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Description and pathology of a new genus and species of fish blood fluke (Digenea: Aporocotylidae Odhner, 1912) infecting white mullet, *Mugil curema* Valenciennes, 1836 (Mugiliformes: Mugilidae) in Mobile Bay (northern Gulf of Mexico) with a phylogenetic analysis

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Abstract

Three fish blood flukes (Aporocotylidae Odhner, 1912) infect mullets (Mugiliformes: Mugilidae): *Cardicola mugilis* Yamaguti, 1970 and *Plethorchis acanthus* Martin, 1975 infect striped mullet, *Mugil cephalus* Linnaeus, 1758 in the Central Pacific Ocean (Hawaiian Islands) and Brisbane River (Australia), respectively; *Cardicola brasiliensis* Knoff & Amato, 1992 infects Lebranche mullet, *Mugil liza* Valenciennes, 1836 from the Southwestern Atlantic Ocean (Brazil). White mullets were cast-netted from the mouth of Deer River, a coastal saltmarsh of Mobile Bay, in the north-central Gulf of Mexico and examined for blood fluke infections. Specimens of *Mugilitrema labowskiae* Warren & Bullard n. gen., n. sp. were found infecting the endocardial surface and inter-trabecular spaces of the atrium, ventricle, and bulbous arteriosus. The new genus and species differ from all other aporocotylids by having the combination of two post-caecal testes, a uterus with straight ascending and descending portions, and a common genital pore. The 28S analysis recovered the new species and *P. acanthus* as sister taxa and Aporocotylidae as monophyletic. Carditis associated with intense infections comprised endocardial hyperplasia, resulting in a thickened cardiac endothelium. Probable dead or deteriorating eggs in the myocardium were encapsulated by granulomas composed of epithelioid histiocytes. Live eggs infected the afferent artery of gill filaments and were associated with varied hyperplasia of the overlying epithelium and haemorrhaging from the afferent artery in high-intensity infections. The new species is the first aporocotylid infecting a mullet from the northwestern Atlantic Ocean and only the second description of demonstrable endocarditis attributed to an adult fish blood fluke infection.

Introduction

Striped mullet (*Mugil cephalus* Linnaeus, 1758) and white mullet (*Mugil curema* Valenciennes, 1836) (Mugiliformes: Mugilidae Jarocki, 1822) each represent economically and culturally important fisheries in the Gulf of Mexico, especially along the Alabama, Mississippi, and Louisiana coasts. Despite many gill and intestinal parasites being reported from these mullets in the region (Overstreet 1971, 1973, 1997; Paperna & Overstreet 1981; Palm & Overstreet 2000; Pulis *et al.* 2013), there is no record of a fish blood fluke (Aporocotylidae Odhner, 1912, *sensu* Warren & Bullard 2023) infection in any mullet from the Gulf of Mexico or northwestern Atlantic Ocean Basin. Presently, the mullets comprise 78 species of 26 genera (Eschmeyer *et al.* 2016). Among these, three nominal fish blood flukes are known: *Cardicola mugilis* Yamaguti, 1970 and *Plethorchis acanthus* Martin, 1975 infect striped mullet in the Central Pacific Ocean (Hawaiian Islands) (Yamguti, 1970) and in the Brisbane River (Australia) (Martin, 1975), respectively; *Cardicola brasiliensis* Knoff & Amato, 1992 infects Lebranche mullet, *Mugil liza* Valenciennes, 1836 in the Southwestern Atlantic Ocean (Brazil) (Knoff & Amato 1992).

Aporocotylid infections are pathogenic in aquaculture when infection intensity is high and if eggs or adults occlude branchial vessels and interfere with oxygen exchange in the gill (Thulin 1980; Ogawa & Fukudome 1994; Munday and Hallegraeff, 1998; Colquitt *et al.* 2001; Bullard & Overstreet 2002, 2008). Damage to the gill epithelium is also reported from eggs, exiting miracidia, or penetrating cercariae (Crespo *et al.* 1992; Kirk & Lewis 1992; Herbert *et al.* 1995;

Bullard & Overstreet 2002, 2008). Further, adult aporocotylids infecting the heart can cause a thickening of the endocardium and endocardial thrombi, which could decrease blood flow (Warren *et al.* 2017). There is limited information regarding pathological effects on cardiac tissue attributed to fish blood fluke infections in wild fish populations. No study has shown that a wild blood fluke infection causes demonstrable, multifocal, or widespread pathological changes to the heart that would decrease heart performance (Warren *et al.* 2017; McElroy *et al.* 2020), but a blood fluke (*Cardallagium* cf. *anthicum*) in the heart of cobias, *Rachycentron canadum* (Linnaeus, 1766) Monod, 1973 (Rachycentridae) from the South China Sea probably could cause mortality in heavy infections, perhaps promulgated by intense cage culture (Warren *et al.* 2017). Because of this and because mullets are an important resource in aquaculture and fisheries settings (primarily in Brazil and Mexico) (Avigliano *et al.* 2015; Pacheco-Almanzar *et al.* 2017; Santana *et al.* 2018; Lima *et al.* 2019), new and existing fish blood fluke histopathological investigations are warranted to contribute to our understanding of the infectious diseases of cage-cultured fishes.

We herein describe a new genus and species of marine fish blood fluke infecting the endocardial surface and inter-trabecular spaces of the atrium, ventricle, and bulbous arteriosus of white mullet captured in the Deer River, a coastal saltmarsh environment of Mobile Bay in the northern Gulf of Mexico. We also present a 28S phylogenetic analysis and pathology study. This study reports the first aporocotylid infecting a mullet from North America and the second description of endocarditis attributed to the adult fish blood fluke infection.

Materials and methods

The hearts of 87 of 108 (81%) white mullet (*M. curema*) from north fork Deer River, a tributary of Mobile Bay, Alabama were infected with adult and juvenile specimens of a new fish blood fluke. Fish were captured by gill net in March 2017 (20 fish) and cast net in March 2021 (88 fish), then transferred on ice and relocated to the laboratory for examination some hours later. At necropsy in the laboratory, hearts and gills were excised intact and examined with the aid of a dissecting microscope and fibre optic light source to isolate fluke specimens and eggs for morphology and nucleotide sequencing. The heart was bisected with fine forceps and scissors to reveal adult blood flukes. Gill filaments were cut with scissors and mounted on glass slides to examine for blood fluke eggs. Adult flukes for morphological study were heat-killed on a glass slide using a butane hand lighter under little or no coverslip pressure as per Bullard *et al.* (2019) and fixed in 10% neutral buffered formalin (n.b.f.). Adult specimens intended for DNA extraction were wet mounted on glass slides and examined to confirm their identity, placed directly into 95% ethanol (EtOH), and stored at -20°C until DNA was extracted (see below).

Adult flukes fixed for morphology were rinsed with distilled water, cleaned with fine brushes to remove any debris, stained overnight in Van Cleave's hematoxylin with two additional drops of Ehrlich's hematoxylin, dehydrated using an EtOH series, cleared in clove oil, and permanently mounted in Canada balsam. Illustrations were completed using an Olympus BX51 microscope (Olympus Corporation of the Americas, Center Valley, PA, USA) equipped with differential interference contrast and a drawing tube. Measurements were made using the camera software Jenoptik Gryphax® version 2.1.0.724 (Jena, Germany) and are reported in micrometres (μm) as the range followed by the mean, standard deviation, and sample size in parentheses. Scientific names, including taxonomic authorities and dates, for fishes follow Eschmeyer *et al.* (2016). Classification and anatomical terms for fish blood flukes follow Warren *et al.* (2023) and Warren & Bullard (2023). Specimens of *P. acanthus* (UWSP-P-10054–10073) from W.E. Martin's collection were available and borrowed from the University of Wisconsin Stevens Point Natural History Museum. We believe these specimens to be the type specimens for *P. acanthus*. Voucher materials were deposited in the National Museum of Natural History's Invertebrate Zoology Collection (United States National Museum, Smithsonian Institution, Washington, DC, USA).

For histopathology, the heart, gill, and viscera from infected fish were fixed in 10% n.b.f and routinely processed. After fixation, 12 gill arches (two per gill from six white mullets), four hearts, and two visceral masses were processed for histopathology sections. Heart and gill were left intact, and each visceral mass was transversally cut into four, 1 cm-long pieces, yielding 24 pieces of tissue, which were then rinsed in water for 2 h, dehydrated in an EtOH series, embedded in paraffin, sectioned at 5 μm, and stained with Gill's 3 hematoxylin and eosin per Luna (1992). Gill arches were decalcified in ethylenediaminetetraacetic acid for one week prior to embedding. Ten slides per portion of tissue were cut yielding over 720 sections on 240 slides. Blood fluke eggs were photographed and measured.

Using two of the EtOH-preserved and microscopically identified blood flukes, DNA was extracted using DNeasyTM Blood and Tissue Kit (Qiagen, Valencia, CA, USA) per the manufacturer's protocol except that the proteinase-K incubation period was extended overnight, and the final elution step used 100 microlitres (μl) of elution buffer to increase the final DNA concentration. Primer choice and the polymerase chain reaction (PCR) amplification cycling profile are outlined in Warren *et al.* (2017) and Warren *et al.* (2021). All PCR reactions were carried out in an MJ Research PTC-200 gradient thermal cycler (BioRad, Hercules, CA, USA). PCR products (12 μl) were verified on a 1% agarose gel and stained with ethidium bromide. PCR products were purified by microcentrifugation with the QIAquick PCR Purification Kit (Qiagen, Valencia, CA, USA) according to the manufacturer's protocols except that the last elution step was performed with autoclaved nanopure H_2O rather than with the provided buffer. DNA sequencing was performed by Genewiz, Incorporated (South Plainfield, NJ, USA). Sequence assembly and analysis of chromatograms were performed with Geneious version 2024.0.2 (http://www.geneious.com). Nucleotide sequence data were deposited in GenBank.

The phylogenetic analysis included two nucleotide sequences of the new species plus selected aporocotylid sequences from GenBank. Sequences were selected based on studies by Warren *et al.* (2019, 2023) and Warren & Bullard (2023). The out-group is represented by species of Elopicolidae Warren & Bullard, 2023 and was informed by results presented by Warren *et al.* (2019, 2023) and Warren & Bullard (2023). Sequence alignment, trimming (1128 (28S) base pairs (bp)), model selection, and alignment reformatting are outlined in Warren *et al.* (2023). Bayesian inference was performed in MrBayes version 3.2.7a (Ronquist & Huelsenbeck 2003) using substitution model averaging ('nst-mixed') and gamma distribution to model rate heterogeneity. Defaults were used in all other parameters. Three independent runs with four Metropolis-coupled chains were run for 5,000,000 generations, sampling the posterior distribution every 1,000 generations.

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Convergence was checked using Tracer v1.6.1 (Rambaut *et al.* 2014) and the 'sump' command in MrBayes: all runs appeared to reach convergence after discarding the first 25% of generation as burn-in. A majority rule consensus tree of the post-burn-in posterior distribution was generated with the 'sumt' command in MrBayes. The inferred phylogenetic tree was visualised using FigTree v1.4.4 (Rambaut *et al.* 2014) and further edited for visualisation purposes with Adobe Illustrator (Adobe Systems;San Jose, CA, USA).

Results

Mugilitrema *n. gen. Warren & Bullard*

Diagnosis (Figures 1a–c; 2a–d)

Body of adult >15 × longer than wide, threadlike, dorsoventrally flat posteriorly, lacking posterolateral protuberance, tapering equally anteriorly, bluntly rounded posteriorly, spined; space between transverse tegumental spine rows 3 × longer than spine row length. Rosethorn-shaped spines absent. Anterior sucker diminutive, appearing concave, accommodating mouth; mouth medioventral, subterminal. Pharynx absent. Oesophagus medial, straight, not looping, extending posteriad one-third body length, with posterior oesophageal swelling, anteromedially connecting with intestine. Intestine medial, H-shaped, lacking diverticula, posterior caeca terminating in middle third of body. Testes two, medial, flanking genitalia anteriorly and posteriorly, deeply lobed. Vasa efferentia coalescing at posterior margin of anterior testis and anterior margin of posterior testis to form large ducts that coalesce to form vas deferens; vas deferens forming from anterior and posterior ducts of vasa efferentia at level of ovary and proximal portion of cirrus sac, extending directly posteriorly and connecting with cirrus sac, ventral to ovary and dorsal to vitelline duct. Auxiliary external seminal vesicle absent. Cirrus sac medial, post-ovarian, post-caecal, inter-testicular; enclosing internal seminal vesicle and cirrus; cirrus everting dorsally in sinistral body half, post-caecal, inter-testicular. Ovary single, medial, post-caecal, inter-testicular, deeply lobed. Oviduct post-caecal; oviducal seminal receptacle present. Laurer's canal absent. Vitellarium diffuse, occupying space from anterior body end to level of ovary. Vitelline duct longitudinal, connecting with oötype posteriorly, extending anteriorly to middle third of oesophagus. Oötype post-ovarian, inter-testicular. Uterus comprising ascending and descending portions, post-ovarian, inter-testicular; uterine eggs large, ovoid, lacking spines or filaments, >90% of uterine width. Metraterm indistinct. Male and female reproductive tracts opening into common atrium and sharing a common pore; common genital pore dorsal, sinistral, post-caecal, and inter-testicular.

Differential diagnosis

Body >15 × longer than wide; space between transverse tegumental spine rows 3 × longer than spine row length. Intestine H-shaped, posterior caeca terminating in middle third of body. Testes two, flanking genitalia anteriorly and posteriorly. Auxiliary external seminal vesicle absent. Cirrus sac post-ovarian, post-caecal, inter-testicular. Ovary post-caecal. Laurer's canal absent. Oötype inter-testicular. Uterine eggs ovoid, lacking filaments, >90% of uterine width. Common genital pore present, post-caecal, inter-testicular.

Type and only nominal species: Mugilitrema labowskiae n. sp. Warren & Bullard.

Etymology: The Latin '*Mugil*' refers to the host genus.

Mugilitrema labowskiae *n. sp. Warren & Bullard*

Description (Figures 1a–c; 2a–d)

Light microscopy of three intact and eight partial whole-mounted adult specimens; USNM collection nos. 1714799–1714808): Body elongate, anterior body thread-like, posterior body at level of genitalia flat, tapering anteriorly, 1,241–1,681 (1,481 ± 223, 3) long, 50–71 (60 ± 8, 10) at greatest width, 17–30 (24 ± 6, 3) × longer than wide (Figures 1a–c); tegumental body spines minute, 18–23 (22 ± 2, 4) from anterior end (Figures 2a–b), 2–4 (2.5 ± 0.4, 20) long, <0.2 wide at base, at limits of light microscopy, extending to posterior body end (Figures 1b–c); tegumental spine rows 3–4 (3.3 ± 0.6, 20) long, 0.4–0.5 (0.4 ± 0.03, 20) wide, regularly spaced apart 5–8 (6 ± 1.2, 10) near anterior sucker; 12–20 (16 ± 3.4, 10) in middle body near intestine; 6–12 (9 ± 2.6, 10) in posterior body at level of posterior testis; 3–5 (4 ± 1, 20) spines per row (Figure 2b), with 106–124 (115 ± 9, 2) per side or total of 217–240 (229 ± 16, 2). Anterior sucker 12–18 (15 ± 3, 3) long, 13–33 (25 ± 8, 4) wide at base, terminal papillae on anterior margin of anterior sucker present, paired, each approximately 1 long and 1 wide; anterior sucker spines small, <1 (1) long, in 5 concentric rows (1) (Figure 2a). Ventrolateral nerve cord and nerve commissure not observed. Mouth small, 3 (2) in diameter, 8 from terminal end of anterior body extremity (Figure 2a). Oesophagus 466–589 (521 ± 60, 4) in total length or 31–38% of body length, 8–13 (10 ± 2, 4) in maximum width (at level just anterior intestine), extending sinuously posteriad along midline, widening posteriorly (Figures 1a, 2a, 2c); anterior oesophageal swelling absent. Intestinal caecal intersection of anterior and posterior caeca 757 or 61% of body length from posterior end; anterior caeca 21–65 (41 ± 22, 3) long or 1–2% of body length, 13–22 (17 ± 4, 3) wide, containing granular material within lumen of some individuals (Figures 1a, 2c); posterior caeca asymmetrical, 123–234 (178 ± 56, 3) long or 10–15% of body length, 15–22 (18 ± 4, 3) wide (Figures 1a, 2c); post-caecal space 602–729 (666 ± 90, 2) long or 48–49% of body length or 13–15% of body length from anterior testis.

Anterior testis 99–212 (155 ± 48, 8) long or 11–13% of body length, 35–59 (49 ± 11, 8) wide or 63–83% of maximum body width, 3–5 × longer than wide, terminating 249–584 (351 ± 95, 9) or 21–47% of body length from posterior body end (Figures 1a–c); posterior testis 107–139 (118 ± 18, 8) long or 7–9% of body length or 65–76% of anterior testis length, 36–63 (51 ± 14, 9) wide or 71–89% of maximum body width, 2–3 × longer than wide, terminating in posterior body extremity (Figures 1a–c). Vasa efferentia difficult to trace in fixed specimens, an interconnecting meshwork of fine ducts entwining throughout testicular tissue, approximately 7–10 (8.5 ± 1.3, 5) wide, containing sperm in all specimens, coalescing in posterior portion of anterior testis and anterior portion of posterior testis to each form larger ducts (Figures 1b–c, 2d); anterior vasa efferentia duct 62–110 (82 ± 25, 4) long or 4–7% of body length, 7–9 (8 ± 1, 4) in maximum width; posterior vasa efferentia duct 101–134 (121 ± 18, 4) long or 1.2–2.2 × longer than anterior duct of vasa efferentia or 8% of body length, 8 (4) in maximum width; vas deferens forming from anterior and posterior ducts of vasa efferentia at level of ovary and proximal portion of cirrus sac, extending directly posterior 3–5 (4 ± 1, 4) or 6–10% of seminal vesicle length, 1–3 (2 ± 1, 4) or 6–18% of cirrus sac width in maximum width, and connecting with cirrus sac, containing sperm in all specimens (Figures 1b–c, 2d). Cirrus sac present, wall approximately 1.5–3 (2.1 ± 0.5, 9) thick, including seminal vesicle, and cirrus; seminal vesicle 41–57 (50 ± 5, 9) long, 9–19 (15 ± 3, 9) wide, 3 × longer then wide

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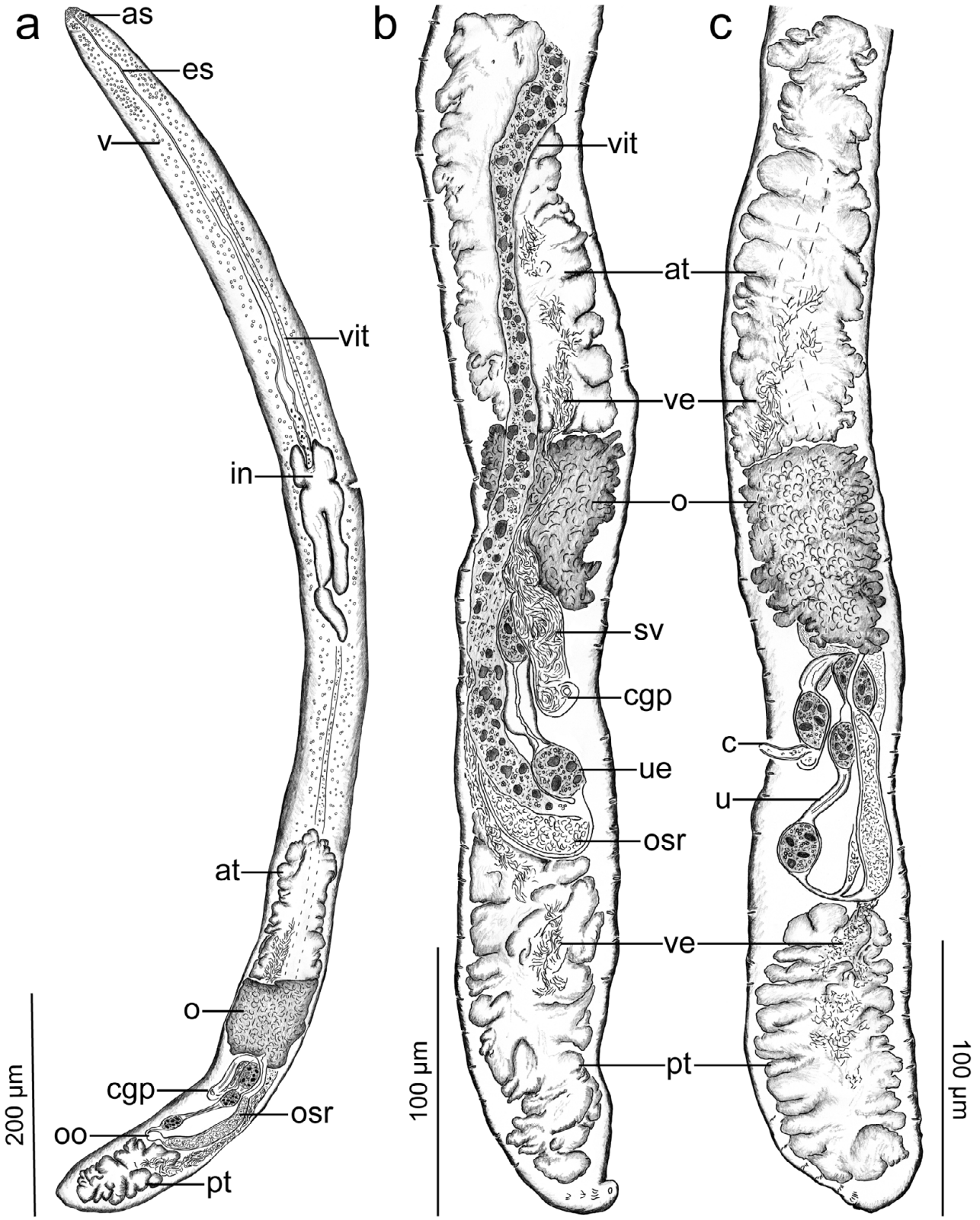

Figure 1. *Mugilitrema labowskiae* Warren & Bullard (Digenea: Aporocotylidae) infecting the heart of white mullet, *Mugil curema* Valenciennes, 1836 (Mugiliformes: Mugilidae) from the mouth of Deer River, a tributary of Mobile Bay, Alabama, (30°32′ 3.401′′ N, 88°6′ 21.113′′ W). Scale values aside bars. (**a**) body of holotype (USNM No. 1714799); (**b**) genitalia of paratype (USNM No. 1714800), ventral; (**c**) genitalia of paratype (USNM No. 1714801), dorsal. Abbreviations: as, anterior sucker; at, anterior testis; c, cirrus; cgp, common genital pore; es, oesophagus; in, intestine; oo, oötype; o, ovary; osr, oviducal seminal receptacle; pt, posterior testis; sv, seminal vesicle; ue, uterine egg; u, uterus; v, vitellarium; ve, vasa efferentia; vit, vitelline duct.

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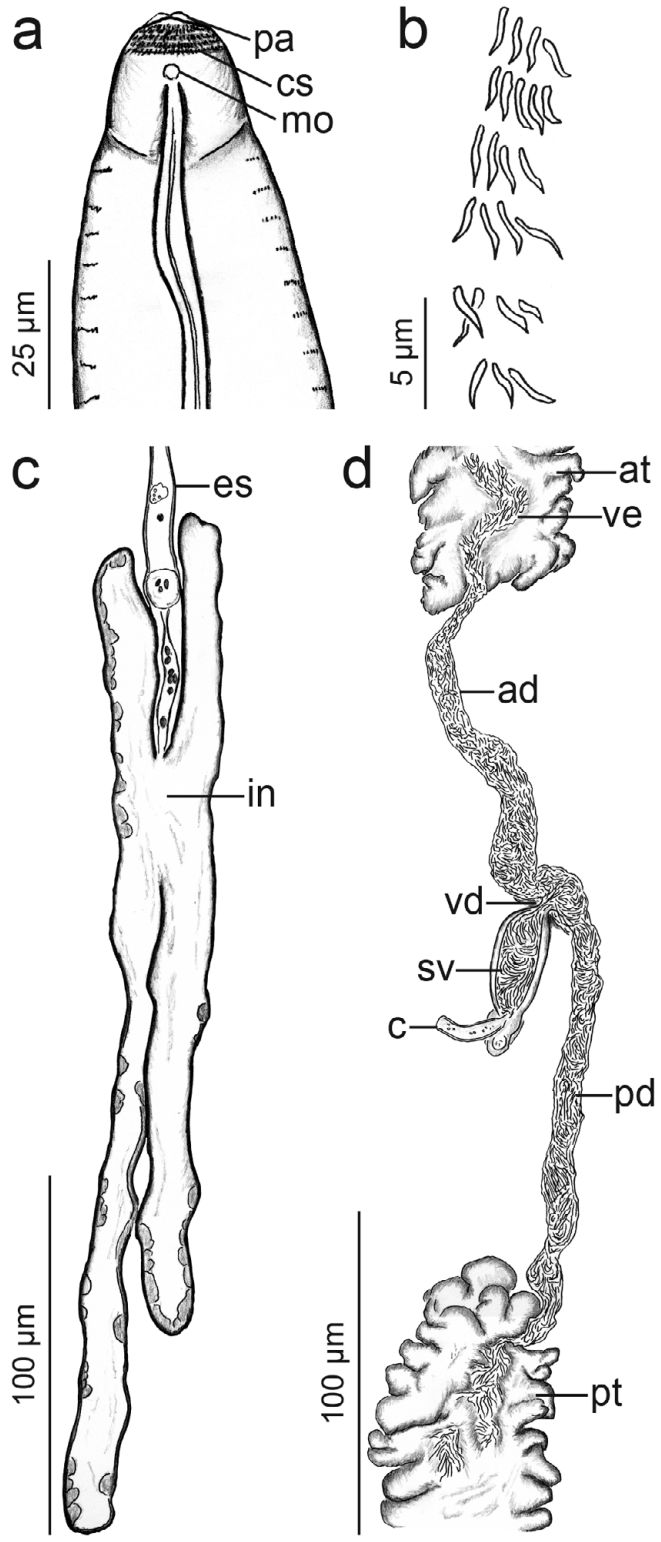

Figure 2. *Mugilitrema labowskiae* Warren & Bullard (Digenea: Aporocotylidae) infecting the heart of white mullet, *Mugil curema* Valenciennes, 1836 (Mugiliformes: Mugilidae) from the mouth of Deer River, a tributary of Mobile Bay, Alabama, (30°32′ 3.401′′ N, 88°6′ 21.113′′ W). Scale values aside bars. (**a**) anterior sucker of holotype (USNM No. 1714799), dorsal; (**b**) lateral tegumental spines of holotype (USNM No. 1714799), ventral; (**c**) intestine of voucher (USNM No. 1714802), ventral; (**d**) male genital system of voucher (USNM No. 1714806) dorsal. Abbreviations: at, anterior testis; ad, anterior vasa efferentia duct; cs, circumoral spines; c, cirrus; es, oesophagus; in, intestine; mo, mouth; pa, papillae; pt, posterior testis; pd, posterior vasa efferentia duct; sv, seminal vesicle; vd, vas deferens; ve, vasa efferentia.

(Figures 2–3, 7); everted cirrus 17–23 (20 ± 4, 5) long or 33–47% of seminal vesicle length, 6 (5) wide, 3 × longer then wide (Figures 1c, 2d). Common genital pore 164–224 (194 ± 30, 4) or 13% of body length from posterior end of the body, 11–13 (12 ± 1.4, 3) from sinistral body margin, 39–41 (40 ± 1.4, 3) from dextral body margin (Figures 1a–c, 2d).

Ovary irregular in shape, 93–111 (102 ± 13, 8) in maximum length or 7% of body length, 57–62 (60 ± 4, 8) wide or 80–87% of body width, 1.5–1.9 × longer than wide, immediately posterior to anterior testis; post-ovarian space 209–273 (241 ± 45, 8) long or 17–22% of body length (Figures 1a–c). Oviduct (including oviducal seminal receptacle) 108–129 (120 ± 11, 8) long; oviducal seminal receptacle 87–104 (94 ± 9, 8) long or 38–42% of oviduct length, 14–18 (15 ± 2, 8) wide. Oötype 10 (1) in diameter. Vitellarium follicular, occupying space ventral and lateral to testis, extending from anterior sucker to ovary; vitelline collecting duct 800–1155 (977 ± 251, 2) long, 13–19 (15 ± 3, 8) wide. Uterus extending directly anteriad from oötype, 90–110 (102 ± 11, 7) long or 7% of body length, 15–17 (16 ± 1, 7) wide, with wall 3–4 (3.7 ± 0.6, 7) thick, containing eggs in all specimens (Figures 1b–c); ascending portion extending anteriad and dorsal to seminal vesicle, curving sinistral to posterior margin of ovary before connecting with descending portion; descending portion 55–60 (57 ± 3, 7) long or 4% of body length, 13–17 (15 ± 1, 7) wide or 14% of body width, with wall 3–4 (3.3 ± 0.6, 7) thick, extending posteriorly before connecting with common genital pore (Figures 1b–c). Uterine eggs 21–28 (26 ± 3, 8) long or 47–49% of descending uterus length, 13–16 (15 ± 1.3, 8) wide or 93–94% of descending uterus width, with wall 1–2 (1.3 ± 0.6, 7) thick, containing dense lipid-like bodies (Figures 1a–c). Excretory vesicle indistinct.

Taxonomic summary

Type host: White mullet, *Mugil curema* Valenciennes, 1836 (Mugiliformes: Mugilidae).

Type locality: Deer River (30°32'02.4"N, 88°06'20.3"W), Mobile Bay, Alabama.

Site of infection: Endocardial surface and inter-trabecular spaces of the atrium, ventricle, and bulbous arteriosus.

Prevalence of infection: 20 of 20 (100%) white mullet sampled in 2017 and 67 of 88 (76%) white mullet sampled in 2021 were infected by 239 adult and juvenile specimens of *M. labowskiae.*

Specimens deposited: Holotype (USNM 1714799), paratypes (USNM 1714800, 1714801), vouchers (USNM 1714802, 1714803, 1714803, 1714804, 1714805, 1714806, 1714807, 1714808).

ZooBank registration: urn:lsid:zoobank.org:act:A26BAE25-6CC1-4827-A61A-AD31A206F8D3

Specimens examined: *Plethorchis acanthus* Martin, 1975 (UWSP-P-10054–10073).

Etymology: The specific epithet '*labowskiae*' is a portmanteau honoring Lauren Jakubowski (Department of Conservation and Natural Resources: Alabama Marine Resource Division) for her contribution and assistance in collecting infected white mullet.

Remarks

Mugilitrema resembles *Psettarium* Goto & Ozaki, 1930, monotypic *Neoparacardicola* Yamaguti, 1970, monotypic *Adelomyllos* Nolan & Cribb, 2004, monotypic *Chaulioleptos* Nolan & Cribb, 2005, and *Phthinomita* Nolan & Cribb, 2006 by having the combination of lateral tegumental spines in transverse rows (vs. single column of spines) (Warren *et al.* 2023), an H-shaped intestine with elongate caeca (vs. inverse U-shaped) (Bullard 2014; Orélis-Ribeiro *et al.*

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2017), and two testes (vs. a single testis or multiple (>2) testes) (Mcintosh 1934; Bullard *et al.* 2008; Santoro *et al.* 2015; Warren *et al.* 2017, 2021) (Figures 1a–c, 2d). Specimens of the new genus differ from these genera by having a common genital pore (vs. separate genital pores) (Yamaguti 1970; Nolan & Cribb 2004, 2005; Cutmore *et al.* 2021).

The new species is most similar to the species of *Psettarium* having two testes (*Psettarium hawaiiensis* (Martin, 1960) Yong, Cutmore, Jones, Gauthier & Cribb, 2018; *Psettarium hustoni* Yong, Cutmore, Jones, Gauthier & Cribb, 2018; *Psettarium martini* Yong, Cutmore, Jones, Gauthier & Cribb, 2018; *Psettarium yoshidae* Yong, Cutmore, Jones, Gauthier & Cribb, 2018) and differs by having an intestine that terminates in the middle third (vs. posterior third) of the body, a vitelline duct that extends to middle third of oesophagus (vs. post-caecal bifurcation), a uterus with straight (vs. convoluted) ascending and descending portions, and a common genital pore (vs. separate genital pores).

The new species differs from *C. mugilis* and *C. brasiliensis* by having two post-caecal testes (vs. single inter-caecal testis), a uterus with straight (vs. convoluted) ascending and descending portions, and a common genital pore (vs. separate genital pores). It differs from *P. acanthus* by having elongate (vs. reduced) anterior caeca, two testes (vs. >100) that are wholly post-caecal (vs. partially inter-caecal), a vitelline duct extending to the middle third of the oesophagus (vs. extending to the post-caecal space), a wholly post-ovarian uterus (vs. extending to the pre-ovarian space), a descending uterus length equal to >20% (vs. >50%) of the body width, and ovoid (vs. fusiform) uterine eggs that lack uterine egg spines or filaments (vs. having spines or filaments).

Phylogenetic results

The amplified 28S fragments representing the new species comprised 1,515 nucleotides (GenBank accession nos. PP960256 and PP960257) and were identical. The new sequence was 15% (178 bp) different from *P. acanthus* with trimming to 100% coverage at 1,200 bp. The new species was recovered sister to *P. acanthus*, supporting a monophyletic mullet-infecting blood fluke clade (Figure 3). Notably, *P. acanthus* infects the body cavity and blood vessels of the mesentery, intestine, and liver (not heart) of striped mullet (*M. cephalus*) from Australia and is morphologically distinct (see Remarks) (Martin 1975; Lest *et al.* 2009). The clade was recovered sister to *Psettarium* spp., monotypic *Neoparacardicola*, monotypic *Adelomyllos*, monotypic *Chaulioleptos*, and

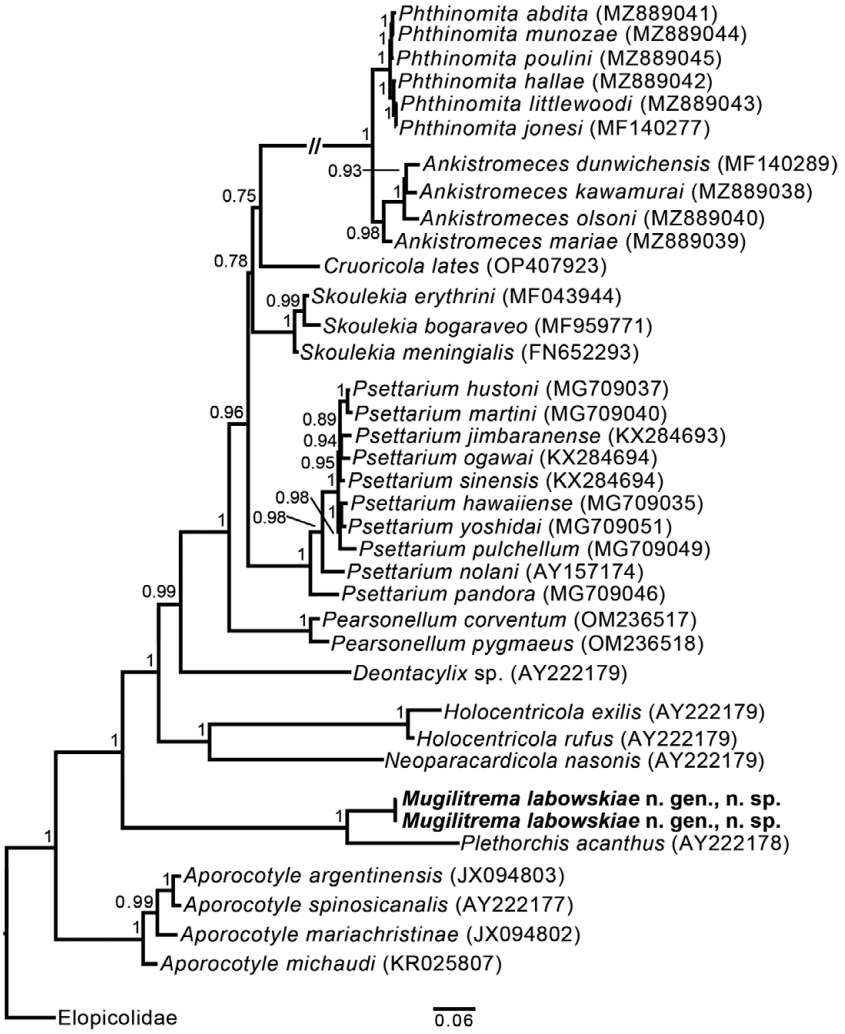

Figure 3. Phylogenetic relationships of species within the Aporocotylidae Odhner, 1912 reconstructed using Bayesian inference analysis using the large ribosomal subunit (28S) gene. Numbers aside nodes indicate posterior probability. The new genus and species are shown in bold.

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Phthinomita spp. (Figure 3). This tree topology is consistent with previous studies concerning the fish blood flukes (Warren *et al.* 2023; Warren & Bullard 2023).

Histology results

Adults of the new species infected the atrium, ventricle, and bulbus arteriosus of the heart and were coelozoic, occupying the luminal spaces of the heart, either adhering to the endocardium and/or within the intertrabecular spaces of the heart (Figures 4a–b). Variable endocardial hyperplasia was observed, ranging from several cells thick with no accompanying endocarditis (Figure 4a) to a significantly thickened endothelium with accompanying endocarditis (Figure 4c) (Warren *et al.* 2017). Endocarditis was characterised by an acute granulomatous inflammatory response composed of eosinophilic granulocytes, mononuclear inflammatory infiltrates, and clusters of free granules, from degranulation of eosinophilic granulocytes, present in proliferating endothelial cells (Figure 4c). No adults were observed in the vessels of the gill or viscera.

Eggs infected the heart and gill, but live/viable eggs were observed in the gill only. In heart, eggs were observed in the cardiac muscle of the myocardium where they were encapsulated by granuloma composed of epithelioid histiocytes (Figure 4d). In gill, eggs were infecting the afferent artery of gill filaments, and each had a miracidium. No egg was observed in a gill lamella (Figures 5a–c). The distribution of eggs in gill filaments was uneven, and the host response varied based on the number of eggs present. In gill filaments with few eggs, no demonstrable host response was observed, with eggs being encapsulated by a granuloma composed of a few layers of epithelioid cells (Figure 5a). In gill filaments with numerous eggs, the encapsulating granuloma was thicker than those surrounding eggs in lightly infected gill filaments, and the overlying epithelium was hyperplastic. Proliferating epithelium extended to the height of the gill lamellae, leaving the lamellae indiscernible from surrounding tissue (Figure 5b). In one gill filament with numerous eggs, the afferent artery was disrupted and haemorrhaging into adjacent tissues was observed (Figure 5c). No eosinophilic granulocytes were observed associated with eggs in the heart or gill.

Discussion

The egg shape of fish blood flukes can vary from triangular, spheroid, crescent, fusiform, and spindle shaped (Martin 1975; Thulin 1980; Nolan & Cribb 2004; Shirakashi *et al.* 2012; Warren & Bullard 2023; Warren *et al.* 2023). The eggs are generally observed in the uterus of the fluke or in the gill epithelium of the fish host; however, several atypical sites (body cavity, mesenteric vessels, intestine, liver) have been identified (Martin 1975; Lester *et al.* 2009). In the present study, eggs in the uterus and gills were ovoid to spheroid (Figures 1a–c). *Plethorchis acanthus* has eggs that are fusiform and were originally reported to have a spine at each pole of the egg (Martin 1975). We did not observe any spine in the uterine eggs of specimens we take to be the type series (UWSP-P-10054–10073). However, Martin (1975) reported eggs of *P. acanthus* from the body cavity, mesenteric vessels, intestine, and liver,

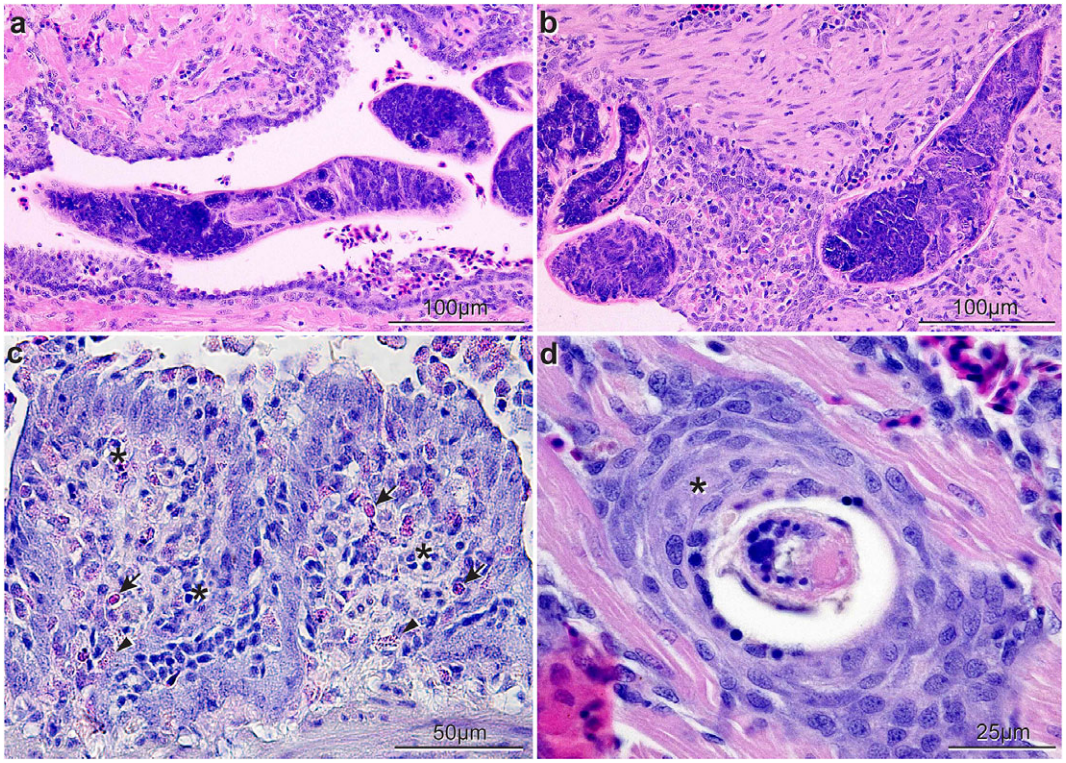

Figure 4. Histological sections (hematoxylin and eosin) of white mullet, *Mugil curema* Valenciennes, 1836 (Mugiliformes: Mugilidae) infected by *Mugilitrema labowskiae* Warren & Bullard (Digenea: Aporocotylidae). (**a**) heart showing adults in the lumen of the ventricle; (**b**) adult in the intertrabecular space of heart; (**c**) endocardium showing hyperplasia of the endocardium and endocarditis composed of eosinophilic granulocytes (arrows), mononuclear inflammatory infiltrates (*), and free granule clusters (triangles); (**d**) granuloma (*) encapsulating egg in cardiac muscle of the myocardium.

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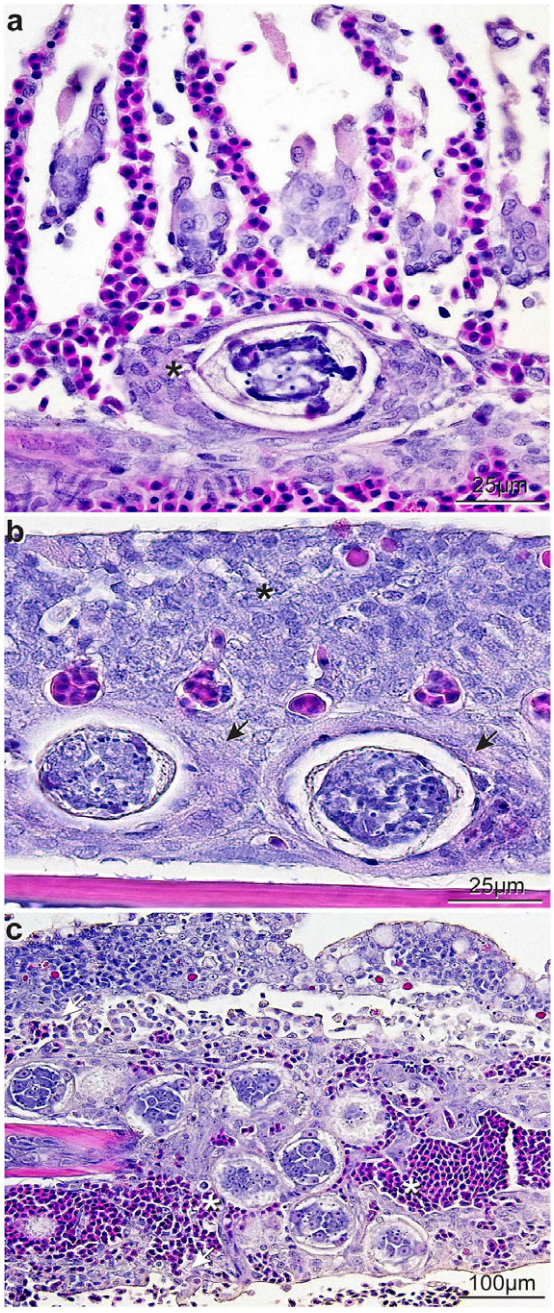

Figure 5. Histological sections (hematoxylin and eosin) of white mullet, *Mugil curema* Valenciennes, 1836 (Mugiliformes: Mugilidae) gill infected by eggs of *Mugilitrema labowskiae* Warren & Bullard (Digenea: Aporocotylidae). (**a**) egg in afferent artery of the gill filament encapsulated by epithelioid cells (*); (**b**) eggs in the afferent artery of the gill showing epithelioid cells (arrows) encapsulating eggs and hyperplasia of the overlying gill epithelium (*); (**c**) eggs in gill showing disruption of the afferent artery (*) and haemorrhaging (arrows).

suggesting that the lack of spined eggs in the uterus could be attributed to the stage of development. The differences in egg morphology between the new species and *P. acanthus* could indicate a functional adaptation. No egg was discovered in the gill epithelium by Martin (1975) or Lester *et al.* (2009), suggesting that eggs exit through an alternative site similar to the blood flukes infecting turtles (Pieper 1953) and mammals (Greer *et al.* 1989). For example, with *P. acanthus*, the presence of spines could assist egg movement through intestinal tissue, or alternatively, the spines could anchor the egg to intestinal material, aiding in host exit. The morphologically divergent and genetic relatedness of the mullet blood flukes suggests that the shape of the egg is related to location of infection site and how eggs and miracidia exit the definitive host rather than aporocotylid ancestry.

Published work characterising the pathological effects of adult blood flukes on the endocardium of fishes (Warren *et al.* 2017) is scarce compared with the effects of blood fluke eggs (Colquitt *et al.* 2001; McElroy *et al.* 2020). Colquitt *et al.* (2001) described fish blood fluke eggs infecting the spongiosa of the heart ventricles as well as gill tissue. Eggs in the heart were encapsulated by a granulomatous response consisting of epithelioid cells and lymphocytes, and the spongiosa was hypertrophied. No pathology associated with the adult worm was documented (Colquitt *et al.* 2001). Warren *et al.* (2017) described endocarditis associated with *Cardallagium* cf. *anthicum* (as *Psettarium* cf. *anthicum*) infecting cultured cobia from Vietnam. The primary results of that study revealed focally thickened endocardium and endocardial thrombi around the adult blood flukes. Loss of muscle striation in the myocardium was also noted and melanin-like pigment suggested the presence of melanomacrophage aggregates (Warren *et al.* 2017). McElroy *et al.* (2020) diagnosed the infection and pathology caused by *Cardicola laruei* Short, 1953 infecting spotted seatrout (*Cynoscion nebulosus* (Cuvier, 1830) Chao, 1978 (Sciaenidae)) from estuaries in South Carolina. The pathology reported was granulomatous myocarditis associated with blood fluke eggs as well as blister-like extrusions on the epicardium. Further, in one blister-like extrusion they observed yellow pigmented cells and suggested these could be melanomacrophages. In contrast to Warren *et al.* (2017), there was no apparent cellular host reaction to the adult blood flukes (McElroy *et al.* 2020). The contrasting cellular host reactions to the adult is interesting and underscores the need for further pathological study.

Endocardial cells are second to hepatocytes in the supply of cytochrome p450, a gene superfamily coding for enzymes involved in the detoxification of blood and the metabolism of fatty acids, steroids, and vitamins (Poppe & Ferguson 2006; Uno *et al.* 2012). Additionally, specialised endocardial cells have a high capacity for endocytosis, removing waste and biological macromolecules from the blood (Poppe & Ferguson 2006). As previously indicated by Warren *et al.* (2017), these properties likely afford the endocardium a role in regulating systemic disease and detoxification of blood, and as a result, changes to the endocardium could increase susceptibility to systemic diseases and reduce ability to phagocytise waste or detoxify pollutants from blood. Future work on how changes to the endocardium impact regulation of infectious disease, detoxification of blood, and metabolism would prove invaluable to better understanding the disease process of a blood fluke infection in a fish host.

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Competing interest. The authors declare that they have no conflict of interest.

Ethical standard. All applicable institutional, national, and international guidelines for the care and use of animals were followed.

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