A NEW APOROCOTYLID (DIGENEA) SPECIES FROM BLOOD VASCULAR SYSTEM OF GAG GROUPER, *MYCTEROGERCA MICROLEPIS* (PERCIFORMES: SERRANIDAE), OFF ALABAMA, WITH AN EMENDATION OF *PEARSONELLUM* OVERSTREET & KØIE, 1989

Stephen A. Bullard
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
A NEW APOROCOTYLID (DIGENEA) SPECIES FROM BLOOD VASCULAR SYSTEM OF GAG GROUPER, MYCTEROPERCA MICROLEPIS (PERCIFORMES: SERRANIDAE), OFF ALABAMA, WITH AN EMENDATION OF PEARSONELLUM OVERSTREET & KØIE, 1989

Stephen A. Bullard
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

ABSTRACT. Pearsonellum lemusi n. sp. (Digenea: Aporocotylidae) infects the blood vascular system of the gag grouper, Mycteropecra microlepis (Perciformes: Serranidae), in the north central Gulf of Mexico, approximately 80 km south of Dauphin Island, Alabama (29°34’09”N, 88°22’16”W). The new species can be most easily differentiated from its only congeners Pearsonellum conventum Overstreet and Köie, 1989 (type species) and Pearsonellum pygmaeus Nolan and Cribb, 2004, both of which infect Australian serranids, by the combination of having a large adult body (3,237 × 370 μm), a cecal intersection comprising an elongated medial channel, anterior ceca >10% of total body length, ovary narrower than testis, and pre-ovarian uterus not looping between testis and ovary. The embryonated eggs of the new species infect gill epithelium, are spheroid, and measure 25–30 μm in diameter. Sympatric Gulf of Mexico serranids were negative for a porocotylid infections: coney, Cephalopholis fulva (n = 1); Nassau grouper, Epinephelus striatus (3); red grouper, Epinephelus morio (32); yellowedge grouper, Epinephelus flavolimbatus (1); rock hind, Epinephelus adscensionis (1); red hind, Epinephelus guttatus (2); Warsaw grouper, Epinephelus nigritus (3); gray seabream, Cephalopholis cruentata (1); black grouper, Mycteropecra bonaci (1), and tatter, Serranus phoebe (2). The new species is the first a porocotylid described from a serranid outside of the southwestern Pacific Ocean. The diagnosis of Pearsonellum Overstreet and Köie, 1989 is herein emended to include anterior sucker having concentric rows of spines anterior to mouth, pharynx absent, esophagus length <1/2 total body length, vas deferens connecting with cirrus sac anteromedially, ovary occupying posterior 1/4–1/3 of body, primary vitelline duct dextral, and oviducal seminal receptacle extending posteriorly in parallel with lateral body margin, not transverse nor constricted anteriorly or posteriorly by sharp bends or kinks.

Only 3 fish blood flukes (Aporocotylidae Odhner, 1912) of 2 genera have been identified from sea basses (Serranidae) (Overstreet and Köie, 1989; Overstreet and Thulin, 1989; Nolan and Cribb, 2004). This host family currently includes approximately 64 genera and 475 species assigned to the 3 subfamilies, i.e., Serraninae, Anthiinae, and Epinephelinae (see Nelson, 2006). Most are oblique reef species with local or regional commercial and recreational value, and they collectively range worldwide in tropical and temperate latitudes. However, little information is currently available on their blood parasites. Herein, we describe the first sea bass a porocotylid, a new species of Pearsonellum Overstreet and Köie, 1989, reported from the Gulf of Mexico and from outside the southwest Pacific Ocean.

MATERIALS AND METHODS
Fish were captured by hook and line from the north central Gulf of Mexico, killed by spinal severance, and kept on ice until returning to the laboratory. Infected fishes first were identified and prioritized for necropsy by excising 5–10 gill filaments, placing them on a slide with a small amount of seawater, coverslipping them, and examining them under ×400 magnification with the aid of a compound microscope DM-2500; Leica Microsystems, Inc., Deerfield, Illinois) equipped with differential interference contrast optical components. Gills infected with a porocotylid eggs were noted, and those fish were examined first to ensure that adult a porocotylids could be removed, heat killed, and immersed in fixative before degrading significantly. The branchial vessels, heat, kidney, viscosa, and body cavity wash were isolated in separate glass bowls; immersed in physiologic saline; and examined for the presence of adult a porocotylids. The fluid from each glass dish was then poured into a settling column, left undisturbed for 10–15 min, and subsequently examined with aid of a stereomicroscope by transferring sediment to a glass petri dish. Flukes for light microscopy were prepared following Bullard (2010a, 2010b). Measurements were obtained by using a calibrated ocular micrometer and are reported in micrometers followed by the number of specimens measured in parentheses. Common and scientific names for fishes follow Froese and Pauly (2010). Higher level fish classification and nomenclature follow Nelson (2006). Nomenclature for Aporocotylidae follows Bullard et al. (2009). Related a porocotylid specimens were borrowed from the U.S. National Parasite Collection (USNPC, Beltsville, Maryland).

DESCRIPTION
Pearsonellum Overstreet & Köie, 1989, emended (Figs. 1–7)

Diagnosis: Adult body spatulate, 5–7× longer than wide, dorsoventrally flattened, lacking posteralateral protuberance associated with male genital pore, ventrally concave, spined; tegumental spines minute, lacking peduncle, with recurved tip, distributing in equally spaced ventrolateral transverse rows along entire length of adult body. Rosethorn-shaped spines absent. Anterior sucker small, weakly muscular, spinous; spines of anterior sucker minute, arranged in concentric rows anterior to mouth; mouth on medioventral surface of anterior sucker. Pharynx absent. Esophagus straight, having total length <1/2 total body length. Intestine with paired anterior and posterior ceca joining medially at midbody; cecal intersection approximately X-shaped; posterior ceca 2–5× longer than anterior ceca. Testis single, primarily dorsal to posterior ceca, ~3–5× longer than wide. Vas deferens connecting with cirrus sac anteromedially. Seminal vesicle present. Auxiliary external seminal present. Cirrus sac well developed, spheroid, sinistral, enveloping seminal vesicle. Male genital pore sinistral, post-ovarian, post-cecal. Ovary single, medial or slightly dextral, post-cecal, post-testicular, narrower than testis, occupying posterior 1/4–1/3 of body. Vitellarium follicular, extending from anterior nerve commissure posteriorly to ends of posterior ceca; primary vitelline duct dextral. Oviduct dextral, extending posteriorly from posteralateral surface of ovary; oviducal seminal receptacle extending posteriorly in parallel with lateral body margin, not transverse (cf. Cardicola spp.) or constructed anteriorly or posteriorly by sharp bends or kinks in duct. Ootype spheroid, posterior to genital pores. Laurer’s canal absent. Uterus extending anterior to ovary, curving ventrally and connecting with metraterm; metraterm conspicuously muscular or not, lateral to ovary. Female genital pore opening at level between male genital pore and ovary. Excretory vesicle small, with 2 distensible anteriorly directed arms and pore; pore dorsal, subterminal. Adults in blood vascular system of sea basses (Serranidae).

Tegumental spines in rows, lacking peduncles. Anterior sucker present, having concentric spine rows anterior to mouth. Esophagus <1/2 body length. Intestine X-shaped; posterior ceca longer than anterior ceca. Testis primarily dorsal to posterior ceca. Auxiliary external seminal vesicle

Received 24 June 2011; revised 17 October 2011; accepted 21 October 2011.
DOI: 10.1645/GE-2901.1

J. Parasitol., 98(2), 2012, pp. 323–327
© American Society of Parasitologists 2012

**Taxonomic summary**

Type species: *Pearsonellum corventum* Overstreet & Koie, 1989, adults from ventricle of heart of leoparded coral grouper, *Plectropomus leopardus* (Lacepède, 1802) (type host); longfin grouper, *Epinephelus quoyanus* (Valenciennes, 1830); white-streaked grouper, *Epinephelus ongus* (Bloch, 1790); and honeycomb grouper, *Epinephelus merra* Bloch, 1793 (see records in Overstreet and Koie, 1989), in addition to blackspotted coral grouper, *Plectropomus laevis* (Lacepède, 1801); maori grouper, *Epinephelus undatostriatus* (Peters, 1866); and chocolate hind, *Cephalopholis boenak* (Bloch, 1790) (see records in Nolan and Cribb, 2004), from the Great Barrier Reef, southwest Pacific Ocean, Australia.

Other species: *Pearsonellum pygmaeus* Nolan and Cribb, 2004, adult specimens from ventricle of the heart of humpback grouper, *Cromileptes altivelis* (Valenciennes, 1828) (type host), from the Great Barrier Reef, southwest Pacific Ocean, Australia.

**Remarks**

The concentric rows of minute spines reported herein are confirmed in the new species as well as *P. pygmaeus* (see Nolan and Cribb, 2004), but they were not included in the original description of the type species, *P. corventum*. Previous results indicate that these spines in other aporocotylids are extremely vulnerable to fixation artifact and may subsequently become detached (Bullard, 2010a). It is possible they may not be present in both juvenile and adult specimens of an aporocotylid species, and further challenging their discovery is that they are minute. However, given the uncanny morphological similarities among *P. corventum* and *P. pygmaeus*, we suspect that *P. corventum* indeed has a spiny anterior sucker. The presence of these spines needs confirmation in newly collected specimens that can be heat-killed after being studied alive. Esophagus length:total body length has been used as a distinguishing feature among *Pearsonellum* spp. The total range of proportions for species of *Pearsonellum* is 29–45%, <1/2 total body length. The new species has an esophagus that is 31–34% of body length. Esophagus length is 29–44% (Overstreet and Koie, 1989) or 35–41% (Nolan and Cribb, 2004) of total body length in *P. corventum* and 44–45% of total body length in *P. pygmaeus* (see Nolan and Cribb, 2004).

Regarding distinguishing features of the male genitalia shared among *Pearsonellum* spp., although seemingly a slight morphological feature, species of *Pearsonellum* have a vas deferens that connects to the anteromedial aspect of the cirrus sac and internal seminal vesicle. This character differentiates them from other aporocotylids, perhaps linked to the presence of an auxiliary external seminal vesicle. Neither of the specimens of the new species studied herein showed strong evidence of the presence of an auxiliary external seminal vesicle; however, based on the relatively poor condition of these specimens and the low sample size, it is expected that the presence of an auxiliary external seminal vesicle will be confirmed upon gathering better quality specimens that may be studied alive using a compound microscope.

The ovary and uterus of *Pearsonellum* spp. are also useful for differentiating the genus from other morphologically similar and closely related aporocotylids. The ovary is medial or slightly dextral and narrower than the testis, and the uterus extends anterior to the ovary in all known species. The distribution of the vitellarium in aporocotylids is sometimes a useful generic feature, but it seems slightly variable among *Pearsonellum* spp., indicating this feature should be used with caution, or not at all. The type species, *P. corventum*, has a vitellarium that extends from the anterior nerve commissure to the distal ends of the posterior ceca (Nolan and Cribb, 2004; see below). The orientation of the oviduct and its seminal receptacle in *Pearsonellum* species is also useful in differentiating these species from other telost aporocotylids. The oviduct extends directly posterior to the posterior margin of the ovary, and its seminal receptacle is straight, not constricted by sharp kinks anteriorly or posteriorly. In species of *Candiviola* Short, 1953, for example, the oviduct can be transverse and its seminal receptacle can be demarcated by sharply convex bends or “kinks.” Such a receptacle oftentimes appears as a significantly expanded bulb located immediately posterior to the ovary (Bullard and Overstreet, 2004; Bullard, 2010a; Bullard et al., 2012; McVay et al., 2011).

**Pearsonellum lemuri n. sp.**

(Figs. 1–7)

Description of adult (measurements and illustrations based on 2 whole-mounted adult specimens comprising the holotype and paratype): Body 2,925–3,237 (2) long, 440–570 (2) wide, or 5.7–6.7% longer than wider, widest at level of cephalic intersection (Fig. 1). Cephalic spines approximately 7–11 long (Fig. 2); spine rows numbering 432 (1) per side of body or a total of 864 rows, 13–15 in breadth at level of midbody (Figs. 2, 5). Nerve cords 18 (2) in width at level of midbody, 65–75 (2) from lateral body margin at level of midbody, joining posteriorly 43 (1) or 1% of body length from posterior end; anterior nerve commissure dorsal, 153–260 (2) or 5–8% of body length from anterior body end, 75 (2) or 13–17% of body width across width of worm; secondary branches and dorsolateral nerve cord not evident (Fig. 1). Anterior sucker 25–45 (2) in width or 6–8% of maximum body width (Figs. 1, 4); anterior sucker spines <1 (2) long, arranged in 6 concentric rows anterior to mouth; mouth 15–20 (2) from anterior margin of anterior sucker, 3 (2) in diameter. Esophagus 897–1,092 (2) long or 31–34% of body length, with wall 3 (2) thick near mouth and 4–5 (2) thick in posterior region, coursing through esophageal gland; esophageal gland diffuse, nearly indistinguishable in whole-mounted specimens of maximum body width. Elongated median cephalic blood vessel connecting anterior and posterior ceca 70–110 (2) long or 2–3% of body length or 8–10% of esophagus length, with wall 5–8 (2) thick; anterior ceca 410–507 (2) long or 38–57% of esophagus length or 13–17% of body length, 30–60 (2) wide or 7–11% of body width, extending directly anteriad from cephalic intersection, not extending laterad beyond ventrolateral nerve cords, lacking diverticula; posterior ceca 920–1,050 (2) long or 92–113% of esophagus length or 31–35% of body length, 25–40 (2) wide or 6–7% of body width, appearing slightly more narrow than anterior ceca, extending directly posteriorly in parallel with lateral body margins, not extending laterally beyond ventrolateral nerve cords, lacking diverticula or secondary rami, neither looping nor extensively curving laterally, lacking swollen or laterally expanded distal extremities (Fig. 1). Testis with anteromedial margin extending more anteriorly than anterodorsal margin, 1,100–1,404 (2) long or 38–43% of body length, 228–380 (2) or 3.7–5.5% longer than wide or 50–67% of body width, an interconnecting network of testicular tissue and vasa efferentia, not extending laterally beyond ventrolateral nerve cords, dorsal to ceca and posterior region of esophagus, extending from level anterior of cephalic intersection to level immediately posterior to distal tips of posterior ceca (Figs. 1, 3). Post-testicular space 990–1,000 (2) or 31–34% of body length (Fig. 1). Vasa efferentia highly branched, 10–13 (2) wide, thin-walled, coalescing ventrally in posterior region of testis to form vas deferens; vas deferens extending posteriorly 450–700 (2) or 15–22% of body length from posterior margin of testis, 8–25 (2) in maximum width, medial, straight, curving sinistrad in distal portion (Figs. 1, 3). Auxiliary external seminal vesicle not clearly evident (likely due to poor fixation or lack of abundant sperm in vesicle). Cirrus sac 500 (2) or 15–17% of body length from posterior body end, 138–163 (2) long, 80–108 (2) wide, 1.5–1.7x longer than wide, thick-walled, with wall incorporating ovod glandular cells, containing relatively large seminal vesicle (Figs. 1, 3). Everted cirrus 60 (2) long, 28 (2) wide, ~2 × longer than wide (Fig. 3). Male genital pore 288–365 (2) from dextral body margin, 85–90 (2) from sinistral body margin, opening 500 (2) or 15–17% of body length from posterior body end (Fig. 3). Ovary lobed, 220–240 (2) wide or 7–8% of body length, 170 (2) wide or 30–39% of body width, 1.3–1.4x longer than wide, near sinistral body margin. Post-ovarian space 750–820 (2) long or 23–28% of body length (Figs. 1, 3). Vitelarium occupying region from anterior nerve commissure to posterior margin of testis, primarily dorsal to gonads and alimentary tract (Fig. 1); primary vitelline duct a thin tube extending directly posteriorly from oviduct, passing into vitelliniferous lateral half of body near posterior margin of testis, ventral to oviduct, connecting with oviduct near midline or slightly dextrally. Oviduct 500–550 (2) long, 20–38 (2) wide in proximal portion, 48 (2) wide in medial and distal portion, curving distally toward midline (Fig. 3). Ootype 35 (1) long, 263–375 (2) or 8–13% of body length from posterior body end, enveloped by a Mehlis’ gland 100 in diameter, posterior to vitellarium in lateral half of body near posterior margin of testis. Oviduct extending anteriad from ootype along midline to 650–850 (2) or 22–26% of body length before curving toward sinistral...
body margin, 40–75 (2) wide, having densely packed developing ova within lumen (Figs. 1, 3, 6); metraterm extending posteriad 450–560 (2), 125–225 (2) wide or 3.0–3.1 \( \times \) width of uterus (Figs. 1, 3). Female genital pore 600–670 (2) or 19–23% of body length from posterior body end. Excretory system not evident. Eggs in branchial epithelium spheroid, 25–30 in diameter (Fig. 7).

**Taxonomic summary**

*Type and only known host:* *Mycteroperca microlepis* (Goode and Bean, 1879) (Perciformes: Serranidae), the gag grouper.

*Site in host:* Adults infecting blood vascular system; eggs with developing miracidia infecting gill epithelium and afferent branchial arterioles.

*Type locality:* Groper Reef (29°34’9.00”N, 88°22’16.00”W), north central Gulf of Mexico, approximately 80 km south of Dauphin Island, Alabama.

*Prevalence and intensity of infection:* Three of 27 (11%); 1 gag had 1 deteriorated specimen (not included in this description), 1 had eggs in gill but no adult specimen, and 1 had eggs in gill and 2 specimens (holotype and paratype of *P. lemusi* n. sp.) in blood.

*Specimens deposited:* Holotype USNPC 105075, paratype USNPC 105076.

**FIGURES 1–3.** *Pearsonellum lemusi* n. sp. (Digenea: Aporocotylidae) from gag grouper, *Mycteroperca microlepis* (Goode and Bean, 1879) (Perciformes: Serranidae), from in north central Gulf of Mexico off Alabama, ventral views. Scale values aside each bar. (1) Body (holotype, USNPC 105075) showing mouth (m), anterior sucker (as), nerve commissure (nc), vitellarium (v), esophagus (e), ventrolateral nerve cord (n), cecal bifurcation (cb), testis (t), ovary (o), metraterm (mt), female genital pore (fp), male genital pore (mp), ootype (oo), and posterior nerve confluence (pnc). (2) Lateral tegumental spine row. (3) Genitalia (holotype, USNPC 105075) showing location of ovary (o), oviduct (ov), oviducal seminal receptacle (osr), ootype (oo), vitelline duct (vt), ascending uterus (au), metraterm (mt), female genital pore (fp), vas deferens (vd), seminal vesicle (sv), and everted cirrus (ec).
The specific epithet lenusi honors Dr. Jason Thomas Lemus for his expertise in collecting fishes from offshore and pelagic sites in the Gulf of Mexico.

Remarks

The new species differs from the 2 other nominal species of Pearsonellum, P. corventum, and P. pygmaeus, by the combination of having a nerve commissure located 5–8% (6–11% in P. corventum; 9–10% in P. pygmaeus) of total body length from the anterior body end, an esophagus length that is 31–34% (35–40% in P. corventum; 44–45% in P. pygmaeus) of total body length, a testis length that is 38–43% (30–44% in P. corventum; 26–27% in P. pygmaeus) of total body length, a cecal intersection comprising an elongated medial channel connecting the anterior and posterior ceca, anterior ceca lengths >10% (3–7% in P. corventum and P. pygmaeus) of total body length, and a uterus not forming a loop between the testis and ovary; the last feature possibly being the most robust and easily identifiable among the 3 species. Another potentially distinguishing feature is that adults of P. lenusi n. sp. reportedly are the largest (3,237 mm) of its congeners, i.e., 1,595 mm in P. corventum and 1,800 mm in P. pygmaeus. The shape of the larvated egg of aporocotylids has been suggested as a useful diagnostic feature for aporocotylid genera, although the larvated egg for most species is unknown (McVay et al., 2011). Eggs of P. lenusi in the gill of gag grouper are spheroid, clearly different from the pyriform eggs of some species of Caridocula (see McVay et al., 2011; Fig. 7). Future descriptions of newly collected specimens representing new species of Pearsonellum and of aporocotylids in other genera should make an effort to document the shape of the “mature” aporocotylid eggs in the gill of their hosts.

DISCUSSION

The new species seems host specific to gag grouper because it was the only host identified among several other necropsied serranids: 1 coney, Cephalopholis fulva (Linnaeus, 1758); 3 Nassau groupers, Epinephelus striatus (Bloch, 1792); 32 red groupers, Epinephelus morio (Valenciennes, 1828); 1 yellowedge grouper, Epinephelus flavolimbatus Poe, 1865; 1 rock hind, Epinephelus adscensionis (Osebeck, 1765); 2 red hind, Epinephelus guttatus (Linnaeus, 1758); 3 Warsaw grouper, Epinephelus nigritus; 1 graysby, Cephalopholis cruentata (Lacepède, 1802); 1 black grouper, Mycteroperca bonaci (Poe, 1860); and 2 tattlers, Serranus phoebe Poe, 1851. However, if the low prevalence in gag grouper is typical for serranid aporocotylids, assessments of host specificity by Pearsonellum spp. would require examination of large numbers of individual fish of each grouper species.

Only 4 aporocotylids are known from serranids, i.e., the 3 species of Pearsonellum plus Adelomyloss teenae Nolan and Cribb, 2004. Pearsonellum lenusi n. sp. is the only nominal serranid aporocotylid documented from beyond the southwestern Pacific Ocean. However, probably several undescribed species exist. Overstreet and Thulin (1989) reported the finding of “digenean eggs containing miracidia of other than P. corventum” that occurred in the ventricle of starry grouper, Epinephelus labriformis (Jenyns, 1840) and E. guttatus from the Pacific Coast of the Republic of Panama and from St. Thomas in the Atlantic Ocean–Caribbean Sea, respectively. In hearts of E. labriformis, the eggs averaged about 32 × 24 μm (comparable to those reported herein for P. lenusi), but most were shrunk and partly degenerated. Interestingly, the eggs observed in the ventricle of E. guttatus appeared operculate to these workers. The collection of additional aporocotylids from these and other serranid species ranging in the eastern Pacific Ocean, Atlantic Ocean, and Gulf of Mexico would allow for additional comparisons and species discovery of serranid aporocotylids.

ACKNOWLEDGMENTS

I thank Jason Lemus (formerly Gulf Coast Research Laboratory [GCRIL], Ocean Springs, Mississippi) for helping collect the infected gag groupers; Robin M. Overstreet (GCRIL) for providing laboratory space, supplies, and logistical support for initial fish collections; Jody Peterson, Eric Pulis, and Stephen Curran for helping collect some of the other serranids necropsied herein; and Eric Hobeg and Pat Pilitt (both USNPC) for ensuring the safe deposition of our type materials. 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 grants 1112729, 1051106, and 1048523 to S.A.B., as well as funds from the Gulf of Mexico Research Initiative (GRI) to S.A.B.

LITERATURE CITED


