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Molecular Characterization of Two Opecoelid Trematodes From Fishes in the Gulf of Mexico, With a Desceiption of a New Species of *Helicometra*

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VERSITA

Molecular characterization of two opecoelid trematodes from fishes in the Gulf of Mexico, with a description of a new species of *Helicometra*

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Abstract

The plagioporine opecoelids *Helicometra fasciata* (Rudolphi, 1819) Odhner, 1902, and *Macvicaria crassigula* (Linton, 1910) Bartoli, Bray, and Gibson, 1989 have been reported from fishes in expansive geographic regions, disjointed from their type localities. New material of *M. crassigula* was collected from near its type locality as well as specimens resembling *Helicometra fasciata* sensu lato from three triglids in the Gulf of Mexico. Comparisons of the ribosomal DNA (rDNA) sequences, comprising the partial 18S rDNA, internal transcribed spacer region (= ITS1, 5.8S, and ITS2), and partial 28S rDNA gene, from *M. crassigula* and *Helicometra fasciata* sensu lato in the Gulf of Mexico were made with sequences deposited in GenBank from those species from the Mediterranean Sea. Results reveal that *M. crassigula* sensu stricto from the Gulf of Mexico is distinct from the two cryptic species of *M. crassigula* sensu lato from the Mediterranean Sea and *Helicometra fasciata* sensu lato in this study differs from *H. fasciata* sequences from the Mediterranean Sea, thus *Helicometra manteri* sp. nov. is described.

Keywords

Digenea, *Macvicaria*, internal transcribed spacer region, Opecoelidae, searobin

Introduction

Helicometra fasciata (Rudolphi, 1819) Odhner, 1902 and *Macvicaria crassigula* (Linton, 1910) Bartoli, Bray, and Gibson, 1989 have been reported from throughout a broad geographic range. Both species have been reported from multiple species of marine teleost hosts; *H. fasciata* has been reported from fish hosts across broad taxonomic ranks, while *M. crassigula* has been primarily reported from sparids. The taxonomic history of both species is tangled, each has several synonyms and likely represents more than one species.

Helicometra fasciata was described with minimal details by Rudolphi (1819) from *Symphodus tinca* (Linnaeus, 1758) (as *Labri tincae* Linnaeus, 1758) off Naples and was subsequently reported from the northeastern Atlantic Ocean, Mediterranean Sea, and Adriatic Sea (e.g. Odhner 1901 [as *Allocreadium fasciatum* [Rudolphi, 1819] Odhner, 1901], Odhner 1902, Stossich 1904 [as *Helicometra gobii* [Stossich, 1813] Stossich, 1904], Nicoll 1910 [as *Helicometra pulchella* [Rudolphi, 1819] Odhner, 1902], Palombi 1929a,b, 1932). *Helicometra fasciata* has also been reported from the southwestern North Atlantic Ocean (Hopkins 1941) and Gulf of Mexico (Manter 1933, 1934, 1947); the eastern South Atlantic Ocean off Gahna (Fischthal and Thomas 1970) and off South Africa (Bray 1987); the Red Sea (Parukhin 1970); and the Pacific Ocean off Mexico (Manter 1940), off Peru (Iannacone *et al*. 2011), from the Great Barrier Reef (Bray and Cribb 1989), off the temperate coast of Australia (Aken'Ova *et al*. 2006), and off Tasmania (Crowcroft 1947). Sekerak and Arai (1974) provide an extensive review of *H. fasciata* (as *Helicometra pulchella* [Rudolphi, 1819] Odhner, 1902) that includes synonyms, host species, and distribution. Madhavi (1975) reported *H. fasciata* from the Bay of Bengal where Meenakshi *et al*. (1993) later described as *Helicometra gibsoni* Meenakshi, Madhavi, and Swarnakumari, 1993 after elucidating its life-cycle. Reversat (1989, 1991) used allozyme analyses to demonstrate that two morphotypes of *H. fasciata* from four host species from Étang de Thau, France, consisted of at least three species, each with different degrees of specificity for their final hosts. Jousson *et al*. (1999) provided the ribosomal DNA (rDNA) internal tran-

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scribed spacer region (= ITS1, 5.8S, and ITS2) from what they identified as *H. fasciata* from *Symphodus rostratus* (Bloch, 1791) and the ITS region for larvae of two species of *Helicometra* from the trochid snail *Jujubinus striatus* (Linnaeus, 1758) [as *Calliostoma striatum* (Linnaeus, 1758)] and the hippolytid shrimp *Hippolyte inermis* Leach, 1814 from off Corsica, France.

Macvicaria crassigula was originally described by Linton (1910) as *Lebouria crassigula* Linton, 1910 off the Dry Tortugas, Florida from *Calamus calamus* (Valenciennes, 1830). Bartoli *et al*. (1989) provided a thorough review of the species, its synonyms, distribution, and definitive hosts. They also discussed the possibility that *M. crassigula* may represent a complex of several species in the Mediterranean Sea. Since then, *M. crassigula* has been reported off Rio de Janeiro, Brazil (Fernandes and Goulart, 1992), and off Tunisia (Gargouri Ben Abdallah and Maamouri, 2008). Jousson *et al*. (2000) used ITS1 sequence data to demonstrate that *M. crassigula* sensu lato in the Mediterranean Sea is a complex of two species, one shared by *Diplodus sargus* (Linnaeus, 1758) and *Diplodus vulgaris* (Geoffroy Saint-Hilaire, 1817) and the other restricted to *Diplodus annularis* (Linnaeus, 1758), but they refrained from naming either species.

Molecular analyses and phylogenetics have greatly influenced our understanding of delineating species-complexes, evaluating traditional taxonomic groups, and estimating relationships among taxa (e.g., Jousson *et al*. 2000, Nolan and Cribb 2005, Olson and Tkach 2005, Yang and Rannala 2012, Curran *et al.* 2013). While these tools are extremely useful, they often have problems estimating relationships between taxa when broadly defined morphological taxa (e.g., *Macvicaria* Gibson and Bray, 1982, and *Neolebouria* Gibson, 1976) are used in the analysis without including the type species (Andres *et al*. 2014). Such problems are often nomenclatural but are necessary when trying to define sub-familial and generic relationships. To this end, we compare the ITS rDNA region of *M. crassigula* collected from near its type locality with the same gene region from the two species in *M. crassigula* sensu lato from the Mediterranean Sea deposited in GenBank and compare the ITS rDNA region from specimens resembling *H. fasciata* sensu lato collected in the Gulf of Mexico from three species of triglids with the sequences of *Helicometra* spp. from the Mediterranean Sea deposited in GenBank.

Materials and Methods

Specimens of the spiny searobin, *Prionotus alatus* Goode and Bean, 1883; the horned searobin, *Bellator militaris* (Goode and Bean, 1886); the streamer searobin, *Bellator egretta* (Goode and Bean, 1896); the jolthead porgy, *Calamus bajonado* (Bloch and Schneider, 1801); and the whitebone porgy, *Calamus leucosteus* (Jordan and Gilbert, 1885), were collected in 2008, 2010, 2011, and 2012 during National Marine Fisheries Service fall pelagic trawl surveys in the northern Gulf of Mexico. Fish were examined for trematodes aboard ship or placed on ice and examined soon after collection. Live trematodes were washed briefly in a 0.8% saline solution, examined briefly, fixed with near-boiling tap water, and placed in either 5% neutral buffered formalin solution or 70% molecular grade ethanol. Other specimens were placed in room-temperature 95% molecular grade ethanol and refrigerated for later sequencing. Specimens were stained in Van Cleave's hematoxylin or aqueous Mayer's hematoxylin, followed by destaining in 1% sodium hydroxide solution; stained specimens were then dehydrated in a graded alcohol series. When specimens stained with Van Cleave's hematoxylin or carmine reached 80% ethanol, they were neutralized with one drop of a dilute lithium carbonate solution and two drops of a 1% butylamine solution. Dehydrated specimens stained with Van Cleave's were cleared in clove oil, specimens stained in Mayer's were cleared in methyl-salicylate, and all were mounted in Damar gum on glass slides. Measurements were made using a compound microscope equipped with a differential interference contrast, a Cannon EOS Rebel T1i camera, and calibrated digital software (iSolutions Lite ©). Measurements are presented as micrometres (μm) and given for the holotype followed by the range of measurements from other specimens in parentheses. Illustrations were made with the aid of a drawing tube and digitally inked using a Wacom tablet (Wacom Co., Vancouver, Washington).

Genomic DNA was extracted from two entire specimens of *H. fasciata* sensu lato from *P. alatus* and one entire specimen each from *B. egretta* and *B. militaris*, and one entire specimen of *M. crassigula* each from *C. bajonado* and from *C. leucosteus* using Qiagen DNAeasy Tissue Kit (Qiagen, Inc., Valencia, California, U.S.A.) following the instructions provided. DNA fragments approximately 2,400 base pairs (bp) long comprising the 3' end of the 18S gene nuclear rDNA gene, internal transcribed spacer (ITS) region ITS1, 5.8S, ITS2, and the 5' end of the partial 28S gene (including variable domains D1-D3) were amplified from the extracted DNA by polymerase chain reaction (PCR) on a PTC-200 Peltier Thermal Cycler using forward primer ITSF (5'-CGCCCGTCGCTACTACCGATTG-3') or S20T2 (5'-GGTAAGTGCAAGTCATAAGC-'9) and reverse primer 1500R (5'-GCTATCCTGAGGGAAACTTCG-3'). Sequencing reactions used the previous primers and the internal forward primers digl2 (5'-AAGCATATCACTAAGCGG-3'), 300F (5'-CAAGTACCGTGAGGGAAAGTTG-3'), and 900F (5'-CCGTCTTGAAACACGGACCAAG-3') and internal reverse primer digl2R (5'-CCGCTTAGTGATATGCTT-3'), 300R (5'-CAACTTTCCCTCACGGTACTTG-3'), and ECD2 (5'-CTTGGTCCGTGTTTCAAGACGGG-3'). The PCR reactions were performed under the protocols described by Tkach *et al*. (2003).

The resulting PCR products were excised from PCR gel using QIAquick Gel Extraction Kit (Qiagen, Inc., Valencia, California, U.S.A.) following kit instructions, cycle-sequenced using ABI BigDye™ chemistry (Applied Biosystems, Inc., Carlsbad, California, U.S.A.), ethanol-precipitated, and run on

Table I. Species of Opecoelidae collected from the Gulf of Mexico and their respective host species, GenBank accession number, and deposition information

Species	Host	Year	Depth (m)	Coordinates	GenBank	USNPC
Helicometra manteri sp. nov.	*Prionotus alatus*	2008	149	27°19′13″N 84°7′28″W	KJ701238	108150
Helicometra manteri sp. nov.	*Bellator egretta*	2008	152	26°47′43″N 84°16′30″W	KJ701239	108152
Macvicaria crassigula sensu stricto	*Calamus bajonado*	2010	57	26°57′02″N 83°45′45″W	KJ701237	108153

an ABI 3130 Genetic Analyzer™. Contiguous sequences were assembled using Sequencher™ (Version 4.10.1, GeneCodes Corp., Ann Arbor, Michigan, U.S.A.) and submitted to GenBank. Newly obtained sequences (Table I) were compared for intraspecific variability along with sequences deposited in GenBank: *H. fasciata* (AJ241793), larval stages of two unidentified species presumed to be in *Helicometra*(AJ241810 and AJ241817), and *Macvicaria crassigula* sensu lato (AJ241803 and AJ277372). Pairwise sequence comparisons were aligned using Bioedit, ver. 7.1.3.0. (Hall, 1999) with gaps treated as missing data.

Results

Molecular results

The trimmed alignment of *M. crassigula* sensu stricto and the two sequences of *M. crassigula* from GenBank consisted of a fragment of the 3‘ end of 18S nuclear rDNA gene (136 bases), ITS1 (706 bases), 5.8S (156 bases), ITS2 (257 bases), and a fragment of the 5‘ end of the 28S gene (61 bases). No intraspecific sequence variation was observed between the two replicates of *M. crassigula* sensu stricto. The 3‘ end of 18S nuclear rDNA gene and the 5.8S gene exhibited no genetic variation among any of the species studied. Comparison of the ITS1 and ITS2 (Table II) revealed that *M. crassigula* sensu stricto was most similar to the sequence of *M. crassigula* sensu lato (AJ241803) shared by *D. sargus* and *D. vulgaris*. Sequence AJ241803 differed from both *M. crassigula* sensu stricto and AJ241803 by one base, and *M. crassigula* sensu stricto differed from AJ277372 by two bases in the partial 28S. The length of the partial 5’ end of the 28S gene of *M. crassigula* sensu stricto was 1,382 bases.

One sequence obtained from a specimen of *H. fasciata* sensu lato from *B. egretta* had an intraspecific variation of two bases (0.4%) in the ITS1 but none in the 3’ end of the 18S, 5.8S, ITS2, and 5’ end of the partial 28S. The trimmed sequence alignment of *H. fasciata* sensu lato and three species of *Helicometra* from GenBank (*H. fasciata* and two unidentified larval specimens of *Helicometra*) consisted of a fragment of the 3‘ end of 18S nuclear rDNA gene (136 bases), ITS-1 (706 bases), 5.8S (156 bases), ITS-2 (257 bases), and a fragment of the 5‘ end of the 28S gene (61 bases). The length of the partial 5’ end of the 28S gene of *H. fasciata* sensu lato was 1,374 bases. Sequences of *H. fasciata* sensu lato obtained from triglids in the Gulf of Mexico represented a unique genotype compared with the other three sequences of *Helicometra* from the Mediterranean Sea (Table III). We therefore describe the following species.

Table II. Pairwise comparisons of percent nucleotide similarity and number of base pair differences (in parentheses) of the ITS-1 (below the diagonal) and ITS-2 (above the diagonal) of the three genotypes of *Macvicaria crassigula* sensu lato

	***M. crassigula* sensu stricto**	**AJ241803**	**AJ277372**
M. crassigula sensu stricto	–	99.2 (2)	98.8 (3)
AJ241803	98.7 (9)	–	98.8 (3)
AJ277372	97.4 (16)	97.9 (13)	–

Table III. Pairwise comparisons of percent nucleotide similarity and number of base pair differences (in parentheses) of the ITS-1 (below the diagonal) and ITS-2 (above the diagonal) of the four species of *Helicometra*

	H. fasciata	**AJ241810**	**AJ241817**	***H. manteri* sp. nov.**
H. fasciata	–	98.3 (5)	97.3 (8)	96.2 (11)
AJ241810	98.1 (9)	–	98.3 (5)	97.3 (8)
AJ241817	98.5 (7)	98.3 (8)	–	96.2 (11)
H. manteri sp. nov.	95.3 (22)–95.7 (20)	95.1 (23)–95.5 (21)	95.7 (20)–96.1 (18)	–

***Helicometra manteri* sp. nov.** (Figs 1–3)

Description based on 10 gravid, wholemounted, unflattened specimens. Body 2,225 (1,989–3,722) long, dorsoventrally flattened, elongate-oval, with frilled lateral and posterior margins, tapered anteriorly, rounded posteriorly, 656 (592–1,194) wide at midbody; body width to body length (BL) ratio 1: 3.4 (1:2.9–3.8). Oral sucker ventrally subterminal, subspherical, 162 (143–264) long, 155 (161–316) wide, associated with conspicuous glands and ducts. Ventral sucker (VS) subspherical, 226 (210–392) long, 283 (251–476) wide, in anterior third of body; VS width to oral sucker width ratio 1: 1.6 (1: 1.4–1.6). Forebody 655 (570–1,100) long, approximately 29% (24–31%) of BL. Prepharynx 8 (4–16) long. Pharynx globular, 110 (74–133) long, 101 (86–152) wide; pharynx to oral sucker width ratio 1: 1.7 (1: 1.6–2.1). Esophagus 214 (105–350) long, slightly sigmoid. Intestinal bifurcation 170 (136–318) anterior to anterior margin of VS. Ceca 1,526 (1,320–2,692) long, narrow, terminating blindly 205 (104–433) from posterior extremity. Postcecal region representing 9% (6–11%) of BL.

Testes 2, tandem, in posterior half of body, nearly contiguous to separated by 36 (5–107), with lobed to irregular margins; anterior testis 175 (152–350) long, 231 (172–496) wide; posterior testis 225 (177–368) long, 208 (125–427) wide. Posttesticular space 512 (512–925) long, representing 23% (22–29%) of BL. Cirrus sac 332 (319–613) long, representing 15% (14–17%) of BL, 65 (60–118) wide at widest portion, medial, fusiform, extending from approximately level of middle of esophagus to mid-level of ventral sucker. Internal seminal vesicle gradually decreasing in diameter anteriorly, sinuous, occupying approximately 2/3 of cirrus sac. Pars prostatica with lightly staining surrounding prostatic cells. Ejaculatory duct 88 (85–182) long, representing 27% (25–30%) of cirrus sac length thick-walled. Genital atrium poorly defined, 5 (indistinct to 9) long. Genital pore median, opening approximately middle of forebody, 395 (339–688) from anterior end.

Ovary with 5–7 lobes, anteroventral and contiguous or nearly so with anterior testis, 167 (112–301) long, 209 (203–447) wide. Mehlis' gland anterior to ovary. Seminal receptacle canalicular, approximately 1/3 to 1/2 size of anterior testis, retort-shaped, anterodorsal to ovary. Laurer's canal opening anterodorsal and sinistral to ovary. Uterus coiling intercecally between anterior margin of ovary and near posterior margin of cirrus sac, then passing dextrally along margin of cirrus-sac to genital atrium without coiling. Vitellarium comprised of 2 lateral fields of small follicles; follicles extending from level in forebody 491 (387–771) from anterior extremity or 22% (15–20%) of BL to near posterior extremity, not confluent in region of uterus and gonads, confluent in posttesticular region, with some dorsal and ventral to ceca, with majority extracecal. Eggs 56 (49–62) long, 20 (14–23) wide, operculate, with unipolar filament approximately equal to 2 times egg length.

Excretory vesicle I-shaped, extending to level of anterior margin of ovary; excretory pore dorsally subterminal.

Taxonomic summary

Type-host: Spiny searobin, *Prionotus alatus* Goode and Bean, 1883 (Scorpaeniformes: Triglidae); other hosts: horned searobin, *Bellator militaris* (Goode and Bean, 1896); streamer searobin, *Bellator egretta* (Goode and Bean, 1896) (Scorpaeniformes: Triglidae).

Site of infection: intestine.

Type-locality: Approximately 150 km west of Sarasota, Florida, 27°19′13″N, 84°7′28″W, from 81 m deep; other localities: from *P. alatu*s 26°47′55″ N, 84°20′23″W from 160 m deep, 28°11′19″N, 94°4′40″ W from 60 m deep, and 26°53′64″ N, 96°40′71″ W from 86 m depth; from *B. militaris* 26°47′55″ N, 84°20′23″W from 160 m deep; from *B. egretta* 26°47′43″ N, 84°16′30″W from 152 m deep, and 26°47′55″N, 84°20′23″W from 160 m deep.

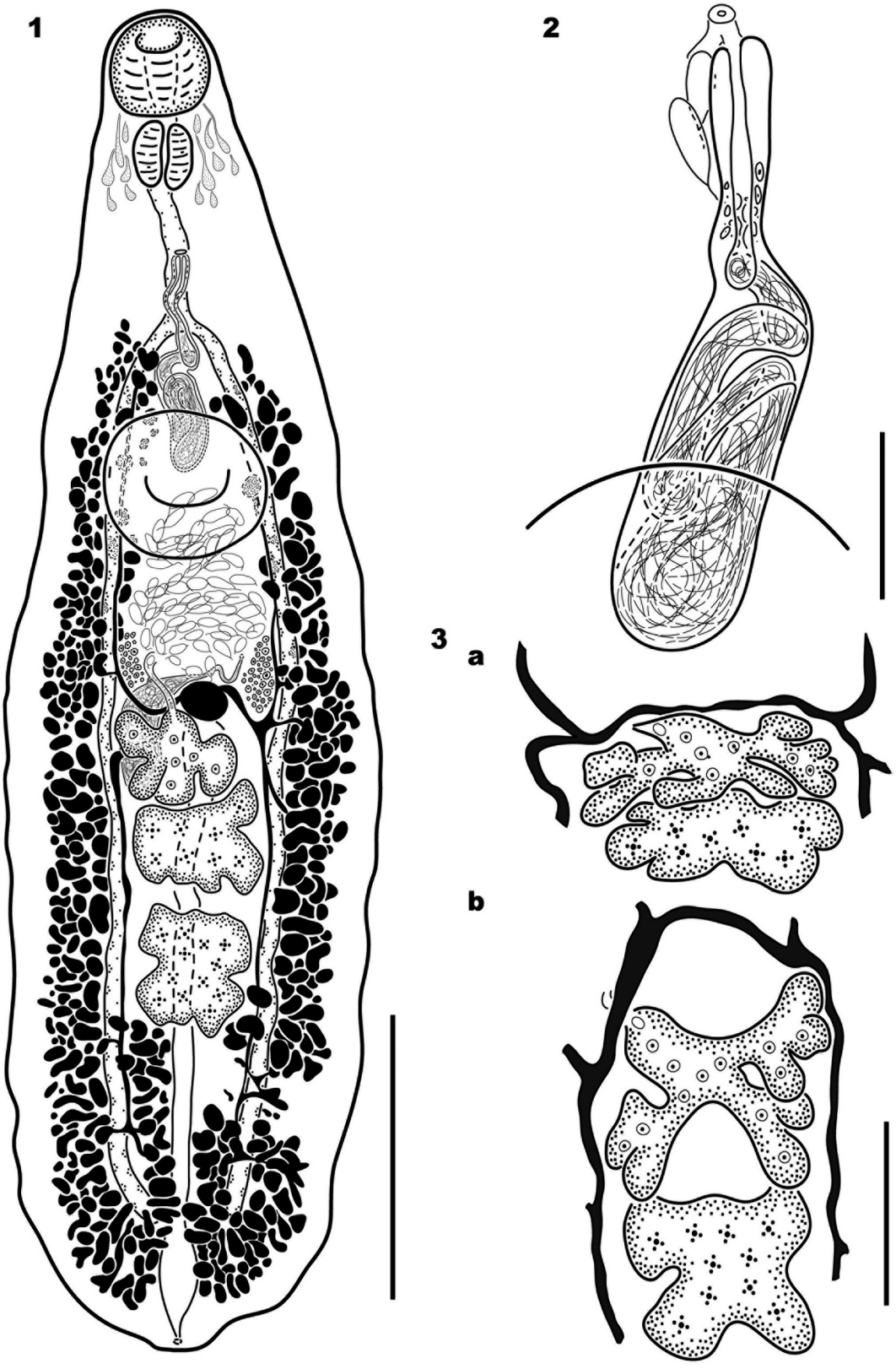
Specimens deposited: U.S. National Parasite Collection (USNPC), Beltsville, Maryland, Holotype (USNPC 108150); Paratypes (USNPC 108151-108152), Harold W. Manter Laboratory Collection, Lincoln, Nebraska P-2014-014, and Gulf Coast Research Laboratory Museum, Ocean Springs, Mississippi, No. 06530-06531.

Etymology: This species is named in honor of the late Dr Harold W. Manter, the first person to report *H. fasciata* sensu lato from the Gulf of Mexico.

Remarks

Specimens of *H. manteri* recovered by us from *Bellator* spp. and by Manter (1933, 1934) from *Bellator militaris* have a slightly more prominently lobed ovary than specimens recovered from *P. alatus* (Fig. 3a,b). Morphometric variation in specimens collected from the three host species is negligible. Manter (1933, 1934) did not report measurements of specimens he collected from off Florida from between 91–110 m (50–60 fathoms) of depth. Sekerak and Arai (1974) examined three specimens reported by Manter (1933) and provided measurements that are of specimens shorter and wider than those we collected. We examined Manter's (1933, 1934) specimens from *B. militaris* and *P. alatus* but morphometric comparisons are not provided because of the apparent differences in fixation techniques between his specimens and ours (e. g. a mean body width to BL ratio of 1:2.5 rather than 1:3.4, a mean posttesticular space representing 20.4% of BL rather than 24.5% of BL, a mean ovary length to ovary width ratio of 1:2.3 rather than 1:1.5, the posterior margin of the cirrus sac in the region of the anterior margin of the ventral sucker rather than at the level of the level of the mid-ventral sucker) .

Helicometra manteri sp. nov. is morphologically most similar to species attributed to *H. fasciata* with tandem testes. The variability exhibited by *H. fasciata* across a broad host and geographic distribution associated with abundant revisions, synonyms, and inconsistent fixation techniques makes most



Figs 1–**3.** *Helicometra manteri* sp. nov. Scale bars: Fig. 1 = 500 μm; Fig. 2 = 100 μm; Fig. 3 = 250 μm. **Fig. 1.** Ventral view, holotype from *Prionotus alatus*. **Fig. 2.** Ventral view, terminal genitalia, dark half circle anterior margin of ventral sucker. **Fig. 3.** Ventral view, ovary of *H. manteri* sp. nov. from *Bellator* spp., displaying more prominent lobes; **a**. Flattened specimen, HWML 0261; **b.** Paratype, USPNC 108151. Scale bars: Fig. 1 = 500 μm; Fig. 2 = 100 μm; Fig. 3 = 250 μm

morphological comparisons of uncertain value. However, *H. manteri* sp. nov. may be differentiated from some *H. fasciata* sensu lato forms by having tandem rather than oblique testes. Additionally, the new species is longer (1,989–3,722 compared with 1,020–1,820), has a shorter cirrus sac (14–17% of BL compared with 25–27% of BL), and a longer posttesticular space (22–29% compared with 14–20% of BL) than *H. fasciata* sensu lato reported by Bray (1989) from off South Africa. *Helicometra manteri* sp. nov. has a slightly shorter forebody (24–31% of BL compared with 27–34% of BL) and cirrus sac (14–17% compared with 16–24% of BL) than *H. fasciata* sensu lato reported by Aken'Ova *et al*. (2006) from off the temperate waters of Australia.

Discussion

Bartoli *et al*. (1989) reviewed *M. crassigula* from five species of sparids off Corsica and mentioned that the morphological variation of specimens from different hosts may indicate the presence of a complex of three species. Jousson *et al*. (2000) used the ITS1 of specimens of *M. crassigula* sensu lato from *D. annularis*, *D. sargus*, and *D. vulgaris* and found that two species of *M. crassigula* sensu lato are present, one species (represented by GenBank sequence number AJ277372) from *D. annularis* and one (represented by GenBank sequence number AJ241803) shared by *D. sargus* and *D. vulgaris*. Neither study discussed the possibility of *M. crassigula* sensu lato from the eastern North Atlantic and Mediterranean Sea being different from *M. crassigula* sensu stricto from the western South Atlantic and Gulf of Mexico, despite the type locality being off the Dry Tortugas, Florida. Jousson *et al*. (2000) suggested that host ecology and habitat, rather than final host phylogeny, were driving factors in the speciation of *M. crassigula* sensu lato. They noted that *D. annularis* inhabits seagrass beds while *D. sargus* and *D. vulgaris* live over rocky substrates, although the three species can be observed together in habitats consisting of both seagrasses and rocks. The three host species of *Calamus* (*C. calamus* [Linton 1910], *C. bajonado* [Overstreet 1969, this study], and *C. leucosteus* [this study]) for *M. crassigula* sensu stricto are all hard bottom reef associated fishes (Sedberry 1989, Carpenter 2002). The closer relationship of *M. crassigula* sensu stricto with *M. crassigula* sensu lato shared by the two species of *Diplodus* may suggest that host habitat, with its corresponding first intermediate hosts, may be more important than geographic distribution in the speciation of members of the *M. crassigula* species complex.

Little is known about the lifecycles of members of the *M. crassigula* complex. No cercarial stage is known. Jousson *et al*. (1999) recovered metacercariae from the urchin *Paracentrotus lividus* (Lamarck, 1816) and the phasianellid gastropod *Tricolia speciosa* (Von Mühlfeldt, 1824) producing sequences that match *M. crassigula* sensu lato from *D. sargus* and *D. vulgaris*. *Paracentrotus lividus* inhabits both seagrass and rocky substrates (Boudouresque and Verlaque 2001), while *T. speciosa* inhabits mainly seagrass (Rueda and Salas 2007). The finding of metacercariae in *T. speciosa* slightly confuses the host-parasite pattern discussed above. We have examined five specimens of the grass porgy, *Calamus arctifrons* Goode and Bean, 1882 from seagrass beds in the Florida Keys without observing any species of *Macvicaria*.

We described *H. manteri* sp. nov. based on sequence differences in the ITS derived from our specimens and those from *Helicometra* spp. reported by Jousson *et al*. (1999). Although the ITS sequence from *H. fasciata* isolated from *Symphodus rostratus* (Bloch, 1791) by Jousson *et al*. (1999) may not represent *H. fasciata* sensu stricto, the three GenBank sequences obtained from Mediterranean species of *Helicometra* by Jousson *et al*. (1999) are all closer to each other than either is to *H. manteri* sp. nov. Using enzyme electrophoresis, Reversat *et al*. (1989, 1991) found that *H. fasciata* consisted of a complex of at least three species. They (1991) considered *H. pulchella* to possess the allele *MDH-1*^{a}, have spherical testes, and be isolated from only *Symphodus cinereus* (Bonnaterre, 1788); *H. gobii* to possess the allele *MDH-1*^{b}, have spherical or lobed testes and be isolated from *Anguilla anguilla* (Linnaeus, 1758), *Gobius niger* Linnaeus, 1758, *S. cinereus*, and *Zosterisessor ophiocephalus* (Pallas, 1814); and *H. fasciata* to possess the alleles *MDH-1*^{c} and *MDH-1*^{d}, have lobed testes, and be isolated from *A. anguilla*, *G. niger*, and *Z. ophiocephalus*. Specimens of *H. fasciata* from the type host, *S. tinca*, have not been analyzed using molecular techniques. Bartoli *et al*. (2005) reported four morphological forms of *Helicometra* in their survey of digeneans from off Corsica, but they refrained from using specific names for species of the *Helicometra* species complex because of the difficulty of distinguishing them. A comprehensive studying including allozyme electrophoresis, sequencing of rDNA, and morphological examination from the type hosts and locality of *H. fasciata*, *H. gobii*, and *H. pulchela* is required to help clarify the *H. fasciata* species complex in the Mediteranean Sea and Northwest Atlantic Ocean. Aken'Ova *et al*. (2006) provided measurements and illustrations for specimens they attributed to *H. fasciata* but with the caveat that they remained suspicious that *H. fasciata* had a cosmopolitan distribution. They also suggested that comparative morphology alone was not sufficient to differentiate all the species of *Helicometra*. Our results agree with this suggestion, and we believe additional species of the *H. fasciata* morphotypes will be revealed from other geographic locations with different sequences.

Hopkins (1941) examined a single specimen he identified as *H. fasciata* sensu lato from *Prionotus carolinus* (Linnaeus, 1771) off Beaufort, North Carolina, USA. We believe that specimen is likely *H. manteri* sp. nov., although Hopkins (1941) reported that the vitellaria extend into the forebody to the level of the middle of the esophagus. Thus, *H. manteri* sp. nov. is probably restricted to specific triglid species from the southwestern Atlantic Ocean and Gulf of Mexico; we have not found it in *Bellator brachychir* (Regan, 1914) from 28°11′19″ N, 94°4′40″W (n = 2); *Prionotus longispinosus* Teague,

1951 from 28° 05′89″N, 90°29′36″W (n = 1), 28°49′93″N, 84°16′25″W (n = 5), 29°43′17″N, 87°03′66″W (n = 6), 27°8′43″ N, 96°40′45″W (n = 3), 27°59′96″N, 95°34′10″ W (n = 1), 28°05′70″N, 95°10′03″W (n = 1), and 28°32′42″N, 89°19′49″W (n = 1); *Prionotus ophryas* Jordan and Swain, 1885from 26°09′72″N, 83°31′09″W (n = 8), *Prionotus paralatus* Ginsburg, 1950 from 26°47′21″N, 84°31′15″W (n = 10) and 28°16′10″N, 93°27′39″W (n = 1); *Prionotus rubio* Jordan, 1886 from 26°09′34″N, 83°31′09″W (n = 1) and 28°24′13″N, 91°33′68″W (n = 7); or *Prionotus stearnsi* Jordan and Swain, 1885 from 28°11′19″N, 94°4′40″W (n = 4), 27°08′24″N, 84°35′29″W (n = 10), and 27°45′12″N, 95°46′17″ W (n = 12). The absence may be partially attributed to under-sampling and differences in the feeding ecology and habitat preferences of the fishes.

We have provided the first molecular test challenging the geographic distribution of *M. crassigula* sensu lato and *H. fasciata* sensu lato. In doing so, we described *H. manteri* and provided sequences from near the type locality for *M. crassigula*. Jousson *et al*. (2000) did not propose a new name for either of the presumed species of *M. crassigula* sensu lato that they detected, likely because specimens of *M. crassigula* from the type locality had not been sequenced. The two sibling species attributed to *M. crassigula* from the Mediterranean Sea should be named on the basis of parallel morphological and molecular studies. By providing these data, we hope future workers can test further the broad geographic range of these species complexes and will be able to describe those and other undetected species, when appropriate.

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