Research Article

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A new genus and species of fish blood fluke, *Achorovermis testisinosus* gen. et sp. n. (Digenea: Aporocotylidae), infecting critically endangered smalltooth sawfish, *Pristis pectinata* (Rhinopristiformes: Pristidae) in the Gulf of Mexico

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Abstract: *Achorovermis testisinosus* gen. et sp. n. (Digenea: Aporocotylidae) infects the heart of the smalltooth sawfish, *Pristis pectinata* Latham (Rhinopristiformes: Pristidae), in the eastern Gulf of Mexico. Specimens of the new genus, along with the other blood flukes that infect batoids are similar by having an inverse U-shaped intestine and a curving testis as well as by lacking tegumental spines. The new genus differs from all of the other blood flukes infecting batoids by having an elongate body ($>50 \times$ longer than wide), a testis having >100 curves, and an ovary wholly anterior to the uterus. It differs from *Ogawaia glaucostegi* Cutmore, Cribb et Yong, 2018, the only other blood fluke infecting a rhinopristiform, by having a body that is $>50 \times$ (vs $<30 \times$) longer than wide, a testis that is $>75 \times$ (vs $<40 \times$) longer than wide and has >100 (vs <70) curves, an ovary wholly anterior to (vs lateral and dorsal to) the seminal vesicle, a uterus wholly posterior to (vs overlapping and lateral to both) the testis and ovary, and a sinuous (vs convoluted) uterus. The new species joins a small group of chondrichthyan blood flukes that lack tegumental spines: *O. glaucostegi*, *Orchispirium heterovitellatum* Madhavi et Rao, 1970, *Myliobaticola richardheardi* Bullard et Jensen, 2008, *Electrovermis zappum* Warren et Bullard, 2019. Blood flukes infecting batoids are further unique by having a curving testis. That is, the blood flukes infecting species within Selachii are morphologically distinct from those infecting species within the Batoidea (excluding *Gymnurahemecus bulbosus* Warren et Bullard, 2019). Based on the morphological similarity, we suspect that the new species shares a recent common ancestor with *O. glaucostegi*. The discovery of the new species brings the total number of chondrichthyan blood flukes to 11 species assigned to nine genera.

Keywords: taxonomy, systematics, Endangered Species Act, Chondrichthyes, Elasmobranchii, North America

Over half (90 of 166 spp. assigned to 40 genera) of the nominal fish blood flukes (Digenea: Aporocotylidae Odhner, 1912; see Bullard et al. 2009) have been discovered in the last 20 years. Of these, 18 have been described from North America (Table 1), including five infecting chondrichthyans (Bullard et al. 2006, Bullard and Jensen 2008, Orélis-Ribeiro et al. 2013, Warren and Bullard 2019, Warren et al. 2019). No blood fluke has been described from a sawfish (Rhinopristiformes: Pristidae) and only one other fish blood fluke, *Ogawaia glaucostegi* Cutmore, Cribb et Yong, 2018, has been described from a rhinopristiform.

The smalltooth sawfish, *Pristis pectinata* Latham (Rhinopristiformes: Pristidae), was the first elasmobranch to be listed as an endangered species under the United States Endangered Species Act (Simpfendorfer 2005), and the International Union for Conservation of Nature (IUCN)

classified it as Critically Endangered in 2006 (Carlson et al. 2013). Gill netting and shrimp trawling contributed to the smalltooth sawfish decline (Seitz and Poulakis 2006). Collectively, 55 parasitic species have been reported from sawfishes (Pristidae: one species of *Anoxypristis* White et Moy-Thomas and four species of *Pristis* Linck): 23 cestodes (Southwell 1927, Watson and Thorson 1976, Campbell and Beveridge 1996, 2009, Beveridge and Campbell 2005, Schaeffner and Beveridge 2012, 2013, Cielocha et al. 2014, Bakenhaster et al. 2018, Cairra et al. 2018); nine monogeneans (Watson and Thorson 1976, Cheung and Nigrelli 1983, Ogawa 1991, Chisholm and Whittington 1997, 2000, Kearn et al. 2010, Kritsky et al. 2017); seven copepods (Ogawa 1991, Morgan et al. 2010, Bakenhaster et al. 2018); four digeneans (Bakenhaster et al. 2018); four isopods (Moreira and Sadowsky 1978, Bakenhaster et al.

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2018); four leeches (Bakenhaster et al. 2018); three nematodes (Bruce et al. 1994, Bakenhaster et al. 2018); and one branchiuran (Bakenhaster et al. 2018). Of these 55, only one innominate aporocotylid (described herein) has been reported (Bakenhaster et al. 2018).

We herein describe a new species of blood fluke infecting the smalltooth sawfish from the eastern Gulf of Mexico. The new species is the first nominal blood fluke reported from a sawfish and the second blood fluke described from a rhinopristiform (Cutmore et al. 2018).

MATERIALS AND METHODS

Four smalltooth sawfish were processed as per Bakenhaster et al. (2018). Three uninfected smalltooth sawfish were collected from Biscayne Bay, Florida on 12 January 2012 and Caloosahatchee River, Florida on 8 November 2018 and 8 January 2019. The single sawfish that was infected was collected from the eastern Gulf of Mexico off Naples, Florida (30°13'22.61"N; 88°3'18.57"W) on 2 July 2016. At necropsy, the heart, gill and spiral intestine were excised intact and separated (heart bisected, gill arches separated, spiral valve opened). All tissues were examined with the aid of a Leica M125 with simultaneous oblique-reflected and polarised substage light conditions to isolate fluke specimens for morphology. The heart was teased apart with forceps to reveal adult blood flukes, and sediment from the heart, gill, and spiral valve was examined with aid of a graduated cylinder after settling. Adult flukes (n = 9) were unmoving and presumed dead, so no attempt was made to relax them prior to their direct transfer to 5% neutral buffered formalin (n = 9).

Adult flukes fixed in formalin were rinsed with distilled water, cleaned with fine brushes to remove any debris, stained overnight in Van Cleave's hematoxylin with several additional drops of Ehrlich's hematoxylin, dehydrated using an ethanol series, cleared in clove oil, permanently mounted in Canada balsam, illustrated using Leica DM 2500 and Leica DMR (Leica, Wetzlar, Germany) microscopes each equipped with differential interference contrast (DIC), measured using an ocular micrometre, and illustrated using a drawing tube. Measurements are reported in micrometres (µm) as the range followed by the mean and sample size in parentheses.

Scientific names, including taxonomic authorities and dates, for fishes follow Eschmeyer et al. (2016). Morphological terms and nomenclature for blood flukes follow Bullard et al. (2006, 2009), Bullard and Jensen (2008), Warren and Bullard (2019), and Warren et al. (2019). Specimens of a related aporocotylid (*Ogawaia glaucostegi*) were borrowed from the Queensland Museum (South Brisbane, Australia). Type and voucher materials of the new species were deposited in the National Museum of Natural History's Invertebrate Zoology Collection (USNM, Smithsonian Institution, Washington, D. C.). Bakenhaster et al. (2018) reported the specimens of the new species described herein but contained a lapse regarding infection intensity, which we correct herein in the taxonomic summary (see below).

RESULTS

Achorovermis Warren et Bullard gen. n. Figs. 1–3

Generic diagnosis: Body extremely elongate, dorsoventrally flattened, having anterior and posterior ends tapering

equally, aspinous. Rosethorn-shaped spines absent. Nervous system indistinct. Anterior sucker aspinous, lacking peduncle, diminutive. Mouth subterminal. Pharynx absent. Oesophagus extending sinuously posteriad along midline for $\leq 1/4$ of body length; posterior oesophageal swelling present. Intestine inverse U-shaped, asymmetrical; posterior caeca slightly shorter than oesophagus, connecting to oesophagus ventrally, lacking diverticulae, terminating in anterior half of body. Testis single, medial, curving, lacking lobed margins, wholly posterior to intestine. Vas deferens short, extending posteriad from testis. Cirrus-sac present, enveloping internal seminal vesicle and cirrus. Internal seminal vesicle distinct, longer than vas deferens. Cirrus short, $<14\%$ of seminal vesicle length, curving sinistrally before everting. Auxiliary external seminal vesicle absent. Common genital pore dorsal, postgonadal, far anterior and sinistral to oötype. Ovary medial, postcaecal; postovarian space comprising $\geq 1/6$ of body length. Vitellarium follicular, diffuse, slightly asymmetrical, filling space between caecal bifurcation and testis. Laurer's canal absent. Oötype dextral, posterior to common genital pore, comprising an inconspicuous ovoid chamber. Uterus postgonadal, not extensively convoluted, extending posteriad from oötype before curving anteriad, crossing midline and extending posteriad; uterine eggs oblong. Uterine seminal receptacle absent. Excretory vesicle small, medial, with arms, visible in posterior most region of body.

Differential diagnosis (see Remarks): Body approx. 50–70 × longer than wide; aspinous, lacking lateral tubercles. Anterior sucker aspinous, lacking peduncle, diminutive. Pharynx absent. Posterior oesophageal swelling present. Intestine inverse U-shaped, asymmetrical; posterior caeca terminating in anterior half of body, lacking diverticulae. Testis single, curving, lacking lobed margins, curving >100 times. Internal seminal vesicle distinct, longer than vas deferens, enveloped by cirrus sac. Cirrus short, $>8\%$ of seminal vesicle length. Common genital pore postcaecal, postgonadal, far anterior and sinistral to oötype. Ovary medial, postcaecal, dorsal to posterior portion of testis, wholly anterior to uterus. Laurer's canal absent. Oötype posterior to common genital pore. Uterus postgonadal, dorsal and flanking seminal vesicle, not extensively convoluted; uterine eggs small, occupying $1/3$ of uterus.

Taxonomic summary

Type species: *Achorovermis testisinuosus* Warren et Bullard sp. n. (Digenea: Aporocotylidae).

Type host: Smalltooth sawfish, *Pristis pectinata* Latham (Rhinopristiformes: Pristidae).

Etymology: '*Achoro*' is for homeless considering the conservation status of its host and '*vermis*' for worm.

Remarks

The new genus is most similar to the other blood flukes that infect batoids (Madhavi and Rao 1970, Bullard and Jensen 2008, Cutmore et al. 2018, Warren and Bullard 2019 [excluding *Gymnurahemecus bulbosus* Warren et Bullard, 2019]) by having an inverse U-shaped intestine and a curving testis as well as by lacking tegumental spines. It dif-

Fig. 1. *Achorovermis testisiniuosus* Warren et Bullard gen. et sp. n. (Digenea: Aporocotylidae) from the heart of the smalltooth sawfish, *Pristis pectinata* Latham (Rhinopristiformes: Pristidae). **A** – body of holotype (USNM No. 1613421), ventral view. **B** – anterior sucker, holotype, ventral view. **Abbreviations:** mo – mouth; os – oesophagus; in – intestine; vit – vitellarium; t – testis; ov – ovary; sv – seminal vesicle; u – uterus; cgp – common genital pore; oo – oötype.

fers from all blood flukes infecting batoids by having the combination of an elongate body ($>50 \times$ longer than wide), a testis having >100 curves, and an ovary wholly anterior to the uterus.

Achorovermis testisiniuosus Warren et Bullard sp. n.

Figs. 1–3

Description of adult (based on nine whole-mounted specimens; USNM coll. nos. 1613421–1613429):

Body 5,580–6,680 (6178; 4) long, 100–160 (116; 4) at greatest width, 49–67 (57; 4) $\times$ longer than wide (Fig. 1A). Nerve commissures and ventrolateral nerve-cords not evident in whole-mounts. Anterior sucker 13–15 (14; 5) long, 16–19 (17; 5) wide, aspinous, centered on mouth. Mouth 2–4 (3; 6) in diameter, 7–10 (8; 4) from terminal end of body (Fig. 1B). Oesophagus 1,030–1,420 (1232; 5) in total length or 15–25% (21%; 5) of body length, 13–20

(16; 5) in maximum width (at level of precaecal dilation), with oesophageal wall gradually thickening and thinning throughout oesophagus (Figs. 1, 2A,B); oesophageal gland enveloping oesophagus anterior to precaecal dilation, 33–73 (50; 6) long or 3–5% (4%; 5) of oesophageal length, 25–53 (31; 6) wide or 29–66% (44%; 5) of body width (Figs. 1A, 2A,B). Caecal bifurcation 1,030–1,480 (1,248; 5) or 15–27% (21%; 4) of body length from anterior body end; caeca extending posteriad in parallel, asymmetrical, dextral caecum 730–1,300 (946; 4) long or 13–19% (16%; 4) of body length, sinistral caecum 520–1,145 (767; 4) long or 9–17% (13%; 4) of body length, mean posterior caeca 13–41 (24; 4) wide or 24–51% (35%; 4) of body width (Figs. 1A, 2B), containing granular material within lumen of some individuals.

Testis 1,950–3,820 (2,703; 6) long or 37–63% (46%; 4) of body length, 20–34 (26; 7) wide, occupying 50–60% (56%; 4) of body width, 78–191 (108; 6) $\times$ longer than wide, postcaecal, curving 109–129 (119; 6) times (Figs. 1A, 2C) until narrowing and becoming confluent with vas deferens. (Figs. 2D, 3); post-testicular space 1,110–1,460 (1,268; 6) long or 19–22% (21%; 4) of body length. Vas deferens 20–50 (30; 6) long, 8–10 (8; 6) wide, emanating from postero-ventral portion of testis, extending posterior for a short distance before connecting to cirrus sac (Figs. 2D, 3). Cirrus-sac having extremely thin wall, 1–3 (2.4; 7) thick (Fig. 3), including seminal vesicle and cirrus; seminal vesicle extending sinuously posteriad, 353–555 (444; 7) long or 6–8% (7%; 4) of body length, 13–20 (15; 7) wide or 14–26% (19%; 4) of body width, running between ascending and descending portions of uterus, ultimately narrowing and curving sinistrally towards body margin (Figs. 1A, 2D, 3); cirrus 40–78 (55; 3) long or 9–14% (11%; 3) of seminal vesicle length, 5–10 (7.6; 5) wide or 33–71% (53%; 5) of seminal vesicle width (Fig. 3); everted cirrus short (Fig. 3), observed in two specimens, 10 and 18 (14; 2) long, 8 and 10 (9; 2) wide, cirrus pore 10–18 (13; 2) in diameter. Common genital pore 630–830 (735; 7) or 9–14% (12; 4) of body length from posterior end of the body, 8–13 (10; 7) from sinistral body margin, 60–88 (76; 7) from dextral body margin (Figs. 2D, 3).

Ovary medial, 123–140 (132; 6) long or 2–3% (2%; 4) of body length, 38–65 (55; 7) wide or 45–52% (49%; 4) of body width, 2–3 (2.3; 6) $\times$ longer than wide, appears as aggregation of ova anterior to male and female reproductive ducts; postovarian space 1,010–1,250 (1,105; 7) long or 17–19% (18%; 4) of body length (Figs. 2D, 3). Oviduct originating from posterior margin of ovary, sharply curving twice before running dextrad and widening to form distal portion (Fig. 3); distal portion of oviduct a narrow tube extending approximately in parallel with and between body margin and ascending uterus, 653–796 (704; 6) long or 11–12% (11.3; 3) of body length, 10–18 (14; 6) in maximum width, curving sinistrally toward medial body line body, then curving dextral before connecting with primary vitelline duct (Figs. 2D, 3). Primary vitelline duct 702–1050 (806; 6) long, 5–10 (8; 6) wide, indistinct anterior of ovary. Laurer's canal not observed. Oötype 25–30 (27; 6) long, 23–30 (26; 6) wide (Figs. 2D, 3).

Fig. 2. *Achrovermis testisnuosus* Warren et Bullard gen. et sp. n. (Digenea: Aporocotylidae) from the heart of the smalltooth sawfish, *Pristis pectinata* Latham (Rhinopristiformes: Pristidae). **A** – anterior body segment (I); **B** – body segment (II); **C** – body segment (III); **D** – Posterior body segment (IV). *Abbreviations:* mo – mouth; os – oesophagus; og – oesophageal gland; cb – caecal bifurcation; vit – vitellarium; t – testis; ve – vasa efferentia; vd – vas deferens; ov – ovary; sv – seminal vesicle; o – oviduct; au – ascending uterus; du – descending uterus; cgp – common genital pore; c – cirrus; v – vitelline duct; oo – oötype.

Uterus extending directly posterior from oötype 618–856 (709; 7) long, 18–28 (24; 7) in maximum width, before looping back anteriorly 133–238 (175; 7) from posterior end and connecting to ascending portion (Fig. 3); total ascending portion 403–698 (548; 7) long or 13–17% (15%; 3) of body length, 30–53 (43; 7) wide (Fig. 3), sharply curving sinistrally to posterior margin of ovary, dorsal to sem-

inal vesicle, before connecting with descending portion; descending portion extends posteriorly 297–416 (360; 7) long or 5–6% (5.7; 3) of body length, 20–35 (28; 7) wide. Uterine eggs 8–13 (10; 7) in diameter or 27–50% (36%; 7) of uterus width, containing many small dense bodies, with thin shell (Fig. 3). Excretory vesicle 10–43 (19; 5) long, 3–15 (7; 5) wide, with arms 93 (1).

Fig. 3 *Achorovermis testisnuosus* Warren et Bullard gen. et sp. n. (Digenea: Aporocotyliidae) from the heart of the smalltooth sawfish, *Pristis pectinata* Latham (Rhinopristiformes: Pristidae). Genitalia of paratype (USNM No. 1613422-1613429), ventral view. **Abbreviations:** t – testis; vd – vas deferens; ov – ovary; sv – seminal vesicle; o – oviduct; au – ascending uterus; du – descending uterus; cgp – common genital pore; ec – everted cirrus; v – vitelline duct; oo – oötype.

Taxonomic summary

Type and only reported host: Smalltooth sawfish, *Pristis pectinata* Latham (Rhinopristiformes: Pristidae).

Site in host: Heart lumen.

Type locality: Off Naples, Florida (30°13'22.61"N; 88°3'18.57"W), eastern Gulf of Mexico, USA.

Prevalence and intensity of infection: One of four (prevalence = 25%) smalltooth sawfish sampled on 12 January 2012, 2 July 2016, 8 November 2018, and 8 January 2019 was infected by 9 specimens of *A. testisnuosus*.

Specimens deposited: Holotype (USNM 1613421), paratypes (USNM 1613422-1613429).

Etymology: The specific epithet '*testisnuosus*' refers to the many curves of the testis.

DISCUSSION

The new species is most similar to *Ogawaia glaucostegi* and the other blood flukes that infect batoids (Madhavi and Rao 1970, Bullard and Jensen 2008, Warren and Bullard 2019 [excluding *Gymnurahemecus bulbosus*, which has C-shaped spines]) by the combination of having a diminutive anterior sucker that lacks spines, an asymmetrical, inverse U-shaped intestine, a curving testis, an internal seminal vesicle and cirrus sac, and a postcaecal common genital pore as well as by lacking lateral tegumental spines. It differs from *O. glaucostegi* by the combination of having a body that is $>50 \times$ (*vs* $<30 \times$) longer than wide, a testis that is $>75 \times$ (*vs* $<40 \times$) longer than wide and has >100 (*vs* <70) curves, an ovary wholly anterior to (*vs* lateral and dorsal to) the seminal vesicle, a uterus wholly posterior to (*vs* overlapping and lateral to both) the testis and ovary, and a sinuous (*vs* convoluted) uterus.

The new species differs from *Orchispirium heterovitellatum* Madhavi et Rao, 1970 and *Myliobaticola richardheardi* Bullard et Jensen, 2008 by the combination of having a body that is >50 (*vs* $3 \times$) longer than wide, a testis with >100 (*vs* 21 and 10, respectively) curves, and a uterus that is sinuous (*vs* extensively convoluted). Further, *O. heterovitellatum* differs by having lateral tubercles along the body, an intestine bearing diverticula, and a testis that is inter-caecal and that has lobed margins. *Achorovermis testisnuosus* lacks lateral tubercles along the body margin, diverticula, and lobed margins along the testis as well as a testis that is wholly postcaecal. *Myliobaticola richardheardi* further differs by having a winding (*vs* sinuous) oesophagus and seminal vesicle.

The new species differs from *Electrovermis zappum* Warren et Bullard, 2019 by the combination of having a body that is $>50 \times$ (*vs* $<40 \times$) longer than wide, a testis that is $>30\%$ (*vs* $<20\%$) of the body length and has >100 (*vs* <40) curves, a seminal vesicle that occupies $<17\%$ (*vs* $>40\%$) of the body, a cirrus that is $<15\%$ (*vs* 65%) of the seminal vesicle length, and an ovary that is $<20\%$ (*vs* $>30\%$) of the body length from the posterior body end.

The new species is the fifth chondrichthyan blood fluke that lacks spines, and all of these species infect batoids (Madhavi and Rao 1970, Bullard and Jensen 2008, Cutmore et al. 2018, Warren and Bullard 2019). Based on the morphological similarity we infer that the new species shares a recent common ancestor with *O. glaucostegi*. The remaining nominal chondrichthyan blood flukes have large C-shaped spines. Four of these infect sharks: *Hyperandrotrema cetorhini* Maillard et Ktari, 1978, *H. walterboegeri* Oréllis-Ribeiro et Bullard, 2013, *Selachohemecus olsoni* Short, 1954, *S. benzi* Bullard, Overstreet et Carlson, 2006;

Table 1. Fish blood flukes (Digenea: Aporocotylidae) described from North America since 2000.

Parasite	Host	Locality	Reference
<i>Acipensericola glacialis</i> Warren et Bullard, 2017	lake sturgeon, <i>Acipenser fulvescens</i> Rafinesque	Lake Winnebago, Wisconsin, USA	Warren et al. 2017
<i>Acipensericola petersoni</i> Bullard, Snyder, Jensen et Overstreet, 2008	American paddlefish, <i>Polyodon spathula</i> (Walbaum)	Mississippi River and Tennessee River (Mississippi River Basin), USA	Bullard et al. 2008
<i>Cardallagium anthicum</i> (as <i>Psettarium</i>) Bullard et Overstreet, 2006	cobia, <i>Rachycentron canadum</i> (Linnaeus)	Northern Gulf of Mexico ~50 km south/southeast of Ocean Springs, Mississippi, USA	Bullard and Overstreet 2006
<i>Cardicola currani</i> Bullard et Overstreet, 2004	red drum, <i>Sciaenops ocellatus</i> (Linnaeus)	Northern Gulf of Mexico, Davis Bayou, Mississippi Sound, Ocean Springs, Mississippi, USA	Bullard and Overstreet 2004
<i>Cardicola langeli</i> Bullard, 2013	sheepshead, <i>Archosargus probatocephalus</i> Walbaum	Northern Gulf of Mexico off Horn Island, Mississippi sound, Mississippi, USA	Bullard 2013
<i>Cardicola nonamo</i> Bullard, 2009	white seaperch, <i>Phanerodon furcatus</i> Girard	Eastern Pacific Ocean, Monterey Bay, California, USA	Bullard 2010
<i>Cardicola palmeri</i> Bullard et Overstreet, 2004	black drum, <i>Pogonias cromis</i> (Linnaeus)	Mississippi Sound off Point Cadet, Biloxi, Mississippi, USA	Bullard and Overstreet 2004
<i>Cardicola parvus</i> Bullard, Baker et de Buron, 2012	Atlantic croaker, <i>Micropogonias undulatus</i> (Linnaeus)	South Atlantic Bight off Cow Island, North Carolina, USA	Bullard et al. 2012
<i>Elaphrobrates euzeti</i> Bullard et Overstreet, 2003	red snapper, <i>Lutjanus campechanus</i> (Poey)	Northern Gulf of Mexico ~50 km south of Ocean Springs, Mississippi, USA	Bullard and Overstreet 2003
<i>Electrovermis zappum</i> Warren et Bullard, 2019	lesser electric ray, <i>Narcine bancroftii</i> (Griffith et Smith)	Northern Gulf of Mexico off Fort Morgan, Alabama, USA	Warren and Bullard 2019
<i>Elopicola franksi</i> Oréllis-Ribeiro et Bullard, 2017	tarpon, <i>Megalops atlanticus</i> (Valenciennes)	North Captiva Island and Bayboro Harbor, off Florida, Gulf of Mexico, USA	Oréllis-Ribeiro et al. 2017
<i>Elopicola nolancribbi</i> Bullard, 2014	lady fish, <i>Elops saurus</i> Linnaeus	Northern Gulf of Mexico off Ship Island, Mississippi sound, Mississippi, USA	Bullard 2014
<i>Gymnurahemecus bulbosus</i> Warren et Bullard, 2019	smooth butterfly ray, <i>Gymnura micrura</i> (Bloch et Schneider)	Northern Gulf of Mexico, Mobile, Alabama, USA	Warren et al. 2019
<i>Hyperandrotrema walterboegeri</i> Oréllis-Ribeiro et Bullard, 2013	shortfin mako shark, <i>Isurus oxyrinchus</i> Rafinesque	Northern Gulf of Mexico, Viosca Knoll, ~123 km south/southwest of Dauphin Island, Alabama, USA	Oréllis-Ribeiro et al. 2013
<i>Littorellicola billhawkinsi</i> Bullard, 2010	Florida pompano, <i>Trachinotus carolinus</i> (Linnaeus)	Northern Gulf of Mexico off Ship Island, Mississippi sound, Mississippi, USA	Bullard 2010
<i>Myliobaticola richardheardi</i> Bullard et Jensen, 2008	Atlantic stingray, <i>Hypanus sabinus</i> (Lesueur, 1824)	Northern Gulf of Mexico off Biloxi, Deer Island, Mississippi Sound, Mississippi, USA	Bullard and Jensen 2008
<i>Pearsonellum lemusi</i> Bullard, 2012	gag grouper, <i>Mycteroperca microlepis</i> (Goode et Bean)	North central Gulf of Mexico, Gulf of Mexico, Groper reef, ~80 km south of Dauphin Island, Alabama, USA	Bullard 2012
<i>Selachohemecus benzi</i> Bullard, Overstreet et Carlson, 2006	blacktip shark, <i>Carcharhinus limbatus</i> (Valenciennes)	Apalachicola Bay, Florida, USA Northern Gulf of Mexico, off Mississippi, USA	Bullard et al. 2006
<i>Achorovermis testisinosus</i> Warren et Bullard gen. et sp. n.	smalltooth sawfish, <i>Pristis pectinata</i> Latham	Eastern Gulf of Mexico off Naples, Florida, USA	present study

one infecting a holocephalan: *Chimaerahemecus trondheimensis* Van der Land, 1967, and one infects a batoid: *Gymnurahemecus bulbosus* (see Short 1954, Van der Land 1967, Maillard and Ktari 1978, Bullard et al. 2006, Oréllis-Ribeiro et al. 2013, Warren et al. 2019).

Shirai (1992, 1996) formalised two lineages of elasmobranchs: Galea (including Orectolobiformes, Lamniformes, and Carcharhiniformes) and Squala (including Chlamydoselachiformes, Hexanchiformes, Echinorhiniformes, Dalatiiformes, Centrophoriformes, Squaliformes, and all batoids). Last et al. (2016) recovered a phylogenetic tree to test batoid interrelationships wherein Rajidae was recovered sister to all other batoids. This contradicts Shirai's analysis that instead recovered Pristidae as such. More recently, Amaral et al. (2018) produced a mitogenomic phylogeny for the elasmobranchs and recovered Batoidea as sister to the Selachii (including Galeomorphii and Squalimorphii). This tree also recovers a clade including Rajoidei sister to Torpedinoidei that is sister to all other batoids.

Based on morphology, the blood flukes of selachians and batoids are likely monophyletic, evidently closely related, and likely share a recent common ancestor. For example,

species of *Hyperandrotrema* and species of *Selachohemecus* infect lamniform and carcharhiniform sharks (Galeomorphii), respectively, and species of both genera have C-shaped spines. *Ogawaia glaucostegi*, *M. richardheardi*, *O. heterovitelatum*, and *E. zappum* all infect batoids (Batoidea) and lack tegumental spines. However, *C. trondheimensis* and *G. bulbosus* are distinct by having C-shaped spines and infecting a holocephalan and a batoid, respectively.

Further, none of the nominal chondrichthyan blood flukes that have C-shaped spines have a curving testis like those infecting batoids (excluding *G. bulbosus*). Further still, *C. trondheimensis*, *H. cetorhini*, *H. walterboegeri*, and *G. bulbosus* have an oötype that is located posterior to all other genitalia, unlike *Selachohemecus* spp. that have an oötype that is dorsal to the ascending uterus (Van der Land 1967, Maillard and Ktari 1978, Bullard et al. 2006, Oréllis-Ribeiro et al. 2013, Warren et al. 2019). Additionally, *S. olsoni* and *S. benzi* differ from the nine other nominal chondrichthyan blood flukes by having an X-shaped (vs inverse U-shaped) intestine. This feature has been observed only in fish blood flukes infecting later branching ray-finned fishes (Actinopterygii) (see Bullard and Overstreet 2006, Bullard 2010). These morphological differences are

predictive of shared ancestry and so far agree with existing molecular phylogenetic analyses that recover monophyletic chondrichthyan blood flukes (Warren and Bullard 2019, Warren et al. 2019).

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