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NEW SPECIES OF *CARDICOLA* (DIGENEA: APOROCOTYLIDAE) FROM HEART OF ATLANTIC CROAKER, *MICROPOGONIAS UNDULATUS* (PERCIFORMES: SCIAENIDAE), OF THE SOUTH ATLANTIC BIGHT

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NEW SPECIES OF *CARDICOLA* (DIGENEA: APOROCOTYLIDAE) FROM HEART OF ATLANTIC CROAKER, *MICROPOGONIAS UNDULATUS* (PERCIFORMES: SCIAENIDAE), OF THE SOUTH ATLANTIC BIGHT

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ABSTRACT: *Cardicola parvus* n. sp. (Digenea: Aporocotylidae) infects the heart of Atlantic croaker, *Micropogonias undulatus* (Linnaeus, 1766) (Perciformes: Sciaenidae), in the South Atlantic Bight off Cow Island (34°38′49″N, 76°33′41″W, type locality) and Figure Eight Island (34°15′48″N, 77°44′27″W), North Carolina, USA, and off Jacksonville Beach (30°08′23″N, 81°20′52″W), Florida, USA. The new species is most easily differentiated from other members of *Cardicola* Short, 1953 by the combination of having a minute adult body (≤1 mm total length) that is 3.1–4.7× longer than wide, widely dispersed ventral tegumental sensory papillae, ~180 tegumental spine rows per side of body, a spheroid anterior sucker that is apparently aspinous, an esophagus that is 38–39% of the body total length, a male genital pore that is anterior to the ootype, a uterus that transitions from ascending to descending portions posterolaterally to the ovary, and a nearly transverse oviducal seminal receptacle. The new species is the second named aporocotylid from a littoral fish of the South Atlantic Bight and the fifth aporocotylid species reported from fishes of the northwestern Atlantic Ocean.

Bullard (2010a) and McVay et al. (2011) presented synoptical information on the diversity and distribution of fish blood flukes (Aporocotylidae Odhner, 1912) (Bullard et al., 2009) assigned to *Cardicola* Short, 1953. As the most species-rich marine and estuarine genus of Aporocotylidae, *Cardicola* probably requires taxonomic revision based on new collections of specimens comprising the type species, *Cardicola cardiocolum* (Manter, 1947) Short, 1953, and related species. Although infections by *Cardicola* spp. have on occasion been mentioned in biotic surveys of littoral fishes (e.g., Nahhas and Short, 1965; Overstreet, 1983; Thoney, 1993), aporocotylid taxonomic diversity among commonly encountered littoral fishes remains underestimated, and new species frequently are discovered subsequent to examinations of a large number of individual conspecific hosts across a range of sizes and ages (Bullard and Overstreet, 2004; Nolan and Cribb, 2006; Bullard and Jensen, 2008; Bullard et al., 2008; Bullard, 2010b; McVay et al., 2011).

Some oceanic regions remain strikingly underexplored for fish blood flukes when considering the biodiversity of potential definitive hosts in that region. For example, only 4 nominal (McIntosh, 1934; Ronald, 1960; Zubchenko, 1980, 1981; Bullard et al., 2004; McVay et al., 2011) and 3 unspecified (Bussieras and Baudin-Laurencin, 1973; Appy and Dadswell, 1979; Thoney, 1993; Bullard et al., 2008) aporocotylids in total reportedly infect 8 fish species in northwestern Atlantic Ocean. Based on the paucity of published records, the diversity of fish species ranging there, and the high level of host specificity exhibited by aporocotylids (Bullard and Overstreet, 2008; Cribb and Bray, 2011), many additional species probably range in the northwestern Atlantic Ocean.

Here, we describe a new species of *Cardicola* based on specimens collected from the heart of the commercially, and recreationally, valued Atlantic croaker, *Micropogonias undulatus* (Linnaeus, 1766) (Perciformes: Sciaenidae), in the south Atlantic Bight off North Carolina and Florida, USA.

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MATERIALS AND METHODS

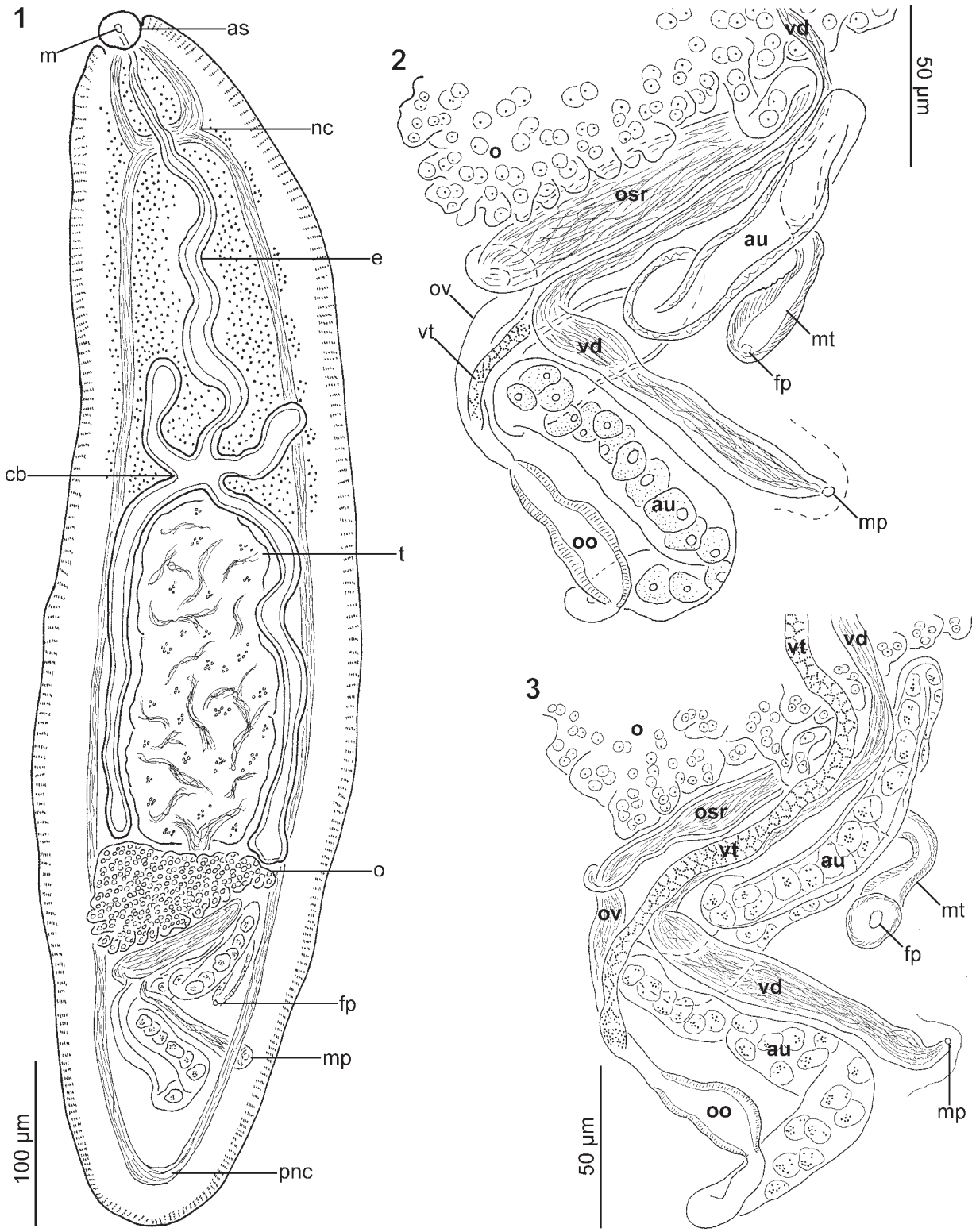
Croakers were captured off North Carolina and Florida during April 2003 by beach seine or trawl. After capture, fish were frozen or killed by spinal severance, placed in a cooler with a small amount of ice, and transported to the laboratory for necropsy. Whether thawed in 10% neutral buffered formalin or 70% ethanol, or examined fresh, the heart of each fish was extracted, placed in a petri dish, immersed in physiologic saline (Bullard, 2010b), bisected to expose its lumen, and examined with the aid of a dissecting microscope. Whole specimens were transferred to, and held in, a vial of 5% neutral buffered formalin, rinsed thoroughly with distilled water, stained overnight in Van Cleave's hematoxylin with several additional drops of Ehrlich's hematoxylin, made basic in 70% ethanol with lithium carbonate and butyl-amine, dehydrated, cleared in clove oil, and permanently mounted in Canada balsam. The specimens for scanning electron microscopy (SEM) were 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 specimens were made with the aid of a Leica DM-2500 microscope equipped with differential interference contrast optical components and a drawing tube. Measurements were obtained by using a calibrated ocular micrometer and are reported in micrometers followed by the number of specimens measured in parentheses. Common and scientific names for fishes follow Froese and Pauly (2010). Fish classification and nomenclature follows Nelson (2006). Nomenclature for Aporocotylidae follows Bullard et al. (2009). Brown (1956) was used to construct the specific epithet. Related aporocotylid specimens were borrowed from the U.S. National Parasite Collection (USNPC, Beltsville, Maryland).

DESCRIPTION

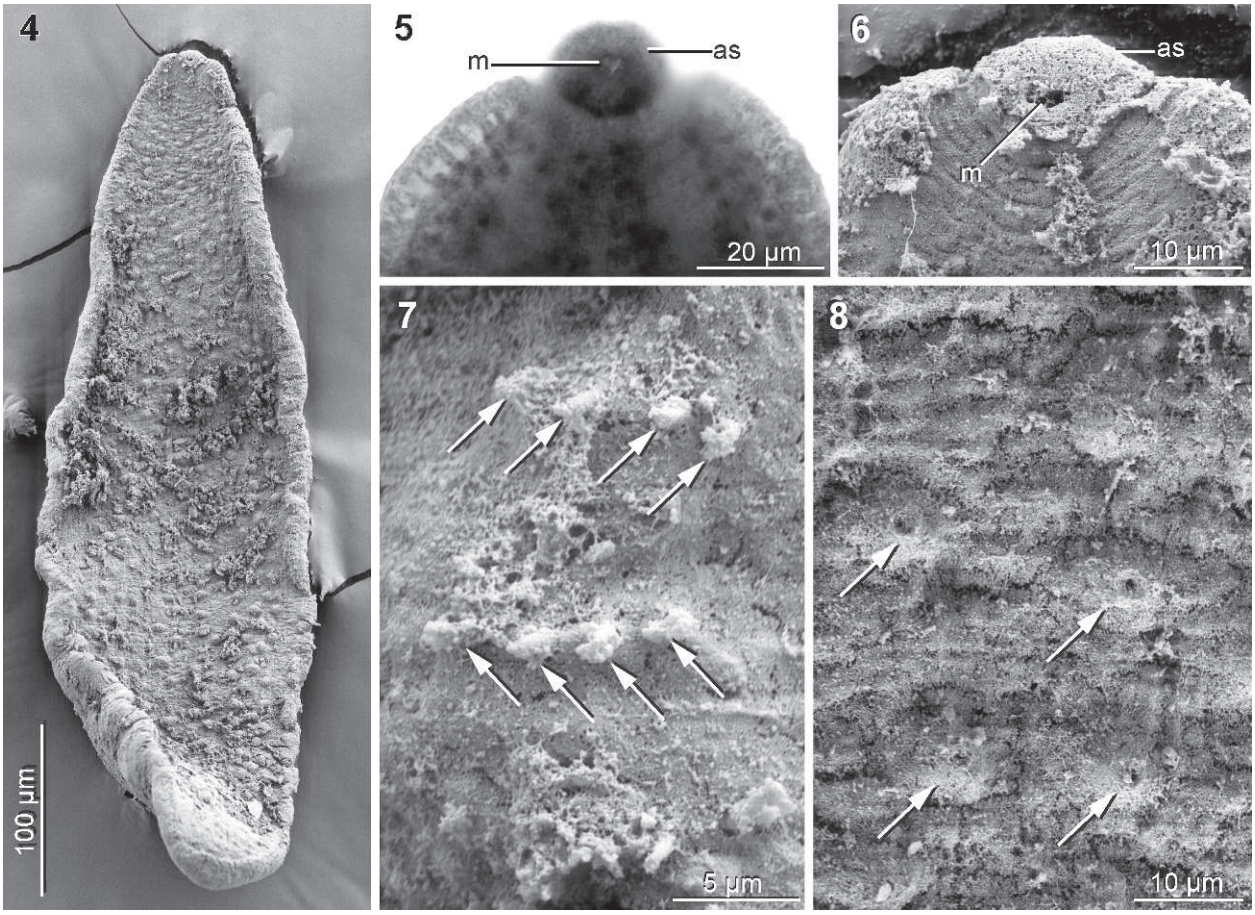
Cardicola parvus n. sp.

(Figs. 1–8)

Diagnosis of adult (measurements and illustrations based on 12 whole-mounted adult specimens including holotype and paratype plus 3 sputter-coated specimens for SEM): Body flat, ventrally concave, elongate oval in shape, 730–990 (11) long, 180–312 (11) or 3.1–4.7× longer than wide, having posterior end slightly more broadly rounded than anterior end, lacking posterolateral body protuberance (Figs. 1, 4); body margin crimped ventrally or straight, spined (Fig. 4); tegumental body spines in ventrolateral transverse rows, not associating with peduncles or protuberances, minute, protruding from tegument only slightly or covered by tegument (Figs. 1, 7), near limits of light microscopy. Tegumental spine rows distributing along ventrolateral body margin for entire body length from level of mouth to extreme posterior body end, distributing in approximately 180 rows per each side of body or a total of approximately 360 rows; spine rows approximately 13 (3) long in middle portion of body, with 5–7 spines per row (Figs. 1, 7). Fused, or rosethorn-shaped, spines lacking. Nervous system difficult to trace in most specimens, comprising at



FIGURES 1–3. *Cardicola parvus* n. sp. (Digenea: Aporocotylidae) from heart of Atlantic croaker, *Micropogonias undulatus* (Linnaeus, 1766) (Perciformes: Sciaenidae), in the South Atlantic Bight. Scale values aside each bar. (**1**) Body (holotype, USNPC 105072) showing mouth (m), anterior sucker (as), nerve commissure (nc), esophagus (e), cecal bifurcation (cb), testis (t), ovary (o), female pore (fp), male pore (mp), and posterior nerve commissure (pnc), ventral view. (**2**) Genitalia (holotype) showing vas deferens (vd), male genital pore (mp), ovary (o), oviducal seminal receptacle (osr), oviduct (ov), vitelline duct (vt), ootype (oo), ascending uterus (au), metraterm (mt), and female pore (fp), ventral view. (**3**) Genitalia (paratype USNPC 105073) showing comparable features as illustrated in Figure 2, ventral view.



FIGURES 4–8. *Cardicola parvus* n. sp. (Digenea: Aporocotylidae) from heart of Atlantic croaker, *Micropogonias undulatus* (Linnaeus, 1766) (Perciformes: Sciaenidae), in the South Atlantic Bight. Scale values aside each bar. (**4**) Body showing sensory papillae distributed about the ventral body surface, ventral view. (**5**) Anterior end showing location of mouth (m) in center of anterior sucker (as), ventral view. Note the lack of spines on sucker. (**6**) Anterior sucker (as) showing location of mouth (m), ventral view. Note the tegument covered by host debris, possibly obscuring the presence of minute spines of sucker. (**7**) Lateral body margin showing orientation of ventrolateral rows (arrows) of spines that protrude from the ventral body surface, lateral view. (**8**) High-magnification view of ventral sensory papillae (arrows) that lack sensory cilia, ventral view.

least a ventrolateral nerve cord plus associated anterior commissure (Fig. 1). Ventrolateral nerve cords paired (Fig. 1); each cord 5–8 (3) wide near midbody at widest level, 30–55 (3) from body margin at midbody, contiguous at body ends, becoming confluent with paired cord 25–30 (3) or 3% of body length from posterior body end; secondary branches and dorsolateral nerve cords not evident (Fig. 1); commissure of ventrolateral nerve cord 80–110 (3) or 11% of body length from anterior body end, 30–38 (3) across width of worm, 7–8 (3) in diameter, perpendicular to long axis of body, coursing dorsal to esophagus (Fig. 1). Ventral tegumental sensory papillae present, approximately 10 in diameter, dispersing over ventral body surface, apparently lacking cilia but having a central pore (Figs. 4, 8). Dorsal tegumental sensory papillae not evident with light or scanning electron microscopy.

Anterior sucker aspinous, comprising a nearly indistinct spheroid structure centering on mouth, 20–23 (3) wide or 6–11% of body width, directing ventrally; mouth a circular pore, 3 (3) in diameter, medioventral, 7–10 (3) from anterior end (Figs. 1, 5, 6). Terminal pre-oral lobe not evident with light microscopy. Pharynx absent. Esophagus 300–393 (5) long or 38–39% of body length, slightly sinuous, widening slightly posteriorly before connecting with cecal bifurcation anteroventrally; esophageal wall thickening slightly from 1–3 (3) near mouth to 5–8 (3) near cecal bifurcation. Esophageal gland extremely diffuse and difficult to distinguish from surrounding parenchyma, appearing to envelop esophagus as much as total length of esophagus, concentrating in area approximately 50–90 wide or 24–29% of body width, not clearly differentiated into anterior and posterior portions probably due to tissue fixation artifact. Intestine X- or H-shaped (Fig. 1), with paired anterior and posterior ceca intersecting medially; intersection of anterior and posterior ceca 300–390 (5) or 38–39% of body length from anterior end (Fig. 1); anterior ceca about equal in length, 50–110 (8) long or 6–14% of body length, 19–23% of esophagus length, 10–30 (3) wide, with each cecum extending anteriad between esophageal gland and ventrolateral nerve cord, smooth, lacking diverticula, containing material within lumen; material granular, dense, brownish-yellow, evenly filling lumen of ceca; posterior ceca not extensively convoluted, approximately equal in length, 190–310 (8) long or 24–33% of body length, 2.2–5.8× length of anterior ceca, each 10–20 (8) wide (Fig. 1).

Genitalia not extending far beyond ventrolateral nerve cords (Fig. 1). Testis approximately rectangular in shape, 190–290 (11) long or 23–30% of body length, 60–120 (11) in maximum width or 33–53% of body width at level of midbody, 0.3–0.5 × wider than long, intercecal, between cecal intersection and distal tips of posterior ceca, with border slightly irregular; posttesticular space 220–340 (11) long or 26–38% of body length. Vasa efferentia difficult to trace in fixed specimens, an interconnecting meshwork of fine ducts entwining throughout testicular tissue, containing spermatozoa, extending primarily along ventral surface of testis, uniting in posterior region of testis to form vas deferens (Fig. 1); vas deferens 125–163 (3) long, 5 (3) wide, extending posteriad from posterior margin of testis, passing ventral and sinistrally to ovary, curving mediad and crossing midline before curving and directing sinistrally, extremely thin-walled, containing sperm in all specimens (Figs. 2, 3). Seminal vesicle oblong, 75–105 (3) long, 13–30 (3) wide or 3.5–6.2× longer than wide, orienting diagonally posteriad. Gland surrounding seminal vesicle indistinct. Everted cirrus small, nipple-like, unarmed, 13 (1) long or 17%

of seminal vesicle length, approximately 8 wide, approximately 1.6× longer than wide, everting dorsally between sinistral ventrolateral nerve cord and body margin; cirrus sac indistinct (Figs. 2, 3).

Ovary medial, not branching or deeply lobed, but having small marginal lobes (Figs. 2, 3), 60–110 (11) long or 8–13% of body length, 70–125 (11) wide or 28–58% of body width, 0.7–1.6× wider than long, medial, occupying space immediately posterior to testis, dorsal to vas deferens, not extending laterally far beyond level of ventrolateral nerve cords, typically extending farther posteriad in dextral half and giving ovary shape of a right triangle (Fig. 1). Postovarian space 160–250 long or 21–30% of body length (Fig. 1). Oviduct extending directly posteriad from posterosinistral margin of ovary, curving dorsally, 5–8 (3) wide, connecting with oviducal seminal receptacle immediately posterior to ovary; oviducal seminal receptacle comprising the proximal portion of the oviduct that is kinked proximally and distally, 90–113 (3) long, 8–20 (3) wide, nearly transverse (extending laterally across body), medial, residing between level of ovary and proximal portion of seminal vesicle, filled with sperm in all specimens (Figs. 1–3); distal portion of oviduct continuing posteriad approximately in parallel with dextral body margin before connecting with ootype (Figs. 1–3). Vitellarium primarily indistinct in our specimens probably due to fixation artifact, evident in anterior body region between and slightly anterior to level of nerve commissure and cecal intersection, observed as extending slightly lateral to ventrolateral nerve cords in some specimens (Fig. 1); secondary collecting ducts indistinct; common collecting duct primarily indistinct in region anterior to ovary, passing ventral to ovary and lateral to oviducal seminal receptacle before extending ventrally along track of vas deferens to unite with oviduct and ootype. Ootype 28–30 (3) long, 10–23 (3) wide, oblong, dextral, anterior to male genital pore, located posterior to level of junction of vas deferens and seminal vesicle, located posterior to or at level of male genital pore and posterior to level of female genital pores (Figs. 2, 3); postootype distance 90–133 (3) or 9–18% of body length (Figs. 1–3). Mehlis' gland indistinct. Uterus running slightly posteriad or laterad from ootype before curving to join ascending uterus; ascending uterus extending 125–200 (3) anteriad before curving several times, 18–20 (3) in maximum width at midlevel of seminal vesicle, primarily occupying space between ootype and ovary, generally at level of seminal vesicle, postcecal, posttesticular, primarily postovarian; transition from ascending to descending uterus not overlapping or anterior to level of ovary; descending uterus a short tube connecting with metraterm, having thickened uterine wall relative to ascending uterus, 68–88 (3) long or 0.4–0.5× length of descending uterus, 10–15 (3) wide or 0.8× width of descending uterus, connecting with proximal portion of metraterm at level of oviducal seminal receptacle and well anterior to level of ootype; metraterm, 32–40 (3) long, 11–17 (3) wide or 2.4–3.1× longer than wide, sinistral to ascending uterus, with wall 2–4 (3) thick (Figs. 2, 3). Uterine eggs lacking thick shell, 7–8 (2) in diameter, filling lumens of ascending and descending uterus (Figs. 2, 3). Female genital pore dorsal, sinistral, postovarian, anteromedial to male genital pore, 138–185 (3) from posterior body end. Excretory system indistinct.

Taxonomic summary

Type and only known host: Micropogonias undulatus (Linnaeus, 1766) (Perciformes: Sciaenidae), the Atlantic croaker.

Sites in host: Lumen of heart.

Type locality: South Atlantic Bight off Cow Island (34°38′49″N, 76°33′41″W), North Carolina.

Other localities: South Atlantic Bight off Figure Eight Island (34°15′48″N, 77°44′27″W), North Carolina, and Jacksonville Beach (30°08′23″N, 81°20′52″W), Florida.

Prevalence and mean intensity of infection: Fifteen of 488 (3.1%) Atlantic croaker had 1–5 flukes each (mean intensity = 1.8 ± 0.5).

Specimen deposited: Holotype USNPC 105072; paratype USNPC 105073-4.

Etymology: The specific Latin epithet *parvus* means little and refers to the small size of the adult fluke.

Remarks

Cardicola is a taxonomically problematic genus that needs careful revision (Bullard, 2010a; McVay et al., 2011). The largest hurdle to accomplishing this task is the redetermination of the morphological features of the type species, *Cardicola cardiocolum* (Manter, 1947) Short, 1953. We provisionally assign the new species to *Cardicola*.

Cardicola parvus n. sp. differs from morphologically similar congeners by the combination of having an adult whose body is minute (total body length of adult ≤1 mm) and 3.1–4.7× longer than wide, a spheroid and evidently aspinous anterior sucker, an esophagus that is 38–39% of the body length, a male genital pore that is anterior to the ootype, a uterus that transitions from ascending to descending portions posterolaterally to the ovary, and a nearly transverse oviducal seminal receptacle (Figs. 1–4).

Bullard (2010a) regarded *Cardicola congruenta* Lebedev and Mamaev, 1968; *Cardicola grandis* Yamaguti, 1970; and *Cardicola ahi* Yamaguti, 1970 as incertae sedis, but they are most easily differentiated from the new species by having a more elongated body that is 6–10× longer than wide. The *Cardicola* spp. that infect siganids and a lutjanid off Australia (*Cardicola milleri* Nolan and Cribb, 2006; *Cardicola coeptus* Nolan and Cribb, 2006; *Cardicola covacinae* Nolan and Cribb, 2006; *Cardicola bartoli* Nolan and Cribb, 2006; *Cardicola watsonensis* Nolan and Cribb, 2006; *Cardicola lafii* Nolan and Cribb, 2006; *Cardicola parilus* Nolan and Cribb, 2006; and *Cardicola tantabiddii* Nolan and Cribb, 2006) can be differentiated from the new species by having a relatively long esophagus that is 40–50% of total body length. The male genital pore is posterior to the ootype in *Cardicola cardiocolum* (Manter, 1947) Short, 1953 (type species); *Cardicola laruei* Short, 1953; *Cardicola coridodacis* Manter, 1954; *Cardicola congruenta* Lebedev and Mamaev, 1968; *Cardicola grandis* Lebedev and Mamaev; 1968, *Cardicola kurochkini* (Parukhin, 1976) Bullard and Overstreet, 2006 (incertae sedis); *Cardicola brasiliensis* Knoff and Amato, 1992; *Cardicola forsteri* Cribb, Daintith, and Munday, 2000; *Cardicola palmeri* Bullard and Overstreet, 2004; *Cardicola currani* Bullard and Overstreet, 2004; *Cardicola ambrosioi* Braichovich, Etchegoin, Timi, and Sardella, 2006; *Cardicola aurata* Holzer, Montero, Reulles, Nolan, Sitja-Bobadilla, Alvarez-Pellitero, Zarza, and Raga, 2008; *Cardicola orientalis* Ogawa, Tanaka, Sugihara, and Takami, 2010; and *Cardicola nonamo* Bullard, 2010, easily differentiating them from *C. parvus* n. sp. *Cardicola whitteni* Manter, 1954 differs from the new species by having an unusual uterus that extends lateral and anterior to the level of the ovary, whereas all other nominal species and the new species possess a uterus that does not extend anterior to the ovary's anterior margin. The specimens of *C. parvus* that we studied had a spheroid anterior sucker that lacked spines; however, we suspect spines are present in well preserved (heat-killed, formalin-fixed) specimens. Some of our specimens were frozen, thawed, and subsequently fixed in 70% ethanol, which may have caused some anterior sucker spines to become detached. These spines can be easily dislodged in poorly fixed or degrading specimens (Bullard, 2010a; McVay et al., 2011).

DISCUSSION

In total, only 4 nominal species of fish blood flukes have been reported from fishes captured in the northwestern Atlantic Ocean: (1) *Aporocotyle simplex* Odhner, 1900 from witch flounder, *Glyptocephalus cynoglossus* (Linnaeus, 1758) (Pleuronectiformes: Pleuronectidae); roundnose grenadier, *Coryphaenoides rupestris* Gunnerus, 1765 (Gadiformes: Macrouridae); and American plaice, *Hippoglossoides platessoides* (Fabricius, 1780) (Pleuronectiformes: Pleuronectidae) (see Ronald, 1960; Zubchenko, 1980l, 1981); (2) *Paradeontacylix sanguinicoloides* McIntosh, 1934 from greater amberjack, *Seriola lalandi* Valenciennes, 1833 (Perciformes: Carangidae) (see McIntosh, 1934); (3) *Cardicola forsteri* Cribb, Daintith, and Munday, 2000 (Perciformes: Scombridae) from *Thunnus thynnus* (see Bullard et al., 2004); and (4) *Cardicola laruei* Short, 1953 from the heart of spotted seatrout, *Cynoscion nebulosus* (Cuvier, 1830) (Perciformes: Sciaenidae), in the St. John River Estuary, Florida (McVay et al., 2011). In addition to an unspecified infection in *M. undulatus*, probably *C. parvus* (see Thoney, 1993), off Cape Hatteras, North Carolina, 2 other unspecified infections of *Cardicola* spp. have been reported from the northwestern Atlantic Ocean: *Acipensericola* sp. from Atlantic sturgeon, *Acipenser brevirostrum* Lesueur, 1818 (Acipenseriformes:

Acipenseridae) in St. John River estuary, Florida (Appy and Dadswell, 1979; Bullard et al., 2008); and *Cardicola* sp. from *Thunnus albacares* in the mid-Atlantic Ocean (Bussieras and Baudin-Laurencin, 1973).

Cardicola parvus may comprise an accurate biological tag that distinguishes stocks of Atlantic croaker in the northwestern Atlantic Ocean and Gulf of Mexico, respectively. Lankford et al. (1999) reported that there was significant genetic heterogeneity between Atlantic Ocean and Gulf of Mexico samples of Atlantic croaker, suggesting a restricted gene flow between Atlantic croaker in the 2 regions. Their findings were consistent with the notion of a single Atlantic croaker stock (challenged by Baker et al. [2007], who used parasitological data to delineate 2 stocks of Atlantic croaker in the northwestern Atlantic Ocean), as well as the existence of a weakly differentiated Gulf of Mexico stock. Qualitatively and, despite examinations of a combined several hundred croaker from the Gulf of Mexico by Ronnie Palmer and Robin M. Overstreet (both Gulf Coast Research Laboratory; R. Overstreet, pers. comm), and one of us (S.A.B.), no blood fluke has been collected to date. The life cycle of *C. parvus* is unknown, but the absence, or low prevalence, of infections among Atlantic croaker in the Gulf of Mexico may simply be attributable to the lack of an appropriate intermediate host for the new species. Future faunal surveys of Atlantic croaker parasites in the Gulf of Mexico should include careful examinations of the vascular system to detect this, or other, flukes infecting the blood vascular systems.

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