

DESCRIPTION OF A NEW SPECIES OF *CRASSICUTIS* MANTER, 1936, PARASITE OF *CICHLASOMA BEANI* JORDAN (OSTEICHTHYES: CICHLIDAE) IN MEXICO, BASED ON MORPHOLOGY AND SEQUENCES OF THE ITS1 AND 28S RIBOSOMAL RNA GENES

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ABSTRACT: A new species of *Crassicutis* Manter, 1936 is described from the Sinaloan cichlid *Cichlasoma beani* (Jordan) (Osteichthyes: Cichlidae) in the upper Río Santiago basin. *Crassicutis choudhuryi* n. sp. differs from most of the other nominal species by having testes located in a symmetrical position. The only other species of the genus that includes some specimens exhibiting this trait is *Crassicutis intermedius* (Szidat 1954), a species found in 5 species of siluriforms and 1 species of characiform in South America. However, this species differs from *Cr. choudhuryi* n. sp. by having testes almost half of the size, and vitelline follicles extending anteriorly to the region between the acetabulum and the intestinal bifurcation. The new species is morphologically very similar to *Crassicutis cichlasomae* Manter, 1936, but clearly differs from this species because of the constantly symmetrical position of the testes. Additionally, *Cr. choudhuryi* n. sp. is found in the Santiago River basin on the Pacific slope of Mexico, parasitizing specifically the endemic *Ci. beani* that does not co-occur with any other cichlid. *Cr. cichlasomae* exhibits more hosts (about 25 species of cichlids only in Mexico) and a wider distribution range that extends from northeastern Mexico southward to Central America, Cuba, and Brazil. To corroborate that our specimens were not conspecific with *Cr. cichlasomae*, sequences of the internal transcribed spacer (ITS1) and the 28S ribosomal RNA genes of individuals from several populations (recently collected in southeastern Mexico) were obtained and compared to the species described herein. Sequence divergence (1.3% for the 28S and 4.0% for the ITS1) gives further support to the erection of a new species.

Cribb and Bray (1999) redefined *Crassicutis* Manter, 1936, while conducting a revision of the Apocreadiidae Skrzabin, 1942. This genus is characterized by consistently lacking tegumental spines, being less elongate, and having an excretory pore opening distinctly dorso-subterminally rather than terminally. The combination of these morphological traits restricts the genus to 7 species, 6 of which are parasites of freshwater fish: *Cr. cichlasomae* (the type species); *Crassicutis bravoae* Jiménez and Caballero y Caballero, 1974; *Crassicutis chuscoi* Pearse; 1920, *Cr. intermedius*; *Crassicutis opisthoseminis* Bravo Hollis and Arroyo, 1962; and *Crassicutis wallini* Pearse, 1920. A single species, *Crassicutis archosargi* Sparks and Thatcher, 1960, parasitizes a brackish water fish. Host and geographic distribution of members of this genus include 3 species from South America, 2 exclusively found in cichlids (*Cr. chuscoi* and *Cr. wallini* from Venezuela), and 1, *Cr. intermedius*, in siluriforms and characids from Argentina, Paraguay, and Brazil. Additionally, 1 species was found in a Central American cichlid (*Cr. opisthoseminis* from northwestern Costa Rica), and 1 species found in cichlids allocated to the Nearctic region in northeastern Mexico, *Cichlasoma bravoae*. The latter species, however, was not considered as valid by Vidal-Martínez et al., (2001), suggesting that “most probably” it is a synonym of *Cr. cichlasomae*. Finally, the genus contains 1 widespread species, *Cr. cichlasomae*, whose host and distributional range include about 25 species of cichlids in 10 states of the Mexican Republic along the Gulf of Mexico slope and the Yucatán Peninsula (see Pérez-Ponce de León et al., 2007, and references therein), southwards to several localities in Central America (Costa Rica and Nicaragua, see Watson, 1976; Aguirre-Macedo et al., 2001; Rodríguez-Ortíz et al., 2004), Cuba (Moravec and Barus, 1972; Vinjoy et al., 1985), and South America (Brazil, see Fernandez and Kohn, 2001). The only brackish water representative of the genus is *Cr. archosargi* that is a parasite of the sparid, *Archosargus probatocephalus* (Walbaum), in Louisiana, Mississippi,

and Texas (Sparks and Thatcher, 1960; Joy, 1971; Overstreet, 1976).

In the last 10 yr, a great effort has been made to document the biodiversity of helminth parasites of freshwater fishes in Mexico. As a part of this ongoing survey, an undescribed species of *Crassicutis* was found and is described herein based on morphological characters, as well as on data on sequence divergence by using ITS1 and 28S ribosomal RNA genes.

MATERIALS AND METHODS

Nineteen specimens of the Sinaloan cichlid, *Ci. beani*, were collected in a tributary of the Río Santiago basin, in the locality of Jesús María Corte, State of Nayarit, in September 2001. The Río Santiago runs from the Lago de Chapala westward to the Pacific coast of Nayarit. Hosts were collected by angling, and examined for helminths 4 hr after capture. Digeneans were collected from the intestines of fish and placed in saline (0.65%). 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. Preserved specimens were stained with Mayer's paracarmine and Gomori's trichrome, cleared in methyl salicylate, dehydrated in graded ethanol series, and mounted as permanent slides using Canada balsam. 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.

For morphological comparisons, specimens deposited at the CNHE and at the Harold W. Manter Laboratory of Parasitology (HWML) were examined. CNHE specimens include *Cr. cichlasomae* ex *Vieja fenestrata* (Günther), *Cichlasoma* sp., *Cichlasoma urophthalmus* (Günther), and *Thorichthys meeki* Brind from the following localities in southeastern Mexico: Lake Catemaco (1266, 1734, 5274) and Los Tuxtlas (5271, 5272), Veracruz State; Puerto Morelos (1297) and Laguna Bakalar (2841), Quintana Roo State; Celestún Lagoon (2845, 2841), Yucatán State; *Cr. bravoae* ex *Herichthys cyanoguttatus* Baird and Girard, Presa Rodrigo Gómez, Nuevo León State (1584); *Cr. opisthoseminis* ex *Cichlasoma* sp., Guanacaste, Costa Rica (763). HWML specimens include *Cr. cichlasomae* ex *Cichlasoma mayorum* Hubbs, Cenote Xtoloc, Chichen-Itza, (801), Yucatán, and *Cichlasoma labiatum* (= *Amphilophus labiatus* Günther) from Nicaragua (800). We also observed specimens from the following regional parasite collections: Colección Helmintológica del CINVESTAV, Mérida, Yucatán, México, (CHCM), 70

Received 14 July 2007; revised 6 July 2007; accepted 9 July 2007.

specimens; Colección Helmintológica del Sureste de México (CIHU-JAT), Universidad Juárez Autónoma de Tabasco, Villahermosa, Tabasco, México, (211 specimens, ex *Ci. urophthalmus*, *Vieja sinspylla*, and *V. fenestrata*). Serapio López provided specimens from his personal collection (20 specimens, ex *Ci. urophthalmus*, *Petenia splendida*, *Thorichthys helleri*, *V. fenestrata*).

Genomic DNA was extracted from individual gravid worms according to the protocols proposed by Hillis et al. (1996). The primer pairs BD1 (5'-GTCGTAACAAGGTTCCGTA-3') and BD2 (5'-TATGCTTAAATTCAGCGGGT-3') (Bowles et al., 1995), 28S1 (5'-AACAGTGC GTGAAACCGCTC-3') (Palumbi, 1996), and LO (5'-GCT ATC CTG AG(AG) GAA ACT TCG-3') (Tkach et al., 2000) were used to amplify approximately 1000 bp of the internal transcribed spacer 1, and approximately 900 bp of the 28S ribosomal RNA gene, respectively. The amplification program for both molecular markers consisted of 1 min at 94 C followed by 35 cycles of 30 sec at 92 C, 45 sec at 55 C, and 1 min at 72 C; reactions were held at 72 C for 10 min to complete elongation and then dropped to 4 C. The PCR products were purified by using QIAquick PCR Purification Kit (Qiagen, Valencia, California). Purified products were sequenced on an ABI PRISM 3100 automated DNA sequencer (Applied Biosystems, Foster City, California) using Big Dye Terminator chemistry, and incorporating the same primers as those used in previous PCRs. For both molecular markers gene sense and antisense strands were sequenced, subsequently assembled, and aligned by eye using the computer program BIOEDIT (v. 3, Hall, 1999).

Pairwise distance matrices were obtained for each molecular marker using PAUP (v. 4.0b10, Swofford, 2002). For comparison, specimens of 2 species of *Crassicutis* were obtained from the United States and Mexico. Specimens of *Cr. cichlasomae* were collected in July, 2006 from *Ci. urophthalmus* in 4 localities, 1 in the State of Tabasco (El Espino), 1 in Campeche (Ulumal), and 2 in Yucatán (El Corchito and Cenote X'lach) in southeastern Mexico (Table I). The 28S and ITS1 ribosomal genes of individual specimens of all those populations were also sequenced following the same protocols. Sequences obtained in this study were submitted to GenBank (accession numbers are presented in Table I). Representative specimens of *Cr. cichlasomae* and *Cr. archosargi* from those collections aforementioned were also deposited at the CNHE.

DESCRIPTION

***Crassicutis choudhuryi* n. sp.**

(Figs. 1–3)

Based on measurements of 18 specimens (12 of them gravid) unless sample size (n) otherwise specified. Body oval, 2,181 (1,040–2,571) long by 1,000 (576–1,351) wide. Tegument smooth. Oral sucker sub-terminal, spherical, 217 (128–258) long by 224 (146–281) wide. Dispersed eye-spot pigment present near pharynx. Acetabulum pre-equatorial, 236 (167–291) long by 248 (162–306) long. Ratio of oral sucker to acetabulum length 1:1.08–1.15. Distance from acetabulum to intestinal bifurcation 228 (69–332, n = 16). Prepharynx short 53 (37–71, n = 5) long. Pharynx 127 (85–151) long by 106 (64–136) wide. Esophagus long, 70 (37–101), followed by the intestinal bifurcation. Cecae narrow, not reaching posterior extremity of body, terminating 321 (249–376, n = 17) of body length from posterior extremity. Testes constantly symmetrical, contiguous or nearly so, smooth to slightly lobed in shape, postequatorial. Left testis 324 (159–416) long by 218 (142–317) wide. Right testis 304 (135–414) long by 214 (125–354) wide. Distance from testes to posterior extremity 703 (292–959). Cirrus sac absent. Seminal vesicle saccate, 251 (99–365, n = 17) long by 159 (39–304, n = 17) wide, occasionally overlapping acetabulum and ovary; distal part tubular, opening into genital pore. Genital pore median, immediately anterior to acetabulum.

Ovary spherical, pretesticular, posterolateral to acetabulum, consistently dextral, roughly midway between right testis and acetabulum, 134 (41–207) long by 141 (52–188) wide. Seminal receptacle saccate, pre-ovarian, longer than and overlapping ovary. Mehlis' gland compact, adjacent to the ovary. Laurer's canal inconspicuous. Vitelline follicles mainly extracecal, with few follicles caecal and intercecal, extending from midway between the pharynx and the intestinal bifurcation level to short distance 146 (63–194) from the posterior extremity. Vitelline follicles confluent in preacetabular and posttesticular regions. Uterus pretesticular, intercaecal, short, with few operculated eggs. Eggs large,

TABLE I. Host, locality, and accession numbers for the species of *Crassicutis* included in the present study.

Species of <i>Crassicutis</i>	Host	Locality	Latitude, Longitude	GenBankNo.		
				28S	ITS1	CNHE
<i>Cr. archosargi</i>	<i>A. probatocephalus</i>	Davis Bayou, Ocean Springs, Mississippi	30°24'36''N, 88°47'51''W	EUI131630	EUI170369	6050
<i>Cr. cichlasomae</i>	<i>Ci. urophthalmus</i>	El Espino, Tabasco	18°14'57''N, 92°49'59''W	EUI131634	EUI170373	5606
<i>Cr. cichlasomae</i>	<i>P. splendida</i>	Río Champotón, Ulumal, Campeche	19°16'43''N, 90°37'26''W	EUI131635	EUI170374	5607
<i>Cr. cichlasomae</i>	<i>Ci. urophthalmus</i>	Cenote X'lach, Dzibilchaltun, Yucatán	21°05'26''N, 89°35'53''W	EUI131632	EUI170371	5609
<i>Cr. cichlasomae</i>	<i>Ci. urophthalmus</i>	Parque Ecológico El Corchito, Progreso, Yucatán	21°16'40''N, 89°38'38''W	EUI131633	EUI170372	5610
<i>Cr. cichlasomae</i>	<i>Ci. trimaculatum</i>	Laguna de Tres Palos, Guerrero	16°47'47''N, 99°44'30''W	EUI131636	EUI170375	5860
<i>Cr. choudhuryi</i> n. sp.	<i>Ci. beani</i>	Río Santiago at Jesús María Corte, Nayarit	21°43'40''N, 104°53'04''W	EUI131631	EUI170370	5603–4

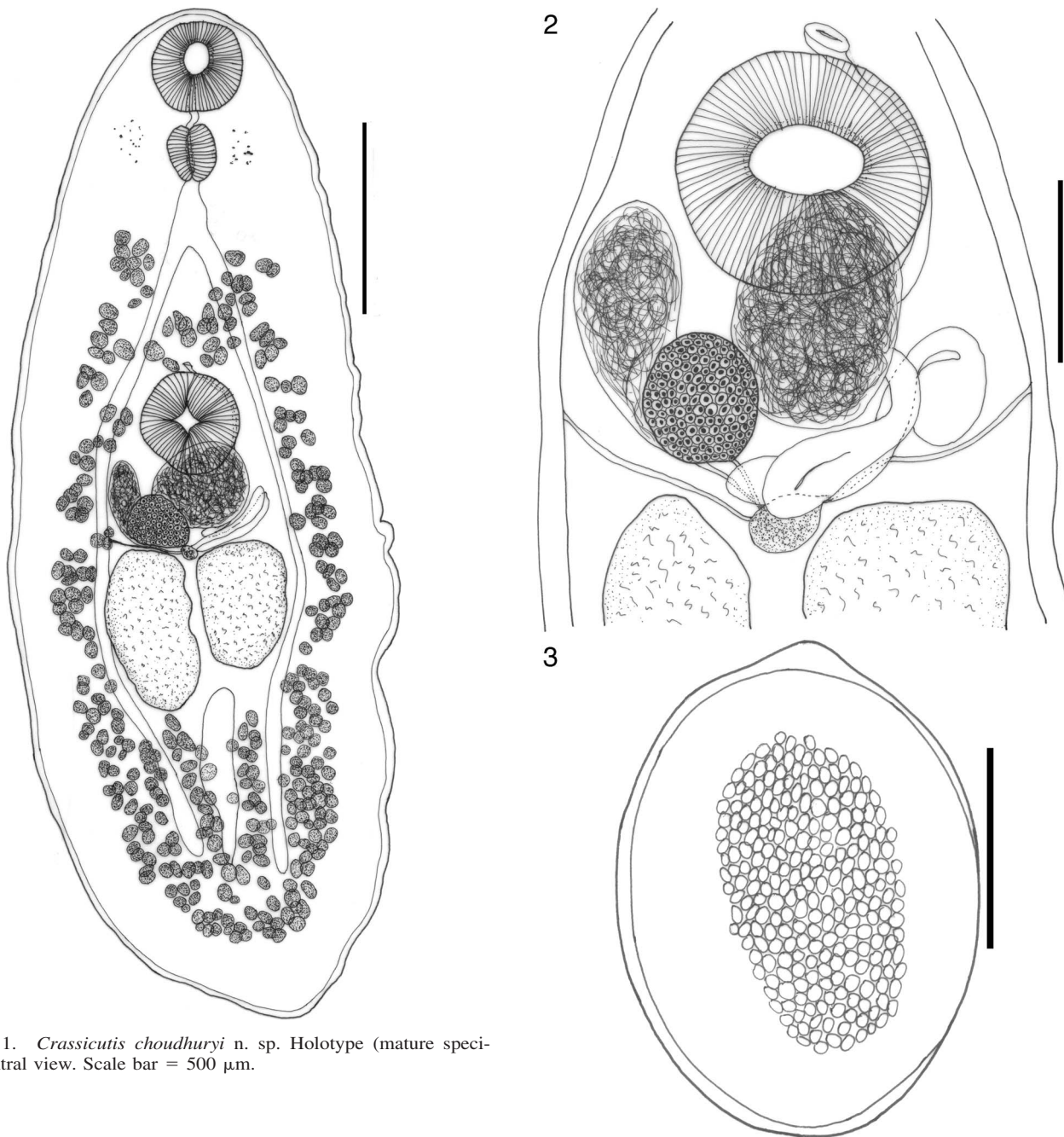


FIGURE 1. *Crassicutis choudhuryi* n. sp. Holotype (mature specimen). Ventral view. Scale bar = 500 μ m.

provided with small knob, 107 (93–117, n = 25) long by 74 (63–99, n = 25) wide. Excretory vesicle I-shaped. Excretory pore distinctly dorsally subterminal, at level of end of caeca.

Taxonomic summary

Type host: *Cichlasoma beani* (Jordan) (Osteichthyes: Cichlidae).

Site of infection: Intestine.

Prevalence of infection: 68.4%.

Mean intensity, and intensity range: 5.8, 1–16.

Type locality: Tributary of the Río Santiago basin, in the locality of Jesús María Corte, State of Nayarit, Mexico (21°43'40"N, 104°53'04"W).

Specimens deposited: Holotype: CNHE 5603. Paratypes: CNHE 5604 (17 specimens).

Etymology: The species is named after Anindo Choudhury in recognition of his important contribution to freshwater fish parasitology in North and Central America.

FIGURES 2–3. (2) Detail of the ovarian complex and testes of *Cr. choudhuryi* n. sp. (paratype). Scale bar = 200 μ m. (3) Egg of *Cr. choudhuryi* n. sp. (paratype). Scale bar = 50 μ m.

Remarks

We compared our specimens with those of *Cr. archosargi* and *Cr. cichlasomae* from several localities in Mexico (Table I). *Crassicutis choudhuryi* n. sp. is readily distinguished from all but 1 congeneric species, *Cr. intermedius*, by having testes constantly located in a symmetrical position. *Crassicutis intermedius* occurs in siluriform (*Cochliodon cochliodon* Kner, *Hypostomus piratatu* Weber, *Hypostomus commersoni* Valenciennes, *Hypostomus boulengeri* Eigenmann and Kennedy, and *Hoplosternum littorale* Hancock) and characiform fishes (*Leporinus copelandi* Steindachner) from South America in the River Plate at Buenos Aires, Argentina; the Guaiba estuary, Brazil; and the Paraguay and Paraná rivers in Paraguay (see Bray et al., 1996, and refer-

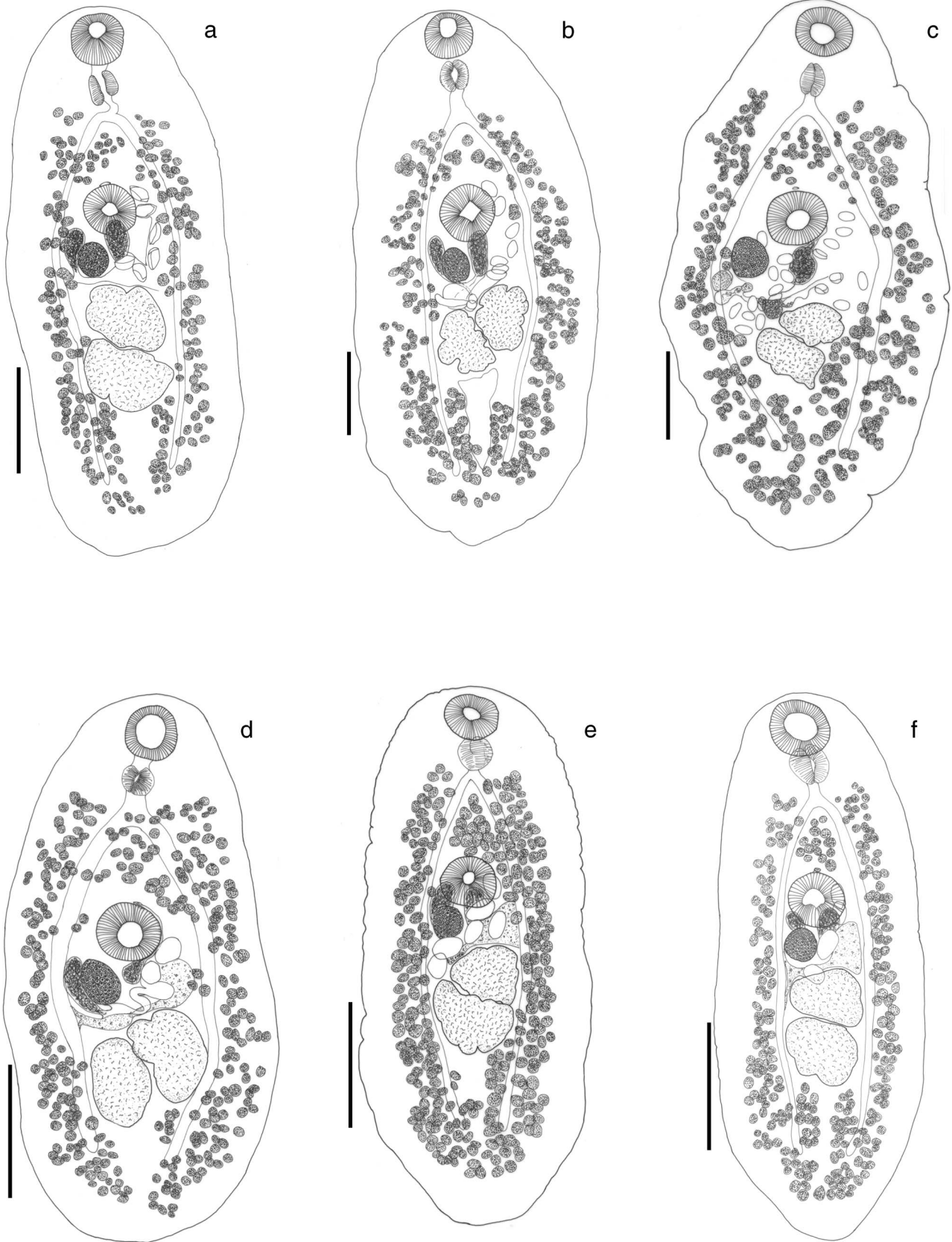


TABLE II. Pairwise distance matrix of ITS1 (above), 405 bp and 28S (below), 789 bp, of 6 isolates belonging to 3 species of *Crassicutis*.

	1	2	3	4	5	6	7
1. <i>C. choudhury</i> n. sp.	—	4.0	4.0	4.0	4.0	4.0	12.0
2. <i>Cr. cichlasomae</i> (Xfach)	1.4	—	0	0	0	0	4.0
3. <i>Cr. cichlasomae</i> (El Corchito)	1.4	0	—	0	0	0	4.0
4. <i>Cr. cichlasomae</i> (El Espino)	1.4	0	0	—	0	0	4.0
5. <i>Cr. cichlasomae</i> (Ulumal)	1.4	0	0	0	—	0	4.0
6. <i>Cr. cichlasomae</i> (Tres Palos)	1.4	0	0	0	0	—	4.0
7. <i>C. archosargi</i> (Ocean Springs)	6.0	6.0	6.0	6.0	6.0	6.0	—

ences therein). However, as noted by Bray et al. (1996), the position of testes is quite variable in specimens collected from different host species, and that species is defined as having testes symmetrical to oblique. In the new species we describe herein, symmetrical position of testes is a constant trait, and none of the specimens we studied showed evidence of a different position of testes. In addition, the new species can be distinguished from *Cr. intermedius* by having much larger testes (almost double sized), and by having vitelline follicles that extend anteriorly to midway between the pharynx and the intestinal bifurcation and are not confluent in that region. Further, the species we describe herein was found in *Ci. beani*, a species that does not occur with any other cichlid. *Cichlasoma beani* represents the cichlid species with the northernmost distribution along the Pacific slope, an area restricted to river basins from the lower Río Yaqui basin in the State of Sonora, southward to lower Río Ameca in Jalisco State (Miller et al., 2005).

We determined 3 reasons to examine numerous specimens of *Cr. cichlasomae*. First, this is the type species for the genus, and the one most commonly found infecting cichlids in Mexico (25 species in localities along 10 states). Second, *Cr. cichlasomae* shows a wide distributional range. According to published records, it has been found in cichlids from Brazil, Costa Rica, Nicaragua, Cuba, and Mexico (Manter, 1936; Bravo and Arroyo, 1962; Watson, 1976; Moravec and Barus, 1972; Vinjoy et al., 1985; Aguirre-Macedo et al., 2001; Vidal-Martínez et al., 2001; Pérez-Ponce de León et al., 2007). Third, this species exhibits some degree of morphological intraspecific variability (Figs. 4a–f). The new species closely resembles *Cr. cichlasomae*. However, of the approximately 120 specimens examined from the CNHE, only 2 exhibited a morphology that suggested either a symmetrical or tandem position of the testes, which definitively shows that testes are constantly located in an oblique position in this species. To further corroborate our observations, we studied another 301 specimens of *Cr. cichlasomae* from regional and personal collections (see Materials and Methods). Of the 301 specimens, 267 exhibited oblique testes, 32 exhibited testes in tandem and only 2 were symmetrical. We observed that the 2 specimens with symmetrical testes were immature. We assume that the tandem or oblique position of the testes is a characteristic of *Cr. cichlasomae* and that some slight difference may occur, most probably as a result of fixation procedures. Scholz et al. (1995) studied the life cycle of *Cr. cichlasomae* in a small swamp in a limestone factory near Mérida, in the Yucatán Peninsula. The developmental stages of this digenean are very well described in that paper, in which Figure 13 (p. 73) illustrates that the newly formed testes in young metacercariae are apparently symmetrical in position, while testes are characteristically oblique in adults, a condition that seems to be modified during development. Therefore, a second assumption could be made that the new species we describe here retains the ontogenetic trait in the adult stage.

There is another species of apocreadiid trematode, *Trematobrien haplochromios* Dollfus, 1950, a parasite of the cichlid *Haplochromis moffati* (Castelnau) from the Congo Republic in Africa (Dollfus, 1950), that might be related, and should be compared with the new species (S.

Curran, pers. comm.). The African species is characterized by having a thick cuticle, and it is also found in cichlids (see Manter, 1962; Cribb and Bray, 1999). This species is morphologically very similar to *Cr. choudhuryi* n. sp. but it possesses a distinctive character, i.e., ceca are fused posteriorly, thus forming a cyclocoel.

DISCUSSION

To further corroborate the validity of the new species and make sure that it does not represent a morphological variation of *Cr. cichlasomae*, we sequenced the ITS1 and the 28S ribosomal RNA genes of individual specimens of *Cr. choudhuryi* n. sp. and also from specimens of other 2 species, i.e., *Cr. cichlasomae* collected within the allegedly “typical” distributional range of the species in southeastern Mexico, in the states of Tabasco, Campeche, and Yucatán; and *C. archosargi* from the brackish water fish *A. probatocephalus* from Mississippi. Needless to say, *C. archosargi* is the only congeneric species that occurs in brackish water fishes (Cribb and Bray, 1999), and is characterized by having testes located in tandem. Additionally we sequenced specimens of *Crassicutis* collected from the cichlid *Cichlasoma trimaculatum* in the Tres Palos Lagoon, a brackish water system near Acapulco, Guerrero State. All specimens collected from this particular locality exhibited testes placed in tandem. Sequence divergence as shown in the pairwise distance matrix (Table II) indicate that all the individuals of *Cr. cichlasomae* (including the specimens from Tres Palos Lagoon) are identical for both molecular markers, and that they differ from the ones corresponding to the new species by 1.4% for the 28S gene, and by 4.0% for the ITS1 gene. With respect to *C. archosargi*, the new species exhibits an even much larger divergence level, by 6% for the 28S gene and by 12% for the ITS1 gene. In addition, *C. archosargi* differs from *Cr. cichlasomae* in 6% for the 28S, and 4.0 for the ITS1. This level of divergence in both ribosomal gene sequences has been used in other studies on digenetic trematodes to establish species differentiation (see van Herwerden et al., 1998, 1999; León-Règagnon et al., 1999; Jousson et al., 2000; Tkach et al., 2000; León-Règagnon and Paredes Calderón, 2002; Razo-Mendivil et al., 2004; Miura et al., 2005; Nolan and Cribb, 2005; Olson and Tkach, 2005). These studies have proved that such molecular markers are useful not only in establishing species limits, but

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FIGURE 4. *Crassicutis cichlasomae* from different hosts and localities. Ventral view. Scale bar = 500 μ m. (a) ex *Cichlasoma labridens*, Río Panuco, San Luis Potosí, Mexico (CNHE 4830). (b) ex *Cichlasoma fenestratum*, Lago de Catemaco, Veracruz (CNHE 1734). (c) ex *Cichlasoma* sp., Puerto Morelos, Quintana Roo, Mexico (CNHE1297). (d) ex *Cichlasoma* sp. Guanacaste, Costa Rica (CNHE 761). (e) ex *Cichlasoma maculicauda*, Bluefields, Nicaragua (CNHE 4193). (f) ex *Cichlasoma trimaculatum*, Laguna de Tres Palos, Guerrero, Mexico (CNHE 5860).

also in uncovering cryptic species (morphologically similar and genetically distinct). Delimiting species of digeneans is often difficult owing to their limited morphological characters, and this may have resulted in a gross underestimation of the true number of species. In this sense, DNA-based identification and discovery of helminth species has implications for our understanding of global biodiversity (Poulin and Morand, 2004) and, in this case, it has a direct implication for efforts to establish the species richness of digeneans in Mexican vertebrates (Pérez-Ponce de León, 2001, 2007). Thus, the validity of the new species herein described is strongly supported as it is based on both morphological and molecular evidence.

Cribb (2005) pointed out that the geographic distribution of *Crassicutis* species is restricted to the Atlantic coast of North and South America. Diagnosis of the genus should indicate, from this point on, that species of this genus are mostly restricted to the Atlantic coast. Some records have been made on the Pacific slope, since records of particular species have been established in cichlids along the Pacific coast of Costa Rica in the province of Guanacaste (see Rodríguez-Ortiz et al., 2004), as well as the record we establish in the present study. Likewise, our finding of a new species of *Crassicutis* poses an interesting question regarding the biogeography of cichlids and their helminth parasites. Recently, Pérez-Ponce de León and Choudhury (2005) discussed the biogeographical implications of the helminth fauna of Mexican cichlids. This group of freshwater fishes apparently utilized the developing Isthmus of Panama in the late Tertiary period to colonize the southern lowlands of Mexico. A few species penetrated farther north along the Pacific coastal drainages, resulting in the presence today of *Ci. beani* (Miller and Smith, 1986). Freshwater members of *Crassicutis* are, without doubt, part of the biogeographical core parasite fauna in cichlids and, with the exception of *Cr. cichlasomae*, all the other species are geographically restricted. The new species we describe herein represents the northernmost record of a species of *Crassicutis*; however, in the absence of a robust phylogenetic analysis among species of *Crassicutis*, it is impossible to establish the sister group relationships between the new species and their congeners. Nonetheless, it can be hypothesized that this species evolved as a peripheral isolate of ancestral populations of *Cr. cichlasomae* that were isolated following the colonization and concomitant speciation of their host in Pacific drainages of Mexico.

ACKNOWLEDGMENTS

We thank Omar Domínguez, Rodolfo Pérez, Jaquelina Bravo, Elizabeth Martínez, Martín García, and Serapio López for their help during field work. Stephen Curran kindly loaned specimens of *C. archosargi* he collected in Mississippi, and provided insights to compare the new species with other apocreadiids. Laura Márquez provided technical assistance with the sequencer and Virginia León-Règagnon provided access to the Molecular Systematics Laboratory. We want to thank the following people for their help with collecting fish: Diana Liz Trejo Torre and Roman Chan Chi, Cenote Xlakah, Zona Arqueológica Dzibilchaltun; and Miguel Angel García Estraga, Sociedad Cooperativa El Corchito, Yucatan. Scott Gardner, Curator, and Agustín Jiménez, Collection Manager, kindly provided specimens from the HWML. Luis García-Prieto kindly provided specimens from the CNHE. Leopoldina Aguirre Macedo provided specimens from the CHCM (Colección Helminológica del CINVESTAV, Mérida, Yucatán, Mexico); Leticia Magaña provided specimens from the CIHUIAT (Colección Helminológica del Sureste de México, Universidad Juárez Autónoma de Tabasco, Villahermosa, Tabasco, México); and Serapio López provided speci-

mens from his personal collection. U.R.M. wishes to thank CONACyT for his postdoctoral fellowship. R.R.V. and H.M.M. wish to thank DGPE and CONACyT respectively for scholarships for accomplishment of their Ph.D. degrees. This study was supported by grants from PAPIIT-UNAM IN220605 and CONACyT 47233 to G.P.P.L.

LITERATURE CITED

- AGUIRRE-MACEDO, M. L., T. SCHOLZ, D. GONZÁLES-SOLÍS, V. M. VIDAL-MARTÍNEZ, P. POSEL, G. ARJONA-TORRES, S. DUMAILO, AND E. SIU-ESTRADA. 2001. Some adult endohelminth parasitizing freshwater fishes from the Atlantic drainages of Nicaragua. *Comparative Parasitology* **68**: 190–195.
- BOWLES, J., D. BLAIR, AND D. P. MCMANUS. 1995. A molecular phylogeny of the human schistosomes. *Molecular Phylogenetics and Evolution* **2**: 103–109.
- BRAVO-HOLLIS, M., AND G. ARROYO. 1962. Tremátodos de peces de Costa Rica. I. Sobre dos especies del género *Crassicutis* Manter, 1936 (Lepocreadiidae Nicoll, 1914) del intestino de *Cichlasoma*. *Revista de Biología Tropical* **10**: 229–235.
- BRAY, R. A., A. DE CHAMBRIER, AND C. VAUCHER. 1996. *Crassicutis intermedius* (Szidat, 1954) n. comb. and *Procaudotestis uruguayensis* Szidat, 1954 (Digenea: Homalometridae) from siluriform fishes in Paraguay. *Systematic Parasitology* **35**: 119–116.
- CRIBB, T. H. 2005. Family Apocreadiidae Skrjabin, 1942. In *Keys to the Trematoda*, Vol. 2, A. A. Bray, R. A. Bray, and D. I. Gibson (eds.). CAB International and The Natural History Museum, London, U.K., p. 621–639.
- , AND R. A. BRAY. 1999. A review of the Apocreadiidae Skrjabin, 1942 (Trematoda: Digenea) and description of Australian species. *Systematic Parasitology* **44**: 1–36.
- DOLLFUS, R. PH. 1950. Trematodes récoltés au Congo Belge par le Professeur Paul Brien. *Annales du Musée du Congo Belge* **1**: 136.
- FERNANDEZ, B. M., AND A. KOHN. 2001. On some trematodes parasites of fishes from Paraná River. *Brazilian Journal of Biology* **61**: 461–466.
- HALL, T. A. 1999. BioEdit: A user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symposium Series* **41**: 95–98.
- HILLIS, D., B. K. MABLE, AND C. MORITZ. 1996. Nucleic acids IV: Sequencing and cloning. In *Molecular Systematics*, D. Hillis, C. Moritz, and B.K. Mable (eds.). Sinauer, Sunderland, Massachusetts, p. 321–383.
- JOUSSON, O., P. BARTOLI, AND J. PAWLOWSKI. 2000. Cryptic speciation among intestinal parasites (Trematoda: Digenea) infecting sympatric host fishes (Sparidae). *Journal of Evolutionary Biology* **13**: 778–785.
- JOY, J. E. 1971. Geographical distribution and host records for the genus *Crassicutis* (Trematoda: Allocreadiidae). *Folia Parasitologica* **18**: 233–234.
- LEÓN-RÈGAGNON, V., D. R. BROOKS, AND G. PÉREZ-PONCE DE LEÓN. 1999. Differentiation of Mexican species of *Haematoloechus* Loos, 1899 (Digenea: Plagiorchiiformes): molecular and morphological evidence. *Journal of Parasitology* **85**: 935–946.
- , AND L. PAREDES-CALDERÓN. 2002. *Haematoloechus danbrooksii* n. sp. (Digenea: Plagiorchioidea) from *Rana vaillanti* from Los Tuxtlas, Veracruz, Mexico. *Journal of Parasitology* **88**: 1215–1221.
- MANter, H. W. 1936. Some trematodes of cenote fish from Yucatán. *Carnegie Institute of Washington Publications* **457**: 33–38.
- . 1962. Notes on the taxonomy of certain digenetic trematodes of South American freshwater fishes. In *Proceedings of the Helminthological Society of Washington* 29: 97–102.
- MILLER, R. R., AND M. L. SMITH. 1986. Origin and geography of the fishes of Central Mexico. *Zoogeography of North American Freshwater Fishes*. In *The Zoogeography of North American freshwater fishes*, C. H. Hocutt and E. O. Wiley (eds.). John Wiley and Sons, New York, New York, p. 491–517.
- , W. L. MINCLEY, AND S. M. NORRIS. 2005. Freshwater fishes of Mexico. The University of Chicago Press, Chicago, Illinois, 490 p.
- MIURA, O., A. M. KURIS, M. E. TORCHIN, R. F. HECHINGER, E. J. DUNHAM, AND S. CHIBA. 2005. Molecular-genetic analyses reveal cryptic species of trematodes in the intertidal gastropod, *Baillaria cumingi* (Crosse). *International Journal for Parasitology* **35**: 793–801.

- MORAVEC, F., AND V. BARUS. 1972. Studies on parasitic worms from Cuban fishes. *Acta Societa Zoologica Bohemoslov* **35**: 56–74.
- NOLAN, M. J., AND T. H. CRIBB. 2005. The use and implications of ribosomal DNA sequencing for the discrimination of digenean species. *Advances in Parasitology* **60**: 101–163.
- OLSON, P. D., AND V. V. TKACH. 2005. Advances and trends in the molecular systematics of the parasitic platyhelminthes. *Advances in Parasitology* **60**: 165–243.
- OVERSTREET, R. M. 1976. A redescription of *Crassicutis archosargi*, a digenean exhibiting an unusual tegumental attachment. *Journal of Parasitology* **62**: 702–708.
- PALUMBI, S. R. 1996. Nucleic acids II. The polymerase chain reaction. In *Molecular systematics*, D. Hillis, C. Moritz, and B. K. Mable (eds.). Sinauer, Sunderland, Massachusetts, p. 321–383.
- PÉREZ-PONCE DE LEÓN, G. 2001. The diversity of digeneans (Platyhelminthes: Cercomeria: Trematoda) in vertebrates in Mexico. *Comparative Parasitology* **68**: 1–8.
- , AND A. CHOUDHURY. 2005. Biogeography of helminth parasites of freshwater fishes in Mexico: The search for patterns and processes. *Journal of Biogeography* **32**: 645–659.
- , L. GARCÍA-PRIETO, AND B. MENDOZA-GARFIAS. 2007. Trematode parasites (Platyhelminthes) of wildlife vertebrates in Mexico. Mag-nolia Press, Auckland, New Zealand. (In press).
- POULIN, R., AND S. MORAND. 2004. Parasite biodiversity. Smithsonian Institution Press, Washington, D.C., 216 p.
- RAZO-MENDIVIL, U., V. LEÓN-RÉGAGNON, AND G. PÉREZ-PONCE DE LEÓN. 2004. Description of two new species of *Glythelmins* Stafford, 1905 (Digenea: Macroderoididae) in *Rana* spp. from Mexico, based on morphology and mtDNA and rDNA sequences. *Systematic Parasitology* **59**: 199–210.
- RODRÍGUEZ-ORTÍZ, B., L. GARCÍA-PRIETO, AND G. PÉREZ-PONCE DE LEÓN. 2004. Checklist of the helminth parasites of vertebrates in Costa Rica. *Revista de Biología Tropical* **52**: 1–41.
- SCHOLZ, T., M. C. PECH-EK, AND R. RODRÍGUEZ-CANUL. 1995. Biology of *Crassicutis cichlasomae* a parasite of cichlid fishes in Mexico and Central America. *Journal of Helminthology* **69**: 69–75.
- SPARKS, A. K., AND V. E. THATCHER. 1960. A new species of *Crassicutis* (Trematoda: Allocreadiidae) from a sparid fish (*A. probatocephalus*) in the Northern Gulf of Mexico. *Transactions of the American Microscopical Society* **79**: 341–342.
- SWOFFORD, D. L. 2002. PAUP 4.0b10. Phylogenetic analysis using parsimony (and other methods), version 4.0b10. Software distributed by Sinauer Associates, Sunderland, Massachusetts.
- TKACH, V., J. PAWLOWSKI, AND V. SHARPILO. 2000. Molecular and morphological differentiation between species of the *Plagiorchis vespertilionis* group (Digenea, Plagiorchiidae) occurring in European bats, with a redescription of *P. vespertilionis* (Müller, 1780). *Systematic Parasitology* **47**: 9–22.
- VAN HERWERDEN, L., D. BLAIR, AND T. AGATSUMA. 1998. Intra- and interspecific variation in nuclear ribosomal internal transcribed spacer 1 of the *Schistosoma japonicum* species complex. *Parasitology* **116**: 311–317.
- , ———, AND ———. 1999. Intra- and interindividual variation in the ITS1 of *Paragonimus westermani* (Trematoda: Digenea) and related species: Implications for phylogenetic studies. *Molecular Phylogenetic Evolution* **12**: 67–73.
- VIDAL-MARTÍNEZ, V., L. AGUIRRE-MACEDO, T. SCHOLZ, D. GONZÁLEZ-SOLÍS, AND E. MENDOZA-FRANCO. 2001. Atlas of the helminth parasites of cichlid fish of Mexico. Academia Press, Praha, Czech Republic, 165 p.
- VINJOY, C. M., T. A. PRIETO, A. E. FAJER, AND R. CARTAYA. 1985. *Crassicutis* sp. (Trematoda: Allocreadiidae) en el tracto intestinal de *Cichlasoma tetracanthus* (biajaca). *Revista de Salud Animal* **7**: 287–290.
- WATSON, D. E. 1976. Digenea of fishes from Lake Nicaragua. In *Investigations of the ichthyofauna of Nicaraguan lakes*, T. B. Thorson (ed.). University of Nebraska Press, Lincoln, Nebraska, p. 251–260.