Cardicola laruei Short, 1953 (Digenea: Aporocotylidae) from Heart of Seatrout, Cynoscion spp., (Perciformes: Sciaenidae) in the Gulf of Mexico and Atlantic Ocean: Taxonomic Redescription, First Observations of Egg and Miracidium, and Comments on Geographic Distribution and Host Specificity

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ABSTRACT: The heart and gill of 125 spotted seatrout, Cynoscion nebulosus (Cuvier, 1830), (Perciformes: Sciaenidae) from 5 Gulf of Mexico localities (Mississippi Sound [n = 18] [30°23′21″N; 88°51′44″W], Apalachicola Bay [n = 17] [29°40′18″N; 85°00′09″W], Suananee Sound and Wacassasa Bay [n = 22] [29°7′55″N; 83°2′24″W], Tampa Bay [n = 58] [27°41′58″N; 82°37′49″W], and Charlotte Harbor [n = 10] [26°43′07″N; 82°11′27″W]; 40 spotted seatrout from 2 northwestern Atlantic Ocean localities (St. John’s River Estuary [n = 28] [30°22′29″N; 81°34′08″W] and Indian River Lagoon [n = 12] [28°5′31″N; 80°36′08″W]; and 103 sand seatrout, Cynoscion arenarius Ginsburg, 1930, from 2 Gulf of Mexico localities (Mississippi Sound [n = 102] and Tampa Bay [n = 1]) were examined for the presence of fish blood flukes (Digenea: Aporocotylidae). One adult aporocotyloid was collected from the heart of each infected spotted seatrout captured in Tampa Bay (6 of 58 infected, 10%) and St. John’s River Estuary (1 of 28 infected, 4%) as well as from the heart of the single sand seatrout from Tampa Bay. We identified these aporocotyloids as Cardicola laruei Short, 1953, based on light and scanning electron microscopy that matched them to the original species description of C. laruei, the type specimens in the United States National Parasite Collection (holotype USNPC 37377; paratypes USNPC 37378–9), and other of Short’s original specimens now in our own collection. The present study is the first reported collection of this aporocotyloid (i) from Tampa Bay, (ii) from the Atlantic Ocean, (iii) from the heart of spotted seatrout, and (iv) since its original description in 1953. Among other significant morphological features previously not ascribed to C. laruei, these specimens have a spheroid anterior sucker with concentric rows of minute spines anterior to the mouth. The fully developed, spindle-shaped egg of C. laruei embeds in the gill epithelium of its fish host proximal to the afferent branchial arterioles and encloses a ciliated miracidium. This report significantly contributes to our knowledge of C. laruei by (i) extending its known geographic distribution to a new ocean basin (Atlantic Ocean), (ii) adding supplemental morphological data derived from light and scanning electron microscopy, (iii) providing the first published observations of nonadult life history stages (egg and miracidium), and (iv) confirming that sympatric, congeneric seatrouts are infected by C. laruei.

KEY WORDS: Cardicola laruei, Aporocotylidae, Sanguinicolidae, Digenea, seatrout, Cynoscion nebulosus, Cynoscion arenarius, heart, Gulf of Mexico, Mississippi, Florida.

Short’s (1953) description of Cardicola laruei Short, 1953 (Digenea: Aporocotylidae) was based on several adult specimens from the heart of the sand seatrout, Cynoscion arenarius Ginsburg, 1930, (Perciformes: Sciaenidae) (type host) plus a single adult specimen of C. laruei from “washes of gut” of a spotted seatrout, Cynoscion nebulosus (Cuvier, 1830), captured in the north-central Gulf of Mexico off Franklin and Wakulla counties, Florida, U.S.A. (Fig. 1) (see also Short’s 1952 abstract: J. Parasitol. 1952; 38:36). Short proposed a new genus, Cardicola Short, 1953, to accommodate C. laruei, and selected as the type species Cardicola cardiocola (Manter, 1947) Short, 1953 (written in Short [1953] as both “Psettarium cardiocolum” and “Cardicola cardiocola,” written in Smith [1997a] as “Cardicola cardiocola”), which infects the heart of jolthead porgy, Calamus bajonado (Bloch and Schneider, 1801) (Perciformes: Sparidae) off Tortugas, Florida (Florida Straights, eastern Gulf of Mexico). In the intervening 57 yr, Cardicola has become the most species-rich genus (nominally) of marine fish blood fluke (Digenea: Aporocotylidae), accommodating many of the taxa that have the combination of a single testis and an “H-shaped” intestine (Smith, 1997a, b, 2002; Bullard and Overstreet, 2004; Bullard et al., 2004; Nolan and Cribb, 2006; Holzer

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Bullard (2010a) provided a recent overview of the taxonomy of *Cardicola* spp. and emphasized that the genus needed revisionary work that should include detailed observations of newly collected specimens of the type species. Like *C. cardiocolum*, few species of *Cardicola* have been morphologically restudied in published works since their original discovery; however, newly collected specimens can help supplement museum materials and help better resolve critical morphological features toward a systematic revision of the genus. In addition, new collections promise to help further characterize host specificity, geographic distribution, and pathological effects of aporocotylids among wild fishes, all of which yields a more nuanced understanding of aporocotylid–fish relationships in the wild.

Perhaps because of the apparent low prevalence of infection by *C. laruei* among seatrouts (*Cynoscion* spp.) outside of Florida waters (present study), and despite it being listed in several faunal surveys (Manter, 1954; Loftin, 1960; Nahhas and Short, 1965; Bullard and Overstreet, 2004; Overstreet et al., 2009), no new taxonomic or biological information about this aporocotylid species has been published since Short’s original work in 1953. In fact, we are not aware of any new specimens being collected since 1951. By integrating a portion of our study into the

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**Figure 1.** Collection localities in the Gulf of Mexico and northwestern Atlantic Ocean. Number of examined and infected spotted seatrout (*Cynoscion nebulosus*) and sand seatrout (*Cynoscion arenarius*), respectively, reported parenthetically.
Florida Fish and Wildlife Conservation Commission’s Fish and Wildlife Research Institute (FWRI) fisheries research and monitoring activities aimed at spotted seatrout, we obtained high-quality opportunistic samples of hearts and gills from seatrouts captured along the Gulf of Mexico and Atlantic coasts of Florida (Fig. 1). We also obtained comparable samples from seatrouts captured in Mississippi Sound (north-central Gulf of Mexico). Some of the Florida samples were infected by *C. laruei*, and we herein use those materials to provide additional biological information on infections of *C. laruei* among wild seatrouts.

Aquaculture of spotted seatrout in the Gulf of Mexico for the purposes of stock enhancement is occurring presently, at least in Texas (Anderson and Karel, 2010), Mississippi (Bullard, personal observations; see also the agency report entitled “Current Status and Opportunities for Marine Stock Enhancement and Aquaculture in Florida,” Feb 2007, Florida Oceans and Coastal Resources Council, Bureau of Seafood and Aquaculture Marketing, Tallahassee, Florida), and Florida (Bakenhaster, personal observations; Kupschus, 2003). Fish blood flukes are known pathogens of fishes in hatchery raceways, ponds, and net pens (Bullard and Overstreet, 2002, 2008). Hence, information on the anatomy of adult and larval stages, geographic distribution, and host specificity of *C. laruei* in spotted seatrout is of potential value to husbandry staff and fish health diagnosticians concerned with the health status of brood stock and juvenile seatrout being released into wild fish populations.

**MATERIALS AND METHODS**

Seatrouts were captured during February 2007 through October 2010 by baited hook and line, cast net, gill net, and beach seine. The number and geographic locality (global positioning system coordinates represent the geographic center of local sampling sites within that water body or adjacent waters) of examined fishes is as follows (Fig. 1): for spotted seatrout (165 individuals total: 125 from Gulf of Mexico and 40 from Atlantic Ocean), 18 from Mississippi Sound off Biloxi, Mississippi, U.S.A. (north-central Gulf of Mexico, 30°23.36'N; 88°51.74'W), 17 from Apalachicola Bay off Apalachicola, Florida (north-central Gulf of Mexico, 29°40.31'N; 85°01.15'W), 22 from Suwannee Sound and Wacassa Bay off Cedar Key, Florida (northeastern Gulf of Mexico, 29°7.92'N; 83°2.40'W), 58 from Tampa Bay near Tampa, Florida (eastern Gulf of Mexico, 27°41.98'N; 82°37.82'W), 10 from Charlotte Harbor off Charlotte Harbor, Florida (eastern Gulf of Mexico, 26°43.13'N; 82°11.46'W), 28 from St. John’s River Estuary near Jacksonville, Florida (northwestern Atlantic Ocean, 30°22.49'N; 81°34.14'W), and 12 from Indian River Lagoon near Melbourne, Florida (northwestern Atlantic Ocean, 28°5.52'N; 80°36.14'W); for sand seatrout (103 individuals total from Gulf of Mexico), 102 from Mississippi Sound off Biloxi (30°23.36'N; 88°51.74'W) and 1 from Tampa Bay (27°41.98'N; 82°37.82'W). The heart (bulbus arteriosus, ventricle, and atrium) and a gill arch of each euthanized seatrout were extracted whole and formalinfixed in the field or the freshly dead, iced fish were dissected and examined for the presence of parasites in the laboratory. During laboratory necropsy, the heart and a gill arch were extracted, placed in a petri dish, immersed in an anticoagulant solution of ~10.0 g sodium chloride and ~2.0 g citrated sodium chloride per liter of distilled water or a solution of full-strength sea water diluted with distilled water to ~8 ppt, and examined for the presence of aporocotylid adults and eggs with the aid of dissecting and compound microscopes, respectively.

Formalin-fixed adult flukes intended as stained, whole-mounted specimens, including those removed from the fish host alive and heat-killed as well as those extracted from formalin-fixed hearts, were rinsed thoroughly with distilled water, stained overnight in Van Cleave’s hematoxylin with several additional drops of Ehrlich’s hematoxylin, dehydrated in a graded series of ethanol to 70% ethanol, made basic with 70% ethanol containing lithium carbonate and butyl-amine, dehydrated further to 100% ethanol, exposed to several drops of xylene for 5 min, and subsequently cleared in clove oil for 1 hr before being permanently mounted in Canada balsam using a glass slide and coverslip. The adult specimen for scanning electron microscopy (SEM) was dehydrated, immersed in hexamethyldisilazane for 30 min, air-dried for 45 min, and sputter-coated with 15 nm of gold–palladium. Illustrations of stained, whole-mounted adult specimens as well as eggs observed in wet-mounted gill filaments were made with the aid of a Leica DM-2500 equipped with differential interference contrast (DIC) optical components and a drawing tube. Photographs of aporocotylid eggs were taken with a digital camera. Measurements of adults and eggs of *C. laruei* were obtained by using a calibrated ocular micrometer and are herein reported as the range in micrometers (μm) followed by the number of specimens measured and mean of measurements in parentheses.

Scientific names for fishes follow Eschmeyer (2010). We use the widely accepted “spotted seatrout” and “sand seatrout” (also commonly referred to in Mississippi as “white trout”) as common names for *C. nebulosus* and *C. arenarius*, respectively (Nelson et al., 2004), rather than the common names “spotted weakfish” and “sand weakfish” reported on FishBase (Froese and Pauly, 2010) because we have never encountered a fisherman or worker who calls these fishes “weakfish.” Higher-level fish classification and nomenclature follows Nelson (2006). Nomenclature for Aporocotylidae follows Bullard et al. (2009).

**RESULTS**

*Cardinola laruei* Short, 1953 (Figs. 2–16)

Redescription of adult and observations of egg and miracidium

Measurements and illustrations based on 16 stained, whole-mounted adult specimens, including newly collected specimens, vouchers, the holotype (United States National
Figures 2–4. Cardicola laruei Short, 1953 (Digenea: Aporocotylidae) from seatrouts (Cynoscion spp.) (Perciformes: Sciaenidae). 2. Body of adult, ventral view, composite illustration based mainly on voucher specimen (USNPC 104646) from heart of spotted seatrout, Cynoscion nebulosus (Cuvier, 1830), captured in Tampa Bay, Florida, U.S.A. (eastern Gulf of Mexico, 27°41.98'N; 82°37.82'W). 3. Genitalia of adult specimen in Figure 2. 4. Eggs from gill epithelium proximal to the afferent branchial arterioles of Cynoscion nebulosus captured in the St. John’s River Estuary near Jacksonville, Florida, U.S.A. (northwestern Atlantic Ocean, 30°22.49'N; 81°34.14'W). ac, anterior cecum; ae, anterior portion of esophagus; as, anterior sucker; au, ascending uterus; dco, distal constriction of oviduct; du, descending uterus; fp, female genital pore; me, metraterm; mg, Mehlis' gland; mp, male genital pore; o, ovary; oc, oviduct connection with ovary; oo, ootype; osr, oviducal seminal receptacle; pc, posterior cecum; pe, posterior portion of esophagus; sv, seminal vesicle; t, testis; tsr, ventrolateral rows of tegumental spines; vd, vas deferens; ve, vasa efferentia; vnc, ventral nerve commissure; vt, vitelline follicles/ducts.
States National Parasite Collection [USNPC] 37377), 2 paratypes (USNPC 37378–9), and 1 sputter-coated adult specimen:

- Body flat, ventrally concave, elongate oval in shape, 980–1,690 (14; 1,339) long, 220–340 (14; 274) wide or 3.1–7.7 (14; 5.0) times longer than wide, widest at level of cecal intersection, having anterior and posterior body ends equally rounded (i.e., neither end markedly more sharply pointed than the other), lacking posterolateral body protuberance (Fig. 2); body margin crimped ventrally or straight depending on disposition of specimen prior to fixation and coverslip pressure upon mounting specimen, slightly ventrally concave in living specimens, extensively spined (Figs. 2, 5–7, 12–16); tegumental body spines distributing in ventrolateral rows, not associating with peduncle or other tegumental protuberance, each spine having recurved distal tip, minute, 5–8 (7; 7) long, 1 (12; 1) wide at base (Figs. 5–7, 14, 16). Tegumental spine rows distributing along body margin for entire body length from level of mouth to extreme posterior body end, confluent posteriorly, discontinuous anteriorly in region of anterior sucker (Figs. 5–7), distributing in approximately 288–314 (5) rows per side of body or a total of approximately 576–628 (5) rows per specimen, 8–13 (15; 11) long and having 4 spines per row in anterior portion of body, 10–13 (15; 13) long and having 5 spines per row in middle portion of body, 8–13 (15; 10) long and having 4 spines per row in posterior portion of body, with number of spines per row increasing from anterior body end mediad, decreasing from medial part of body posteriad. Fused or rose-thorn–shaped spines lacking. Tegumental papillae not evident. Nervous system difficult to trace in most specimens, comprising at least paired ventrolateral nerve cords and associated anterior commissure; dorsolateral nerve cords and commissure not evident in whole-mounted specimens (Fig. 2). Ventrolateral nerve cords paired; each cord 13 (13; 13) wide near midbody at widest level, 43–58 (10; 49) from body margin at midbody, coursing near ceca, contiguous at body ends, becoming confluent with paired cord 25–33 (11; 29) or approximately 2% of body length from anterior body end, having secondary branches 5–15 (9; 11) wide at base and extending both laterad and mediad; commissure of ventrolateral nerve cord 95–143 (10; 113) or 6–11% of body length from anterior body end, 43–80 (10; 64) across width of worm, 10–23 (10; 18) in diameter, perpendicular to long axis of body, coursing dorsal to esophagus (Fig. 2).

Anterior sucker comprising a nearly indistinct spheroid structure centering on mouth, spinous (Figs. 2, 5–7, 12, 13, 15), 10–13 (12; 4) in diameter by SEM, directing ventrally (Figs. 13, 15); mouth a circular pore, approximately 1 in diameter, medioventral, posterior to concentric rows of minute spines distributing in anterior half of anterior sucker. Terminal preoral lobe absent. Pharynx absent. Esophagus 485–765 (13; 622) long or 45–52% of body length, slightly sinuous along entire length, having distinctive anterior and posterior portions; anterior portion comprising approximately 70% of esophagus length, 8–23 (8; 12) in maximum width between mouth and commissure of ventrolateral nerve cord, widening in posterior portion to 20–35 (11; 26) in maximum width, thick-walled at level of tips of anterior ceca; posterior portion of esophagus constricted, markedly more narrow than anterior portion, connecting to cecal intersection medioventrally, thin-walled (Fig. 2). Esophageal gland difficult

Figures 5–7. Anterior sucker of adult specimens of Cardicola laruei Short, 1953 (Digenea: Aporocotylidae) from heart of spotted seatrout, Cynoscion nebulosus (Cuvier, 1830), (Perciformes: Sciaenidae) captured in Tampa Bay, Florida, U.S.A. (eastern Gulf of Mexico, 27°41.98′N; 82°37.82′W). Light microscopy using differential interference contrast optical components. 5. Ventral view of adult specimen showing location of anterior sucker (As) concentric rows of spines (arrows), mouth (*), and tegumental spine rows (Tsr) on ventrolateral body surface. 6. Ventral view of another specimen showing same features. 7. Frontal view of anterior sucker showing same features.
to detect in whole-mounted specimens, comprising an opaque field of glandular cells surrounding posterior two thirds of anterior portion of esophagus, 80–118 (4; 97) maximum width at level of tips of anterior ceca, not extending lateral to ventrolateral nerve cords. Intestine fitting the so-called “H-shaped” or “X-shaped” configuration reported in the literature, having paired anterior and posterior ceca intersecting medially; intersection of anterior and posterior ceca 495–770 (14; 640) or 45–53% of body length from anterior end; anterior ceca 225–400 (8; 313) long or 21–27% of body length, 43–57% of esophagus length, 20–55 (9; 31) wide, with each cecum extending anteriad between esophagus and nerve cords, smooth, lacking diverticula; posterior ceca convoluted, extending posteriad medial to respective ventrolateral nerve cord, 250–490 (11; 387) long or 24–34% of body length, 0.92–1.48 times length of respective anterior cecum, 15–35 (11; 27) in maximum width at distal extremity (Fig. 2).

Testis irregular in shape, longer than wide, 173–320 (10; 248) long or 15–22% of body length, 93–148 (9; 121) in maximum width or 40–51% of body length at level of midbody, intercecal, not extending anterior to cecal intersection in any specimen, having border slightly irregular or occasionally slightly lobed but never branching, enclosing rod-like refractive processes typical of Cardicola spp. (see Bullard and Overstreet, 2004); posttesticular space 310–535 (14; 406) long or 27–33% of body length (Figs. 2, 3) Vasa efferentia difficult to trace in whole-mounted specimens, an interconnecting network of fine ducts entwining throughout testicular tissue, approximately 8–10 (7; 9) in diameter, extending primarily along ventral surface of testis, uniting in posterior margin of testis to form vas deferens; vas deferens 183–245 (5; 206) long, 7–14 (5; 10) wide in maximum width where vasa efferentia join, arching posterolaterally from posterior margin of testis before curving medially and narrowing to connect with seminal

vesicle; seminal vesicle comprising a thin-walled distal expansion of vas deferens, 93–120 (5; 108) long, 18–28 (5; 21) wide, directing posteriad and slightly laterad, containing sperm. Everted cirrus small, nipple-like, unarmed, everting dorsally at level of ventrolateral nerve cord or between sinistral ventrolateral nerve cord and body margin; cirrus sac indistinct (Figs. 2, 3).

Ovary medial, irregular in shape, with dextral portion extending slightly more posteriad than sinistral portion in most specimens, not branching, slightly lobed along margins, 103–190 (11; 145) long or 10–13% of body length, 108–158 (11; 132) wide or 41–58% of body width, 0.7–1.5 times longer than wide, medial, occupying space immediately posterior to testis, dorsal to vas deferens, intercecal and not extending laterally beyond level of ventrolateral nerve cords, containing refractive rod-like dorsoventral processes similar to those of testis; postovarian space 210–375 (14; 274) long or 17–23% of body length (Figs. 2, 3). Oviduct 303–368 (4; 334) in total length, 10 (4) wide in proximal portion immediately posterior to ovary, with proximal portion comprising a thin tube emanating from posterosinistral margin of ovary and extending laterally and posteriad, having conspicuous kink upon curving mediad and before expanding laterally to form oviducal seminal receptacle; oviducal seminal receptacle massive, diagonal, comprising middle portion of oviduct, elongate oval in shape, having a conspicuous kink at distal end, 155–225 (8; 186) long, 28–50 (8; 44) wide, residing between level of ovary and seminal vesicle; distal

portion of oviduct comprising a narrow tube recurving dorsolaterad before extending posteriad and connecting to ootype (Fig. 3). Vitellarium follicular, primarily filling space between level of alimentary tract and lateral body margin, extending lateral slightly beyond nerve cords, not extending far posteriad beyond level of distal tips of posterior ceca (Fig. 2); common collecting duct apparent in whole-mounted specimens medioventral to ovary, 193–248 (4; 225) in length from level of testis to distal end. Ootype 23–40 (7; 31) long, 13–18 (7; 16) wide, elongate or nearly spherical in shape, dextral, located well posterior to junction of vas deferens and seminal vesicle, at level of male genital pore (Figs. 2, 3). Uterus extending directly posterior from ootype 28–40 (8; 32) or 2–4% of body length, curving dorsally and anteriad before connecting to ascending portion of uterus; ascending portion of uterus extending 238–305 (8; 275) or 15–29% of body length anteriad before recurving twice medially and immediately posterior to oviducal seminal receptacle, postcecal, postgonadal, dorsal to seminal vesicle, curving dorsally before connecting with descending portion of uterus; descending portion of uterus a simple short duct connecting ascending uterus with metraterm, extending 38–78 (8; 54) or 2–8% of body length posteriad, 5–8 (6; 7) in maximum width; metraterm postcecal, postgonadal, 23–38 (8; 28) long, 13–23 (8; 16) in maximum width, having wall approximately 3 (4) thick. Probable egg anlagen residing in lumen of ascending uterus, 9–30 (2; 20) long, 10–11 (2; 11) wide, subspherical (Fig. 2). Female genital pore dorsal, sinistral, postvarian, anteromedial to male genital pore (Figs. 2, 3). Excretory system difficult to trace; bladder evident at level of posterior commissure of ventrolateral nerve cords (Fig. 2).

Egg (in gill of C. nebulosus) embedding in gill epithelium proximal to afferent branchial arterioles, spindle-shaped, 92–130 (3; 115) long, 12–19 (3; 16) in maximum width at middle or 6.8–7.6 times longer than wide, orienting approximately in parallel to long axis of gill filament (Fig. 4); smaller eggs having refractive granular material, lacking miracidium; larger eggs either vacant (hatched?) (Fig. 9) or enclosing miracidium (Fig. 10); miracidium within egg appearing to have ciliated tegument (although difficult to detect pattern of cilia on body surface), orienting in parallel with long axis of egg body, having terminal mouth, containing excretory concretions in middle of body and brownish-colored spheroid bodies in posterior body half (Figs. 10, 11); cilia most obvious about anterior body end.

**Taxonomic summary**

*Type host:* Sand seatrout, *Cynoscion arenarius* Ginsburg, 1930 (Perciformes: Sciaenidae).

*Other host:* Spotted seatrout, *Cynoscion nebulosus* (Cuvier, 1830).

*Sites in hosts:* Adults in heart lumen of sand seatrout and in “washings of the gut” of spotted seatrout; adults in ventricle and atrium of heart in sand seatrout and spotted seatrout (present study); eggs in gill epithelium proximal to afferent branchial arterioles of spotted seatrout (present study).

*Type locality:* Northern Gulf of Mexico off Florida panhandle, Alligator Harbor, Florida (north-central Gulf of Mexico, near 29°54.60’N; 84°23.71’W).

*Other localities:* Tampa Bay near Tampa, Florida (eastern Gulf of Mexico, 27°41.98’N; 82°37.82’W), and St. John’s River Estuary near Jacksonville, Florida (northwestern Atlantic Ocean, 30°22.49’N; 81°34.14’W).

*Prevalence and intensity of infection* (Fig. 1): Six of 58 (7%) spotted seatrout in Tampa Bay had 1 fluke each (6 of 125 [5%] spotted seatrout from the Gulf of Mexico, all from Tampa Bay, were infected); the only sand seatrout examined from Tampa Bay had 1 fluke (1 of 103 [1%] sand seatrout from the Gulf of Mexico were infected); 1 of 28 (4%) spotted seatrout in the St. John’s River Estuary had 1 fluke (1 of 40 [3%] spotted seatrout from the northwestern Atlantic Ocean were infected).

*Specimens of Cardicola laruei examined herein:* One stained whole-mounted adult specimen (holotype, USNPC 37377) plus 2 stained, whole-mounted adult specimens (paratypes, USNPC 37378–9) from the heart of sand seatrout captured in Alligator Harbor, Florida (north-central Gulf of Mexico, near 29°54.60’N; 84°23.71’W) (collected by R. Short during 3–17 July 1951); 5 stained, whole-mounted adult specimens on 1 slide (reputed to be part of Short’s original type series; donated to SAB by F. Nahhas) from an unspecified site in “trout” (host scientific name not specified on slide) captured in Alligator Harbor (north-central Gulf of Mexico, near 29°54.60’N; 84°23.71’W) (collected by R. Short during 3–17 July 1951); 2 stained, whole-mounted adult specimens from an unspecified site in sand seatrout captured in Apalachee Bay, Florida (north-central Gulf of Mexico, 29°59.18’N; 84°23.71’W); 5 stained, whole-mounted adult specimens from heart of spotted seatrout captured in Tampa Bay near...
Tampa, Florida (eastern Gulf of Mexico, 27°41.98′N; 82°37.82′W) (newly collected specimens derived from present study) (2 specimens deposited: USNPC 104646); 1 sputter-coated adult specimen for SEM from heart of spotted seatrout captured in Tampa Bay near Tampa, Florida (eastern Gulf of Mexico, 27°41.98′N; 82°37.82′W) (present study); 1 damaged adult specimen (not mounted) from heart of sand seatrout in Tampa Bay near Tampa, Florida (eastern Gulf of Mexico, 27°41.98′N; 82°37.82′W) (present study); 1 adult specimen from heart of spotted seatrout in St. John’s River Estuary near Jacksonville, Florida (northwestern Atlantic Ocean, 30°22.49′N; 81°34.14′W) (present study) (USNPC 104647).

Remarks

In most regards, the original description of *C. laruei*, which was based on 9 whole-mounted specimens (8 from heart of sand seatrout and 1 from gut washings of spotted seatrout) plus observations of a number of living specimens from the heart of sand seatrout, matched the type specimens we studied as well as our newly collected specimens. Some characters delineated by Short (1953) deserve emphasis because they likely represent generic features for aporocotylids whereas additional characters warrant discussion herein because our observations of them differ from Short’s (1953, original wording in italics). As Short (1953) indicated, “Margins of body recurved ventrally,” the single SEM specimen we studied clearly demonstrates that the lateral tegumental spine rows are directed ventrally by the body’s concavity (Figs. 12–16), probably allowing the fluke to insert the hooked spines into the endothelial lining of the heart for attachment and simultaneous locomotion (Bullard and Overstreet, 2003, 2004, 2008). Although the tegumental spine rows (Figs. 2, 5, 12–16) appear slightly submarginal in figure 3 of Short (1953), we concur that this likely comprises artifact because Short’s specimens were mounted under coverslip pressure. The position of spine rows, i.e., as lateral or ventral (submarginal), may be diagnostic for some aporocotylid genera (Bullard, 2010b). Of minor note also is that our SEM specimen had spines that protruded 6–8 μm, which is significantly more than “about 3 microns beyond body surface,” perhaps because the fluke’s tegument had contracted slightly during SEM processing and thereby revealed more of the spine than normally would be exposed in a living fluke (Figs. 14, 16). Although Short (1953) reported that there were “occasionally only two, or even a single spine representing a row,” we did not observe any row of 1 or 2 spines in the newly collected materials, and our spine counts returned a 4–5–4 pattern for spines per row from anterior to posterior. The holotype indeed has a few rows comprising a single spine. Hence, we suspect that, simply, and as is common among aporocotylids, some of Short’s specimens, e.g., the holotype, may have been deteriorated and lost spines during routine specimen processing.

Three of the newly collected adult specimens of *C. laruei* clearly have a spheroid anterior sucker bearing concentric rows of minute spines anterior to the ventral, subterminal mouth (Figs. 2, 5–7, 12, 13, 15). Short (1953) did not report these features, although he did note that the mouth was ventral, subterminal. The anterior sucker and its spination are vulnerable to fixation artifact and are best visualized in living specimens (Bullard, 2010a, b), and, in lieu of living specimens, reliable elucidation of this feature requires the study of well-fixed, heat-killed, specimens extracted immediately from fresh-dead fish. The sputter-coated specimen we studied using SEM was not handled in this way, which may explain why the minute spines in the sucker were not evident. Although the presence of a spheroid anterior sucker that bears spines is a potentially valuable generic feature for aporocotylids (Bullard and Overstreet, 2003; Nolan and Cribb, 2006), the spines of the anterior sucker and the anterior sucker itself were not evident in type materials of *C. laruei* but clearly evident in our smaller adult specimens only, which introduces the possibility that the anterior sucker spines become less obvious or are “lost” as the fluke grows. A study of living specimens of *C. laruei* across a range of adult sizes could help further elucidate this character. Further, we did not observe the “numerous tubercles on ventral surface of body,” and although Short (1953) included this characteristic in his description of *C. laruei*, he did not illustrate them. Such features have been described in SEM studies of other species of *Cardicola* (see Bullard and Overstreet, 2004).

We emphasize that esophagus shape is a potentially useful generic feature for aporocotylids, and Short (1953) insightfully noted that “posterior 1/5 to 1/4 of esophagus narrower than anterior part.” We confirmed this in our newly collected specimens (Fig. 2), and note that this is indeed a distinguishing feature among species of *Cardicola*. Also in regard to the esophagus, Short noted, “wider anterior portion possessing hair-like projections on inner surface and
surround by cells which are evidently glandular; narrower posterior part lacking gland cells and hair-like projections.” We observed that the esophageal lumen indeed appeared to possess a wispy quality, but we were unable to further differentiate this feature because the “hair-like projections” appear to be at the limits of light microscopy using DIC (×2,000 magnification = ×1,000 objective-ocular magnification with ×2 magnification changer). Published literature regarding aporocotylid digestion physiology is non-existent, but future ultrastructural studies of aporocotylid gut could not only document diet constituents and nutrient absorption in aporocotylids but serve to differentiate aporocotylid genera by revealing differences in the fine structure of the esophageal wall and associated features of the “esophageal gland,” whose functions are presently indeterminate.

Our observations largely confirm Short’s (1953) original observations of *C. laruei* regarding the female genitalia, especially the oviduct and uterus. Semantically, the “fusiform chamber for storage of spermatozoa” is herein referred to as an oviducal seminal receptacle. The track of the uterus, detailed by Short (1953) as “Uterus... with two loops to about level of midpoint of posterior margin of ovary,” appears useful for differentiating species of *Cardicola*. We herein emphasize that the relative lengths of the ascending and descending portions of the uterus as well as the number of curves of the uterus seem valuable as diagnostic characters among congeners and other members of Aporocotylidae. Further, we confirm the presence of a short, muscular metraterm. Regarding uterine eggs of *C. laruei*, Short (1953) stated, “uterus usually containing several thin-shelled eggs of rhomboidal, oval or spindle shape.” Although we did observe uterine contents that are likely to be egg anlagen (Figs. 2, 3), we did not observe “spindle-shaped” eggs in the uterus, only in the gill epithelium of the fish host (Figs. 4, 8–11).

Several aporocotylids are known to have both a ventrolateral and a dorsolateral pair of nerve cords, each having anterior commissures and becoming confluent posteriorly (Bullard and Overstreet, 2004; Bullard, 2010a, b). As detailed for other species of *Cardicola*, the nervous system of *C. laruei* comprises obvious ventrolateral nerve cords, detailed by Short (1953) as “Lateral nerve trunks conspicuous, joining posteriorly and united anteriorly by commissure dorsal to oesophagus.” However, perhaps attributable to the small size of this species, we were unable to locate the dorsolateral nerve cords or their anterior commissure in type and newly collected specimens.

Herein, for *C. laruei* we report the first description of a fully developed egg, i.e., one enclosing a ciliated miracidium (Figs. 4, 8–11). Although the shape of the fully developed egg of aporocotylids could be a useful generic feature (Bullard, personal observations), few have been described. We know of 4 species of *Cardicola* whose eggs have been photographed in wet-mounted gill filaments (not histologically sectioned), which is preferable to histological sections for showing the actual shape of the fully developed egg and allows the researcher to confirm the presence of the ciliated miracidium within the egg as well as the orientation of the egg in the gill tissue: (i) *Cardicola currani* Bullard and Overstreet, 2004, in red drum, *Sciaenops ocellatus* (Linnaeus, 1766) (Sciaenidae) in the Gulf of Mexico (Bullard and Overstreet, 2004); (ii) *Cardicola aurata* Holzer, Montero, Repullés, Nolan, Sitja-Bodabilla, Alvarez-Pellitero, Zarza, and Raga, 2008, from the “afferent gill vessels” of gilthead seabream, *Sparus aurata* Linnaeus, 1758 (Perciformes: Sparidae) in the Mediterranean Sea (Holzer et al., 2008); (iii) *Cardicola ambrosioi* Braicovich, Etchegoin, Timi, and Sardella, 2006, in Brazilian flathead, *Percophis brasiliensis* Quoy and Gaimard, 1825 (Perciformes: Percophidae) in the southwestern Atlantic Ocean (Braicovich et al., 2006); and (iv) *Cardicola orientalis* Ogawa, Tanaka, Sugihara, and Takami, 2010, in Pacific bluefin tuna, *Thunnus orientalis* (Temminck and Schlegel, 1844) (Perciformes: Scombridae) in the northwestern Pacific Ocean (Ogawa et al., 2010). In some species it appears that developing eggs are spheroid and lack miracidia, but at least in the case of *C. currani* the fully developed egg is spheroid (see figure 29 of Bullard and Overstreet, 2004). Interestingly, as a result, at least 2 putative species of *Cardicola* have markedly different egg morphologies: spheroid in *C. currani* and spindle-shaped in *C. laruei* (Figs. 3, 7–10). Regarding other *Cardicola* spp., the shape of the fully developed egg is unknown to us but certainly could help test the hypothesis that egg shape is a reliable generic character among aporocotylids; in which case the present taxonomy of *Cardicola* would require revision. Perhaps significant is that specimens of *C. currani* do not have a spheroid anterior sucker with spines, in addition to other morphological differences (Bullard and Overstreet, 2004). Moreover, of course it would be extremely informative to know the shape of the fully developed egg of *C. cardiocolum*, type species of *Cardicola* (see Manter, 1947), in the gill of its type host.
No aporocotylid has been reported from seatrout outside of the type locality for *C. laruei* (see Short, 1953), and to our knowledge, with exception to the present study, no specimen of *C. laruei* had been collected since Short’s work in the early 1950s (Fig. 1). The St. John’s River estuary near Jacksonville, Florida (northwestern Atlantic Ocean, 30°22.49′N; 81°34.14′W), is the first record of *C. laruei* in the Atlantic Ocean and only the second record of a species of *Cardicola* from the northwestern Atlantic Ocean. The previous report was that of *Cardicola forsteri* Cribb, Daintith, and Munday, 2000, in heart of northern bluefin tuna, *Thunnus thynnus* (Linnaeus, 1758), (Perciformes: Scombridae) from off North Carolina, U.S.A. (Bullard et al., 2004). Tampa Bay is a significant new geographic locality record for *C. laruei* in the Gulf of Mexico, extending the reported range of *C. laruei* to the eastern Gulf of Mexico and South Florida waters. Short (1953) documented infections of *C. laruei* in 13 of 16 (81%) sand seatrout and 2 of 8 (25%) spotted seatrout, with the number of flukes per infected host ranging from 1 to 34. In the present study, we found a total of only 8 specimens of *C. laruei* distributed evenly (1 specimen per infected seatrout) in 6 and 1 spotted seatrout in the Gulf of Mexico and Atlantic Ocean, respectively, and in one sand seatrout in the Gulf of Mexico. The prevalence of infection by *C. laruei* among spotted seatrout in Florida was 6 of 107 (6%) in the Gulf of Mexico and 1 of 40 (3%) in the Atlantic Ocean. Considering all examined fish herein, *C. laruei* had a mean prevalence in spotted and sand seatrouts (combined) of 7 of 228 (2%) in the Gulf of Mexico and 1 of 40 (3%) in the northwestern Atlantic Ocean. The type host for *C. laruei* is sand seatrout, and our collection efforts were heavily biased toward spotted seatrout in both the Gulf of Mexico and northwestern Atlantic Ocean off Florida because our samples primarily were derived from a fisheries independent monitoring program targeting spotted seatrout. However, our results indicate that *C. laruei* is at least rare or does not range off Mississippi (0 of 120 seatrouts infected) and that it is relatively common in Tampa Bay, Florida (6 of 58 [10%] seatrouts infected), although still well below the prevalence reported for *C. laruei* in sand seatrout in the type locality (Short, 1953) (Fig. 1).

**DISCUSSION**

Our results confirm that *C. laruei* infects the heart of two sympatric, congenic seatrouts (spotted and sand seatrouts) in the Gulf of Mexico off Florida (Short, 1953; Nahhas and Short, 1965; Overstreet, 1983). In addition to heart of the type host sand seatrout, Short’s (1953) original treatment of *C. laruei* reported a single specimen from the “washings of gut of *C. nebulosus*.” The present disposition of that specimen is indeterminate, and prior to the present study no other specimen of *C. laruei* was known from spotted seatrout. The level of host specificity exhibited by species of *Cardicola* is largely indeterminate because (i) few species have been collected since their original descriptions, (ii) no published survey has specifically documented the distribution of any species of *Cardicola* within a community of wild marine fishes, (iii) proportionally few workers seem to routinely search in fish blood or body cavity for the presence of aporocotylids, and, we speculate, (iv) negative parasitological data are probably underreported in the primary literature. Willing to report negative data, Bullard and Overstreet (2004) sampled 278 individual drums (Sciaenidae) of 10 genera and 13 species (including seatrouts) from Louisiana, Mississippi, and Texas, U.S.A., and failed to find an infection of *Cardicola palmeri* Bullard and Overstreet, 2004, or *C. currani* in any host other than the respective type hosts, i.e., black drum, *Pogonias cromis* (Linnaeus, 1766), (Perciformes: Sciaenidae) and red drum.

Although published host records for nominal species of *Cardicola* seem to indicate that most of these flukes are strictly host-specific to a single fish species, several *Cardicola* spp. do in fact infect phylogenetically closely related hosts (i.e., fishes of the same genus or family). In addition to *C. laruei*, which infects congenic seatrouts, several examples of one aporocotylid species infecting congenic fish hosts with overlapping geographic distributions exist. Yamaguti (1970) reported *Cardicola ahi* Yamaguti, 1970, from “gill washings” and “probably blood vessels” of 2 tunas (*Thunnus* spp.) from the central Pacific Ocean off Hawaii: yellowfin tuna, *Thunnus albacares* (as *Neothunnus macropterus*) (type host) and bigeye tuna, *Thunnus obesus* (as *Parathunnus sibi*). Another tuna aporocotylid, *Cardicola forsteri* Cribb, Daintith, and Munday, 2000, infects another 2 tunas: southern bluefin tuna, *Thunnus maccoyii* (Castelnau, 1872) (type host), from the southwest Pacific Ocean off Rabbit and Louth islands, Australia (Cribb et al., 2000) and northern bluefin tuna, *Thunnus thynnus* (Linnaeus, 1758), in the northwestern Atlantic Ocean off North Carolina (Bullard et al., 2004). Additionally, *C. forsteri* eggs in gill and adults in heart have been reported from northern bluefin...
tuna in the Adriatic Sea (Maldineo and Tudor, 2004; Bullard and Overstreet, 2008) and Mediterranean Sea (Aiken et al., 2006), respectively. Cardicola chaetodontis Yamaguti, 1970, infects 11 butterflyfishes (Chaetodon spp.): gill and heart of millet butterflyfish, Chaetodon miliaris Quoy and Gaimard, 1825 (type host), and bluestripe butterflyfish, Chaetodon fremblii Bennett, 1828, off Hawaii (Yamaguti, 1970) plus 9 butterflyfishes from the southwest Pacific Ocean off Australia (Nolan and Cribb, 2006). Cardicola nonamo Bullard, 2010, infects 2 surfperches (Embiotocidae) in the eastern Pacific Ocean off California: heart of white seaperch, Phanerodon furcatus Girard, 1854 (type host) and branchial vessels of rubberlip seaperch, Rhacochilus toxotes Agassiz, 1854 (Bullard, 2010a).

Although these records prove that the same species of Cardicola can infect multiple fish host species, “host specificity” nevertheless remains indeterminate for most marine fish aporocotylids, and for the vast majority of marine helminths for that matter. Host specificity assessments should involve sampling large numbers of individuals representing a variety of congeneric fishes within the geographic area of study. As an aside, host diet and parasite life cycle have been used to help explain the distribution and host specificity of trophically transmitted platyhelminths in the marine environment (Jensen and Bullard, 2010); however, unlike many digeneans and cestodes, aporocotylids reportedly are not transmitted via trophic linkages between intermediate (mollusk or polychaete) and definitive hosts (fish) (Martin, 1952; Oglesby, 1961; Smith, 1972; Køie, 1982; Køie and Peterson, 1988; Smith, 1997a). In this way, aporocotylids collectively may serve as useful models for studying factors that mediate definitive host specificity among non–trophically transmitted, endoparasitic platyhelminths in the aquatic environment.

The spotted seatrout is a highly valued commercial and recreational fish species in the Gulf of Mexico off Texas, Louisiana, Mississippi, Alabama, and Florida, U.S.A., as well as along the Atlantic coast of Florida (Johnson and Seaman, 1986; Brown-Peterson and Warren, 2001; Ward et al., 2007). Genetic studies on the population structure of spotted seatrout in the Gulf of Mexico and northwestern Atlantic Ocean have employed various tools and reported various results; however, independent lines of evidence derived from fish morphology, physiology, and molecular sequence data seemingly provide evidence of biologically significant regional differentiation (Ward et al., 2007) and, thus, genetically recognizable populations (MacKenzie, 2002; Lester and MacKenzie, 2009) of spotted seatrout. Supporting this notion, tagging studies strongly suggest that spotted seatrout are life-long residents of a single estuary and concomitantly move only short distances during their lives (Overstreet, 1983; Johnson and Seaman, 1986; Brown-Peterson and Warren, 2001). Ward et al. (2007) reported genetic differentiation of spotted seatrout by using allele frequencies and 5 microsatellite markers among spotted seatrout collected from the Laguna Madre (Gulf of Mexico) to the St. John’s River (Atlantic Ocean). Spotted seatrout from Florida waters were “strongly differentiated” from those of Texas and Louisiana; however, within Florida, spotted seatrout from the St. John’s River (Atlantic Ocean) were more similar to those from Charlotte Harbor (Gulf of Mexico) than to those of neighboring Tampa Bay (Gulf of Mexico) (Fig. 1). This result coupled with another study (Wiley and Chapman, 2003) suggested that two major distributional breaks affect the population structure of spotted seatrout in the Gulf–Atlantic region: the first distributional break is along the Atlantic Coast in the vicinity of the border of Georgia and Florida and the second one is along the Florida Gulf coast between Charlotte Harbor and Tampa Bay (Fig. 1).

Following the seminal works of Sindermann (1961), Kabata (1963), and Margolis (1965), numerous parasitologists have utilized ichthyoparasite survey data in the hopes of discriminating genetically defined groups of fish, i.e., a “self-reproducing population” or “stock” (Johnson and Seaman, 1986; Brown-Peterson and Warren, 2001; MacKenzie, 2002; Waldman, 2004; Ward et al., 2007; Lester et al., 2009). Although spotted seatrout have a wide geographic distribution and show evidence of population structure, to date no published study has examined the use of parasites as biological tags (or “stock markers”) of spotted seatrout populations in the Gulf of Mexico and Atlantic Ocean. Lester and MacKenzie (2009) strongly cautioned against the flippant use of ichthyoparasite diversity and prevalence data in fisheries management. Respecting the pitfalls of that practice, and because we sampled few fishes (i.e., 165 spotted seatrout total) for the presence of blood flukes, we can only propose that, with additional sampling, seatrouts in the Gulf–Atlantic region may comprise a fruitful model system with which to test concordance of ichthyoparasite data and fish population genetics. Given our understanding of the stock structure of spotted seatrout (see above), we
would expect an insignificant difference in prevalence of C. laruei among spotted seatrouts captured in Tampa Bay and in Mississippi Sound; however, we did not detect an infection in 102 sand seatrout and 18 spotted seatrout in Mississippi. The lack of reported infections there is noteworthy, especially near the Gulf Coast Research Laboratory (GCRL, Ocean Springs, Mississippi), because these seatrouts periodically have been examined in moderate to large numbers since the early 1970s. There is also the possibility that the stock structure of seatrout has nothing to do with parasite distribution. The required mollusk or polychaete intermediate host for C. laruei and the life span of C. laruei in its definitive fish host are both indeterminate; however, if the intermediate host’s geographic distribution is limited to the eastern Gulf of Mexico, one might expect that a group of susceptible seatrout having low vagility would exhibit significantly different prevalences of C. laruei regardless of fish stock genetic structure.

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