NEW GENUS AND SPECIES OF FISH BLOOD FLUKE (DIGENEA: APOROCOTYLIDEA ODHNER, 1912) FROM STOPLIGHT PARROTFISH, SPARISOMA VIRIDE (BONNATERRE, 1788), (LABRIDA: SCARINA) IN THE CARIBBEAN SEA

Stephen A. Bullard,∗ Ernest H. Williams, Jr.; and Lucy Bunkley-Williams†

Aquatic Parasitology Laboratory, Department of Fisheries and Allied Aquacultures, College of Agriculture, Auburn University, 203 Swingle Hall, Auburn, Alabama 36849. e-mail: ash.bullard@auburn.edu

Received 20 January 2012; revised 7 June 2012; accepted 11 June 2012.

1 To whom correspondence should be addressed.
‡ Department of Marine Sciences, University of Puerto Rico, P.O. Box 9000, Mayagüez, Puerto Rico 00861-9000.
§ Department of Biology, University of Puerto Rico, P.O. Box 9000, Mayagüez, Puerto Rico 00861-9000.

DOI: 10.1645/GE-3099.1

ABSTRACT: Primisanguis caribeensis n. gen., n. sp. (Digenaea: Aporocotylidae) infects the stoplight parrotfish, Sparisoma viride (Bonnaterre, 1788), (Labridae: Scarinae) in the Caribbean Sea off La Parguera (17°58’26”N, 67°02’47”W), Puerto Rico. It is most easily distinguished from other nominal aporocotylids by the combination of having an adult body that is approximately 5–10 times longer than wide, a sinistral posterolateral body protuberance, tegumental body spines that are straight (lacking recurved tip) and wrap dorsoventrally around the body margin, posterior ceca that are 2–5 times the anterior ceca length, a single testis that extends lateral to both the ceca and nerve cords, an ovary abutting the posterior margin of the testis, a symmetrical vitellarium and mediolateral vitelline duct, a post-cecal and post-gonadal ootype, and a proximal uterus that is extensively convoluted posterior to the ootype and which functions as a uterine seminal receptacle. The new genus lacks a spiny anterior sucker, pharynx, auxiliary external seminal vesicle, cirrus stylet, oviducal seminal receptacle, and Laurer’s canal. The new species is morphologically most similar to the other aporocotylids that have a posterolateral body protuberance, including species of Psittarium Goto and Ozaki, 1930, Aporocotyle Odhner, 1900, Ankistromeces Nolan and Cribb, 2004, Phthinomita Nolan and Cribb, 2006, and Lisoterricole Bullard, 2010, but it can be differentiated from them, at the least, by lack of a spiny anterior sucker in the adult and by the combination of having dorsoventral rows of tegumental spines, a testis dorsal to the posterior ceca, and a uterine seminal receptacle posterior to the ootype. The new species is the first aporocotylid reported from the Caribbean Sea or from a parrotfish of Sparisoma.

F ew fish blood flukes (Aporocotylidae Odhner, 1912) are known to infect parrotfishes. To date, only 3 species, all assigned to Braya Nolan and Cribb, 2006, have been described, and each of these infects a parrotfish from Australia’s Great Barrier Reef (Nolan and Cribb, 2006a), i.e., Braya psittacus Nolan and Cribb, 2006 from blue-barred parrotfish, Scarus ghobban Forskål, 1775, Braya yantschi Nolan and Cribb, 2006 from steephead parrotfish, Chlorurus microrhinos (Bleeker, 1854), and Braya juxi Nolan and Cribb, 2006 from bridled parrotfish, Scarus frenatus Lacepède, 1802. Herein, we describe a new species of Aporocotylidae from a parrotfish in the Caribbean Sea and propose a new genus to accommodate the new species. The new species is the first named blood fluke from a fish captured in the Caribbean Sea.

MATERIALS AND METHODS

Parrotfish were sampled opportunistically from commercial net fisher- men in La Parguera (17°58’26”N, 67°02’47”W), Puerto Rico, segregated by fish species, and necropsied to remove the heart, viscera, and gill. As a result of the collecting conditions in the fish market where fish were examined, all tissues from conspecific parrotfishes were fixed immediately in 10% neutral buffered formalin and pooled in respective plastic sample bags. Hence, the site of infection in the host, as well as the prevalence and intensity of infection, could not be obtained. Later, the formalin-fixed contents of each sample bag were examined for the presence of fish blood flukes with the aid of a dissecting microscope. Aporocotylid specimens for staining purposes were transferred to, and held in, a vial of 5% neutral buffered formalin for at least 48 hr, then rinsed thoroughly with distilled water and cleaned with fine brushes to remove any debris. These specimens were stained overnight in Van Cleave’s hematoxylin with several additional drops of Ehrlich’s hematoxylin; they were made basic at 70% ethanol with lithium carbonate and butyl-amine, dehydrated, cleared in clove oil, and permanently immersed in hexamethyldisilazane for 30 min, air dried for 45 min, and sputter-coated with 15 nm gold palladium (Bullard and Jensen, 2008). Illustrations of stained, whole-mount specimens were made with the aid of a Leica DM-2500 (Leica, Wetzlar, Germany) equipped with differential interference contrast optical components and a drawing tube. Measurements were obtained using a calibrated ocular micrometer and are reported in micrometers (μm). If fewer than 7 specimens were measured, the number measured is indicated in parentheses following the range.

Common and scientific names for fishes follow Eschmeyer (2010). Higher-level fish classification and nomenclature follows Nelson (2006) for ordinal relationships and considered Bellwood (1994), Bernardi et al. (2000), and Westneat and Alfaro (2003) for taxonomy of parrotfishes, i.e., parrotfishes belong to Labridae. Nomenclature for Aporocotylidae follows Bullard et al. (2009). Brown (1956) was used to help construct the genus name and specific epithet. Specimens of related aporocotylids were borrowed from the United States National Parasite Collection (Beltsville, Maryland, USA; USNPC) and the holotype and paratypes of the new species were deposited there.

DESCRIPTION

Primisanguis n. gen.
(Figs. 1–6)

Diagnosis: Body of adult flat, approximately 5–10 × longer than wide, bearing sinistral posterolateral body protuberance in posterior 1/5 of body, spined. Tegumental body spines distributed along lateral body surface from level of mouth to posterior body end, distributed in rows wrapping dorsoventrally around body margin (not entirely ventrolateral), straight, lacking recurved tip. Rosethorn-shaped spines and marginal tegumental peduncles supporting spines lacking. Nervous system comprising nerve cords and anterior commissure. Spheroidea anterior sucker absent in adult. Mouth not associated with obvious muscular or spiny anterior sucker. Pharynx absent. Intestine comprising paired anterior and posterior ceca joining medially and formingecal intersection, lacking diverticula or secondary rami; posterior ceca asymmetrical; posterior ceca 2–5 times anterior ceca length. Testis single, lacking deep lobes, dorsal to ceca, approximately 4 times longer than wide, occupying breadth of body from cecal intersection to distal tips of posterior ceca and extending lateral to ceca and nerve cords. Seminal vesicle a laterally-expanded distal portion of vas deferens, post-cecal, post-gonadal, directing sinistrad. Axillary external seminal vesicle and stylet associated with cirrus absent. Ovary single, medial or slightly dextral, lacking deep lobes, dorsal to tips of posterior ceca, post-testicular, abutting posterior margin of testis. Oviduct an elongate and narrow duct emanating from postero medial margin of ovary, uninterrupted by vitelline duct immediately before ootype; ooviducal...
Seminal receptacle not evident. Vitellarium follicular, symmetrical, extending from about level of anterior nerve commissure to ovary, dorsal and ventral to gonads and alimentary tract. Primary vitelline duct medial. Ootype spheroid, dextral, post-cecal, post-gonadal. Laurer’s canal absent. Uterus highly convoluted, comprising proximal and distal portions; proximal portion of uterus an extensively convoluted mass residing between ootype and ducts (arms) of excretory system, having dense aggregation of sperm and functioning as uterine seminal receptacle; distal portion of uterus narrow and thin-walled relative to proximal portion of uterus. Metraterm sinistral, post-cecal, post-testicular. Female genital pore dorsal, anteromedial to male genital pore.

**Differential diagnosis:** Adult body 5–10 times longer than wide; sinistral posterolateral body protuberance present. Tegumental body spines straight, distributing in rows wrapping dorsally. Posterior ceca 2–5 times anterior ceca length. Testis single, distributing from cecal intersection to tips of posterior ceca, lateral to ceca and nerve cords. Ovary single, abutting posterior margin of testis. Vitellarium symmetrical. Ootype post-cecal, post-gonadal. Proximal uterus extensively convoluted posterior to ootype and functioning as uterine seminal receptacle. Spheroid anterior sucker, pharynx, auxiliary external seminal vesicle, cirrus stylet, and Laurer’s canal absent.

**Taxonomic summary**

Type and only known species: *Primisanguis caribbeanensis* n. sp.

Etymology: The genus name is intended to emphasize that it is the first (*primus*) fluke reported from the blood (*sanguis*) of a fish captured from within the Caribbean Sea.

**Figures 1–2.** *Primisanguis caribbeanensis* n. gen., n. sp. (Digenea: Aporocotylidae) from stoplight parrotfish, *Sparisoma viride* (Bonnaterre, 1788), (Labridae: Scarinae) in the Caribbean Sea off La Parguera (17°58’26″N, 67°02’47″W), Puerto Rico. (1) Body of holotype (USNPC No. 105936) showing mouth (m), nerve commissure (nc), esophagus (e), sinistral anterior cecum (sc), nerve chord (ncd), esophageal gland (eg), cecal bifurcation (cb), testis (t), primary vitelline duct (vt), ovary (o), female pore (fp), posterolateral protuberance (pp), ootype (oo), and posterior extent of the uterus (pu); ventral view. (2) Genitalia showing location of testis (t), vasa efferentia (ve), distal tips of dextral (dxc) and sinistral (sc) posterior ceca, ovary (o), vitelline duct (vt), oviduct (ov), ootype (oo), glandular region (gr), uterine seminal receptacle (usr), excretory vesicle (ev), metraterm (mt), female pore (fp), seminal vesicle (sv), and posterolateral protuberance (pp), ventral view.
**Primsanguis caribbeanensis** n. sp. (Figs. 1–6)

Description of adult (measurements and illustrations based on 7 whole-mounted adult specimens comprising the holotype, paratype, and 5 vouchers plus 3 sputter-coated adult specimens): Body posterior end more broadly rounded than anterior end, 4,320–6,846 long, 489–1,060 wide or 5.7–8.8 times longer than wide, with widest portion of body in posterior body half, lacking sensory papillae on dorsum; posterolateral body protuberance approximately 200 wide at base, reflected dorsally, post-cecal, post-testicular, residing at level of posterior margin of ovary (Figs. 1, 2, 6). Tegumental body spines each 5–7 long, each approximately 1 wide, extending approximately 2 from tegument; spine rows numbering 372–453 (4) per side of body or a total of 744–906 (4) rows, with 6–21 spines per row in anterior region of body, 6–27 spines per row in medial region of body, and 10–17 spines per row in posterior region of body (Figs. 1, 3–5). Nerve cords conspicuous in lateral and anterior regions of body, 10–20 in maximum width at mid-body, 75–144 from lateral body margin at mid-body, becoming confluent with paired cord posteriorly 25–80 (3) or 1% of body length from posterior body end; anterior nerve commissure 214–249 (6) or 3–4% of body length from anterior body end, 99–149 (6) across width of worm, 15–25 (6) in maximum diameter (Fig. 1). Mouth ventral, subterminal, 7–15 in diameter, not associating with spines visible with light microscopy in adult (Figs. 1, 3). Esophagus medial, slightly sinusous to straight, extending posteriorly along midline 1,522–2,185 or 28–35% of body length, approximately equal in width anteriorly and posteriorly, 15–50 (6) wide just posterior to mouth, 25–65 (6) just anterior to cecal intersection, with laterally expanded medial portion; medial portion of esophagus residing between tips of anterior ceca; 55–124 (6) in maximum width or 0.9–3.3 times width of anterior portion of esophagus, coursing through main body of esophageal gland; esophageal gland 1,301–1,669 (3) long or 21–25% of body length or 75–87% of esophagus length, 172–174 (3) wide or 21–25% of body width (Fig. 1). Intestine containing refractive, brownish-gold granular material in lumen; anterior ceca 712–1,326 or 11–21% of body length, extending anteriorly >1/2 esophagus length; 40–80 wide or 36–63% of esophagus maximum width, not extending laterally beyond ventrolateral nerve cords; posterior ceca 1,841–3,504 long or 45–52% of body length, 2.5–4.5 times longer than respective anterior cecum, with left cecum longer in all 7 specimens studied, 25–99 wide anteriorly or 0.4–1.3 times wider than respective anterior cecum; distal swelling of posterior ceca expanding to 45–149 or 1.4–3.0 times wider than cecum; post-cecal distance 811–1,277 or 16–20% of body length (Fig. 1).

Testis 2,136–3,260 long or 39–49% of body length, 652–897 wide or 2.6–4.6 times longer than wide or 73–85% of body width, 80–100% length of posterior ceca; post-testicular space 908–2,200 or 17–32% of body length (Figs. 1, 2). Vasa efferentia highly branched, narrow, thin-walled, coalescing ventrally in posterior region of testis to form vas deferens; vas deferens extending directly posteriad 372–497 (6) from posterior margin of testis, 15–40 (6) in maximum width, primarily medial, crossing midline before curving more sharply and connecting with seminal vesicle; seminal vesicle 273–517 long or 6–8% body length, 65–149 wide or 6–20% of body width, with wall 3–5 (6) thick, orienting approximately perpendicular to long axis of body; intensely basophilic glandular cells enveloping seminal vesicle from insertion of vas deferens to distal end. Cirrus 149–249 long or 38–62% of seminal vesicle maximum length, 99–199 wide or 1–1.7 times longer than wide; male genital pore at distal end of posterolateral body protuberance, 614–1,141 or 14–18% of body length from posterior body end.

Ovary slightly asymmetrical with lateral region extending more or less posteriorly, lacking lobes and having more or less smooth borders, 174–497 (6) long or 3–8% of the body length, 249–507 (6) wide or 28–48% of the body width, as wide or slightly more narrow than testis maximum width, residing in posterior 1/4 of body; post-ovarian space approximately 800 long or 13% of body length (Figs. 1, 2). Oviduct extending posteriorly primarily in dextral half of body, curving sharply at mid-length, but lacking clear evidence of oviducal seminal receptacle, 491–597 long, 35–50 in maximum width. Primary vitelline duct thin tube extending directly posteriorly along midline, ventral to gonads, tracking from vicinity of cecal intersection, extending 497–845 (6) posteriorly from posterior margin of ovary and connecting with oviduct near midline or slightly dextrally and immediately before ootype, approximately equal in diameter for entire length, but having slightly laterally expanded distal portion, 25–75 (6) in maximum width. Ootype 62–109 in diameter, centering on obvious Mehlis’ gland 149–199 (5) in diameter, posterior to posterolateral protuberance, with obvious ducts from Mehlis’ gland converging on wall; post-ovotype space <10% of body length from posterior end. Uterus extending posteriorly from ootype to 60–149 or 1–2% of body length from posterior body end, dividing into proximal (descending) and distal (ascending) portions; proximal (descending) portion of uterus extensively coiled in posterior region of body between ootype and excretory bladder, looping at least 4 times in that region, 60–149 in maximum width; distal (ascending) portion of uterus extending sinuously anteriad from proximal portion of uterus in sinistral body half, traversing midline and narrowing considerably, convoluted posterolateral to ovary, apparently lacking sperm, approximately 100 in maximum width; contents of distal uterus

**Figures 3–6.** Primsanguis caribbeanensis n. gen., n. sp. (Digenea: Aporocotylidae) from stoplight parrotfish, Sparisoma viride (Bonneraterre, 1788), (Labridae: Scarinae) in the Caribbean Sea off La Parguera (17°58′ 26″N, 67°02′ 47″W). Puerto Rico, scanning electron micrographs. (3) Anterior end showing mouth (arrow) and anterior extent of lateral tegumental spine rows, ventral view. Note that the crenulation of ventral body surface is an artifact. (4) Dorso-ventral rows (arrows) of lateral tegumental spines, dorsal view. (5) Ventral view of lateral tegumental spine rows showing detail of the distal tips of spines, which are straight rather than recurved. Note that some spines have detached, leaving pore-like holes in the tegument (arrows). (6) Sinistral body margin and dorso-ventral rows showing posterolateral protuberance (pp), area of protuberance lacking dorsolateral rows of spines (arrow), and vicinity of female genital pore (fp), dorsal view.
comprising oblong masses of ova 25–47 long and 25–37 wide, having an estimated 5–15 ova per mass. Metraterm at level of posterior margin of ovary, shorter than seminal vesicle, anterior to seminal vesicle, thin-walled and weakly muscular. Female genital pore 771–1,277 or 16–20% of body length from posterior body end, 17–35 in diameter. Excretory vesicle Y-shaped, 25–50 (4) long, 12–25 (4) wide, with wall 3 (2) thick, with pore 3–7 (2) in diameter, opening 25–27 (2) from posterior body end.

**Taxonomic summary**

*Type and only known host:* Sparisoma viride (Bonnetatre, 1788) (Labridae: Scarinae), stoplight parrotfish.

*Type locality:* Caribbean Sea off La Parguera (17°58′26″N, 67°02′47″W), Puerto Rico.

*Specimens deposited:* United States National Parasite Collection (Beltsville, Maryland), USNPC No. 105936 (holotype) and USNPC No. 105937 (paratype).

*Etymology:* The specific epithet is for the sea that includes the type locality.

**Remarks**

Among the most obvious morphological characters of the new genus is the postero lateral body protuberance associated with the male genital pore. This is a feature unique to the male genitalia of fish blood flukes, with no comparable tegumental extension being associated with the female genitalia in any nominal species. Homology of these protuberances is indeterminate but, nevertheless, the presence or absence of this feature seems helpful in differentiating aporocotylid genera. Given the intuitive adaptive significance of a tegumental extension that increases the "copulatory reach" of a worm, it is easy to envision how such a protuberance could have evolved multiple times, independently, within a lineage of flukes that must remain firmly attached ventrally to the endothelial lining of blood vessels during copulation. In specific, *Primisanguis* n. gen. and *Psettarium* Goto and Ozaki, 1930 (see Goto and Ozaki, 1929; Manter, 1940; Bullard and Overstreet, 2006), and probably at least some species of *Aproctocotyle* Odhner, 1900 (see Villalba and Fernández, 1986), *Ankistronecetes* Nolan and Cribb, 2004 (see Nolan and Cribb, 2004), *Phthinomita* Nolan and Cribb, 2006 (see Nolan and Cribb, 2006b), *Skouplekia* Alma-Bermejo, Montero, Raga, and Holzer, 2011, and *Littorellulica* Bullard, 2010 (see Holmes, 1971; Bullard, 2010a) have a body protuberance about the male genital pore. *Braya* Nolan and Cribb, 2006 is the only aporocotylid genus previously known to include species that infect parrotfishes, and 3 species, *B. yantschi* Nolan and Cribb, 2006, may possess a protuberance (fig. 53 of Nolan and Cribb (2006b)).

*Primisanguis* can be easily differentiated from *Psettarium japonicum* (Goto and Ozaki, 1929) Goto and Ozaki, 1930 (type species of *Psettarium*) and other species of *Psettarium* (see Bullard and Overstreet, 2006) by having a testis that extends from the cecal intersection to the anterior margin of the ovary, i.e., *P. japonica* has a wholly post-cecal testis. *Primisanguis* differs from *Ankistronecetes*, *Phthinomita*, and *Skouplekia*, at the very least by having an adult body approximately 5–10 times longer than wide, a single testis that is approximately symmetrical and primarily occupies the space between the posterior ceca, a spheroid ootype, and a uterus that extends posterior and anterior to the ootype (Short, 1953; Bullard and Overstreet, 2004; Bullard, 2010b; McVay et al., 2011; Bullard et al., 2012). However, and although several other morphological features are distinct (see above), the new genus is most easily differentiated from *Cardicola* by having a relatively large adult body, a postero lateral body protuberance, tegumental spine rows that wrap dorsoventrally around the body margin, and straight spines that lack recurved distal tips. *Cardicola* is in need of taxonomic revision but includes species that have ventrolateral tegumental spine rows and that lack a posterolateral body protuberance.

**DISCUSSION**

The new species does not resemble the blood flukes that infect Australian parrotfishes (i.e., species of *Braya*, see Remarks), suggesting that Atlantic and Pacific parrotfishes were acquired as hosts independently. *Sparisoma* and the related genera *Cryptotoxus* and *Nicholsina* include species that range in the tropical regions of the Atlantic Ocean and immediately adjacent waters. Bernardi et al. (2000) hypothesized that these parrotfishes may have been geographically isolated from Indo-Pacific parrotfishes by closure of the eastern Tethys Sea 13–15 mya; Bellwood (1994) postulated that the common ancestor of *Sparisoma* spp. diverged from other parrotfishes approximately 5–14 mya. The present report, however, is only the first fish blood fluke reported from the Caribbean Sea, and future records from parrotfishes there, and elsewhere, may help shed light on the biogeography of the parasite group.

The new species seems host-specific to the stoplight parrotfish, *Sparisoma viride*, but its occurrence in Caribbean Sea parrotfishes was low. We examined 8 parrotfish species in 2 genera from the Caribbean Sea and Gulf of Mexico subsequent to the initial discovery of infections in stoplight parrotfish but did not detect another infection, i.e., 1 emerald parrotfish, *Nicholsina usta usta* (Valenciennes, 1840), 1 midnight parrotfish, *Sparus coelestins* Valenciennes, 1840, 6 rainbow parrotfish, *Sparus guacamaia* Cuvier, 1829, 2 princess parrotfish, *Sparus taeniopeterus* Desmarest, 1831, 1 queen parrotfish, *Sparus vetula* Bloch and Schneider, 1801, 5 retialt parrotfish, *Sparisoma chrysopterum* (Bloch and Schneider, 1801), 1 redfin parrotfish, *Sparisoma rubripinne* (Valenciennes, 1840), and 57 stoplight parrotfish. It is, however, typical for some blood fluke infections to have an extremely low prevalence in their definitive hosts, and these numbers offer little insight into the potential species diversity of aporocotylids in parrotfishes off North America and within the Caribbean Sea. Noteworthy, however, is that one of us (EHW, pers. obs.) has necropsied several thousand fish representing hundreds of Caribbean species over approximately 38 yr, and the present report is the only aporocotylid infection observed to date. Perhaps this indicates that the Caribbean aporocotylid fauna may not be as diverse as that of the Indo-Pacific and Great Barrier Reef of Australia (Nolan and Cribb, 2006b).

**ACKNOWLEDGMENTS**

We thank Eric Hoberg and Pat Pilott (both at USNPC) for ensuring the safe deposition of our type materials and Kirsten Jensen (University of Kansas, Lawrence, Kansas) for taking the SEM micrographs. This work is a contribution of the Southeastern Fish Parasite and Disease Cooperative Project and was supported in part by National Science Foundation-Division of Environmental Biology grant numbers NSF-DEB 1112729, NSF-DEB 1051106, and NSF-DEB 1048523 to SAB.

**LITERATURE CITED**


Bernard, G., D. R. Robertson, K. E. Clifton, and E. Azzuro. 2000. Molecular systematics, zoogeography, and evolutionary biology of...


Bullard, S. A. 2010a. Littorellicola billhawkinsi n. gen., n. sp. (Digenea: Aporocotylidae) from the myocardial lacunae of Florida pompano, Trachinotus carolinus (Carangidae) in the Gulf of Mexico, with a comment on interrelationships and functional morphology of intrabrachial aporocotylids. Parasitology International 59: 587–598.


