	17.54%	14.54%	16.67%	15.62%	1.00%	1.00%
P. mirabilis OR282832	20.92%	23.76%	21.86%	18.09%	15.60%	16.31%	15.36%	1.26%	1.75%
P. veracruzensis OR282833	20.57%	23.40%	20.40%	18.45%	16.31%	15.60%	14.58%	9.20%	8.71%
P. veracruzensis OR282834	20.92%	23.76%	21.14%	18.66%	16.67%	15.25%	14.58%	9.45%	8.68%
P. veracruzensis OR282835	20.92%	23.76%	20.65%	18.91%	16.67%	15.25%	14.29%	8.96%	8.68%
P. veracruzensis OR282836	20.92%	23.76%	20.90%	19.15%	16.67%	15.25%	13.99%	9.20%	8.93%
Protomicrocotyle **sp. 4** ES81	21.28%	24.11%	20.65%	19.46%	16.31%	17.02%	15.77%	2.49%	1.99%
Protomicrocotyle **sp. 4** ES82	21.28%	24.11%	20.65%	19.70%	16.67%	17.02%	15.77%	2.49%	2.48%
Protomicrocotyle **sp. 4** ES83	21.28%	24.11%	20.65%	19.46%	16.31%	17.02%	15.77%	2.49%	1.99%
Protomicrocotyle **sp. 4** ES87	21.63%	24.47%	20.90%	19.70%	16.67%	16.67%	15.48%	2.49%	2.48%
Protomicrocotyle **sp. 5** ES19	20.57%	23.40%	21.89%	18.61%	15.96%	15.25%	14.88%	7.96%	7.94%
Protomicrocotyle **sp. 5** ES20	20.57%	23.40%	21.89%	18.86%	16.31%	14.89%	14.58%	8.21%	8.19%
Protomicrocotyle **sp. 5** ES21	20.57%	23.40%	21.89%	18.61%	15.96%	15.25%	14.88%	7.96%	7.94%

Table 3. 3. *Continuation.*

	P. mirabilis OR282823	*P. mirabilis* OR282824	*P. mirabilis* OR282825	*P. mirabilis* OR282826	*P. mirabilis* OR282827	*P. mirabilis* OR282828	*P. mirabilis* OR282829	*P. mirabilis* OR282830	*P. mirabilis* OR282831
P. mirabilis OR282824	1.28%								
P. mirabilis OR282825	0.74%	0.77%							
P. mirabilis OR282826	1.00%	1.02%	0.75%						
P. mirabilis OR282827	0.50%	0.77%	0.00%	0.50%					
P. mirabilis OR282828	1.24%	1.28%	0.99%	0.25%	0.75%				
P. mirabilis OR282829	1.24%	0.77%	0.99%	0.75%	0.75%	0.74%			
P. mirabilis OR282830	0.99%	1.02%	0.74%	0.50%	0.50%	0.50%	0.50%		
P. mirabilis OR282831	1.25%	1.29%	0.50%	1.26%	0.50%	1.00%	1.25%	1.00%	
P. mirabilis OR282832	2.01%	1.03%	1.25%	1.52%	1.26%	1.50%	0.75%	1.25%	1.50%
P. veracruzensis OR282833	9.45%	9.21%	8.71%	9.52%	8.73%	9.70%	9.70%	9.45%	9.27%
P. veracruzensis OR282834	9.43%	9.46%	9.18%	9.27%	8.73%	9.68%	9.68%	9.43%	9.75%
P. veracruzensis OR282835	9.43%	8.95%	8.68%	9.27%	8.48%	9.68%	9.68%	9.43%	9.25%
P. veracruzensis OR282836	9.68%	9.21%	8.93%	9.52%	8.73%	9.93%	9.93%	9.68%	9.50%
Protomicrocotyle **sp. 4** ES81	2.23%	2.81%	1.99%	2.26%	1.75%	2.48%	2.48%	2.23%	2.50%
Protomicrocotyle **sp. 4** ES82	2.73%	2.81%	2.48%	1.75%	2.24%	1.99%	2.48%	2.23%	3.00%
Protomicrocotyle **sp. 4** ES83	2.23%	2.81%	1.99%	2.26%	1.75%	2.48%	2.48%	2.23%	2.50%
Protomicrocotyle **sp. 4** ES87	2.73%	2.81%	2.48%	2.26%	2.24%	2.48%	2.48%	2.23%	3.00%

Table 3. 3. *Continuation.*

Protomicrocotyle **sp. 5** ES19	8.44%	7.93%	7.69%	8.27%	7.48%	8.19%	8.44%	8.19%	7.75%
Protomicrocotyle **sp. 5** ES20	8.68%	8.18%	7.94%	8.52%	7.73%	8.44%	8.68%	8.44%	8.00%
Protomicrocotyle **sp. 5** ES21	8.44%	7.93%	7.69%	8.27%	7.48%	8.19%	8.44%	8.19%	7.75%
	P. mirabilis OR282832	*P. veracruzensis* OR282833	*P. veracruzensis* OR282834	*P. veracruzensis* OR282835	*P. veracruzensis* OR282836	*Protomicrocotyle* **sp. 4** ES81	*Protomicrocotyle* **sp. 4** ES82	*Protomicrocotyle* **sp. 4** ES83	*Protomicrocotyle* **sp. 4** ES87
P. veracruzensis OR282833	10.05%								
P. veracruzensis OR282834	10.53%	0.75%							
P. veracruzensis OR282835	10.03%	0.25%	0.50%						
P. veracruzensis OR282836	10.28%	0.50%	0.74%	0.25%					
Protomicrocotyle **sp. 4** ES81	3.26%	7.46%	7.44%	7.44%	7.69%				
Protomicrocotyle **sp. 4** ES82	3.26%	7.96%	7.94%	7.94%	8.19%	0.48%			
Protomicrocotyle **sp. 4** ES83	3.26%	7.46%	7.44%	7.44%	7.69%	0.00%	0.48%		
Protomicrocotyle **sp. 4** ES87	3.26%	7.96%	7.94%	7.94%	8.19%	0.48%	0.48%	0.48%	
Protomicrocotyle **sp. 5** ES19	8.77%	4.73%	4.96%	4.47%	4.71%	7.43%	7.92%	7.43%	7.92%
Protomicrocotyle **sp. 5** ES20	9.02%	4.98%	5.21%	4.71%	4.96%	7.67%	8.17%	7.67%	8.17%
Protomicrocotyle **sp. 5** ES21	8.77%	4.73%	4.96%	4.47%	4.71%	7.43%	7.92%	7.43%	7.92%

Table 3. 3. *Continuation.*

	Protomicrocotyle **sp. 5** ES19	*Protomicrocotyle* **sp. 5** ES20	*Protomicrocotyle* **sp. 5** ES21
Protomicrocotyle **sp. 5** ES20	0.25%		
Protomicrocotyle **sp. 5** ES21	0.00%	0.25%	

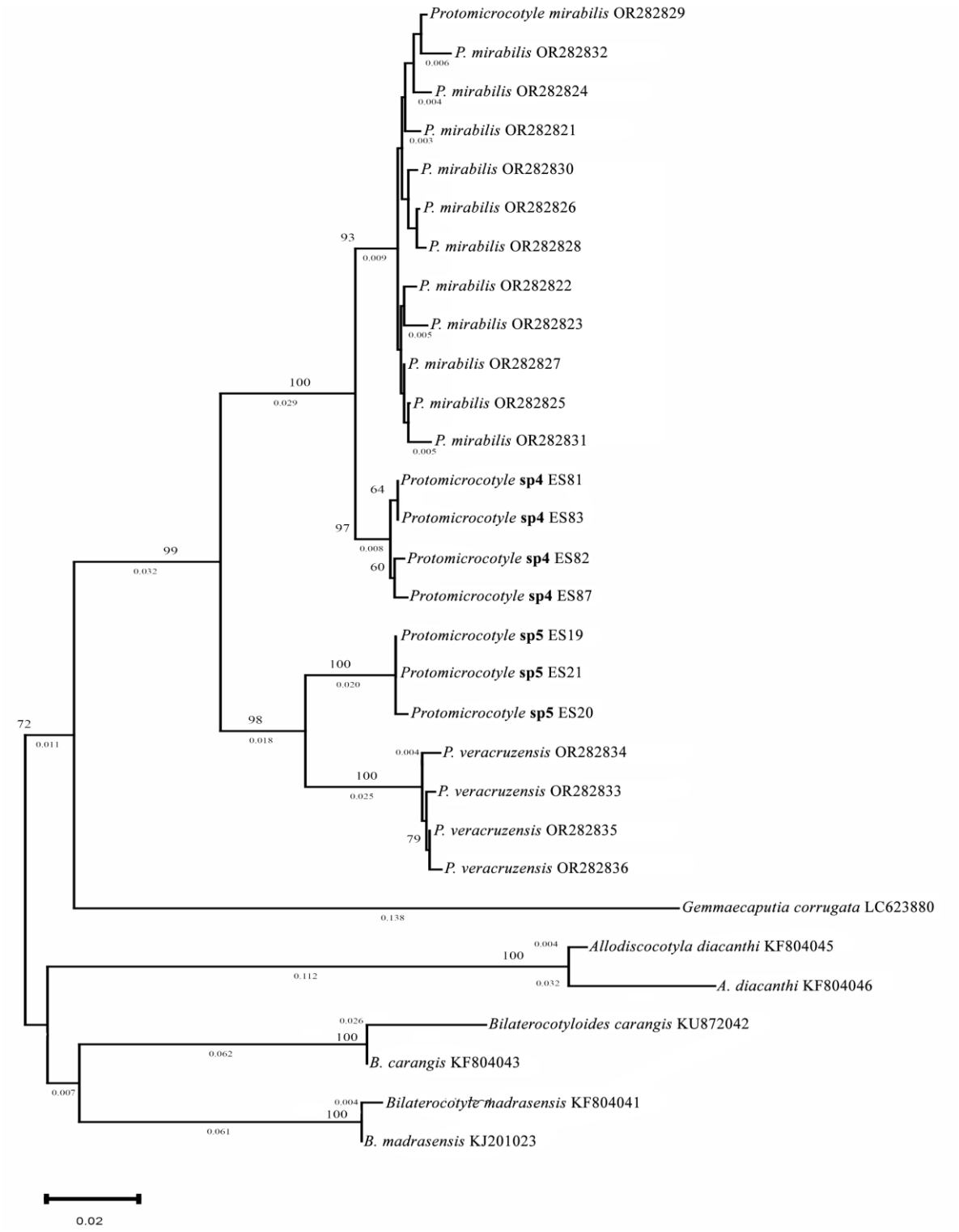

Figure 3. 1. Neighbor-joining phenogram topology of the partial sequences of the CO1 from specimens of *Protomicrocotyle* with bootstrap and distance values.

The 28S matrix comprised a total of 33 sequences, of which 24 were generated in the present study. The length of the final alignment was 769 bp. The intraspecific p-distance with 28S from *P. mirabilis* was the identical sequences to 0.68%; *P. veracruzensis* was identical sequences; *Protomicrocotyle* **sp. 4** was identical sequences to 0.27%; and *Protomicrocotyle* **sp. 5** was identical sequences (Table 3.4). Furthermore, interspecific variation between *P. mirabilis* and *P. veracruzensis* was 0.81% to 1.49%; *P. mirabilis* and *Protomicrocotyle* **sp. 4** was 0.67% to 1.63%; and *P. mirabilis* and *Protomicrocotyle* **sp. 5** was 0.94% to 1.61%. The interspecific variation between *P. veracruzensis* and *Protomicrocotyle* **sp. 4** was 1.76% to 1.89%, and *P. veracruzensis* and *Protomicrocotyle* **sp. 5** was 0.40% to 0.41%. The interspecific variation between *Protomicrocotyle* **sp. 4** and *Protomicrocotyle* **sp. 5** was 1.35% to 2.01% (Table 3.4). The NJ tree shows that the sequences of *Protomicrocotyle* **sp. 4** cluster with those of *P. mirabilis* and those of *Protomicrocotyle* **sp. 5** cluster with *P. veracruzensis*, indicating similarity between specimens of the already described species and undescribed specimens (Figure 3.2).

The 18S matrix comprised a total of 14 sequences, of which 10 were generated in the present study. The species in the 18S data matrix had an intraspecific variation of *P. mirabilis* sequences of identical sequences. Furthermore, interspecific variation of *P. mirabilis* is observed with *P. veracruzensis* of 0.13% to 0.14%. The intraspecific variation of *P. veracruzensis* are identical sequences, while the interspecific variation is 0.13% to 0.14% with *P. mirabilis* (Table 3.5). The NJ tree shows similarity between the sequences of *P. mirabilis* and *P. veracruzensis* (Figure 3.3).

Table 3. 4. Intraspecific and interspecific genetic distances estimated for partial sequences of 28S of species of *Protomicrocotyle*. Pairwise uncorrected p-distances are expressed as percentages (%).

	Allodiscocotyla diacanthi KF804038	*A. diacanthi* KF804040	*Metacamopia oligoplites* AF382038	*Bilaterocotyle madrasensis* KF804029	*B. madrasensis* KF804037	*Bilaterocotyloides carangis* KJ201022	*Neomicrocotyle pacifica* AF382043	*Neomicrocotyle* sp. KF378589	*Lethacotyle vera* KF378588
Allodiscocotyla diacanthi KF804038									
A. diacanthi KF804040	3.50%								
Metacamopia oligoplites AF382038	0.94%	3.98%							
Bilaterocotyle madrasensis KF804029	5.25%	8.61%	5.65%						
B. madrasensis KF804037	5.25%	8.61%	5.65%	0.00%					
Bilaterocotyloides carangis KJ201022	5.29%	7.21%	5.59%	0.44%	0.44%				
Neomicrocotyle pacifica AF382043	6.06%	9.42%	6.46%	2.42%	2.42%	2.94%			
Neomicrocotyle sp. KF378589	5.93%	8.00%	5.93%	2.20%	2.20%	2.79%	0.14%		
Lethacotyle vera KF378588	6.21%	8.00%	6.21%	3.03%	3.03%	3.38%	1.79%	1.65%	
Protomicrocotyle mirabilis OR282885	5.25%	8.38%	5.85%	2.55%	2.55%	3.23%	2.69%	2.48%	2.20%
P. mirabilis OR282886	5.14%	8.34%	5.79%	2.43%	2.43%	3.10%	2.83%	2.63%	2.35%

Table 3. 4. *Continuation.*

P. mirabilis OR282887	5.26%	8.46%	5.91%	2.56%	2.56%	3.24%	2.69%	2.48%	2.21%
P. mirabilis OR282888	5.26%	8.23%	5.67%	2.56%	2.56%	3.24%	2.70%	2.48%	2.21%
P. mirabilis OR282889	5.68%	7.38%	5.64%	2.97%	2.97%	3.08%	3.38%	2.62%	2.34%
P. mirabilis OR282890	5.57%	7.47%	5.57%	2.85%	2.85%	3.09%	3.26%	2.62%	2.34%
P. mirabilis OR282891	5.31%	7.29%	5.26%	2.59%	2.59%	3.23%	2.72%	2.48%	2.20%
P. mirabilis OR282892	5.27%	7.97%	5.41%	2.56%	2.56%	3.23%	2.70%	2.48%	2.20%
P. mirabilis OR282893	5.46%	7.38%	5.46%	2.73%	2.73%	3.10%	3.14%	2.63%	2.35%
P. mirabilis OR282894	5.43%	7.46%	5.43%	2.71%	2.71%	3.09%	3.12%	2.62%	2.34%
P. mirabilis ES65	5.19%	7.24%	5.19%	2.46%	2.46%	3.09%	2.86%	2.62%	2.35%
P. mirabilis ES66	5.34%	7.39%	5.34%	2.60%	2.60%	3.24%	2.73%	2.49%	2.21%
P. veracruzensis OR282895	5.38%	8.61%	5.52%	2.96%	2.96%	3.67%	2.82%	2.62%	2.48%
P. veracruzensis OR282896	5.38%	8.61%	5.52%	2.96%	2.96%	3.67%	2.82%	2.62%	2.48%
P. veracruzensis OR282897	5.38%	8.61%	5.52%	2.96%	2.96%	3.67%	2.82%	2.62%	2.48%
P. veracruzensis OR282898	5.41%	8.38%	5.41%	2.97%	2.97%	3.68%	2.83%	2.62%	2.48%

Table 3. 4. *Continuation.*

Protomicrocotyle ES81	**sp.**	**4**	6.63%	8.05%	6.58%	3.92%	3.92%	3.96%	3.92%	3.03%	2.89%
Protomicrocotyle ES82	**sp.**	**4**	6.50%	8.05%	6.44%	3.78%	3.78%	3.96%	3.78%	3.03%	2.75%
Protomicrocotyle ES87	**sp.**	**4**	6.51%	8.08%	6.46%	3.79%	3.79%	3.96%	3.79%	3.03%	2.89%
Protomicrocotyle ES88	**sp.**	**4**	6.38%	8.08%	6.33%	3.66%	3.66%	3.96%	3.66%	3.03%	2.75%
Protomicrocotyle ES19	**sp.**	**5**	5.59%	7.43%	5.40%	3.13%	3.13%	3.82%	3.27%	3.03%	2.89%
Protomicrocotyle ES20	**sp.**	**5**	5.52%	8.63%	5.97%	3.09%	3.09%	3.82%	3.23%	3.03%	2.89%
Protomicrocotyle ES21	**sp.**	**5**	5.59%	7.56%	5.39%	3.13%	3.13%	3.82%	3.27%	3.03%	2.89%
Protomicrocotyle ES22	**sp.**	**5**	5.59%	7.56%	5.39%	3.13%	3.13%	3.82%	3.27%	3.03%	2.89%

	Protomicrocotyle mirabilis OR282885	*P. mirabilis* OR282886	*P. mirabilis* OR282887	*P. mirabilis* OR282888	*P. mirabilis* OR282889	*P. mirabilis* OR282890	*P. mirabilis* OR282891	*P. mirabilis* OR282892	*P. mirabilis* OR282893
P. mirabilis OR282886	0.13%								
P. mirabilis OR282887	0.00%	0.13%							
P. mirabilis OR282888	0.00%	0.14%	0.00%						
P. mirabilis OR282889	0.67%	0.54%	0.68%	0.68%					

Table 3. 4. *Continuation.*

P. mirabilis OR282890	0.54%	0.41%	0.54%	0.54%	0.00%				
P. mirabilis OR282891	0.00%	0.14%	0.00%	0.00%	0.13%	0.14%			
P. mirabilis OR282892	0.00%	0.14%	0.00%	0.00%	0.68%	0.54%	0.00%		
P. mirabilis OR282893	0.41%	0.27%	0.41%	0.41%	0.00%	0.00%	0.14%	0.41%	
P. mirabilis OR282894	0.41%	0.27%	0.41%	0.41%	0.14%	0.14%	0.14%	0.41%	0.14%
P. mirabilis ES65	0.14%	0.00%	0.14%	0.14%	0.00%	0.00%	0.14%	0.14%	0.00%
P. mirabilis ES66	0.27%	0.14%	0.27%	0.27%	0.14%	0.14%	0.27%	0.27%	0.14%
P. veracruzensis OR282895	0.81%	0.94%	0.81%	0.81%	1.49%	1.36%	0.82%	0.81%	1.23%
P. veracruzensis OR282896	0.81%	0.94%	0.81%	0.81%	1.49%	1.36%	0.82%	0.81%	1.23%
P. veracruzensis OR282897	0.81%	0.94%	0.81%	0.81%	1.49%	1.36%	0.82%	0.81%	1.23%
P. veracruzensis OR282898	0.81%	0.95%	0.81%	0.81%	1.49%	1.36%	0.82%	0.81%	1.23%
Protomicrocotyle **sp. 4** ES81	1.47%	1.63%	1.49%	1.49%	1.07%	1.09%	0.94%	1.49%	1.09%
Protomicrocotyle **sp. 4** ES82	1.21%	1.36%	1.22%	1.22%	0.80%	0.81%	0.67%	1.22%	0.82%
Protomicrocotyle **sp. 4** ES87	1.34%	1.50%	1.36%	1.36%	1.08%	1.09%	0.94%	1.36%	1.09%
Protomicrocotyle **sp. 4** ES88	1.08%	1.22%	1.09%	1.09%	0.81%	0.81%	0.67%	1.08%	0.82%
Protomicrocotyle **sp. 5** ES19	1.08%	1.09%	0.95%	0.95%	1.08%	1.09%	1.08%	0.95%	1.10%
Protomicrocotyle **sp. 5** ES20	1.06%	1.08%	0.94%	0.94%	1.61%	1.49%	1.08%	0.94%	1.36%

Table 3. 4. *Continuation.*

Protomicrocotyle **sp. 5** ES21	1.08%	1.09%	0.95%	0.95%	1.08%	1.09%	1.08%	0.95%	1.09%
Protomicrocotyle **sp. 5** ES22	1.08%	1.09%	0.95%	0.95%	1.08%	1.09%	1.08%	0.95%	1.09%
	P. mirabilis OR282894	*P. mirabilis* ES65	*P. mirabilis* ES66	*P. veracruzensis* OR282895	*P. veracruzensis*OR282896	*P. veracruzensis* OR282897	*P. veracruzensis* OR282898	*Protomicrocotyle* **sp. 4** ES81	*Protomicrocotyle* **sp. 4** ES82
P. mirabilis ES65	0.00%								
P. mirabilis ES66	0.14%	0.14%							
P. veracruzensis OR282895	1.22%	0.95%	0.82%						
P. veracruzensis OR282896	1.22%	0.95%	0.82%	0.00%					
P. veracruzensis OR282897	1.22%	0.95%	0.82%	0.00%	0.00%				
P. veracruzensis OR282898	1.22%	0.95%	0.82%	0.00%	0.00%	0.00%			
Protomicrocotyle **sp. 4** ES81	1.22%	1.09%	1.09%	1.89%	1.89%	1.89%	1.89%		
Protomicrocotyle **sp. 4** ES82	0.95%	0.82%	0.82%	1.89%	1.89%	1.89%	1.89%	0.27%	
Protomicrocotyle **sp. 4** ES87	1.22%	1.09%	1.09%	1.76%	1.76%	1.76%	1.76%	0.00%	0.27%

Protomicrocotyle ES88	**sp.**	**4**	0.95%	0.82%	0.82%	1.76%	1.76%	1.76%	1.76%	0.27%	0.00%

Table 3. 4. *Continuation.*

Protomicrocotyle ES19	**sp.**	**5**	1.09%	1.09%	1.23%	0.41%	0.41%	0.41%	0.41%	1.35%	1.49%
Protomicrocotyle ES20	**sp.**	**5**	1.36%	1.09%	1.23%	0.40%	0.40%	0.40%	0.40%	2.01%	2.01%
Protomicrocotyle ES21	**sp.**	**5**	1.09%	1.09%	1.23%	0.41%	0.41%	0.41%	0.41%	1.48%	1.48%
Protomicrocotyle ES22	**sp.**	**5**	1.09%	1.09%	1.23%	0.41%	0.41%	0.41%	0.41%	1.48%	1.48%

			Protomicrocotyle **sp. 4** ES87	*Protomicrocotyle* **sp. 4** ES88	*Protomicrocotyle* **sp. 5** ES19	*Protomicrocotyle* **sp. 5** ES20	*Protomicrocotyle* **sp. 5** ES21	*Protomicrocotyle* **sp. 5** ES22
Protomicrocotyle ES88	**sp.**	**4**	0.27%					
Protomicrocotyle ES19	**sp.**	**5**	1.35%	1.49%				
Protomicrocotyle ES20	**sp.**	**5**	1.88%	1.88%	0.00%			
Protomicrocotyle ES21	**sp.**	**5**	1.48%	1.48%	0.00%	0.00%		
Protomicrocotyle ES22	**sp.**	**5**	1.48%	1.48%	0.00%	0.00%	0.00%	

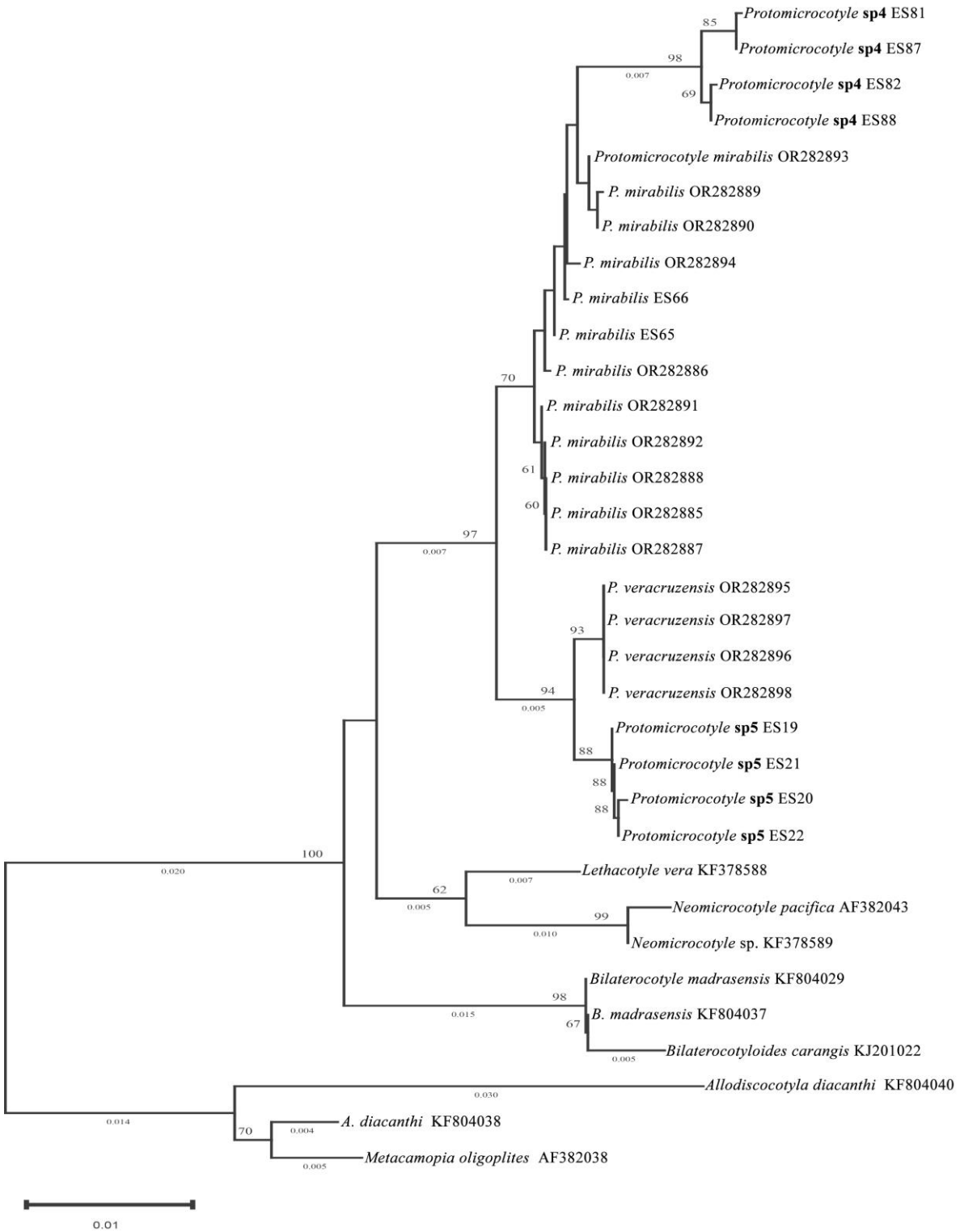

Figure 3. 2. Neighbor-joining phenogram topology of the partial sequences of the 28S from specimens of *Protomicrocotyle* with bootstrap and distance values.

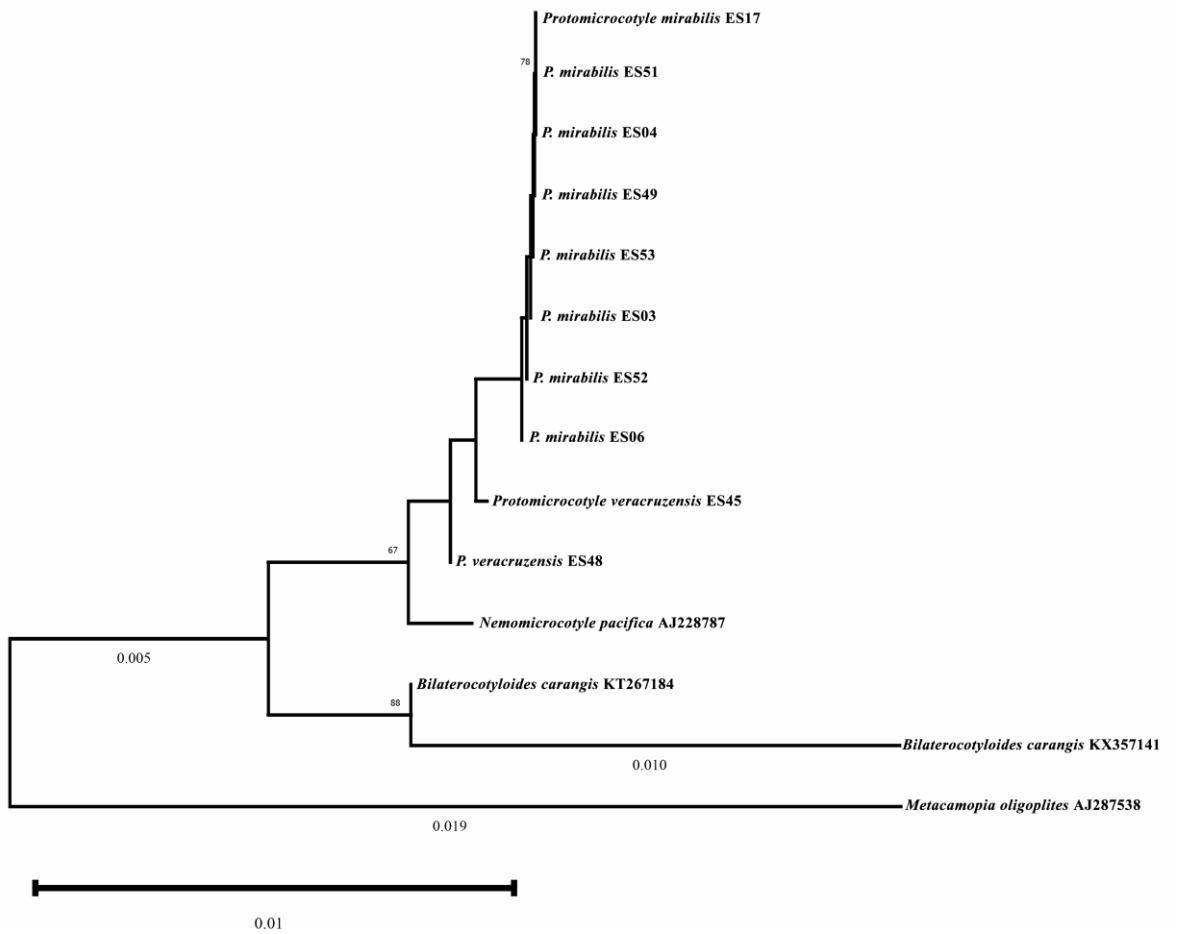

Figure 3. 3. Neighbor-joining phenogram topology of the partial sequences of the 18S from specimens of *Protomicrocotyle* with bootstrap and distance values.

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Table 3. 5. Intraspecific and interspecific genetic distances estimated for 18S of species of *Protomicrocotyle*. Pairwise uncorrected p-distances are expressed as percentages (%).

Species, GenBank accession number and identifier of the sequence	*Metacamopia oligoplites* AJ287538	*Neomicrocotyle pacifica* AJ228787	*Bilaterocotyloides carangis* KT267184	*B.* carangis KX357141
Metacamopia oligoplites AJ287538				
Neomicrocotyle pacifica AJ228787	2.54%			
Bilaterocotyloides carangis KT267184	2.67%	0.89%		
B. carangis KX357141	3.69%	2.08%	0.96%	
Protomicrocotyle mirabilis ES03	2.84%	0.27%	0.54%	1.67%
P. mirabilis ES04	2.98%	0.39%	0.78%	1.93%
P. mirabilis ES06	2.81%	0.27%	0.53%	1.66%
P. mirabilis ES17	3.70%	1.02%	1.41%	1.93%
P. mirabilis ES49	2.85%	0.27%	0.54%	1.67%
P. mirabilis ES51	3.00%	0.39%	0.78%	1.93%
P. mirabilis ES52	2.82%	0.27%	0.54%	1.67%
P. mirabilis ES53	2.85%	0.27%	0.54%	1.67%
P. veracruzensis ES45	2.85%	0.26%	0.91%	2.08%
P. veracruzensis ES48	2.71%	0.14%	0.68%	1.84%

Table 3. 5. *Continuation.*

	Protomicrocotyle mirabilis ES03	*P. mirabilis* ES04	*P. mirabilis* ES06	*P. mirabilis* ES17	*P. mirabilis* ES49	*P. mirabilis* ES51	*P. mirabilis* ES52	*P. mirabilis* ES53	*P. veracruzensis* ES45	*P. veracruzensis* ES48
Protomicrocotyle mirabilis ES03										
P. mirabilis ES04	**0.00%**									
P. mirabilis ES06	**0.00%**	**0.00%**								
P. mirabilis ES17	**0.00%**	**0.00%**	**0.00%**							
P. mirabilis ES49	**0.00%**	**0.00%**	**0.00%**	**0.00%**						
P. mirabilis ES51	**0.00%**	**0.00%**	**0.00%**	**0.00%**	**0.00%**					
P. mirabilis ES52	**0.00%**	**0.00%**	**0.00%**	**0.00%**	**0.00%**	**0.00%**				
P. mirabilis ES53	**0.00%**	**0.00%**	**0.00%**	**0.00%**	**0.00%**	**0.00%**	**0.00%**			
P. veracruzensis ES45	0.13%	0.13%	0.13%	0.13%	0.14%	0.13%	0.13%	0.14%		
P. veracruzensis ES48	0.14%	0.14%	0.14%	0.14%	0.14%	0.14%	0.14%	0.14%	**0.00%**	

The **bold values** represent the intraspecific variation of *P. mirabilis a*nd *P. veracruzensis*.

Morphological variables and codifications of characters

The list of morphological variables and the codification of characters are presented in the following list. For more details of continuous and meristic data see the chapter II.

Continuous characters

0. Body length (LT).
1. Body width (AT).
2. Length of oral opening (LAO).
3. Oral opening width (AAO).
4. Pharyngeal length (LF).
5. Pharyngeal width (AF).
6. Length of esophagus (LEGO).
7. Width of esophagus (AEGO).
8. Right pseudo-sucker length (LPD).
9. Width of right pseudo-sucker (APD).
10. Length of left pseudo-sucker cup (LPI).
11. Width of left pseudo-sucker (API).
12. Haptor length (LHA).
13. Haptor width (AHA).
14. Long right lateral haptoral lappet (LLDAL).
15. Long left lateral haptoral lappet (LLIAL).
16. Long central haptoral lappet (LCAL).
17. Larval haptoral lappet (AAL).
18. Length of first clamp (LPP).
19. Width of first clamp (APP).
20. Length of second clamp (LSP).
21. Width of second clamp (ASP).
22. Length of third clamp (LTP).
23. Width of third clamp (ATP).
24. Length of fourth clamp (LCP).

25. Width of fourth clamp (ACP).
26. Distance from the cecal bifurcation to the anterior end (DBCEA).
27. Distance from genital pore to anterior end (DPGEA).
28. Distance from vaginal vestibule to anterior end (DVVEA).
29. Distance from the genital pore to the vaginal vestibule (DPGVV).
30. Right side hook length (Anchor) (LGLD).
31. Right side hook width (AGLD).
32. Right side hook opening length (LAGLD).
33. Internal root length of right lateral hook (LRIGLD).
34. Width of internal root of the right lateral hook (ARIGLD).
35. Right side hook handle length (LMGLD).
36. External root length of the right lateral hook (LREGLD).
37. Right side hook main part length (LPPGLD).
38. Right Lateral Hook Tip Length (LPGLD).
39. Left lateral hook length (anchor) (LGLI).
40. Left lateral hook width (AGLI).
41. Left side hook opening length (LAGLI).
42. Internal root length of the left lateral hook (LRIGLI).
43. Width of internal root of the right lateral hook (ARIGLI).
44. Left Side Hook Handle Length (LMGLI).
45. External root length of the left lateral hook (LREGLI).
46. Length of main part of the left lateral hook (LPPGLI).
47. Left lateral hook tip length (LPGLI).
48. Right middle hook length (Anchor) (LGMD).
49. Hook width middle right (AGMD).
50. Right middle hook opening length (LAGMD).
51. Internal root length of the right middle hook (LRIGMD).
52. Width of internal root of the right middle hook (ARIGMD).
53. Right middle hook handle length (LMGMD).
54. External root length of the right middle hook (LREGMD).
55. Length of main part of right middle hook (LPPGMD).

56. Right middle hook tip length (LPGMD).
57. Left middle hook length (Anchor) (LGMI).
58. Left middle hook width (AGMI).
59. Left middle hook opening length (LAGMI).
60. Internal root length of the left middle hook (LRIGMI).
61. Width of the internal root of the left middle hook (ARIGMI).
62. Left middle hook handle length (LMGMD).
63. External root length of the left middle hook (LREGMI).
64. Length of main part of left middle hook (LPPGMD).
65. Left middle hook tip length (LPGMD)
66. Total length of right center hook (LTGCD).
67. Right center hook width (ATGCD).
68. Total length of left center hook (LTGCI).
69. Left center hook width (ATGCI).
70. Average length of the testicles (LPT).
71. Average width of the testicles (APT).
72. Length of male copulatory organ (LOCM).
73. Width of male copulatory organ (AOCM).
74. Length of spines of the male copulatory organ (LEOCM).
75. Width of spines of the male copulatory organ (AEOCM).
76. Long of the ovary (LOV).
77. Ovary width (AOV).
78. Length of vaginal vestibule (LVV).
79. Vaginal vestibule width (AVV).
80. Length of spines of the vaginal vestibule (LEVV).
81. Width of spines of the vaginal vestibule (AEVV).
82. Egg length (LH).
83. Egg width (AH).
84. Filament length (anterior region) (LFDHRA).
85. Filament width (anterior region) (AFDHRA).
86. Filament length (posterior region) (LFDHRP).

87. Filament width (posterior region) (AFDHRP).

Meristic characteristics

88. Number of spines in the vaginal vestibule.

89. Number of spines in the male copulatory organ.

90. Number of testes.

Argumentation of characters

91. Shape of body.

0= No fusiform, L or V shaped as *Metacamopia lebedevi* (Nguyen et al., 2020), and L shaped as *Vallisiopsis contorta* (Subhapradha, 1951).

1= Fusiform as *Protomicrocotyle* (Ramalingam, 1960; Bravo-Hollis, 1979; Kritsky et al., 2011; Ramírez-Cruz et al., 2023).

92. Shape of body in *Protomicrocotyle*.

0= Fusiform-narrowly body. The width of the body is less than the width of the haptoral lappet as *Protomicrocotyle mirabilis* (Kritsky et al., 2011; Ramírez-Cruz et al., 2023).

1= Fusiform-broadly. The width of the body is greater than the width of the haptoral lappet as *P. manteri* and *P. veracruzensis* (Bravo-Hollis, 1979; Kritsky et al., 2011; Ramírez-Cruz et al., 2023).

The body of monogeneans presents a great diversity of shapes, which are considered within the taxonomic examination for the identification and determination of the species (Bychowsky, 1961; Yamaguti, 1963; Schmidt et al., 2009).

93. Body symmetry.

0= Absent, as *Metacamopia lebedevi* (Nguyen et al., 2020), and *Vallisiopsis contorta* (Subhapradha, 1951).

1= Bilateral symmetry, as *Bilaterocotyle chirocentrosus* (Chauhan, 1945), *Gemmaecaputia corrugata* (Kamio & Nitta, 2022).

2= Partial bilateral symmetry, as *Lethacotyle* (Manter & Prince, 1953; Justine et al., 2013), *Neomicrocotyle* (Ramalingam, 1960; Bravo-Hollis & Salgado-Maldonado, 1985; Pulido-Flores, 1997; Aréchiga-Ramos et al., 2023), *Protomicrocotyle* (Yamaguti, 1953; Bravo-Hollis, 1966; Bravo-Hollis, 1979; Kritsky et al., 2011; Ramírez-Cruz et al., 2023).

Bilateral symmetry is commonly present in most monogenean species (Bychowsky, 1961), in species of the genus *Gemmaecaputia* this morphological characteristic is present, while in *M. lebedevi* it is absent, this is mainly due to the lateral growth in the middle part of the body of this species, which causes the body to be divided into two sections (Nguyen et al., 2020). In the genera *Neomicrocotyle* and *Protomicrocotyle*, the characteristic of bilateral symmetry is partial, since a portion of the haptor is asymmetric.

94. Body divided in regions.

0= The body is divided into four well-defined regions. The prohaptor, trunk, trunk extension, and haptor, as in *M. levedevi* (Nguyen et al., 2020).

1= The body is divided into three regions. The prohaptor, trunk, and, haptor, as in species of *Protomicrocotyle* (Bravo-Hollis, 1979; Kritsky et al., 2011).

The body of monogeneans is generally divided into three regions: the anterior region, known as the prohaptor, in which some attachment structures are found; the middle region or trunk, in which the reproductive organs are found; and the haptor, which is the main fixation structure and where structures such as hooks, suckers or pseudo-suckers, and clamps are found (Bychowsky, 1961; Yamaguti, 1963; Schmidt et al., 2009). The fourth region is anterior to the hind body and consists of a twisted bulb region in the middle of the body, as *M. levedevi* (Nguyen et al., 2020).

95. Position of the oral opening.

0= Subterminal

1= Terminal

The position of the oral opening can be found in the anterior region without reaching the anterior end of the body, as in *Protomicrocotyle mirabilis* (Kritsky et al., 2011) or be at the anterior end as in *P. manteri* and *Gemmaecaputia corrugata* (Bravo-Hollis, 1966; Kamio and Nitta, 2022).

96. Prohaptor with retractile muscular glandular organ.

0= Absent, as *Allodiscocotyla diacanthi* (Unnithan, 1962; Nguyen et al., 2020), *Gemmaecaputia corrugata* (Kamio & Nitta, 2022), *P. mirabilis* (Kritsky et al., 2011), and *P. veracruzensis* (Ramírez-Cruz et al., 2023).

1= Present, as *P. manteri* (Bravo-Hollis, 1966).

The retractile muscular glandular organ is an anatomical structure found in the prohaptor region that is involved in attachment to the host and feeding (Bychowsky, 1961; Yamaguti, 1963). Within *Protomicrocotyle*, (Bravo-Hollis, 1966) reports it to *P. manteri*.

97. Presence of glandular cells in the prohaptor region.

0= Absent, as *Protomicrocotyle* (Yamaguti, 1953; Bravo-Hollis, 1966; Bravo-Hollis, 1979; Kritsky et al., 2011; Ramírez-Cruz et al., 2023).

1= Present, as *Gemmaecaputia corrugata* (Kamio & Nitta, 2022).

The gland cells comprise a cluster of some closely apposed cells situated in a median plane of the prohaptor, anterior to the buccal pseudo-suckers and dorsal to the mouth opening. These gland cells provide temporary attachment during the leech-like locomotion of the monogenean (Halton et al., 1974; El-Naggar & Kearn, 1983). These anatomical structures have not been observed in *Protomicrocotyle* species, while they have been observed in *G. corrugata* (Kamio & Nitta, 2022).

98. Shape of glandular cells.

0= Absent, as *Protomicrocotyle* (Yamaguti, 1953; Bravo-Hollis, 1966; Bravo-Hollis, 1979; Kritsky et al., 2011; Ramírez-Cruz et al., 2023).

1= Present, as *Gemmaecaputia corrugata* (Kamio & Nitta, 2022).

(Kamio & Nitta, 2022) described this anatomical structure like rice grain-shaped.

99. Number of glandular cells.

0= Absent, as *Protomicrocotyle* (Yamaguti, 1953; Bravo-Hollis, 1966; Bravo-Hollis, 1979; Kritsky et al., 2011; Ramírez-Cruz et al., 2023).

1= 14 to 19 in number, as *Gemmaecaputia corrugata* (Kamio & Nitta, 2022).

100. Esophagus.

0= Without diverticula, as *Protomicrocotyle carangis* (Pillai & Pillai, 1978), and *Allodiscocotyla dicanthi* (Nguyen et al., 2020).

1= With diverticula, as *Protomicrocotyle* (Yamaguti, 1953; Kritsky et al., 2011).

The diverticula are small glandular extensions present in the esophagus; the condition zero refers to the absence (Nguyen et al., 2020), and the condition one to the presence (Yamaguti, 1953; Kritsky et al., 2011).

101. Position of the cecal bifurcation.

0=Anterior to male copulatory organ, as *Gotocotyla acanthura* (Parona & Perugia, 1986)

1= Posterior to male copulatory organ, as *P. manteri* (Bravo-Hollis, 1966), *P. mirabilis* (Kritsky et al., 2011), *P. veracruzensis* (Ramírez-Cruz et al., 2023).

2= At the level of the genital pore, as *Protomicrocotyle celebesensis* (Yamaguti, 1953).

102. Extension of the intestinal ceca.

0= The intestinal caeca does not reach the haptor, as *Gemmaecaputia corrugata* (Chauhan, 1945).

1= The intestinal caeca extends to the haptor, surpassing the haptoral lappet, as Protomicrocotyle (Kritsky et al., 2011; Justine et al., 2013)

103. Tegument with transverse striations on the body.

0= Absent, as *Metacamopia* (Takemoto et al., 1996; Nguyen et al., 2020).

1= Present, as *Protomicrocotyle* (Yamaguti, 1953; Bravo-Hollis, 1966; Bravo-Hollis, 1979; Kritsky et al., 2011; Ramírez-Cruz et al., 2023).

Transverse striae are extensions of the integument in the form of rings that are found along the body or only in some regions (anterior, middle, or posterior) (Buchmann & Bresciani, 2006b).

104. Transverse striations distribution in the body.

0= Absent

1= Transverse striae with distribution along the body, as *Allodiscocotyla chorinemi* (Unnithan, 1962).

2= Transverse striae with distribution from the region of the germarium to the region of the posterior end of the haptor, as *Protomicrocotyle mirabilis* (Kritsky et al., 2011).

3= Transverse striae are present only in the haptor region, as in *Lethacotyle vera* (Justine et al., 2013).

105. Shape of the transverse striae.

0= Absent

1= Scale-shaped, as *Protomicrocotyle mirabilis* (Kritsky et al., 2011), and *Vallisiopsis contorta* (Subhapradha, 1951).

2= Lobed shape, as *P. manteri* and *P. veracruzensis* (Bravo-Hollis, 1966; Ramírez-Cruz et al., 2023).

The condition one stretch marks have a small triangular extension that gives them a serrated appearance. The striae of condition two have lobed prolongation.

106. Haptor symmetry.

0= Bilateral symmetry, as *Allodiscocotyla chorinemi* (Unnithan, 1962), and *Gemmaecaputia corrugata* (Kamio & Nitta, 2022).

1= Asymmetric haptor, as *Lethacotyle* (Manter & Prince, 1953; Justine et al., 2013), *Neomicrocotyle* (Ramalingam, 1960; Bravo-Hollis & Salgado-Maldonado, 1985; Pulido-Flores, 1997; Aréchiga-Ramos et al., 2023), *Protomicrocotyle* (Yamaguti, 1953; Bravo-Hollis, 1966; Bravo-Hollis, 1979; Kritsky et al., 2011; Ramírez-Cruz et al., 2023), and *Vallisiopsis contorta* (Subhapradha, 1951).

107. Haptor differentiates from the rest of the body.

0= The haptor is not different from the rest of the body, *Gemmaecaputia corrugata* (Kamio & Nitta, 2022), and *Protomicrocotyle* **sp. 2**.

1= The haptor is differentiated from the rest of the body, as *Lethacotyle* (Manter & Prince, 1953; Justine et al., 2013), *Neomicrocotyle* (Ramalingam, 1960; Bravo-Hollis & Salgado-Maldonado, 1985; Pulido-Flores, 1997; Aréchiga-Ramos et al., 2023), *Protomicrocotyle* (Yamaguti, 1953; Bravo-Hollis, 1966; Bravo-Hollis, 1979; Kritsky et al., 2011; Ramírez-Cruz et al., 2023), and *Vallisiopsis contorta* (Subhapradha, 1951).

The haptor is differentiated from the rest of the body with a constriction that marks the division of the trunk from the haptor.

108. Shape of the haptor.

0= Triangular shape, as *Allodiscocotyla* (Unnithan, 1962), *Gotocotyla acanthura* (Parona & Perugia, 1986), and *Metacamopia oligoplites* (Takemoto et al., 1996).

1= Fan-shaped, as *Gemmaecaputia corrugata* (Kamio & Nitta, 2022).

2= Protomicrocotylidae type, as *Protomicrocotyle*, *Neomicrocotyle*, *Lethacotyle* (Ramalingam, 1960; Bravo-Hollis, 1966; Bravo-Hollis, 1979; Kritsky et al., 2011; Justine et al., 2013).

3= Vallisiopsis type, as *Youngiopsis australis* (Young, 1968), and *Vallisiopsis contorta* (Subhapradha, 1951).

The haptor is located in the posterior region of the body of the monogeneans. In this anatomical structure are the structures that help the monogeneans remain attached to the host (Yamaguti, 1963; Buchmann & Bresciani, 2006b).

109. Heel-shaped projection on the haptor.

0= Absent, as *Gemmaecaputia corrugata* (Kamio & Nitta, 2022), *Neomicrocotyle* (Ramalingam, 1960; Bravo-Hollis & Salgado-Maldonado, 1985; Pulido-Flores, 1997; Aréchiga-Ramos et al., 2023), and *Protomicrocotyle* (Yamaguti, 1953; Bravo-Hollis, 1966; Bravo-Hollis, 1979; Kritsky et al., 2011; Ramírez-Cruz et al., 2023).

1= Present, as *Metacamopia oligoplites* (Takemoto et al., 1996).

It is an extension of the body present in the haptor region in *Metacamopia oligoplites* (Takemoto et al., 1996).

110. Cup-shaped accessory adhesive organ.

0= Absent

1= Present, as *Y australis* (Young, 1968).

Formed mainly from a modification of the tegument and musculature near the junction of the fore-body and hind-body, this structure has the function of adhering to the gill filaments and helping with the fixation and locomotion of the monogenean at the site of infection. Within the family Protomicrocotylidae it is reported in *Y australis* (Young, 1968).

111. Haptoral groove.

0= Absent, as *Allodiscocotyla chorinemi* (Unnithan, 1962), and *Gemmaecaputia corrugata* (Kamio & Nitta, 2022).

1= Present, as *Lethacotyle* (Manter & Prince, 1953; Justine et al., 2013), *Neomicrocotyle* (Ramalingam, 1960; Bravo-Hollis & Salgado-Maldonado, 1985; Pulido-Flores, 1997; Aréchiga-Ramos et al., 2023), *Metacamopia* (Takemoto et al., 1996; Nguyen et al., 2020), *Protomicrocotyle* (Yamaguti, 1953; Bravo-Hollis, 1966; Bravo-Hollis, 1979; Kritsky et al., 2011; Ramírez-Cruz et al., 2023), and *Vallisiopsis contorta* (Subhapradha, 1951).

The haptoral groove is a horizontal depression present between the posterior region of the first clamp and the anterior region of the second clamp on the posterior part of the body of the monogenean (Ramalingam, 1960; Bravo-Hollis, 1966; Bravo-Hollis, 1979; Kritsky et al., 2011; Justine et al., 2013).

112. Number of haptoral grooves.

0= Absent

1= One, as *Neomicrocotyle* (Ramalingam, 1960; Bravo-Hollis & Salgado-Maldonado, 1985; Pulido-Flores, 1997; Aréchiga-Ramos et al., 2023), and *Protomicrocotyle* (Yamaguti, 1953; Bravo-Hollis, 1966; Bravo-Hollis, 1979; Kritsky et al., 2011; Ramírez-Cruz et al., 2023).

2= Three, as *Metacamopia* (Takemoto et al., 1996; Nguyen et al., 2020), and *Vallisiopsis contorta* (Subhapradha, 1951).

113. Types of clamps.

0= Without clamps, as *Lethacotyle* (Manter & Prince, 1953; Justine et al., 2013).

1= Microcotylid clamps, as *Neomicrocotyle* (Ramalingam, 1960; Bravo-Hollis & Salgado-Maldonado, 1985; Pulido-Flores, 1997; Aréchiga-Ramos et al., 2023).

2= Gastrocotylid clamps, as *Protomicrocotyle* (Yamaguti, 1953; Bravo-Hollis, 1966; Bravo-Hollis, 1979; Kritsky et al., 2011; Ramírez-Cruz et al., 2023).

The clamps are attachment structures found in the haptor (Yamaguti, 1963; Buchmann & Bresciani, 2006b; Maggenti et al., 2008). In species of the Protomicrocotylidae family, two types of clamps are found: the "gastrocotylid" type (with an accessory oblique sclerite) and the "microcotylid" type (without an accessory oblique sclerite) (Justine et al., 2013).

114. Number of clamps.

0= Without clamps, as *Lethacotyle* (Manter & Prince, 1953; Justine et al., 2013).

1= Four, as *Neomicrocotyle* (Ramalingam, 1960; Bravo-Hollis & Salgado-Maldonado, 1985; Pulido-Flores, 1997; Aréchiga-Ramos et al., 2023), and *Protomicrocotyle* (Yamaguti, 1953; Bravo-Hollis, 1966; Bravo-Hollis, 1979; Kritsky et al., 2011; Ramírez-Cruz et al., 2023).

2= Six, as *Bilaterocotyle chirocentrosus* (Chauhan, 1945).

3= Eight, as *Gemmaecaputia corrugata* (Kamio & Nitta, 2022), *Metacamopia* (Takemoto et al., 1996; Nguyen et al., 2020), and *Vallisiopsis contorta* (Subhapradha, 1951).

115. Arrangement of the clamps.

0= Without clamps, as *Lethacotyle* (Manter & Prince, 1953; Justine et al., 2013).

1= Unilateral, as *Neomicrocotyle* (Ramalingam, 1960; Bravo-Hollis & Salgado-Maldonado, 1985; Pulido-Flores, 1997; Aréchiga-Ramos et al., 2023), and *Protomicrocotyle* (Yamaguti, 1953; Bravo-Hollis, 1966; Bravo-Hollis, 1979; Kritsky et al., 2011; Ramírez-Cruz et al., 2023).

2= Bilateral, as *Allodiscocotyla* (Unnithan, 1962), *Bilaterocotyle* (Chauhan, 1945), *Bilaterocotyloides*, *Gemmaecaputia* (Kamio & Nitta, 2022), *Metacamopia* (Takemoto et al., 1996; Nguyen et al., 2020), and *Vallisiopsis contorta* (Subhapradha, 1951).

116. Pedunculed clamps.

0= Without clamps, as *Lethacotyle* (Manter & Prince, 1953; Justine et al., 2013).

1= Clamp with muscular peduncle absent, as *Neomicrocotyle* (Ramalingam, 1960; Bravo-Hollis & Salgado-Maldonado, 1985; Pulido-Flores, 1997; Aréchiga-Ramos et al., 2023).

2= Partly sessile clamps, as *Allodiscocotyla chorinemi* (Unnithan, 1962).

2= Clamps with muscular peduncles present, as *Protomicrocotyle* (Yamaguti, 1953; Bravo-Hollis, 1966; Bravo-Hollis, 1979; Kritsky et al., 2011; Ramírez-Cruz et al., 2023), and *Vallisiopsis contorta* (Subhapradha, 1951).

The peduncles are muscular bundles that support the clamps and allow them to be mobile (Bravo-Hollis, 1966). Condition zero refers to clamps not supported by a peduncle, and condition one refers to clamps supported by a peduncle.

117. Haptoral lappet.

0= Absent

1= Present

The haptoral lappet is an extension of the haptor that extends and forms a secondary structure and is generally armed with one to four pairs of hooks of different shapes (hook-like or anchor-like). In the genus *Gemmaecaputia* this structure is not mentioned (Kamio & Nitta, 2022). In other genera, it appears as a small tongue-shaped extension, as in the genera *Allodiscocotyla* (Unnithan, 1962), and *Metacamopia* (Unnithan, 1962; Takemoto et al., 1996; Nguyen et al., 2020), or as an oval extension, as in some genera of the family Protomicrocotylidae (Ramalingam, 1960; Bravo-Hollis, 1966; Bravo-Hollis, 1979; Kritsky et al., 2011).

118. Shape of the haptoral lappet.

0= Absent, as *Gemmaecaputia corrugata* (Kamio & Nitta, 2022).

1= Oval and transversely elongated, as *Protomicrocotyle* (Yamaguti, 1953; Bravo-Hollis, 1966; Bravo-Hollis, 1979; Kritsky et al., 2011; Ramírez-Cruz et al., 2023).

2= Oval and transversely elongated with anchor-shaped lobes at the lateral ends, as *Neomicrocotyle* (Unnithan, 1962).

3= Tongue shaped, as *Allodiscocotyla* (Unnithan, 1962), and *Metacamopia* (Takemoto et al., 1996; Nguyen et al., 2020).

119. Number of pairs of hooks present in the haptoral lappet.

0= Absent, *Gemmaecaputia corrugata* (Kamio & Nitta, 2022).

1= A pair of hooks, as *Allodisoocotyla chorinemi* (Unnithan, 1962), and *Metacamopia lebedevi* (Nguyen et al., 2020).

2= Two pairs of hooks

3= Three pairs of hooks, as *Neomicrocotyle* (Unnithan, 1962), and *Protomicrocotyle* (Yamaguti, 1953; Bravo-Hollis, 1966; Bravo-Hollis, 1979; Kritsky et al., 2011; Ramírez-Cruz et al., 2023).

120. Hook-like“crochet en fléau”.

0= Absent, as *Gemmaecaputia corrugata* (Kamio & Nitta, 2022), and *Protomicrocotyle* (Yamaguti, 1953; Bravo-Hollis, 1966; Bravo-Hollis, 1979; Kritsky et al., 2011; Ramírez-Cruz et al., 2023).

1= Present, as s *Allodiscocotyla* (Unnithan, 1962), and *Metacamopia* (Takemoto et al., 1996; Nguyen et al., 2020).

121. Gastrocotylid-like “crochet en fléau”.

0= Absent, *Gemmaecaputia corrugata* (Kamio & Nitta, 2022)

1= Present, as s *Allodiscocotyla* (Unnithan, 1962), *Metacamopia* (Takemoto et al., 1996; Nguyen et al., 2020), and *Protomicrocotyle* (Yamaguti, 1953; Bravo-Hollis, 1966; Bravo-Hollis, 1979; Kritsky et al., 2011; Ramírez-Cruz et al., 2023).

122. Stubby sclerites.

0= Absent, Absent, as *Gemmaecaputia corrugata* (Kamio & Nitta, 2022), and *Protomicrocotyle* (Yamaguti, 1953; Bravo-Hollis, 1966; 1979; Kritsky et al., 2011; Ramírez-Cruz et al., 2023).

1= Present, as *Allodiscocotyla* (Unnithan, 1962), and *Metacamopia* (Takemoto et al., 1996; Nguyen et al., 2020).

123. Vagina number.

0= One vagina, as *Allodiscocotyla* (Unnithan, 1962), *Gemmaecaputia corrugata* (Kamio & Nitta, 2022), *Neomicrocotyle* (Unnithan, 1962), and *Protomicrocotyle* (Yamaguti, 1953; Bravo-Hollis, 1966; Bravo-Hollis, 1979; Kritsky et al., 2011; Ramírez-Cruz et al., 2023).

1= Two vaginas, as *Metacamopia* (Takemoto et al., 1996; Nguyen et al., 2020).

The vagina is normally found in the anterior region of the body, it can be single or paired and have spines of different sizes or present without these structures (Yamaguti, 1963).

124. Shape of the vagina.

0= Simple vagina, as *Allodiscocotyla* (Unnithan, 1962), *Bilaterocotyle chirocentrosus* (Chauhan, 1945), *Gemmaecaputia corrugata* (Kamio & Nitta, 2022), and *Metacamopia* (Takemoto et al., 1996; Nguyen et al., 2020).

1= Tubular-saccular, as *Protomicrocotyle ivoriensis* (Wahl, 1972).

2= Distal part of vagina armed, forming the vaginal vestibule, as species of *Neomicrocotyle* (Ramalingam, 1960; Bravo-Hollis & Salgado-Maldonado, 1985; Pulido-Flores, 1997; Aréchiga-Ramos et al., 2023), *Lethacotyle* (Manter & Prince, 1953; Justine et al., 2013), and

Protomicrocotyle (Yamaguti, 1953; Bravo-Hollis, 1966; Bravo-Hollis, 1979; Kritsky et al., 2011; Ramírez-Cruz et al., 2023).

125. Vaginal vestibule.

0= Absent, as *Allodiscocotyla* (Unnithan, 1962), *Bilaterocotyle chirocentrosus* (Chauhan, 1945), *Gemmaecaputia corrugata* (Kamio & Nitta, 2022), *Metacamopia* (Takemoto et al., 1996; Nguyen et al., 2020), *Protomicrocotyle ivoriensis* (Wahl, 1972).

1= Present, as *Neomicrocotyle* (Ramalingam, 1960; Bravo-Hollis & Salgado-Maldonado, 1985; Pulido-Flores, 1997; Aréchiga-Ramos et al., 2023), *Lethacotyle* (Manter & Prince, 1953; Justine et al., 2013), and *Protomicrocotyle* (Yamaguti, 1953; Bravo-Hollis, 1966; Bravo-Hollis, 1979; Kritsky et al., 2011; Ramírez-Cruz et al., 2023).

Refers to the presence of sclerotized spines, whose arrangement, shape, size, and number can be variable between genera and species (Bychowsky, 1961; Yamaguti, 1963; Buchmann & Bresciani, 2006b).

126. Shape of the vaginal vestibule.

0= Absent, as *Allodiscocotyla* (Unnithan, 1962), *Bilaterocotyle chirocentrosus* (Chauhan, 1945), *Gemmaecaputia corrugata* (Kamio & Nitta, 2022), *Metacamopia* (Takemoto et al., 1996; Nguyen et al., 2020), *Protomicrocotyle ivoriensis* (Wahl, 1972).

1= Cone-shaped, as *Lethacotyle vera* (Justine et al., 2013).

2= Oval-Globular, as *Bilaterocotyloides, Lethacotyle, Neomicrocotyle, Protomicrocotyle* (Chauhan, 1945; Ramalingam, 1960; Yamaguti, 1963; Bravo-Hollis, 1966; Young, 1968; Bravo-Hollis, 1979; Al-Zubaidy, 2013; Justine et al., 2013).

127. Shape of the spines of the vaginal vestibule.

0= Absent

1= Elongated and have a rounded apical end with small projections, as *Protomicrocotyle nayaritensis* (Bravo-Hollis, 1979), and *P. manteri* (Bravo-Hollis, 1966).

2= Elongated and have a flat apical end with small projections, as *P. mirabilis* (Kritsky et al., 2011).

The spines of the vaginal vestibule can be mostly elongated and have a rounded apical end with small projections, as in *P. manteri* (Bravo-Hollis, 1966), or elongated and have a flat apical end with small projections, as in *P. mirabilis* (Kritsky et al., 2011).

128. Question mark-shaped ovary.

0= Absent

1= Present in *M. levedevi* (Nguyen et al., 2020).

129. Oval ovary

0= Absent, as *Allodiscocotyla* (Unnithan, 1962), and *Metacamopia* (Takemoto et al., 1996; Nguyen et al., 2020).

1= Present, as *Gemmaecaputia corrugata* (Kamio & Nitta, 2022), *P. mirabilis*, , *Protomicrocotyle* sp. 3, *Protomicrocotyle* sp. 4.

130. Lobed Ovary.

0= Absent, as *Allodiscocotyla* (Unnithan, 1962), and *Metacamopia* (Takemoto et al., 1996; Nguyen et al., 2020).

1= Present, *Lethacotyle vera* (Justine et al., 2013), *P. veracruzensis*.

131. U-shaped ovary.

0= Absent

1= Present, as *Protomicrocotyle mannarensis*, *P. madrasensis*, and *P. minutum* (Ramalingam, 1960), *P. manteri* (Bravo-Hollis, 1966).

132. U inverted shaped ovary.

0= Absent

1= Present, as *Neomicrocotyle carangis* (Pillai & Pillai, 1978), and *Protomicrocotyle ivoriensis* (Wahl, 1972).

Monogeneans have a single ovary, which can be located anterior or posterior to the testicles and can present different shapes (Yamaguti, 1963; Buchmann & Bresciani, 2006b). The Characters 40, 41, 42, and 43 are based on the proposal of (Boeger & Kritsky, 1993).

133. Cirrus of the male copulatory organ (MCO).

0= Unarmed, as species of Protomicrocotylidae family (Koratha, 1955a; Ramalingam, 1960; Bravo-Hollis, 1966; Bravo-Hollis, 1979; Kritsky et al., 2011; Ramírez-Cruz et al., 2023).

1= Armed, as *Allodiscocotyla chorinemi* (Unnithan, 1962), *M. lebedevi* (Nguyen et al., 2020), and *Metacamopia oligoplites* (Takemoto et al., 1996).

The male copulatory organ (MCO) in monogeneans has the function of ensuring the transfer of sperm from one monogenean to another. This structure is very diverse and complex (Kearn & Whittington, 2015). It is made up of the copulatory organ that, together with the uterus (in

polyopisthocotyleans), opens into a common muscular genital atrium, which can be armed with a great variety of hooks & spines of different sizes or unarmed.

The MCO can only be a cirrus armed with a variable number of spines, as in *Metacamopia oligoplites* (Takemoto et al., 1996), *M. lebedevi* (Nguyen et al., 2020), and *Allodiscocotyla chorinemi* (Unnithan, 1962) or it may be an unarmed cirrus embedded in a cup-shaped or tubular structure with armor of hooks or spines in the apical region. The hooks of the genital bulb form a ring with their tips directed inwards and located in the genital atrium (Rohde, 1975), as in members of the Protomicrocotylidae family (Koratha, 1955a; Ramalingam, 1960; Bravo-Hollis, 1966; Bravo-Hollis, 1979; Kritsky et al., 2011; Ramírez-Cruz et al., 2023).

134. Shape of the cirrus.

0= A simple cirrus duct and a bulb armed with spines are formed in the distal portion, as *Neomicrocotyle* (Ramalingam, 1960; Bravo-Hollis & Salgado-Maldonado, 1985; Pulido-Flores, 1997; Aréchiga-Ramos et al., 2023), *Lethacotyle* (Manter & Prince, 1953; Justine et al., 2013), and *Protomicrocotyle* (Yamaguti, 1953; Bravo-Hollis, 1966; Bravo-Hollis, 1979; Kritsky et al., 2011; Ramírez-Cruz et al., 2023).

1= Elongate to fusiform, as *Allodiscocotyla chorinemi* (Unnithan, 1962), *M. lebedevi* (Nguyen et al., 2020), and *Metacamopia oligoplites* (Takemoto et al., 1996).

135. Shape of distal portion of cirrus.

0= Absent

1=Tubular shape, *Bilaterocotyloides*

2= Elongate to fusiform, as *Gemmaecaputia corrugata* (Kamio & Nitta, 2022).

3= Cup-shaped, as *Lethacotyle* (Manter & Prince, 1953; Justine et al., 2013), *Neomicrocotyle* (Ramalingam, 1960; Bravo-Hollis & Salgado-Maldonado, 1985; Pulido-Flores, 1997; Aréchiga-Ramos et al., 2023), *Protomicrocotyle* (Yamaguti, 1953; Bravo-Hollis, 1966; Bravo-Hollis, 1979; Kritsky et al., 2011; Ramírez-Cruz et al., 2023).

136. Shape of the spines distal portion of cirrus.

0= Absent.

1= Sickle-shaped, *Neomicrocotyle* (Ramalingam, 1960; Bravo-Hollis & Salgado-Maldonado, 1985; Pulido-Flores, 1997; Aréchiga-Ramos et al., 2023), and *Protomicrocotyle* (Yamaguti, 1953; Bravo-Hollis, 1966; Bravo-Hollis, 1979; Kritsky et al., 2011; Ramírez-Cruz et al., 2023).

137. Position of the testes.

0= Anterior to ovary, as *Allodiscocotyla* (Unnithan, 1962; Nguyen et al., 2020), *M. lebedevi* (Nguyen et al., 2020), *Neomicrocotyle* (Ramalingam, 1960; Bravo-Hollis & Salgado-Maldonado, 1985; Pulido-Flores, 1997; Aréchiga-Ramos et al., 2023), and *Protomicrocotyle* (Yamaguti, 1953; Bravo-Hollis, 1966; Bravo-Hollis, 1979; Kritsky et al., 2011; Ramírez-Cruz et al., 2023).

1= In a position anterior and posterior to the ovary (both sides), as *Gemmaecaputia corrugata* (Kamio & Nitta, 2022), *Metacamopia oligoplites* (Takemoto et al., 1996), and *Youngiopsis australis* (Young, 1968).

The testes are found in the middle region of the body, they are generally intercecal and their position with respect to the oviduct and ovary can vary, being anterior to these anatomical structures, posterior to them or found above and below them.

138. Shape of the testes.

0= Broadly elliptoid (globular), as *Protomicrocotyle mirabilis* (Kritsky et al., 2011; Ramírez-Cruz et al., 2023), *Metacamopia lebedevi* (Nguyen et al., 2020).

1= Sub-globular (irregular shape), as *Gemmaecaputia corrugata* (Kamio & Nitta, 2022)

2= Depressed elliptoid, the testes are wider than long, as *Protomicrocotyle manteri* (Bravo-Hollis, 1966), *P. veracruzensis* (Ramírez-Cruz et al., 2023).

139. Arrangement of the testes.

0= One field, as *Neomicrocotyle* (Ramalingam, 1960).

1= Two fields well defined, with one row in each field, as *Protomicrocotyle mirabilis* (Kritsky et al., 2011; Ramírez-Cruz et al., 2023), *Metacamopia lebedevi* (Nguyen et al., 2020).

2= Two fields, each field with one or two interleaved rows, forming an irregular column, as *P. veracruzensis* (Ramírez-Cruz et al., 2023).

The arrangement of the testes can be found as a single field as in *Neomicrocotyle* (Ramalingam, 1960), in two fields, forming two well-defined longitudinal rows as in *Protomicrocotyle mirabilis* (Kritsky et al., 2011), or with two longitudinal rows with two testes in each row in some parts of the testicular arrangement (Yamaguti, 1953; Ramírez-Cruz et al., 2023).

140. Extension of the vitelline follicles.

0= The follicles extending from level of intestinal bifurcation to posterior end, without reaching the haptor (Justine et al., 2013).

1= The follicles extending from level of intestinal bifurcation to haptor (Chauhan, 1945; Yamaguti, 1953).

Morphology, molecular and total evidence analyses

Morphology

Phylogenetic analysis with continuous and discrete morphological data resulted in two trees with a CI of 0.706 and an RI of 0.852 with 287 steps (Figure 3.4). The topology of the tree shows that the genus *Protomicrocotyle* is polyphyletic, although this result could be mainly due to the lack of continuous data, since taxa such as *P. ivoriensis*, *P. carangis*, *P. celebensis*, *P. minutum*, *P. madrasensis* and *P. mannarensis* do not have full information from these data, as well as other taxa included in the analysis (Figure 3.4).

Based on the results of the morphological analysis, *Neomicrocotyle pacifica* is shown as the sister group of *Protomicrocotyle* (the taxa with the complete data), with *P. nayaritensis* as an independent lineage (Figure 3.4). In the cladogram, the formation of two large groups of *Protomicrocotyle* can be observed (Figure 3.4). The first group is made up of the species *Protomicrocotyle mirabilis*, *P. manteri*, and the morphotypes *Protomicrocotyle* **sp. 2**, *Protomicrocotyle* **sp. 3**, and *Protomicrocotyle* **sp. 5**. The second group is made up of *P. veracruzensis* and the morphotypes *Protomicrocotyle* **sp. 1** and *Protomicrocotyle* **sp. 4** (Figure 3.4).

The oval ovary shape is the synapomorphy that supports clade I (Character 129). In this clade, the species *P. mirabilis* is identified as a lineage that is supported by synapomorphies. These include the presence of transverse striae, which are distributed from the region of the germarium to the region of the posterior end of the haptor (104, 2), as well as the arrangement of the testes (140, 2). The *Protomicrocotyle* **sp. 3** is corroborated by the presence of the lobed ovary (130, 2) and the arrangement of the testes (138, 2). These taxa also exhibit the following synapomorphies: the shape of the transverse striae (105, 1) and the shape of the spines of the vaginal vestibule (127, 2) (Figure 3.5). *Protomicrocotyle* **sp. 5** represents a polytomy within clade I and exhibits synapomorphies including the shape of the body (92, 0), the position of the oral opening (95, 1), and the shape of the testes (138, 2).

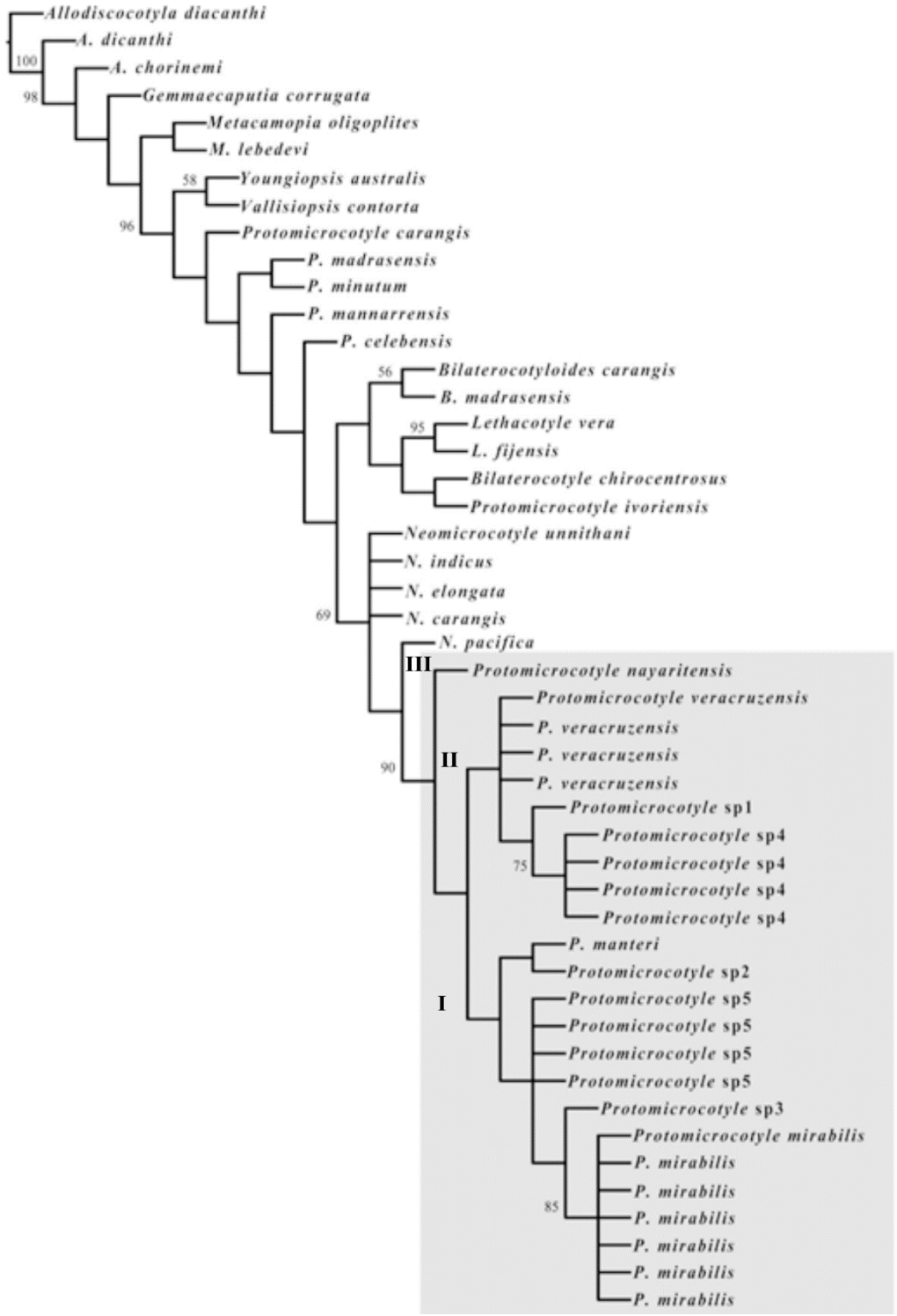

Figure 3. 4. Strict consensus cladogram of MP obtained of 2 trees with morphological data for representatives of the Protomicrocotylidae family. The numbers I, II, and III are used to correspond with the three distinct clades of *Protomicrocotyle*. CI: 0.706, RI: 0.852, 287 steps. Shaded area represents the species of *Protomicrocotyle* from Mexico. The bootstrap values are displayed; values less than 50 are not included in the cladogram.

The second group within Clade I comprises the species *P. manteri* and *Protomicrocotyle* **sp. 2**. Both taxa exhibit the synapomorphy of a prohaptor with a retractile muscular glandular organ (96, 1). The synapomorphies that support *Protomicrocotyle* **sp. 2** are as follows: the haptor differentiates from the rest of the body (107, 0), the shape of the haptoral lappet (118, 1) and the extension of the vitellogenous glands (140, 1). In *P. manteri*, the synapomorphies are as follows: the width of the central hook (69), the ovary is lobuled and in U-shaped (130, 0 and 131, 1) (Figure 3.5).

Clade II is comprised of *P. veracruzensis*, *Protomicrocotyle* **sp. 1**, and *Protomicrocotyle* **sp. 4**. The three taxa in question exhibit a synapomorphy in the arrangement of their testes (139, 2). The following synapomorphies support the classification of *Protomicrocotyle* **sp. 4**: the arrangement of the testes (139, 2) and the extension of the vitellogenous glands (140, 0). The following synapomorphies support *Protomicrocotyle* **sp. 1**: width of the internal root of the right middle hook (52), width of the internal root of the left middle hook (61), prohaptor with retractile muscular glandular organ (96, 1), and shape of the testicles (138, 1).

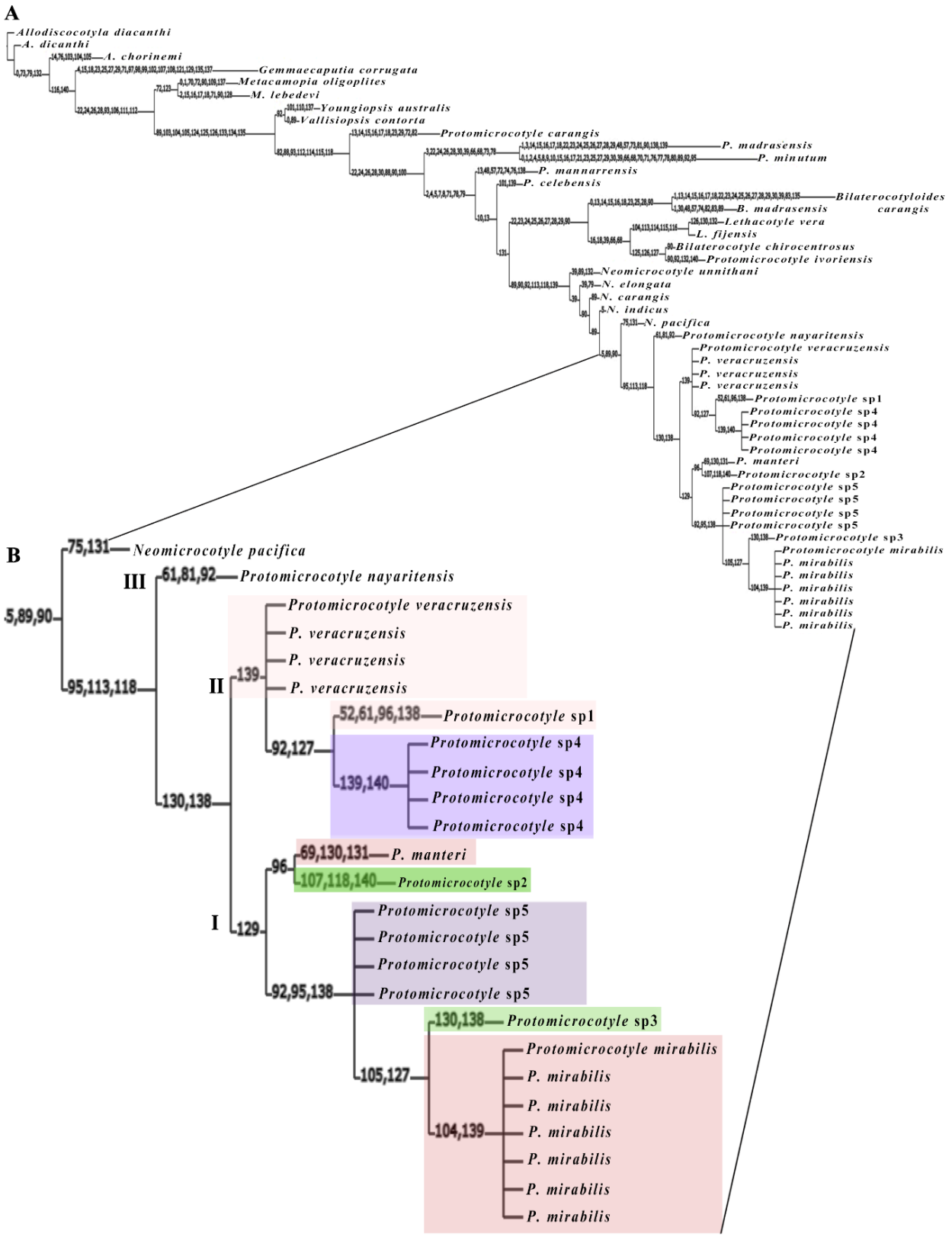

Figure 3. 5. A. The cladogram illustrates the synapomorphies observed within each of the identified clades. **B.** The numbers I, II, and III are used to correspond with the three distinct clades of *Protomicrocotyle*. The shaded area represents the terminal lineages.

Molecular

The phylogenetic analysis of the molecular data with the matrix combined of CO1, 28S and 18S genetic data resulted in a total of 13 trees, with a CI of 0.908 and an RI of 0.981 with 1300 steps. The topology of the tree demonstrates that the *Protomicrocotyle* group is not monophyletic (Figure 3.6). *Protomicrocotyle* morphotypes have been shown to constitute a group that is independent of the *P. mirabilis* and *P. veracruzensis* species (Figure 3.6). However, the independent analysis of each of the molecular markers indicates that they are monophyletic (Figures 3.1, 3.2).

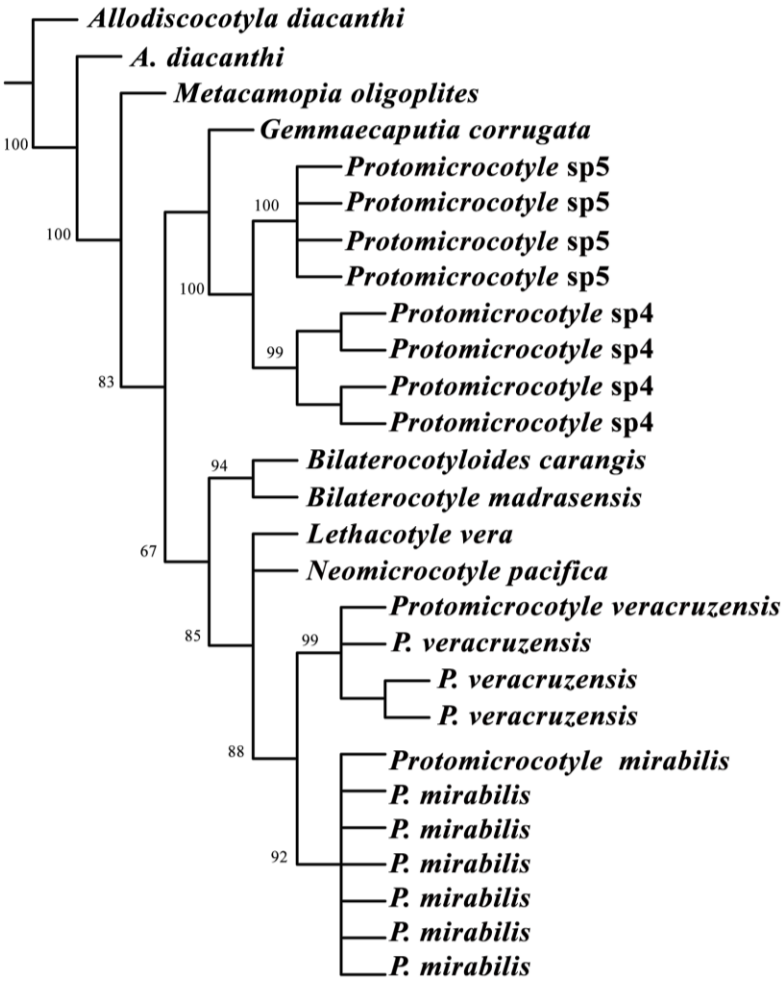

Figure 3. 6. Strict consensus cladogram of MP obtained of 14 trees with molecular data for representatives of the Protomicrocotylidae family. CI: 0.908, RI: 0.981, 1300 steps. The bootstrap values are displayed; values less than 50 are not included in the cladogram.

Total Evidence

The analysis of the morphological data combined with the molecular data resulted in 4 trees with a CI of 0.766 and an RI of 0.846 with a total of 769 steps (Figure 3.7). The topology of the cladogram shows that the genus *Protomicrocotyle* from Mexico is monophyletic, since the species such as *P. madrasensis*, *P. minutum*, *P. celebensis* and *P. ivoriensis* are not resolved. As with the analysis of the morphological data, the topology of the tree shows *Neomicrocotyle pacifica* as sister group to *Protomicrocotyle*. The first group is formed by *P. mirabilis* and *Protomicrocotyle* **sp. 3**. The second group is made up of *P. veracruzensis*, *P. manteri*, *P. nayaritensis* and *Protomicrocotyle* **sp. 2**. The third group is made up of *Protomicrocotyle* **sp. 4** and *Protomicrocotyle* **sp. 5** both morphotypes correspond to the same species host and locality (Figure 3.7).

Figure 3.8 illustrates the morphological and molecular synapomorphies that substantiate the formation of each group. The number of spines in the male copulatory organ (89) and the number of testes (90) are synapomorphies that maintain *N. pacifica* as the sister group of the species of *Protomicrocotyle* from Mexico. The monophyly of the species *Protomicrocotyle* from Mexico is supported by morphological synapomorphies, including the body shape (92), the gastrocotylid-type clamp (113), and the shape of the haptoral lappet, which is oval and transversely elongated (118). Additionally, molecular character 1042 provides further evidence for this grouping.

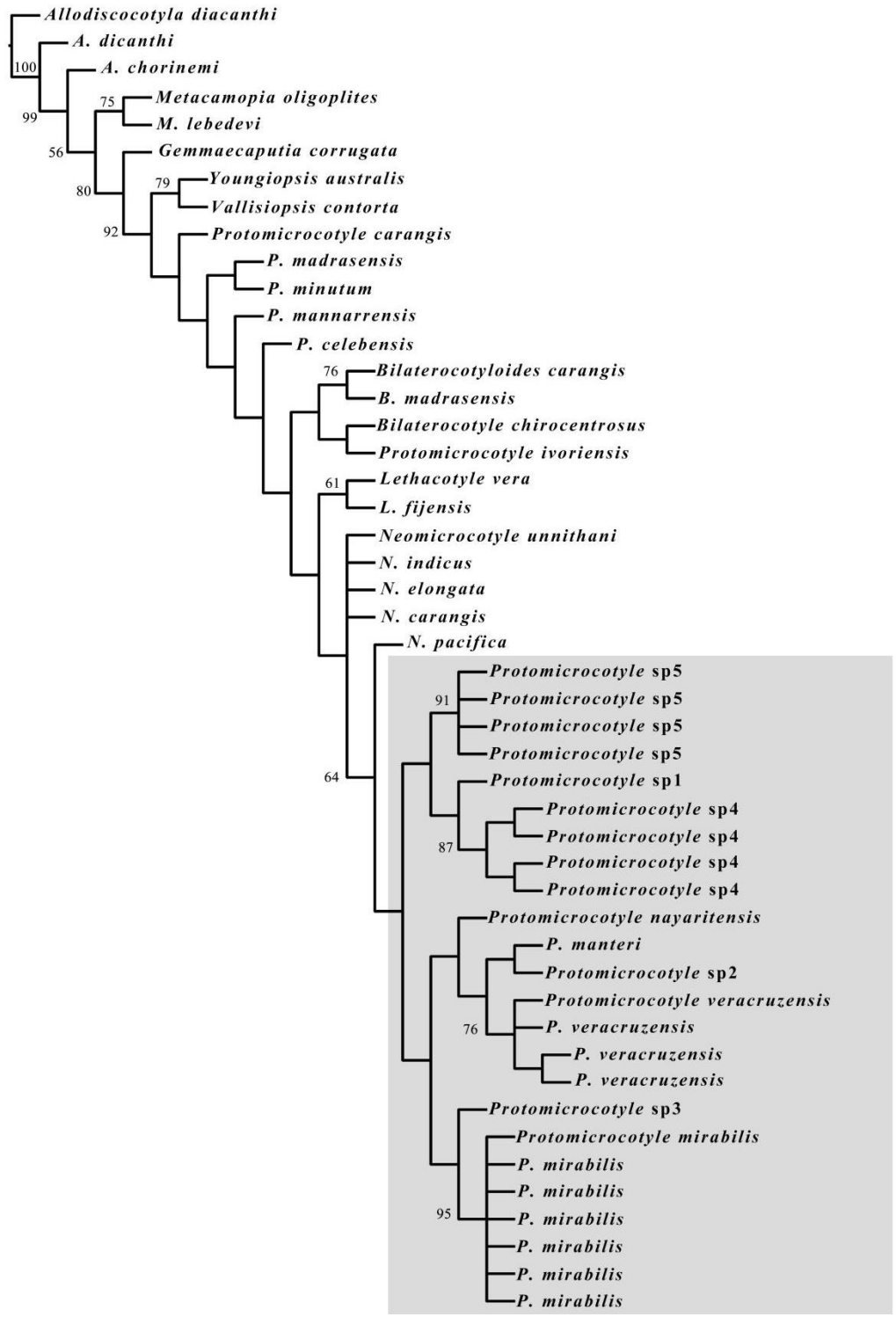

Figure 3. 7. Strict consensus cladogram obtained of 4 trees with morphological and molecular data for representatives of the Protomicrocotylidae family. CI: 0.766, RI; 0.846, 769 steps. Shaded area represents the species of *Protomicrocotyle* from Mexico. The bootstrap values are displayed; values less than 50 are not included in the cladogram.

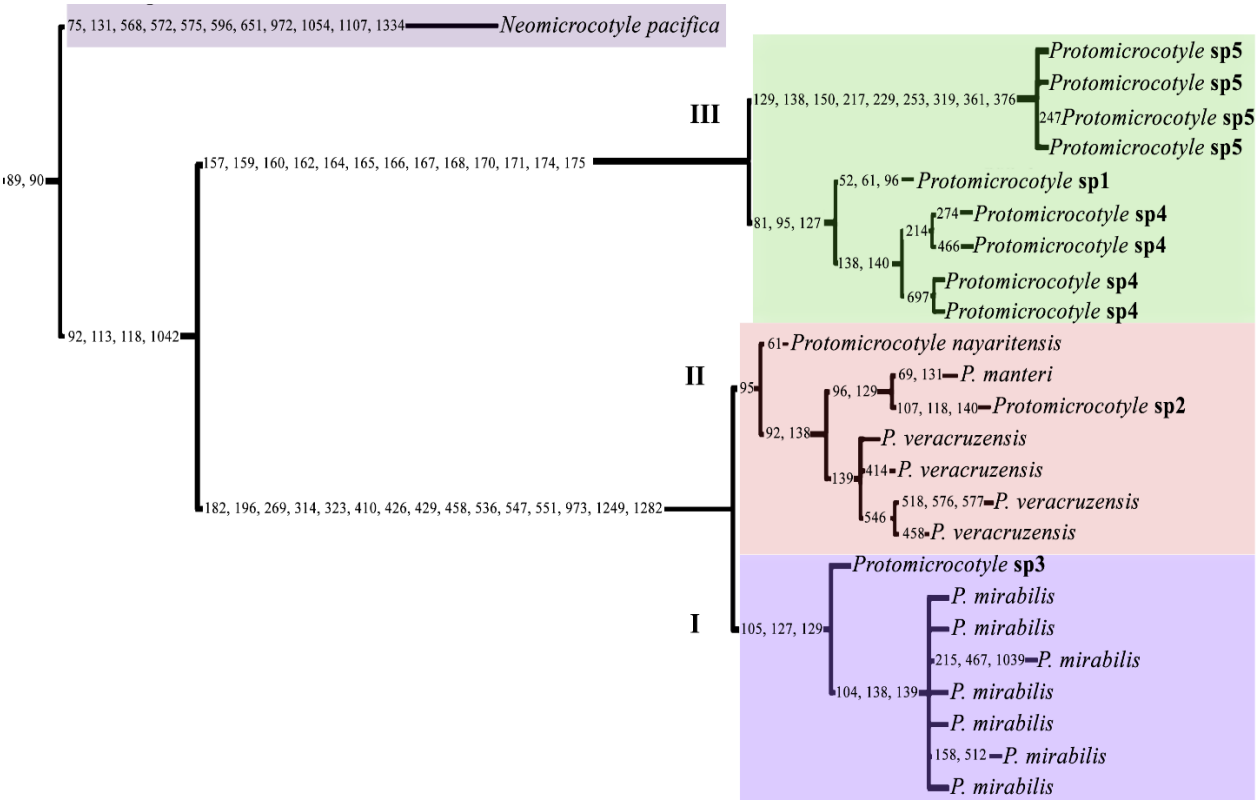

Figure 3. 8. Morphological and molecular synapomorphies observed within each of the identified clades in the cladogram on MP for total evidence. The numbers I, II, and III are used to correspond with the three distinct clades of *Protomicrocotyle*. Morphological synapomorphies are values less than 140. Values from 141 onwards correspond to molecular synapomorphies. The shaded area represents the terminal lineages.

4. Discussion

Monogeneans represent the second most diverse group within Platyhelminthes in Mexico (García-Prieto et al., 2014; Mendoza-Garfias et al., 2017). A number of phylogenetic studies have been conducted using morphological characters (Justine, 1991a; Boeger & Kritsky, 1993; 2001; Zamparo et al., 2001). An examination of the morphology and partial 18S and 28S sequences indicates that the class is a non-monophyletic group (Justine, 1991a; Justine, 1991b; Mollaret et al., 1997; Brabec et al., 2023). Brabec et al. (2023) present two distinct topologies of the phylogenetic relationships of Platyhelminthes, proposing Monopisthocotylea and Polyopisthocotylea at the class level.

At the family level, various studies have addressed the phylogenetic relationships, diversity, and intraspecific variability of monogenean species using morphological and molecular characters (Blasco-Costa et al., 2012; Mendoza-Palmero et al., 2015; Tambireddy et al., 2016; Moreira et al., 2019; Morsy et al., 2021; Nejat et al., 2023). As previously noted, the findings offer insights into the relationships between parasites and their hosts, which are valuable for understanding biogeography and host-parasite coevolution across diverse biogeographic regions (Moreira et al., 2019). In the case of the order Mazocraeidea, which comprises 29 families (WoRMS, 2024), different species have been integrated into phylogenetic studies using morphological characters (Boeger & Kritsky, 1993; 2001), included species of the superfamily Protomicrocotyloidea Unnithan 1962, which comprises the Allodiscocotylidae, Chauhaneidae, and Protomicrocotylidae was proposed as monophyletic (Boeger & Kritsky, 1993; 2001). With regard to the Protomicrocotylidae family, there is a paucity of systematic work that analyzes the phylogenetic relationships of all the taxa that comprise it. The studies conducted by (Boeger & Kritsky, 1993; 2001) integrate data exclusively from the genus *Neomicrocotyle*.

In recent studies, molecular data analysis has been employed to ascertain the phylogenetic relationships of certain taxa that constitute the order Mazocraeidea (Tambireddy et al., 2016). In the aforementioned study, a number of taxa from various families were subjected to analysis. From the Protomicrocotylidae family, partial sequences of *Bilaterocotyloides*, *Neomicrocotyle pacifica*, *Lethacotyle vera*, and *Neomicrocotyle* sp. from CO1 and 28s were incorporated and examined using the Neighbor-joining, maximum parsimony, maximum likelihood, and Bayesian inference methods. The resulting topologies indicate that the Protomicrocotylidae family is monophyletic with respect to the other taxa within the order under analysis. Allodiscocotylidae family

representing a sister group. Other studies, such as those conducted by (Kamio & Nitta, 2022) and (Kamio et al., 2023), have demonstrated phylogenetic analyses in which species of Protomicrocotylidae are integrated, thereby substantiating the monophyly of the family and the classification of Allodiscocotylidae as a sister group. Consequently, the outgroup selected for the present study was that of taxa of Allodiscocotylidae.

In this study, a comprehensive examination of the morphology of *Protomicrocotyle* was conducted, and novel partial sequences of CO1, 28S, and 18S were generated for species within this genus for the first time. The partial sequences of each gene were employed in the genetic distance analyses and for the analysis with the NJ phylogenetic inference method based on genetic distances. The newly generated sequences enabled the addition of new information regarding the intra- and interspecific variation of this group of monogeneans. The degree of intraspecific variation observed with CO1 among *P. mirabilis* (0.74% to 1.28%), *P. veracruzensis* (0.50%), *Protomicrocotyle* **sp. 4** (0.48%), and *Protomicrocotyle* **sp. 5** (0.25%) (Table 3.3), is considered to be relatively low in comparison to that observed within microcotylid species (Mladineo et al., 2009), and mazocreids (5.6%) (Yan et al., 2016). The degree of interspecific variation between *P. mirabilis* and *P. veracruzensis* (9.21% to 9.63) is high, while *P. veracruzensis* and *Protomicrocotyle* **sp. 5** (4.73% to 5.20%) is medium low. In contrast, the degree of variation between *P. mirabilis* and *Protomicrocotyle* **sp. 4** (1.98% to 2.81) is relatively low (Table 3.3). Furthermore, lower levels of interspecific variation have been reported between species of *Microcotyle*, with figures of 0.7% (Lablack et al., 2022), 4.5% (Ayadi et al., 2017; Víllora-Montero et al., 2020), 6.1% (Ono et al., 2020). The phenogram of NJ with CO1 revealed that the genetic sequences of *P. mirabilis*, *P. veracruzensis, Protomicrocotyle* **sp. 4**, and *Protomicrocotyle* **sp. 5** form a cluster with high statistical support (Figure 3.1). However, the relationships between the species of *Bilaterocotyle* and *Bilaterocotyloides* remain poorly resolved (Figure 3.1). In (Kamio & Nitta, 2022), the partial sequence of *Gemmaecaputia corrugata* appears to be the most closely related to the *Protomicrocotyle* sequences, and the phylogenetic tree presented indicates that *G. corrugata* and *Pseudochauhanea macrorchis* (Chauhaneidae) form a sister group and the species of Allodiscocotylidae are separated into two groups. Consequently, the interrelationships among the taxa in the families Allodiscocotylidae, Chauhaneidae, and Protomicrocotylidae remain unresolved when the CO1 partial sequences are utilized alone. It would therefore be advisable to incorporate additional data and undertake a phylogenetic analysis of these families.

At the species level, the utilization of a fragment of the CO1 (650 bp) has been proposed as a universal marker or DNA barcode for the global biological identification of animal species (Hebert et al., 2003; Ratnasingham & Hebert, 2007; Antil et al., 2023). The CO1 genes are highly conserved, typically haploid, devoid of introns, and exhibit limited recombination. They are also less susceptible to insertions, deletions, or other large-scale rearrangements that introduce more ambiguous variation in the sequence. The mitochondrial genome evolves at a faster rate than the nuclear genome. Consequently, mitochondrial genome sequences are more informative for the differentiation or distinction of closely related species (Hebert et al., 2003; Mueller, 2006). These characteristics have led to its use as a marker in a variety of biological groups, including helminths (Ferri et al., 2009; Prosser et al., 2013; Nyman et al., 2021; Thaenkham et al., 2022b). The available CO1 sequence information for Mazocraeidea species is insufficient, necessitating the accumulation of molecular studies for the purpose of phylogenetic analysis with morphological and molecular data (Tambireddy et al., 2016).

Various fragments of the nuclear ribosomal DNA, like the genes for 18S, 5.8S and 28S rRNA, and the internal transcribed spacers ITS-1 and ITS-2, evolve at different rates, making them suitable for assessing genetic divergence at various levels (Hillis & Dixon, 1991; Vanhove et al., 2013). In monogeneans, various portions of the rDNA are considered to adequately mirror differences between morphologically recognized species (Vanhove et al., 2013). They are also useful in identifying cryptic species (Pouyaud et al., 2006; Ondračková et al., 2020). As a consequence, these sequence fragments are often included in species descriptions and redescriptions (Morsy et al., 2021; Rothman et al., 2022; Kamio et al., 2023; Pinacho-Pinacho et al., 2023; Ramírez-Cruz et al., 2023). In the Protomicrocotylidae family, only limited data is available for the 28S and 18S markers, specifically for *Bilaterocotyle madrasensis*, *Bilaterocotyloides carangis*, *Neomicrocotyle pacifica*, *Lethacotyle vera*, *Protomicrocotyle mirabilis*, *P. veracruzensis*, *Neomicrocotyle* sp., *Protomicrocotyle* **sp. 4**, and *Protomicrocotyle* **sp. 5**. Information on *Protomicrocotyle* species was produced as a result of this research study.

Intraspecific variation with 28S of *P. mirabilis* was 0% to 0.41%, *P. veracruzensis* were 0%, *Protomicrocotyle* **sp. 4** was 0% to 0.13%, and *Protomicrocotyle* was 0% (Table 3.2). As demonstrated by Ramírez-Cruz et al. (2023), the variation of *P. mirabilis* was 0% to 0.14. In the present study, with the addition of new sequences, the variation increases to 0.41% (Table 3.4). As illustrated in the 28S phenogram (Figure 3.4), the sequences identified as *P. mirabilis*, based

on the morphological characteristics of the species (Kritsky et al., 2011; Ramírez-Cruz et al., 2023), are the sequences were grouped in a pectinate manner, though with low support values (Figure 3.2). The sequences identified as *Protomicrocotyle* **sp. 4** were found at the top of the phenogram, forming a group separated from the *P. mirabilis* sequences with a high support value (Figure 3. 2).

Comparative sequence analyses of the species of Protomicrocotylidae showed that both partial CO1, 28S and 18S genes can be successfully used for species identification (Rahmouni et al., 2017; Torres-Carrera et al., 2020; Pinacho-Pinacho et al., 2021; Zago et al., 2021; Kamio et al., 2023; Ramírez-Cruz et al., 2023) and for phylogenetic inference (Justine et al., 2013; Tambireddy et al., 2016; Kamio & Nitta, 2022). Nevertheless, the number of sequences accessible for representatives of the family remains relatively limited, despite the recent surge in the utilization of CO1 DNA data for the identification and discovery of monogenic species. Consequently, it is imperative to persist in the generation of molecular data that is supplementary to morphological information in the description of novel species and for phylogenetic studies.

This study generated the first phylogenetic hypothesis for Protomicrocotylidae based on morphological and molecular data and a combined total evidence data matrix. The strict consensus cladograms of morphological and total evidence data indicate that the *Protomicrocotyle* species from Mexico constitute a monophyletic group, with *Neomicrocotyle pacifica* identified as a sister group (Figures 3.4, 3.7). However, the topology of the clades that comprise the *Protomicrocotyle* groups differs when the morphological (Figure 3.4) and total evidence data (Figure 3.7) are considered. The support values obtained with the morphology cladogram for this set of species of *Protomicrocotyle* are 90. However, only the clades comprising *P. mirabilis* and *Protomicrocotyle* **sp. 4** exhibit a high support value, at 75 and 85, respectively. The remaining *Protomicrocotyle* clades do not demonstrate a high support value (Figure 3.4).

In the strict consensus cladogram of total evidence, the statistical support for *Protomicrocotyle* from Mexico is 64 (Figure 3.7). The internal clades that maintain a high support value are *P. mirabilis* (95), *P. veracruzensis* (76), *Protomicrocotyle* **sp. 4** (87), and *Protomicrocotyle* **sp. 5** (91) (Figure 3.7). The synapomorphies that support the monophyly of *Protomicrocotyle* are the body shape (92, 1, 2), the gastrocotylid-type clamp (113, 2), and the shape of the haptoral lappet, which is oval and transversely elongated (118, 1). With respect to the aforementioned synapomorphies, the clamps are an important and characteristic part of the

anatomy of polyopisthocotylean monogeneans, and are clearly the main organ used for attachment to the host (Bychowsky, 1961; Yamaguti, 1963; Lebedev, 1986). The classification of polyopisthocotylean monogeneans is mainly based on clamp structure (Lebedev, 1986). The two distinct clamp types: the first, a gastrocotylid clamp, was observed to possess an additional sclerite, while the second, a microcotylid type, lacked this sclerite (Lebedev, 1986; Justine et al., 2013), but protomicrocotylids are unique in that this structure changes relative to each genus within the family: *Protomicrocotyle* has clamps of the gastrocotylid-type (Koratha, 1955a; Caballero y Caballero & Bravo-Hollis, 1965a; Bravo-Hollis, 1966; Wahl, 1972; Bravo-Hollis, 1979; Kritsky et al., 2011; Ramírez-Cruz et al., 2023), and *Neomicrocotyle* has clamps of the microcotylid type (Ramalingam, 1960; Lebedev, 1986; Pulido-Flores, 1997; Aréchiga-Ramos et al., 2023).

The topology of the total evidence cladogram (Figure 3.8) demonstrates that *Protomicrocotyle* from Mexico is divided into three groups: clade I of *P.* mirabilis and *Protomicrocotyle* **sp. 3**, which form independent lineages; and clade II of *P. veracruzensis* and *P. manteri*. The topology of the total evidence cladogram demonstrates that *P. manteri* and *Protomicrocotyle* **sp. 2** are shown as sister groups of *P. veracruzensis*, and in turn, *P. nayaritensis* is shown as a sister group of them (Figure 3.8). This clade encompasses species distributed in the Pacific Ocean (*P. manteri* and *P. nayaritensis*) and the Gulf of Mexico (*P. veracruzensis* and *Protomicrocotyle* **sp. 2**). In this context, *P. nayaritensis* is identified as the sister group of *P. manteri*, *Protomicrocotyle* **sp. 2**, and *P. veracruzensis*. Clade III is comprised of the morphotypes of *Protomicrocotyle* **sp. 4**, *Protomicrocotyle* **sp. 1**, and *Protomicrocotyle* **sp. 5** (Figure 3.8).

Clades I, II, and III are distinguished by molecular synapomorphies, while the terminal lineages exhibit both morphological and molecular synapomorphies (Figure 3.8). Clade I is characterized by the presence of synapomorphies, including scale-shaped transverse striae (105, 1), spines elongated and rounded apical ends with small projections (127, 2), and an oval-shaped ovary (129, 1). This clade comprises the terminal lineages of *P. mirabilis* and the *Protomicrocotyle* **sp. 4** morphotype. The *P. mirabilis* lineage, in turn, presents autapomorphies such as transverse striae with distribution from the region of the germarium to the region of the posterior end of the haptor (104, 2). The testes are broadly elliptoid (globular) in shape (138, 0) and arranged in two distinct fields, with one row in each (139, 1) (Figure 3.8).

In clade II, the position of the oral opening as terminal (95, 1) is the synapomorphy that groups *P. veracruzensis*, *Protomicrocotyle* **sp. 2**, *P. manteri*, and *P. nayaritensis* (Figure 3.8). *Protomicrocotyle veracruzensis*, *Protomicrocotyle* **sp. 2**, and *P. manteri* conform a clade that exhibit synapomorphies, such a fusiform-broadly shaped body (92, 1) and a depressed elliptoid stem of the testes (138, 2). Clade III presents molecular synapomorphies, and in the terminal lineages, morphological and molecular synapomorphies are presented (Figure 3.8), which provide additional information that will contribute to the future description of these *Protomicrocotyle* morphotypes.

With regard to the group of species originating from Mexico, further molecular data is necessary for *P. manteri* and *P. nayaritensis*, as they are species distributed in the Pacific Ocean (Bravo-Hollis, 1966; Bravo-Hollis, 1979). Therefore, biological material is required to obtain DNA sequences. Previously, *Protomicrocotyle* **sp. 1**, *Protomicrocotyle* **sp. 2**, and *Protomicrocotyle* **sp. 3** had been identified as *P. mirabilis* (*Protomicrocotyle* **sp. 1**) and *P. manteri* (*Protomicrocotyle* **sp. 2** and *Protomicrocotyle* **sp. 3**) in certain localities within the Gulf of Mexico (Caballero y Caballero & Bravo-Hollis, 1965a). However, following a comprehensive examination of their morphology, it was determined that they should be classified as distinct morphotypes of *Protomicrocotyle*. Consequently, it is imperative to conduct sampling in the localities of Sontecomapan and Jicacal in the state of Veracruz and Isla Mujeres in the state of Quintana Roo to procure fresh material and process it to obtain genetic sequences.

Phylogenetic trees provide an indirect record of the speciation events that led to present-day species. By constructing species-level phylogenies and comparing the geographical distribution of sister taxa, the relative contribution of the different speciation modes can be inferred (Barraclough & Nee, 2001). Speciation is the evolutionary process through which populations evolve to become distinct species, producing morphologically distinct species (Mayden, 1999). There are three recognized basic modes of speciation: allopatric, sympatric, and parapatric (Morrone, 2013; Thaenkham et al., 2022a). The sympatric speciation refers to the formation of new species in the absence of either a geographical or a physical barrier that isolates the population of the ancestral species. Sympatric speciation occurs within the range of an ancestral species where geography does not play a causal role in species formation (Wiley and Lieberman, 2011; Morrone, 2013).

The total evidence phylogenetic hypothesis posits that the evolutionary pattern of *Protomicrocotyle* in the oceans of Mexico is occurring through sympatric speciation by colonization of a new host. This is exemplified by *P. mirabilis*, which is found in *C. hippos*, and its morphotype *Protomicrocotyle* **sp. 3**, which is parasitizing *C. latus*. However, it is necessary to integrate molecular and biogeographic information of this morphotype to corroborate this hypothesis. The process of speciation by host change is one of the most predominant documented processes in parasites (Price, 1980; McCoy, 2003; Le Gac & Giraud, 2004). This is also the case in monogeneans, where it has been widely documented (Desdevises et al., 2002a; Huyse & Volckaert, 2005; Mandeng et al., 2015; Rodríguez-González et al., 2015).

The morphotypes *Protomicrocotyle* **sp. 4** and *Protomicrocotyle* **sp. 5** are considered sympatric species, as they are found in the same host species and in the same geographic locality, as well as in sympatric distribution with *P. mirabilis*. The rapid environmental changes experienced by parasites within their host and breeding sites provide a greater opportunity for diversification than is afforded to free-living organisms (Huyse et al., 2005). Fluctuating environmental conditions may play a pivotal role in host speciation. However, phenotypic variations (plasticity) may result, particularly in the context of fluctuating environmental conditions (Thaenkham et al., 2022a).

The Taxon Pulse model posits that cycles of isolation and expansion in the distribution of communities, coupled with the environmental dynamics of the planet, facilitate the formation of new species. As a result of these processes, communities structured in this way become complex mosaics, the result of the mixing of communities due to episodes of expansion and isolation (Halas et al., 2005; Hoberg & Brooks, 2010). Under this model, parasite lineages ought to show alternating geographical patterns of expansion and isolation, just like freeliving species (Hoberg & Brooks, 2008). This model may provide an explanation for the topology of species within clade II (Figure 3.7, 3.8), as *P. nayaritensis* is depicted as the sister clade *P. manteri* and *Protomicrocotyle* **sp. 2**, and as a derived species, *P. veracruzensis* (Figure 3.8). The species *P. nayaritensis* and *P. manteri* are found in the Pacific Ocean, while *Protomicrocotyle* **sp. 2** and *P. veracruzensis* are found in the Gulf of Mexico and the Caribbean Sea. It can be inferred that the emergence of the Panama Bridge led to the diversification of the ancestral species. The Taxon Pulse Hypothesis permits the extrapolation of how the interactions of aquatic organisms oscillate

over time. An understanding of this historical process can facilitate the comprehension of patterns of diversity.

The coded morphological characters were selected based on the available information in the literature and the comparison of specimens from different collections. They were considered taxonomically diagnostic for this group of monogeneans. The combination of continuous, meristic, and discrete morphological data with molecular data allows for the formulation of a preliminary hypothesis regarding *Protomicrocotyle* from Mexico. Despite the integration of taxa from the same genus and other genera that comprise the Protomicrocotylidae family, the lack of data introduces uncertainty regarding the evolutionary process of the family, resulting in the Protomicrocotyle genus being polyphyletic (Figure 3.7). It is therefore necessary to analyses the morphology of the taxa in detail and include genetic sequences in order to construct a robust family hypothesis that will allow precise conclusions to be drawn about the evolutionary pattern of this family.

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CAPÍTULO IV

1. Conclusiones generales

- Como parte del presente estudio se describió una nueva especie de *Protomicrocotyle* en la especie de pez *Caranx latus*, y se generó nueva información morfológica para la especie *P. mirabilis* y otros morfotipos del género.
- Este estudio representa el primer estudio morfológico de *Protomicrocotyle*. Con base a los análisis morfométricos de análisis de componentes principales, discriminante y el de coordenadas principales se logra separar cinco nuevos morfotipos de *Protomicrocotyle*: *Protomicrocotyle* **sp. 1** de *Caranx hippos* en la localidad de Sontecomapan, Veracruz; *Protomicrocotyle* **sp. 2** de *C. hippos* y *Caranx* sp., de las localidades de Jicacal, Veracruz e Isla Cozumel, Quintana Roo; *Protomicrocotyle* **sp. 3** de *C. latus* de la bahía de Chetumal, Quintana Roo; *Protomicrocotyle* **sp. 4** y *Protomicrocotyle* **sp. 5** de *C. hippos* de Tecolutla, Veracruz.
- Se generaron secuencias parciales del gen mitocondrial CO1 y de los genes nucleares 28S y 18S por primera vez para *Protomicrocotyle*. Con base al análisis de estas secuencias se generó información sobre la divergencia intra e interespecífica de *Protomicrocotyle*.
- Este estudio representa el primer análisis filogenético con datos morfológicos y moleculares analizados de forma independiente y con evidencia total. La hipótesis filogenética de evidencia total muestra que las especies de *Protomicrocotyle* y los morfotipos de México forman un grupo monofilético, y que su especie hermana es *Neomicrocotyle pacifica*.

Anexo A.

New species of *Protomicrocotyle* (Monogenea: Protomicrocotylidae), and new information on *P. mirabilis*, parasites of *Caranx* spp. from Veracruz, Mexico.

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Original Article

New species of *Protomicrocotyle* (Monogenea: Protomicrocotylidae), and new information on *P. mirabilis*, parasites of *Caranx* spp. from Veracruz, México

Uma nova espécie de *Protomicrocotyle* (Monogenea: Protomicrocotylidae), e novas informações sobre *P. mirabilis*, parasitos de *Caranx* spp. de Veracruz, México

Edgar Salvador Ramírez-Cruz[1]; Scott Monks[1]; Norma Leticia Manríquez-Morán[2]; Juan Violante-González[3]; Griselda Pulido-Flores[1]*

[1]Laboratorio de Morfología Animal, Centro de Investigaciones Biológicas, Instituto de Ciencias Básicas e Ingeniería, Universidad Autónoma del Estado de Hidalgo, Pachuca, Hidalgo, México
[2]Laboratorio de Sistemática Molecular, Centro de Investigaciones Biológicas, Instituto de Ciencias Básicas e Ingeniería, Universidad Autónoma del Estado de Hidalgo, Pachuca, Hidalgo, México
[3]Centro de Ciencias de Desarrollo Regional, Facultad de Ecología Marina, Universidad Autónoma de Guerrero, Acapulco, Guerrero, Mexico

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Abstract

During a study of the helminth parasites of carangid fish of the Gulf of Mexico, *Protomicrocotyle mirabilis* and a new member of that genus were found. The aim of the present study is to provide new morphological and sequences of 28s rDNA and CO1 mtDNA for *P. mirabilis* and describe the new species. Between 2005–2022, 73 specimens of *Caranx* spp. were purchased from local fishermen of the littoral waters of the Gulf of Mexico. *Protomicrocotyle veracruzensis* sp. nov. is most similar to *P. mirabilis* than to *P. ivoriensis*, the only members of the genus known from the Greater Atlantic Ocean Basin. *Protomicrocotyle veracruzensis* sp. nov. can be distinguished from those two species by the arrangement and number of testes. Measurement data on the haptoral armature for the new species is provided and the potential value and need for comparative data from these structures of other members of the genus is discussed. The results of the molecular analysis and the morphometric analysis of 91 characters confirmed that this new species belongs to *Protomicrocotyle*.

Keywords: Monogenea, marine fishes, *Protomicrocotyle veracruzensis* sp. nov., CO1, 28S, neighbor-joining.

Resumo

Encontraram-se durante um estudo dos helmintos parasitas de peixes carangídeos do Golfo do México, *Protomicrocotyle mirabilis* e um novo membro do gênero. O objetivo do presente estudo é fornecer novos dados morfológicos e sequências de 28s rDNA e CO1 mtDNA para *P. mirabilis* e descrever a nova espécie. Entre 2005-2022, 73 espécimes de *Caranx* spp. foram comprados de pescadores locais das águas litorâneas do Golfo do México. *Protomicrocotyle veracruzensis* sp. nov. é mais semelhante a *P. mirabilis* do que a *P. ivoriensis*, os únicos membros do género conhecidos da Grande Bacia do Oceano Atlântico. *Protomicrocotyle veracruzensis* sp. nov. pode ser distinguido dessas duas espécies pela disposição e número de testículos. Neste estudo são fornecidos dados de medidas da armadura haptoral para a nova espécie, discutido o valor potencial e a necessidade de dados comparativos destas estruturas de outros membros do gênero. Os resultados da análise molecular e da análise morfométrica de 91 caracteres confirmaram que essa nova espécie pertence ao gênero *Protomicrocotyle*.

Palavras-chave: Monogenea, peixes marinhos, *Protomicrocotyle veracruzensis* sp. nov., CO1, 28S, associação de vizinho.

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*Corresponding author: Griselda Pulido-Flores. E-mail: gpulido@uaeh.edu.mx

Introduction

The Carangidae is one of the most morphologically diverse families of fishes (Nelson, 2006). The family is comprised of approximately 140 species, that are assigned to four tribes of 32 genera (Jacobina et al., 2014). Systematic analyses of the genus *Caranx* Lacépède, 1801 has shown that some recognized species have wide geographic distribution and regional populations with cryptic taxonomic features indicating that they constitute species complexes (Jacobina et al., 2014). In the Gulf of Mexico, the most common species of *Caranx* are *Caranx hippos*, *Caranx crysos*, and *Caranx latus* (López-Herrera et al., 2021). However, there are some registers of *Caranx ruber*, *Caranx bartholomaei,* and *Caranx lugubris* (Froese & Pauly, 2022).

Members of the family Carangidae are usual hosts for monogenean parasites. Several species of Protomicrocotylidae Johnston & Tiegs, 1922 commonly infect the gills of carangid fishes (Poche, 1926; Yamaguti, 1963; Mendoza-Garfias et al., 2017). *Protomicrocotyle* was establish by Johnston & Tiegs (1922), who transferred *Acanthodiscus mirabile* MacCallum, 1918 as *Protomicrocotyle mirabilis* (MacCallum, 1918) Johnston & Tiegs, 1922 to be the type species of the genus. *Protomicrocotyle mirabilis* had been collected from a fish, *Caranx hippos*, found in the New York Aquarium (MacCallum, 1918). However, although the study had many detailed descriptions (Johnston & Tiegs, 1922), it lacked precise morphological information for the subfamily-level taxon and for *P. mirabilis*.

Koratha (1955) provided more information on the species than Johnston & Tiegs (1922) and extended the distribution of *P. mirabilis* to Port Aransas, Texas, in the northwest Gulf of Mexico. In addition, he provided measurements and discussed the arguments by Sproston (1946) for moving the genus to Vallisiinae Price, 1943, Discocotylidae Price, 1936. Unnithan (1962) established the Superfamily Protomicrocotyloidea (Unnithan, 1962) for Protomicrocotylidae and placed *Protomicrocotyle*, along with several other genera, in the Protomicrocotylinae in a new family. Protomicrocotylidae currently is comprised of nine genera: *Abortipedia* Unnithan, 1963; *Bilaterocotyle* Chauhan, 1945; *Bilaterocotyloides* Ramalingam, 1961; *Chauhanocotyle* Khoche and Dad, 1975; *Lethacotyle* Manter & Price, 1953; *Neomicrocotyle* Ramalingam, 1960; *Protomicrocotyle* Johnston & Tiegs, 1922; *Vallisiopsis* Subhapradha, 1951; and *Youngiopsis* Lebedev, 1972 and approximately 42 species are currently assigned to the family.

Protomicrocotyle mirabilis has been reported from the Atlantic Ocean basin [as defined by Craig (2019)] from *C. hippos* from Ebrié Lagoon, Côte d'Ivoire, Western Africa; a description of that material was provided by Wahl (1972). The species also was reported from the Caribbean waters of Quintana Roo, Mexico by Bravo-Hollis (1989) who found it in *C. crysos*, *C. latus*, and *Caranx* sp.. *Protomicrocotyle mirabilis* has been reported several times in the Gulf of Mexico; Caballero y Caballero & Bravo-Hollis (1965) reported the species from *C. latus* collected from the waters off Tuxpan, Northern Veracruz; Bravo-Hollis (1989) reported the species in *C. hippos* and *Trachinotus carolinus*, collected from the Laguna de Sontecomapan and Las Cabañas, respectively, in Southern Veracruz, and Caballero y Caballero & Bravo-Hollis (1967) reported it from Campeche, Campeche, in *C. hippos*. *Protomicrocotyle ivoriensis* Wahl, 1972, the only other species known from the Atlantic Ocean basin, was described from specimens collected from *C. hippos* from Ebrié Lagoon, Côte d'Ivoire, Western Africa; *Protomicrocotyle manteri* Bravo-Hollis, 1966 and *Protomicrocotyle nayaritensis* Bravo-Hollis, 1979 have been described from the Pacific Coast of Mexico (Bravo-Hollis, 1966; Bravo-Hollis, 1979), and five other species have been described as parasites of fishes collected from the Pacific Ocean and the Indian Ocean basins (*Protomicrocotyle carangis* Pillai & Pillai, 1978, *Protomicrocotyle celebesensis* Yamaguti, 1953, *Protomicrocotyle madrasensis* Ramalingam, 1960, *Protomicrocotyle mannarensis* Ramalingam, 1960, and *Protomicrocotyle minutum* Ramalingam, 1960 (Yamaguti, 1953; Ramalingam, 1960; Pillai & Pillai, 1978).

In recent years, molecular approaches have been widely used for the exact identification of monogeneans and treatment of some unsolved taxonomical questions when only morphological methods were used (i.e. diagnosis, the discovery of cryptic species, delimitation of phenotypic variation, phylogeny) (Mendoza-Palmero et al., 2015; Tambireddy et al., 2016; Razo-Mendivil et al., 2016; Ondračková et al., 2020; Azizi et al., 2021; Ayadi et al., 2022). However, our present knowledge of molecular identification of the protomicrocotylids remains very limited due to the lack of genetic data. Currently, only four species of Protomicrocotylidae have been characterized with 28S, and two with cytochrome c oxidase subunit I (CO1) genes (Olson & Littlewood, 2002; Justine et al., 2013; Tambireddy et al., 2016) and genetic data for these species are available in GenBank® (Sayers et al., 2020) database. However, molecular data for many members of Mazocraeidea Bychowsky, 1937 are still lacking.

As part of the ongoing study of the helminths of marine fishes, individuals of *C. hippos*, and *C. latus* were collected from the coastal waters of Veracruz, Mexico (2005–2022) (Figure 1; Table 1) and necropsied for helminths. This study presents updated information on *P. mirabilis* [*sensu stricto* Kritsky et al. (2011)] and the description of a new species of *Protomicrocotyle*, a gill parasite of *C. latus* from Casitas and Puerto de Veracruz, Veracruz, Mexico. An integrative approach, including morphometrical categorization and molecular analyses of the cytochrome c oxidase subunit I and 28S rDNA was used to characterize these monogeneans.

Figure 1. Geographic locations of the localities in the Coastal waters off Veracruz, Mexico, where fishes were collected: (1) Tuxpan; (2) Tecolutla; (3) Casitas; and (4) Puerto de Veracruz.

Table 1. List of species of fish sampled in the Coastal waters off Veracruz, Mexico.

Locality (number)	Species	Number of fish collected	Infected with *P. mirabilis*	Infected with *P. veracruzensis* sp. nov.	Collection date	Coordinates
Tuxpan (1)	*Caranx hippos*	4	4	–	11 Mar 2010	20° 58′ 09.2″ N; 97° 19′ 43.4 "W
		6	5	–	12 Nov 2013	
		12	8	–	19 Aug 2019	
Tecolutla (2)	*C. hippos*	1	–	–	27 May 2015	20° 29′ 00.8″ N; 97° 00′ 07.2″ W
		14	14	–	12 Nov 2021	
Casitas (3)	*C. hippos*	22	18	–	14 Nov 2021	20° 15′ 31.5″ N; 96° 47′ 49.5″ W
	C. latus	2	–	1	4 May 2005	
		3	–	–	19 Aug 2019	
Puerto de Veracruz (4)	*C. hippos*	8	7	–	24 May 2022	19° 13′ 11.2″ N; 96° 09′ 24.4″ W
	C. latus	1	–	1	24 May 2022	

The number in the parenthesis is represented in Figure 1.

Materials and Methods

Collection localities

The present study was carried out using fish collected from four localities (port cities) with the following formal names: Tuxpan de Rodriguez Cano, Tecolutla, Casitas, and Veracruz; all in the state of Veracruz de Ignacio de la Llave (INEGI, 2023). To avoid confusion, they will be referred to using the commonly used names: Tuxpan, Tecolutla, Casitas, and Puerto de Veracruz, respectively. The state will be referred to simply as Veracruz or as the State of Veracruz.

Specimen collection

During the period from 2005–2022, 73 specimens of *Caranx* were purchased from local fishermen, who caught them in littoral waters of the Gulf of Mexico, offshore of the four localities in the State of Veracruz, Mexico: 67 specimens of *C. hippos*, crevalle jack (jurel amarillo- Mexican common name) and six of *C. latus*, horse-eye jack (jurel blanco) (Figure 1; Table 1). The external body surface of each fish was examined for helminths using a magnifying glass and gill arches were excised, placed in a Petri dish with seawater, and examined using a stereomicroscope (Leica Zoom 2000). Members of *C. hippos* were found to be infected with *P. mirabilis* and members of *C. latus* were infected with an undescribed species of that genus (Table 1). Each fish was infected with only a single species of monogenean.

Monogeneans, dead at the time of collection, were removed from gill filaments and transferred temporarily to dishes containing seawater. When all worms had been collected, they were fixed with Alcohol-Formalin-Acetic Acid (AFA) at room temperature for at least 12 h and then transferred for storage to 70% ethyl alcohol for morphological studies [following Pulido-Flores & Monks (2005)]; other specimens were fixed and stored in 96-100% ethyl alcohol for molecular studies.

Morphological and morphometric analysis

Specimens were stained using Gomori's trichrome, Mayer's carmalum, or Delafield's hematoxylin, dehydrated in an ethanol series, cleared in methyl salicylate, and mounted individually as whole-mounts on slides in Canada balsam. Morphometric comparisons were made using the information available in the literature for species of *Protomicrocotyle* reported in the Pacific and Atlantic Oceans and specimens borrowed from two collections (cited below). Specimens were examined using a compound optical microscope equipped with differential interference contrast (DIC) optics and drawings were made with the aid of a drawing tube. Measurements were made using an ocular micrometer; all measurements are given in micrometers as the mean followed in parentheses by the range and the number of structures (n) measured. For comparison with the shape of the haptoral lappet of other species, the length of the lappet was measured at three positions: right side lateral haptoral lappet (rt), opposite to the clamps; the middle of the lappet (mid); and the left side of the lappet (lf), the side closest to the clamps (Figure 2A).

Measurements of the anchors follow, in part, those taken by Mariniello et al. (2004), Amine & Euzet (2005), Vaughan & Christison (2012), and Řehulková et al. (2013): the total length and width of each anchor (a, b, respectively), length of the opening (c), the length and the width of the internal root (d, e, respectively), and the lengths of the external root (f), the length of the external blade (g), the length of the internal blade (h), and the length of the point (i) were taken (Figure 2B, 2C). Terminology of the clamp sclerites followed Boeger & Kritsky (1993) and Bouguerche et al. (2020). The shapes (forms) of structures were classified according to Clopton (2004). The infection parameters were calculated in accordance with Bautista-Hernández et al. (2015) and Bush et al. (1997). For comparative purposes, some type and voucher specimens from the Colección Nacional de Helmintos (CNHE) were examined: *Protomicrocotyle mirabilis* (CNHE-82; 111; 161; 162; 163; 164; 165; 166); *P. manteri* (CNHE-21; 134; 344; 345; 346; 347; 348; 3114; 3115) and *P. nayaritensis* (CNHE-158; 159). Specimens were deposited in the CNHE, Harold W. Manter Laboratory Collection (HWML), and the Colección de Helmintos (CHE), Universidad Autónoma del Estado de Hidalgo.

In order to corroborate preliminary assignment of specimens collected found infecting fish from the localities of Tuxpan, Tecolutla, Casitas, and Puerto de Veracruz, Veracruz to *P. mirabilis*, the measurements provided by Kritsky et al. (2011) and those of the specimens of this study were compared to determine if there was a significant difference using a two-sample independent Mann-Whitney test. This test can be used to determine if two independent groups are from the same group (MacFarland & Yates, 2016), this case, the same species.

For the morphometric analyses, 91 morphological variables from 150 specimens of *Protomicrocotyle* were analyzed (88 continuous, and three meristic). The measurements were obtained from specimens that had been identified previously as members of *P. mirabilis* (CHE: Ver-10-42; Ver-19-318; 317; 341; 348; 349; Ver-15-126; Ver-21-10; Ver-22-162; Ver-22-169: CNHE: 82; 111), *P. manteri* (CNHE: 346; 348; 3114; 3115), *P. nayaritensis* (CNHE: 158; 159), *Neomicrocotyle pacifica* (Meserve, 1938 Yamaguti, 1968) (CNHE: 371; 372; 3116) and the specimens of *P. mirabilis* and the new species collected as part of this study were used for the construction of a Principal Component Analysis (PCA) and Discriminant Analyses (DA). Individual values were transformed from micrometers to log for statistical analyses. The PCA was used to order and visualize possible clustering among the morphometric characters by evaluating the PCA with the objective of reducing the number of variables introduced (Palacio et al., 2020). The DA determines to what degree the analyzed variables, measurements of objects or individuals, best explain the attribution of the difference of the groups to which said objects or individuals belong (Torrado Fonseca & Berlanga Silvente, 2013; Palacio et al., 2020). The analyzes were performed using Past 4.06b (Hammer et al., 2001).

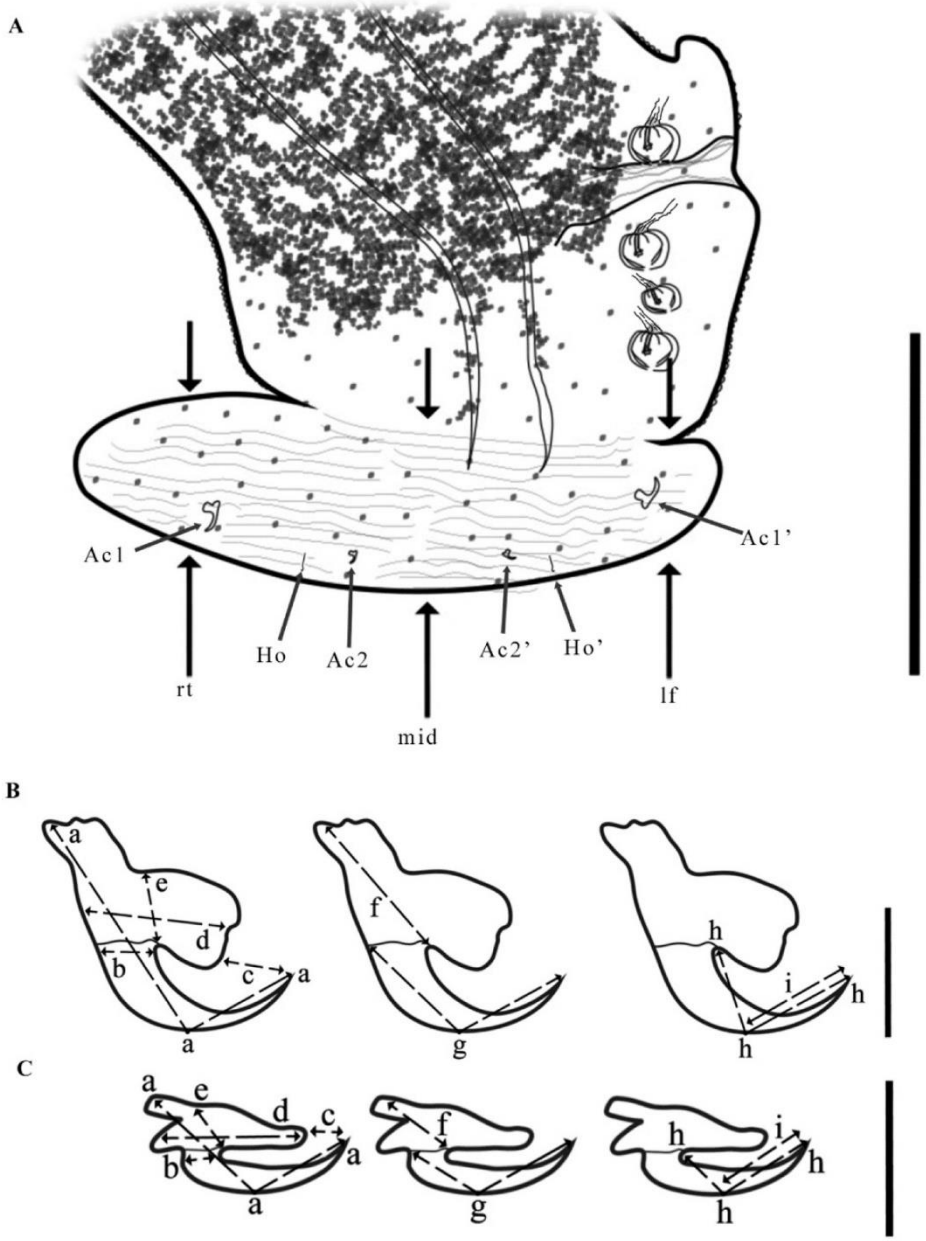

Figure 2. Scheme of measurements of the haptoral lappet and sclerotized structures. A. Haptoral lappet; rt = right side lateral haptoral lappet, length; mid = middle haptoral lappet length; lf = left side lateral haptoral lappet, length; Ac1 = right lateral anchor; Ac1' = left lateral anchor; Ac2 = right medial anchor; Ac2' = left medial anchor; Ho = right central hook; Ho' = left central hook. Bars 350 µm. B. Lateral anchor, Bar 15 µm. C. Median anchor, Bar 17 µm; a = anchor, total length; b = anchor, width; c = opening; d, e = internal root (length and width, respectively); f = external root; g = length external blade; h = length internal blade; i = point.

Molecular study

Fish (*C. hippos*) that had been collected from the four localities in 2019 and 2022 (Table 1) were found to be infected with *P. mirabilis* [*sensu stricto* (Kritsky et al., 2011)]. An undescribed species was found to infect *C. latus* from Puerto de Veracruz. Specimens of *P. mirabilis* and the new species were prepared for molecular analyses. Genomic DNA was extracted using a DNeasy Blood and Tissue Kit (Qiagen, Hilden, Germany) following the manufacturer's protocol.

CO1 fragments were amplified using published primers JB3-F (ASmit1) (5'-TTTTTTGGGCATCCTGAGGTTTAT-3') and JB4-R (ASmit2) (5'-TAAAGAAAGAACATAATGAAAATG-3') (Bowles et al., 1993; Tambireddy et al., 2016), and the primers LSU5-F (5'-TAGGTCGACCCGCTGAAYTTAAGC-3') and EC-D2-R (5'-CCTTGGTCCGTGTTTCAAGACGGG-3') were used for 28S rDNA amplification (Littlewood et al., 1997; Tkach et al., 2003). The Polymerase Chain Reactions (PCR) were performed in a total of 25 µL, consisting of 4 µL of template DNA, 4.85 µL of master solution (0.15 µL of Taq DNA polymerase (5 µ/mL; BioTecMol), 1 µL dNTPs (2.5 mM; Promega), 0.2 µL of each primer (10 nM), 1.8 µL of 5x PCR buffer (BioTecMol), 1.5 µL of $MgCl_2$ (25 mM; BioTecMol)), and 16.15 µL of distilled water.

The cycling conditions included initial denaturation at 94°C for 5 min, followed by 38 cycles of 94°C for 30 s, 45°C for 30 s, and 72°C for 1:10 min, and a final extension of 7:00 min at 72°C. PCR products were visualized in an electrophoresis agarose gel and were purified using polyethylene glycol (PEG) protocol and were sequenced using BigDye Terminator v3.1 Cycle Sequencing Kit in 10 µL reactions and 3730xl DNA Analyzer-Thermo Fisher Scientific using the JB3-F and LSU5-F primers, respectively, performed at the Instituto de Biología, UNAM, Mexico. Sequence data and electropherograms were inspected and edited using Pregap4 and Gap4 modules by Staden software V.1.6 (Staden, 1996).

Sequences obtained in the present study for CO1 and 28S regions were aligned with sequences from other protomicrocotylid retrieved from GenBank and *Allodiscocotyla diacanthi* Unnithan, 1962 (Allodiscocotylidae Tripathi, 1959 was used as the outgroup, based on the phylogenetic analysis by Tambireddy et al. (2016) (Table 2). Average *p-distance* between conspecific sequences from GenBank and collected samples (Table 2) were calculated in MEGA 11 (Tamura et al., 2021). A distance matrix was used for clustering analysis and the presentation of tree topology. The Neighbor-Joining (NJ) method was used to builds a tree from a matrix of pairwise evolutionary distances relating to the set of taxa being studied, therefore, the algorithm of the method finds the pairs of sequences that minimize the total length of the topology of the tree in each iteration (Gascuel & Steel, 2006; Saitou & Nei, 1987). The NJ analyses from CO1 and 28S was performed in MEGA 11 with bootstrap analysis based on 1000 resampling of each data set. The trees were edited in Adobe® Photoshop®.

Table 2. List of monogeneans included in the genetic distances analyses and Unweighted Pair Group Method with Arithmetic Mean analyses, and GenBank accession numbers of sequences from the partial CO1 and 28S genes. New sequences obtained for the present study are in bold.

Species of monogeneans	Host	GenBank ID		Reference for sequences
		CO1	28S	
Protomicrocotylidae				
Protomicrocotyle mirabilis	*Caranx hippos*	**OR282821– OR282832**	**OR282885– OR282894**	Present study
Protomicrocotyle veracruzensis sp. nov.	*C. latus*	**OR282833– OR282836**	**OR282895– OR282898**	Present study
Neomicrocotyle pacifica (Meserve, 1938) Yamaguti, 1968	*C. hippos*	–	AF382043	Olson & Littlewood (2002)
Neomicrocotyle sp.	*C. sexfasciatus*	–	KF378589	Justine et al. (2013)
Lethacotyle vera Justine, Rahmouni, Gey, Schoelinck and Hoberg, 2013*	*C. papuensis*	–	KF378588	Justine et al. (2013)
Bilaterocotyloides carangis Ramalingam, 1961	*Megalaspis cordyla*	KF804043	–	Tambireddy et al. (2016)
		–	KJ201022	Tambireddy et al. (2016)
Bilaterocotyloides madrasensis Radha, 1966	*M. cordyla*	KF804041	KF804029	Tambireddy et al. (2016)
		–	KF804037	Tambireddy et al. (2016)
Outgroup				
Allodiscocotylidae				
Allodiscocotyla diacanthi Unnithan, 1962	*Scomberoides commersonnianus*	KF804045	KF804033	Tambireddy et al. (2016)
		KF804046	KF804038	Tambireddy et al. (2016)

*Reported as *Lethacotyle* sp. in GenBank.

Results

Morphology study

Class Monogenea van Beneden, 1858

Subclass Polypisthocotylea Odhner, 1912

Order Mazocraeidea Bychowsky, 1937

Family Protomicrocotylidae Johnston & Tiegs, 1922

Genus *Protomicrocotyle* Johnston & Tiegs, 1922

Protomicrocotyle mirabilis (MacCallum, 1918) Johnston & Tiegs, 1922

Description. Based on 81 specimens, stained, and mounted to be viewed from ventral side of the body.

Body (Figure 3A), Table 3. Body fusiform [see Clopton (2004) for names of shapes], 2861 (805–4673, n = 79) long (including haptoral lappet) and 295 (134–537, n = 80) wide at level of middle of testicular field. Tegument with annular ridges in the region media of the body and posterior to the ovary that extend to posterior region of body, these striations on the margin of the body give it a serrated appearance (Figure 3A). Prohaptoral suckers anterolateral, muscular, oval; right oral sucker 44 (13–68, n = 77) long, 31 (10–48, n = 77) wide, and left sucker 44 (14–67, n = 77) long, 31 (11–48, n = 77) wide. Buccal cavity subterminal, ventral, ovoid-broadly, 26 (11–52, n = 70) long, 31 (12–67, n = 73) wide. Pharynx, posterior to buccal cavity, muscular, broadly elliptoid, 42 (14–64, n = 77) long, 35 (14–52, n = 77) wide. Esophagus long, with lateral diverticula, 495 (295–670, n = 66) long (Figure 3A). Cecal bifurcation posterior to male copulatory organ (MCO), 606 (380–775, n = 66) from anterior end of body. Intestinal caecum lateral to midline and reproductive organs, extending into haptor; caecum with numerous short and lateral ramifications (Figure 3A).

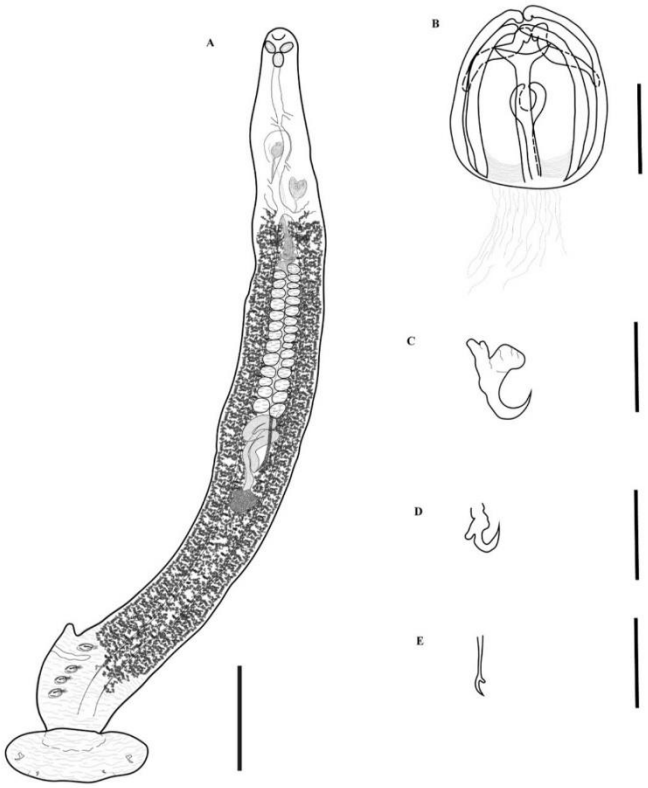

Figure 3. *Protomicrocotyle mirabilis*. A. Whole worm (ventral view), Bar 500 µm. B. Details of clamp, Bar 32 µm. C. Lateral anchor, Bar 32 µm. D. Median anchor, Bar 32 µm. E. Hook, Bar 32 µm.

Table 3. Morphometric comparison of different records of *Protomicrocotyle mirabilis* (taxonomic identity corroborated) and *P.* cf. *mirabilis* (taxonomic identity not corroborated). Measurements are presented in micrometers.

Species	*Protomicrocotyle mirabilis*	*P. mirabilis*	*P. mirabilis*
Locality	Veracruz (this study)	Florida Bay, Everglades National Park, Florida	New York Aquarium, USA
Host	*C. hippos*	*Caranx hippos*	*C. hippos*
Site	Gill filaments	Gill lamellae	Gill lamellae
	µm	µm	µm
Total length	2861 (805–4673, n =79)	2240 (1470–3080)	**3128**
Total width	295 (134–537, n =80)	322 (214–449)	200
Mouth length	26 (11–52, n =70)	16	–
Mouth width	31 (12–67, n =73)	25	–
Cecal bifurcation to anterior end	606 (380–775, n =66)	**346**	–
Pharynx length	40 (14–64, n =77)	32	–
Pharynx width	35 (14–52, n =77)	47 (39–57)	–
Prohaptoral sucker length (right)	44 (13–68, n =77)	**23**	**31**
Prohaptoral sucker width (right)	31 (10–48, n =77)	**32**	**22**
Prohaptoral sucker length (left)	44 (14–67, n =77)	**24**	**30**
Prohaptoral sucker width (left)	31 (11–48, n =77)	**33**	**17**
Clamp length	45.7 (14.2–65, n =71)*	57 (49–67)	**55**
Haptor length	477 (165–815, n =76)	**338**	
Haptor width	255 (70–425, n =76)	**161**	**110**
Right side of haptoral lappet length	117 (60–217, n =72)	**64**	**73**
Left side of haptoral lappet length	120 (40–202, n =73)	**72**	**61**
Central haptoral lappet length	125 (40–214, n =74)	**88**	**134**
Haptoral lappet width	437 (105–785, n =75)	241	330
Lateral anchor length	41.5 (22.5–65, n = 153)*	33 (29–37)	–
Lateral anchor width	5 (2–7.5, n = 153)*	**3**	–
Medial anchor length	27.5 (9–37, n = 151)*	21 (19–22)	–
Medial anchor width	4 (2–6, n = 151)*	**2**	–
Hook length	17 (7–23, n = 144)*	18 (16–19)	–
Hook width	3 (1–3, n = 138)*	**2**	–
Number of testes	31 (21–38, n =77)	27 (23–33)	14–35
Testes length	45 (17–79, n =81)	43 (28–54)	**34.6**
Testes width	50 (14–86, n =80)	43 (28–54)	**49.3**
Testes-Ratio length: wide	1:1.1	1:1	1:1.4
Male copulatory organ length	54 (22–82, n =80)	**77**	–
Male copulatory organ width	41 (11–66, n =80)	50 (42–56)	–
Genital pore to anterior end distance	370 (140–660, n =81)	250	**452**
Vaginal vestibule length	69 (28–94, n =80)	82 (66–99)	**52.5**
vaginal vestibule width	51 (19–77, n =80)	59 (41–75)	**48**
Vaginal opening to anterior end	464 (175–740, n =80)	**330**	**567**
Vaginal opening to genital pore	112 (40–180, n =80)	**88**	**134**
Egg length	157 (88–206, n =41)	180 (167–193)	**183**
Egg width	49 (24–68, n =41)	68 (66–70)	**85.5**
Number of spines in male copulatory organ (MCO)	22 (16–29, n =71)	19 (16–21)	**20**
Number of spines in vaginal vestibule	45 (31–65, n =63)	**49**	–
Reference	Present study	Kritsky et al. (2011)	MacCallum (1918)

*Average of all anatomical structures measured. Values in bold were taken of the line drawings.

Table 3. Continued...

Species	*P. mirabilis*	*P.* cf. *mirabilis*	*P.* cf. *mirabilis*
Locality	Tuxpan, Veracruz	Sontecomapan, Veracruz, Mexico	Las Cabanas, Veracruz, Mexico
Host	*C. latus*	*C. hippos*	*C. hippos*
Site	Gill lamellae	Gill lamellae	Gill lamellae
	µm	µm	µm
Total length	1254–2451	1482–3706	1881–2052
Total width	149–387	171–399	285–342
Mouth length	**19**	–	–
Mouth width	**44**	–	–
Cecal bifurcation to anterior end	**375**	–	–
Pharynx length	**43**	–	–
Pharynx width	**44**	–	–
Prohaptoral sucker length (right)	**44**	–	–
Prohaptoral sucker width (right)	**29**	–	–
Prohaptoral sucker length (left)	**44**	–	–
Prohaptoral sucker width (left)	**27**	–	–
Clamp length	48–52	–	–
Haptor length	**342**	–	–
Haptor width	149–298	–	–
Right side of haptoral lappet length	**49**	–	–
Left side of haptoral lappet length	**44**	–	–
Central haptoral lappet length	**71**	–	–
Haptoral lappet width	283	–	–
Lateral anchor length	–	–	–
Lateral anchor width	–	–	–
Medial anchor length	–	–	–
Medial anchor width	–	–	–
Hook length	–	–	–
Hook width	–	–	–
Number of testes	20–30	18–29	17–26
Testes length	37–41	–	–
Testes width	33–59	–	–
Testes-Ratio length: wide	1:0.8	–	–
Male copulatory organ length	**49**	–	–
Male copulatory organ width	**38**	–	–
Genital pore to anterior end distance	231	–	–
Vaginal vestibule length	**88**	–	–
vaginal vestibule width	**60**	–	–
Vaginal opening to anterior end	268–402	–	–
Vaginal opening to genital pore	**77**	–	–
Egg length	–	–	–
Egg width	–	–	–
Number of spines in male copulatory organ (MCO)	18–20	17–19	16–17
Number of spines in vaginal vestibule	–	–	–
Reference	Caballero y Caballero & Bravo-Hollis (1965)	Bravo-Hollis (1989)	Bravo-Hollis (1989)

*Average of all anatomical structures measured. Values in bold were taken of the line drawings.

Table 3. Continued...

Species	*P.* cf. *mirabilis*	*P.* cf. *mirabilis*	*P.* cf. *mirabilis*	*P.* cf. *mirabilis*	*P.* cf. *mirabilis*
Locality	Chetumal, Quintana Roo, Mexico	Isla Cozumel, Quintana Roo, Mexico	Isla Mujeres, Quintana Roo, Mexico	Port Aransas, Texas, USA	Ebrié Lagon, near Abidjan
Host	*C. latus*	*Caranx* sp.	*C. crysos*	*C. hippos*	*C. hippos*
Site	Gill lamellae	Gill lamellae	Gill lamellae	Gill lamellae	Gill lamellae
				µm	µm
Total length	1938–3192	2565–3078	2223–2280	5400	2000–2900
Total width	171–285	228–399	470–620	275	250–550
Mouth length	–	–	–	–	–
Mouth width	–	–	–	–	–
Cecal bifurcation to anterior end	–	–	–	–	**345**
Pharynx length	–	–	–	–	–
Pharynx width	–	–	–	–	–
Prohaptoral sucker length (right)	–	–	–	–	–
Prohaptoral sucker width (right)	–	–	–	–	–
Prohaptoral sucker length (left)	–	–	–	–	–
Prohaptoral sucker width (left)	–	–	–	–	–
Clamp length	–	–	–	60	60
Haptor length	–	–	–	–	**138**
Haptor width	–	–	–	–	–
Right side of haptoral lappet length	–	–	–	–	**166**
Left side of haptoral lappet length	–	–	–	–	**178**
Central haptoral lappet length	–	–	–	–	**154**
Haptoral lappet width	–	–	–	200	**416**
Lateral anchor length	–	–	–	–	–
Lateral anchor width	–	–	–	–	–
Medial anchor length	–	–	–	–	–
Medial anchor width	–	–	–	–	–
Hook length	–	–	–	–	–
Hook width	–	–	–	–	–
Number of testes	20–25	20–21	24–25	–	28–32
Testes length	–	–	–	–	75–85
Testes width	–	–	–	–	45–55
Testes-Ratio length: wide	–	–	–	–	1:0.6
Male copulatory organ length	–	–	–	–	**107**
Male copulatory organ width	–	–	–	–	**71**
Genital pore to anterior end distance	–	–	–	–	**309**
Vaginal vestibule length	–	–	–	–	**71**
vaginal vestibule width	–	–	–	–	**95**
Vaginal opening to anterior end	–	–	–	–	**300**
Vaginal opening to genital pore	–	–	–	–	–
Egg length	–	–	–	–	–
Egg width	–	–	–	–	–
Number of spines in male copulatory organ (MCO)	18–19	16–20	18–19	–	15–20
Number of spines in vaginal vestibule	–	–	–	–	–
Reference	Bravo-Hollis (1989)	Bravo-Hollis (1989)	Bravo-Hollis (1989)	Koratha (1955)	Wahl (1972)

*Average of all anatomical structures measured. Values in bold were taken of the line drawings.

Haptor (Figure 3A). Haptor asymmetrical, comprised of four lateral clamps on the left side and a terminal haptoral lappet; (no asymmetry of these structures observed); 477 (165–815, n = 76) long, 255 (70–425, n = 76) wide at the level of the haptoral groove. Clamps located ventrally, each with a short muscle peduncle, positioned on left side of worm (on right side of drawing of worm in ventral view) (Figure 3B). First clamp (anterior to haptoral groove) 46 (12–66, n = 65) long, 35 (11–52, n = 65) wide; second clamp 46 (14–67, n = 71) long, 37 (13–73, n = 71) wide; third clamp 46 (12–65, n = 74) long, 37 (13–56, n = 74) wide; fourth clamp 46 (19–66, n = 72) long, 36 (12–54, n = 72) wide. Clamps typical of Family Gastrocotylidae. Small haptoral groove, with raised edges, between the first and second clamps (Figure 3A). Haptoral lappet elongate ovate, wider than long. Length of lappet measured in three regions (Figure 2A); right side of haptoral lappet (rt) 117 (60–217, n = 72) long, middle region of haptoral lappet (mid) 125 (40–214, n = 74) long, left side of haptoral lappet (lf) 120 (40–202, n = 73) long. Haptoral lappet 437 (105–785, n = 75) wide, armed with two pairs of anchors, and one pair of hooks (Figure 3C, 3D, 3E).

Male reproductive structures (Figures 3A, 4A). Testes 31 (21–38, n = 77) in number, very broadly elliptoid, intercecal, anterior to descending ducts of germarium, arranged in two parallel fields, each field with one regular column of testes. Testes 45 (17–79, n = 81) long, 50 (14–86, n = 80) wide. Length to width ratio L:W= 1:1.1 (Figure 3A). Vas deferens dorsal to testes, running in zigzag pattern from anterior part of testes to MCO. Male copulatory organ 54 (22–82, n = 80) long, 41 (11–66, n = 80) wide, subspherical, armed with 22 (16–29, n = 71) spines (Figure 4A). Spines of MCO hooklike, 36 (16–50, n = 78) long, 3 (2–6, n = 78) wide, arranged in a circle on anterior part of MCO (Figure 4B). Genital atrium ventral, near midline, anterior to cecal bifurcation, opening at 370 (140–660, n = 81) from anterior end of body.

Female reproductive structures (Figure 4C, 4E). Germarium intercecal, post-testicular, comprised of germarial bulb with immature oöcytes, 116 (36–199, n = 75) long, 69 (17–124, n = 75) wide, with a wide ascending duct and an irregularly descending duct that form loops that contain mature oöcytes. The oviduct ends in the oötype; uterus ascends from oötype to the genital atrium (Figure 4E). Vaginal pore ventral, anterior to vaginal vestibule. Vaginal vestibule 69 (28–94, n = 80) long, 51 (19–77, n = 80) wide, located 464 (175–740, n = 80) from anterior end of body and 112 (40–180, n = 80) from the genital pore, lateral to midline on the side opposite to that having the haptoral clamps (Figure 4C). Vaginal vestibule armed with approximately 45 (31–65, n = 63) spines 34 (13–50, n = 76) long, 3 (1–5, n = 76) wide; the spines of the anterior region of the vestibule are larger and those of the middle and basal region smaller. These spines have small spine-like extensions on the distal region of each spine (Figure 4D). The vaginal duct descends from the vaginal vestibule to the germarium and connects to the oötype in the ventral region of the germarium between descending duct and germarium bulb. Seminal receptacle not observed. Vitelline glands in two lateral fields, starting just posterior to vaginal vestibule, overlapping ceca anteriorly and posteriorly and the lateral margin of the testes, uniting posterior to germarium, extending to posterior region of body but not reaching the haptoral lappet (Figure 3A).

Eggs (Figure 4F). Uterus contains 2 (1–3, n = 41) eggs. Eggs elongate, elliptoid, with long polar filaments at each pole; length, not including polar filaments, 157 (88–206, n = 41), 49 (24–68, n = 41) wide. Filament in anterior end (as positioned in uterus) 217 (49–308, n = 19) long, 5(2–8, n = 19) wide, and posterior end 187 (84–317, n = 26) long, 5 (4–8, n = 26) wide.

Taxonomic summary

Type Host: *Caranx hippos* (Carangidae).

Common name: crevalle jack.

Additional hosts: *Caranx latus* (Carangidae) (Caballero y Caballero & Bravo-Hollis, 1965).

Type locality: New York Aquarium, New York (MacCallum, 1918).

Additional localities: Everglades National Park, Florida (Kritsky et al., 2011); Littoral waters off the Gulf of Mexico off Tuxpan, Tecolutla, Casitas and Puerto de Veracruz, Veracruz, Mexico (this study) (Figure 1; Table 1).

Site of infection: Gill filaments.

Abundance, prevalence, mean intensity of infection and range of intensity: General infection characterization: *C. hippos*, 7.21 of abundance, 56 infected fish of 67 examined (83.58%), 8.63 and 1–65. Tuxpan: 10.64 of abundance, 17 infected fish of 22 examined (77.27%), 13.76 and 3–44; Tecolutla: 11 of abundance, 14 infected fish of 15 examined (93.33%), 11.79 and 4–59; Casitas: 13.27 of abundance, 18 infected fish of 22 examined (81.82%), 16.22 and 1–65, and; Puerto de Veracruz: 14.25 of abundance, 7 infected fish of 8 examined (87.50%), 16.29 and 2–53.

Specimens deposited: vouchers CNHE-12822 to 12825, HWML-216985 to 216994, CHE-P00147.

Genbank accession numbers: Cytochrome C Oxidase Subunit I (CO1) OR282821 to OR282832; 28S rDNA OR282885 to OR282894

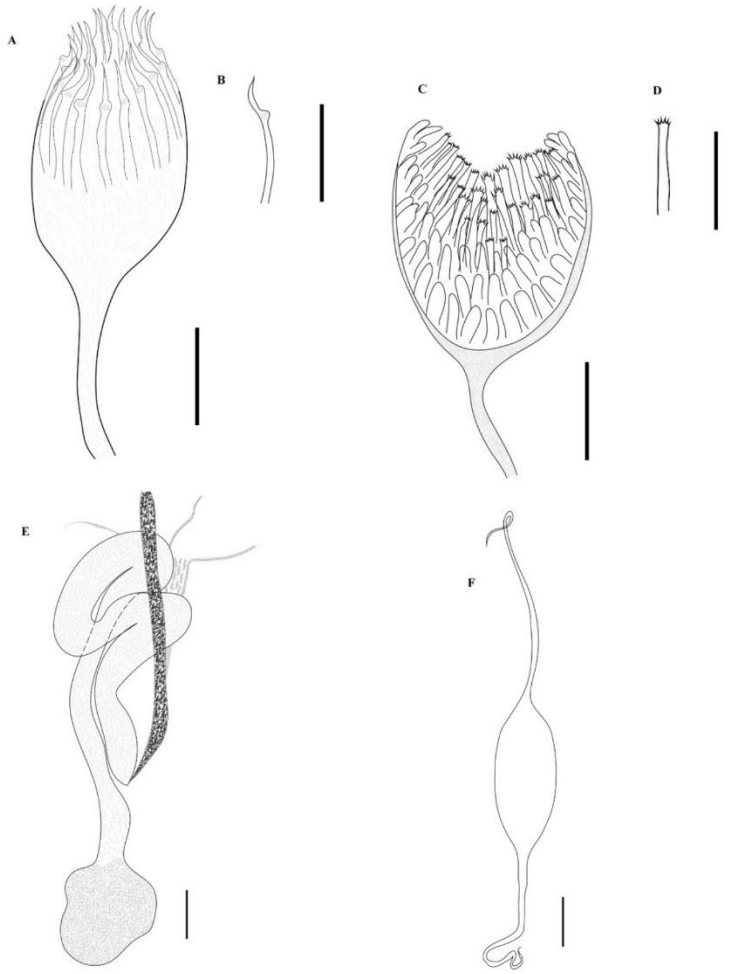

Figure 4. Female and male reproductive organs of *Protomicrocotyle mirabilis*. A. Male copulatory organ, Bar 32 µm. B. Spine of male copulatory organ, Bar 32 µm. C. Vaginal vestibule, Bar 32 µm. D. Spine of vaginal vestibule, Bar 32µm. E. Female reproductive organs, Bar 50 µm. F. Egg, Bar 50 µm.

Remarks

Members of *Protomicrocotyle* can be characterized by a suite of characters that include: having an asymmetrical haptor with four Gastrocotylidae-type clamps in a longitudinal row on the side opposite to the vaginal vestibule; clamps with accessory sclerites; the haptoral lappet is transversely elongate (wider than long), armed with two pairs of anchors (larger anchors positioned laterally with smaller anchors between the larger ones), and one pair of hooks (located between the smaller paired anchors); the esophagus and ceca have diverticula; vitellaria and ceca may or may not extend into the haptor; the testes are relatively numerous, with variations in shape, size, and arrangement, but always are anterior to the female complex; the MCO bulbous, may be muscular, and is provided with a crown of numerous spines; genital pore ventral to esophagus; ovary consisting of a germarium and a tubular duct, winding or not, post-testicular; genitointestinal canal present, crossing ovary or not; eggs with filament at each pole; vagina opening ventrally to the right or left, posterior to genital pore, armed or not, with numerous spines of different shapes; vitellaria extends lateral and dorsal to ceca (Yamaguti, 1963). They are parasites of marine teleost's, principally of fishes of Carangidae (Yamaguti, 1963). The material described herein has the diagnostic morphological characteristics of this genus. The reason for reporting this information in detail is the necessity to corroborate the identity of the specimens collected from Tuxpan so that the molecular data could be linked to *P. mirabilis*, so it could be compared to that of the new species.

The results of the Mann-Whitney test indicated that there are no significant differences [*Z (U)* = 0.729; *P* = 0.465] in the measurements between the specimens of *Protomicrocotyle mirabilis* from Florida, as described by Kritsky et al. (2011), and those specimens collected from Veracruz (Figure 1; Table 1). In accordance with the redescription by Kritsky et al. (2011) and the specimens collected as part of this study, *P. mirabilis* is characterized by having the characters of the genus and of the species, as detailed above. Measurements for *P. mirabilis* presented in this work are shown in comparison with that reported by Kritsky et al. (2011) in Table 3. Comparing the measurements in Table 3, it is possible to observe variation with respect to some variables, but as demonstrated by the Mann-Whitney test, there are no significant differences. However, it is worth mentioning that there are some characters that are outside the ranges established by Kritsky et al. (2011) in their redescription of *P. mirabilis*, such as body length, average testes size, the number of spines in the male reproductive organ, the vaginal vestibule, and the number of testes, among other variables (Table 3) of the specimens in this study are larger than what was mentioned by Kritsky et al. (2011). Therefore, it is necessary to carry out studies of specimens from other locations within the different biogeographical provinces that make up the Gulf of Mexico (Carolina Province and Caribbean Province) (Briggs & Bowen, 2012) and of the different ecoregions of which these provinces are pertain (Lara-Lara et al., 2008; Mendelssohn et al., 2017) that include the analysis of morphological and molecular characteristics. In this manner, existing variation in morphology can be attributed to intraspecific variation or if it reveals a complex of cryptic species. However, the current information is interpreted as a confirmation that *P. mirabilis* (*sensu stricto* Kritsky et al. (2011)) is widely distributed within the Gulf of Mexico. Verified locality reports include Tuxpan [this study and Caballero y Caballero & Bravo-Hollis (1965)], Tecolutla (this study), Casitas (this study), Puerto de Veracruz (this study) and Campeche [this study and Caballero y Caballero & Bravo-Hollis (1967)]. Previous records from other localities in the Gulf of Mexico (Bravo-Hollis, 1989; Mendoza-Garfias et al., 2017; Montoya-Mendoza et al., 2017), Caribbean Sea (Bravo-Hollis, 1989), South America (Boada et al., 2012; Vianna et al., 2020) and the Republic of Côte d'Ivoire (Wahl, 1972) could not be corroborated from existing material. Additional material that includes specimens for comparative morphological and molecular analyses must be collect in order to establish the limits of the distribution of *P. mirabilis*.

Protomicrocotyle veracruzensis sp. nov.

Description (Figures 2, 5, 6, 7; Tables 4, 5). Based on 24 adult specimens, stained, and mounted to be viewed from ventral side of the body. Measurements and data of other species are presented in Table 4.

Body (Figures 2A, 5A, 7A). Body narrowly fusiform, 3887 (3209–4709, n = 24) long (including haptoral lappet) and 804 (586–1098, n = 24) wide at level of middle of testicular field (Figure 5A). Tegument with annular ridges posterior to the ovary that extend to posterior region of body, reaching the haptoral lappet (Figure 5A-a inset). Prohaptoral suckers anterolateral, muscular, oval; right oral sucker 76 (47–97, n = 24) long, 48 (32–59, n = 24) wide, and left sucker 82 (67–94, n = 24) long, 49 (32–58, n = 24) wide (Figures 2A, 5B). Buccal cavity subterminal, ventral, 35 (25–44, n = 24) long, 52 (38–72, n = 24) wide. Pharynx 54 (46–58, n = 24) long, 52 (48–59, n = 24) wide, muscular, and orbicular in shape. Esophagus long, with lateral diverticula. Cecal bifurcation posterior to male copulatory organ (MCO), 285 (190–405, n = 24) from anterior end of body. Intestinal ceca lateral to midline and reproductive organs, extending into haptor; ceca with numerous short and lateral ramifications (Figures 2A, 5A).

Haptor (Figures 2A, 2C, 5A, 5E, 7A, 7C). Haptor asymmetrical, comprised of four lateral clamps on the left side and a terminal haptoral lappet; (no asymmetry of these structures observed); 630 (515–685, n = 22) long, 575 (415–980, n = 24) wide. Clamps located ventrally, each with a short peduncle (Figures 2A, 5A, 5C), positioned on left side of worm (on right side of drawing of worm in ventral view). First clamp (anterior to haptoral groove) 65 (46–74, n = 22) long, 54 (32–74, n = 22) wide; second clamp 65 (48–76, n = 20) long, 58 (42–76, n = 20) wide; third clamp 63 (49–76, n = 22) long, 57 (42–72, n = 22) wide; fourth clamp 63 (46–72, n = 22) long, 57 (38–76, n = 22) wide. Clamps typical of Gastrocotylidae; each clamp formed by a ventral arm of median spring, accessory skeletal piece, ventral and dorsal jaws of right side, ventral and dorsal jaws of left side, dorsal arm of dorsal jaw, dorsal arm of ventral jaw and ventral and dorsal oblique sclerite (Figure 5B). Small haptoral groove, with raised edges, between the first and second clamps (Figures 2A, 5A, 7A, 7C). Haptoral lappet elongate ovate, wider than long. Length of lappet measured in three regions (Figure 2A); right side of haptoral lappet (rt) 173 (136–240, n = 22) long, middle region of haptoral lappet (mid) 206 (164–259, n = 22) long, left side of haptoral lappet (lf) 194 (124–259, n = 22) long. Haptoral lappet 769 (580–920, n = 22) wide (Figure 2A), armed with two pairs of anchors, and one pair of hooks (Figures 2A, 2C, 5A, 5C, 5E, 7C; Table 5).

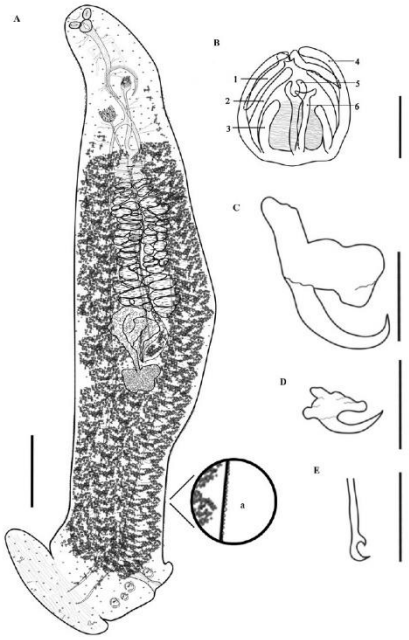

Figure 5. *Protomicrocotyle veracruzensis* sp. nov. (holotype). A. Whole worm (ventral side up), a = inset showing marginal folds, Bar 500 µm. B. Details of clamp, 1 = oblique sclerite; 2 = dorsal jaw;, 3 = dorsal arm of the ventral jaw; 4 = ventral jaw; 5 = accessory skeletal piece; 6 = ventral arm of median spring, Bar 30 µm. C. Lateral anchor, Bar 15 µm. D. Median anchor, Bar 20 µm. E. Hook, Bar 15 µm.

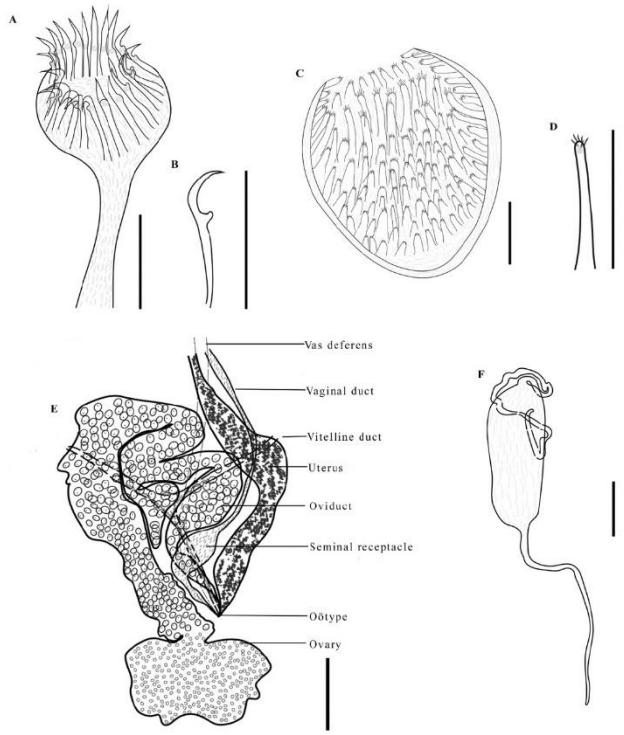

Figure 6. *Protomicrocotyle veracruzensis* sp. nov. A. Male copulatory organ, Bar 50 µm. B. Spine of male copulatory organ, Bar 40 µm. C. Vaginal vestibule, Bar 25 µm. D. Spine of vaginal vestibule, Bar 40 µm. E. Female reproductive organs, Bar 200 µm. F. Egg, Bar 80 µm.

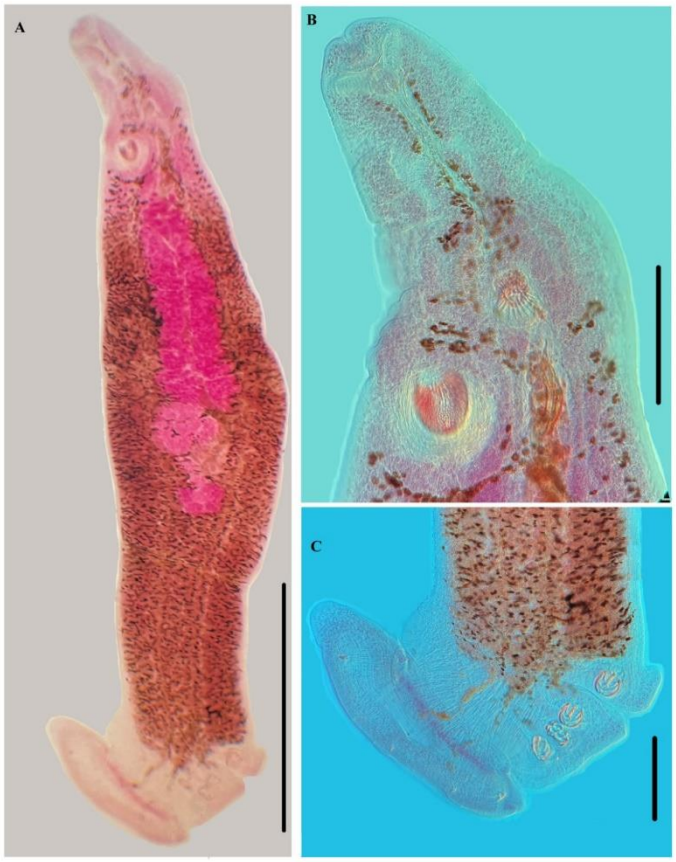

Figure 7. Photograph of *Protomicrocotyle veracruzensis* sp. nov. (Holotype). A. Whole specimen (ventral view), Bar 125 µm. B. Prohaptor, Bar 500 µm. C. Haptor, Bar 500 µm.

Table 4. Comparative morphological characteristics of the species of the genus *Protomicrocotyle*. Measurements are presented in micrometers.

Species	***Protomicrocotyle veracruzensis* sp. nov.**	***P. mirabilis* (redescription)**
Hosts	***Caranx latus***	***C. hippos* and *C. latus***
Location	**Casitas and Puerto de Veracruz, Veracruz, México**	**Florida, EUA and Veracruz, Mexico**
Total length	3858 (3209–4709)	805–4673
Total Width	804 (586–1098)	134–537
Haptor width	575 (415–980)	70–425
Right side of haptoral lappet length	173 (136–240)	60–217
Left side of haptoral lappet length	194 (124–259)	40–202
Central haptoral lappet length	206 (164–259)	40–214
Haptoral lappet width	769 (580–920)	105–785
Clamps length	64 (47–74)*	14.2–67
Number of testes	47 (36–69)	14–38
Testes length	47 (31–59)	17–79
Testes width	142 (96–176)	14–86
Testes-Ratio length: width	01:03	1:0.8–1:1.1
Vaginal opening to anterior end	610 (440–730)	175–740
Vaginal opening to genital pore	506 (340–615)	140–660
Number of spines in MCO	23 (16–28)	16–29
Number of spines in vestibule vaginal	49 (33–59)	31–65
Reference	Present study	Present study

*Data obtained from the average length of the four clamps. Values in **bold** were taken of the line drawings. MCO = male copulatory organ.

Table 4. Continued...

Species	***P. manteri***	***P. nayaritensis***	***P. ivoriensis***
Hosts	***Trachinotus paitensis***	***C. caninus***	***C. hippos***
Location	**La Paz, BCS, Mexico**	**Nayarit, Mexico**	**Ivory Coast**
Total length	1360–3680	6820–8580	3200–4600
Total Width	400–800	770–1990	600–1200
Haptor width	**590**	1050	442
Right side of haptoral lappet length	**136**	**250**	**173**
Left side of haptoral lappet length	**121**	**150**	**178**
Central haptoral lappet length	**181**	**300**	**134**
Haptoral lappet width	**348**	643–801	500
Clamps length	50–55	45–48	44
Number of testes	22–33	46–48	50
Testes length	60.2	88	58.8
Testes width	116.5	129	99.5
Testes-Ratio length: width	01:01.8	01:01.4	01:01.6
Vaginal opening to anterior end	84–155	1072–1258	442
Vaginal opening to genital pore	289–471	858–1001	365
Number of spines in MCO	33–38	48–54	25–26
Number of spines in vestibule vaginal	15	22–46	–
Reference	Bravo-Hollis (1966)	Bravo-Hollis (1979)	Wahl (1972)
Species	***P. celebensis***	***P. madrasensis***	***P. mannarensis***
Hosts	***Caranx* sp.**	***C. affinis***	***C. sexfasciatus***
Location	**Indonesia**	**India**	**India**
Total length	4000–5100	2390	2880–3640
Total Width	600–830	410	480–1140
Haptor width	–	300	434
Right side of haptoral lappet length	**111**	**250**	**130**
Left side of haptoral lappet length	**111**	**250**	**152**
Central haptoral lappet length	**166**	**112**	**213**
Happtoral lappet width	–	240	320–440
Clamps length	–	32–48	30–46
Number of testes	40–55	64	42–52
Testes length	–	33–38	21–33
Testes width	–	58–81	67–181
Testes-Ratio length: width	–	1:1.7-1:2.1	1:3-1:5.4
Vaginal opening to anterior end	500–830	462	526
Vaginal opening to genital pore	375–630	362	447
Number of spines in MCO	16–22	24	24
Number of spines in vestibule vaginal	–	31	37
Reference	Yamaguti (1953)	Ramalingam (1960)	Ramalingam (1960)
Species	***P. minutum***	***P. carangis***	
Hosts	***C. sexfasciatus***	***C. sansun***	
Location	**India**	**India**	
Total length	1500	2540–3100	
Total Width	300	460–540	
Haptor width	239	–	
Right side of haptoral lappet length	**65**	–	
Left side of haptoral lappet length	**65**	–	
Central haptoral lappet length	**119**	–	
Haptoral lappet width	360	320–410	
Clamps length	52	48	
Number of testes	34	20–24	
Testes length	14	–	
Testes width	34–44	–	
Testes-Ratio length: width	1:2.4-1:3.1	–	
Vaginal opening to anterior end	304	–	
Vaginal opening to genital pore	260	–	
Number of spines in MCO	12	22	
Number of spines in vestibule vaginal	19	14–16	
Reference	Ramalingam (1960)	Ramalingam (1960)	

*Data obtained from the average length of the four clamps. Values in **bold** were taken of the line drawings. MCO = male copulatory organ.

Table 5. Measurements (in µm) of haptoral armature (anchors, and hooks) of *Protomicrocotyle veracruzensis* sp. nov.

	Right lateral anchor (Ac1)	Left lateral anchor (Ac1')	Right medial anchor (Ac2)	Left medial anchor (Ac2')	Right central hook (Ho)	Left central hook (Ho')
Total length (a)	43 (38–50, n = 24)	42 (40–47, n = 24)	27 (25–29, n = 20)	27 (24–29, n = 20)	18 (17–19, n = 22)	18 (17–19, n = 22)
Total width (b)	5 (5–7, n = 24)	6 (5–7, n = 24)	4 (2–5, n = 22)	3 (2–4, n = 20)	2 (2–2, n = 22)	2 (2–2, n = 22)
Opening length (c)	10 (7–13, n = 24)	10 (7–13, n = 23)	5 (4–8, n = 22)	6 (4–11, n = 20)	-	-
Internal root length (d)	16 (11–19, n = 24)	15 (11–17, n = 24)	7 (5–11, n = 22)	9 (7–12, n = 20)	-	-
Internal root width (e)	7 (5–10, n = 24)	7 (5–8, n = 24)	3 (2–4, n = 22)	3 (2–5, n = 20)	-	-
External root length (f)	16 (13–20, n = 24)	15 (12–19, n = 24)	9 (7–13, n = 22)	9 (7–11, n = 20)	-	-
External blade length (g)	23 (18–29, n = 24)	24 (17–34, n = 24)	16 (13–19, n = 22)	18 (14–20, n = 20)	-	-
Internal blade length (h)	20 (16–25, n = 24)	20 (13–31, n = 24)	13 (11–17, n = 22)	15 (12–20, n = 20)	-	-
Point length (i)	13 (5–19, n = 24)	15 (8–24, n = 24)	9 (5–12, n = 22)	11 (8–13, n = 20)	-	-

The letters in the parenthesis are represented in Figure 2. *n*: number of specimens measured.

Male reproductive structures (Figures 5A, 6A, 6B, 7A, 7B). Testes 47 (36–69, n = 23) in number, depressed elliptoid (and some are shallowly elliptoid), intercecal, anterior to descending ducts and germarium, arranged in two parallel fields, each field with one or two interspersed testes wide, forming an irregular column. Testes 47 (31–59, n = 24) long, 142 (96–176, n = 24) wide (Figure 5A), shallowly-doliform to shallowly-elliptoid in shape. Vas deferens dorsal to testes, running in zigzag pattern from anterior part of testes to MCO. Male copulatory organ 83 (74–91, n = 24) long, 68 (55–82, n = 24) wide, subspherical (Figures 3A, 6A, 6B, 7A, 7B), armed with 23 (16–28, n = 23) spines. Spines of MCO hooklike, 43 (38–47, n = 24) long, 3 (2–5, n = 24) wide, arranged in a circle on anterior part of MCO (Figure 6A, 6B). Each spine with small knob in the anterior region just posterior to curved tip, knob and curved tip directed outwards (Figure 6B). Genital atrium ventral, near midline, anterior to cecal bifurcation, opening at 506 (340–615, n = 24) from anterior end of body (Figure 5A).

Female reproductive structures (Figures 5A, 6C, 6E, 7A, 7B). Germarium intercecal, post-testicular, comprised of germarial bulb with immature oöcytes, 161 (133–197, n = 24) long, 188 (149–232, n = 24) wide, with a wide ascending duct and an irregularly descending duct that form loops that contain mature oöcytes. Descending duct united to the oötype just anterior to the ovary (Figure 6E). Vaginal duct/seminal receptacle and vitelline duct connect to oötype; uterus ascends from oötype to the genital atrium (Figure 6E). Vaginal pore ventral, anterior to vaginal vestibule. Vaginal vestibule 92 (78–103, n = 24) long, 72 (49–96, n = 24) wide (Figures 5A, 6C, 6D, 7A, 7B), located 610 (440–730, n = 24) from anterior end of body and 203 (130–225, n = 24) from the genital pore, lateral to midline on the side opposite to that having the haptoral clamps. Vaginal vestibule armed with 49 (33–59, n = 23) spines 42 (38–46, n = 24) long, 4 (2–5, n = 24) wide; basal spines smallest, uppermost spines largest (Figure 6C). Each spine elongated in shape with 5 (4–6, n = 24) small needle-like spikes at the distal end (Figure 6D). Vaginal duct descends from vaginal vestibule to germarium; small elongate seminal receptacle 25 (18–33, n = 4) long, 15 (8–21, n = 4) wide, at terminal end of vaginal duct, connected to ventral side of germarium (Figure 6E). Vitelline glands in two lateral fields, starting just posterior to vaginal vestibule, overlapping ceca anteriorly and posteriorly and the lateral margin of the testes, uniting posterior to germarium, extending to posterior region of body but not reaching the haptoral lappet (Figures 2A, 5A, 7A, 7C). Vitellogenic ducts from each field unite in the region dorsal to the germarium, forming a single vitelline channel that leads to and connects to the oötype (Figures 5A, 6E).

Eggs (Figure 6F). Uterus contains 3 (1–8, n = 13) eggs. Eggs elongate, elliptoid, with long polar filaments at each pole; length, not including polar filaments, 182 (128–229, n = 15), 57 (47–72, n = 15) wide. Filament in anterior end (as positioned in uterus) 237 (210–250, n = 7) long, 5 (4–6, n = 7) wide, and posterior end 191 (137–247, n = 5) long, 4 (2–6, n = 5) wide.

Taxonomic summary

Type host: *Caranx latus* (Carangidae).

Common name: Horse-eye jack.

Type locality: Littoral waters of Gulf of Mexico off Casitas (20° 15′ 31.5″ N; 96° 47′49.5″ W), Veracruz, Mexico; collected in May 2005.

Site of infection: Gill filaments

Other locality: Littoral waters of Gulf of Mexico off Puerto de Veracruz (19° 13′ 11.2″ N; 96° 09′ 24.4″ W), Veracruz, Mexico; collected in May 2022.

Etymology: The specific epithet is derived from the name of the state of Veracruz, Mexico, where these specimens were collected.

Abundance, prevalence, mean intensity of infection and range of intensity: General infection characterization: *C. latus*, 12.17 of abundance, 2 infected fish of 6 examined (33.33%), 36.50 and 14–59. Casitas: 2.80 of abundance, 1 infected fish of 5 examined (20%), 14 and 14; Puerto de Veracruz: 59 of abundance, 1 infected fish of 1 examined (100%), 59 and 59.

Specimen deposit: holotype CNHE-12819; paratypes CNHE-12820 to 12821, HWML-216978 to 216984 and 2166998, CHE-P00148.

ZooBank registration: D3E641BF-5A28-447E-A097-47813D3CA67C

Genbank accession numbers: Cytochrome C Oxidase Subunit I (CO1) OR282833 to OR282836; 28S rDNA OR282895 to OR282898.

Remarks

Within the suite of characters of the genus that were mentioned above, several differences in character states have been used to distinguish between species of *Protomicrocotyle*: the number of testes (Yamaguti, 1953; Bravo-Hollis, 1966; Pillai & Pillai, 1978); the number and shape of spines on the male copulatory organ (Yamaguti, 1953; Ramalingam, 1960; Pillai & Pillai, 1978; Kritsky et al., 2011); and the number of vaginal spines (Ramalingam, 1960; Wahl, 1972; Pillai & Pillai, 1978). Employing these characters, *P. veracruzensis* sp. nov. has 47 (36–69 testes; *P. mirabilis* 27 (23–33 testes) (Kritsky et al., 2011), *P. carangis* (20–24) (Pillai & Pillai, 1978), *P. manteri* (22–33) (Bravo-Hollis, 1989), *P. minutum* (34) (Ramalingam, 1960) have less testes and the new species and *P. celebesensis* (40–57) (Yamaguti, 1953), *P. ivoriensis* (50) (Wahl, 1972), *P. madrasensis* (64) (Ramalingam, 1960), *P. mannarensis* (42–52) (Ramalingam, 1960) and *P. nayaritensis* (46–48) (Bravo-Hollis, 1979) appear to have a number of testes that overlap with that of the new species, although detailed data is lacking in some species of each group. The male copulatory organ of *P. veracruzensis* sp. nov. has 23 (16–28) spines: the MCO of *P. minutum* has less (12 spines) (Ramalingam, 1960) and *P. manteri* and *P. nayaritensis* have more (33–38 and 48–54 spines, respectively); for the other species, *P. carangis* (22), *P. celebesensis* (16–22), *P. ivoriensis* (25–26), *P. madrasensis* (24), *P. mannarensis* (24), and *P. mirabilis* (16–21), the number of spines overlaps with that of the new species. The vagina of the new species has 49 (33–59) spines; *P. carangis* (14–16), *P. madrasensis* (31), *P. mannarensis* (37), *P. manteri* (15), and *P. minutum* (19) all have less vaginal spines, *P. mirabilis* (49) and *P. nayaritensis* (22–46) have numbers of vaginal spines that overlap with the new species, and there is no information for *P. celebesensis* and *P. ivoriensis*.

Some authors have mentioned the arrangement of the testes in their descriptions, but often they have mention only that the testes are arranged in two fields/rows (Yamaguti, 1953; Ramalingam, 1960; Pillai & Pillai, 1978; Bravo-Hollis, 1979). The testes of *P. mirabilis*, *P. carangis*, and *P. mannarensis* are arranged in a single row on each side of the midline (MacCallum, 1918; Ramalingam, 1960; Wahl, 1972; Pillai & Pillai, 1978; Kritsky et al., 2011). The testes of *P. veracruzensis* sp. nov., *P. minutum*, and *P. nayaritensis* are arranged in rows with 1–2 adjacent testes on each side (Ramalingam, 1960; Bravo-Hollis, 1966), in *P. madrasensis* they appear to be in row with 2 adjacent testes on each side (Ramalingam, 1960), and those of *P. ivoriensis* have rows with 1–3 adjacent testes (Wahl, 1972).

Of the known species of *Protomicrocotyle,* the new species is most similar to the species from the Atlantic Ocean basin, *P. mirabilis* and *P. ivoriensis*. Using the structures mentioned above, the new species can be distinguished from *P. mirabilis* by having more testes (36–69 vs. 23–33, respectively); there is overlap in the number of testes of *P. ivoriensis* (36–69 vs. 50, respectively) by having less testes, although, if the number of testes reported for the latter species was invariant, or an average, is not known. The new species also can be distinguished from these two species by the arrangement of the testes; the new species has 1–2 adjacent testes in the lateral rows vs. *P. mirabilis*, which has testes in a single-file row in each field and *P. ivoriensis* has groups of 1–3 adjacent testes in each row. The relation between the length and width (L: W) of the testes in the new species is L: W: 1:3 and in *P. mirabilis* is L: W: 1:1.1, showing that the testes of the new species are larger than those of *P. mirabilis*. The relationship between the length and width of the testes of the new species is also greater than in *P. ivoriensis* (1:3 vs. 1:1.6), *P. madrasensis* (1:3 vs. 1:1.7-2.1), *P. manteri* (1:3 vs. 1:1.8), *P. nayaritensis* (1:3 vs. 1:1.4), smaller than *P. mannarensis* (1:3 vs. 1:3-5.41) and in range with *P. minutum* (1:3 vs. 1:2.4-3.1). Complete detailed comparative data for the new species and the other nine valid species is given in Table 4.

Morphometric analyses

Results of the principal components analysis (PCA) show an accumulated variance of 52.75 (Table 6A) of the first two components and the morphological variables that most influence the multidimensional arrangement of the first component. They are, in order of importance: the width of the body, the width of the ovary, the width of the haptor, the average width of the testes, the distance from the vaginal vestibule to the genital pore and the total longitude of the body. The variables that most influenced the second component are, in order of importance: the number of spines of the vaginal vestibule and the length of the spines of the vaginal vestibule. Eigenvalues and accumulated variance for first two principal components are presented in Table 6A.

In the Discriminant Analysis (DA), the first two factor axes explained 88.52% of the total variation (Table 6B). The first factor (eigenvalue 55.73, 46.88 of variation) alone was the main discriminant function, which arranged the specimens of *Protomicrocotyle* and *Neomicrocotyle* with the morphological variables, in order of importance: the number of spines in the male copulatory organ and the length of the spines of the vaginal vestibule. The second factor (eigenvalue 49.49, 41.64 of variation) has a value almost equal to the first factor, and separated the specimens of *Protomicrocotyle* and *Neomicrocotyle* with the morphological variables, in order of importance: the width of the body, number of spines of the vaginal vestibule, number of testes, longitude of the male copulatory organ and the width of the ovary. The DA, like the PCA, in the multidimensional plane, separated the five groups that correspond to the specimens of *P. mirabilis*, *P. manteri*, *P. nayaritensis*, *Neomicrocotyle pacifica* and the new species, *P. veracruzensis* sp. nov. (Figure 8A, 8B). An important finding was that Factor 1, of the DA, separated the specimens of the Atlantic species (*P. mirabilis* and *P. veracruzensis* sp. nov.) from the specimens of the Pacific species (*P. manteri*, *P. nayaritensis*, and *Neomicrocotyle pacifica*). Finally, Factor 2, separated the specimens of *Protomicrocotyle* from *Neomicrocotyle pacifica* (Figure 8B).

Molecular study

Sequences (16 of CO1 and 14 of 28S), belonging to *Protomicrocotyle mirabilis* (OR282821–OR282832; OR282885–OR282894) and the new species (OR282833–OR282836; OR282895–OR282898) were obtained in the present study (Table 2). The sequences of CO1 and sequences of 28S from GenBank® (Sayers et al., 2020) of *Bilaterocotyloides carangis* Ramalingam, 1961, *Bilaterocotyloides madrasensis* Radha, 1966, and *Allodiscocotyla diacanthi* Unnithan, 1962 were used in the distance's genetic analyses and Neighbor-Joining (Table 2). The final alignment of CO1 consisted in 20 sequences, with a length of 403 bp. The 14 sequences of 28S were aligned with 8 sequences of 28S from GenBank (*Neomicrocotyle pacifica* (Meserve, 1938) Yamaguti, 1968; *Neomicrocotyle* sp.; *Lethacotyle vera* Justine, Rahmouni, Gey, Schoelinck & Hoberg, 2013; *B. carangis*; *B. madrasensis*; and *A. diacanthi*) (Table 2). The final alignment consisted of the 22 partial sequences of the 28S region, with a length of 750 bp.

The intraspecific genetic variation between specimens of *P. mirabilis* was 0% to 2.01% for CO1 and 0% to 0.14% for 28S. Between specimens of *P. veracruzensis* sp. nov., the variation was 0.25% to 0.75% for CO1 and 0% for 28S. The interspecific genetic variation between specimens of *P. mirabilis* and *P. veracruzensis* sp. nov. was 8.48% to 10.53% with CO1, and 0.81% to 0.95% with 28S (Figure 9A, 9B; Table 7A, 7B).

Table 6. Cumulative variance of (**A**) the first two principal components and (**B**) factor values of discriminant analyses.

A			
Principal Component	**Eigenvalue**	**% variance**	**% Accumulate variance**
1	0.81	44.10	44.10
2	0.16	8.651	52.75

B			
Factor	**Eigenvalue**	**% variance**	**% Accumulate variance**
1	55.73	46.88	46.88
2	49.49	41.64	88.52

The NJ analysis using CO1 and 28S sequences of all species of Protomicrocotylidae currently in GenBank and *Allodiscocotyla diacanthi* from GenBank as outgroup (Table 2) resulted in the identification of a single group of *Protomicrocotyle* with support of 99% with CO1 and 95% with 28S (Figure 9A, 9B). *Protomicrocotyle mirabilis* and *P. veracruzensis* sp. nov. form each one an independent group with the support of 100% with CO1 (Figure 9A), and 92% and 99% with 28S (Figure 9B), respectively. *Protomicrocotyle* nested within members of the family Protomicrocotylidae with high support with both molecular markers (Figure 9A, 9B).

Table 7. Intraspecific and interspecific genetic distances estimated for (**A**) mitochondrial cytochrome c oxidase subunit I (CO1) and (**B**) 28S rDNA of *Protomicrocotyle veracruzensis* sp. nov. and other species of Protomicrocotylidae. Pairwise corrected *p-distances* are expressed as percentages (%).

A								
	KF804045	**KF804046**	**KF804041**	**KF804043**	**OR282821***	**OR282822***	**OR282823***	**OR282824***
KF804045								
KF804046	3.55%							
KF804041	19.86%	22.34%						
KF804043	18.09%	20.21%	12.41%					
OR282821*	20.21%	23.05%	16.67%	15.25%				
OR282822*	20.21%	23.05%	16.31%	14.89%	**1.00%**			
OR282823*	20.21%	23.05%	16.31%	14.89%	**1.24%**	**0.74%**		
OR282824*	20.92%	23.76%	16.31%	14.89%	**0.77%**	**1.28%**	**1.28%**	
OR282825*	20.21%	23.05%	16.31%	14.89%	**0.50%**	**0.50%**	**0.74%**	**0.77%**
OR282826*	20.21%	23.05%	16.31%	15.25%	**0.75%**	**0.75%**	**1.00%**	**1.02%**
OR282827*	20.21%	23.05%	16.31%	14.89%	**0.50%**	**0.50%**	**0.75%**	**0.77%**
OR282828*	20.21%	23.05%	16.31%	15.25%	**1.00%**	**0.99%**	**1.24%**	**1.28%**
OR282829*	20.57%	23.40%	16.67%	15.25%	**1.00%**	**0.99%**	**1.24%**	**0.77%**
OR282830*	20.21%	23.05%	16.31%	14.89%	**0.75%**	**0.74%**	**0.99%**	**1.02%**
OR282831*	19.86%	22.70%	16.67%	14.54%	**1.00%**	**1.00%**	**1.25%**	**1.29%**
OR282832*	20.92%	23.76%	16.31%	15.60%	**1.26%**	**1.75%**	**2.01%**	**1.03%**
OR282833**	20.57%	23.40%	15.60%	16.31%	*9.20%*	*8.71%*	*9.45%*	*9.21%*
OR282834**	20.92%	23.76%	15.25%	16.67%	*9.45%*	*8.68%*	*9.43%*	*9.46%*
OR282835**	20.92%	23.76%	15.25%	16.67%	*8.96%*	*8.68%*	*9.43%*	*8.95%*
OR282836**	20.92%	23.76%	15.25%	16.67%	*9.20%*	*8.93%*	*9.68%*	*9.21%*
	OR282825*	**OR282826***	**OR282827***	**OR282828***	**OR282829***	**OR282830***	**OR282831***	**OR282832***
OR282826*	**0.75%**							
OR282827*	**0.00%**	**0.75%**						
OR282828*	**0.99%**	**0.25%**	**1.00%**					
OR282829*	**0.99%**	**0.75%**	**1.00%**	**0.74%**				
OR282830*	**0.74%**	**0.50%**	**0.75%**	**0.50%**	**0.50%**			
OR282831*	**0.50%**	**1.26%**	**0.50%**	**1.00%**	**1.25%**	**1.00%**		
OR282832*	**1.25%**	**1.52%**	**1.26%**	**1.50%**	**0.75%**	**1.25%**	**1.50%**	
OR282833**	*8.71%*	*9.52%*	*8.73%*	*9.70%*	*9.70%*	*9.45%*	*9.27%*	*10.05%*
OR282834**	*9.18%*	*9.27%*	*8.98%*	*9.68%*	*9.68%*	*9.43%*	*9.75%*	*10.53%*
OR282835**	*8.68%*	*9.27%*	*8.48%*	*9.68%*	*9.68%*	*9.43%*	*9.25%*	*10.03%*
OR282836**	*8.93%*	*9.52%*	*8.73%*	*9.93%*	*9.93%*	*9.68%*	*9.50%*	*10.28%*
	OR282833**	**OR282834****	**OR282835****	**OR282836****				
OR282834**	**0.75%**							
OR282835**	**0.75%**	**0.50%**						
OR282836**	**0.75%**	**0.74%**	**0.25%**					

The values in **bold** represents the intraspecific divergence in *Protomicrocotyle mirabilis* and *P. veracruzensis* sp. nov; The values in *italics* represents the interspecific divergence between *P. mirabilis* and *P. veracruzensis* sp. nov. *Sequences of *Protomicrocotyle mirabilis*; **Sequences of *Protomicrocotyle veracruzensis* sp. nov.

Table 7. Continued...

B								
	KF804033	**KF804038**	**KF804029**	**KF804037**	**KJ201022**	**AF382043**	**KF378589**	**KF378588**
KF804033								
KF804038	0.00%							
KF804029	5.25%	5.25%						
KF804037	5.25%	5.25%	0.00%					
KJ201022	5.29%	5.29%	0.44%	0.44%				
AF382043	6.06%	6.06%	2.42%	2.42%	2.94%			
KF378589	5.93%	5.93%	2.20%	2.20%	2.79%	0.14%		
KF378588	6.21%	6.21%	3.03%	3.03%	3.38%	1.79%	1.65%	
OR282885*	5.25%	5.25%	2.55%	2.55%	3.23%	2.69%	2.48%	2.20%
OR282886*	5.14%	5.14%	2.43%	2.43%	3.10%	2.83%	2.63%	2.35%
OR282887*	5.26%	5.26%	2.56%	2.56%	3.24%	2.69%	2.48%	2.21%
OR282888*	5.26%	5.26%	2.56%	2.56%	3.24%	2.70%	2.48%	2.21%
OR282889*	5.15%	5.15%	2.44%	2.44%	3.08%	2.84%	2.62%	2.34%
OR282890*	5.17%	5.17%	2.45%	2.45%	3.09%	2.85%	2.62%	2.34%
OR282891*	5.31%	5.31%	2.59%	2.59%	3.23%	2.72%	2.48%	2.20%
OR282892*	5.27%	5.27%	2.56%	2.56%	3.23%	2.70%	2.48%	2.20%
OR282893*	5.20%	5.20%	2.46%	2.46%	3.10%	2.87%	2.63%	2.35%
OR282894*	5.16%	5.16%	2.44%	2.44%	3.09%	2.85%	2.62%	2.34%
OR282895**	5.38%	5.38%	2.96%	2.96%	3.67%	2.82%	2.62%	2.48%
OR282896**	5.38%	5.38%	2.96%	2.96%	3.67%	2.82%	2.62%	2.48%
OR282897**	5.38%	5.38%	2.96%	2.96%	3.67%	2.82%	2.62%	2.48%
OR282898**	5.41%	5.41%	2.97%	2.97%	3.68%	2.83%	2.62%	2.48%
	OR282885*	**OR282886***	**OR282887***	**OR282888***	**OR282889***	**OR282890***	**OR282891***	**OR282892***
OR282886*	**0.13%**							
OR282887*	**0.00%**	**0.13%**						
OR282888*	**0.00%**	**0.14%**	**0.00%**					
OR282889*	**0.14%**	**0.00%**	**0.14%**	**0.14%**				
OR282890*	**0.14%**	**0.00%**	**0.14%**	**0.14%**	**0.00%**			
OR282891*	**0.00%**	**0.14%**	**0.00%**	**0.00%**	**0.14%**	**0.14%**		
OR282892*	**0.00%**	**0.14%**	**0.00%**	**0.00%**	**0.14%**	**0.14%**	**0.00%**	
OR282893*	**0.14%**	**0.00%**	**0.14%**	**0.14%**	**0.00%**	**0.00%**	**0.14%**	**0.14%**
OR282894*	**0.14%**	**0.00%**	**0.14%**	**0.14%**	**0.14%**	**0.14%**	**0.14%**	**0.14%**
OR282895**	*0.81%*	*0.94%*	*0.81%*	*0.81%*	*0.95%*	*0.95%*	*0.82%*	*0.81%*
OR282896**	*0.81%*	*0.94%*	*0.81%*	*0.81%*	*0.95%*	*0.95%*	*0.82%*	*0.81%*
OR282897**	*0.81%*	*0.94%*	*0.81%*	*0.81%*	*0.95%*	*0.95%*	*0.82%*	*0.81%*
OR282898**	*0.81%*	*0.95%*	*0.81%*	*0.81%*	*0.95%*	*0.95%*	*0.82%*	*0.81%*
	OR282893*	**OR282894***	**OR282895****	**OR282896****	**OR282897****	**OR282898****		
OR282894*	**0.14%**							
OR282895**	*0.96%*	*0.95%*						
OR282896**	*0.96%*	*0.95%*	**0.00%**					
OR282897**	*0.96%*	*0.95%*	**0.00%**	**0.00%**				
OR282898**	*0.96%*	*0.95%*	**0.00%**	**0.00%**	**0.00%**			

The values in **bold** represents the intraspecific divergence in *Protomicrocotyle mirabilis* and *P. veracruzensis* sp. nov; The values in *italics* represents the interspecific divergence between *P. mirabilis* and *P. veracruzensis* sp. nov. *Sequences of *Protomicrocotyle mirabilis*; **Sequences of *Protomicrocotyle veracruzensis* sp. nov.

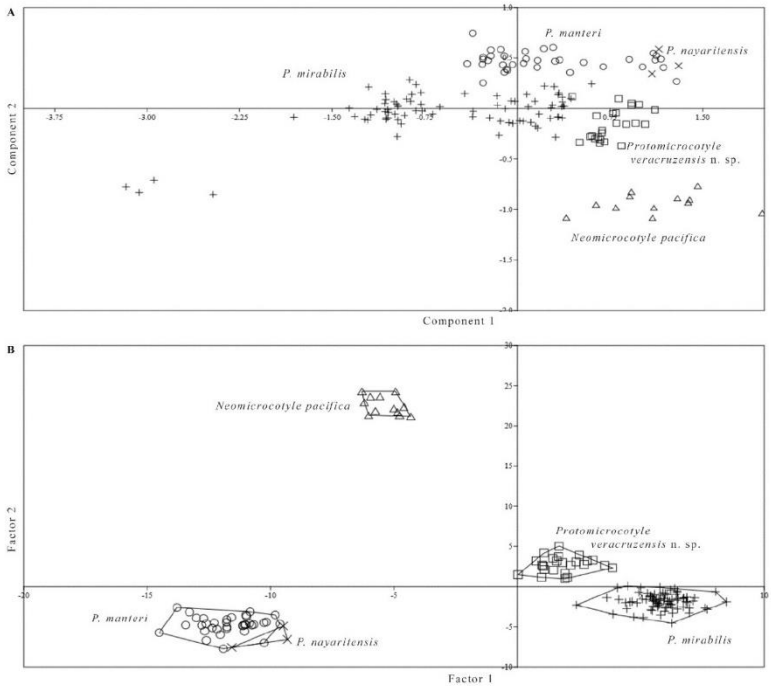

Figure 8. Morphometric analyses of the characteristics of species of *Protomicrocotyle*. A. Principal components analyses. B. Discriminant analyses.

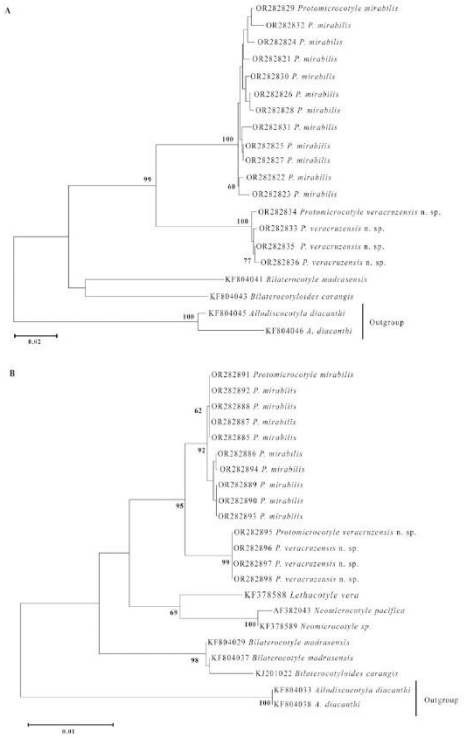

Figure 9. Cluster analysis showing similarities between specimens of *Protomicrocotyle* and other protomicrocotylids. A. Dendrogram of sequences of cytochrome c oxidase subunit I. B. Dendrogram of sequences of 28S rDNA. Note: Values in bold are the bootstrap values.

Discussion

As with many groups of helminths, the time span since the first member of the group was described by MacCallum (1918) and today's descriptions encompass many advances in the development of our understanding of the characteristics that reveal the limits between species. For groups that are not well-studied, like the genus *Protomicrocotyle*, a suite of characters are most useful for the identification of members on a group level instead of a single diagnostic feature. This has resulted in descriptions which mention only the characters that are known at that time in order to distinguish the new species from a particular congener. The same features, as the number of testes, the number of spines in the MCO, and the number of spines in the vagina were used to distinguish the new species from others of the genus. However, these and other values often overlapped with particular species in such a way that no single character could distinguish the new species from the other species of *Protomicrocotyle*. For better understanding of the intra- and interspecific variation, Table 4, with the measurements of the 10 known species, was developed and included herein. Most of the measurements of the previously known species were taken from published descriptions (see Table 4 for citations of the works that were consulted). Possibly because some authors were only reporting a new locality or host record, many of those articles did not provide a full description, only mentioning those characters that helped them assign their material. The limitations of that material and those descriptions were not sufficient to confirm the assignment of those specimens to a particular species (Table 3). In those cases, new material and molecular studies will be needed for confirmation of their identity.

One area of interest is in the use of the measurements of the hard parts (hooks, anchors, spines, etc.) as informative characters in descriptions of new taxa; a few examples of the use of the measurements of these structures in recent studies of monogeneans are mentioned herein. Vaughan & Christison (2012), proposed a measurement scheme for the haptoral armament of members of *Callorhynchocotyle* Suriano & Incorvaia, 1982 and used the measurements in a systematic study of the members of that genus. Most recently, Vaughan et al. (2021) used the same scheme and provided the measurements of the haptoral armament of *Scyliorhinocotyle narvaezae* Vaughn, Christison & Hansen, 2021. These works prompted us to provide the measurements for the haptoral armament in this study (Table 5). It is hoped that similar measurement data will be provided by authors in future descriptions of new species of *Protomicrocotyle*.

In the present study, the seminal receptacle of *P. veracruzensis* sp. nov. was observed and described, although it could not be distinguished in all specimens. This structure often is not mentioned in descriptions of other members of the genus. In most cases, whether the structure is present or not, or whether it was just not mention, cannot be determined. However, Ramalingam (1960) described and illustrated the seminal receptacles of *P. madrasensis*, *P. mannarensis*, and *P. minutum*. A review detailed of the material of the remaining members of the genus is necessary in order to verify the presence or absence of the seminal receptacle in those species. In most cases, collections of new material will be required.

In a similar manner, many authors have not mentioned whether the clamps are sessile or pedunculate. Because the data is incomplete or conflicting, this feature was not used in the comparisons. Of the nine previously-known species, four authors have reported that three species of *Protomicrocotyle* have clamps with peduncles: *P. nayaritensis*, *P. mirabilis*, and *P. manteri* [see Bravo-Hollis (1979), Caballero y Caballero & Bravo-Hollis (1965), MacCallum (1918), and Bravo-Hollis (1966), respectively]. Three authors have reported species that have clamps that are sessile: *P. mirabilis*, by Kritsky et al. (2011), contrary to previous reports by Caballero y Caballero & Bravo-Hollis (1965) and MacCallum (1918); *P. madrasensis*, *P. mannarensis*, and *P. minutum* by Ramalingam (1960); and *P. carangis* by Pillai & Pillai (1978). The presence or absence of peduncles for *P. mirabilis* was not mentioned by Johnston & Tiegs (1922), Koratha (1955), Wahl (1972), and Bravo-Hollis (1989), and for *P. celebesensis* by Yamaguti (1953), and Barton et al. (2009). Wahl (1972) did not mention peduncles for *P. ivoriensis*; however, in the drawing of *P. ivoriensis* the clamps are shown to have peduncles [Wahl (1972), his Figure 2a]. The clamps of the new species, *P. veracruzensis* sp. nov., have peduncles similar to those of *P. ivoriensis*, and specimens of *P. mirabilis* reported as part of this study had clamps with peduncles. In all these species, the peduncles are short [as mentioned by MacCallum (1918)], which might explain why they were not given emphasis by some authors.

Finally, the orientation of the clamps has been mentioned only by Kritsky et al. (2011), who said that they could be dextral or sinistral, but they did not mention their relationship to other structures. The clamps of specimens of *P. mirabilis* and *P. veracruzensis* sp. nov., were observed to be on the right in some worms and on the left side of other worms, but always they were opposite to the vaginal vestibule. The male copulatory organ also always was opposite to the vaginal vestibule, and always on the same side as the clamps. This has not been mentioned by previous author.

In Mexico, two species of *Protomicrocotyle* have been described, *P. manteri* and *P. nayaritensis* in the Pacific Ocean (Bravo-Hollis, 1966, 1979), and one, *P. mirabilis*, has been recorded in the Gulf of Mexico (Caballero y Caballero & Bravo-Hollis, 1965; Kritsky et al., 2011); other reports from the Caribbean Sea (Caballero y Caballero & Bravo-Hollis, 1967; Bravo-Hollis, 1989; Kritsky et al., 2011) and from Ivory Coast, Africa (Wahl, 1972) could not be evaluated properly because new material must be collected for comparative morphological and molecular studies.

This work adds a fourth species reported from Mexico; in this study, *P. mirabilis* and *P. veracruzensis* sp. nov. are the first members of the genus to be characterized with morphological and molecular data. With this new species, tenth member to the genus, the helminthological record of monogenean parasites of *C. latus* is now seven species: *Ahpua piscicola* Caballero y Caballero & Bravo-Hollis, 1973, *Allopyragraphorus winteri* (Caballero y Caballero & Bravo-Hollis, 1965) Bravo-Hollis & Salgado-Maldonado, 1983, *Cemocotyle noveboracensis* Price, 1962, *Cemocotylella elongata* (Meserve, 1938) Price, 1962, *P. mirabilis*, *P. veracruzensis* sp. nov., and *Pseudomazocraes selene* Hargis, 1957. Fifteen species have been reported from *C. hippos: Axine* sp., *Ahpua piscicola*, *Allopyragraphorus caballeroi* (Zerecero, 1960) Yamaguti, 1963, *A. hippos* (Hargis, 1956) Yamaguti, 1963, *A. winteri*, *Cemocotyle carangis* (MacCallum, 1913) Sproston, 1946, *C. noveboracensis* Price, 1962, *Cemocotylella elongata* (Meserve, 1938) Price, 1962, *N. pacifica*, *Pseudomazocraes monsivaisae* Caballero y Caballero & Bravo Hollis, 1955, *P. riojai* (Caballero y Caballero & Bravo-Hollis, 1963) Lebedev, 1970, *P. selene*, *P. mirabilis*, *Salinacotyle mexicana* (Caballero y Caballero & Bravo-Hollis, 1963) Lebedev, 1984 and, *Zeuxapta seriolae* (Meserve, 1938) Price, 1962 [see Mendoza-Garfias et al. (2017)].

The use of morphological and molecular information has been a useful tool in the description of new species of Monogenea (Aguiar et al., 2017; Camargo & Santos, 2020; Torres-Carrera et al., 2020; Zago et al., 2021; Ayadi et al., 2022; Dmitrieva et al., 2022), and the relevance of the integrative taxonomy approach in the description and redescription of species of helminth has recently been highlighted. This study, with the molecular characterization of the CO1 and 28S genes of *P. mirabilis* and *P. veracruzensis* sp. nov. can be added to this list.

The molecular analysis revealed that the level of interspecific genetic variation between *P. mirabilis* and *P. veracruzensis* sp. nov. with CO1 sequence data is distinctly higher than that of the interspecific genetic variation with 28S (Table 7), as one would expect; other studies show the same pattern of high interspecific genetic variation between members of species of the same genus (Ayadi et al., 2017; Camargo & Santos, 2020; Torres-Carrera et al., 2020). In the genus, *Microcotyle* Van Beneden & Hesse, 1863, intraspecific nucleotide divergence of 0% to 1.4% has been reported in *Microcotyle visa* Bouguerche, Gey, Justine & Tazerouti, 2019, and an interspecific difference of 9.5% to 10.7% between *Microcotyle isyebi* Bouguerche, Gey, Justine & Tazerouti, 2019 and *M. visa* (Bouguerche et al., 2019). However, percentages of genetic divergence within and among taxa cannot be interpreted until a more complete molecular database is available (Torres-Carrera et al., 2020). Even taking this into account, the use of morphological data and the combination of different molecular markers are convincing evidence sufficient to differentiate *P. veracruzensis* sp. nov. from the other known members of the genus. As well, the same integrative approach corroborates evidence of the presence of *P. mirabilis* in the localities sampled in this study and the previous report of the species from Tuxpan by (Caballero y Caballero & Bravo-Hollis, 1965) and Campeche (Caballero y Caballero & Bravo-Hollis, 1967).

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Ethics declaration

The authors declare to follow ethical standards of the Universidad Autónoma del Estado de Hidalgo for scientific publication.

Conflict of interest

The authors declare that they have no conflict of interest.

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Anexo B

Supplementary material

Matrix of morphological and molecular data used in the phylogenetic analyses in TNT.

Allodiscocotyla_dicanthi01 3.10-3.12 2.20-2.30 2.32-2.34 2.30 2.29 2.38
1.48-1.68 2.48-2.52 ? ? ? 1.45-1.51 1.30-1.34 1.3
1.38 ? ? ? ? ? ? 1.65-1.77 ? ? ? ?
? ? ? ? ? ? ? ? ? ? ? ?
? ? ? ? ? ? ? ? ? ? ? ?
? ? ? ? ? ? ? ? ? ? ? ?
? ? ? ? ? 1.45 1.56 1.88-1.90 1.18 ? ?
2.35 1.88-1.65 1.78 1.2 ? ? ? ? ? ? ? ?
0 2.21 1.3

Allodiscocotyla_dicanthi02 3.10-3.12 2.20-2.30 2.32-2.34 2.30 2.29 2.38
1.48-1.68 2.48-2.52 ? ? ? 1.45-1.51 1.30-1.34 1.3
1.38 ? ? ? ? ? ? 1.65-1.77 ? ? ? ?
? ? ? ? ? ? ? ? ? ? ? ?
? ? ? ? ? ? ? ? ? ? ? ?
? ? ? ? ? ? ? ? ? ? ? ?
? ? ? ? ? 1.45 1.56 1.88-1.90 1.18 ? ?
2.35 1.88-1.65 1.78 1.2 ? ? ? ? ? ? ? ?
0 2.21 1.3

Allodiscocotyla_chorinemi 3.18-3.41 2.30-2.40 ? ? ? ? ? ?
2.18-2.52 ? ? ? ? 1.30-1.48 1.26-1.32 1.48 1.26
1.56 1.38 ? ? ? 1.79-1.86 1.65-1.76 1.79-1.86 1.65-
1.76 1.79-1.86 1.65-1.76 1.79-1.86 1.65-1.76 ? ? ? ? ?
? ? ? ? ? ? ? ? ? ? ? ?
? ? ? ? ? ? ? ? ? ? ? ?
? ? ? ? ? ? ? ? ? ? 1.85-
1.98 4.48-1.52 0.60-0.70 ? 2.45-2.68 1.48-1.65 2.00-2.11 1.60-
1.78 ? ? ? ? ? ? ? 0 ? 1.26-1.30

Metacamopia_lebedevi 3.28-3.37 2.31-2.45 2.69-2.70 ? ? ? ?
? ? 2.49 ? ? ? ? 1.40-1.46 1.34 1.38 1.34
1.38 ? ? ? 1.53 1.43 1.41 1.48 1.53 1.43 1.41 1.48 ?
? ? ? ? ? ? ? ? ? ? ? ? ?
? ? ? ? ? ? ? ? ? ? ? ? ?
? ? ? ? ? ? ? ? ? ? ? ? ?
1.51 1.53 2.27-2.28 1.20-1.61 0.68-0.72 ? ? ? ? ?
? ? ? ? ? ? ? ? ? 2.14 1.08

Metacamopia_ologoplites 3.52-3.77 2.90-3.16 2.28-2.58 ? ? ? ?
? ? ? ? ? ? 1.66-1.69 1.57-1.63 1.66-1.83

1.54-1.76 1.66-1.83 1.55-1.76 ? ? ? ? ? ? ?
? ? ? ? ? ? ? ? ? ? ? ? ?
? ? ? ? ? ? ? ? ? ? ? ? ?
? ? ? ? ? ? ? ? ? ? ? ? ?
? ? ? ? ? 1.64 1.74 2.54 1.20-1.61 ? ? ?
? ? ? ? ? 2.37-2.50 1.64-1.90 ? ? ? ?
? ? 1.41-1.74

Gemmaecaputia_corrugata 3.04-3.42 2.40-2.62 ? ? 1.7-2.08 2.48-2.67 ? ? ? ? ? ? ? ? ? 1.24 1.51 1.6
1.72 ? ? ? 1.80-1.94 1.80-1.92 1.80-1.94 1.80-1.92
1.80-1.94 1.80-1.92 1.80-1.94 1.78-1.88 ? ? ? ? ?
? ? ? ? ? ? ? ? ? ? ? ? ?
? ? ? ? ? ? ? ? ? ? ? ? ?
? ? ? ? ? ? ? ? ? 1.44-1.57 1.92-2.00 1.80-2.08 1.00-1.30 ? ? 2.30-2.51 1.78-2.04 ? ? ?
? ? ? ? ? ? ? ? ? 1.20-1.28

Protomicrocotyle_mirabilis1 1.46-14.39 1.22-10.15 1.80-10.49 1.34-4.66 1.52-10.78 1.34-9.69 1.32-10.64 1.91-10.83 1.54-10.20 1.70-10.54 1.49-7.99
1.18-5.40 1.14-5.62 1.51-5.87 1.63-5.61 1.62-5.79 1.46-5.35
1.54-5.86 1.55-5.30 1.40-8.08 1.56-7.95 1.69-7.85 1.65-6.06
1.34-6.00 1.76-5.91 1.37-5.99 1.68-6.00 1.55-5.86 1.73-6.03
1.53-5.84 1.62-6.12 1.31-2.28 1.03-3.61 1.04-4.29 0.96-2.80
1.92-4.38 0.87-4.20 1.87-4.18 1.72-3.69 1.71-6.14 1.24-2.37
1.23-3.61 1.50-4.01 1.16-2.65 1.47-4.96 1.29-4.00 1.75-4.33
1.48-3.87 1.73-5.05 1.02-2.13 0.90-2.62 1.07-2.55 0.62-2.01
0.08-6.23 1.07-2.69 1.31-4.20 1.29-3.60 2.10-4.62 0.93-2.20
0.81-2.61 1.08-2.63 0.65-2.08 1.35-4.57 0.98-2.72 1.23-4.36
1.21-3.83 1.87-3.94 0.54-1.48 2.05-3.75 0.48-2.92 1.39-6.38
1.26-6.76 1.80-6.15 2.03-5.37 1.54-5.65 0.83-2.33 1.32-8.05
1.21-7.37 1.76-6.93 1.60-6.41 1.77-5.25 0.84-2.37 1.93-8.35
1.22-6.62 0.98-9.83 0.91-2.95 1.14-9.32 0.98-3.14 1.45-6.37
1.80-4.43 1.54-5.33

Protomicrocotyle_mirabilis2 1.46-14.39 1.22-10.15 1.80-10.49 1.34-4.66 1.52-10.78 1.34-9.69 1.32-10.64 1.91-10.83 1.54-10.20 1.70-10.54 1.49-7.99
1.18-5.40 1.14-5.62 1.51-5.87 1.63-5.61 1.62-5.79 1.46-5.35
1.54-5.86 1.55-5.30 1.40-8.08 1.56-7.95 1.69-7.85 1.65-6.06
1.34-6.00 1.76-5.91 1.37-5.99 1.68-6.00 1.55-5.86 1.73-6.03
1.53-5.84 1.62-6.12 1.31-2.28 1.03-3.61 1.04-4.29 0.96-2.80
1.92-4.38 0.87-4.20 1.87-4.18 1.72-3.69 1.71-6.14 1.24-2.37
1.23-3.61 1.50-4.01 1.16-2.65 1.47-4.96 1.29-4.00 1.75-4.33
1.48-3.87 1.73-5.05 1.02-2.13 0.90-2.62 1.07-2.55 0.62-2.01
0.08-6.23 1.07-2.69 1.31-4.20 1.29-3.60 2.10-4.62 0.93-2.20
0.81-2.61 1.08-2.63 0.65-2.08 1.35-4.57 0.98-2.72 1.23-4.36
1.21-3.83 1.87-3.94 0.54-1.48 2.05-3.75 0.48-2.92 1.39-6.38
1.26-6.76 1.80-6.15 2.03-5.37 1.54-5.65 0.83-2.33 1.32-8.05

1.21-7.37 1.76-6.93 1.60-6.41 1.77-5.25 0.84-2.37 1.93-8.35
1.22-6.62 0.98-9.83 0.91-2.95 1.14-9.32 0.98-3.14 1.45-6.37
1.80-4.43 1.54-5.33

Protomicrocotyle_mirabilis3 1.46-14.39 1.22-10.15 1.80-10.49 1.34-4.66 1.52-10.78 1.34-9.69 1.32-10.64 1.91-10.83 1.54-10.20 1.70-10.54 1.49-7.99
1.18-5.40 1.14-5.62 1.51-5.87 1.63-5.61 1.62-5.79 1.46-5.35
1.54-5.86 1.55-5.30 1.40-8.08 1.56-7.95 1.69-7.85 1.65-6.06
1.34-6.00 1.76-5.91 1.37-5.99 1.68-6.00 1.55-5.86 1.73-6.03
1.53-5.84 1.62-6.12 1.31-2.28 1.03-3.61 1.04-4.29 0.96-2.80
1.92-4.38 0.87-4.20 1.87-4.18 1.72-3.69 1.71-6.14 1.24-2.37
1.23-3.61 1.50-4.01 1.16-2.65 1.47-4.96 1.29-4.00 1.75-4.33
1.48-3.87 1.73-5.05 1.02-2.13 0.90-2.62 1.07-2.55 0.62-2.01
0.08-6.23 1.07-2.69 1.31-4.20 1.29-3.60 2.10-4.62 0.93-2.20
0.81-2.61 1.08-2.63 0.65-2.08 1.35-4.57 0.98-2.72 1.23-4.36
1.21-3.83 1.87-3.94 0.54-1.48 2.05-3.75 0.48-2.92 1.39-6.38
1.26-6.76 1.80-6.15 2.03-5.37 1.54-5.65 0.83-2.33 1.32-8.05
1.21-7.37 1.76-6.93 1.60-6.41 1.77-5.25 0.84-2.37 1.93-8.35
1.22-6.62 0.98-9.83 0.91-2.95 1.14-9.32 0.98-3.14 1.45-6.37
1.80-4.43 1.54-5.33

Protomicrocotyle_mirabilis4 1.46-14.39 1.22-10.15 1.80-10.49 1.34-4.66 1.52-10.78 1.34-9.69 1.32-10.64 1.91-10.83 1.54-10.20 1.70-10.54 1.49-7.99
1.18-5.40 1.14-5.62 1.51-5.87 1.63-5.61 1.62-5.79 1.46-5.35
1.54-5.86 1.55-5.30 1.40-8.08 1.56-7.95 1.69-7.85 1.65-6.06
1.34-6.00 1.76-5.91 1.37-5.99 1.68-6.00 1.55-5.86 1.73-6.03
1.53-5.84 1.62-6.12 1.31-2.28 1.03-3.61 1.04-4.29 0.96-2.80
1.92-4.38 0.87-4.20 1.87-4.18 1.72-3.69 1.71-6.14 1.24-2.37
1.23-3.61 1.50-4.01 1.16-2.65 1.47-4.96 1.29-4.00 1.75-4.33
1.48-3.87 1.73-5.05 1.02-2.13 0.90-2.62 1.07-2.55 0.62-2.01
0.08-6.23 1.07-2.69 1.31-4.20 1.29-3.60 2.10-4.62 0.93-2.20
0.81-2.61 1.08-2.63 0.65-2.08 1.35-4.57 0.98-2.72 1.23-4.36
1.21-3.83 1.87-3.94 0.54-1.48 2.05-3.75 0.48-2.92 1.39-6.38
1.26-6.76 1.80-6.15 2.03-5.37 1.54-5.65 0.83-2.33 1.32-8.05
1.21-7.37 1.76-6.93 1.60-6.41 1.77-5.25 0.84-2.37 1.93-8.35
1.22-6.62 0.98-9.83 0.91-2.95 1.14-9.32 0.98-3.14 1.45-6.37
1.80-4.43 1.54-5.33

Protomicrocotyle_mirabilis5 1.46-14.39 1.22-10.15 1.80-10.49 1.34-4.66 1.52-10.78 1.34-9.69 1.32-10.64 1.91-10.83 1.54-10.20 1.70-10.54 1.49-7.99
1.18-5.40 1.14-5.62 1.51-5.87 1.63-5.61 1.62-5.79 1.46-5.35
1.54-5.86 1.55-5.30 1.40-8.08 1.56-7.95 1.69-7.85 1.65-6.06
1.34-6.00 1.76-5.91 1.37-5.99 1.68-6.00 1.55-5.86 1.73-6.03
1.53-5.84 1.62-6.12 1.31-2.28 1.03-3.61 1.04-4.29 0.96-2.80
1.92-4.38 0.87-4.20 1.87-4.18 1.72-3.69 1.71-6.14 1.24-2.37
1.23-3.61 1.50-4.01 1.16-2.65 1.47-4.96 1.29-4.00 1.75-4.33
1.48-3.87 1.73-5.05 1.02-2.13 0.90-2.62 1.07-2.55 0.62-2.01
0.08-6.23 1.07-2.69 1.31-4.20 1.29-3.60 2.10-4.62 0.93-2.20

0.81-2.61 1.08-2.63 0.65-2.08 1.35-4.57 0.98-2.72 1.23-4.36
1.21-3.83 1.87-3.94 0.54-1.48 2.05-3.75 0.48-2.92 1.39-6.38
1.26-6.76 1.80-6.15 2.03-5.37 1.54-5.65 0.83-2.33 1.32-8.05
1.21-7.37 1.76-6.93 1.60-6.41 1.77-5.25 0.84-2.37 1.93-8.35
1.22-6.62 0.98-9.83 0.91-2.95 1.14-9.32 0.98-3.14 1.45-6.37
1.80-4.43 1.54-5.33

Protomicrocotyle_mirabilis6 1.46-14.39 1.22-10.15 1.80-10.49 1.34-4.66 1.52-10.78 1.34-9.69 1.32-10.64 1.91-10.83 1.54-10.20 1.70-10.54 1.49-7.99
1.18-5.40 1.14-5.62 1.51-5.87 1.63-5.61 1.62-5.79 1.46-5.35
1.54-5.86 1.55-5.30 1.40-8.08 1.56-7.95 1.69-7.85 1.65-6.06
1.34-6.00 1.76-5.91 1.37-5.99 1.68-6.00 1.55-5.86 1.73-6.03
1.53-5.84 1.62-6.12 1.31-2.28 1.03-3.61 1.04-4.29 0.96-2.80
1.92-4.38 0.87-4.20 1.87-4.18 1.72-3.69 1.71-6.14 1.24-2.37
1.23-3.61 1.50-4.01 1.16-2.65 1.47-4.96 1.29-4.00 1.75-4.33
1.48-3.87 1.73-5.05 1.02-2.13 0.90-2.62 1.07-2.55 0.62-2.01
0.08-6.23 1.07-2.69 1.31-4.20 1.29-3.60 2.10-4.62 0.93-2.20
0.81-2.61 1.08-2.63 0.65-2.08 1.35-4.57 0.98-2.72 1.23-4.36
1.21-3.83 1.87-3.94 0.54-1.48 2.05-3.75 0.48-2.92 1.39-6.38
1.26-6.76 1.80-6.15 2.03-5.37 1.54-5.65 0.83-2.33 1.32-8.05
1.21-7.37 1.76-6.93 1.60-6.41 1.77-5.25 0.84-2.37 1.93-8.35
1.22-6.62 0.98-9.83 0.91-2.95 1.14-9.32 0.98-3.14 1.45-6.37
1.80-4.43 1.54-5.33

Protomicrocotyle_mirabilis7 1.46-14.39 1.22-10.15 1.80-10.49 1.34-4.66 1.52-10.78 1.34-9.69 1.32-10.64 1.91-10.83 1.54-10.20 1.70-10.54 1.49-7.99
1.18-5.40 1.14-5.62 1.51-5.87 1.63-5.61 1.62-5.79 1.46-5.35
1.54-5.86 1.55-5.30 1.40-8.08 1.56-7.95 1.69-7.85 1.65-6.06
1.34-6.00 1.76-5.91 1.37-5.99 1.68-6.00 1.55-5.86 1.73-6.03
1.53-5.84 1.62-6.12 1.31-2.28 1.03-3.61 1.04-4.29 0.96-2.80
1.92-4.38 0.87-4.20 1.87-4.18 1.72-3.69 1.71-6.14 1.24-2.37
1.23-3.61 1.50-4.01 1.16-2.65 1.47-4.96 1.29-4.00 1.75-4.33
1.48-3.87 1.73-5.05 1.02-2.13 0.90-2.62 1.07-2.55 0.62-2.01
0.08-6.23 1.07-2.69 1.31-4.20 1.29-3.60 2.10-4.62 0.93-2.20
0.81-2.61 1.08-2.63 0.65-2.08 1.35-4.57 0.98-2.72 1.23-4.36
1.21-3.83 1.87-3.94 0.54-1.48 2.05-3.75 0.48-2.92 1.39-6.38
1.26-6.76 1.80-6.15 2.03-5.37 1.54-5.65 0.83-2.33 1.32-8.05
1.21-7.37 1.76-6.93 1.60-6.41 1.77-5.25 0.84-2.37 1.93-8.35
1.22-6.62 0.98-9.83 0.91-2.95 1.14-9.32 0.98-3.14 1.45-6.37
1.80-4.43 1.54-5.33

Protomicrocotyle_veracruzensis1 2.27-14.34 1.74-11.48 2.29-10.82 1.28-5.35
2.23-10.82 1.68-10.91 2.05-11.22 1.88-11.58 1.90-10.73 1.96-11.08
1.36-9.35 1.58-5.56 1.38-6.41 1.95-6.17 2.10-5.85 1.35-6.97
1.73-5.92 1.40-7.00 1.86-5.87 1.81-8.60 1.76-8.73 1.38-9.03
1.26-6.62 1.77-6.24 1.33-6.56 1.79-6.29 1.41-6.50 1.84-6.24
1.53-6.45 1.82-6.27 1.69-6.15 1.40-2.28 1.26-3.48 1.51-4.18
1.28-2.78 1.78-4.56 1.58-3.93 1.64-4.38 1.17-4.17 1.74-6.14

1.42-2.33	1.32-3.53	1.62-4.02	1.36-2.66	1.78-4.67	1.47-4.00
1.59-4.52	1.29-4.20	1.44-5.18	1.16-2.12	1.07-2.46	0.81-3.03
0.72-2.12	1.66-4.03	0.86-3.21	1.42-3.87	1.32-3.56	2.22-4.48
1.26-1.92	0.94-2.73	0.62-3.41	0.59-2.00	1.70-4.05	0.79-3.24
1.43-4.00	1.47-3.49	2.03-3.78	0.66-1.38	2.00-3.79	0.65-1.38
1.36-6.77	1.29-8.21	1.97-6.67	1.60-6.52	1.81-5.86	0.89-2.29
1.93-8.26	1.67-8.61	1.49-7.79	1.88-6.89	1.82-5.59	1.03-2.29
1.73-8.74	1.75-6.51	1.87-8.95	1.06-2.72	0.57-9.75	0.36-3.28
1.66-6.56	1.75-4.51	1.89-6.22			
Protomicrocotyle_veracruzensis2		2.27-14.34	1.74-11.48	2.29-10.82	1.28-5.35
2.23-10.82	1.68-10.91	2.05-11.22	1.88-11.58	1.90-10.73	1.96-11.08
1.36-9.35	1.58-5.56	1.38-6.41	1.95-6.17	2.10-5.85	1.35-6.97
1.73-5.92	1.40-7.00	1.86-5.87	1.81-8.60	1.76-8.73	1.38-9.03
1.26-6.62	1.77-6.24	1.33-6.56	1.79-6.29	1.41-6.50	1.84-6.24
1.53-6.45	1.82-6.27	1.69-6.15	1.40-2.28	1.26-3.48	1.51-4.18
1.28-2.78	1.78-4.56	1.58-3.93	1.64-4.38	1.17-4.17	1.74-6.14
1.42-2.33	1.32-3.53	1.62-4.02	1.36-2.66	1.78-4.67	1.47-4.00
1.59-4.52	1.29-4.20	1.44-5.18	1.16-2.12	1.07-2.46	0.81-3.03
0.72-2.12	1.66-4.03	0.86-3.21	1.42-3.87	1.32-3.56	2.22-4.48
1.26-1.92	0.94-2.73	0.62-3.41	0.59-2.00	1.70-4.05	0.79-3.24
1.43-4.00	1.47-3.49	2.03-3.78	0.66-1.38	2.00-3.79	0.65-1.38
1.36-6.77	1.29-8.21	1.97-6.67	1.60-6.52	1.81-5.86	0.89-2.29
1.93-8.26	1.67-8.61	1.49-7.79	1.88-6.89	1.82-5.59	1.03-2.29
1.73-8.74	1.75-6.51	1.87-8.95	1.06-2.72	0.57-9.75	0.36-3.28
1.66-6.56	1.75-4.51	1.89-6.22			
Protomicrocotyle_veracruzensis3		2.27-14.34	1.74-11.48	2.29-10.82	1.28-5.35
2.23-10.82	1.68-10.91	2.05-11.22	1.88-11.58	1.90-10.73	1.96-11.08
1.36-9.35	1.58-5.56	1.38-6.41	1.95-6.17	2.10-5.85	1.35-6.97
1.73-5.92	1.40-7.00	1.86-5.87	1.81-8.60	1.76-8.73	1.38-9.03
1.26-6.62	1.77-6.24	1.33-6.56	1.79-6.29	1.41-6.50	1.84-6.24
1.53-6.45	1.82-6.27	1.69-6.15	1.40-2.28	1.26-3.48	1.51-4.18
1.28-2.78	1.78-4.56	1.58-3.93	1.64-4.38	1.17-4.17	1.74-6.14
1.42-2.33	1.32-3.53	1.62-4.02	1.36-2.66	1.78-4.67	1.47-4.00
1.59-4.52	1.29-4.20	1.44-5.18	1.16-2.12	1.07-2.46	0.81-3.03
0.72-2.12	1.66-4.03	0.86-3.21	1.42-3.87	1.32-3.56	2.22-4.48
1.26-1.92	0.94-2.73	0.62-3.41	0.59-2.00	1.70-4.05	0.79-3.24
1.43-4.00	1.47-3.49	2.03-3.78	0.66-1.38	2.00-3.79	0.65-1.38
1.36-6.77	1.29-8.21	1.97-6.67	1.60-6.52	1.81-5.86	0.89-2.29
1.93-8.26	1.67-8.61	1.49-7.79	1.88-6.89	1.82-5.59	1.03-2.29
1.73-8.74	1.75-6.51	1.87-8.95	1.06-2.72	0.57-9.75	0.36-3.28
1.66-6.56	1.75-4.51	1.89-6.22			
Protomicrocotyle_veracruzensis4		2.27-14.34	1.74-11.48	2.29-10.82	1.28-5.35
2.23-10.82	1.68-10.91	2.05-11.22	1.88-11.58	1.90-10.73	1.96-11.08
1.36-9.35	1.58-5.56	1.38-6.41	1.95-6.17	2.10-5.85	1.35-6.97
1.73-5.92	1.40-7.00	1.86-5.87	1.81-8.60	1.76-8.73	1.38-9.03

1.26-6.62 1.77-6.24 1.33-6.56 1.79-6.29 1.41-6.50 1.84-6.24
1.53-6.45 1.82-6.27 1.69-6.15 1.40-2.28 1.26-3.48 1.51-4.18
1.28-2.78 1.78-4.56 1.58-3.93 1.64-4.38 1.17-4.17 1.74-6.14
1.42-2.33 1.32-3.53 1.62-4.02 1.36-2.66 1.78-4.67 1.47-4.00
1.59-4.52 1.29-4.20 1.44-5.18 1.16-2.12 1.07-2.46 0.81-3.03
0.72-2.12 1.66-4.03 0.86-3.21 1.42-3.87 1.32-3.56 2.22-4.48
1.26-1.92 0.94-2.73 0.62-3.41 0.59-2.00 1.70-4.05 0.79-3.24
1.43-4.00 1.47-3.49 2.03-3.78 0.66-1.38 2.00-3.79 0.65-1.38
1.36-6.77 1.29-8.21 1.97-6.67 1.60-6.52 1.81-5.86 0.89-2.29
1.93-8.26 1.67-8.61 1.49-7.79 1.88-6.89 1.82-5.59 1.03-2.29
1.73-8.74 1.75-6.51 1.87-8.95 1.06-2.72 0.57-9.75 0.36-3.28
1.66-6.56 1.75-4.51 1.89-6.22

Protomicrocotyle_sp. 1 2.24-12.52 2.14-7.90 ? 1.63-4.26 2.15-8.86
1.44-8.05 1.91-8.02 ? 2.01-8.59 2.15-9.05 1.70-7.19 1.04-
3.46 1.09-4.60 1.35-4.79 1.47-4.48 1.73-3.76 1.51-3.86 1.83-3.78
1.55-3.80 2.11-5.92 2.38-5.53 2.07-5.84 1.99-4.19 1.90-3.93
2.17-4.08 1.44-4.37 1.90-4.30 1.36-4.46 1.85-4.35 1.82-4.05
2.23-4.41 0.61-1.80 1.31-2.58 1.24-2.61 0.38-2.22 0.99-3.44
1.36-2.48 1.63-3.57 1.31-2.58 2.04-4.59 0.76-2.18 1.46-2.44
1.15-2.70 0.59-2.01 1.01-3.52 1.36-2.48 1.65-3.65 1.48-2.31
1.87-3.59 0.70-1.38 0.87-2.35 0.76-2.18 0.69-0.69 0.89-3.14
0.81-2.54 1.30-3.46 1.20-2.32 1.97-3.34 0.70-1.38 1.26-2.09
0.59-2.01 0.69-0.69 0.85-3.04 0.59-2.01 1.32-3.32 1.20-2.32
1.59-2.57 0.69-0.69 1.76-2.44 0.69-0.69 2.01-5.31 1.74-5.38
2.13-4.99 1.89-4.13 2.31-3.51 0.70-1.38 1.07-7.53 1.69-5.54
2.02-5.23 1.32-5.68 1.88-3.82 1.10-1.10 1.28-8.03 1.98-5.29 ?
? 0.95-9.72 0.88-3.15 2.04-6.32 1.88-4.18 2.11-5.57

Protomirocotyle_sp. 2 1.41-14.10 1.65-11.90 2.75-9.54 1.12-5.43 1.46-
10.76 1.83-11.05 2.45-9.65 2.54-10.08 1.73-9.86 1.19-10.88 1.77-8.85
1.23-4.74 0.99-6.08 2.37-5.77 1.71-6.38 1.52-6.61 1.45-5.85
1.57-6.52 1.40-5.93 2.10-7.67 1.65-8.01 1.62-8.11 1.71-5.73
2.38-5.46 1.71-5.74 2.37-5.66 1.70-5.69 2.32-5.65 1.78-5.64
1.73-6.20 2.24-5.74 1.19-2.54 1.21-3.62 1.60-3.72 1.25-2.69
1.39-5.28 1.78-3.93 1.02-5.24 1.19-4.68 1.45-6.45 1.30-2.43
0.94-3.71 1.83-3.57 0.70-3.59 1.64-5.00 1.86-3.79 1.54-4.87
1.46-4.56 2.19-4.59 1.31-2.13 1.30-2.55 1.27-2.62 0.70-2.57
2.05-3.83 1.36-2.63 1.83-3.76 1.66-3.48 2.16-4.65 1.20-2.15
1.04-2.67 1.23-2.68 0.69-2.18 1.49-4.50 1.51-2.57 1.87-3.87
1.73-3.55 1.53-4.20 1.10-1.10 1.91-3.83 1.10-1.10 1.10-6.49
1.38-8.69 1.70-6.99 2.15-5.92 1.68-5.87 0.64-2.24 1.17-8.39
1.76-8.63 2.08-7.09 1.77-7.02 2.18-5.21 1.29-2.03 1.65-9.02
1.24-6.96 1.43-9.33 0.93-3.32 0.45-9.96 0.58-2.72 1.92-5.90
1.83-4.37 1.57-4.94

Protomicrocotyle_sp. 3 4.18-12.03 1.52-11.45 3.84-9.04 1.96-4.46 1.99-
10.81 1.66-10.19 1.99-10.27 3.85-9.39 0.88-11.01 3.15-9.61 1.37-8.93

1.03-5.18 1.28-5.16 2.43-5.48 1.97-5.81 2.42-5.00 1.72-5.18
2.20-5.12 2.23-4.81 1.75-8.28 1.41-8.46 1.55-8.43 1.96-5.01
2.94-4.95 1.45-5.64 2.76-5.20 2.19-5.14 1.45-6.48 2.01-5.66
1.83-5.85 2.79-5.12 1.19-2.39 1.80-3.33 1.33-3.64 1.56-2.47
2.29-4.13 1.62-3.96 1.89-4.15 1.43-4.14 2.60-5.34 1.42-2.33
1.26-3.34 1.91-3.30 1.43-2.24 2.78-3.69 1.83-3.59 2.16-4.00
1.64-4.06 1.98-4.63 1.34-2.15 1.04-2.70 1.31-2.65 0.56-1.95
2.18-3.71 1.56-2.47 2.00-3.63 2.14-2.95 2.40-4.47 1.34-2.15
1.19-2.39 0.91-2.91 0.62-2.15 1.91-4.15 1.07-3.21 2.29-3.49
1.91-3.26 1.89-3.90 1.10-1.10 2.23-3.58 0.61-1.42 2.06-6.20
1.37-8.13 1.86-6.88 1.93-6.00 2.35-5.11 0.62-2.15 2.67-7.53
1.40-8.35 2.19-7.10 1.95-7.10 2.03-5.27 1.25-2.06 1.99-8.52
1.99-6.56 1.05-9.49 1.35-2.54 ? ? 1.96-5.64 2.19-4.02
2.72-5.36

Protmomicrocotyle_sp. 4(01)1.93-12.93 1.88-10.45 ? ? 1.80-9.83 2.31-
9.15 2.62-9.03 ? 1.66-9.45 1.81-9.89 1.57-8.02 2.26-4.34 1.15-
5.63 1.70-5.88 2.50-5.12 1.89-5.65 2.04-4.58 2.08-5.51 1.99-4.72
1.77-7.45 2.09-7.24 1.81-7.67 1.21-6.17 1.97-5.55 1.50-5.52
2.37-5.31 1.37-6.06 1.90-5.64 1.57-5.79 1.63-6.01 2.48-5.21
1.35-2.14 1.47-3.13 1.90-3.36 1.45-2.31 1.38-4.78 1.57-3.37
2.02-4.06 1.72-3.45 2.50-5.39 1.18-2.31 1.39-3.45 2.10-3.22
1.35-2.54 2.51-3.88 1.80-3.33 2.28-3.73 2.04-3.24 2.42-4.14
0.61-1.80 1.26-2.09 1.15-2.43 1.10-1.10 2.09-3.57 0.94-2.53
1.89-3.26 1.18-3.46 2.36-4.23 0.59-2.01 0.38-2.22 0.76-2.18
1.10-1.10 2.00-3.60 1.43-2.26 2.15-3.13 1.25-3.42 2.19-3.26 ?
1.90-3.58 ? 1.31-5.85 1.78-7.21 1.95-5.70 2.26-5.35 2.20-
4.54 0.66-2.11 1.27-7.35 1.61-7.17 1.93-6.74 2.13-5.75 2.58-3.89
1.10-1.10 2.39-7.72 2.15-5.61 ? ? ? ? 1.93-6.00
1.97-4.44 1.60-5.76

Protmomicrocotyle_sp. 4(02)1.93-12.93 1.88-10.45 ? ? 1.80-9.83 2.31-
9.15 2.62-9.03 ? 1.66-9.45 1.81-9.89 1.57-8.02 2.26-4.34 1.15-
5.63 1.70-5.88 2.50-5.12 1.89-5.65 2.04-4.58 2.08-5.51 1.99-4.72
1.77-7.45 2.09-7.24 1.81-7.67 1.21-6.17 1.97-5.55 1.50-5.52
2.37-5.31 1.37-6.06 1.90-5.64 1.57-5.79 1.63-6.01 2.48-5.21
1.35-2.14 1.47-3.13 1.90-3.36 1.45-2.31 1.38-4.78 1.57-3.37
2.02-4.06 1.72-3.45 2.50-5.39 1.18-2.31 1.39-3.45 2.10-3.22
1.35-2.54 2.51-3.88 1.80-3.33 2.28-3.73 2.04-3.24 2.42-4.14
0.61-1.80 1.26-2.09 1.15-2.43 1.10-1.10 2.09-3.57 0.94-2.53
1.89-3.26 1.18-3.46 2.36-4.23 0.59-2.01 0.38-2.22 0.76-2.18
1.10-1.10 2.00-3.60 1.43-2.26 2.15-3.13 1.25-3.42 2.19-3.26 ?
1.90-3.58 ? 1.31-5.85 1.78-7.21 1.95-5.70 2.26-5.35 2.20-
4.54 0.66-2.11 1.27-7.35 1.61-7.17 1.93-6.74 2.13-5.75 2.58-3.89
1.10-1.10 2.39-7.72 2.15-5.61 ? ? ? ? 1.93-6.00
1.97-4.44 1.60-5.76

Protmomicrocotyle_sp. 4(03)	1.93-12.93	1.88-10.45	?	?	1.80-9.83	2.31-9.15	2.62-9.03	?
1.66-9.45	1.81-9.89	1.57-8.02	2.26-4.34	1.15-5.63	1.70-5.88	2.50-5.12	1.89-5.65	
2.04-4.58	2.08-5.51	1.99-4.72						
	1.77-7.45	2.09-7.24	1.81-7.67	1.21-6.17	1.97-5.55	1.50-5.52		
	2.37-5.31	1.37-6.06	1.90-5.64	1.57-5.79	1.63-6.01	2.48-5.21		
	1.35-2.14	1.47-3.13	1.90-3.36	1.45-2.31	1.38-4.78	1.57-3.37		
	2.02-4.06	1.72-3.45	2.50-5.39	1.18-2.31	1.39-3.45	2.10-3.22		
	1.35-2.54	2.51-3.88	1.80-3.33	2.28-3.73	2.04-3.24	2.42-4.14		
	0.61-1.80	1.26-2.09	1.15-2.43	1.10-1.10	2.09-3.57	0.94-2.53		
	1.89-3.26	1.18-3.46	2.36-4.23	0.59-2.01	0.38-2.22	0.76-2.18		
	1.10-1.10	2.00-3.60	1.43-2.26	2.15-3.13	1.25-3.42	2.19-3.26	?	
	1.90-3.58	?	1.31-5.85	1.78-7.21	1.95-5.70	2.26-5.35	2.20-4.54	0.66-2.11
1.27-7.35	1.61-7.17	1.93-6.74	2.13-5.75	2.58-3.89				
	1.10-1.10	2.39-7.72	2.15-5.61	?	?	?	?	1.93-6.00
	1.97-4.44	1.60-5.76						

Protmomicrocotyle_sp. 4(04)	1.93-12.93	1.88-10.45	?	?	1.80-9.83	2.31-9.15	2.62-9.03	?
1.66-9.45	1.81-9.89	1.57-8.02	2.26-4.34	1.15-5.63	1.70-5.88	2.50-5.12	1.89-5.65	
2.04-4.58	2.08-5.51	1.99-4.72						
	1.77-7.45	2.09-7.24	1.81-7.67	1.21-6.17	1.97-5.55	1.50-5.52		
	2.37-5.31	1.37-6.06	1.90-5.64	1.57-5.79	1.63-6.01	2.48-5.21		
	1.35-2.14	1.47-3.13	1.90-3.36	1.45-2.31	1.38-4.78	1.57-3.37		
	2.02-4.06	1.72-3.45	2.50-5.39	1.18-2.31	1.39-3.45	2.10-3.22		
	1.35-2.54	2.51-3.88	1.80-3.33	2.28-3.73	2.04-3.24	2.42-4.14		
	0.61-1.80	1.26-2.09	1.15-2.43	1.10-1.10	2.09-3.57	0.94-2.53		
	1.89-3.26	1.18-3.46	2.36-4.23	0.59-2.01	0.38-2.22	0.76-2.18		
	1.10-1.10	2.00-3.60	1.43-2.26	2.15-3.13	1.25-3.42	2.19-3.26	?	
	1.90-3.58	?	1.31-5.85	1.78-7.21	1.95-5.70	2.26-5.35	2.20-4.54	0.66-2.11
1.27-7.35	1.61-7.17	1.93-6.74	2.13-5.75	2.58-3.89				
	1.10-1.10	2.39-7.72	2.15-5.61	?	?	?	?	1.93-6.00
	1.97-4.44	1.60-5.76						

Protmomicrocotyle_sp. 5(01)	2.81-13.83	2.95-9.63	2.65-10.35	1.79-4.64	2.62-10.56	3.07-9.10	2.70-10.48	2.79-10.70
2.59-10.04	2.69-10.39	2.31-8.08						
	2.35-4.74	1.35-6.19	2.47-5.54	2.44-5.33	2.13-5.81	2.67-4.89		
	2.25-5.76	2.24-5.39	2.45-7.88	2.05-8.21	2.54-8.03	2.28-5.19		
	2.60-5.41	2.49-5.21	2.85-5.29	2.24-5.48	2.66-5.53	2.02-5.77		
	2.67-5.51	2.60-5.43	1.48-2.16	2.08-2.08	1.70-3.74	1.39-2.67		
	2.20-4.39	1.41-3.66	1.91-4.28	1.35-4.27	2.84-5.17	1.49-2.14		
	0.99-3.08	1.72-3.84	1.45-2.58	2.28-4.22	1.44-3.57	2.28-3.83		
	1.48-3.88	2.18-4.55	0.74-2.16	0.80-2.49	1.05-2.64	0.70-2.17		
	2.10-3.82	1.04-2.76	1.70-3.99	1.83-3.30	2.34-4.29	0.89-2.16		
	0.73-2.49	0.70-2.71	0.56-1.95	2.28-3.66	1.34-2.15	2.00-3.63		
	2.15-3.06	2.00-3.72	1.10-1.10	2.18-3.62	1.10-1.10	1.82-6.17		
	1.68-7.15	2.26-6.10	2.39-5.42	2.10-5.43	1.29-2.13	1.97-8.14		
	1.79-7.48	2.47-6.99	2.57-6.32	2.45-5.03	1.42-2.16	1.55-8.97		

1.71-6.42 1.56-8.98 1.58-2.43 1.97-9.42 1.06-2.83 1.69-5.97
1.04-5.10 2.37-5.86

Protmomicrocotyle_sp. 5(02)2.81-13.83 2.95-9.63 2.65-10.35 1.79-4.64 2.62-10.56 3.07-9.10 2.70-10.48 2.79-10.70 2.59-10.04 2.69-10.39 2.31-8.08
2.35-4.74 1.35-6.19 2.47-5.54 2.44-5.33 2.13-5.81 2.67-4.89
2.25-5.76 2.24-5.39 2.45-7.88 2.05-8.21 2.54-8.03 2.28-5.19
2.60-5.41 2.49-5.21 2.85-5.29 2.24-5.48 2.66-5.53 2.02-5.77
2.67-5.51 2.60-5.43 1.48-2.16 2.08-2.08 1.70-3.74 1.39-2.67
2.20-4.39 1.41-3.66 1.91-4.28 1.35-4.27 2.84-5.17 1.49-2.14
0.99-3.08 1.72-3.84 1.45-2.58 2.28-4.22 1.44-3.57 2.28-3.83
1.48-3.88 2.18-4.55 0.74-2.16 0.80-2.49 1.05-2.64 0.70-2.17
2.10-3.82 1.04-2.76 1.70-3.99 1.83-3.30 2.34-4.29 0.89-2.16
0.73-2.49 0.70-2.71 0.56-1.95 2.28-3.66 1.34-2.15 2.00-3.63
2.15-3.06 2.00-3.72 1.10-1.10 2.18-3.62 1.10-1.10 1.82-6.17
1.68-7.15 2.26-6.10 2.39-5.42 2.10-5.43 1.29-2.13 1.97-8.14
1.79-7.48 2.47-6.99 2.57-6.32 2.45-5.03 1.42-2.16 1.55-8.97
1.71-6.42 1.56-8.98 1.58-2.43 1.97-9.42 1.06-2.83 1.69-5.97
1.04-5.10 2.37-5.86

Protmomicrocotyle_sp. 5(03)2.81-13.83 2.95-9.63 2.65-10.35 1.79-4.64 2.62-10.56 3.07-9.10 2.70-10.48 2.79-10.70 2.59-10.04 2.69-10.39 2.31-8.08
2.35-4.74 1.35-6.19 2.47-5.54 2.44-5.33 2.13-5.81 2.67-4.89
2.25-5.76 2.24-5.39 2.45-7.88 2.05-8.21 2.54-8.03 2.28-5.19
2.60-5.41 2.49-5.21 2.85-5.29 2.24-5.48 2.66-5.53 2.02-5.77
2.67-5.51 2.60-5.43 1.48-2.16 2.08-2.08 1.70-3.74 1.39-2.67
2.20-4.39 1.41-3.66 1.91-4.28 1.35-4.27 2.84-5.17 1.49-2.14
0.99-3.08 1.72-3.84 1.45-2.58 2.28-4.22 1.44-3.57 2.28-3.83
1.48-3.88 2.18-4.55 0.74-2.16 0.80-2.49 1.05-2.64 0.70-2.17
2.10-3.82 1.04-2.76 1.70-3.99 1.83-3.30 2.34-4.29 0.89-2.16
0.73-2.49 0.70-2.71 0.56-1.95 2.28-3.66 1.34-2.15 2.00-3.63
2.15-3.06 2.00-3.72 1.10-1.10 2.18-3.62 1.10-1.10 1.82-6.17
1.68-7.15 2.26-6.10 2.39-5.42 2.10-5.43 1.29-2.13 1.97-8.14
1.79-7.48 2.47-6.99 2.57-6.32 2.45-5.03 1.42-2.16 1.55-8.97
1.71-6.42 1.56-8.98 1.58-2.43 1.97-9.42 1.06-2.83 1.69-5.97
1.04-5.10 2.37-5.86

Protmomicrocotyle_sp. 5(04)2.81-13.83 2.95-9.63 2.65-10.35 1.79-4.64 2.62-10.56 3.07-9.10 2.70-10.48 2.79-10.70 2.59-10.04 2.69-10.39 2.31-8.08
2.35-4.74 1.35-6.19 2.47-5.54 2.44-5.33 2.13-5.81 2.67-4.89
2.25-5.76 2.24-5.39 2.45-7.88 2.05-8.21 2.54-8.03 2.28-5.19
2.60-5.41 2.49-5.21 2.85-5.29 2.24-5.48 2.66-5.53 2.02-5.77
2.67-5.51 2.60-5.43 1.48-2.16 2.08-2.08 1.70-3.74 1.39-2.67
2.20-4.39 1.41-3.66 1.91-4.28 1.35-4.27 2.84-5.17 1.49-2.14
0.99-3.08 1.72-3.84 1.45-2.58 2.28-4.22 1.44-3.57 2.28-3.83
1.48-3.88 2.18-4.55 0.74-2.16 0.80-2.49 1.05-2.64 0.70-2.17
2.10-3.82 1.04-2.76 1.70-3.99 1.83-3.30 2.34-4.29 0.89-2.16
0.73-2.49 0.70-2.71 0.56-1.95 2.28-3.66 1.34-2.15 2.00-3.63

2.15-3.06 2.00-3.72 1.10-1.10 2.18-3.62 1.10-1.10 1.82-6.17
1.68-7.15 2.26-6.10 2.39-5.42 2.10-5.43 1.29-2.13 1.97-8.14
1.79-7.48 2.47-6.99 2.57-6.32 2.45-5.03 1.42-2.16 1.55-8.97
1.71-6.42 1.56-8.98 1.58-2.43 1.97-9.42 1.06-2.83 1.69-5.97
1.04-5.10 2.37-5.86

Protomicrocotyle_nayaritensis 2.79-15.16 1.98-11.83 3.98-10.29 1.63-6.42
3.38-10.25 2.46-10.84 3.42-9.93 3.54-10.97 3.04-10.58 2.52-11.62
2.20-9.63 2.28-4.85 1.94-6.21 2.25-5.64 2.71-4.91 2.81-5.52
1.91-5.74 3.02-5.29 2.08-5.62 3.01-7.36 1.82-8.84 2.63-8.64
2.18-5.34 2.42-5.41 2.42-5.50 2.97-5.05 2.29-5.34 3.73-4.40
2.00-5.62 3.69-4.36 1.98-6.31 1.55-2.22 1.71-2.77 1.97-3.88
1.71-2.77 3.02-3.96 2.02-3.60 2.43-4.01 2.16-3.73 3.20-5.26
1.95-1.95 1.22-4.00 2.94-2.94 1.95-2.89 2.17-4.62 2.30-3.24
2.38-4.44 1.88-3.94 ? ? ? ? ? ? ? ? ?
2.87-3.92 1.53-1.53 1.76-1.76 1.30-3.09 1.76-1.76 2.16-3.73
1.33-2.78 1.94-3.73 1.59-3.60 2.25-3.42 0.62-1.29 2.09-3.67
0.56-1.50 2.74-6.57 2.73-7.16 2.77-6.45 2.60-6.26 2.69-4.13
1.22-1.22 2.55-9.80 1.50-8.79 2.07-7.86 2.88-6.41 1.81-5.26
1.76-1.76 2.31-7.86 1.81-6.29 2.61-8.30 1.00-3.06 ? ?
1.58-4.83 2.03-5.83 2.60-4.64

Protomicrocotyle_manteri 3.02-13.22 2.54-10.56 3.85-8.65 2.51-4.00 2.92-
9.82 2.73-9.64 2.84-9.44 3.79-9.09 3.02-9.03 3.13-9.59 2.79-7.71
2.31-4.43 2.61-4.51 3.11-4.58 3.09-4.37 2.58-5.34 2.59-4.26
2.59-5.30 2.72-4.18 2.41-7.62 2.59-7.50 2.57-7.73 2.85-4.49
3.27-4.35 2.88-4.51 3.13-4.53 2.86-4.50 3.16-4.55 2.95-4.42
3.11-4.58 3.36-4.96 1.75-1.98 2.08-2.88 2.46-3.18 1.82-2.45
2.90-3.75 2.33-3.27 2.76-3.63 2.47-3.32 3.24-5.00 1.70-2.02
1.89-2.80 2.43-3.18 1.82-2.35 2.98-3.71 2.22-3.41 2.77-3.61
2.56-3.11 2.90-3.81 1.08-2.46 1.64-2.08 1.76-2.32 1.36-1.80
2.52-3.41 1.82-2.26 2.46-3.23 2.17-2.98 3.04-3.71 1.46-1.71
1.60-2.10 1.72-2.24 1.30-1.57 2.55-3.36 1.72-2.38 2.42-3.20
2.14-3.02 2.60-3.23 1.08-1.24 2.63-3.17 1.14-1.26 2.97-5.22
2.55-6.79 2.88-5.53 2.89-5.30 2.86-3.79 1.18-1.31 2.82-7.57
2.38-7.82 3.12-5.46 2.82-5.25 2.73-3.81 1.61-1.85 2.75-7.56
2.49-5.45 1.93-9.05 1.54-2.28 2.62-8.60 1.61-2.15 2.31-3.59
3.06-4.57 2.83-4.08

Protomicrocotyle_minutum 3.18 2.48 2.34 1.51 2.26 2.38 2.56 2.53 2.42
2.48 1.94 ? ? 1.59 1.64 1.73 1.65 1.73 1.64 ? ?
2.15 1.38 1.77 1.34 1.84 1.38 1.88 1.36 1.82 1.38 ? ? ?
? ? ? ? ? 1.38 ? ? ? ? ? ? ?
? 1.15 ? ? ? ? ? ? ? ? 1.15 ? ?
? ? ? ? ? ? 0.90 ? 0.90 ? 1.15 1.57-
1.64 1.68 1.60 0.40 ? 1.56-1.69 2.27 1.18-1.46 1.65 1.44 0.88 ?
? ? ? ? ? 1.28 1.08 1.53

Protomicrocotyle_mannarrensis				3.46-3.56		2.68-3.06		2.75	1.60	2.43-2.59				
	2.64	2.51-2.64		2.86	2.65	2.72	2.23	?	?	1.52-1.54		1.61-		
1.62	1.72	1.60	1.72	1.60	?	?	2.11-2.23		1.60	1.73	1.64	1.82		
	1.62	1.83	1.60	1.75	1.52-1.57		?	?	?	?	?	?	?	
	?	1.52-1.57		?	?	?	?	?	?	?	?	1.30-		
1.34	?	?	?	?	?	?	?	?	1.30-1.34		?	?	?	
	?	?	?	?	?	1.15-1.23		?	1.15-1.23		?	1.32-		
1.52	1.83-2.26		1.58	1.49	0.11	?	1.78-1.90		2.21-2.08		2.15	1.91		
	1.73	0.92	?	?	?	?	?	?	1.57	1.38	1.62-1.72			
Protomicrocotyle_madrasensis				3.53	4.42	2.53	1.40	2.34	2.48	2.38	2.65			
	2.56	2.67	2.05	?	?	1.58-1.61		1.57	1.51	1.40	1.48	1.37	?	
	?	2.08-2.04		1.20	1.57	1.14	1.70	1.14	1.70	1.18	1.70	1.45	?	
	?	?	?	?	?	?	?	1.45	?	?	?	?	?	
	?	?	?	1.23	?	?	?	?	?	?	?	?		
	1.23	?	?	?	?	?	?	?	?	0.95	?	0.95	?	
	1.52-1.58		1.76-1.91		1.82	1.70	?	?	2.10-2.25		2.05	1.74		
	1.63	1.52	0.67	?	?	?	?	?	?	1.49	1.38	1.81		
Protomicrocotyle_carangis				3.40-3.49		2.66-2.73		?	?	?	?	?	?	
	?	?	?	?	?	1.75	1.70	1.81	1.72	1.81	1.72	?	?	
	?	1.68	1.78	1.68	1.78	1.68	1.78	1.68	1.78	1.58	?	?	?	
	?	?	?	?	?	1.58	?	?	?	?	?	?	?	
	?	1.08-1.15		?	?	?	?	?	?	?	?	1.08-		
1.15	?	?	?	?	?	?	?	?	1.20-1.26		?	1.20-		
1.26	?	?	?	1.56-1.58		?	?	?	?	?	?	?	?	
	?	2.23	1.83	?	?	?	?	1.15-1.20		1.34	1.30-1.38			
Protomicrocotyle_celebensis				3.60-3.71		2.78-2.92		?	?	?	?	?	?	
	2.57-2.80		?	2.70-2.92		?	?	1.84	1.57-1.70		1.43-1.64			
	1.48-1.68		1.43-1.65		1.48-1.68		?	?	?	?	?	?	?	
	?	?	?	?	?	?	?	?	?	?	?	?	?	
	?	?	?	?	?	?	?	?	?	?	?	?	?	
	?	?	?	?	?	?	?	?	?	?	?	?	?	
	?	?	?	?	?	?	?	?	?	?	?	?	?	
	?	?	?	?	2.22-2.32		1.77-1.90		2.38-2.48		?	?	?	
	?	1.20-1.34		1.60-1.74										
Protomicrocotyle_ivoriensis				3.50-3.66		2.78-3.08		2.32	1.28	2.86	2.65	2.70		
	2.65	2.56	2.65	2.40	?	?	1.83	1.76	1.58	1.78	1.28	1.76	?	
	?	2.13	1.40	1.61	1.43	1.61	1.36	1.58	1.58	1.58	1.34	?	?	
	?	?	?	?	?	?	1.34	?	?	?	?	?	?	
	?	?	0.95-1.00		?	?	?	?	?	?	?	?		
	0.95-1.00		?	?	?	?	?	?	?	?	0.60	?		
	0.60	?	1.77	2.00	1.46	1.58	1.65	0.3	2.36	2.32	2.54	2.06	?	
	?	2.34	1.81	2.26	?	2.49	?	?	1.40-1.41		1.70			
Neomicrocotyle_pacifica				2.75-14.03		2.65-11.68		2.28-10.44		2.29-3.84		2.92-		
10.66	2.74-10.63		2.84-10.56		2.46-10.84		2.43-10.19		2.42-10.60		2.69-8.55			

1.86-4.74 2.35-5.23 2.49-5.98 2.48-5.91 2.22-5.60 1.97-5.04
2.22-5.58 2.00-5.03 2.22-9.25 2.30-9.28 2.41-9.11 2.56-5.49
2.75-6.03 2.67-5.36 2.76-6.02 2.68-5.25 2.92-5.91 2.62-5.18
3.04-5.65 2.78-5.37 1.70-2.07 1.70-3.23 2.62-3.38 1.71-2.43
2.15-4.79 2.10-3.07 2.22-4.34 2.07-3.58 2.87-5.21 1.48-1.88
1.76-3.26 2.56-3.35 1.73-2.34 2.64-4.21 2.19-2.63 2.52-4.14
1.65-3.99 2.98-3.74 1.34-1.71 1.29-2.11 1.37-2.53 1.18-1.59
2.44-3.42 1.42-1.70 2.31-3.25 2.33-2.85 3.04-3.78 1.44-1.94
1.39-2.17 1.41-2.35 1.20-1.72 2.54-3.24 1.54-2.02 2.36-3.18
2.27-2.88 1.90-3.52 ? 2.05-3.26 ? 2.27-6.43 2.55-6.43
2.71-7.78 2.61-6.07 2.90-6.82 1.59-2.06 2.04-8.58 2.17-8.71
2.58-6.70 2.78-5.63 2.18-2.95 1.56-1.85 3.50-6.75 2.01-6.17
0.94-9.84 1.18-3.36 1.82-9.07 1.50-2.78 3.53-5.38 2.61-3.33
3.30-6.96

Neomicrocotyle_carangis ? ? ? ? ? ? ? ? ? ?
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Neomicrocotyle_elongata 3.63-3.83 3.11-3.24 ? ? ? ? ? ?
2.75-2.96 ? ? 1.96 1.90 ? ? 1.81 1.64 1.81 1.64 ?
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2.40 1.77-1.90 ? ? ? ? ? 1.26 2.11-2.16

Neomicrocotyle_indicus ? ? ? ? ? 0.19 ? ? ? ?
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Neomicrocotyle_unnithani ? ? ? ? ? ? ? ? ? ?
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Bilaterocotyle_chirocentrocus ? ? ? ? ? ? ? ? ? ?
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Bilaterocotyle_madrasensis 3.06-3.38 2.9 ? ? ? ? ? ? ?
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1.51 1.60 1.51 1.60 1.51 1.60 1.51 1.60 1.56 ? ? ? ?
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1.14 ? ? ? ? ? ? ? ? 1.14 ? ? ?
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1.08 ? ? ? ? ? ? ? 2.3 1.78 ? ? ?
? ? 1.32 1.26-1.30

Bilaterocotyloides_carangis 3.30-3.47 2.52-2.68 ? ? ? ? ? ?
2.62-2.72 ? ? ? ? 0.19 0.18 0.19 0.14 0.19 0.14 ?
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2.19 ? 1.30-1.34 ? ? ? ? ? ? ? 2.33
1.86 ? ? ? ? ? 1.38-1.41 1.20-1.30

Lethacotyle_fijensis 3.50-3.58 2.82-2.89 2.78 ? ? ? ? 2.94 ?
? ? ? ? 1.81 1.7 1.69 1.72 1.69 1.72 ? ? ?
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Lethacotyle_vera ? ? ? ? ? ? ? ? ? ? ? ?
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1.38 1.54

Vallisiopsis_contorta 3.54 ? ? ? ? ? ? ? ? ? ?
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1.32 ?

Youngiopsis_australis 3.14-3.47 2.73-3.17 ? ? ? ? ? ?
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? 1.76-1.81 1.76-1.81 1.76-1.81 1.76-1.81 1.76-1.81 1.76-
1.81 1.76-1.81 1.76-1.81 1.76-1.81 1.76-1.81 ? ? ? ? ?
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1.47 1.23

& [num]

Allodiscocotyla_dicanthi01 1 0 1 1 0 0 0 0 0 0
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0 0 0 0 0 1 1 1 0 0 0 1 0
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Allodiscocotyla_dicanthi02 1 0 1 1 0 0 0 0 0 0
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3 2 2 1 3 1 1 1 1 0 0 0 0
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Allodiscocotyla_chorinemi 1 0 1 1 0 0 0 0 0 0
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3 2 2 1 3 1 1 1 1 0 0 0 0
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0

Metacamopia_lebedevi 0 0 0 0 0 0 0 0 0 0
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3 2 ? 1 3 1 1 1 1 1 0 0 0

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Metacamopia_ologoplites 0 0 0 0 0 0 0 0 0 0
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1

Gemmaecaputia_corrugata 1 0 1 1 0 0 1 1 1 0
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3 2 3 ? ? ? 0 0 0 0 0 0 0
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1

Protomicrocotyle_mirabilis1 1 0 2 1 0 0 0 0 0 1
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1 1 3 1 1 3 0 1 0 0 2 1 2
2 0 1 1 0 0 0 0 3 1 0 0 1
1

Protomicrocotyle_mirabilis2 1 0 2 1 0 0 0 0 0 1
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1 1 3 1 1 3 0 1 0 0 2 1 2
2 0 1 1 0 0 0 0 3 1 0 0 1
1

Protomicrocotyle_mirabilis3 1 0 2 1 0 0 0 0 0 1
1 1 1 2 1 1 1 2 0 0 1 1 2
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2 0 1 1 0 0 0 0 3 1 0 0 1
1

Protomicrocotyle_mirabilis4 1 0 2 1 0 0 0 0 0 1
1 1 1 2 1 1 1 2 0 0 1 1 2
1 1 3 1 1 3 0 1 0 0 2 1 2
2 0 1 1 0 0 0 0 3 1 0 0 1
1

Protomicrocotyle_mirabilis5 1 0 2 1 0 0 0 0 0 1
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1 1 3 1 1 3 0 1 0 0 2 1 2
2 0 1 1 0 0 0 0 3 1 0 0 1
1

Protomicrocotyle_mirabilis6 1 0 2 1 0 0 0 0 0 1
1 1 1 2 1 1 1 2 0 0 1 1 2
1 1 3 1 1 3 0 1 0 0 2 1 2
2 0 1 1 0 0 0 0 3 1 0 0 1
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Protomicrocotyle_mirabilis7 1 0 2 1 0 0 0 0 0 1
1 1 1 2 1 1 1 2 0 0 1 1 2
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2 0 1 1 0 0 0 0 3 1 0 0 1
1

Protomicrocotyle_veracruzensis1 1 1 2 1 1 0 0 0 0
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2 1 1 3 1 1 3 0 1 0 0 2 1
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2 1

Protomicrocotyle_veracruzensis2 1 1 2 1 1 0 0 0 0
1 1 1 1 1 2 1 1 2 0 0 1 1
2 1 1 3 1 1 3 0 1 0 0 2 1
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2 1

Protomicrocotyle_veracruzensis3 1 1 2 1 1 0 0 0 0
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2 1

Protomicrocotyle_veracruzensis4 1 1 2 1 1 0 0 0 0
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2 1

Protomicrocotyle_sp. 1 1 0 2 1 1 1 0 0 0 1
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1 1 3 1 1 3 0 1 0 0 2 1 2
2 0 0 1 0 0 0 0 3 1 0 1 2
1

Protomirocotyle_sp. 2 1 1 2 1 1 1 0 0 0 1
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1 0 1 1 0 0 0 0 3 1 0 2 0
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Protomicrocotyle_sp. 3 1 0 2 1 0 0 0 0 0 1
1 1 1 1 1 1 1 2 0 0 1 1 2
1 1 3 1 1 3 0 1 0 0 2 1 2
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1

Protmomicrocotyle_sp. 4(01)1 0 2 1 1 0 0 0 0 1
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1 1 3 1 1 3 0 1 0 0 2 1 2
2 0 0 1 0 0 0 0 3 1 0 2 1
0

Protmomicrocotyle_sp. 4(02) 1 0 2 1 1 0 0 0 0 1
1 1 1 1 2 1 1 2 0 0 1 1 2
1 1 3 1 1 3 0 1 0 0 2 1 2
2 0 0 1 0 0 0 0 3 1 0 2 1
0

Protmomicrocotyle_sp. 4(03) 1 0 2 1 1 0 0 0 0 1
1 1 1 1 2 1 1 2 0 0 1 1 2
1 1 3 1 1 3 0 1 0 0 2 1 2
2 0 0 1 0 0 0 0 3 1 0 2 1
0

Protmomicrocotyle_sp. 4(04) 1 0 2 1 1 0 0 0 0 1
1 1 1 1 2 1 1 2 0 0 1 1 2
1 1 3 1 1 3 0 1 0 0 2 1 2
2 0 0 1 0 0 0 0 3 1 0 2 1
0

Protmomicrocotyle_sp. 5(01) 1 0 2 1 0 0 0 0 0 1
1 1 1 1 2 1 1 2 0 0 1 1 2
1 1 3 1 1 3 0 1 0 0 2 1 2
1 0 1 1 0 0 0 0 3 1 0 0 0
1

Protmomicrocotyle_sp. 5(02) 1 0 2 1 0 0 0 0 0 1
1 1 1 1 2 1 1 2 0 0 1 1 2
1 1 3 1 1 3 0 1 0 0 2 1 2
1 0 1 1 0 0 0 0 3 1 0 0 0
1

Protmomicrocotyle_sp. 5(03) 1 0 2 1 0 0 0 0 0 1
1 1 1 1 2 1 1 2 0 0 1 1 2
1 1 3 1 1 3 0 1 0 0 2 1 2
1 0 1 1 0 0 0 0 3 1 0 0 0
1

Protmomicrocotyle_sp. 5(04) 1 0 2 1 0 0 0 0 0 1
1 1 1 1 2 1 1 2 0 0 1 1 2
1 1 3 1 1 3 0 1 0 0 2 1 2
1 0 1 1 0 0 0 0 3 1 0 0 0
1

Protomicrocotyle_nayaritensis 1 0 2 1 1 0 0 0 0
1 1 1 1 1 2 1 1 2 0 0 1 1
2 1 1 3 1 1 3 0 1 0 0 2 1

2 1 0 0 0 0 0 0 0 3 1 0 1
0 1

Protomicrocotyle_manteri 1 1 2 1 1 1 0 0 0 1
1 1 1 1 2 1 1 2 0 0 1 1 2
1 1 3 1 1 3 0 1 0 0 2 1 2
1 0 1 0 1 0 0 0 3 1 0 2 0
1

Protomicrocotyle_minutum 1 1 2 1 1 0 0 0 0 1
1 1 1 1 2 1 1 2 0 0 1 1 2
1 1 3 1 1 3 0 1 0 0 2 1 2
1 0 0 0 1 0 0 0 3 1 0 1 1
1

Protomicrocotyle_mannarrensis 1 0 2 1 0 0 0 0 0
1 1 1 1 1 2 1 1 2 0 0 1 1
2 1 1 3 1 1 3 0 1 0 0 2 1
2 1 0 0 0 1 0 0 0 3 1 0 2
1 1

Protomicrocotyle_madrasensis 1 0 2 1 0 0 0 0 0
1 1 1 1 1 2 1 1 2 0 0 1 1
2 1 1 3 1 1 3 0 1 0 0 2 1
2 1 0 0 0 1 0 0 0 3 1 0 2
0 1

Protomicrocotyle_carangis 1 0 2 1 0 0 0 0 0 0
1 1 1 1 2 1 1 2 0 0 1 1 2
1 1 3 1 1 3 0 1 0 0 2 1 2
? 0 0 0 1 0 0 0 3 1 0 1 1
1

Protomicrocotyle_celebensis 1 0 2 1 0 0 0 0 0 1
2 1 1 1 2 1 1 2 0 0 1 1 2
1 1 3 1 1 3 0 1 0 0 2 1 2
? 0 0 0 1 0 0 0 3 1 0 1 2
1

Protomicrocotyle_ivoriensis 1 1 2 1 0 0 0 0 0 1
1 1 1 1 2 1 1 2 0 0 1 1 2
1 1 3 1 1 3 0 1 0 0 1 0 0
0 0 0 0 0 1 0 0 3 1 0 1 1
0

Neomicrocotyle_pacifica 1 1 2 1 0 0 0 0 0 1
1 1 1 1 2 1 1 2 0 0 1 1 1
1 1 3 1 2 3 0 1 0 0 2 1 2
1 0 0 0 1 0 0 0 3 1 0 1 0
1

Neomicrocotyle_carangis 1 1 2 1 0 0 0 0 0 1
1 1 1 1 2 1 1 2 0 0 1 1 1
1 1 3 1 2 3 0 1 0 0 2 1 2
? 0 0 0 0 1 0 0 3 1 0 1 0
1

Neomicrocotyle_elongata 1 1 2 1 0 0 0 0 0 1
1 1 1 1 2 1 1 2 0 0 1 1 1
1 1 3 1 2 3 0 1 0 0 2 1 2
? 0 0 0 0 0 0 0 3 1 0 1 0
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Neomicrocotyle_indicus 1 1 2 1 0 0 0 0 0 1
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1 1 3 1 2 3 0 1 0 0 2 1 2
? 0 0 0 0 0 0 0 3 1 0 1 0
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Neomicrocotyle_unnithani 1 1 2 1 0 0 0 0 0 1
1 1 1 1 2 1 1 2 0 0 1 1 1
1 1 3 1 2 3 0 1 0 0 2 1 2
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Bilaterocotyle_chirocentrocus 1 0 2 1 0 0 0 0 0 0
1 1 1 1 1 2 1 1 2 0 0 1 1
2 2 2 3 1 1 3 0 1 0 0 0 0
0 0 0 0 0 0 0 0 0 3 1 0 1
1 1

Bilaterocotyle_madrasensis 1 0 2 1 0 0 0 0 0 1
1 1 1 1 2 1 1 2 0 0 1 1 2
2 2 3 1 1 3 0 1 0 0 2 1 2
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Bilaterocotyloides_carangis 1 0 2 1 0 0 0 0 0 1
1 1 1 1 2 1 1 2 0 0 1 1 2
2 2 3 1 1 3 0 1 0 0 2 1 2
1 0 0 0 0 0 0 0 1 1 0 1 1
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Lethacotyle_fijensis 1 0 2 1 0 0 0 0 0 1 1
1 1 3 2 1 1 2 0 0 1 1 0 0
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Lethacotyle_vera 1 0 2 1 0 0 0 0 0 1 1
1 1 3 2 1 1 2 0 0 1 1 0 0

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Vallisiopsis_contorta 0 1 0 0 0 0 0 0 0 0 1
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2 3 1 3 2 0 1 0 0 2 1 2 ?
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Youngiopsis_australis 0 1 0 0 0 0 0 0 0 0
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3 2 3 1 3 2 0 1 0 0 2 1 2
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Allodiscocotyla_dicanthi01
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TCGGTATACCAACAGGTGTGAAGGTTATTGCTTGGGTGAGAATGTTAAGTAAAGCTG
GTATATATCGAAGGGACCCCATAGTGTGATGGTTACTTTCTTTTGTCTTTCTATTTAC
CCTAGGGGGAATAACAGGTCTAATTTTATCTTGTTCTAGTGTTGATATG?????????????
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Allodiscocotyla_dicanthi02
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GTATATATCGAAGGGACCCCATAGTGTGATGGTTACTTTCTTTTGTCTTTCTATTTAC
CCTAGGGGGAATAACAGGTCTAATTTTATCTTGTTCTAGTGTTGATATG?????????????
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Allodiscocotyla_chorinemi
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Metacamopia_lebedevi
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Metacamopia_ologoplites
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Gemmaecaputia_corrugata
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TTCTTTAGTGCCGTAACTGCTTTAATAGGGATTCCCACCGGAGTTAAGGTAATAGCT
TGAGTTTCCATGCTAAGCAATGCGGCTGTCTATCGACAAGACCCTCTAACACTCTGG
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Protomicrocotyle_mirabilis1
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GT?CCTTTCTTTA?

Protomicrocotyle_mirabilis2
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Protomicrocotyle_mirabilis3
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Protomicrocotyle_mirabilis4
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TTCTTTAGAGCAGTAACTGCTTTAATAGGTATACCAACAGGTGTTAAGGTTATAGCT
TGGGTTTCCATGCTTAGAAAATCAGGTATTTATCGAAGAGAACCTATAGTTTGGTGG
CTTATATCTTTTATCTTCTTATTTACACTAGGAGGAATTACTGGGTTAATACTTTCTTG
TTCTAGAGTAGACATGATATTACACGATAGTTGATTTGTAGTAGCACATTTTCATTAT
GT?TTTTTCT????

Protomicrocotyle_mirabilis5

??????????ATTTGGTATAGTTAGTCACATTTGTATAGAGATAAGCAACAAATC
GACTCCTTTAGGGTATGTGGGGATGGTTTTTGCCATGTTTTCTATAGTTGTTTTAGGG
TTCATAGTTTGAGCTCATCACATGTTTACAGTAGGTATGGATTTAAAGAGTAAAACT
TTCTTTAGAGCAGTAACTGCTTTAATAGGTATACCAACAGGTGTTAAGGTTATAGCT
TGGGTTTCCATGCTTAGAAAATCAGGTATTTATCGAAGAGAACCTATAGTTTGGTGG
CTTATATCTTTTATTTTCTTATTTACACTAGGAGGAATTACCGGGTTAATACTTTCTTG
TTCTAGAGTAGACATGATATTACACGATAGTTGATTTGTAGTAGCACATTTTCATTAT
GT?CTTTTCTTTA?

Protomicrocotyle_mirabilis6

??????????ATTTGGTATAGTTAGTCACATTTGTATAGAGATAAGCAACAAATC
GACTCCTTTAGGGTATGTAGGGATGGTTTTTGCCATGTTTTCTATAGTTGTTTTAGGG
TTCATAGTTTGAGCTCATCACATGTTTACAGTAGGTATGGATTTAAAGAGTAAAACT
TTCTTTAGAGCAGTAACTGCTTTAATAGGTATACCAACAGGTGTTAAGGTTATAGCT
TGGGTTTCCATGCTTAGAAAATCAGGTATTTATCGAAGAGAACCTATAGTTTGGTGG
CTTATATCTTTTATCTTCTTATTTACACTAGGAGGAATTACTGGGTTAATACTTTCTTG
TTCTAGAGTAGACATGATATTACACGATAGTTGATTTGTAGTAGCACATTTTCATTAT
GT?TTTTTCT????

Protomicrocotyle_mirabilis7

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Protomicrocotyle_veracruzensis1

????????????TTTGGTATAGTTAGTCATATTTGTGTAGAGATAAGTAATAAATCC
ACTCCTTTGGGGTATGTAGGTATGGTTTTTGCTATGTTTTCTATAGTTGTTTTAGGTTT
TATAGTTTGAGCTCATCACATGTTTACAGTGGGTATGGACTTAAAGAGTAAAACTTT
CTTTAGGGCAGTAACTGCTTTAATAGGAATACCCACGGGGGTTAAGGTAATAGCTTG
GGTTTCTATGCTTAGAAAATCAGGCATTTATCGAAGAGAGCCTATAGTTTGGTGGCT
AATCTCTTTTATTTTTTTATTTACCTTAGGGGGTATTACTGGATTGATACTCTCTTGTT
CTAGGGTAGATATGATATTACACGATAGTTGATTTGTAGTGGCGCATTTTCATTATGT
?TCTTTCTTTA?

Protomicrocotyle_veracruzensis2

?????????GGTTTTGGTATAGTTAGTCATATTTGTGTAGAGATAAGTAATAAATC
CACTCCTTTGGGGTATGTAGGTATGGTTTTTGCTATGTTTTCTATAGTTGTTTTAGGTT
TTATAGTTTGAGCTCATCACATGTTTACAGTGGGTATGGACTTAAAGAGTAAAACTT

TCTTTAGGGCAGTAACTGCTTTAATAGGAATACCCACGGGGGTTAAGGTAATAGCTT
GGGTTTCTATGCTTAGAAAATCAGGCATTTATCGAAGAGAGCCTATAGTTTGGTGGC
TAATCTCTTTTATTTTTTTATTTACCTTAGGAGGTATTACTGGATTGATACTCTCTTGT
TCTAGGGTAGATATGATATTACACGATAGTTGGTTTGTAGTGGCGCATTTTCATTATG
T?TCTTTCTTTA?

Protomicrocotyle_veracruzensis3

???????????TTTTGGTATAGTTAGTCATATTTGTGTAGAGATAAGTAATAAATCC
ACTCCTTTGGGGTATGTAGGTATGGTTTTTGCTATGTTTTCTATAGTTGTTTTAGGTTT
TATAGTTTGAGCTCATCACATGTTTACAGTGGGTATGGACTTAAAGAGTAAAACTTT
CTTTAGGGCAGTAACTGCTTTAATAGGAATACCCACGGGGGTTAAGGTAATAGCTTG
GGTTTCTATGCTTAGAAAATCAGGCATTTATCGAAGAGAGCCTGTAGTTTGGTGGCT
AATCTCTTTTATTTTTTTATTTACCTTAGGAGGTATTACTGGATTGATACTCTCTTGTT
CTAGGGTAGATATGATATTACATGATAGTTGATTTGTAGTGGCGCATTTTCATTATGT
TCCTTTCTTTA?

Protomicrocotyle_veracruzensis4

???TCATCTCGTTTTGGTATAGTTAGTCATATTTGTGTAGAGATAAGTAATAAA
TCCACTCCTTTGGGGTATGTAGGTATGGTTTTTGCTATGTTTTCTATAGTTGTTTTAGG
TTTTATAGTTTGAGCTCATCACATGTTTACAGTGGGTATGGACTTAAAGAGTAAAAC
TTTCTTTAGGGCAGTAACTGCTTTAATAGGAATACCCACGGGGGTTAAGGTAATAGC
TTGGGTTTCTATGCTTAGAAAATCAGGCATTTATCGAAGAGAGCCTATAGTTTGGTG
GCTAATCTCTTTTATTTTTTTATTTACCTTAGGAGGTATTACTGGATTGATACTCTCTT
GTTCTAGGGTAGATATGATATTACACGATAGTTGATTTGTAGTGGCGCATTTTCATTA
TGT?CCTTTCTTTA?

Protomicrocotyle_sp. 1

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Protomirocotyle_sp. 2

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Protomicrocotyle_sp. 3

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Protmomicrocotyle_sp. 4(01)

TCATCCTAGGATTTGGTATAGTTAGTCACATTTGTATAGAGATAAGCAACAAA

TCGACTCCTTTAGGGTATGTAGGGATGGTTTTTGCCATGTTTTCTATAGTTGTTTTAG
GGTTCATAGTTTGAGCTCATCACATGTTTACAGTAGGTATGGATTTAAAGAGTAAAA
CTTTCTTTAGGGCAGTAACTGCTTTAATAGGTATACCCACGGGTGTTAAGGTAATAG
CTTGGGTTTCCATGCTTAGAAAATCAGGTATTTATCGAAGAGAACCTATAGTTTGGT
GGCTTATATCTTTTATTTTCTTATTTACACTAGGAGGAATTACTGGGTTAATACTTTC
TTGTTCTAGGGTAGAAATGATATTACACGATAGTTGATTTGTAGTGGCACATTTTCAT
TATGTCCTTTCTTTA???

Protmomicrocotyle_sp. 4(02)

TCATCCTAGGATTTGGTATAGTTAGTCACATTTGTATAGAGATAAGCAACAAA
TCGACTCCTTTAGGGTATGTGGGGATGGTTTTTGCCATGTTTTCTATAGTTGTTTTAG
GGTTCATAGTTTGAGCTCATCACATGTTTACAGTAGGTATGGATTTAAAGAGTAAAA
CTTTCTTTAGGGCAGTAACTGCTTTAATAGGTATACCCACGGGTGTTAAGGTAATAG
CTTGGGTTTCCATGCTTAGAAAATCAGGTATTTATCGAAGAGAACCTATAGTTTGGT
GGCTTATATCTTTTATTTTCTTATTTACACTAGGAGGAATTACCGGGTTAATACTTTC
TTGTTCTAGGGTAGAAATGATATTACACGATAGTTGATTTGTAGTGGCACATTTTCAT
TATGTCCTTTCTTTA???

Protmomicrocotyle_sp. 4(03)

TCATCCTAGGATTTGGTATAGTTAGTCACATTTGTATAGAGATAAGCAACAAA
TCGACTCCTTTAGGGTATGTAGGGATGGTTTTTGCCATGTTTTCTATAGTTGTTTTAG
GGTTCATAGTTTGAGCTCATCACATGTTTACAGTAGGTATGGATTTAAAGAGTAAAA
CTTTCTTTAGGGCAGTAACTGCTTTAATAGGTATACCCACGGGTGTTAAGGTAATAG
CTTGGGTTTCCATGCTTAGAAAATCAGGTATTTATCGAAGAGAACCTATAGTTTGGT
GGCTTATATCTTTTATTTTCTTATTTACACTAGGAGGAATTACTGGGTTAATACTTTC
TTGTTCTAGGGTAGAAATGATATTACACGATAGTTGATTTGTAGTGGCACATTTTCAT
TATGTCCTTTCTTTA???

Protmomicrocotyle_sp. 4(04)

TCATCCTAGGATTTGGTATAGTTAGTCACATTTGTATAGAGATAAGCAACAAA
TCGACTCCTTTAGGGTATGTGGGGATGGTTTTTGCCATGTTTTCTATAGTTGTTTTAG
GGTTCATAGTTTGAGCTCATCATATGTTTACAGTAGGTATGGATTTAAAGAGTAAAA
CTTTCTTTAGGGCAGTAACTGCTTTAATAGGTATACCCACGGGTGTTAAGGTAATAG
CTTGGGTTTCCATGCTTAGAAAATCAGGTATTTATCGAAGAGAACCTATAGTTTGGT
GGCTTATATCTTTTATTTTCTTATTTACACTAGGAGGAATTACTGGGTTAATACTTTC
TTGTTCTAGGGTAGAAATGATATTACACGATAGTTGATTTGTAGTGGCACATTTTCAT
TATGTCCTTTCTTTA???

Protmomicrocotyle_sp. 5(01)

?????????CTTTTGGTATAGTTAGTCAAATTTGTTTAGAGATAAGAAATAAATCG
ACTCCTTTGGGGTATGTAGGTATGGTTTTTGCTATGTTTTCTATAGTTGTTTTAGGTTT
TATAGTTTGAGCTCATCACATGTTTACAGTGGGTATGGATTTAAAGAGTAAAACTTT
CTTTAGTGCAGTAACTGCTTTAATAGGAATACCAACGGGGGTTAAGGTAATAGCTTG
GGTTTCTATGCTTAGAAAATCAGGAATTTATCGAAGAGACCCTATAGTTTGGTGGCT
AATATCTTTTATTTTTTTATTCACTTTAGGAGGAATTACTGGGTTGATACTTTCTTGTT
CTAGGGTAGATATGATATTACATGATAGTTGATTTGTAGTGGCCCATTTTCATTATGT
TTTTTCTTTA???

Protmomicrocotyle_sp. 5(02)

?????????CTTTTGGTATAGTTAGTCAAATTTGTTTAGAGATAAGAAATAAATCG
ACTCCTTTGGGGTATGTAGGTATGGTTTTTGCTATGTTTTCTATAGTTGTCTTAGGTTT
TATAGTTTGAGCTCATCACATGTTTACAGTGGGTATGGATTTAAAGAGTAAAACTTT
CTTTAGTGCAGTAACTGCTTTAATAGGAATACCAACGGGGGTTAAGGTAATAGCTTG
GGTTTCTATGCTTAGAAAATCAGGAATTTATCGAAGAGACCCTATAGTTTGGTGGCT
AATATCTTTTATTTTTTTATTCACTTTAGGAGGAATTACTGGGTTGATACTTTCTTGTT
CTAGGGTAGATATGATATTACATGATAGTTGATTTGTAGTGGCCCATTTTCATTATGT
TTTTTCTTTA???

Protmomicrocotyle_sp. 5(03)

?????????CTTTTGGTATAGTTAGTCAAATTTGTTTAGAGATAAGAAATAAATCG
ACTCCTTTGGGGTATGTAGGTATGGTTTTTGCTATGTTTTCTATAGTTGTTTTAGGTTT
TATAGTTTGAGCTCATCACATGTTTACAGTGGGTATGGATTTAAAGAGTAAAACTTT
CTTTAGTGCAGTAACTGCTTTAATAGGAATACCAACGGGGGTTAAGGTAATAGCTTG
GGTTTCTATGCTTAGAAAATCAGGAATTTATCGAAGAGACCCTATAGTTTGGTGGCT
AATATCTTTTATTTTTTTATTCACTTTAGGAGGAATTACTGGGTTGATACTTTCTTGTT
CTAGGGTAGATATGATATTACATGATAGTTGATTTGTAGTGGCCCATTTTCATTATGT
TTTTTCTTTA???

Protmomicrocotyle_sp. 5(04)

?????????CTTTTGGTATAGTTAGTCAAATTTGTTTAGAGATAAGAAATAAATCG
ACTCCTTTGGGGTATGTAGGTATGGTTTTTGCTATGTTTTCTATAGTTGTTTTAGGTTT
TATAGTTTGAGCTCATCACATGTTTACAGTGGGTATGGATTTAAAGAGTAAAACTTT
CTTTAGTGCAGTAACTGCTTTAATAGGAATACCAACGGGGGTTAAGGTAATAGCTTG
GGTTTCTATGCTTAGAAAATCAGGAATTTATCGAAGAGACCCTATAGTTTGGTGGCT
AATATCTTTTATTTTTTTATTCACTTTAGGAGGAATTACTGGGTTGATACTTTCTTGTT
CTAGGGTAGATATGATATTACATGATAGTTGATTTGTAGTGGCCCATTTTCATTATGT
TTTTTCTTTA???

Protomicrocotyle_manteri

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Protomicrocotyle_minutum

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Protomicrocotyle_mannarrensis

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Protomicrocotyle_madrasensis

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Protomicrocotyle_carangis

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Protomicrocotyle_celebensis

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Protomicrocotyle_ivoriensis

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Neomicrocotyle_pacifica

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Neomicrocotyle_carangis

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Neomicrocotyle_elongata

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Neomicrocotyle_indicus

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Neomicrocotyle_unnithani

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Bilaterocotyle_chirocentrocus

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Bilaterocotyle_madrasensis

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GGTGTTTGCTATGTTTTCTATTGTTGTCTTGGGTTTTATAGTTTGAGCCCATCATATGT
TTACGGTCGGTATGGATTTAAAGAGTAAAACATTTTTTAGAGCTGTCACTGCATTAA
TAGGTATACCGACAGGCGTAAAGGTAATAGCCTGGGTGTCTATGCTTAGTAACTCTG
GTATTTATCGTAGTGATCCCATAGTATGGTGGTTACTATCTTTTATTTTTCTGTTTACG
TTAGGAGGTATTACGGGTCTTATACTATCTTGTTCTAGAGTAGATATG????????????
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Bilaterocotyloides_carangis

??AT
GGTTTTTGCAATGTTTTCTATAGTTGTTTTGGGGTTTATAGTTTGAGCGCATCACATG
TTCACAGTGGGGATGGATTTAAAGAGTAAAACATTTTTTAGAGCTGTTACCGCATTG
ATAGGTATCCCAACAGGTGTAAAGGTGATAGCTTGGGTTTCTATGCTCAGTAAAGCT
GGTATATATCGTAGTGACCCCATAGTGTGGTGGTTGTTATCTTTTATCTTCTTATTTA
CGTTAGGGGGTATTACTGGTCTTATATTATCTTGTTCTAGAGTAGATATG????????????
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Lethacotyle_fijensis

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Lethacotyle_vera

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Vallisiopsis_contorta

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Youngiopsis_australis

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Allodiscocotyla_dicanthi01

???????ATGGGATCCAATAAAATGCCCCTGTATTTCCCCTACTAACG?GCGAGT
GAACAGGGATTAGCCCATCACCGAAGCCTGCGTCCTTTTGGGCGTTTGGCAATGTGG
TGTTTAGGTCTATTGCTTTCGTATACTGCTTCGTTCCAAGTCCAATCATGAATATGGC
ATTTGAATAGCCCAGAGAGGGTGAAAGGCCCGTATGGACGGAGTGGTGTGTGACTG
CATTGACCGTGGAGTCGGGTTGTTTGAGAATGCAGCCCAAAATTGGTGGCAAACTCC
ATCTAAGGCTAAATACTGGCACGAGTCCGATAGCGAACAAGTACCGTGAGGGAAAG
TTGAAAAGTACTCTGAAGAGAGAGTATACTATGCGTGAAACCGATCATAGGCAAGC
GGGTGTAGTCAAGCTGCATGCTGTGGGAAATTGGCGGATCAGAATGGCGTGAGCTT
GGGCATATTTGGTCTGGTTATACGTGCGTCTGTTTGGGTTTGTTCGGCTCGCAAGGGT
CGGATGTGTGAAGTGTCATACGATGCTGCATACCTGTAACTGGTTTGTCCTGCTGGT
CCGTTTACTTTCTCATAGCGTATGTCACGACCGTCGCAACTGTCGGTCCACTTCGTCT
AAACCGTAGCGCGAGT?GCCTTGTGTTTCTTGCGTTGTGGGGG?CAGGTAGCTGGTTT
?GGTTTCGGCCAGGCTAGTGTTATCGCTGGGCGGAGTGT?TATCTGTGCCGTACGGCG
GGGCTGGCGGTGCGTGGTACCGGGCGTGCTAAAGTGTG??

Allodiscocotyla_dicanthi02

???????ATGGGATCCAATAAAATGCCCCTGTATTTCCCCTACTAACG?GCGAGT
GAACAGGGATTAGCCCATCACCGAAGCCTGCGTCCTTTTGGGCGTTTGGCAATGTGG
TGTTTAGGTCTATTGCTTTCGTATACTGCTTCGTTCCAAGTCCAATCATGAATATGGC
ATTTGAATAGCCCAGAGAGGGTGAAAGGCCCGTATGGACGGAGTGGTGTGTGACTG

CATTGACCGTGGAGTCGGGTTGTTTGAGAATGCAGCCCAAAATTGGTGGCAAACTCC
ATCTAAGGCTAAATACTGGCACGAGTCCGATAGCGAACAAGTACCGTGAGGGAAAG
TTGAAAAGTACTCTGAAGAGAGAGTATACTATGCGTGAAACCGATCATAGGCAAGC
GGGTGTAGTCAAGCTGCATGCTGTGGGAAATTGGCGGATCAGAATGGCGTGAGCTT
GGGCATATTTGGTCTGGTTATACGTGCGTCTGTTTGGGTTTGTTCGGCTCGCAAGGGT
CGGATGTGTGAAGTGTCATACGATGCTGCATACCTGTAACTGGTTTGTCCTGCTGGT
CCGTTTACTTTCTCATAGCGTATGTCACGACCGTCGCAACTGTCGGTCCACTTCGTCT
AAACCGTAGCGCGAGT?GCCTTGTGTTTCTTGCGTTGTGGGGG?CAGGTAGCTGGTTT
?GGTTTCGGCCAGGCTAGTGTTATCGCTGGGCGGAGTGT?TATCTGTGCCGTACGGCG
GGGCTGGCGGTGCGTGGTACCGGGCGTGCTAAAGTGTG??

Allodiscocotyla_chorinemi

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Metacamopia_lebedevi

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Metacamopia_ologoplites

TATCCATAAGCGGAGGAAAAGAACTACCAGG??ATTCCCCTAGTAACG?GCGA
GTGAACAGGGATTAGCCCAGCACCGAAGCCTGCGTCCGTTTGGGCGTTTGGCAATGT
GGTGTTTAGGTCTATTGCTTTCGTATACTGCTTCGTTCCAAGTCCAATCATGAATATG
GCATTTGTATGGCCCAGAGAGGGTGAAAGGCCCGTATGGACGGAGTGGTGTGTGAC
TGCATTGACCGTGGAGTCGGGTTGTTTGAGAATGCAGCCCAAAGTTGGTGGTAAACT
CCATCTAAGGCTAAATACTGGCACGAGTCCGATAGCGAACAAGTACCGTGAGGGAA
AGTTGAAAAGTACTCTGAAGAGAGAGTAAACAGTGCGTGAAACCGATCAGAGGCAA
GCGGGTGTAGTCAAGCTGCAAGCTGTGGGAAATTGGCGGATCAGAATGGCGTGAGC
TTGGGCATATCTGGTCTGGTTATAGGTGCGTCTGTTTGGGCGTGTTCGGCTCGCAAG
GGTCGGATGTGTGAAGTGTCATACGATGCTGCATACCTGTAACTGGTTTGTCCTGCT
GGTCCGTTTACTTTCTCATAGCGTATGTCACGACCGTCGCAACTGTCGGTCCACTTCG
TCTAAACCGTAGCGCGAGT?GCCTTGTGTTTCTTGCGTTGTGGGGG?CAGGTAGCTGG

TTT?GGTTTCGGCCAGATTAGTGTTATCGCTGGGCGGAGTGG?TATCTGTGCGGTACG
GCGGGGCTGGCGGTGCGTGGTACCGGGCGTGCTAAAGTGTGT?

Gemmaecaputia_corrugata

????????????????????ACTAACCAGG??ATTCCCCTAGTAACG?GCGAGTGAACA
GGGATTAGCCCAGCACCGAAGCCTGCGTCCGTTTGGGCGTTAGGCAATGTGGTGTTT
AGGTCTATCGCTTTTGTATACTACTTCGTTCCAAGTCCAAACTTGAATATGGCCCATG
TACGGCCCAGAGAGGGTGAAAGGCCCGTGGGGACGAGGTGGTGTGCGACTGCGTGG
ACCGTGGAGTCGGGTTGTTTGAGAATGCAGCCCAAAGTTGGTGGTAAACTCCATCTA
AGGCTAAATACTGGCACGAGTCCGATAGCGAACAAGTACCGTGAGGGAAAGTTGAA
AAGTACTCTGAAGAGAGAGTAAACAGTGCGTGAAACCGCTTAGAGGCAAACGGGTG
TAGTCAAGCTGCAAGCCGTGGGAAATTTGCGGATCAGAATGGCGTGAGCCTGAGCA
TACTTGATCTGGTTGTTGGCGCGTCTGGTAAGGCGTGTTCGGCTCGCAAGAGTCGGA
TGAGTGCAGTGTCATATGATGCGGCTTGCCAGTAGCTGGAATGTTCTGCTGGTCCGA
TGACTTTCTCATGGCGTATGTCACGACCGTTGCGACTGTCGGTCCGCTTCGTCTAAAC
CGTAGCGTGAGTAGCCTTGTGTTTCTTGCGTTGCGGGGG?CAGGTAGCTAATCT?GGT
TTCGGCCGGGTTGGTGTTATCGCTGGGCGGAGTGG?TATCTGTGCAGGACAGCGGGG
CTGGCGGTGCGTGGTACCGGGCGTGCTAAAGTGTGT?

Protomicrocotyle_mirabilis1

????????????????????AGAACTAACCAGG??ATTCCCCTAGTAACG?GCGAGTGAAC
AGGGATTAGCCCAGCACCGAAGCCTGCGTCTTTTTGGGCGTTAGGCAATGTGGTGTT
TAGGTTTATTGCTTTCTTATGCTGCTTCGTTCCAAGTCCAAACTTGAATATGGCATTT
GTATGGCCCAGAGAGGGTGAAAGGCCCGTATGGACGGAGTGGTGTTTGACTGCGTA
GACCGTGGAGTCGGGTTGTTTGAGAATGCAGCCCAAAGTTGGTGGTAAACTCCATCT
AAGGCTAAATACTGGCACGAGTCCGATAGCGAACAAGTACCGTGAGGGAAAGTTGA
AAAGTACTCTGAAGAGAGAGTAAACAGTGCGTGAAACCGATTAGAGGCAAACGGGT
GTAGTCAAGCTGCAAGCTGTGGGAAATTGGCGGATCAGAATGGCGTGAGCTTGAGC
ATATTTGATCTGGTTGTAGGTACGTCTGGTTGGGCGTATTCGGCTCGCAAGGGTCGG
ATGTGTGAAGTGTCATACGATGCTGCATGCCTATAACTGGTTTGTTCTGCTGGTCCGT
TTACTTTCTCATAGCGTATGTCACGACCGTCGCAGCTGTCGGTCCGCTTCGTCTAAAC
CGTAGCGTGAGTAGCCTTGTGTTTCTCGCGTTGCGGGGG?CAGGTAGCCAATTT?GGT
TTCGGCTAGGTTGGTGTAATCGCTGGGCGGAGTGG?TACTTGTGCAGTACGGCGGGG
CTGGCGGTGCGTGGTACCGGGCGTG???????????

Protomicrocotyle_mirabilis2

????????????????????AGAACTAACCAGG??ATTCCCCTAGTAACG?GCGAGTGAAC
AGGGATTAGCCCAGCACCGAAGCCTGCGTCTTTTTGGGCGTTAGGCAATGTGGTGTT
TAGGTTTATTGCTTTCTTATGCTGCTTCGTTCCAAGTCCAAACTTGAATATGGCATTT
GTATGGCCCAGAGAGGGTGAAAGGCCCGTATGGACGGAGTGGTGTTTGACTGCGTA
GACCGTGGAGTCGGGTTGTTTGAGAATGCAGCCCAAAGTTGGTGGTAAACTCCATCT
AAGGCTAAATACTGGCACGAGTCCGATAGCGAACAAGTACCGTGAGGGAAAGTTGA
AAAGTACTCTGAAGAGAGAGTAAACAGTGCGTGAAACCGATTAGAGGCAAACGGGT
GTAGTCAAGCTGCAAGCTGTGGGAAATTGGCGGATCAGAATGGCGTGAGCTTGAGC
ATATTTGATCTGGTTGTAGGTACGTCTGGTTGGGCGTATTCGGCTCGCAAGGGTCGG
ATGTGTGAAGTGTCATACGATGCTGCATGCCTATAACTGGTTTGTTCTGCTGGTCCGT
TTACTTTCTCATAGCGTATGTCACGACCGTCGCAGCTGTCGGTCCGCTTCGTCTAAAC
CGTAGCGTGAGTAGCCTTGTGTTTCTCGCGTTGCGGGGGGCAGGTAGCCAATTT?GG

TTTCGGCTAGGTTGGTGTAATCGCTGGGCGGAGTGG?TACTTGTGCAGTACGGCGGG
GCTGGCGGTGCGTGGTACCGGGCGTGC??????????

Protomicrocotyle_mirabilis3

??????????????????AAGAACTAACCAGG??ATTCCCCTAGTAACG?GCGAGTGAAC
AGGGATTAGCCCAGCACCGAAGCCTGCGTCTTTTTGGGCGTTAGGCAATGTGGTGTT
TAGGTTTATTGCTTTCTTATGCTACTTCGTTCCAAGTCCAAACTTGAATATGGCATTT
GTATGGCCCAGAGAGGGTGAAAGGCCCGTATGGACGGAGTGGTGTTTGACTGCGTA
GACCGTGGAGTCGGGTTGTTTGAGAATGCAGCCCAAAGTTGGTGGTAAACTCCATCT
AAGGCTAAATACTGGCACGAGTCCGATAGCGAACAAGTACCGTGAGGGAAAGTTGA
AAAGTACTCTGAAGAGAGAGTAAACAGTGCGTGAAACCGATTAGAGGCAAACGGGT
GTAGTCAAGCTGCAAGCTGTGGGAAATTGGCGGATCAGAATGGCGTGAGCTTGAGC
ATATTTGATCTGGTTGTAGGTACGTCTGGTTGGGCGTATTCGGCTCGCAAGGGTCGG
ATGTGTGAAGTGTCATACGATGCTGCATGCCTATAACTGGTTTGTTCTGCTGGTCCGT
TTACTTTCTCATAGCGTATGTCACGACCGTCGCAGCTGTCGGTCCGCTTCGTCTAAAC
CGTAGCGTGAGTAGCCTTGTGTTTCTCGCGTTGCGGGGG?CAGGTAGCCAATTT?GGT
TTCGGCTAGGTTGGTGTAATCGCTGGGCGGAGTGG?TACTTGTGCAGTACGGCGGGG
CTGGCGGTGCGTGGTACCGGGCGTGCT??????????

Protomicrocotyle_mirabilis4

??????????????????????????CCAGG??ATTCCCCTAGTAACG?GCGAGTGAACAGG
GATTAGCCCAGCACCGAAGCCTGCGTCTTTTTGGGCGTTAGGCAATGTGGTGTTTAG
GTTTATTGCTTTCTTATGCTGCTTCGTTCCAAGTCCAAACTTGAATATGGCATTTGTA
TGGCCCAGAGAGGGTGAAAGGCCCGTATGGACGGAGTGGTGTTTGACTGCGTAGAC
CGTGGAGTCGGGTTGTTTGAGAATGCAGCCCAAAGTTGGTGGTAAACTCCATCTAAG
GCTAAATACTGGCACGAGTCCGATAGCGAACAAGTACCGTGAGGGAAAGTTGAAAA
GTACTCTGAAGAGAGAGTAAACAGTGCGTGAAACCGATTAGAGGCAAACGGGTGTA
GTCAAGCTGCAAGCTGTGGGAAATTGGCGGATCAGAATGGCGTGAGCTTGAGCATA
TTTGATCTGGTTGTAGGTACGTCTGGTTGGGCGTATTCGGCTCGCAAGGGTCGGATG
TGTGAAGTGTCATACGATGCTGCATGCCTATAACTGGTTTGTTCTGCTGGTCCGTTTA
CTTTCTCATAGCGTATGTCACGACCGTCGCAGCTGTCGGTCCGCTTCGTCTAAACCGT
AGCGTGAGTAGCCTTGTGTTTCTCGCGTTGCGGGGG?CAGGTAGCCAATTT?GGTTTC
GGCTAGGTTGGTGTAATCGCTGGGCGGAGTGG?TACTTGTGCAGTACGGCGGGGCTG
GCGGTGCGTGGTACCGGGCGTGC??????????

Protomicrocotyle_mirabilis5

??????????????????AAGAACTAACCAGG??ATTCCCCTAGTAACG?GCGAGTGAAC
AGGGATTAGCCCAGCACCGAAGCCTGCGTCTTTTTGGGCGTTAGGCAATGTGGTGTT
TAGGTTTATTGCTTTCTTATGCTGCTTCGTTCCAAGTCCAAACTTGAATATGGCATTT
GTATGGCCCAGAGAGGGTGAAAGGCCCGTATGGACGGAGTGGTGTTTGACTGCGTA
GACCGTGGAGTCGGGTTGTTTGAGAATGCAGCCCAAAGTTGGTGGTAAACTCCATCT
AAGGCTAAATACTGGCACGAGTCCGATAGCGAACAAGTACCGTGAGGGAAAGTTGA
AAAGTACTCTGAAGAGAGAGTAAACAGTGCGTGAAACCGATTAGAGGCAAACGGGT
GTAGTCAAGCTGCAAGCTGTGGGAAATTGGCGGATCAGAATGGCGTGAGCTTGAGC
ATATTTGATCTGGTTGTAGGTACGTCTAGTTGGGCGTATTCGGCTCGCAAGGGTCGG
ATGTGTGAAGTGTCATACGATGCTGCATGCCTATAACTGGTTTGTTCTGCTGGTCCGT
TTACTTTCTCATAGCGTATGTCACGACCGTCGCAGCTGTCGGTCCGCTTCGTCTAAAC
CGTAGCGTGAGTAGCCTTGTGTTTCTCGCGTTGCGGGGG?CAGGTAGCCAATTT?GGT

TTCGGCTAGGTTGGTGTAATCGCTGGGCGGAGTGG?TACTTGTGCAGTACGGCGGGG
CTGGCGGTGCGTGGTACCGGGCGTG???????????

Protomicrocotyle_mirabilis6

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Protomicrocotyle_mirabilis7

??????????????????????GAACTAACCAGG??ATTCCCCTAGTAACG?GCGAGTGAACA
GGGATTAGCCCAGCACCGAAGCCTGCGTCTTTTTGGGCGTTAGGCAATGTGGTGTTT
AGGTTTATTGCTTTCTTATGCTACTTCGTTCCAAGTCCAAACTTGAATATGGCATTTG
TATGGCCCAGAGAGGGTGAAAGGCCCGTATGGACGGAGTGGTGTTTGACTGCGTAG
ACCGTGGAGTCGGGTTGTTTGAGAATGCAGCCCAAAGTTGGTGGTAAACTCCATCTA
AGGCTAAATACTGGCACGAGTCCGATAGCGAACAAGTACCGTGAGGGAAAGTTGAA
AAGTACTCTGAAGAGAGAGTAAACAGTGCGTGAAACCGATTAGAGGCAAACGGGTG
TAGTCAAGCTGCAAGCTGTGGGAAATTGGCGGATCAGAATGGCGTGAGCTTGAGCA
TATTTGATCTGGTTGTAGGTACGTCTGGTTGGGCGTATTCGGCTCGCAAGGGTCGGA
TGTGTGAAGTGTCATACGATGCTGCATGCCTATAACTGGTTTGTTCTGCTGGTCCGTT
TACTTTCTCATAGCGTATGTCACGACCGTCGCAGCTGTCGGTCCGCTTCGTCTAAACC
GTAGCGTGAGTAGCCTTGTGTTTCTCGCGTTGCGGGGG?CAGGTAGCCAATTT?GGTT
TCGGCTAGGTTGGTGTAATCGCTGGGCGGAGTGG?TACTTGTGCAGTACGGCGGGGC
TGGCGGTGCGTGGTACCGGGCGTGCTAAAGTGTG??

Protomicrocotyle_veracruzensis1

??????????????????GAAGAACTAACCAGG??ATTCCCCTAGTAACG?GCGAGTGAA
CAGGGATTAGCCCAGCACCGAAGCCTGCGTCCATTTGGGCGTTAGGCAATGTGGTGT
TTAGGTTTATTGCTTTCTTATGCTACTTCGTTCCAAGTCCAAACTTGAATATGGCATT
TGTATGGCCCAGAGAGGGTGAAAGGCCCGTATGGACGGAGTGGTGTTTGACTGCGT
AGACCGTGGAGTCGGGTTGTTTGAGAATGCAGCCCAAAGTTGGTGGTAAACTCCATC
TAAGGCTAAATACTGGCACGAGTCCGATAGCGAACAAGTACCGTGAGGGAAAGTTG
AAAAGTACTCTGAAGAGAGAGTAAACAGTGCGTGAAACCGATTAGAGGCAAACGG
GTGTAGTCAAGCTGCAAGCTGTGGGAAATTGGCGGATCAGAATGGCGTGAGCTTGA
GCATATTTGATCTGGTTGTAGGTACGTCTAGTTGGGCGTATTCGGCTCGCAAGGGTC
GGATGTGTGAAGTGTCATACGATGCTGCATGCCTATAACTGGTTTGTTCTGCTGGTC
CGTTTACTTTCTCATAGCGTATGTCACGACCGTCGCAGCTGTCGGTCCGCTTCGTCTA
AACCGTAGCGTGAGTAGCCTTGTGTTTCTCGCGTTGCGGGGG?CAGGTAGCCAATTT?
GGTTTCGGCTAGATTGGTGTTAACGCTGGGCGGAGTGG?TACTTGTGCAGTACGGCG
GGGCTGGCGGTGCGTGGTACCGGGCGTGCTAAAGTG????

Protomicrocotyle_veracruzensis2

??????????????????AAGACTAACCAGG??ATTCCCCTAGTAACG?GCGAGTGAAC
AGGGATTAGCCCAGCACCGAAGCCTGCGTCCATTTGGGCGTTAGGCAATGTGGTGTT
TAGGTTTATTGCTTTCTTATGCTACTTCGTTCCAAGTCCAAACTTGAATATGGCATTT
GTATGGCCCAGAGAGGGTGAAAGGCCCGTATGGACGGAGTGGTGTTTGACTGCGTA
GACCGTGGAGTCGGGTTGTTTGAGAATGCAGCCCAAAGTTGGTGGTAAACTCCATCT
AAGGCTAAATACTGGCACGAGTCCGATAGCGAACAAGTACCGTGAGGGAAAGTTGA
AAAGTACTCTGAAGAGAGAGTAAACAGTGCGTGAAACCGATTAGAGGCAAACGGGT
GTAGTCAAGCTGCAAGCTGTGGGAAATTGGCGGATCAGAATGGCGTGAGCTTGAGC
ATATTTGATCTGGTTGTAGGTACGTCTAGTTGGGCGTATTCGGCTCGCAAGGGTCGG
ATGTGTGAAGTGTCATACGATGCTGCATGCCTATAACTGGTTTGTTCTGCTGGTCCGT
TTACTTTCTCATAGCGTATGTCACGACCGTCGCAGCTGTCGGTCCGCTTCGTCTAAAC
CGTAGCGTGAGTAGCCTTGTGTTTCTCGCGTTGCGGGGGGCAGGTAGCCAATTT?GG
TTTCGGCTAGATTGGTGTTAACGCTGGGCGGAGTGG?TACTTGTGCAGTACGGCGGG
GCTGGCGGTGCGTGGTACCGGGCGTGCTA?????????

Protomicrocotyle_veracruzensis3

????AGGGCGGGGAGGAAAGAACTAACCAGG??ATTCCCCTAGTAACG?GCGAG
TGAACAGGGATTAGCCCAGCACCGAAGCCTGCGTCCATTTGGGCGTTAGGCAATGT
GGTGTTTAGGTTTATTGCTTTCTTATGCTACTTCGTTCCAAGTCCAAACTTGAATATG
GCATTTGTATGGCCCAGAGAGGGTGAAAGGCCCGTATGGACGGAGTGGTGTTTGAC
TGCGTAGACCGTGGAGTCGGGTTGTTTGAGAATGCAGCCCAAAGTTGGTGGTAAACT
CCATCTAAGGCTAAATACTGGCACGAGTCCGATAGCGAACAAGTACCGTGAGGGAA
AGTTGAAAAGTACTCTGAAGAGAGAGTAAACAGTGCGTGAAACCGATTAGAGGCAA
ACGGGTGTAGTCAAGCTGCAAGCTGTGGGAAATTGGCGGATCAGAATGGCGTGAGC
TTGAGCATATTTGATCTGGTTGTAGGTACGTCTAGTTGGGCGTATTCGGCTCGCAAG
GGTCGGATGTGTGAAGTGTCATACGATGCTGCATGCCTATAACTGGTTTGTTCTGCT
GGTCCGTTTACTTTCTCATAGCGTATGTCACGACCGTCGCAGCTGTCGGTCCGCTTCG
TCTAAACCGTAGCGTGAGTAGCCTTGTGTTTCTCGCGTTGCGGGGG?CAGGTAGCCA
ATTT?GGTTTCGGCTAGATTGGTGTTAACGCTGGGCGGAGTGGGTACTTGTGCAGTA
CGGCGGGGCTGGCGGTGCGTGGTACCGGGCGTGCTA?????????

Protomicrocotyle_veracruzensis4

??????????????????????TAACCAGG??ATTCCCCTAGTAACG?GCGAGTGAACAG
GGATTAGCCCAGCACCGAAGCCTGCGTCCATTTGGGCGTTAGGCAATGTGGTGTTTA
GGTTTATTGCTTTCTTATGCTACTTCGTTCCAAGTCCAAACTTGAATATGGCATTTGT
ATGGCCCAGAGAGGGTGAAAGGCCCGTATGGACGGAGTGGTGTTTGACTGCGTAGA
CCGTGGAGTCGGGTTGTTTGAGAATGCAGCCCAAAGTTGGTGGTAAACTCCATCTAA
GGCTAAATACTGGCACGAGTCCGATAGCGAACAAGTACCGTGAGGGAAAGTTGAAA
AGTACTCTGAAGAGAGAGTAAACAGTGCGTGAAACCGATTAGAGGCAAACGGGTGT
AGTCAAGCTGCAAGCTGTGGGAAATTGGCGGATCAGAATGGCGTGAGCTTGAGCAT
ATTTGATCTGGTTGTAGGTACGTCTAGTTGGGCGTATTCGGCTCGCAAGGGTCGGAT
GTGTGAAGTGTCATACGATGCTGCATGCCTATAACTGGTTTGTTCTGCTGGTCCGTTT
ACTTTCTCATAGCGTATGTCACGACCGTCGCAGCTGTCGGTCCGCTTCGTCTAAACC
GTAGCGTGAGTAGCCTTGTGTTTCTCGCGTTGCGGGGG?CAGGTAGCCAATTT?GGTT
TCGGCTAGATTGGTGTTAACGCTGGGCGGAGTGG?TACTTGTGCAGTACGGCGGGGC
TGGCGGTGCGTGGTACCGGGCGTGCT??????????

Protomicrocotyle_sp. 1

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Protomirocotyle_sp. 2

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Protomicrocotyle_sp. 3

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Protmomicrocotyle_sp. 4(01)

CCAGAAATTCCCCTAGTAACGGCGAGTGAACAGGGATTAGCCCAGCACCGAA
GCCTGCGTCTTTTTGGGCGTTAGGC?AATGTGGTG?TTTAGGTTTATTGCTTTCTTATG
CTGCTTCGTT?CCAAGTCCAAACTTGAATATGGCATTTGTATGGCCCAGAGAGGGTG
AAAGGCCCGTATGGACGGAGTGGTGTTTGACTGCGTAGACCGTGGAGTCGGGTTGTT
TGAGAATGCAGCCCAAAGTTGGTGGTAAACTCCATCTAAGGCTAAATACTGGCACG
AGTCCGATAGCGAACAAGTACCGTGAGGGAAAGTTGAAAAGTACTCTGAAGAGAGA
GTAAACAGTGCGTGAAACCGATTAGAGGCAAACGGGTGTAGTCAAGCTGCAAGCTG
TGGGAAATTGGCGGATCAGAATGGCGTGAGCTTGAGCATATTTGATCTGGTTGTAGG
TACGTCTTGTTGGCCGTATTCGGCTCGCAAGGGTCGGATGTGTGAAGTGTCATACGA
TGCTGGATGCCTATAACTGGTTTGTTCTGCTGGTCCGTTTACTTTCTCATAGCGTATG
TCACGACCGTCGCAGCTGTCGGTCCGCTTCGTCTAAACCGTAGCGTGAGTAGCCTTG

TGTTTCTCGCGTTGC?GGGGGCAGGTAGCCAATTTGGTTTCGGCTAGGTTGGTGTAAT
CGCTGGGCGGAGT?GGTACTTGTGCAGTACGGCGGGGCTGGCGGTGCGTGGTACCGG
GCGTGCTAAAGTGT??

Protmomicrocotyle_sp. 4(02)

??????????????????????????????CCAGAAATTCCCCTAGTAACGGCGAGTGAACAGG
GATTAGCCCAGCACCGAAGCCTGCGTCTTTTTGGGCGTTAGGC?AATGTGGTG?TTTA
GGTTTATTGCTTTCTTATGCTACTTCGTT?CCAAGTCCAAACTTGAATATGGCATTTGT
ATGGCCCAGAGAGGGTGAAAGGCCCGTATGGACGGAGTGGTGTTTGACTGCGTAGA
CCGTGGAGTCGGGTTGTTTGAGAATGCAGCCCAAAGTTGGTGGTAAACTCCATCTAA
GGCTAAATACTGGCACGAGTCCGATAGCGAACAAGTACCGTGAGGGAAAGTTGAAA
AGTACTCTGAAGAGAGAGTAAACAGTGCGTGAAACCGATTAGAGGCAAACGGGTGT
AGTCAAGCTGCAAGCTGTGGGAAATTGGCGGATCAGAATGGCGTGAGCTTGAGCAT
ATTTGATCTGGTTGTAGGTACGTCTTGTTGGCCGTATTCGGCTCGCAAGGGTCGGAT
GTGTGAAGTGTCATACGATGCTGGATGCCTATAACTGGTTTGTTCTGCTGGTCCGTTT
ACTTTCTCATAGCGTATGTCACGACCGTCGCAGCTGTCGGTCCGCTTCGTCTAAACC
GTAGCGTGAGTAGCCTTGTGTTTCTCGCGTTGC?GGGGGCAGGTAGCCAATTTGGTTT
CGGCTAGGTTGGTGTAATCGCTGGGCGGAGT?GGTACTTGTGCAGTACGGCGGGGCT
GGCGGTGCGTGGTACCGGGCGTGCTAAAGTGT??

Protmomicrocotyle_sp. 4(03)

??????????????????????????????AGAAATTCCCCTAGTAACGGCGAGTGAACAGG
GATTAGCCCAGCACCGAAGCCTGCGTCTTTTTGGGCGTTAGGC?AATGTGGTG?TTTA
GGTTTATTGCTTTCTTATGCTGCTTCGTT?CCAAGTCCAAACTTGAATATGGCATTTGT
ATGGCCCAGAGAGGGTGAAAGGCCCGTATGGACGGAGTGGTGTTTGACTGCGTAGA
CCGTGGAGTCGGGTTGTTTGAGAATGCAGCCCAAAGTTGGTGGTAAACTCCATCTAA
GGCTAAATACTGGCACGAGTCCGATAGCGAACAAGTACCGTGAGGGAAAGTTGAAA
AGTACTCTGAAGAGAGAGTAAACAGTGCGTGAAACCGATTAGAGGCAAACGGGTGT
AGTCAAGCTGCAAGCTGTGGGAAATTGGCGGATCAGAATGGCGTGAGCTTGAGCAT
ATTTGATCTGGTTGTAGGTACGTCTTGTTGGCCGTATTCGGCTCGCAAGGGTCGGAT
GTGTGAAGTGTCATACGATGCTGGATGCCTATAACTGGTTTGTTCTGCTGGTCCGTTT
ACTTTCTCATAGCGTATGTCACGACCGTCGCAGCTGTCGGTCCGCTTCGTCTAAACC
GTAGCGTGAGTAGCCTTGTGTTTCTCGCGTTGC?GGGGGCAGGTAGCCAATTTGGTTT
CGGCTAGGTTGGTGTAATCGCTGGGCGGAGT?GGTACTTGTGCAGTACGGCGGGGCT
GGCGGTGCGTGGTACCGGGCGTGCTAAAGTGT??

Protmomicrocotyle_sp. 4(04)

??????????????????????????????AGAAATTCCCCTAGTAACGGCGAGTGAACAGG
GATTAGCCCAGCACCGAAGCCTGCGTCTTTTTGGGCGTTAGGC?AATGTGGTG?TTTA
GGTTTATTGCTTTCTTATGCTACTTCGTT?CCAAGTCCAAACTTGAATATGGCATTTGT
ATGGCCCAGAGAGGGTGAAAGGCCCGTATGGACGGAGTGGTGTTTGACTGCGTAGA
CCGTGGAGTCGGGTTGTTTGAGAATGCAGCCCAAAGTTGGTGGTAAACTCCATCTAA
GGCTAAATACTGGCACGAGTCCGATAGCGAACAAGTACCGTGAGGGAAAGTTGAAA
AGTACTCTGAAGAGAGAGTAAACAGTGCGTGAAACCGATTAGAGGCAAACGGGTGT
AGTCAAGCTGCAAGCTGTGGGAAATTGGCGGATCAGAATGGCGTGAGCTTGAGCAT
ATTTGATCTGGTTGTAGGTACGTCTTGTTGGCCGTATTCGGCTCGCAAGGGTCGGAT
GTGTGAAGTGTCATACGATGCTGGATGCCTATAACTGGTTTGTTCTGCTGGTCCGTTT
ACTTTCTCATAGCGTATGTCACGACCGTCGCAGCTGTCGGTCCGCTTCGTCTAAACC

GTAGCGTGAGTAGCCTTGTGTTTCTCGCGTTGC?GGGGGCAGGTAGCCAATTTGGTTT
CGGCTAGGTTGGTGTAATCGCTGGGCGGAGT?GGTACTTGTGCAGTACGGCGGGGCT
GGCGGTGCGTGGTACCGGGCGTGCTAAAGTGT??

Protmomicrocotyle_sp. 5(01)

??????????????????????????????????ATTCCCCTAGTAACGGCGAGTGAACAGGGA
TTAGCCCAGCACCGAAGCCTGCGTCCATTTGGGCGTTAGGC?AATGTGGTG?TTTAGG
TTTATTGCTTTCTTATGCTACTTCGTT?CCAAGTCCAAACTTGAATATGGCATTTGTAT
GGCCCAGAGAGGGTGAAAGGCCCGTATGGACGGAGTGGTGTTTGACTGCGTAGACC
GTGGAGTCGGGTTGTTTGAGAATGCAGCCCAAAGTTGGTGGTAAACTCCATCTAAGG
CTAAATACTGGCACGAGTCCGATAGCGAACAAGTACCGTGAGGGAAAGTTGAAAAG
TACTCTGAAGAGAGAGTAAACAGTGCGTGAAACCGATTAGAGGCAAACGGGTGTAG
TCAAGCTGCAAGCTGTGGGAAATTGGCGGATCAGAATGGCGTGAGCTTGAGCATAT
TTGATCTGGTTGTAGGTACGTCTGGTTGGGCTTATTCGGCTCGCAAGGGTGGGATGT
GTGAAGTGTCATACGATGCTGCATGCCTATAACTGGTTTGTTCTGCTGGTCCGTTTAC
TTTCTCATAGCGTATGTCACGACCGTCGCAGCTGTCGGTCCGCTTCGTCTAAACCGTA
GCGTGAGTAGCCTTGTGTTTCTCGCGTTGC?GGGGGCAGGTAGCCAATTTGGTTTCGG
CTAGATTGGTGTTAACGCTGGGCGGAGT?GGTACTTGTGCAGTACGGCGGGGCTGGC
GGTGCGTGGTACCGGGCGTGCTAAAGTGTTT

Protmomicrocotyle_sp. 5(02)

??????????????????A??AGAACTAACCAGGATTCCCCTAGTAACGGCGAGTGAAC
AGGGATTAGCCCAGCACCGAAGCCTGCGTCCATTTGGGCGTTAGGC?AATGTGGTG?
TTTAGGTTTATTGCTTTCTTATGCTACTTCGTT?CCAAGTCCAAACTTGAATATGGCAT
TTGTATGGCCCAGAGAGGGTGAAAGGCCCGTATGGACGGAGTGGTGTTTGACTGCG
TAGACCGTGGAGTCGGGTTGTTTGAGAATGCAGCCCAAAGTTGGTGGTAAACTCCAT
CTAAGGCTAAATACTGGCACGAGTCCGATAGCGAACAAGTACCGTGAGGGAAAGTT
GAAAAGTACTCTGAAGAGAGAGTAAACAGTGCGTGAAACCGATTAGAGGCAAACG
GGTGTAGTCAAGCTGCAAGCTGTGGGAAATTGGCGGATCAGAATGGCGTGAGCTTG
AGCATATTTGATCTGGTTGTAGGTACGTCTGGTTGGGCTTATTCGGCTCGCAAGGGT
GGGATGTGTGAAGTGTCATACGATGCTGCATGCCTATAACTGGTTTGTTCTGCTGGT
CCGTTTACTTTCTCATAGCGTATGTCACGACCGTCGCAGCTGTCGGTCCGCTTCGTCT
AAACCGTAGCGTGAGTAGCCTTGTGTTTCTCGCGTTGC?GGGGGCAGGTAGCCAATT
TGGTTTCGGCTAGATTGGTGTTAACGCTGGGCGGAGT?GGTACTTGTGCAGTACGGC
GGGGCTGGCGGTGCGTGGTACCGGGCGTGCTAAAGTGTTT

Protmomicrocotyle_sp. 5(03)

??????????????????????????????????GATTCCCCTAGTAACGGCGAGTGAACAGGG
ATTAGCCCAGCACCGAAGCCTGCGTCCATTTGGGCGTTAGGC?AATGTGGTG?TTTAG
GTTTATTGCTTTCTTATGCTACTTCGTT?CCAAGTCCAAACTTGAATATGGCATTTGTA
TGGCCCAGAGAGGGTGAAAGGCCCGTATGGACGGAGTGGTGTTTGACTGCGTAGAC
CGTGGAGTCGGGTTGTTTGAGAATGCAGCCCAAAGTTGGTGGTAAACTCCATCTAAG
GCTAAATACTGGCACGAGTCCGATAGCGAACAAGTACCGTGAGGGAAAGTTGAAAA
GTACTCTGAAGAGAGAGTAAACAGTGCGTGAAACCGATTAGAGGCAAACGGGTGTA
GTCAAGCTGCAAGCTGTGGGAAATTGGCGGATCAGAATGGCGTGAGCTTGAGCATA
TTTGATCTGGTTGTAGGTACGTCTGGTTGGGCTTATTCGGCTCGCAAGGGTGGGATG
TGTGAAGTGTCATACGATGCTGCATGCCTATAACTGGTTTGTTCTGCTGGTCCGTTTA
CTTTCTCATAGCGTATGTCACGACCGTCGCAGCTGTCGGTCCGCTTCGTCTAAACCGT

AGCGTGAGTAGCCTTGTGTTTCTCGCGTTGC?GGGGGCAGGTAGCCAATTTGGTTTCG
GCTAGATTGGTGTTAACGCTGGGCGGAGT?GGTACTTGTGCAGTACGGCGGGGCTGG
CGGTGCGTGGTACCGGGCGTGCTAAAGTGTTT

Protmomicrocotyle_sp. 5(04)

??????????????????????????????????GATTCCCCTAGTAACGGCGAGTGAACAGGG
ATTAGCCCAGCACCGAAGCCTGCGTCCATTTGGGCGTTAGGC?AATGTGGTG?TTTAG
GTTTATTGCTTTCTTATGCTACTTCGTT?CCAAGTCCAAACTTGAATATGGCATTTGTA
TGGCCCAGAGAGGGTGAAAGGCCCGTATGGACGGAGTGGTGTTTGACTGCGTAGAC
CGTGGAGTCGGGTTGTTTGAGAATGCAGCCCAAAGTTGGTGGTAAACTCCATCTAAG
GCTAAATACTGGCACGAGTCCGATAGCGAACAAGTACCGTGAGGGAAAGTTGAAAA
GTACTCTGAAGAGAGAGTAAACAGTGCGTGAAACCGATTAGAGGCAAACGGGTGTA
GTCAAGCTGCAAGCTGTGGGAAATTGGCGGATCAGAATGGCGTGAGCTTGAGCATA
TTTGATCTGGTTGTAGGTACGTCTGGTTGGGCTTATTCGGCTCGCAAGGGTGGGATG
TGTGAAGTGTCATACGATGCTGCATGCCTATAACTGGTTTGTTCTGCTGGTCCGTTTA
CTTTCTCATAGCGTATGTCACGACCGTCGCAGCTGTCGGTCCGCTTCGTCTAAACCGT
AGCGTGAGTAGCCTTGTGTTTCTCGCGTTGC?GGGGGCAGGTAGCCAATTTGGTTTCG
GCTAGATTGGTGTTAACGCTGGGCGGAGT?GGTACTTGTGCAGTACGGCGGGGCTGG
CGGTGCGTGGTACCGGGCGTGCTAAAGTGTTT

Protomicrocotyle_manteri

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Protomicrocotyle_minutum

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Protomicrocotyle_mannarrensis

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Protomicrocotyle_madrasensis

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Protomicrocotyle_carangis

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Protomicrocotyle_celebensis

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Protomicrocotyle_ivoriensis

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Neomicrocotyle_pacifica

TCAATAAGCGGAGGAAAAGAAACTAACCAGG??ATTCCCTTAGTAACG?GCGA
GTGAACAGGGATTAGCCCAGCACCGAAGCCTGCGTCTGTTTAGGCGTTAGGCAATGT
GGTGTTTAGGTTTATTGCTTTCGTATGCTACTTCGTTCCAAGTCCAAACTTGAATATG
GCATTTGTATGGCCCAGAGAGGGTGAAAGGCCCGTATGGACGGAGTGGTGTTTGAC
TGCGTAGACCGTGGAGTCGGGTTGTTTGAGAATGCAGCCCAAAGTTGGTGGTAAACT
CCATCTAAGGCTAAATACTGGCACGAGTCCGATAGCGAACAAGTACCGTGAGGGAA
AGTTGAAAAGTACTCTGAAGAGAGAGTAAACAGTGCGTGAAACCGATTAGAGGCAA
ACGGGTGTAGTCAAGCTGCAAGTCGTGGGAAATTGGCGGATCAGAATGGCGTGAGC
TTGAGCATATTTGATCTGGTTGTAGGTACGTCTAGTCAGGCGTGTTCGACTCGCGAG
GGTCGGATGTGTGAAGTGTCATACGATGCTGCATGCCTATAATTAGTTTGTTCTGCT
GGTCCGTTTACTTTCTCATGGCGTATGTCACGACCGTCGCAGCTGTCGGTCCGCTTCG
TCTAAACCGTAGCGTGAGTAGCCTTGTGTTTCTCGCGTTGCGGGGG?CAGGTAGCCA
ATTT?GGTTTCGGCCGGGTTGGTGTTATCGCTGGGCGGAGTGG?TATCTGTGCAGTAC
GGCGGGGCTGGCGGTGCGTGGTACCGGGCGTGCTAAAGTGTCT?

Neomicrocotyle_carangis

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Neomicrocotyle_elongata

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Neomicrocotyle_indicus

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Neomicrocotyle_unnithani

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Bilaterocotyle_chirocentrocus

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Bilaterocotyle_madrasensis

??????AATCCGTTTAATAA?ACTAACCAGG??ATTCCCCTAGTAACG?GCGAGTG
AACAGGGATTAGCCCAGCACCGAAGCCTGCGTCCGTTTGGGCGTAAGGCAATGTGG
TGTTTAGGTTTATTGCTTTCGTATGCTGCTTCGTTCCAAGTCCAAACTTGAATATGGC
ATTTGTATGGCCCAGAGAGGGTGAAAGGCCCGTGTGGACGGAGTGGTGTTCGACTG
CGTAGACCGTGGAGTCGGGTTGTTTGAGAATGCAGCCCAAAGTTGGTGGTAAACTCC
ATCTAAGGCTAAATACTGGCACGAGTCCGATAGCGAACAAGTACCGTGAGGGAAAG
TTGAAAAGTACTCTGAAGAGAGAGTAAACAGTGCGTGAAACCGATTAGAGGCAAAC
GGGTGTAGTCAAGCTGCAAGTCGTGGGAAATTGGCGGATCAGAATGGCGTGAGCTT
GAGCATATTTGATCTGGTTTTAGGTGCGTCTGGTAAGGCGTGTTCGGCTCGCAAGGG
TCGGATGTGTGAAGTGTCATACGATGCTGCATGCCTAAAACTGGTTTGTTCTGCTGG
TCCGTTTACTTTCTCATGGCGTATGTCACGACCGTCGCAGCTGTCGGTCCGCTTCGTC

TAAACCGTAGCGTGAGTAGCCTTGTGTTTCTCGCGTTGCGGGGG?CAGGTAGCCAAT
TT?GGTTTCGGCCAGGTTGGTGTAATCGCTGGGCGGAGTGG?TATCTGTGCAGTACGG
CGGGGCTGGCGGTGCGTGGTACCGGGCGTGCTAAAGTGTGT?

Bilaterocotyloides_carangis

????????GGCTTTAGGTATCACTAATCCATCTATTCCCCTAGTAACGCGCGAGTG
AACAGGGATTAGCCCAGCACCGAAGCCTGCGTCCGTTTGGGCGTAAGGCAATGTGG
TGTTTAGGTTTATTGCTTTCGTATGCTGCTTCGTTCCAAGTCCAAACTTGAATATGGC
ATTTGTATGGCCCAGAGAGGGTGAAAGGCCCGTGTGGACGGAGTGGTGTTCGACTG
CGTAGACCGTGGAGTCGGGTTGTTTGAGAATGCAGCCCAAAGTTGGTGGTAAACTCC
ATCTAAGGCTAAATACTGGCACGAGTCCGATAGCGAACAAGTACCGTGAGGGAAAG
TTGAAAAGTACTCTGAAGAGAGAGTAAACAGTGCGTGAAACCGATTAGAGGCAAAC
GGGTGTAGTCAAGCTGCAAGTCGTGGGAAATTGGCGGATCAGAATGGCGTGAGCTT
GAGCATATTTGATCTGGTTTTAGGTGCGTCTGGTAAGGCGTGTTCGGCTCGCAAGGG
TCGGATGTGTGAAGTGTCATACGATGCTGCATGCCTAAAACTGGTTTGTTCTGCTGG
TCCGTTTACTTTCTCATGGCGTATGTCACGACCGTCGCAGCTGTCGGTCCACTTCGTC
TAAACCGTAGCGTGAGTAGCCTTGTGTTTCTCGCGTTGCGGGGG?CAGGTAGCCAGT
TTTGGTTTCGGCCAGGCTGGTGTAATCGCTGGGCGGAGTGG?TATCTGTGCAGTACG
GCGGGGCTGGCGGTGCGTGGTACCGGGCGTGCTAAAGTGTGT?

Lethacotyle_fijensis

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Lethacotyle_vera

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AGCCCAGCACCGAAGCCTGCGTCTTTTTGGACGTTAGGCAATGTGGTGTTTAGGTTT
ATTGCTTTCTTATGCTACTTCGTTCCAAGTCCAAACTTGAATATGGCATTTGTATGGC
CCAGAGAGGGTGAAAGGCCCGTATGGACGGAGTGGTGTTCGACTGCGTAGACCGTG
GAGTCGGGTTGTTTGAGAATGCAGCCCAAAGTTGGTGGTAAACTCCATCTAAGGCTA
AATACTGGCACGAGTCCGATAGCGAACAAGTACCGTGAGGGAAAGTTGAAAAGTAC
TCTGAAGAGAGAGTAAACAGTGCGTGAAACCGATTAGAGGCAAACGGGTGTAGTCA
AGCTGCAAGCTGTGGGAAATTGGCGGATCAGAATGGCGTGAGCTTGAGCATATTTG
ATCTGGTTGTAGGTACGTCTAGTCAGGCGTGTTCGGCTCGTGAGGGTCGGATGTGTG
AAGTGTCATACGATGCTGCATGCCTATAATTGGTTTGTTCTGCTGGTCCGTTTACTTT
CTCATGGCGTATGTCACGACCGTCGCAGCTGTCGGTCCACTTCGTCTAAACCGTAGC
GTGAGTAGCCTTGTGTTTCTCGCGTTGCGGGGG?CAGGTAACCAATTT?GGTTTCGGC
CGGGTTGGTGTTATCGCTGGGCGGAGTGG?TATTTGTGCAGTACGGCGGGGCTGGCG
GTGCGTGGTACCGGGCGTGCTAAAGTGTGT?

Vallisiopsis_contorta
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Youngiopsis_australis
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Allodiscocotyla_dicanthi01
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Allodiscocotyla_dicanthi02
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Allodiscocotyla_chorinemi

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Metacamopia_lebedevi

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Metacamopia_ologoplites

CCATGCATGCCTAAGTATGTGCTCCAGTAAAGTGAAACCACGAATGGCTCAT
TAAATCAGCTATTATTTATTAGATCATACCTGTTACATGGATAACTGTAGTAATTCTA
GAGCTAATACATGCCTCGAGTCCGTGACCCGCGAGGGGATCGGTGCACTTATTAGA
ACAAGACCATCCGGGCGCTCGCAAGAGTACTCGGCCATGTCGTGTTGACTCTGGATA
ATTT?TACAGATCGCAGTCGGCCTTGTGTCGGCGACAGGTCTATCAAATGTCTGCCCT
ATCAACTTACGATGGTAGGTAACCTGCCTACCATG??GTGATAACGGGTAACGGGGA
ATCAGGGTTCGATTCCGGAGAGGGAGCCTGAGAA?ACGGCTACCACATCCAAGGAA
GGCAGCAGGCGCGCAAATTACCCACTCCCGGCACGGGGAGGTAGTGACGAAAAATA
ACGATGCGGAACTCTTTGAGGATCCGTAATCGGAATGAGT?ACAGTCTAAAGCCTTT
AACGAGG?ATCAATTGGAGGGCAAGTCTGGTGCCAGCAGCCGCGGTAACTCCAGCT
CCAATAGCGTATATTAAAGTTGCTGCAGTTAAAA?AGCTCGTAGTTGGATCTTGACG
GCACTGTCGCTGATCGAGTTTCGTTTGCGAATGGAGCGACCACCGGTTGTCTTCGTA
AGCTACGTACTTGACTTGTTGATGGTGCAGTCTTTCAGTCGTATCTGGTCCTTTCGGG
GATCAGGTGCC?TTTTGACGGATGAGCTTGCCTTGACCGTCAATT?

Gemmaecaputia_corrugata

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Protomicrocotyle_mirabilis1

??ATGCATGCCTAAGTATGTGCTCCAGTAAAGTGAAACCACGAATGGCTCATT
AAATCAGCTATTATTCATTAGATCATACCCGTTACATGGATAACTGTAGTAATTCTA
GAGCTAATACATGCCTCGAATCCGTGACCCGCGAGGGAATCGGTGCACTTATTAGA
ACAAGACCAGCCGGGCACTCGCAAGAGTACTCGACCATGTCGTGTTGACTCTGGAT
AATTTCTACAGATCGCAGTCGGCCTTGTGTCGGCGACAGTTCTTTCAAATGTCTGCCC
TATCAACTTACGATGGTAGGTAACCTGCCTACCATGGTG??ATAACGGGTAACGGGG
AATCAGGGTTCGATTCCGGAGAGGGAGCCTGAGAAA?CGGCTACCACATCCAAGGA
AGGCAGCAGGCGCGCAAATTACCCACTCCCGGCACGGGGAGGTAGTGACGAAAAAT
AACGATGCGGGACTCTCTGAGGACCCGTAATCGGAATGAGTA?CAGTCTAAAGCCTT
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CCAATAGCGTATATTAAAGTTGCTGCAGTTAAAAA?GCTCGTAGTTGGATCTTGACG
GCACTGTCGCTGATCGAGTTTCGTGTGCGAATGGAGCAGCCACCGGTTGTCTTCGTG
AGCTACGTACTTGACTTGTTGATGGTGCAGTCTTTCAGCCGTGTCTGATCCTTTCGGG
GGTCAGGTGCCCTTTTGTCGGA????????????????????????

Protomicrocotyle_mirabilis2

??ATGCATGCCTAAGTATGTGCTCCAGTAAAGTGAAACCACGAATGGCTCATT
AAATCAGCTATTATTCATTAGATCATACCCGTTACATGGATAACTGTAGTAATTCTA
GAGCTAATACATGCCTCGAATCCGTGACCCGCGAGGGAATCGGTGCACTTATTAGA
ACAAGACCAGCCGGGCACTCGCAAGAGTACTCGACCATGTCGTGTTGACTCTGGAT
AATTTCTACAGATCGCAGTCGGCCTTGTGTCGGCGACAGTTCTTTCAAATGTCTGCCC
TATCAACTTACGATGGTAGGTAACCTGCCTACCATGGTG??ATAACGGGTAACGGGG
AATCAGGGTTCGATTCCGGAGAGGGAGCCTGAGAAA?CGGCTACCACATCCAAGGA
AGGCAGCAGGCGCGCAAATTACCCACTCCCGGCACGGGGAGGTAGTGACGAAAAAT
AACGATGCGGGACTCTCTGAGGACCCGTAATCGGAATGAGTA?CAGTCTAAAGCCTT
TAACGAGG?ATCAATTGGAGGGCAAGTCTGGTGCCAGCAGCCGCGGTAACTCCAGCT
CCAATAGCGTATATTAAAGTTGCTGCAGTTAAAAA?GCTCGTAGTTGGATCTTGACG
GCACTGTCGCTGATCGAGTTTCGTGTGCGAATGGAGCAGCCACCGGTTGTCTTCGTG
AGCTACGTACTTGACTTGTTGATGGTGCAGTCTTTCAGCCGTGTCTGATCCTTTCGGG
GGTCAGGTGCCCTTTTGTCGGA????????????????????????

Protomicrocotyle_mirabilis3

??ATGCATGCCTAAGTATGTGCTCCAGTAAAGTGAAACCACGAATGGCTCATT
AAATCAGCTATTATTCATTAGATCATACCCGTTACATGGATAACTGTAGTAATTCTA
GAGCTAATACATGCCTCGAATCCGTGACCCGCGAGGGAATCGGTGCACTTATTAGA

ACAAGACCAGCCGGGCACTCGCAAGAGTACTCGACCATGTCGTGTTGACTCTGGAT
AATTTCTACAGATCGCAGTCGGCCTTGTGTCGGCGACAGTTCTTTCAAATGTCTGCCC
TATCAACTTACGATGGTAGGTAACCTGCCTACCATGGTG??ATAACGGGTAACGGGG
AATCAGGGTTCGATTCCGGAGAGGGAGCCTGAGAAA?CGGCTACCACATCCAAGGA
AGGCAGCAGGCGCGCAAATTACCCACTCCCGGCACGGGGAGGTAGTGACGAAAAAT
AACGATGCGGGACTCTCTGAGGACCCGTAATCGGAATGAGTA?CAGTCTAAAGCCTT
TAACGAGG?ATCAATTGGAGGGCAAGTCTGGTGCCAGCAGCCGCGGTAACTCCAGCT
CCAATAGCGTATATTAAAGTTGCTGCAGTTAAAAA?GCTCGTAGTTGGATCTTGACG
GCACTGTCGCTGATCGAGTTTCGTGTGCGAATGGAGCAGCCACCGGTTGTCTTCGTG
AGCTACGTACTTGACTTGTTGATGGTGCAGTCTTTCAGCCGTGTCTGATCCTTTCGGG
GGTCAGGTGCCCTTTTGTCGGA??????????????????????????

Protomicrocotyle_mirabilis4

??ATGCATGCCTAAGTATGTGCTCCAGTAAAGTGAAACCACGAATGGCTCATT
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AATTTCTACAGATCGCAGTCGGCCTTGTGTCGGCGACAGTTCTTTCAAATGTCTGCCC
TATCAACTTACGATGGTAGGTAACCTGCCTACCATGGTG??ATAACGGGTAACGGGG
AATCAGGGTTCGATTCCGGAGAGGGAGCCTGAGAAA?CGGCTACCACATCCAAGGA
AGGCAGCAGGCGCGCAAATTACCCACTCCCGGCACGGGGAGGTAGTGACGAAAAAT
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TAACGAGG?ATCAATTGGAGGGCAAGTCTGGTGCCAGCAGCCGCGGTAACTCCAGCT
CCAATAGCGTATATTAAAGTTGCTGCAGTTAAAAA?GCTCGTAGTTGGATCTTGACG
GCACTGTCGCTGATCGAGTTTCGTGTGCGAATGGAGCAGCCACCGGTTGTCTTCGTG
AGCTACGTACTTGACTTGTTGATGGTGCAGTCTTTCAGCCGTGTCTGATCCTTTCGGG
GGTCAGGTGCCCTTTTGTCGGA??????????????????????????

Protomicrocotyle_mirabilis5

??ATGCATGCCTAAGTATGTGCTCCAGTAAAGTGAAACCACGAATGGCTCATT
AAATCAGCTATTATTCATTAGATCATACCCGTTACATGGATAACTGTAGTAATTCTA
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ACAAGACCAGCCGGGCACTCGCAAGAGTACTCGACCATGTCGTGTTGACTCTGGAT
AATTTCTACAGATCGCAGTCGGCCTTGTGTCGGCGACAGTTCTTTCAAATGTCTGCCC
TATCAACTTACGATGGTAGGTAACCTGCCTACCATGGTG??ATAACGGGTAACGGGG
AATCAGGGTTCGATTCCGGAGAGGGAGCCTGAGAAA?CGGCTACCACATCCAAGGA
AGGCAGCAGGCGCGCAAATTACCCACTCCCGGCACGGGGAGGTAGTGACGAAAAAT
AACGATGCGGGACTCTCTGAGGACCCGTAATCGGAATGAGTA?CAGTCTAAAGCCTT
TAACGAGG?ATCAATTGGAGGGCAAGTCTGGTGCCAGCAGCCGCGGTAACTCCAGCT
CCAATAGCGTATATTAAAGTTGCTGCAGTTAAAAA?GCTCGTAGTTGGATCTTGACG
GCACTGTCGCTGATCGAGTTTCGTGTGCGAATGGAGCAGCCACCGGTTGTCTTCGTG
AGCTACGTACTTGACTTGTTGATGGTGCAGTCTTTCAGCCGTGTCTGATCCTTTCGGG
GGTCAGGTGCCCTTTTGTCGGA??????????????????????????

Protomicrocotyle_mirabilis6

????????GCCTAAGTATGTGCTCCAGTAAAGTGAAACCACGAATGGCTCATTAA
ATCAGCTATTATTCATTAGATCATACCCGTTACATGGATAACTGTAGTAATTCTAGA
GCTAATACATGCCTCGAATCCGTGACCCGCGAGGGAATCGGTGCACTTATTAGAACA

AGACCAGCCGGGCACTCGCAAGAGTACTCGACCATGTCGTGTTGACTCTGGATAATT
TCTACAGATCGCAGTCGGCCTTGTGTCGGCGACAGTTCTTTCAAATGTCTGCCCTATC
AACTTACGATGGTAGGTAACCTGCCTACCATGGTG??ATAACGGGTAACGGGGAATC
AGGGTTCGATTCCGGAGAGGGAGCCTGAGAAA?CGGCTACCACATCCAAGGAAGGC
AGCAGGCGCGCAAATTACCCACTCCCGGCACGGGGAGGTAGTGACGAAAAATAACG
ATGCGGGACTCTCTGAGGACCCGTAATCGGAATGAGTA?CAGTCTAAAGCCTTTAAC
GAGG?ATCAATTGGAGGGCAAGTCTGGTGCCAGCAGCCGCGGTAACTCCAGCTCCA
ATAGCGTATATTAAAGTTGCTGCAGTTAAAAA?GCTCGTAGTTGGATCTTGACGGCA
CTGTCGCTGATCGAGTTTCGTGTGCGAATGGAGCAGCCACCGGTTGTCTTCGTGAGC
TACGTACTTGACTTGTTGATGGTGCAGTCTTTCAGCCGTGTCTGATCCTTTCGGGGGT
CAGGTGCC?TTTTGTCGG??????????????????????????

Protomicrocotyle_mirabilis7

CCATGCATGCCTAAGTATGTGCTCCAGTAAAGTGAAACCACGAATGGCTCAT
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GAGCTAATACATGCCTCGAATCCGTGACCCGCGAGGGAATCGGTGCACTTATTAGA
ACAAGACCAGCCGGGCACTCGCAAGAGTACTCGACCATGTCGTGTTGACTCTGGAT
AATTTCTACAGATCGCAGTCGGCCTTGTGTCGGCGACAGTTCTTTCAAATGTCTGCCC
TATCAACTTACGATGGTAGGTAACCTGCCTACCATGGTG??ATAACGGGTAACGGGG
AATCAGGGTTCGATTCCGGAGAGGGAGCCTGAGAAA?CGGCTACCACATCCAAGGA
AGGCAGCAGGCGCGCAAATTACCCACTCCCGGCACGGGGAGGTAGTGACGAAAAAT
AACGATGCGGGACTCTCTGAGGACCCGTAATCGGAATGAGTA?CAGTCTAAAGCCTT
TAACGAGG?ATCAATTGGAGGGCAAGTCTGGTGCCAGCAGCCGCGGTAACTCCAGCT
CCAATAGCGTATATTAAAGTTGCTGCAGTTAAAAA?GCTCGTAGTTGGATCTTGACG
GCACTGTCGCTGATCGAGTTTCGTGTGCGAATGGAGCAGCCACCGGTTGTCTTCGTG
AGCTACGTACTTGACTTGTTGATGGTGCAGTCTTTCAGCCGTGTCTGATCCTTTCGGG
GGTCAGGTGCCCTTTTGTCGGATGAGCTAGCCCTTGACCGTCGAT?

Protomicrocotyle_veracruzensis1

?CATGCATGCCTAAGTATGTGCTCCAGTAAAGTGAAACCACGAATGGCTCATT
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GAGCTAATACATGCCTCGAATCCGTGACCCGCGAGGGAATCGGTGCACTTATTAGA
ACAAGACCAGCCGGGCACTCGCAAGAGTACTCGACCATGTCGTGTTGACTCTGGAT
AATTTCTACAGATCGCAGTCGGCCTTGTGTCGGCGACAGTTCTTTCAAATGTCTGCCC
TATCAACTTACGATGGTAGGTAACCTGCCTACCATGGTG??ATAACGGGTAACGGGG
AATCAGGGTTCGATTCCGGAGAGGGAGCCTGAGAAA?CGGCTACCACATCCAAGGA
AGGCAGCAGGCGCGCAAATTACCCACTCCCGGCACGGGGAGGTAGTGACGAAAAAT
AACGATGCGGGACTCTCTGAGGACCCGTAATCGGAATGAGTA?CAGTCTAAAGCCTT
TAACGAGG?ATCAATTGGAGGGCAAGTCTGGTGCCAGCAGCCGCGGTAACTCCAGCT
CCAATAGCGTATATTAAAGTTGCTGCAGTTAAAAA?GCTCGTAGTTGGATCTTGACG
GCACTGTCGCTGATCGAGTTTCGTGTGCGAATGGAGCAACCACCGGTTGTCTTCGTG
AGCTACGTACTTGACTTGTTGATGGTGCAGTCTTTCAGCCGTGTCTGATCCTTTCGGG
GGTCAGGTGCC?TTTTGTCGGATGAGCTAGCCTTGACCGTCGATTG

Protomicrocotyle_veracruzensis2

?CATGCATGCCTAAGTATGTGCTCCAGTAAAGTGAAACCACGAATGGCTCATT
AAATCAGCTATTATTCATTAGATCATACCCGTTACATGGATAACTGTAGTAATTCTA
GAGCTAATACATGCCTCGAATCCGTGACCCGCGAGGGAATCGGTGCACTTATTAGA

ACAAGACCAGCCGGGCACTCGCAAGAGTACTCGACCATGTCGTGTTGACTCTGGAT
AATTTCTACAGATCGCAGTCGGCCTTGTGTCGGCGACAGTTCTTTCAAATGTCTGCCC
TATCAACTTACGATGGTAGGTAACCTGCCTACCATGGTG??ATAACGGGTAACGGGG
AATCAGGGTTCGATTCCGGAGAGGGAGCCTGAGAAA?CGGCTACCACATCCAAGGA
AGGCAGCAGGCGCGCAAATTACCCACTCCCGGCACGGGGAGGTAGTGACGAAAAAT
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CCAATAGCGTATATTAAAGTTGCTGCAGTTAAAAA?GCTCGTAGTTGGATCTTGACG
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AGCTACGTACTTGACTTGTTGATGGTGCAGTCTTTCAGCCGTGTCTGATCCTTTCGGG
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Protomicrocotyle_veracruzensis3

?CATGCATGCCTAAGTATGTGCTCCAGTAAAGTGAAACCACGAATGGCTCATT
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AATTTCTACAGATCGCAGTCGGCCTTGTGTCGGCGACAGTTCTTTCAAATGTCTGCCC
TATCAACTTACGATGGTAGGTAACCTGCCTACCATGGTG??ATAACGGGTAACGGGG
AATCAGGGTTCGATTCCGGAGAGGGAGCCTGAGAAA?CGGCTACCACATCCAAGGA
AGGCAGCAGGCGCGCAAATTACCCACTCCCGGCACGGGGAGGTAGTGACGAAAAAT
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TAACGAGG?ATCAATTGGAGGGCAAGTCTGGTGCCAGCAGCCGCGGTAACTCCAGCT
CCAATAGCGTATATTAAAGTTGCTGCAGTTAAAAA?GCTCGTAGTTGGATCTTGACG
GCACTGTCGCTGATCGAGTTTCGTGTGCGAATGGAGCAACCACCGGTTGTCTTCGTG
AGCTACGTACTTGACTTGTTGATGGTGCAGTCTTTCAGCCGTGTCTGATCCTTTCGGG
GGTCAGGTGCC?TTTTGTCGGATGAGCTAGCCTTGACCGTCGATTG

Protomicrocotyle_veracruzensis4

?CATGCATGCCTAAGTATGTGCTCCAGTAAAGTGAAACCACGAATGGCTCATT
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ACAAGACCAGCCGGGCACTCGCAAGAGTACTCGACCATGTCGTGTTGACTCTGGAT
AATTTCTACAGATCGCAGTCGGCCTTGTGTCGGCGACAGTTCTTTCAAATGTCTGCCC
TATCAACTTACGATGGTAGGTAACCTGCCTACCATGGTG??ATAACGGGTAACGGGG
AATCAGGGTTCGATTCCGGAGAGGGAGCCTGAGAAA?CGGCTACCACATCCAAGGA
AGGCAGCAGGCGCGCAAATTACCCACTCCCGGCACGGGGAGGTAGTGACGAAAAAT
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CCAATAGCGTATATTAAAGTTGCTGCAGTTAAAAA?GCTCGTAGTTGGATCTTGACG
GCACTGTCGCTGATCGAGTTTCGTGTGCGAATGGAGCAACCACCGGTTGTCTTCGTG
AGCTACGTACTTGACTTGTTGATGGTGCAGTCTTTCAGCCGTGTCTGATCCTTTCGGG
GGTCAGGTGCC?TTTTGTCGGATGAGCTAGCCTTGACCGTCGATTG

Protomicrocotyle_sp. 1

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Protomirocotyle_sp. 2

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Protomicrocotyle_sp. 3

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Protmomicrocotyle_sp. 4(01)

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Protmomicrocotyle_sp. 4(02)

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Protmomicrocotyle_sp. 5(04)

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Protomicrocotyle_manteri

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Protomicrocotyle_minutum

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Protomicrocotyle_mannarrensis

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Protomicrocotyle_madrasensis

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Protomicrocotyle_carangis

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Protomicrocotyle_celebensis

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Protomicrocotyle_ivoriensis

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Neomicrocotyle_pacifica

CCATGCATGCCTAAGTATGTGCTCCAGTAAAGTGAAACCACGAATGGCTCAT
TAAATCAGCTATTATTCATTAGATCATACCCGTTACATGGATAACTGTAGTAATTCTA
GAGCTAATACATGCCTCGAATCCGTGACCCGCGAGGGAATCGGTGCACTTATTAGA
ACAAGACCAGCCGGGCACTCGCAAGAGTACTCGACCATGTCGTGTTGACTCTGGAT
AATTTCTACAGATCGCAGTCGGCCTTGTGTCGGCGACAGTTCTTTCAAATGTCTGCCC
TATCAACTTACGATGGTAGGTAACCTGCCTACCATG??GTGATAACGGGTAACGGGG
AATCAGGGTTCGATTCCGGAGAGGGAGCCTGAGAA?ACGGCTACCACATCCAAGGA
AGGCAGCAGGCGCGCAAATTACCCACTCCCGGCACGGGGAGGTAGTGACGAAAAAT
AACGATGCGGGACTCTCTGAGGACCCGTAATCGGAATGAGT?ACAGTCTAAAGCCTT
TAACGAGGCATCAATTGGAGGGCAAGTCTGGTGCCAGCAGCCGCGGTAACTCCAGC
TCCAATAGCGTATATTAAAGTTGCTGCAGTTAAAA?AGCTCGTAGTTGGATCTTGAC
GGCACTGTCGCTGATCGAGTTTCGTGTGCGAATGGAGCAACCACCGGTTGTCTTCGT
GAGCTACGTACTTGACTTGTTGATGGTGCAGTCTTTCAGCCGTGTCTGGTCCTTTCGG
GGGTCAGGTGCC?TTTTGTCGGATGAGCTAGCCTTGACCGTCAATT?

Neomicrocotyle_carangis

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Neomicrocotyle_elongata

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Neomicrocotyle_indicus

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Neomicrocotyle_unnithani

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Bilaterocotyle_chirocentrocus

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Bilaterocotyle_madrasensis

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Bilaterocotyloides_carangis

CCATGCATGCCTAAGTATGTGCTCCAGTAAAGTGAAACCACGAATGGCTCAT
TAAATCAGCTATTATTCATTAGATCATACCCGTTACATGGATAACTGTAGTAATTCTA
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ACAAGACCAGCCGGGCACTCGCAAGAGTACTCGACCATGTCGTGTTGACTCTGGAT
AATTTCTACAGATCGCAGTCGGCCTTGTGTCGGCGACAGTTCTTTCAAATGTCTGCCC
TATCAACTTACGATGGTAGGTAACCTGCCTACCATG??GTGATAACGGGTAACGGGG
AATCAGGGTTCGATTCCGGAGAGGGAGCCTGAGAA?ACGGCTACCACATCCAAGGA
AGGCAGCAGGCGCGCAAATTACCCACTCCCGGCACGGGGAGGTAGTGACGAAAAAT
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CCAATAGCGTATATTAAAGTTGCTGCAGTTAAAA?AGCTCGTAGTTGGATCTTGACG
GCACTGTCGCTGATCGAGTTTCGTGTGCGAATGGAGCGGCCACCGGTCGTCTTCGTG
AGCTACGTACTTGACTTGTTGATGGTGCAGTCTTTCAGCCGTGTCTGATCCTTTCGGG
GGTCAGGTGCC?TTTTGTCGGATGAGCTAGCCTTGACTGTCAATT?

Lethacotyle_fijensis

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Lethacotyle_vera

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Vallisiopsis_contorta

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Youngiopsis_australis

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