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A NEW CRYPTOGONIMID (DIGENEA) FROM THE MAYAN CICHLID, *CICHLASOMA UROPHTHALMUS* (OSTEICHTHYES: CICHLIDAE), IN SEVERAL LOCALITIES OF THE YUCATÁN PENINSULA, MEXICO

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ABSTRACT: *Oligogonotylus mayae* n. sp. is described from the intestine of the Mayan cichlid *Cichlasoma urophthalmus* (Günther) in Ría Lagartos, Ría Celestún, and Estero Progreso, Yucatán State. This is the second species described for *Oligogonotylus* Watson, 1976, the other being *O. manteri* Watson, 1976. The new species is readily distinguished from *O. manteri* by the anterior extension of the vitelline follicles. In *O. manteri*, vitelline follicles are found entirely in the hindbody, extending posteriorly to mid-testicular level. Vitelline follicles in the new species extend from the anterior margin of posterior testis to the region between the ventral sucker and the pharynx. Comparison of approximately 1,850 bases of ribosomal DNA (ITS1, ITS2, 5.8S, and 28S), and 400 bases of cytochrome c oxidase subunit I (*cox*1) strongly supports the status of *O. mayae* as a new species, as compared to *O. manteri* collected from cichlids in other localities of Mexico, Belize, and Guatemala.

Oligogonotylus was erected by Watson (1976) to include *O. manteri* as a parasite of *Cichlasoma nicaraguense* (=*Hypsophrys nicaraguensis* Günther), *C. labiatum* (=*Amphilophus labiatus* Günther), *C. citrinellum* (=*A. citrinellus* Günther), *C. rostratum* (=*A. rostratus* Gill), and *C. maculicauda* (=*Vieja maculicauda* Regan) from Lake Nicaragua. Aguirre-Macedo et al. (2001) later found this species in *Vieja maculicauda* and *Parachromis managuensis* (Günther) in Atlantic drainages of Nicaragua. In Mexico, adults of this digenean have been reported from at least 15 species of fishes in 33 localities of southeastern Mexico, corresponding to 6 States (Campeche, Chiapas, Quintana Roo, Tabasco, Veracruz, and Yucatán (see Pérez-Ponce de León et al., 2007 and references therein). Eleven of the 15 host species are members of the Cichlidae and, accordingly, this digenean could be considered as a part of the biogeographical core helminth fauna of cichlids (Pérez-Ponce de León and Choudhury, 2005). The Mayan cichlid *C. urophthalmus* (Günther) seems to be the preferential host for *O. manteri*, as it has been found in 25 of the 33 localities where the parasite has been recorded. This species of cichlid has a wide distribution in southeastern Mexico (Miller et al., 2005).

Because this species was recorded for the first time by Watson (1976), it has been assumed that all the cryptogonimid parasites of middle-American cichlids possessing a longitudinal row of 5 to 8 gonotyls, with an spinose body, and lacking a circumoral crown of spines, correspond with the monotypic *O. manteri* (Pineda-López et al., 1985; Scholz et al., 1994, 1995; Salgado-Maldonado et al., 1997; Vidal-Martínez et al., 2001). Only 2 other cryptogonimid species have been described that possess multiple gonotyls, namely *Multigonotylus micropteri* Premvati, 1967 from the largemouth bass, *Micropterus salmoides* (Lacepede), in Florida and *Polycryptocylix leonilae* Lamothe-Argumedo, 1970 from the red snapper, *Lutjanus guttatus* (Steindachner), off the coast of Oaxaca on the Pacific slope of Mexico (Premvati, 1967; Lamothe-Argumedo, 1970).

As part of an ongoing survey of the helminth parasites of middle-American freshwater fishes, several specimens of *Oligogonotylus* were collected from the intestine of cichlid species in several localities of Mexico, Belize, and Guatemala. After examining the morphological characters in some detail, we noted that 2 morphotypes could be distinguished among the specimens we collected. Specimens deposited in museum collections were studied to further corroborate the existence of 2 morphotypes and, in addition to that, morphological differences were correlated with genetic differences using data on sequence divergence of ribosomal DNA (ITS1, ITS2, and 28S), and mitochondrial (*cox*1) genes. Based on both sources of evidence, in this paper a new species of *Oligogonotylus* is described as a parasite of *C. urophthalmus* in 3 locations on the Yucatán Peninsula.

MATERIALS AND METHODS

Between June 2006 and March 2007, 429 specimens of cichlids (representing 16 species) were collected in several localities of Mexico, Belize, and Guatemala (Fig. 1). In particular, 106 cryptogonimids belonging to *Oligogonotylus* were obtained from 2 species of cichlids, *C. urophthalmus* (172) and *C. istlanum* (Jordan & Snyder) (8), collected from 8 localities in Mexico, 1 in Belize, and 1 in Guatemala (Table I). Hosts were captured by angling and examined for helminths 4 hr after capture. Digeneans were collected and placed in a 0.65 % saline solution. Some worms were relaxed in hot (near boiling) tap water and fixed in 70% ethanol while others were immediately placed in 100% ethanol for DNA extraction.

Unflattened specimens were stained with Mayer's paracarmine, Ehrlich's hematoxylin, and Gomori's trichrome, cleared in methyl salicylate, and mounted in Canada balsam. Several specimens were permanently mounted between coverslips and held in Cobb slides. Drawings were made with the aid of a drawing tube attached to the microscope. Measurements are presented in micrometers (μm) with the mean followed by the range (in parentheses). Specimens were deposited in the Colección Nacional de Helmintos (CNHE), Instituto de Biología, UNAM, (Table I). For morphological comparisons, specimens of *O. manteri* deposited at the CNHE, the United States National Parasite Collection (USNPC), CHCM (Colección Helmintológica del CINVESTAV, Mérida, Yucatán, and the personal collection of Dr. Serapio López Jimenez (Universidad Juárez Autónoma de Tabasco, Villahermosa, Tabasco) were examined as follows: CNHE: ex *C. urophthalmus* from the following localities in southeastern Mexico: Villahermosa, Tabasco (1510); Ría Celestún, Yucatán (1270 and 1274). USNPC: ex *C. nicaraguensis* (061324.00, paratype). CHCM (53 specimens): ex *C. urophthalmus*, Estero del Pargo, and Boca Peralta, Campeche; ex *Vieja synspila* (Hubbs) Champotón, Campeche. Serapio López' personal Collection (11 specimens): ex *C. urophthalmus*, El Espino, Camellones Chontales, Vicente Guerrero, Tabasco, ex *C. managuensis* (Günther), *Petenia splendida* (Günther) Pantanos de Centla, Tabasco.

Genomic DNA of 17 gravid worms from 8 of 10 localities (Table I) was extracted either with phenol/chloroform procedures (Hillis et al., 1996) or by using the DNeasy Blood & Tissue Kit (Qiagen, Valencia,

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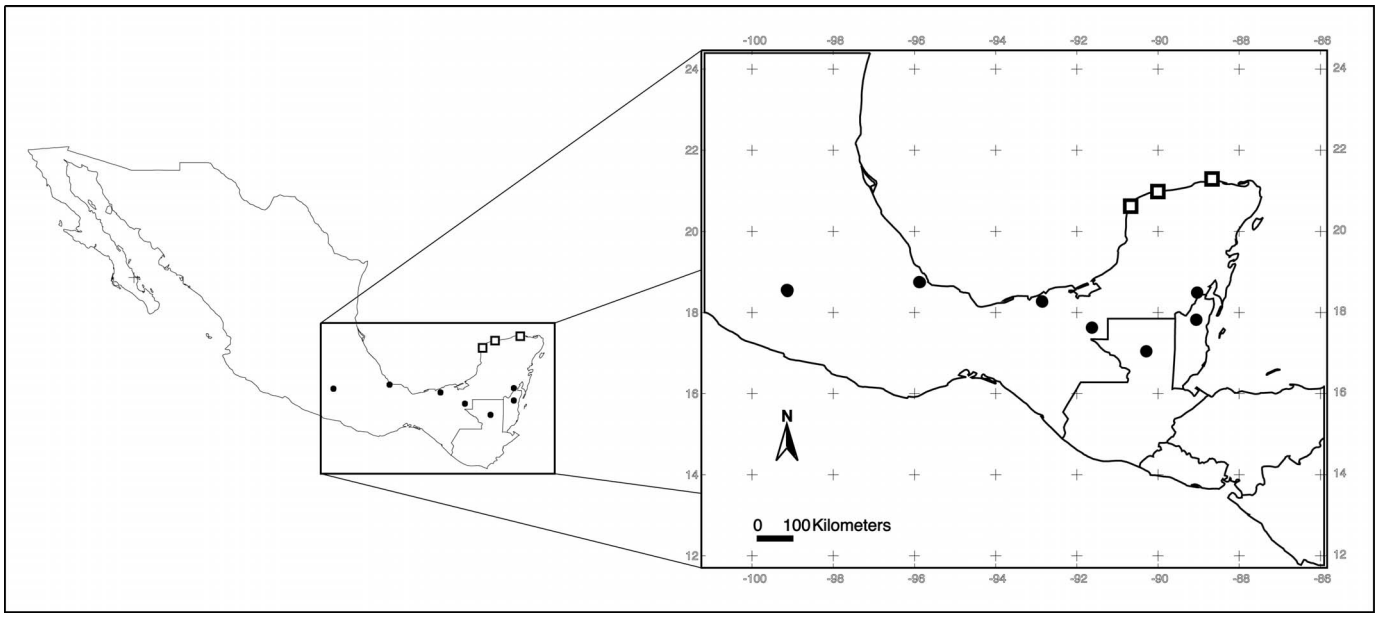


FIGURE 1. Map of Mexico showing the localities where *Oligogonotylus manteri* (●), and the new species, *O. mayae* (□), are found.

California) following manufacturer's instructions. Mitochondrial (cithocrome oxidase c subunit I, *cox*1) and nuclear (internal transcribed spacers 1 and 2, 5.8 gene, and large subunit 28S) markers were amplified by PCR in a final volume of 25 μl. Primers employed to obtain partial fragments of mitochondrial and nuclear markers were: *cox*1, JB3 (5′-TTT TTT GGG CAT CCT GAG GTT TAT-3′) and JB4.5 (5′-TAA AGA AAG AAC ATA ATG AAA ATG-3′), ~400bp, (Morgan and Blair, 1998); ITS1 + 5.8S + ITS2, BD1 (5′-GTCGTAACAAGGTTT CCGTA-3′) and BD2 (5′-TAT GCT TAA ATT CAG CGG GT-3′), ~1000 bp, (Bowles et al., 1995); and 28S, 28sl (5′-AACAGTGCGTG AAACCGCTC-3′) (Palumbi, 1996) and LO (5′-GCT ATC CTG AG(AG) GAA ACT TCG-3′), ~850 bp (Tkach et al., 2000). With the exception of annealing temperatures, reaction conditions used were the same regardless of primer set employed. An initial denaturation at 94 C for 5 min was followed by 35 cycles of 92 C for 30 sec, primers annealed for 45 sec at 47 C (*cox*1) or at 55 C (ITS1, ITS2 and 28S), and extension at 72 C for 90 sec; the mixes were held at 72 C for 10 min to complete elongation and then dropped to 4 C. The PCR products were purified by using Montage PCR centrifugal filter devices (Millipore, Bedford, Massachusetts). Mitochondrial and nuclear purified products were sequenced on an ABI PRISM 310 automated DNA sequencer (Applied Biosystems, Foster City, California) using the Big Dye Terminator™ (Applied Biosystems, Foster City, California) chemistry and incorporating the same primers as those used in previous PCRs. Sense and anti-sense strands were sequenced for all molecular markers and subsequently assembled and aligned manually using the software BIOEDIT (version 7.0.5.3; Hall, 1999).

Uncorrected genetic distances were obtained for each data set using PAUP (version 4.0b10; Swofford, 2002), and these are shown as pairwise distance matrices (Tables II–IV). ITS1 and ITS2 were analyzed separately, without including the 5.8S gene (121 bp), because it is highly conservative. The sequences generated in this study were submitted to GenBank (accession numbers are presented in Table I). The alignment of each set of molecular data has been deposited in EBI and is available by anonymous FTP from http://ftp.ebi.ac.uk in directory /pub/databases/embl/align under the numbers ALIGN_001244 (28S), ALIGN_001245 (ITS1, 5.8S and ITS2) and ALIGN_1246 (*cox*1).

DESCRIPTION

Oligogonotylus mayae n. sp.

(Figs. 2a–c, 3e–f)

Diagnosis (based on 18 adult specimens): Body elongate 964 (628–1,339) long, 279 (223–337) wide; widest at ovarian level. Anterior and posterior ends rounded. Tegument spinose, with spines becoming shorter and less dense in the posterior region; without circumoral crown of spines. Scattered diffuse eyespot remnants present. Oral sucker spherical, sub-terminal 120 (69–172) long, 170 (131–204) wide. Ventral sucker spherical 114 (83–145) long, 133 (101–158) wide, pre-equatorial 223 (127–354) from oral sucker, enclosed in a ventrogenital sac with genital pore in the middle; genital pore close to the anterior margin of ventral sucker. Oral sucker/ventral sucker length and width ratio 1:0.96, 1:0.77, respectively. Longitudinal row of 6–8 sucker-like gonotyls located between the posterior margin of oral sucker and the anterior margin of the ventral sucker, increasing in size from anterior to posterior. Prepharynx inconspicuous 54 (33–77) long, 68 (51–85) wide, possessing pharyngeal glands. Pharynx well-developed, muscular 93 (67–131) long, 85 (61–131) wide. Esophagus thin, longer than pharynx, 109 (58–153) long, 36 (20–64) wide. Caeca nearly reach the posterior end of body, terminating 128 to 140 from end.

Testes 2, rounded to oval, in tandem, intercecal; anterior testis 114 (79–159) long, 122 (92–188) wide; posterior testis larger 136 (105–168) long, 122 (79–183) wide. Seminal vesicle undivided, mostly posterolateral to ventral sucker. Cirrus sac and cirrus lacking. Ovary median, pre-testicular, equatorial, intercecal, lobated, 103 (69–145) long, 119 (75–173) wide. Seminal receptacle median, pre-ovarian, ovoid, 116 (74–149) long, 102 (32–146) wide. Mehlis' gland and Laurer's canal not visible in whole mounts. Vitellarium consisting of small, irregularly shaped follicles; follicles arranged in lateral fields partly overlapping caeca or extracecal. Vitelline fields not confluent, extending along the middle part of body (42–43% of body length) between anterior margin of posterior testis and region between esophagus and pharynx; in some specimens, vitelline follicles reach posterior margin of pharynx. Uterus tubular with several loops filling most of posterior part of body. Eggs small 19 (14–22) long, 8 (7–10) wide. Excretory pore terminal; excretory vesicle Y-shaped, arms wide reaching level of esophagus.

Taxonomic summary

Type host: Mayan cichlid, *Cichlasoma urophthalmus* (Günther) (Osteichthyes: Cichlidae).

Site of infection: Intestine.

Type locality: Ría Celestún, Yucatán (20°54′15.5″N, 90°20′34.4″W).

Other localities: Estero Progreso at Corchito (21°16′40.6″W, 89°38′38.8″N), Ría Lagartos (21°35′54.1″W, 88°09′24.6″N), Yucatán.

Prevalence of infection: Ría Celestún (23.07%), Estero Progreso at Corchito (18.75%), Ría Lagartos (27.27%).

Intensity range: Ría Celestún (1–5), Estero Progreso at Corchito (2–4), Ría Lagartos (2–5).

TABLE I. Host, locality, and accession numbers for the species of *Oligogonotylus* included in the present study (*Holotype).

Host	Locality	Geographical coordinates	GenBank no.	Vouchers
C. istlanum	Rio Amacuzac at Huixastla, Morelos	18°28′34.1′′N, 99°08′34.7′′W	EU662170–1 (28S), EU662179–80 (*cox1*), EU662193 (ITS1, 5.8, ITS2).	CNHE 6130
C. urophthalmus	Río Papalopapan at Tlacotalpan, Veracruz	18°42′13.4′′N, 95°45′27.9′′W	EU662169 (28S), EU662178 (*cox1*), EU662191–2 (ITS1, 5.8, ITS2).	CNHE 6131
C. urophthalmus	Lago El Espino, Tabasco	18°14′57′′N, 92°49′59′′W	EU662172 (28S), EU662194 (ITS1, 5.8, ITS2).	CNHE 6132
C. urophthalmus	Río Escondido at Ucum, Quintana Roo	18°30′21.4′′N, 88°30′52.7′′W	–	CNHE 6133
C. urophthalmus	Laguna de Canitzan, Tabasco	17°35′15.4′′N, 91°23′29′′W	–	CNHE 6134
C. urophthalmus	Crooked Three Lagoon, Belize	17°46′30.8′′N, 88°31′22.7′′W	EU662173 (28S), EU662181 (*cox*1), EU662195 (ITS1, 5.8, ITS2).	CNHE 6135
C. urophthalmus	Lago Petén, Guatemala	16°55′45′′N, 89°53′27′′W	EU662174 (28S), EU662182 (*cox*1), EU662196 (ITS1, 5.8, ITS2).	CNHE 6136
C. urophthalmus	Ría Celestún, Yucatán	20°54′15.5′′N, 90°20′34.4′′W	EU662175 (28S), EU662183–5 (*cox*1), EU662197 (ITS1, 5.8, ITS2).	CNHE 6126*–6127
C. urophthalmus	Estero Progreso at Corchito, Yucatán	21°16′40.6′′N, 89°38′38.8′′W	EU662176 (28S), EU662186–7 (*cox*1), EU662198 (ITS1, 5.8, ITS2).	CNHE 6128
C. urophthalmus	Ría Lagartos, Yucatán	21°35′54.1′′N, 88°09′24.6′′W	EU662177 (28S), EU662188–89 (*cox*1), EU662199 (ITS1, 5.8, ITS2).	CNHE 6129

TABLE II. Pairwise differences among *cox*1 sequences.

Isolate	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
1. *O. manteri* Ver1	–																
2. *O. manteri* Ver2	0	–															
3. *O. manteri* Ver3	0	0	–														
4. *O. manteri* Mor1	0.28	0.28	0.28	–													
5. *O. manteri* Mor2	0.56	0.56	0.56	0.28	–												
6. *O. manteri* Mor3	0.56	0.56	0.28	0.28	0	–											
7. *O. manteri* Bel1	3.09	3.09	3.09	3.37	3.65	3.65	–										
8. *O. manteri* Gua1	3.09	3.09	3.09	3.37	3.65	3.65	0.11	–									
9. *Oligogonotylus* Cel1	9.55	9.55	9.55	9.83	9.55	9.55	10.1	9.55	–								
10. *Oligogonotylus* Cel2	9.55	9.55	9.55	9.83	9.55	9.55	10.1	9.55	0	–							
11. *Oligogonotylus* Cel3	9.83	9.83	9.83	10.1	9.83	9.83	10.3	9.83	0.28	0.28	–						
12. *Oligogonotylus* Cor1	9.27	9.27	9.27	9.55	9.83	9.83	9.83	9.27	0.56	0.56	0.84	–					
13. *Oligogonotylus* Cor2	9.83	9.83	9.83	10.1	9.83	9.83	10.3	9.83	0.56	0.56	0.84	0.56	–				
14. *Oligogonotylus* Cor3	9.55	9.55	9.55	9.83	9.55	9.55	10.1	9.55	0.28	0.28	0.56	0.28	0.28	–			
15. *Oligogonotylus* RLag1	9.55	9.55	9.55	9.83	9.55	9.55	10.1	9.55	0	0	0.28	0.56	0.56	0.28	–		
16. *Oligogonotylus* RLag2	9.83	9.83	9.83	10.1	9.83	9.83	10.3	9.83	0.28	0.28	0.56	0.84	0.84	0.56	0.28	–	
17. *Oligogonotylus* RLag3	9.27	9.27	9.27	9.55	9.83	9.83	9.83	9.27	0.28	0.28	0.56	0.28	0.84	0.56	0.28	0.56	–

TABLE III. Pairwise differences among isolates, calculated from the ITS1 below the diagonal and ITS2 above.

Isolate	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
1. *O. manteri* Ver1	–	0	0	0	0	0	0	0	0	0	0	1.35	1.35	1.35	1.35	1.35	1.35	1.35	1.35	1.35
2. *O. manteri* Ver2	0	–	0	0	0	0	0	0	0	0	0	1.35	1.35	1.35	1.35	1.35	1.35	1.35	1.35	1.35
3. *O. manteri* Ver3	0	0	–	0	0	0	0	0	0	0	0	1.35	1.35	1.35	1.35	1.35	1.35	1.35	1.35	1.35
4. *O. manteri* Mor1	0	0	0	–	0	0	0	0	0	0	0	1.35	1.35	1.35	1.35	1.35	1.35	1.35	1.35	1.35
5. *O. manteri* Mor2	0	0	0	0	–	0	0	0	0	0	0	1.35	1.35	1.35	1.35	1.35	1.35	1.35	1.35	1.35
6. *O. manteri* Mor3	0	0	0	0	0	–	0	0	0	0	0	1.35	1.35	1.35	1.35	1.35	1.35	1.35	1.35	1.35
7. *O. manteri* Tab1	0	0	0	0	0	0	–	0	0	0	0	1.35	1.35	1.35	1.35	1.35	1.35	1.35	1.35	1.35
8. *O. manteri* Tab2	0	0	0	0	0	0	0	–	0	0	0	1.35	1.35	1.35	1.35	1.35	1.35	1.35	1.35	1.35
9. *O. manteri* Tab3	0	0	0	0	0	0	0	0	–	0	0	1.35	1.35	1.35	1.35	1.35	1.35	1.35	1.35	1.35
10. *O. manteri* Bel1	0.16	0.16	0.16	0.16	0.16	0.16	0.16	0.16	0.16	–	0	1.35	1.35	1.35	1.35	1.35	1.35	1.35	1.35	1.35
11. *O. manteri* Gua1	0	0	0	0	0	0	0	0	0	0.16	–	1.35	1.35	1.35	1.35	1.35	1.35	1.35	1.35	1.35
12. *Oligogonotylus* Cel1	4.94	4.93	4.94	4.94	4.94	4.94	4.94	4.94	4.94	4.78	4.93	–	0	0	0	0	0	0	0	0
13. *Oligogonotylus* Cel2	4.94	4.93	4.94	4.94	4.94	4.94	4.94	4.94	4.94	4.78	4.93	0	–	0	0	0	0	0	0	0
14. *Oligogonotylus* Cel3	4.94	4.93	4.94	4.94	4.94	4.94	4.94	4.94	4.94	4.78	4.93	0	0	–	0	0	0	0	0	0
15. *Oligogonotylus* Cor1	4.94	4.93	4.94	4.94	4.94	4.94	4.94	4.94	4.94	4.78	4.93	0	0	0	–	0	0	0	0	0
16. *Oligogonotylus* Cor2	4.94	4.93	4.94	4.94	4.94	4.94	4.94	4.94	4.94	4.78	4.93	0	0	0	0	–	0	0	0	0
17. *Oligogonotylus* Cor3	4.94	4.93	4.94	4.94	4.94	4.94	4.94	4.94	4.94	4.78	4.93	0	0	0	0	0	–	0	0	0
18. *Oligogonotylus* RLag 1	4.94	4.93	4.94	4.94	4.94	4.94	4.94	4.94	4.94	4.78	4.93	0	0	0	0	0	0	–	0	0
19. *Oligogonotylus* RLag2	4.94	4.93	4.94	4.94	4.94	4.94	4.94	4.94	4.94	4.78	4.93	0	0	0	0	0	0	0	–	0
20. *Oligogonotylus* RLag3	4.94	4.93	4.94	4.94	4.94	4.94	4.94	4.94	4.94	4.78	4.93	0	0	0	0	0	0	0	0	–

TABLE IV. Pairwise differences among isolates from the 28S.

Isolate	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
1. *O. manteri* Ver1	–																			
2. *O. manteri* Ver2	0	–																		
3. *O. manteri* Ver3	0	0	–																	
4. *O. manteri* Mor1	0.12	0.12	0.12	–																
5. *O. manteri* Mor2	0.12	0.12	0.12	0	–															
6. *O. manteri* Mor3	0	0	0	0.12	0.12	–														
7. *O. manteri* Tab1	0.12	0.12	0.12	0	0	0.12	–													
8. *O. manteri* Tab2	0.12	0.12	0.12	0	0	0.12	0	–												
9. *O. manteri* Tab3	0.12	0.12	0.12	0	0	0.12	0	0	–											
10. *O. manteri* Bel1	0.12	0.12	0.12	0	0	0.12	0	0	0	–										
11. *O. manteri* Gua1	0.12	0.12	0.12	0	0	0.12	0	0	0	0	–									
12. *Oligogonotylus* Cel1	1.75	1.75	1.75	1.63	1.63	1.75	1.63	1.63	1.63	1.63	1.63	–								
13. *Oligogonotylus* Cel2	1.75	1.75	1.75	1.63	1.63	1.75	1.63	1.63	1.63	1.63	1.63	0	–							
14. *Oligogonotylus* Cel3	1.75	1.75	1.75	1.63	1.63	1.75	1.63	1.63	1.63	1.63	1.63	0	0	–						
15. *Oligogonotylus* Cor1	1.75	1.75	1.75	1.63	1.63	1.75	1.63	1.63	1.63	1.63	1.63	0	0	0	–					
16. *Oligogonotylus* Cor2	1.75	1.75	1.75	1.63	1.63	1.75	1.63	1.63	1.63	1.63	1.63	0	0	0	0	–				
17. *Oligogonotylus* Cor3	1.75	1.75	1.75	1.63	1.63	1.75	1.63	1.63	1.63	1.63	1.63	0	0	0	0	0	–			
18. *Oligogonotylus* RLag 1	1.75	1.75	1.75	1.63	1.63	1.75	1.63	1.63	1.63	1.63	1.63	0	0	0	0	0	0	–		
19. *Oligogonotylus* RLag2	1.75	1.75	1.75	1.63	1.63	1.75	1.63	1.63	1.63	1.63	1.63	1.63	0	0	0	0	0	0	–	
20. *Oligogonotylus* RLag3	1.75	1.75	1.75	1.63	1.63	1.75	1.63	1.63	1.63	1.63	1.63	1.63	0	0	0	0	0	0	0	–

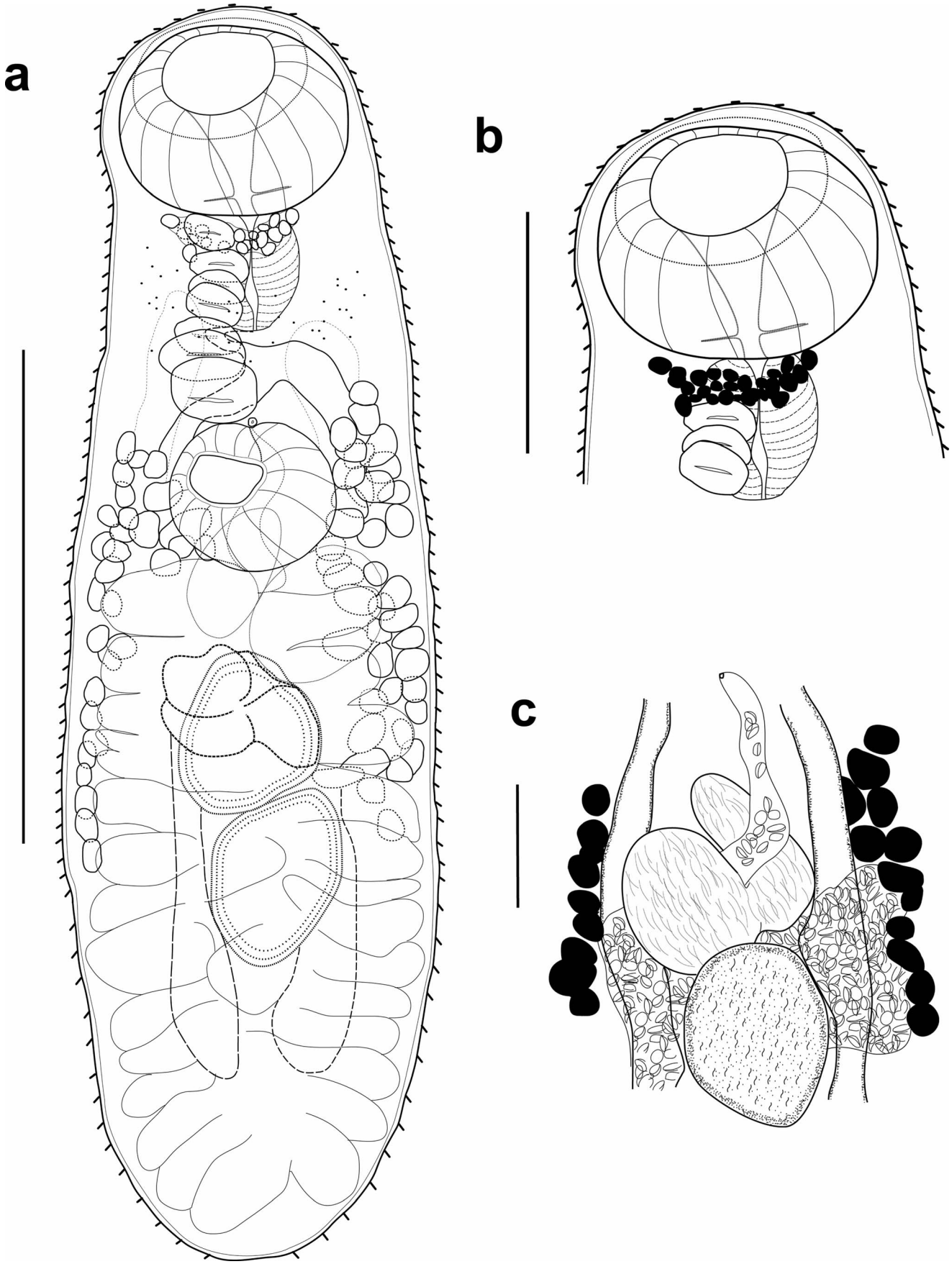
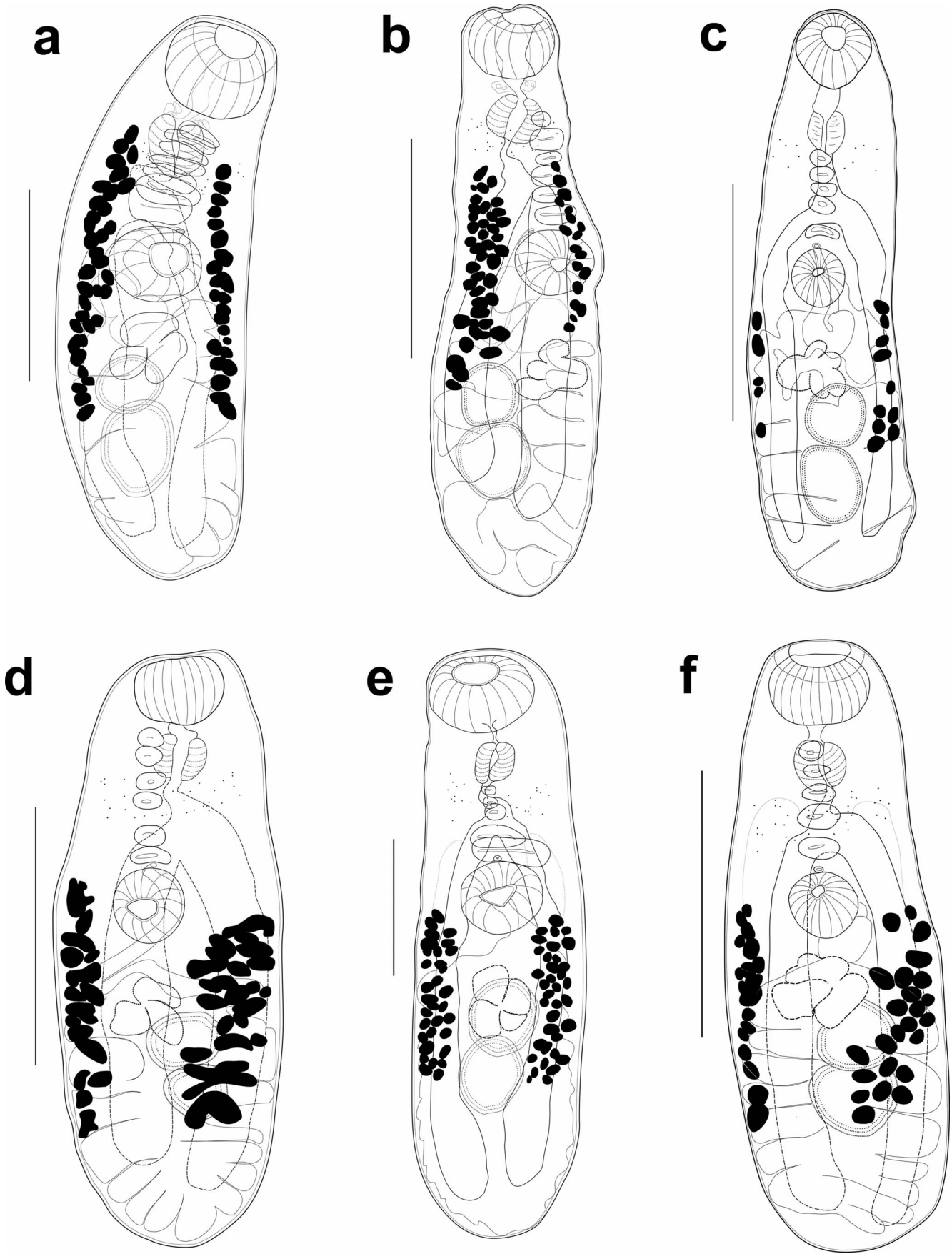


FIGURE 2. *Oligogonotylus mayae* n. sp. (**a**) Holotype (gravid specimen); ventral view. Bar = 500 μm. (**b**) Detail of the pharyngeal glands; ventral view. Bar = 200 μm. (**c**) Detail of the terminal genitalia; dorsal view. Bar = 200 μm.


a
b
c
d
e
f

Specimen deposition: Holotype: CNHE 6126. Paratypes: CNHE 6127–6129.

Etymology: The specific epithet refers to the Maya civilization, a Mesoamerican civilization that lived in southeastern Mexico (c. 250 to 900).

REMARKS

Light microscopy study of several individuals of *Oligogonotylus* collected from 10 localities, 8 in Mexico, 1 in Belize, and 1 in Guatemala, allowed us to detect morphological differences between the specimens from Veracruz, Tabasco, Quintana Roo, and Morelos (Mexico), Crooked Three Lagoon (Belize), and Lago Petén (Guatemala) with respect to those from 3 localities in the Yucatán Peninsula (Ría Celestún, Estero Progreso at Corchito, and Ría Lagartos). The main difference between individuals from Yucatán in relation to those from the aforementioned localities, and also those from the type locality in Lake Nicaragua, Nicaragua, is the anterior extension of the vitelline follicles. Individuals from all those localities possess vitelline follicles distributed entirely in the hindbody and reaching anteriorly to the posterior margin of the ventral sucker (herein designated as Morphotype I, corresponding with the type-species, *O. manteri*). In contrast, individuals from 3 localities of Yucatán exhibit vitelline follicles that extend anteriorly to the level of the intestinal bifurcation, between the pharynx and esophagus (herein designated as Morphotype II) (Figs. 2a–c, 3a–b). Watson (1936) did not describe the presence of scattered diffuse eyespots remnants around the pharynx; however, observation of the paratype (USNPC 945-1) shows that diffuse eyespots are actually present, as well as in other specimens of *O. manteri*, and that they are also present in the new species. The observation of specimens from museum collections revealed that both morphotypes were present; however, no previous attempt had been made to differentiate between the 2 species.

The only species of *Oligogonotylus* previously described is *O. manteri*. The new species differs from *O. manteri* by possessing follicles that extend between the anterior margin of the posterior testis and the region between the esophagus and the pharynx. Previous descriptions and redescriptions of *O. manteri* show that vitelline follicles extend between the posterior margin of the ventral sucker and the posterior end of the posterior testis. *Oligogonotylus mayae* n. sp. is, in general terms, a larger and more robust species than *O. manteri*.

Molecular analyses

cox1: The alignment of the *cox*1 for 17 isolates included 356 base pairs (bp), and no gaps were required to align these sequences. A pairwise distance matrix (Table II) showed some level of intraspecific genetic variability among individuals allocated to Morphotype I (*O. manteri*) from 3 localities in Mexico, 1 in Belize, and 1 in Guatemala. Genetic divergence between individuals of Morphotype I, only from Mexico (Tabasco, Veracruz, and Morelos), ranged from 0.28 to 0.56%. Additionally, when comparing the sequences of distinct individuals from Mexico with those collected in Belize and Guatemala, the genetic divergence varied from 3.0 to 3.65%. However, divergence levels among individuals of Morphotype I, with respect to those of Morphotype II (Ría Celestún, Estero Progreso at Corchito, and Ría Lagartos), reached higher values, from 9.55 to 10.39%. Intraspecific variability was also observed among the 9 isolates corresponding to the 3 localities at which Morphotype II was collected, with values ranging from 0.28 to 0.84%.

ITS1 and ITS2: The sequences of the ITS1 and ITS2 produced an alignment of 880 bp. The length of the ITS1 of most isolates ranged from 607 to 610 nucleotides. However, the length of the fragment of 2 isolates of *O. manteri*, 1 from Río Papaloapan, Tlacotalpan, Veracruz, Mexico and 1 from Lago Peten, Guatemala, was of 658 bp because they have an insert of 47 nucleotides. Consequently, the ITS1 alignment was of 658 bp. Sequence variation for this molecular marker among isolates of the Morphotype I showed a very low level of intraspecific divergence, with 0.16% (Table III). When comparing these isolates with those from the Morphotype II, the genetic divergence reaches values from 4.7 to 4.9%. In contrast, there was no intraspecific variation among isolates of the Morphotype II, even though they were collected in 3 different localities along the Yucatán Peninsula. The ITS2 alignment consisted of 222 bp. No intraspecific variation was observed among individuals allocated to either Morphotype I or Morphotype II; however, among morphotypes, genetic divergence was 1.35%.

28S: The sequenced fragment of this gene included the domains D2 and D3 as identified by Littlewood and Johnston (1995); the entire alignment was 798 bp. A low intraspecific genetic divergence was observed among isolates of Morphotype I from Morelos, Tabasco, Veracruz (Mexico), Belize, and Guatemala, with 0.12%. Divergence level between isolates of Morphotype I and II reached values of 1.63 to 1.75%. No intra-individual differences were observed among isolates of the Morphotype II (Table IV).

Our results, which are based on both sources of evidence (morphology and genetic distance, see below), clearly indicate that the so-called Morphotype II corresponds with the new species we describe herein. Molecular evidence was generated using different markers, including ribosomal (ITS1, ITS2 and 28S) and mitochondrial (*cox*1) markers. The level of divergence we found by using these genes corresponds with a series of studies that have been conducted on several digenetic trematodes (see Adlard et al., 1993; van Herwerden et al., 1998, 1999; Jousson et al., 2000; Tkach and Sharpilo, 2000; León-Règagnon and Paredes-Calderón, 2002; Razo-Mendivil et al., 2004; Miura et al., 2005; Nolan and Cribb, 2005; Olson and Tkach, 2005; Pérez-Ponce de León et al., 2008).

DISCUSSION

Interestingly, our results confirmed the presence of *O. manteri* in species of cichlids from strictly freshwater localities and, in this paper, 5 new locality records (3 in Mexico, 1 in Guatemala, and 1 in Belize) and 1 new host record (*C. istlanum*) are reported. There seems to be a habitat and host preference for the new species of *Oligogonotylus* because it is confined to brackish water environments and to the Mayan cichlid *C. urophthalmus*. The 3 localities where the new species was found correspond to this type of habitat, and it is noteworthy that there is no (ribosomal genes), or very low (mitochondrial gene), genetic divergence among isolates from each locality, as well as between isolates from all localities. Specimens of *Oligogonotylus* from Estero el Pargo, Champotón, and Boca Peralta, in Campeche, borrowed from the Colección Parasitológica del CINVESTAV-Mérida correspond, in parti, with the new species. However, no specimens were collected for molecular study from these localities, and this observation needs further corroboration. Additionally, specimens from the CNHE collected in *C. urophthalmus* from Ría Celestún, Yucatán (nos. 1270 and 1274) correspond with the new species we describe herein. Finally, *O. manteri* and *O. mayae* are considered as members of the core helminth fauna of cichlids and represent key elements in explaining the historical biogeography of this host–parasite association in Middle America.

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FIGURE 3. Morphotypes of *Oligogonotylus* spp. (**a**) Morphotype II, Ría Lagartos, Yucatán ex *C. urophthalmus*. (**b**) Morphotype II, Estero Progreso at Corchito, Yucatán ex *C. urophthalmus*. (**c**) Morphotype I (paratype of *O. manteri*, USNPC No. 061324.00, Lago Nicaragua, Nicaragua, ex *Cichlasoma nicaraguensis*. (**d**) Morphotype I, Papaloapan River at Tlacotalpan, Veracruz ex *C. urophthalmus*. (**e**) Morphotype I, Basas River at Huisaxtla, Morelos ex *C. urophthalmus*. (**f**) Morphotype I, El Espino, Tabasco ex *C. uropluthalmus*. Bar = 500 μm.

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