

BLOOD FLUKES (DIGENEA: APOROCOTYLIDAE) OF STINGRAYS (MYLIOBATIFORMES: DASYATIDAE): *ORCHISPIRIUM HETEROVITELLATUM* FROM *HIMANTURA IMBRICATA* IN THE BAY OF BENGAL AND A NEW GENUS AND SPECIES OF APOROCOTYLIDAE FROM *DASYATIS SABINA* IN THE NORTHERN GULF OF MEXICO

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ABSTRACT: We redescribe *Orchispirium heterovitellatum* based on the holotype and 3 original voucher specimens collected from the mesenteric blood vessels of scaly whiprays *Himantura imbricata* (Bloch and Schneider, 1801) (as *Dasyatis imbricatus*) captured in the western Bay of Bengal off Waltair, India. We amend the diagnosis of *Orchispirium* to include anterior sucker present, testis looping, cirrus sac enveloping large internal seminal vesicle, oviducal seminal receptacle present, and metraterm short and thin-walled. We describe *Myliobaticola richardheardi* n. gen., n. sp. based on live observations, light microscopy, and scanning electron microscopy of adult specimens collected from between the cardiac trabeculae of Atlantic stingrays *Dasyatis sabina* (Lesueur, 1824) captured in Mississippi Sound (type locality), Mississippi, and Apalachicola Bay, Florida. The new species has a minute, aspinous body lacking lateral tubercles; an aspinous and eversible anterior sucker lacking a peduncle; a posterior esophageal swelling; an inverse U-shaped intestine; smooth ceca terminating in the anterior body half; a looping testis lacking lobes; a cirrus sac enveloping a large internal seminal vesicle; a medial and primarily post-testicular ovary; an oviducal seminal receptacle; a postgonadal uterus flanking the internal seminal vesicle; a short and thin-walled metraterm; and a common genital pore. It lacks a pharynx and Laurer's canal. No other named aporocotylids infect a member of cohort Batoidea or have the combination of an aspinous body, an aspinous anterior sucker, a posterior esophageal swelling, an inverse U-shaped intestine, a looping testis, a cirrus sac enveloping a large internal seminal vesicle, and a common genital pore; these observations indicate that *O. heterovitellatum* and *M. richardheardi* are closely related. The discovery of a second species representing a second genus of Aporocotylidae in diamond stingrays (Dasyatidae) suggests that Batoidea is an undersampled host group for aporocotylid infections.

A total of only 5 species of fish blood flukes (Aporocotylidae Odhner, 1912) has been named and described from the estimated 970 extant species belonging to the craniate class Chondrichthyes (see Smith, 1997; Bullard et al., 2006; Nelson, 2006): (1) *Selachohemecus olsoni* Short, 1954, from the heart of the Atlantic sharpnose shark *Rhizoprionodon terraenovae* (Richardson, 1836) (as *Scoliodon terra-novae*) in the Gulf of Mexico (Short, 1954; Bullard et al., 2006); (2) *Selachohemecus benzi* Bullard, Overstreet, and Carlson, 2006, from the heart and kidney of the blacktip shark *Carcharhinus limbatus* (Müller and Henle, 1839) in the Gulf of Mexico (Bullard et al., 2006); (3) *Hyperandrotrema cetorhini* Maillard and Ktari, 1978, from the blood vascular system and heart of the basking shark *Cetorhinus maximus* (Gunnerus, 1765) in the Mediterranean Sea and North Sea (Smith, 1972; Maillard and Ktari, 1978); (4) *Chimaerohemecus trondheimensis* van der Land, 1967, from the dorsal aorta of the rabbit fish *Chimaera monstrosa* Linnaeus, 1758 (type host) in the North Sea (van der Land, 1967; Lockyer et al., 2003) and from the dorsal aorta and postcardinal vein around the kidney of the spookfish *Hydrolagus mitsukurii* (Jordan and Snyder, 1904) in the Western Pacific Ocean (Kamegai et al., 2002; see Karlsbakk et al. 2002 for a record of *Chimaerohemecus* sp. from the atrial trabeculae of the smalleyed rabbitfish *Hydrolagus affinis* (de Brito Capello, 1868) in the North Atlantic Ocean); and (5) *Orchispirium heterovitellatum* Madhavi and Hanumantha Rao, 1970, from the mesenteric blood vessels of the scaly whipray *Himantura imbricata* (Bloch and Schneider, 1801) (as *Dasyatis imbricatus*) in the Bay of Bengal (Madhavi and Hanumantha Rao, 1970, 1971). With the excep-

tion of *O. heterovitellatum* (see Madhavi and Hanumantha Rao, 1971) and *S. olsoni* (see Bullard et al., 2006), the published accounts for each of these aporocotylids are limited to original species descriptions and subsequent parasitological surveys; nothing has been published on the life history, pathology, growth, or diet of these flukes. In fact, only 1 publication focused on a nontaxonomic aspect, i.e., the chemical nature of the eggshell of *O. heterovitellatum* (see Madhavi and Hanumantha Rao, 1971). Herein, we redescribe *O. heterovitellatum* based on the holotype and 3 original voucher specimens and compare that species with a new aporocotylid, for which we also propose a new genus, from the heart of the Atlantic stingray *Dasyatis sabina* (Lesueur, 1824) in the Gulf of Mexico.

MATERIALS AND METHODS

The original voucher specimens of *O. heterovitellatum* we studied herein were collected on 11 September 1967 from the western Bay of Bengal off Waltair, India, by R. Madhavi and K. Hanumantha Rao and subsequently loaned to SAB by R. Madhavi. Batoids were captured by otter trawl, speargun, stick spear, Hawaiian sling, hook-and-line, seine, and dip net in Mississippi Sound (off Deer Island [30°22'22"N, 88°50'00"W], Horn Island [30°15'04"N, 88°42'42"W], and Ship Island [30°14'29"N, 88°53'05"W]), in Apalachicola Bay (29°54'54"N, 84°20'06"W), Florida, and in the north-central Gulf of Mexico (29°04'35"N, 84°26'56"W and adjacent waters) during July 1999–September 2007. Most batoids were killed by spinal severance in the field and kept on ice, or they were transported back to the laboratory and killed by exposure to MS-222. To obtain healthy, adult aporocotylids for live observations, several Atlantic stingrays were captured with hand nets, placed in a cooler containing seawater, transported to the laboratory, and maintained alive until necropsy. The heart of each stingray was extracted, placed in a Petri dish, bisected to expose its lumen, immersed in an anticoagulant solution of ~10.0 g NaCl and ~2.0 g NaCl-citrate per liter of distilled water or a solution of full strength seawater diluted with distilled water to 12 ppt, and examined with the aid of a dissecting microscope. After that initial examination, the myocardium was teased apart with fine forceps to expose flukes. Observations of living flukes were made with the aid of a compound microscope

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at $\times 400$ – $1,000$ magnification immediately after the host was killed. Flukes intended as stained, whole mounted specimens were killed with heat from an ethanol-burner flame under little or no coverslip pressure, transferred to and held in a vial of 5% neutral buffered formalin for at least 48 hr, rinsed thoroughly with distilled water, stained overnight in Van Cleave's hematoxylin with several additional drops of Ehrlich's hematoxylin, made basic at 70% ethanol with lithium carbonate and butyl-amine, dehydrated, cleared in clove oil, and mounted in Canada balsam. The 2 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. Drawings were made with the aid of a drawing tube and facilitated by differential interference contrast optical components. Measurements are reported in micrometers (μm) and given as ranges with the sample size in parentheses. Ordinal names and assignments of families and genera within Batoidea follow McEachran and Aschliman (2004) and Nelson (2006), and common names for host species follow Froese and Pauly (2006).

DESCRIPTIONS

Orchispirium Madhavi and Hanumantha Rao, 1970

(Figs. 1–12)

Diagnosis: Body flat, ventrally concave, elongate, 3–4 times longer than wide, aspinous; lateral tubercles distributing along anterolateral body margin, unevenly spaced, directing laterad, papilla-like, i.e., unlike peduncles of *Selachohemecus* Short, 1954. Rosethorn-shaped spines absent. Ventrolateral nerve cords present. Anterior sucker aspinous, lacking peduncle, diminutive, centered on mouth; mouth ventral, subterminal. Pharynx absent. Esophagus medial, extending sinuously posteriad approximately $\frac{1}{3}$ body length; esophageal gland enveloping esophagus from level of anterior nerve commissure to posterior end of esophagus; posterior esophageal swelling medial, comprising a laterally expanded and bulbous portion of esophagus. Intestine inverse U-shaped, with long posterior ceca only; posterior ceca may bear diverticula flanking cecal bifurcation, terminating in middle $\frac{1}{3}$ of body, straight or slightly sinuous. Testis single, looping extensively, crossing midline repeatedly, having smooth anterior margins and lobed posterior margins; testicular field 2–3 times longer than wide, not extending laterad beyond posterior ceca, not extending anteriorly beyond level of cecal bifurcation. Vas deferens short, medial, extending posteriad from testis; internal seminal vesicle longer than vas deferens, enveloped by cirrus sac. Auxiliary external seminal vesicle absent. Cirrus appendix-like. Ovary single, dextral, lobed, post-cecal, post-testicular, adjacent to vas deferens, occupying posterior $\frac{1}{4}$ of body. Vitellarium asymmetrical, dendritic, having follicles extending posteriad in dextral half of body, an extensive network of narrow interconnecting branching bands having granular vitelline material distributing among genitalia and alimentary tract. Primary vitelline duct dextral, ventral to ovary, extending directly posteriad and in parallel with dextral body margin before connecting to oviduct, lacking distally expanded vitelline reservoir. Oviduct dextral, a narrow duct extending posteriad from ventromedial surface of ovary; oviducal seminal receptacle present, comprising proximal portion of oviduct ventral to ovary. Laurer's canal absent. Ootype spherical, post-cecal, post-gonadal. Uterus post-testicular, flanking internal seminal vesicle; proximal portion of ascending uterus may contain sperm and serve as seminal receptacle; uterine eggs oblong, vacuous, having membranous shell. Metraterm short, thin-walled. Male and female reproductive tracts sharing common atrium and pore, lacking posterolateral protuberance of body associated with pore; pore dorsal, sinistral, post-gonadal, post-cecal. Excretory vesicle Y-shaped, thin-walled; excretory pore dorsal, subterminal. In mesenteric blood vessels of myliobatiforms.

Differential diagnosis: Body 3–4 times longer than wide, aspinous; lateral tubercles distributing along anterolateral body margin. Anterior sucker aspinous, lacking peduncle. Pharynx absent. Posterior esophageal swelling present. Intestine inverse U-shaped. Testis looping extensively. Vas deferens extending posteriad from testis; internal seminal vesicle longer than vas deferens, enveloped by cirrus sac. Auxiliary external seminal vesicle absent. Ovary single, dextral, lobed, post-cecal, post-testicular. Vitellarium asymmetrical, dendritic, having follicles extending posteriad in dextral half of body. Primary vitelline duct lacking distally expanded vitelline reservoir. Oviducal seminal receptacle comprising proximal portion of oviduct ventral to ovary. Laurer's canal absent. Ootype spherical, post-cecal, post-gonadal. Uterus post-testicu-

lar, flanking internal seminal vesicle; uterine eggs oblong, vacuous, having membranous shell. Metraterm short, thin-walled. Male and female reproductive tracts sharing common atrium and pore, lacking posterolateral protuberance of body associated with pore; pore dorsal, sinistral, post-gonadal, post-cecal. Excretory pore dorsal, subterminal.

Type and only nominal species: *Orchispirium heterovitellatum* Madhavi and Hanumantha Rao, 1970.

Remarks

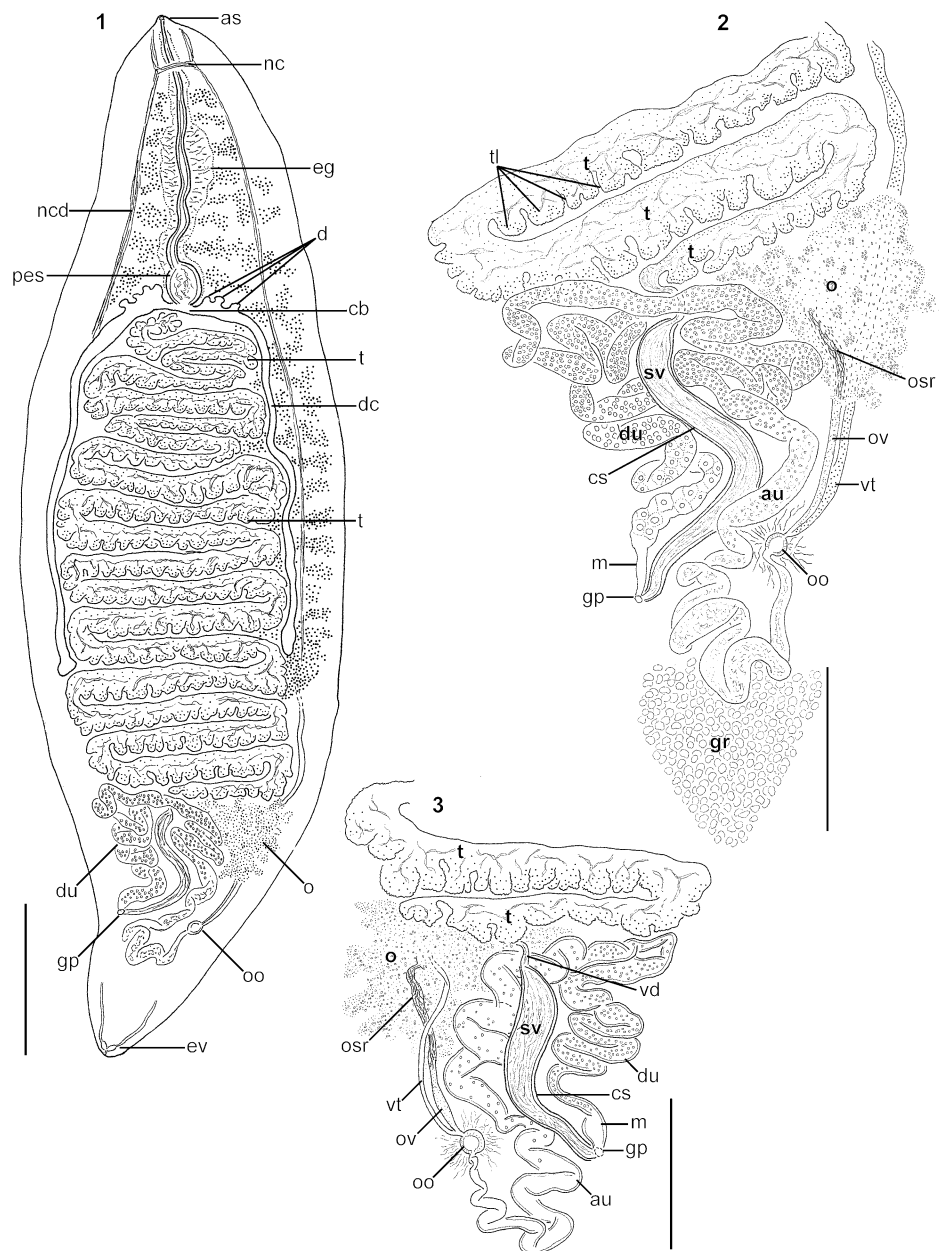
Orchispirium is most easily differentiated from all other accepted aporocotyloid genera by having a looped testis (Figs. 1–4, 11); no other accepted aporocotyloid genus reportedly has this feature. It resembles *Chimaerohemecus* van der Land, 1967, *Hyperandrotrema* Maillard and Ktari, 1978, *Acipensericola* Bullard, Snyder, Jensen, and Overstreet, 2008, and *Paracardicoloides* Martin, 1974, by having an inverse U-shaped intestine (Figs. 1, 4) (Martin, 1974; Bullard et al., 2008). However, in addition to having a looped testis, it further differs from each of those genera by having an aspinous body (Figs. 1, 4–7) and a diminutive, aspinous anterior sucker (Figs. 5–6), as well as by lacking a Laurer's canal (Figs. 1–3). *Chimaerohemecus* and *Hyperandrotrema* each have robust, C-shaped lateral tegumental spines and a Laurer's canal, and *Acipensericola* and *Paracardicoloides*, most notably, each have a large bowl-shaped anterior sucker that is demarcated from the body proper by a peduncle. As indicated by the specific epithet "*heterovitellatum*," Madhavi and Hanumantha Rao (1970) noted that most vitelline follicles were dextral in the type species, and they included "vitellarium asymmetry" as a generic feature. We concur that the symmetry of the vitellarium is an important generic feature (Figs. 1, 4); at least 1 other chondrichthyan aporocotyloid has an asymmetrical vitellarium, i.e., *Selachohemecus benzi*.

Orchispirium heterovitellatum Madhavi and Hanumantha Rao, 1970

(Figs. 1–12)

Diagnosis of adult (measurements and illustrations based on the holotype and 3 original voucher specimens): Body 2,925–3,470 (4) long, 936–1,092 (4) wide, 3.0–3.3 times longer than wide, with maximum width at level of midbody, lacking obvious sensory papillae on dorsal or ventral surface (Figs. 1, 4). Lateral tubercles 7–12 (2) wide at base, 5–7 (2) tall, comprising 2–3 disorganized rows in anterior body region, becoming reduced or absent posteriorly; muscle fibers in tubercles indistinct (Fig. 7); tegumental extensions near lateral tubercles non-sclerotized, plank-shaped, approximately 2 long and 1 wide, distributing anterior to nerve commissure only. Nerve commissure 95–174 (4) or 3–5% of body length from anterior body end, 55–100 (4) across width of worm or 5–11% of body width, 12–15 (4) in diameter, perpendicular to long axis of body (Figs. 1, 4). Anterior sucker 25–30 (4) long, 27–50 (4) wide, 1–1.7 times wider than long (Figs. 1, 4–6). Mouth a minute pore approximately 3 wide (Figs. 5, 6). Esophagus 800–900 (4) or 24–29% of total body length, 10–15 (4) wide near mouth, with wall 2–3 (4) thick near mouth and thickening to 7–10 (4) near cecal bifurcation (Figs. 1, 4, 8, 9). Esophageal gland 200–235 (3) long, 100–113 (3) wide, concentrating about medial portion of esophagus 350 (1) or 11% of body length from anterior body end (Fig. 8). Posterior esophageal swelling 85–127 (4) long or 10–13% of esophagus length, 75–105 (4) wide or 1.0–1.5 times longer than wide, having wall 7 thick (Figs. 1, 4, 9). Ceca bifurcating 810–969 (4) or 23–29% of body length from anterior body end (Figs. 1, 4, 9); posterior ceca each 1,053–1,287 (4) long or 34–44% of body length, 25–50 (4) wide, terminating lateral to testis loops (Fig. 10); diverticula extending 30–40 (2) anteriorly (Fig. 1); post-cecal space 900–1,443 (4) long or 31–42% of body length (Figs. 1, 4).

Testis cord 50–100 (4) wide, occupying intercecal space 1,227–1,833 (4) long or 44–55% of body length, 515–858 (4) wide or 1.3–1.4 times longer than wide, comprising testicular tissue and network of vasa efferentia, curving 20 or 21 (4) times or looping 10 or 11 (4) times, bearing lobes extending posteriad from posterior margin of testis cord and laterally from curved portion of testis cord only, lacking anteriorly directed lobes, lacking refractive acini (Figs. 1–4, 11); post-testicular space 500–900 (4) long or 17–27% of body length (Figs. 1, 4). Vas deferens extending 75–153 (3) or 2–5% of body length posteriad from testicular mass, 13 (1) in maximum width, joining cirrus sac and internal seminal vesicle medially and immediately posterior to posterior margin of testis (Figs. 2, 3); internal seminal vesicle 400–503 (4) long or 14–

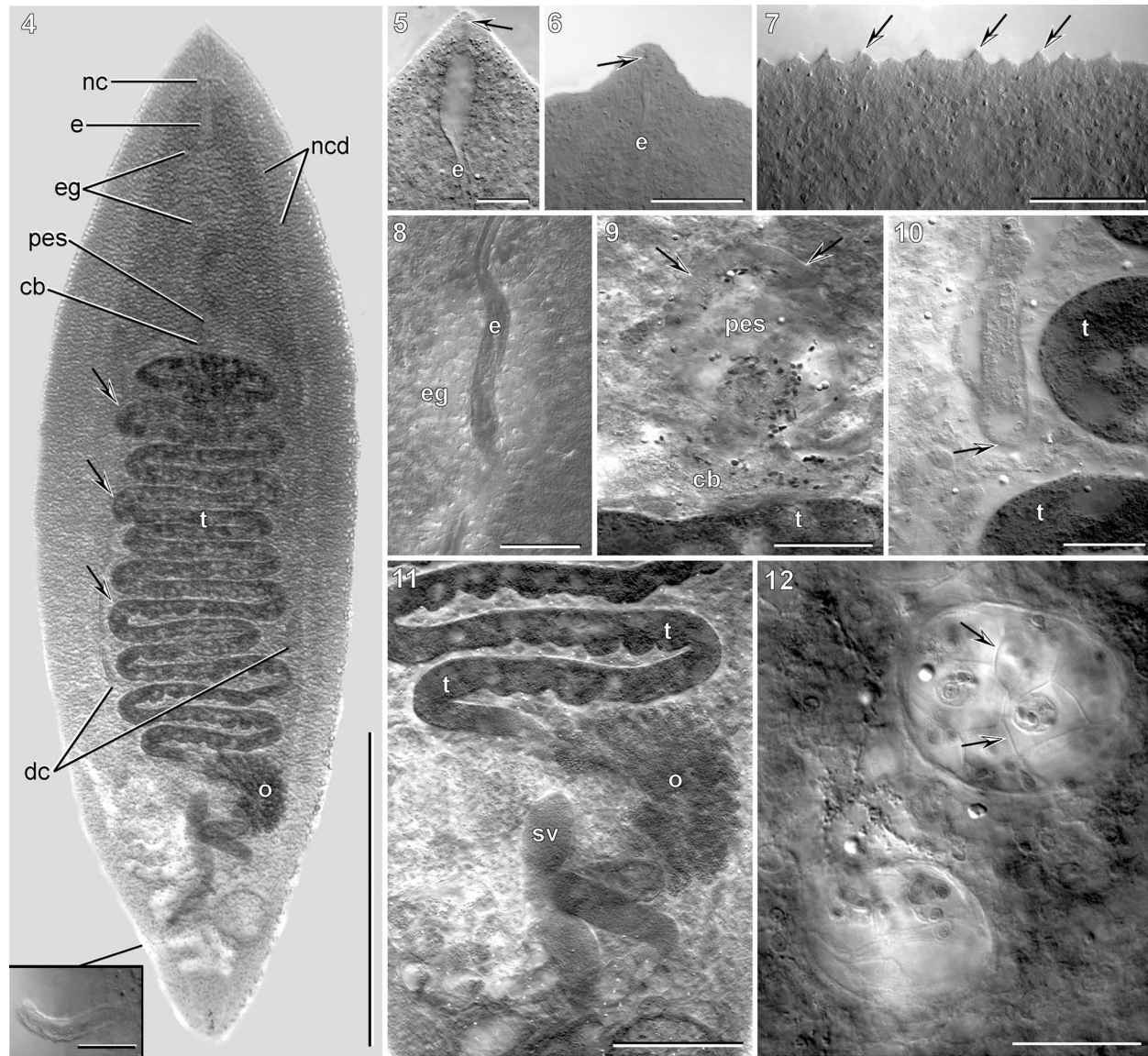


FIGURES 1–3. *Orchispirium heterovitellatum* Madhavi and Hanumantha Rao, 1970 (Digenea: Apocotylidae), from the mesenteric blood vessels of the scaly whipray *Himantura imbricata* (Bloch and Schneider, 1801) (Myliobatiformes: Dasyatidae) in the Bay of Bengal. Composite illustrations based on holotype (USNPC 70566) and 3 original voucher specimens. (1) Body of adult showing location of anterior sucker (as), nerve commissure (nc), esophageal gland (eg), ventrolateral nerve cord (ncd), posterior esophageal swelling (pes), diverticula (d), cecal bifurcation (cb), looped testis (t), dextral posterior cecum (dc), ovary (o), descending uterus (du), genital pore (gp), ootype (oo), and excretory vesicle (ev), dorsal view. Bar = 500 μm . (2) Genitalia showing location of testis (t), testicular lobes (tl), ovary (o), internal seminal vesicle (sv), cirrus sac (cs), genital pore (gp), descending uterus (du), ascending uterus (au), oviduct (ov), oviducal seminal receptacle (osr), vitelline duct (vt), ootype (oo), metraterm (m), and glandular region (gr), dorsal view. Bar = 300 μm . (3) Genitalia showing comparable features and vas deferens (vd) as depicted in Figure 2, ventral view. Bar = 300 μm .

16% of body length, 30–50 (4) wide, S-shaped, narrowing distally (Figs. 1–4, 11). Cirrus sac with wall 3–5 (2) thick (Figs. 2, 3); everted cirrus 50 (1) long, 8 (1) wide (Fig. 4). Common genital pore 372–397 (3) or 10–11% of body length from posterior body end (Figs. 1–4).

Ovary 250–325 (4) long or 8–10% of body length, 125–240 (4) wide or 13–23% of body width, 1.2–2.4 times longer than wide, posterolateral to male reproductive tract, diffuse, lacking clearly defined membranous margin in holotype (Figs. 1–4, 11); post-ovarian space 250–

510 (4) long or 9–15% of body length (Figs. 1, 4). Oviduct straight, 183–250 (4) long or 4–8% of body length, 7–13 (4) wide (Figs. 1–3). Oviducal seminal receptacle 7–25 (2) wide, comprising a slightly laterally expanded portion of oviduct ventral to ovary (Figs. 2, 3). Primary vitelline duct 445 (2) long or 14% of body length, 15–25 (2) wide, difficult to trace anterior to ovary in whole mounted specimens, following track of oviduct and coursing near dextral body margin and oviduct. Ootype a clearly delineated spheroid chamber with surrounding glan-



FIGURES 4–12. *Orchispirium heterovitellatum* Madhavi and Hanumantha Rao, 1970 (Digenea: Aporocotylidae), from the mesenteric blood vessels of the scaly whipray *Himantura imbricata* (Bloch and Schneider, 1801) (Myliobatiformes: Dasyatidae) in the Bay of Bengal. Light micrographs, all dorsal view of holotype (USNPC 70566) except Figures 6 and 8, which are dorsal views of an original voucher specimen. (4) Body, showing locations of nerve commissure (nc), esophagus (e), ventrolateral nerve cord (ncd), esophageal gland (eg), posterior esophageal swelling (pes), cecal bifurcation (cb), distal ends of ceca (dc), testis (t), ovary (o), and loops of testis (arrows). Bar = 1 mm. Inset magnifies everted cirrus of holotype, bar is 20 μ m. (5) Anterior body end showing location of mouth (arrow) and esophagus (e). Note that anterior sucker is not evident and that anterior end of esophagus is expanded to form a vesicle-like structure. Bar = 20 μ m. (6) Anterior body end showing location of esophagus (e) and mouth (arrow) in center of anterior sucker. Bar = 30 μ m. (7) Lateral tubercles (arrows) along lateral body margin showing absence of tegumental spines. Bar = 50 μ m. (8) Esophageal gland (eg) surrounding esophagus (e). Bar = 25 μ m. (9) Posterior esophageal swelling (pes) immediately anterior to cecal bifurcation (cb) and testis (t). Arrows mark thickened wall of posterior esophageal swelling. Bar = 50 μ m. (10) Distal end of sinistral posterior cecum (arrow) lateral to loops of testis (t). Bar = 50 μ m. (11) Genital region showing posterior loops of testis (t), ovary (o), and seminal vesicle (sv). Bar = 150 μ m. (12) Uterus showing vacuous uterine eggs with thin, pliable shells (arrows). Bar = 25 μ m.

dular region, 23–30 (4) in diameter, 395–440 (4) or 11–14% of body length from posterior body end, dextral, residing near level of genital pore (Figs. 1–3). Uterus extending short distance sinuously posterior from ootype before curving anterodorsally; ascending uterus 600–750 (2) long, 45–63 (4) in maximum width, extending sinuously anterior, arching sinistral before coursing dorsal to vas deferens, extending along posterior surface of testis (Figs. 2, 3, 12); descending uterus convoluted, 350–600 (2) long, 30–40 (4) wide, having more eggs than proximal portion of ascending uterus, meandering posterior before coiling and meeting with metraterm (Figs. 2, 3); uterine eggs 10–20 (10) in diam-

eter (Fig. 12); metraterm 72 (1) long, 42 (1) wide (Figs. 2, 3). Excretory vesicle 10 (1) long, 10 (1) wide, with arms 13 (1) long and 7 (1) wide (Fig. 1).

Taxonomic summary

Type and only known host: *Himantura imbricata* (Bloch and Schneider, 1801) (as *D. imbricatus*) (Myliobatiformes: Dasyatidae), the scaly whipray.

Site: Adults in mesenteric blood vessels.

Type locality: Western Bay of Bengal, waters off Waltair (17°43'42"N, 83°20'53"E), India.

Specimens examined: Holotype (USNPC 70566) and 3 original voucher specimens (personal collection of Rokkam Madhavi).

Remarks

Comparison with original description: The original description of *O. heterovitelatum* was based on a combination of whole mounts, serial sections, and living specimens, and it mostly matched the holotype and 3 original voucher specimens that we studied. However, some observations in the present study contradict both the original account (Madhavi and Hanumantha Rao, 1970) and a subsequent diagnosis of the genus (Smith, 2002).

Anterior sucker: Madhavi and Hanumantha Rao (1970) reported "suckers and pharynx absent," as did Smith (2002). Although difficult to delineate in the holotype (Fig. 5), the original voucher specimens we studied have a distinct apical structure associated with the mouth that we regard as a type of anterior sucker (Figs. 1, 6), albeit one that is diminutive relative to that of many other non-aporocotylid digeneans and even some aporocotylids. For example, *Elaphrobates euzeti* Bullard and Overstreet, 2003, has a spheroid anterior sucker with concentric spine rows distributing anterior to the mouth (Bullard and Overstreet, 2003; we accept *Elaphrobates* Bullard and Overstreet, 2003, and reject its synonymy with *Cardicola* Short, 1953, as proposed by Nolan and Cribb 2006) and *Acipensericola petersoni* Bullard, Snyder, Jensen, and Overstreet, 2008, has a spinous, bowl-shaped anterior sucker that is centered on the mouth and demarcated from the body by a peduncle. We suspect that the presence of an aspinous, eversible anterior sucker that lacks a peduncle is a synapomorphy for batoid aporocotylids. However, as with many aporocotylids, observing living specimens is probably helpful because the sucker is not evident in all fixed specimens (e.g., Figs. 5, 6).

Lateral tubercles: The wavy body margin of *O. heterovitelatum* depicted in figure 1 of Madhavi and Hanumantha Rao (1970) and figure 51.20 of Smith (2002) is stylized such that the length and width of the lateral tubercles are greatly exaggerated (Figs. 1, 4, 7). Nevertheless, we concur that the presence of lateral tubercles is an important feature that does not comprise fixation artifact. Homology of the lateral tubercles, however, is indeterminate. If biased by a consideration of the host's phylogenetic affiliation in Chondrichthyes, the lateral tubercles at least superficially resemble the peduncles that support the large, C-shaped lateral tegumental spines of shark and chimaera aporocotylids (i.e., *C. trondheimensis*, *H. cetorhini*, and *Selachohemecus* spp.). The lateral tubercles of *O. heterovitelatum* are also largest and most numerous in the anterior body region, similar to the relative size and distribution of peduncles of *H. cetorhini*, *C. trondheimensis*, and *S. benzi*. However, the lateral tubercles of *O. heterovitelatum* and the peduncles of the shark and chimaera aporocotylids are hardly comparable upon closer examination. Thus, the former are aspinous and seemingly lack muscle (Fig. 7), whereas the latter have muscle fibers that allow the flukes to support and articulate robust, C-shaped spines (Bullard et al., 2006). Although not immediately intuitive in light of the host's phylogenetic affiliation, another possible homolog to the lateral tubercles of *O. heterovitelatum* might comprise the mound-like tegumental features associated with the spines of some of the cypriniform aporocotylids that previously have been attributed to *Sanguinicola* Plehn, 1905 (see Ejsmont, 1926; Simon-Martin and Rojo-Vazquez, 1984; Simon-Martin et al., 1988).

Lateral tegumental spines: We regard the absence of lateral tegumental spines as a reliable feature even though Madhavi and Hanumantha Rao (1970) excluded it from their diagnosis; they indicated "spines not evident, probably lost" in their description of *O. heterovitelatum*. Likewise, Smith (2002) included "spines lost?" in his diagnosis of the genus. Hence, both accounts suggest the presence of spines. Although it is not uncommon for a number of spines to become detached and separated from degraded aporocotylids, we speculate that this species truly lacks spines. Madhavi and Hanumantha Rao (1970) did not report a spine in any living specimen, and none of the 4 fixed, whole mounted specimens (including the holotype) that we examined appeared significantly degraded. Moreover, even if somewhat degraded we doubt that no spine would remain attached to a specimen or embedded within the balsam of any whole mount. Noteworthy is that crystalline, refractive debris surrounded the voucher specimens we observed

and, superficially, some bits of this debris may deceptively appear to be detached lateral tegumental spines.

Testis loops: The testis was described by Madhavi and Hanumantha Rao (1970) as "a transversely coiled tube" and by Smith (2002) as "coiled"; however, it is looped. Thus, rather than the testis being wound into rings laid within or on top of one another or wound spirally around another structure, the testis clearly forms non-overlapping loops that are tightly compressed (Figs. 1, 4, 11). This feature distinguishes *Orchispirium* from all other accepted aporocotylid genera.

Oviduct and oviducal seminal receptacle: The oviduct emanates from the ventral aspect of the ovary rather than the posterior margin of the ovary. Madhavi and Hanumantha Rao (1970) stated that the uterus serves as the seminal receptacle in *O. heterovitelatum*, but Smith (2002), apparently rejecting that feature, included "seminal receptacle absent" in his diagnosis of *Orchispirium*. The proximal portion of the oviduct has sperm, and we regard the proximal portion of the oviduct ventral to the ovary as a type of oviducal seminal receptacle (Figs. 2, 3). Some sperm was present in the proximal portion of the ascending uterus (Fig. 2) of 1 of the original voucher specimens, but whether or not it is a dedicated sperm receptacle is uncertain. Although this portion of the uterus is markedly expanded in the holotype (see Madhavi and Hanumantha Rao, 1970), it is not expanded in another specimen (Figs. 1–3).

Metraterm: The metraterm comprises a delicate, thin-walled structure rather than the thick-walled structure implied by the stylized illustrations of Madhavi and Hanumantha Rao (1970) and Smith (2002).

Myliobaticola n. gen.

(Figs. 13–30)

Diagnosis: Body minute, flat, ventrally concave, equal in width for most of length, aspinous, lacking lateral tubercles. Rosethorn-shaped spines absent. Anterior sucker aspinous, lacking peduncle, diminutive, centered on mouth, strongly eversible; mouth ventral, subterminal in fixed specimens. Pharynx absent. Esophagus medial, extending sinusously posteriad approximately 1/3 body length; posterior esophageal swelling present. Intestine inverse U-shaped, with short posterior ceca only; posterior ceca shorter than esophagus, terminating in anterior half of body, lacking diverticula. Testis single, looping, crossing midline repeatedly, lacking lobed margins; testicular field 1–3 times longer than wide, medial, not extending breadth of posterior ceca, between ovary and ceca. Vas deferens short, extending posteriad from testis; internal seminal vesicle longer than vas deferens, enveloped by cirrus sac. Auxiliary external seminal vesicle absent. Everted cirrus nipple-like. Ovary single, medial, primarily post-testicular. Vitellarium dendritic, diffuse, an extensive network of narrow interconnecting branching bands having granular vitelline material distributing among genitalia and alimentary tract. Oviduct dextral, a narrow duct extending sinusously posteriad in parallel with body margin; oviducal seminal receptacle present, comprising proximal portion of oviduct immediately posterior to ovary, delineated anteriorly and posteriorly by constrictions of oviduct proper. Laurer's canal absent. Ootype indistinct. Uterus post-gonadal, flanking internal seminal vesicle; uterine eggs oblong, vacuuous, having membranous shell. Metraterm short, thin-walled. Male and female reproductive tracts sharing common atrium and pore, lacking posterolateral protuberance of body associated with pore; pore dorsal, sinistral, post-gonadal, post-cecal. In heart of myliobatiforms.

Differential diagnosis: Body minute, aspinous, lacking lateral tubercles. Anterior sucker aspinous, lacking peduncle, diminutive, strongly eversible. Pharynx absent. Posterior esophageal swelling present. Intestine inverse U-shaped; posterior ceca terminating in anterior half of body, lacking diverticula. Testis looping, lacking lobed margins. Internal seminal vesicle longer than vas deferens, enveloped by cirrus sac. Ovary medial, primarily post-testicular. Oviducal seminal receptacle present, delineated anteriorly and posteriorly by constrictions in oviduct proper. Laurer's canal absent. Uterus post-gonadal, flanking internal seminal vesicle; uterine eggs oblong, vacuuous, having membranous shell. Metraterm short, thin-walled. Male and female reproductive tracts sharing common atrium and pore, lacking posterolateral protuberance of body associated with pore; pore dorsal, sinistral, post-gonadal, post-cecal.

Taxonomic summary

Type and only known species: *Myliobaticola richardheardi* n. sp.

Etymology: The name "*Myliobaticola*" is for the host order, Myliobatiformes, and the Latin "*cola*," meaning inhabitant or dweller.

Remarks

Myliobaticola and *Orchispirium* probably share a recent, common ancestor because they are the only accepted aporocotyloid genera having a looped testis. Additional similarities among these genera include the presence of an aspinous body, an aspinous anterior sucker, an inverse U-shaped intestine, a posterior esophageal swelling, and a common genital pore (Figs. 1–4, 13–16). The configuration of the terminal male genitalia further likens the genera: both *Orchispirium* and *Myliobaticola* have a massive internal seminal vesicle that is sinuous and enveloped by the cirrus sac and that meets with a diminutive cirrus and common genital atrium (Figs. 1–3, 13–15). Another similarity between the species of these monotypic genera is the presence of vacuous eggs having a thin, pliable, membranous shell (Figs. 12, 30). Although as a group aporocotyloids are regarded as having pliable eggshells, we are not aware of any other named aporocotyloids having eggs that are as vacuous and as thin-shelled as those of *O. heterovittellatum* and the type species of *Myliobaticola*. We suspect that the presence of this type of egg is an ecological synapomorphy for these genera and that it may be related to their life history. Despite these similarities, *Myliobaticola* is most easily differentiated from *Orchispirium* by lacking lateral tubercles and by having a minute body, proportionally short posterior ceca, a testis that lacks lobes, and a medial ovary. *Orchispirium* has lateral tubercles, a large body, proportionally long posterior ceca, a testis with posteriorly-directed lobes, and a dextral ovary. Although these monotypic genera seem closely related also by their hosts' phylogenetic affiliation in Myliobatiformes, they differ in the site of infection and their geographic distribution: *Orchispirium* includes a species that infects the mesenteric vessels of a stingray in the Indian Ocean and the type species of *Myliobaticola* infects the heart of a stingray in the Gulf of Mexico.

Myliobaticola richardheardi n. sp. (Figs. 13–30)

Diagnosis of adult (measurements and illustrations based on 10 heat-killed, whole mounted specimens, 2 SEM prepared specimens, and several live specimens): Body extremely small with some specimens having body ~4 times width of host erythrocyte (Figs. 13, 16, 24), 365–650 (9) long, 65–130 (9) wide, 3.2–9.3 times longer than wide (Figs. 13, 16); minute dimples distributing over dorsal body surface (Fig. 21); sensory papillae naked, lacking cilia (Figs. 21, 22). Nerve commissures and ventrolateral nerve cords not evident in whole mounted specimens or living specimens. Anterior sucker 9–17 (5) long, 10–20 (5) wide, 0.7–1.0 times longer than wide, capable of erratic probing movements (Figs. 23–25). Mouth 1 (5) wide, 4–9 (4) or 1–2% of body length from anterior body end in fixed specimens (Figs. 24, 25). Esophagus 135–265 (6) long or 25–42% body length, 2–5 (4) wide near mouth, 4–8 (4) wide in posterior region, with esophageal wall 1 (3) thick near mouth, 3 (3) thick in posterior region (Fig. 13). Esophageal gland indistinct. Posterior esophageal swelling thin-walled, 15 (1) long and 10 (1) wide, occupying space immediately anterior to cecal bifurcation in posterior portion of esophagus (Figs. 13, 17), containing brownish-colored granular material in living specimens; cecal bifurcation 138–276 (5) or 0.26–0.46 times body length from anterior body end (Figs. 13, 16, 17). Posterior ceca 30–35 (2) long, 10 (2) wide, thin-walled, having broadly rounded distal tips; postcecal space 230–467 (5) long or 57–74% of total body length (Figs. 13, 17).

Testicular mass 60–100 (4) long, 33–43 (4) wide, 1.5–2.7 times longer than wide, comprising testicular tissue and vasa efferentia, curving 9–10 (3) times, slightly dorsal to ceca or entirely post-cecal in anterior region, meandering posteriad and directing slightly sinistrad before narrowing and becoming confluent with vas deferens (Figs. 13–16, 18); post-testicular space 168–325 (4) long or 39–52% of body length (Figs. 13, 16). Internal seminal vesicle markedly wider than vas deferens, extending sinuously posteriad, curving 9–10 (4) times, 105–150 (4) long or 22–30% of body length, 10–15 (4) in maximum width, filled with sperm in all specimens, having most active and dense aggregation of sperm in proximal portion in living specimens, slanting toward sinistral body margin (Figs. 13–16, 19, 28, 29). Cirrus sac indistinct in fixed whole mounted specimens, clearly visible in living specimens (Figs. 28, 29), enveloping entire length of internal seminal vesicle (Figs. 14, 15). Cirrus 7–14 (3) long, 3–7 (3) wide, 2.0–3.3 times longer than wide (Fig. 26). Common genital pore 10–15 (5) from sinistral body margin, 50–70 (5) from dextral body margin, 50–75 (5) or 11–13%

of body length from posterior body end (Figs. 13–16, 20, 22, 26); sensory papillae distributing near common genital pore, 3–4 in number (Fig. 22).

Ovary 20–30 (5) long or 4–6% of body length, 35–60 (5) wide or 39–62% of body width, 1.3–2.0 times wider than long, appearing as loose aggregation of ova anterior to male and female reproductive ducts; post-ovarian space 158–213 (5) or 33–40% of body length (Figs. 13, 16). Oviduct short, originating from posterior margin of ovary, twisting proximally and running dextrad before widening as oviducal seminal receptacle (Figs. 14, 15, 19); oviducal seminal receptacle 30–38 (5) long, 7–20 (5) in maximum width, 2.8–4.4 times longer than wide, orienting diagonally and directing toward dextral body margin, filled with sperm in all specimens, twisting distally and narrowing as distal portion of oviduct; distal portion of oviduct a narrow tube extending approximately in parallel with and between body margin and internal seminal vesicle, 85–163 (5) long or 20–29% of body length, 3–5 (5) in maximum width, convoluted, indistinct for part of its length in most whole mounted specimens, curving toward midline in posterior region before expanding to form probable ootype (Figs. 13–15, 19, 28). Probable ootype residing in posterior extremity of oviduct lateral to common genital pore, lacking constrictions demarcating chamber. Vitellarium refractive (Fig. 27). Primary vitelline duct and Mehlis' gland indistinct in whole mounted specimens and living specimens. Ascending uterus 100–175 (4) long or 1.0–1.4 times longer than distal portion of oviduct, 7–30 (4) wide, extending anteriorly between oviduct and internal seminal vesicle from level of common genital pore to oviducal seminal receptacle, widening and arching dorsally over proximal portion of internal seminal vesicle (Figs. 13–15), containing sperm in some specimens; descending uterus 100–175 (5) long or 90–100% length of ascending uterus, 6–10 (5) wide, more narrow than ascending uterus, extending posteriad between internal seminal vesicle and sinistral body margin, curving as many as 5 times, containing well-developed eggs in lumen (Figs. 14, 15). Uterine eggs 6–7 (5) long, 4–6 (5) wide (Fig. 19). Metatrerm 75 (2) long, 17 (2) wide (Figs. 14, 15, 29). Ejected eggs approximately 20–30 long, 10–15 wide, with cellular material concentrating at 1 end of egg (Fig. 30). Excretory system indistinct.

Taxonomic summary

Type and only known host: *Dasyatis sabina* (Lesueur, 1824) (Myliobatiformes: Dasyatidae), the Atlantic stingray.

Site: Adults in intertrabecular spaces of heart.

Type locality: Deer Island (30°22'22"N, 88°50'00"W), Mississippi Sound, Northern Gulf of Mexico off Biloxi, Mississippi.

Other localities: Horn Island (30°15'04"N, 88°42'42"W) and Ship Island (30°14'29"N, 88°53'05"W), Mississippi Sound, Northern Gulf of Mexico; Apalachicola Bay (29°54'54"N, 84°20'06"W), Northern Gulf of Mexico near Panama City Beach, Florida.

Specimens deposited: Holotype USNPC 101303. Paratype USNPC 101304.

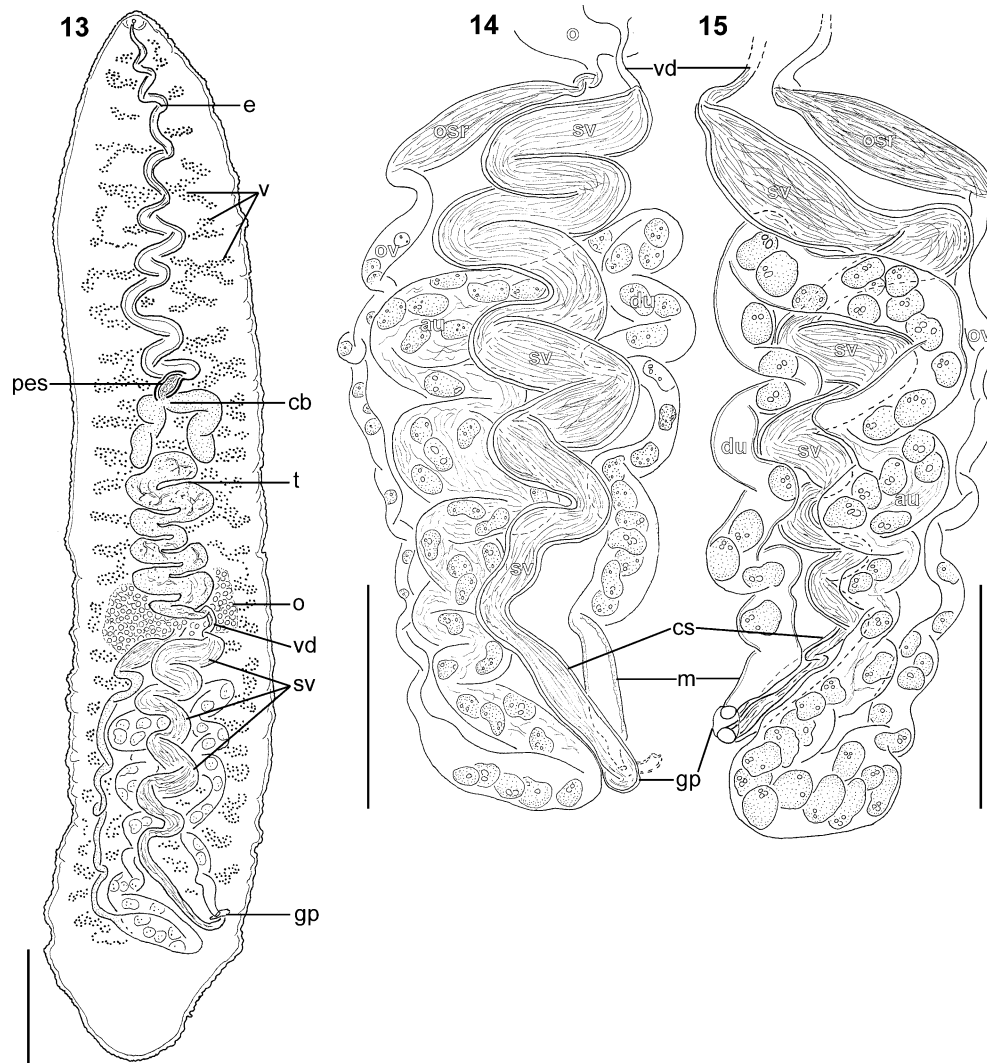
Prevalence of infection: Fifteen of 54 (28%) off Mississippi and 1 of 5 (20%) off Florida.

Etymology: The specific name *richardheardi* honors our friend and admired colleague Prof. Richard Willis Heard, Jr. (Gulf Coast Research Laboratory, University of Southern Mississippi) for his lifelong dedication as a naturalist who has amassed an encyclopedic knowledge of the identities and biology of parasitic and free-living invertebrates of the Northern Gulf of Mexico.

Remarks

The microhabitat of *M. richardheardi* in the heart of *D. sabina* was difficult to confirm because of the small size of the adult flukes. Intact adult flukes were revealed only after pieces of the bisected heart were teased apart and irrigated with physiologic saline, thereby washing dislodged flukes into the Petri dish. Nevertheless, no specimen of *M. richardheardi* applied its flat body to the luminal surface of the atrium, ventricle, or bulbus arteriosus, and in this regard it differs from some larger, spinous aporocotyloids, e.g., *Cardicola palmeri* Bullard and Overstreet, 2004, and *Cardicola currani* Bullard and Overstreet, 2004, that infect the luminal surfaces of the heart.

The body, especially the anterior end, of *M. richardheardi* is highly contractile. Locomotion in this species seems to be achieved by a contraction of circular muscles, which narrows and lengthens the body, followed by a contraction of longitudinal muscles, which widens the



FIGURES 13–15. *Myliobaticola richardheardi* n. gen., n. sp. (Digenea: Aporocotylidae) from the heart of the Atlantic stingray *Dasyatis sabina* (Lesueur, 1824) (Myliobatiformes: Dasyatidae) in the Northern Gulf of Mexico. Composite illustrations based on whole mounts and living specimens. (13) Whole body showing location of esophagus (e), vitellarium (v), posterior esophageal swelling (pes), cecal bifurcation (cb), testis (t), ovary (o), vas deferens (vd), internal seminal vesicle (sv), and genital pore (gp), ventral view. Bar = 50 μ m. (14) Genitalia showing location of ovary (o), vas deferens (vd), internal seminal vesicle (sv), oviducal seminal receptacle (osr), oviduct (ov), ascending uterus (au), descending uterus (du), cirrus sac (cs), distal portion of uterus comprising the metraterm (m), and genital pore (gp), ventral view. Bar = 50 μ m. (15) Genitalia showing same features as in Figure 14, dorsal view. Bar = 50 μ m.

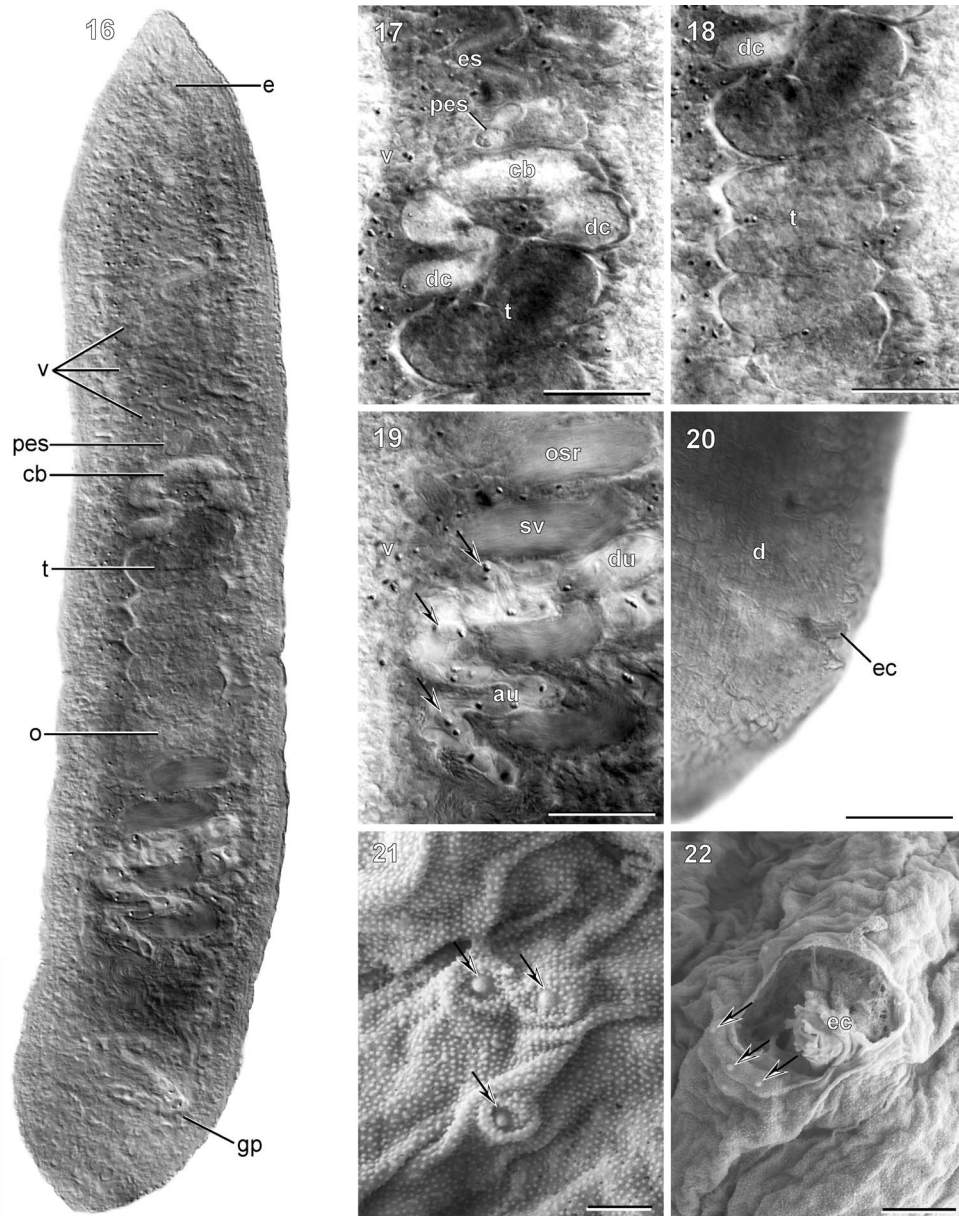
body and causes it to become bulb-like (Fig. 23). These movements at least superficially resembled those described for lumbricid oligochaetes (see fig. 13.12 of Brusca and Brusca 2003); no specimen of *M. richardheardi* made a wavelike undulation of its body margin like that reported for species of *Cardicola* and *Elaphrobates* (see Bullard and Overstreet, 2003, 2004).

DISCUSSION

We advocate including observations of living aporocotylids in species descriptions whenever possible. Although studying living aporocotylids is challenging in areas without electricity or in cramped spaces that cannot accommodate a microscope, such observations can enrich the resulting species description, especially if adults of the species are tiny, i.e., <1 mm. For example, several features of *M. richardheardi* were best demonstrated by living specimens and not readily apparent in fixed,

whole mounted specimens: (1) absence of lateral tegumental spines, (2) absence of lateral tubercles, (3) presence of anterior sucker (Figs. 24, 25), (4) presence of posterior esophageal swelling (Fig. 13), (5) shape of posterior ceca, (6) extent of vas deferens (Figs. 14, 16, 28), (7) limits of internal seminal vesicle (Fig. 28), (8) presence of cirrus sac (Figs. 28, 29), (9) distribution of vitellarium (Fig. 27), (10) limits of metraterm (Fig. 29), and (11) presence and location of the common genital atrium and pore (Fig. 26).

Seemingly distinct modes of locomotion and attachment exist among the heart-dwelling aporocotylids; some crawl, some burrow, and some are sedentary. Based on our observations of live specimens, adults of *M. richardheardi* lodge themselves between cardiac trabeculae (see Remarks) instead of actively crawling over the luminal surface of the heart. In this regard, locomotion in

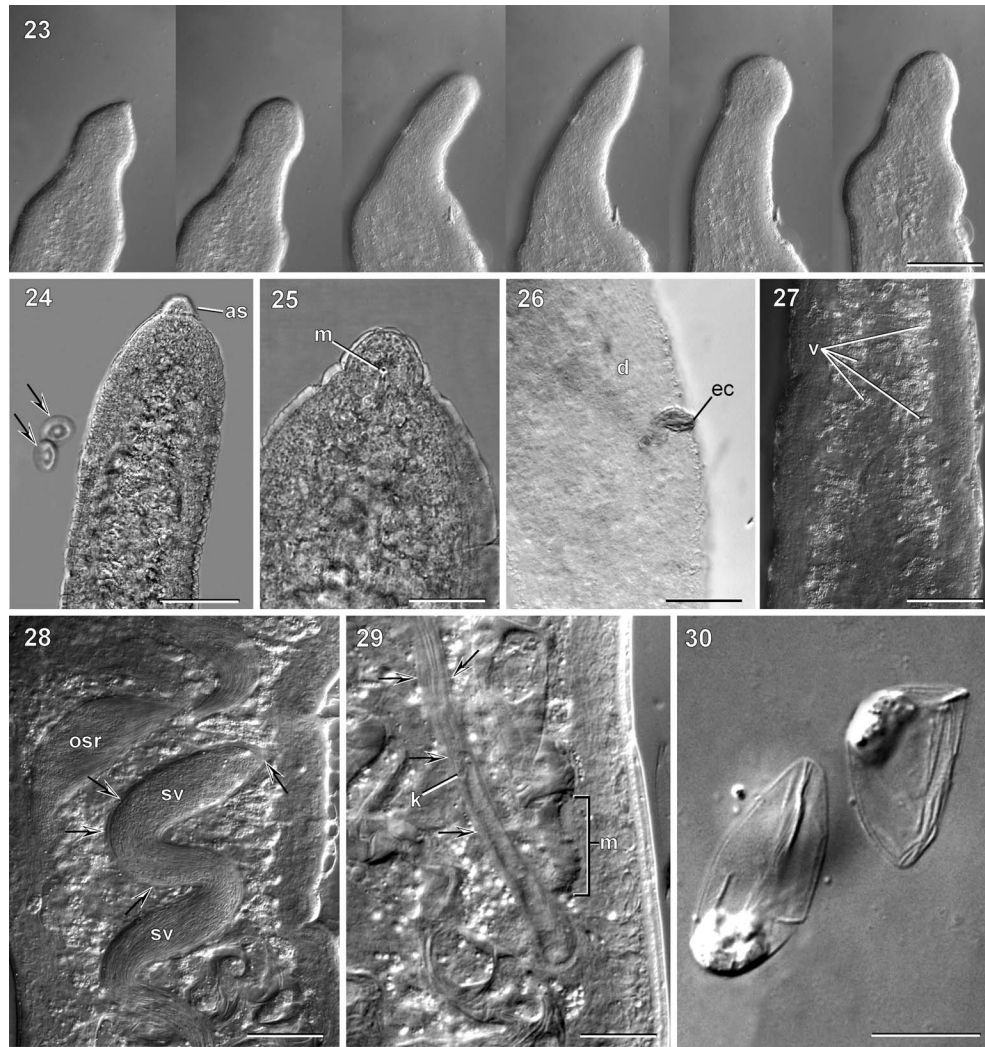


FIGURES 16–22. *Myliobaticola richardheardi* n. gen., n. sp. (Digenea: Apocotylidae) from the heart of the Atlantic stingray *Dasyatis sabina* (Lesueur, 1824) (Myliobatiformes: Dasyatidae) in the Northern Gulf of Mexico. Light micrographs of holotype, except Figures 21 and 22, which are scanning electron micrographs. (16) Body showing location of esophagus (e), vitellarium (v), posterior esophageal swelling (pes), cecal bifurcation (cb), testis (t), ovary (o), and genital pore (gp), ventral view. Bar = 100 μ m. (17) Esophagus (es), posterior esophageal swelling (pes), cecal bifurcation (cb), vitellarium (v), distal ends of posterior ceca (dc), and anterior region of looped testis (t), ventral view. Bar = 25 μ m. (18) Distal end of dextral posterior cecum (dc) terminating just anterior to anterior margin of looped testis (t), ventral view. Bar = 25 μ m. (19) Genital region showing oviducal seminal receptacle (osr), proximal (= anterior) region of internal seminal vesicle (sv), ascending uterus (au), descending uterus (du), vitellarium (v), and uterine eggs (arrows), ventral view. Bar = 25 μ m. (20) Posterolateral body margin showing submarginally everted cirrus (ec) of dorsal body surface (d), dorsal view. Bar = 25 μ m. (21) Nonciliated probable sensory papillae (arrows). Bar = 1 μ m. (22) Common genital pore showing remnants of partially everted cirrus (ec) and triplet (arrows) of probable sensory papillae about base of cirrus, dorsolateral view. Bar = 2 μ m.

adults of *M. richardheardi* differs from the “crawling” documented in species of *Cardicola* and *Elaphrobatas* that actively move about within the cardiac lumen of drums (Perciformes: Sciaenidae) and snappers (Perciformes: Lutjanidae), respectively. These drum and snapper flukes use their ventrolateral rows of tegumental spines to grip heart tissue in concert with coordinated, wave-like undulations of the lateral body margin (Bullard and Ov-

erstree, 2003, 2004). Still other adult apocotylids are sedentary, anchoring themselves in the cardiac lumen by threading their body through the host’s myocardium, e.g., *Psettarium anthicum* Bullard and Overstreet, 2006, in the heart of cobia *Rachycentron canadum* (Linnaeus, 1766) (Perciformes: Rachycentridae) (see Bullard and Overstreet, 2006).

Myliobaticola richardheardi may be specific to the heart of *D.*



FIGURES 23–30. *Myliobaticola richardheardi* n. gen., n. sp. (Digenea: Aporocotylidae) from the heart of the Atlantic stingray *Dasyatis sabina* (Lesueur, 1824) (Myliobatiformes: Dasyatidae) in the Northern Gulf of Mexico. Light micrographs of wet-mounted, live specimens. (23) Series showing movement of anterior body end. Bar = 50 μm . (24) Anterior body region showing anterior sucker (as) and 2 host erythrocytes (arrows), ventral view. Bar = 50 μm . (25) Mouth (m) in center of anterior sucker. Note that sucker lacks spines and is not clearly delineated from anterior body region by a neck or peduncle, ventral view. Bar = 25 μm . (26) Posterolateral body margin showing everted cirrus (ec) of dorsal body surface (d), dorsal view. Bar = 20 μm . (27) Middle body region showing the distribution of the vitellarium (v) about the looping testis, ventral view. Bar = 50 μm . (28) Posterior body end centered on proximal region of internal seminal vesicle (sv) showing oviducal seminal receptacle (osr) and cirrus sac (arrows), ventral view. Bar = 20 μm . (29) Posterior body end centered on distal portion of internal seminal vesicle and metraterm (m). Note cirrus sac (arrows) and kink (k) associated with the distal portion of the internal seminal vesicle, ventral view. Bar = 10 μm . (30) Eggs recently ejected from the metraterm. Bar = 10 μm .

sabina. We did not find an infection in another batoid (Table I), and we did not find a specimen in an extra-cardiac site in *D. sabina*. Still, we suspect that some of these batoids may harbor additional new aporocotylids because most of our examinations focused on the heart. Hence, we are not sure that some of those potential hosts were not infected in other regions of the blood vascular system, e.g., the mesenteric blood vessels, branchial blood vessels, or body cavity. Further, given the low prevalence of infection by *M. richardheardi* (0.20–0.28) in the 2 geographic regions where we collected it, we probably did not examine enough individuals of most of those batoids to detect an infection, if present. For these reasons, further examination of Gulf of Mexico batoids is justified. Moreover, additional collections of batoid aporocotylids from extracardiac sites as well as the heart can help test

the hypothesis that microhabitat in the definitive host comprises an ecological characteristic that differentiates aporocotylid genera. We find it noteworthy that the batoid aporocotylids occupy different ecological niches in their definitive hosts, with *O. heterovitelatum* and *M. richardheardi* maturing in the mesenteric blood vessels and heart, respectively.

Many unnamed aporocotylids could potentially infect members of Batoidea (see McEachran and Aschliman, 2004; including electric rays [Torpediniformes], sawfishes [Pristiformes], guitarfishes and skates [Rajiformes], and stingrays [Myliobatiformes]) that range in other ocean basins, or at least the scarcity of published host records suggests that these fishes are seldom examined for the presence of aporocotylid infections. We know of only 3 previous records (Bazikalova, 1932; Madhavi and

TABLE I. Batoids examined for aporocotyloid infections in the present study.

Host		n	Prevalence	Locality
Myliobatiformes				
Myliobatidae				
<i>Myliobatis freminvillii</i>	bullnose eagle ray	1	0.00	Northern Gulf of Mexico
<i>Rhinoptera bonasus</i>	cownose ray	13	0.00	Northern Gulf of Mexico, off Mississippi
<i>Mobula hypostoma</i>	lesser devil ray	4	0.00	Northern Gulf of Mexico, off Florida
		1	0.00	Northern Gulf of Mexico, off Mississippi
<i>Aetobatus narinari</i>	spotted eagle ray	1	0.00	Northern Gulf of Mexico, off Mississippi
Dasyatidae				
<i>Dasyatis americana</i>	southern stingray	2	0.00	Northern Gulf of Mexico, off Mississippi
<i>Dasyatis sabina</i>	Atlantic stingray	54	0.28	Northern Gulf of Mexico, off Mississippi
		5	0.20	Northern Gulf of Mexico, off Florida
<i>Dasyatis say</i>	bluntnose stingray	13	0.00	Northern Gulf of Mexico, off Mississippi
Urolophidae				
<i>Urobatis jamaicensis</i>	yellow stingray	11	0.00	Eastern Gulf of Mexico, off Florida Keys
Rajiformes				
Rajidae				
<i>Dipturus olseni</i>	spreadfin skate	18	0.00	Northern Gulf of Mexico
<i>Fenestraja sinusmexicanus</i>	pygmy skate	3	0.00	Northern Gulf of Mexico
<i>Leucoraja garmani</i>	freckled skate	4	0.00	Northern Gulf of Mexico
<i>Leucoraja lentiginosa</i>	speckled skate	1	0.00	Northern Gulf of Mexico
<i>Raja eglanteria</i>	clearnose skate	13	0.00	Northern Gulf of Mexico
<i>Raja texana</i>	roundel skate	3	0.00	Northern Gulf of Mexico

Hanumantha Rao, 1970, 1971). The present study, representing the fourth such record, is the first report of a batoid aporocotyloid in 37 yr. *Himantura imbricata* and *D. sabina*, both of Dasyatidae, are the only batoids that have been confirmed as aporocotyloid hosts, representing a rather pitiful sampling of the roughly 534 extant batoid species allocated to 4 orders, 17 families, and 72 genera (Nelson, 2006). One report suggests that another order within Batoidea, i.e., Rajiformes, includes an aporocotyloid host. Bazikalova (1932) reported specimens of "Aporocotyloidea (?) gen. sp." from the "lumen of the intestine" of a thorny skate *Amblyraja radiata* (Donovan, 1808) (Rajiformes: Rajidae). Smith (1972) dismissed the record and suspected that these worms were detached cestode proglottids. Although we concur that the site of infection reported by Bazikalova (1932) is dubious, in light of the present study we are not sure that these worms are not aporocotyloids. Moreover, we find it hard to accept that one could mistake a proglottid for an aporocotyloid, especially considering that this host was probably infected with cestodes comprising in-hand comparative material. Thus, it seems more likely that a worker might overlook a blood fluke altogether rather than specifically misidentify 1 among many other neighboring cestode specimens in the dish. Based on that logic, we submit the possibility that, like adults of another batoid aporocotyloid, i.e., *O. heterovitellatum*, Bazikalova's putative aporocotyloids infected the mesenteric blood vessels and during necropsy contaminated the container holding the thorny skate's alimentary tract. Indeed, aporocotyloids can be difficult to isolate since they infect blood. Further, they can reside in sinuses and lumens that are difficult to individually separate and contain. For example, the first named aporocotyloid, *Aporocotyle simplex* Odhner, 1900, was originally thought to be a gill ectoparasite (Odhner, 1900) until it was recollected several years later (Odhner, 1911). In any event, the thorny skate and its

rajiform kin certainly deserve future attention regarding the presence of aporocotyloid infections.

We cannot pigeonhole either of the named batoid aporocotyloids, *O. heterovitellatum* and *M. richardheardi*, as "marine" or "freshwater" blood flukes because their hosts are euryhaline and their life cycles are indeterminate. Berra (2001) cited several reports of stingrays of *Himantura* and *Dasyatis* that enter freshwater rivers in Asia, Africa, New Guinea, and Australia. Most relevant to the present study, *D. sabina*, the type host for *M. richardheardi*, commonly ranges in low-salinity, nearshore waters of Mississippi Sound (Bullard, pers. obs.) and reportedly can thrive in freshwater (Piermarini and Evans, 1998, 2000, 2001). Likewise, *H. imbricata*, the type host for *O. heterovitellatum*, ranges in estuarine mangroves and tolerates low salinities (Froese and Pauly, 2006). Hence, rather than being regarded as marine fishes with marine aporocotyloids, these dasyatid hosts could be exposed to riverine or estuarine mollusks, or perhaps polychaetes, shedding aporocotyloid cercariae. Although no record of a blood fluke exists from any of the ~20 species of so-called "river stingrays" (Myliobatiformes: Potamotrygonidae), which range in the Atlantic drainages of South American rivers only (Berra, 2001), the discovery and subsequent description of an aporocotyloid from a potamotrygonid would be informative regarding the natural history of batoid aporocotyloids as it pertains specifically to the freshwater or marine origins of the group.

Rather than being an anomalous finding, aporocotyloids that infect batoids may comprise a taxonomically diverse and closely related group. The shared suite of unique morphological characters exhibited by *O. heterovitellatum* and *M. richardheardi* certainly does not discourage the notion that members of Batoidea harbor aporocotyloids that share a recent common ancestor.

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