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Proterometra epholkos sp. n. (Digenea: Azygiidae) from Terrapin Creek, Alabama, USA: Molecular characterization of life cycle, redescription of *Proterometra albacauda*, and updated lists of host and geographic locality records for *Proterometra* spp. in North America

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ABSTRACT

Proterometra epholkos sp. n. asexually reproduces in the stream dwelling prosobranch, *Elimia* cf. *modesta* (Cerithioidea: Pleuroceridae) and infects the buccal cavity epithelium of spotted bass, *Micropterus punctulatus* (Perciformes: Centrarchidae) in the Coosa River (Terrapin Creek; N33°51′36.56″, W85°31′28.15″; Cleburne County, Alabama, USA). We characterize cercariae and adults of the new species using morphology and molecular sequence data and redescribe its morphologically similar congener *Proterometra albacauda* based on the holotype and paratype (USNPC Nos. 61229-30). The new species can be distinguished most easily from *P. albacauda* by the combination of having cercariae with long mamillae (>100 μm) that encircle the tail stem anteriorly, that are restricted to 1 lateral column per body margin at midbody, and that are absent from the medial surface of the tail stem as well as by having adults with a partly extracecal uterus, a transverse metraterm occupying the space between the oral sucker and prostatic sac, and a vitellarium that is longer than the ceca and extends anteriad to the level of or beyond the posterior margin of the oral sucker. Sequence data from the ribosomal internal transcribed spacer 2 (ITS2; 251 bp) did not reject the notion that the cercariae and adults we collected simultaneously from those infected, sympatric, individual snails and fish in Terrapin Creek were conspecific. Also provided herein for species of *Proterometra* are (i) taxonomic keys for cercariae and adults based on morphological and behavioral characteristics sourced from the published literature, (ii) updated lists of host records (prosobranchs and fishes) and geographic locality records for *Proterometra* spp., and (iii) synopses and assessments of the morphological features previously used to differentiate them. *Proterometra macrostoma* (type species), *Proterometra melanophora*, and *Proterometra hodgesiana* are *species inquirendae*; requiring new collections from type localities and hosts concomitant with neotype designations. *P. macrostoma* seems a repository for conspicuous, furcocystocercous cercariae shed from freshwater prosobranchs in eastern North American rivers and streams. The specific epithet "*pinguis*" associated with specimens purportedly infecting *Esox lucius* and deposited by JF Mueller is a nomen nudum. *Proterometra guangzhouensis*, *Proterometra sillagae*, *Proterometra brachyura*n, and *Proterometra lamellorchis* are *incertae sedis*. Significant barriers to characterizing biodiversity and distributions (host range and geographic distribution) of *Proterometra* spp. comprise a paucity of data on adult morphology, dubious species-level identification or a lack of information regarding prosobranch hosts, lack of molecular data for putative comparisons among fluke 'strains' and species as well as between cercariae and adults, lack of consistency in terminology, and indeterminate homology for key morphological features. Uncertainty about the providence and identity of, or absence of, accessioned museum materials of *P. macrostoma*, *Proterometra catenaria*, and *P. hodgesiana* together represent another fundamental problem. The present study comprises the first description of a new species of *Proterometra* in nearly 20 years, first report of a species of the genus from the Coosa River (Mobile–Tensaw River Basin) and from these host species, and first use of molecular sequence data to elucidate a life cycle for a species of *Proterometra*.

1. Introduction

Proterometra Horsfall, 1933 (Digenea: Azygiidae) is readily distinguished from the remaining 3 genera of Azygiidae, i.e., *Azygia* Looss 1899, *Otodistomum* Stafford 1904, and *Leuceruthrus* Marshall and Gilbert

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Table 1
Snail hosts for cercariae of *Proterometra* spp. Horsfall, 1933[a] (Digenea: Azygiidae).

Species	Host[b]	Locality[c]	Author (year) [Ref.]
Proterometra macrostoma (Faust, 1918) Horsfall, 1933[d]	Campeloma*subsolidum*	Univ. IL zoological laboratory, Homer, IL	Faust (1918) [3]
	Lithasia*L. obovata* (as *Goniobasis depygis*)	McCormicks Creek, IN (OH)	Cable (1939) [4]
	Elimia floridensis	Wekiva River, FL (SAG)	Hunter & Wigington (1972) [5]
	Elimia*E. livescens*	Vermillion River, IL (GL)	Horsfall (1933) [1]
		Des Plaines River, IL (UM)	Horsfall (1934) [6]
		Indian River, MI (GL)	Lushbaugh (1968) [7]
		Sandusky River, OH (GL)	Lushbaugh (1968) [7]
		Carp Lake River, MI (GL)	Riley & Uglem (1995) [8]
		Olentangy River, OH (UM)	Riley & Uglem (1995) [8]
		Clear Creek, IN (OH)	Krist (2000) [9]
	Elimia *semicarinata* (as Goniobasis*G. pulchella*)	Univ. IL zoological laboratory, Homer, IL	Faust (1918) [3]
	E. semicarinata	Elkhorn Creek, KY (OH)	Lewis et al. (1989) [10]; Riley & Uglem (1995) [8]; Rosen et al. (2005; 2011) [11,12]
	Elimia spp.	Great Lakes (GL)	Dickerman (1945) [13]
		Elkhorn Creek, KY (OH)	Uglem & Lee (1985) [14]
	Pleurocera*P. acuta*	Oconomowoc River, WI (UM)	Horsfall (1933) [1]
		ns	Horsfall (1934) [6]
	Pleurocera spp.	Great Lakes (GL)	Dickerman (1945) [13]
	ns	Texas	Smith (1936) [15]
		Rutherford County, TN (TN [Stones River, Christmas Creek, Bushnell Creek, McKnight Brook]) (T)	Viyanant & Dunn (1975) [16]
Cercaria fusca Pratt, 1919[d,e]	*E. livescens*	Oneida River, NY (GL)	Pratt (1919) [17]
"Forked-Tailed Cercaria" Cahn, 1927[e]	*P. acuta*	Oconomowoc River, WI (UM)	Cahn (1927) [18]
"Cystocercous cercariae of the mirabilis group" Dickerman, 1931[e]	*E. livescens* (as *G. livescens correcta*)	Des Plaines River, IL (UM)	Dickerman (1931) [19]
Proterometra melanophora (Smith, 1932) Smith, 1936[d]	Prosobranch (as Goniobasis*G. opaca*)[f]	Cooley Creek, AL (SAG)[f]	Smith (1936) [15]
	Elimia spp. (as *Goniobasis* sp.)	"Alabama"	Smith (1932 abstract) [20]
Proterometra hodgesiana (Smith, 1932) Smith, 1936	Prosobranch (as *G. vicina*)[f]	Big Sandy Creek, AL (SAG)[f]	Smith (1936) [15]
		Miller Springs, AL (SAG)	Smith (1936) [15]
	Elimia spp. (as *Goniobasis* sp.)	"Alabama"	Smith (1932 abstract) [20]
		Warrior River, AL (SAG)	Horsfall (1934) [6]
Proterometra catenaria Smith, 1934[e]	Elimia*E. catenaria*	St. John's River, FL (SAG)	Smith (1934) [21]
		Suwannee River, FL (SAG)	Smith (1934) [21]
		Apalachicola River, FL (SAG)	Smith (1934) [21]
		Blue Springs, FL (SAG)	Anderson & Anderson (1967) [22]
	Elimia dooleyensis	Choctawhatchee River, AL (SAG)	Smith (1934) [21]
Proterometra sagittaria Dickerman, 1946	*E. livescens*	Carp River, MI (GL)[g]	Anderson and Anderson (1969) [23]
	Elimia and *Pleurocera* snails	Lake Erie, OH (GL)[f]	Dickerman (1946) [24]
		Maumee River, OH (GL)	Dickerman (1946) [24]
		Sandusky River, OH (GL)	Dickerman (1946) [24]
Proterometra dickermani Anderson, 1962	*E. livescens*[f]	Ocqueoc River, MI (GL)[f]	Anderson (1962) [25]; Lushbaugh (1968) [7]; Uglem et al. (1990) [26]
		Looking Glass River, MI (GL)	Lushbaugh (1968) [7]
Proterometra septimae Anderson and Anderson, 1967	*E. catenaria*[f]	Blue Springs, FL (SAG)[f]	Anderson & Anderson (1967) [22]
Proterometra albacauda Anderson and Anderson, 1967	*E. catenaria*[f]	Blue Springs, FL (SAG)[f]	Anderson & Anderson (1967) [22]
		Ogeechee River, GA (SAG)	Aliff et al. (1977) [27]
Proterometra edneyi Uglem and Aliff, 1984	*E. semicarinata*[f]	Elkhorn Creek, KY (OH)[f]	Uglem & Aliff (1984) [28]; Lewis et al. (1989) [10]
Proterometra autraini LaBeau and Peters, 1995	*E. livescens*[f]	Au Train River, MI (GL)[f]	LaBeau & Peters (1995) [29]
Proterometra epholkos sp. n.	*Elimia* cf. *modesta*[f]	Terrapin Creek, AL (SAG)[f]	Present study

ns = not specified.
[a] The genus group name "*Cercaria*" is a synonym of *Proterometra*.
[b] *Goniobasis* is a junior subjective synonym of *Elimia*; original host identifications in parentheses.
[c] Water resources regions (Seaber et al. 1987) for collection localities in parentheses. TG = Texas Gulf; GL = Great Lakes; UM = Upper Mississippi, OH = Ohio; T = Tennessee; SAG = South Atlantic Gulf.
[d] Type host or locality indeterminate or unspecified by original author.
[e] Conspecific with *P. macrostoma* sensu Horsfall (1934).
[f] Type host or type locality.
[g] Introduced population.

1905, by having testes that are transverse, abreast, and positioned near the posterior extremity of the body as well as having a uterine field and vitellarium that each extend anteriad beyond the prostatic sac, occupying the space between it and the oral sucker [1,2]. No infection by an accepted species of *Proterometra* has been documented from beyond North America (in chronological order): *Proterometra macrostoma* (Faust, 1918) Horsfall, 1933 (type species; *species inquirenda*), *Proterometra melanophora* (Smith, 1932) Smith 1936 (herein treated as *species inquirenda* but considered a junior subjective synonym of *P. macrostoma* by other authors), *Proterometra hodgesiana* (Smith, 1932) Smith, 1936 (*species inquirenda*), *Proterometra catenaria* Smith, 1934, *Proterometra sagittaria* Dickerman, 1946, *Proterometra dickermani* Anderson, 1962, *Proterometra albacauda* Anderson and Anderson, 1967, *Proterometra septimae* Anderson and Anderson, 1967, *Proterometra edneyi* Uglem and Aliff, 1984, and *Proterometra autraini* LaBeau and Peters, 1995 (Tables 1, 2). We concur with LaBeau and Peters [29] and

Gibson [38] in excluding *Proterometra guangzhouensis* Lu (1992), *Proterometra sillagae* Wu (1997), *Proterometra brachyura*n Wu (1997), and *Proterometra lamellorchis* Wu (1997) from *Proterometra* and herein consider these species *incertae sedis*. Species of *Proterometra* are endemic to North American freshwater environments, and only one report details an infection west of the main stem of the Mississippi River [30] (Table 2).

Since Dickerman [33], experimental infections of *Proterometra* spp. (8 of 10 species) have indicated that these flukes undergo asexual reproduction in freshwater prosobranch snails, primarily of *Elimia* but also *Pleurocera acuta* [1,6], *Lithasia obovata* [4; see Graff [39] for synonymy], and *Campeloma subsolidum* [3], that range in rivers and streams draining to the Great Lakes, Gulf of Mexico, and Atlantic Ocean (Table 1). Of note is that, in an abstract, Dickerman [40] stated that he succeeded in feeding cercariae to "fish and turtles;" however, no subsequent work, including Dickerman [13,24], mentions a turtle host for any species of *Proterometra*. These flukes have a macroscopic, 3–22 mm long, furcocystocercous cercaria that can be progenetic [25,29,41] and flamboyantly swims in the water column, perhaps luring the fish definitive host to bite and/or swallow it [22,35]. The cercarial body (="distome") is typically withdrawn inside a cavity (="tail cavity") within the anterior or midbody portion of the tail stem before cercarial emergence (="shedding") from the gastropod. No encysted metacercaria has been reported for any species of *Proterometra*, leading some to regard the 2-host life cycle of these flukes as truncated [42]. Adults develop rapidly (if the cercaria is not already egg-bearing, progenetic) upon infecting the epithelial surfaces of the buccal cavity, esophagus, esophageal sphincter, and occasionally gut [22] of sunfishes (*Lepomis*) and basses (*Micropterus*) (Perciformes: Centrarchidae) as well as representatives of five other families of primary division freshwater fishes in North America (Table 2).

Historically, these flukes have been differentiated based on primarily the habitus and behavior of their charismatic cercariae, which first were described by Faust [3] as "*conspicuous objects*" and which have likewise attracted aquatic biologists within and beyond parasitology since the early 20th century. Perhaps because few parasitologists specifically examine the buccal cavity and esophageal sphincter of fishes for digenean infections and perhaps because of the appeal of rather focusing on the charismatic free-living cercariae, adults of *Proterometra* spp. have not garnered the same level of anatomical scrutiny as their cercariae (Tables 3–6). As a result, relatively little information is available on diagnostic features of adults of *Proterometra* spp. Some authors consider adult specimens to be indistinguishable across species, e.g., Anderson and Anderson [22] stated that, "*adults [sic] of all seven (species of Proterometra) are very similar and their separation would be questionable were it not for the striking differences between their cercariae*." Another gap comprises the fact that molecular data have yet to be employed in differentiating species of *Proterometra*; one sequence (28S) exists in GenBank (*Proterometra* sp.) [43].

Towards contributing to the knowledge of *Proterometra* spp., the addition of morphological features of adults and cercariae, and the development of molecular taxonomic information, we herein use morphology and molecules to explore the biodiversity and life history of these azygiids in Alabama. Also provided herein for species of *Proterometra* are taxonomic keys for cercariae and adults based on morphological and behavioral characteristics sourced from their respective original taxonomic works and select museum materials, updated lists of hosts (prosobranchs and fishes) and geographic localities for *Proterometra* spp., and synopses and assessments of the morphological features previously used to differentiate them.

2. Materials and methods

Prosobranch snails (hereafter "snails") were collected by hand while snorkeling in Terrapin Creek (South Fork; N33°51′36.56″; W85°31′28.15″; Cleburne County, Alabama, USA) on 22 May 2013 and immediately thereafter transported to the laboratory in 20-L plastic buckets filled with ambient stream water and aerated using battery-powered aerators and airstones. They were identified as *Elimia* cf. *modesta* (Lea, 1845) as per Thompson [44] by having (i) 4 rust colored bands visible through aperture, (ii) broadly conical shell, (iii) shoulder whorls smooth, and (iv) adults lacking carina. In the laboratory, snails were isolated individually in cell culture well plates modified with perforated lids and bottoms, allowing water flow through each well, and immersed in 40-L aquaria filled with aerated, filtered, and de-chlorinated tap water [9]. Snails were monitored for 36 h and indicated as infected by the presence of swimming, naturally-shed cercariae within their particular well. Infected snails immediately were relocated to 1-L glass jars filled with aerated aquarium water. The total volume of each container was changed semiweekly. Swimming cercariae were collected with a large bore plastic pipette and transferred to a glass jar wherein their behavior could be observed without aeration current before being preserved for morphology and molecular biology. Fluke specimens for morphology were wet-mounted on glass slides and heat killed under slight coverslip pressure with heat from an EtOH burner flame or isolated and heat killed within a dish flooded with freshwater heated to 60 °C. Killed specimens were then transferred to a vial of 10% neutral buffered formalin. Specimens for molecular biology were pipetted from the aquaria alive and immediately preserved in a vial of 95% EtOH and stored at −20 °C. Germinal sacs and intramolluscan cercariae were separated from soft tissues of infected snails by transverse fracturing of each shell using a 150 mm Bessey workbench vice before teasing apart snail soft tissues from parasites in a petri dish filled with stream water and with the aid of a dissecting microscope. Tissue vouchers (snail soft tissue) were placed in a vial of 95% EtOH and stored at −20 °C. Shell vouchers for each infected snail were placed in a vial of 70% EtOH and deposited in the United States National Museum (USNM), Smithsonian Institution, Washington DC, USA.

Fish hosts were cast-netted from Terrapin Creek on 22 May 2013 and 24 October 2013, maintained alive in a cooler filled with ambient stream water and aerated using battery-powered aerators and airstones, transported to the laboratory, killed by spinal severance, measured, and identified as spotted bass (*Micropterus punctulatus* [Rafinesque, 1819]) as per Boschung and Mayden [45] by having (i) black lateral stripe with bars or blotches extending along lateral line, (ii) >13 scale rows on cheek, (iii) tooth patch on tongue, (iv) pyloric ceca un-branched, and (v) rows of spots beneath level of lateral line. Shears were used to hemisect the jaw and the buccal cavity to reveal epithelial surfaces before inspection with the aid of a stereoscope at 50× magnification. Flukes were removed using fine forceps or brushes and preserved as cercariae were (see above).

All flukes, adults and cercariae, intended for morphology were left in fixative for at least 48 h and subsequently rinsed overnight in distilled water, stained overnight in Van Cleave's hematoxylin with several additional drops of Ehrlich's hematoxylin, made basic in 70% ethanol with lithium carbonate and butyl-amine, dehydrated, cleared in clove oil, and permanently mounted on glass slides using Canada balsam. Measurements, and illustrations of stained, whole-mounted specimens were made with aid of a Leica DM-2500 equipped with differential interference contrast (DIC) optical components and a drawing tube. Measurements are herein reported in micrometers (μm) followed by the mean and number of specimens measured for that feature in parentheses.

Specimens for molecular biology comprised 1 adult and 2 cercariae. Total genomic DNA from collected specimens was extracted using a DNeasy™ Blood and Tissue kit (Qiagen) according to the manufacturer's protocol. PCR was carried out using the forward primer "GA1" (5′-AGA ACA TCG ACA TCT TGA AC-3′) (3′ end of 5.8S rDNA) [46] and the reverse primer "ITS2.2" (5′-CCT GGT TAG TTT CTT TTC CTC CGC-3′) (5′ end of 28S rDNA) [47] and sequenced as per Nolan and Cribb [48]. The PCR products were purified using the QIAquick Gel Extraction Kit (Qiagen) following the manufacturer's protocols. DNA sequencing was performed by Lucigen with an ABI 3730xl sequencer at Lucigen Corp. (Madison, WI) using the same primers as used in the PCR. Chromatograms were analyzed with BioNumerics® version 7.0 (Applied Maths, Sint-Martens-Latem, Belgium), and amplification primer sequences were trimmed after consensus sequences between both strands were

Table 2
Fish hosts for species of *Proterometra* Horsfall, 1933 (Digenea: Azygiidae).

Species	Host	Locality[a]	Author (year) (Ref.)
Proterometra macrostoma (Faust, 1918) Horsfall, 1933[b]	Astyanax*A. mexicanus*	Upper San Marcos River, Texas (TG)	Underwood & Dronen (1984) [30]
	Ambloplites rupestris	ns	Horsfall (1933) [1]
		Upper San Marcos River, Texas (TG)	Underwood & Dronen (1984) [30]
		Lake Superior, Lake Ontario (GL)	Dechtiar and Lawrie (1988) [31]; Dechtiar and Christie (1988) [32]
	Lepomis auritus	Upper San Marcos River, Texas (TG)	Underwood & Dronen (1984) [30]
	Lepomis*L. cyanellus*	ns	Horsfall (1933) [1]
	Lepomis*L. gibbosus*[c]	Des Plaines River, IL (UM)	Dickerman (1934) [33]
		Bass Lake, MI (GL)	Horsfall (1934) [6]
		ns	Dickerman (1946) [24]
		Lake Huron, Lake Ontario (GL)	Dechtiar et al. (1988) [34]; Dechtiar & Christie (1988) [32]
	Lepomis gulosus	ns	Horsfall (1933) [1]
		Elkhorn Creek, KY (OH)	Riley & Uglem (1995) [8]; Rosen et al. (2005) [35]
		Upper San Marcos River, Texas (TG)	Underwood & Dronen (1984) [30]
	Lepomis humilis	ns	Horsfall (1933) [1]
	Lepomis macrochirus	ns	Horsfall (1933) [1]
		Des Plaines River, IL (UM)	Dickerman (1934) [33]
		Elkhorn Creek, KY (OH)	Riley & Uglem (1995) [8]; Rosen et al. (2005) [35]
		Bass Lake, MI (GL)	Horsfall (1934) [6]
		ns	Krist (2000) [9]; Dickerman (1934) [33]
		Wekiva River, FL (SAG)	Hunter & Wigington (1972) [5]
		Upper San Marcos River, Texas (TG)	Underwood & Dronen (1984) [3]
	Lepomis megalotis	Elkhorn Creek, KY (OH)	Riley & Uglem (1995) [8]; Rosen et al. (2005) [35]
	Lepomis microlophus	Upper San Marcos River, Texas (TG)	Underwood & Dronen (1984) [30]
	Lepomis punctatus	Upper San Marcos River, Texas (TG)	Underwood & Dronen (1984) [30]
	Micropterus dolomieu	ns	Horsfall (1933) [1]
	Micropterus*M. salmoides*	ns	Horsfall (1933) [1]
		Bass Lake, MI (GL)	Horsfall (1934) [6]
		Elkhorn Creek, KY (OH)	Riley & Uglem (1995) [8]
		Upper San Marcos River, Texas (TG)	Underwood & Dronen (1984) [30]
	Pomoxis*P. annularis*	ns	Horsfall (1933) [1]
	Pomoxis*P. nigromaculatus*	ns	Horsfall (1933) [1]
		Des Plaines River, IL (UM)	Dickerman (1934) [3]
	Centrarchidae spp.	ns	Smith (1936) [15]

(continued on next page)

Table 2 *(continued)*

Species	Host	Locality[a]	Author (year) (Ref.)
"Forked-Tailed Cercaria"[d] (=*P. macrostoma*)	Centrarchidae spp.	ns	Cahn (1927) [18]
Proterometra melanophora[d]	*M. salmoides*[e]	Cooley Creek, (SAG)[e]	Smith (1936) [15]
Proterometra catenaria Smith, 1934[b]	Centrarchidae spp.[c]	ns	Smith (1934) [21]
	L. cyanellus[c]	Blue Springs, FL (SAG)	Smith (1934) [21]; Anderson & Anderson (1967) [22]
	L. gibbosus[c]	ns	Anderson & Anderson (1967) [22]
Proterometra hodgesiana (Smith, 1932) Smith, 1936[b]	Centrarchidae spp.[c]	ns	Smith (1936) [15]
	L. cyanellus[c]		Smith (1936) [15]
Proterometra sagittaria Dickerman, 1946[b]	*L. gibbosus*[c]	ns	Dickerman (1946) [24]
	"Fish"	Maumee River, OH (GL)	Dickerman (1946) [24]
Proterometra dickermani Anderson, 1962[b,f]	*L. gibbosus*[c]	ns	Anderson (1962) [25]; Anderson & Anderson (1963) [36]
	L. macrochirus[c]	ns	Anderson (1962) [25]
	M. salmoides[c]	ns	Anderson & Anderson (1963) [36]
Proterometra septimae Anderson and Anderson, 1967	*L. gibbosus*[c,e]	ns	Anderson & Anderson (1967) [22]
Proterometra albacauda Anderson and Anderson, 1967	Noturus*N. gyrinus*	Ogeechee River, GA (SAG)	Aliff et al. (1977) [27]
	L. auritus	Ogeechee River, GA (SAG)	Aliff et al. (1977) [27]
	L. gibbosus[c,e]	Douglas Lake, MI	Anderson & Anderson (1967) [22]
		Ogeechee River, GA (SAG)	Aliff et al. (1977) [27]
	L. gulosus	Ogeechee River, GA (SAG)	Aliff et al. (1977) [27]
	L. macrochirus	Ogeechee River, GA (SAG)	Aliff et al. (1977) [27]
	L. megalotis	Ogeechee River, GA (SAG)	Aliff et al. (1977) [27]
	L. microlophus	Ogeechee River, GA (SAG)	Aliff et al. (1977) [27]
	P. annularis	Ogeechee River, GA (SAG)	Aliff et al. (1977) [27]
Proterometra edneyi Uglem and Aliff, 1984	*Cottus carolinae*	Elkhorn Creek, KY (OH)	Uglem & Aliff (1984) [28]
	Etheostoma spectabile[e]	Elkhorn Creek, KY (OH)[e]	Uglem & Aliff (1984) [28]
	Etheostoma blennioides	Elkhorn Creek, KY (OH)	Uglem & Aliff (1984) [28]
	Etheostoma caeruleum	Elkhorn Creek, KY (OH)	Uglem & Aliff (1984) [28]
	Etheostoma flabellare	Elkhorn Creek, KY (OH)	Uglem & Aliff (1984) [28]
Proterometra autraini LaBeau and Peters, 1995	*M. salmoides*[c,g]	Au Train River, MI (GL)	Spence & Peters (1971) [37]
	Lota*L. lota*	Au Train River, MI (GL)	LaBeau & Peters (1995) [29]
	Cottus bairdii[c,e]	Au Train River, MI (GL)[e]	LaBeau & Peters (1995) [29]
		Dead River, MI (GL)	LaBeau & Peters (1995) [29]
	A. rupestris	Au Train River, MI (GL)	LaBeau & Peters (1995) [29]
	L. macrochirus[c]	Redberry Lake, MI (GL)	LaBeau & Peters (1995) [29]
	M. dolomieu	Au Train River, MI (GL)	LaBeau & Peters (1995) [29]
	Perca*P. flavescens*[c]	Au Train River, MI (GL)	LaBeau & Peters (1995) [29]
Proterometra epholkos sp. n.	*M. punctulatus*[e]	Terrapin Creek, AL (SAG)[e]	Present study

ns = not specified.

[a] Water resources regions (Seaber et al. 1987) for published collection localities in parentheses; Texas Gulf (TG); Great Lakes (GL); Upper Mississippi (UM), Ohio (OH); South Atlantic Gulf (SAG).

[b] Type host or locality unspecified by original author.

Table 3
Cercarial morphology of *Proterometra* spp.

Species	Tail stem L × W[a]	Furcae L × W[a]	Color	Furcae shape	Shedding	Distome position in tail stem[b]	Spines per mamilla	Egg #[a]	Swim[c]	Author (year) (Ref.)
P. epholkos sp. n.	4.9–7.3 (6) × 1.6–2.6 (2)	1.1–1.3 (1.3) × 1.4–1.8 (1.6)	Amber	Obcordate	Late PM–early AM	A/+	0–6	2–42 (20)	✓	Present study
P. macrostoma	2–6.8 × 0.6–1.7	0.3–1.5 × 0.5–1.6	Yellow	Obcordate	PM	A–M/+	0–5	Max. 50	✓	Horsfall (1934) [6]; Dickerman (1934) [33]
P. catenaria	9–16 long; 5.2–8.2 × 1.1–1.3[d]	1.0–1.3 × ns	White	Ovate	Early PM	A–M/+	10–20	"A few ova"	✓	Smith (1934) [21]; Anderson and Anderson (1967) [22]
P. hodgesiana	3.8 × ns	0.8 × 0.7	Clear	Obcordate	AM	A	Aspinous	ns	×	Smith (1936) [15]
P. sagittaria	12–18 × 1–1.2	1–3 × 0.3–0.5	No pigment	Lanceolate	AM	A/+	Aspinous	20–30	✓	Dickerman (1946) [24];
P. dickermani	3.2 × 0.3	0.7 × 0.3	Brown/yellow	Obcordate	Late PM–early AM	Ex./×	Aspinous	Max. 293	✓	Anderson (1962) [25]
P. albacauda	3.5–6.5 × 1–1.8	0.8 × 1.2	White	Obcordate	ns	A/+	0–5	0–10	✓	Anderson and Anderson (1967) [22]; present study
P. septimae	3.4–4.6 × 0.8–1.8	0.7–0.9 × ns	Brown w/ white spots	Ovate, ends incurled	ns	A/+	6–7	ns	✓	Anderson and Anderson (1967) [22]
P. edneyi	2.9–3.4 (3.2) × 0.4–0.5 (0.5)	0.7–0.8 (0.8) × 0.4–0.6 (0.5)	Amber	Obcordate	PM	M/+[e]	0–1	16–25	×	Uglem and Aliff (1984) [28]
P. autraini	4.3–5.5 (4.8) × 1.3–1.8 (1.5)	1.1–1.4 (1.2) × 0.8–1.43 (1)	Light gold	Obcordate	Diurnal	Ex.–A/+ –	0–8	200–300	✓	LaBeau and Peters (1995) [29]

ns = not specified.
[a] All measurements in millimeters (mm); means in parentheses.
[b] Extruded (Ex.) from tail stem, within anterior portion (A) of tail stem, within middle portion (M) of tail stem; within a cavity (+), external to a cavity (−), cavity not reported or reported as absent (×).
[c] ✓ = swimming × = not swimming.
[d] Smith's (1936) original description described length of the cercaria as 9–16 mm. Anderson and Anderson (1967) published supplemental observations for *P. catenaria* and described the fixed cercaria as 5.2–8.2 mm long.
[e] Based on Fig. 2 of Uglem and Aliff (1984).

generated. IUPAC ambiguity codes were used for coding polymorphic sites, i.e., M should be read as the presence of A and C, rather than as an ambiguous reading between A or C. These positions were identified in the chromatogram by the occurrence of overlapping peaks, where both signals differ from each other by less than 20% [49,50]. To further rule out the inclusion of low quality reads as polymorphisms, we added the restriction that double peaks had to occur on the same position on both forward and reverse strands. The ITS2 region was identified using the annotation tool of "ITS2 Database" [51]. All sequences were deposited as GenBank accession numbers KM503118 and KM503119.

Nomenclature of snails, fish, and digeneans is as follows. Common names for snails follow Johnson et al. [52]. Higher-level gastropod nomenclature and classification follows Graff [39], Johnson et al. [52], Burch and Tottenham [53], and Burch [54,55]. Pre-1995 works assigned species of *Elimia* to *Goniobasis*, but we herein treat the latter genus as a junior subjective synonym of *Elimia* [56]. Common names for fishes follow Boschung and Mayden [45], and higher-level fish classification follows Nelson [57]. Prior to Horsfall [1], wherein she proposed *Proterometra*, species of *Proterometra* were assigned to the collective group name *Cercaria* [3,17,20] (see International Code of Zoological Nomenclature [ICZN] page 70, article 67.14). Subsequent to the establishment of *Proterometra*, published works assigned the adults and cercariae separately, with the former being assigned to *Proterometra* and the latter to *Cercaria* [1,6,15,16,24,33]. To provide taxonomic resolution within *Proterometra* we discuss conspecificity of species assigned to *Cercaria* and treat those species as junior subjective synonyms of their conspecific adults assigned to *Proterometra*.

Type materials were borrowed from the United States National Parasite Collection (USNPC), Beltsville, Maryland, USA, courtesy of Dr. Eric Hoberg and Pat Pilitt, and type materials of the new species were deposited in the USNM.

3. Results

3.1. *P. albacauda* Anderson & Anderson, 1967 (Figs. 1–6)

Diagnosis of adult based on light microscopy of holotype (USNPC No. 61229). Body of adult 2160 long, 1240 wide or 1.7× longer than wide, anterior end narrower than posterior end; tegument approximately 10 thick; tegumental projections (not illustrated) dense anteriorly, sparse at level of and posterior to ventral sucker, not associated with rim of oral sucker or ventral sucker (Fig. 1). Excretory pore medial, terminal. Nervous system indistinct. Oral sucker subterminal, 160 or 7% of body length from anterior body end, 640 long or 31% of body length, 670 wide or 54% of body width, posterior margin 440 from anterior margin of ventral sucker. Ventral sucker in posterior half of body, with anterior margin 1240 or 57% of body length from anterior body end, 330 long or 15% of body length, 370 wide or 30% of body width, 52% of oral sucker length, 56% of oral sucker width. Mouth opening ventrally, 55 long, 238 wide, 4.3× wider than longer. Pharynx ovoid, muscular, immediately posterior to oral sucker, 105 long or 5% of body length, 125 wide or 1.2× wider than longer (Fig. 1). Esophagus extending posteriad from mouth 470 before bifurcating immediately posterior to pharynx, with esophageal branches arching posterolaterad before joining with intestinal ceca; each esophageal branch extending posterolaterad 305 (dextral) and 245 (sinistral), constricted distally by a muscular sphincter; intestinal ceca confluent with esophageal branches, appearing inverse U-shaped inclusive of esophageal branches, comprising paired dextral and sinistral ceca; dextral cecum 1240 long or 57% of body length, beginning 940 or 44% of body length from anterior end of body, post-cecal space, 110 or 5% of body length from posterior end; sinistral cecum 1340 long or 62% of body length, beginning 800 or 37% of body length from anterior end of body, post-cecal space 90 or 4% of body length.

Testes 2 in number, transverse, abreast, oblique, ovoid to pyriform; dextral testis 335 long or 15% of body length, 180 wide or 15% of body width, post testicular space 160 or 7% of body length; sinistral testis

Table 4
Adult morphology of *Proterometra* spp.

Species	Body L × W[a]	Body color	OS:VS ratio[b]	Testes shape	Uterus[b]	Extent of vitellarium[b]	Eggs[c,d]	Author (year) (Ref.)
P. epholkos sp. n.	1980–2300 (2189) × 820–1280 (1169)	Orange	2:1	Ovoid	Extracecal, convoluted from posterior region of body to posterior third of OS	Lateral, posterior quarter of OS to posterior margin of body	51 × 26, 78 × 44	Present study
P. macrostoma	1800–2100 × 1000–1200	"Light tan /pink"	2:1	Ovoid	Intercecal, loose irregular loops from ovary to posterior margin of OS	Lateral, posterior margin of OS to region of testes	65 × 34, 95 × 75	Horsfall (1933,1934) [1,6]; Dickerman (1934) [33]
P. catenaria	1700–1830 × 1180–1300	Pale yellow	2.5:1	Pyriform	,Extracecal, anterior loops even with vitellaria	Lateral, between anterior & posterior 1/3 of OS to posterior margin of VS	52 × 28, 59 × 34	Smith (1934) [21]; Anderson and Anderson (1967) [22]
P. hodgesiana	ns	ns	ns	ns	ns	ns	ns	Smith (1936) [15]
P. sagittaria	1500–2000 × 900–1000	Orange	2:1	"Elongate pyriform"	Intercecal, loose transverse coils from ovary to OS	Lateral, OS posterior margin to posterior region of testes	67 × 35, 79 × 44	Dickerman (1946) [24]
P. dickermani	1760–1870 × 1010–1180	"Tan w/ pink tinge"	1.7:1	Ovoid	Intercecal, coils extend anterior to posterior margin of OS	Lateral, mid-level of OS to or beyond posterior margin of testes	74 × 47, 98 × 66	Anderson (1962) [25]
P. albacauda	1990–2200 × 1230–1380	Yellow/tan	2:1	Pyriform	Intercecal, convoluted between ovary and posterior margin of OS	Lateral, posterior margin of OS to posterior fourth of testes	62 × 34, 75 × 42	Anderson and Anderson (1967) [22]
P. septimae	1610–1860 × 1150–1270	Pale orange	2.7:1	Ovoid	Extracecal,extends to mid level of OS	Lateral, center of OS to center of VS	65 × 26, 73 × 36	Anderson and Anderson (1967) [22]
P. edneyi	550–1010 (737) × 330–610 (450)	ns	2:1	Elongate, elliptical	Intercecal, one loop between VS and OS	Lateral, OS posterior margin to anterior one half of testes	71 × 40, 77 × 42	Uglem and Aliff (1984) [28]
P. autraini	1920–2160 (2030) × 1320–1540 (1430)	Light orange	2:1	Elongate elliptical	Intercecal, convoluted, extends to posterior third of OS	Lateral, posterior level of OS to anterior level of testes	75 × 42, 92 × 64	LeBeau and Peters (1995) [29]

ns = not specified.
[a] Measurements in micrometers (μm); mean in parentheses.
[b] OS = oral sucker; VS = ventral sucker.
[c] Measurements in millimeters (mm).
[d] Smallest egg (L × W), largest egg (L × W).

225 or 10% of body length, 140 wide or 11% of body width, post testicular space 55 or 3% of body length (Fig. 1). Vasa efferentia approximately 5 wide, coalescing into 2 main collecting ducts extending anteriad from testes and lateral to ventral sucker before connecting with seminal vesicle (=a robust vas deferens enveloped by prostatic sac) dorsally and in posterior half of prostatic sac (Figs. 1, 2). Prostatic sac medial, anterior margin 90 from posterior margin of oral sucker, posterior margin 90 from anterior margin of ventral sucker, occupying space between pharynx and ventral sucker, 250 long, 280 wide, having a highly muscular wall incorporating myriad gland-like cells, enveloping seminal vesicle and pars prostatica. Seminal vesicle thin walled, highly convoluted, having swollen proximal region and narrow distal region; proximal region of seminal vesicle extending sinuously anteriad, S-shaped, 448 long, 60 in maximum width; distal region highly convoluted, 220 long, 23 in maximum width, connected to pars prostatica via a minute duct. Minute duct (=possibly "verschlussapparat" of Looss [58] in Horsfall [6], page 321) 25 long, thin-walled, appearing to pierce proximal surface of ejaculatory duct (=thick walled pars prostatica) (Fig. 2). Pars prostatica 263 long, 38 wide proximally, 13 wide distally, dorsolateral to seminal vesicle, arched, widest and thick-walled in proximal half, narrow and thin-walled distally, extending posteriad before exiting prostatic sac posteriorly. Ejaculatory duct (=continuation of pars prostatica external to prostatic sac) extending posteriad from prostatic sac and becoming confluent with hermaphroditic duct, thin walled for entire length (lacking gland-like cells or muscular wall), 60 long or 22% of pars prostatica length, 15 in maximum width. Confluence of male and female genitalia indistinct (Fig. 2). Sinus organ indistinct in holotype. Hermaphroditic pore sinistral, anterior to genital pore, occupying space between prostatic sac and ventral sucker, directing ventrally before opening into genital atrium (="genital sinus" of Horsfall [1]) (Fig. 2). Genital atrium connecting hermaphroditic pore and genital pore. Genital pore immediately anterior to ventral sucker, medial, posterior to prostatic sac, opening ventrally in posterior half of body. Cervical groove indistinct.

Ovary medial, intercecal for entire breadth, immediately anterior to testes, 140 long or 6% of body length, 230 wide or 19% of body width or 1.6× wider than long; post-ovary space, 275 long or 13% of body length; germarium present (not illustrated), a chamber occupying center portion of ovary, becoming confluent with proximal portion of oviduct at level of muscular sphincter; oviduct thin-walled, lacking sperm in proximal portion, emanating from mid-dorsal surface of ovary, extensively convoluted and extending sinuously anteriad before curving dextrad and becoming confluent with Laurer's canal, 225 long from confluence with Laurer's canal to ootype (Fig. 1). Laurer's canal narrow proximally, swollen medially with sperm (=perhaps functioning as a "rudimentary seminal receptacle", sensu [2]), indistinct for most of its course dorsal and ventral to ovary in holotype, opening dorsally and posterior to ventral sucker (Fig. 3). Ovovitelline duct indistinct. Ootype dorsal to ovary, directing sinistrad, anterior to testes, 73 long, 40 in maximum width; Mehlis gland indistinct. Uterus extensively convoluted, not extending lateral to ceca, occupying space between posterior margin of oral sucker and ovary, comprising a field 1000 long or 46% of body length and 1100 wide or 88% of body width, passing ventral sucker sinistrally, thin-walled for entire length, 80 in maximum width, with hundreds of eggs, with distal portion comprising a metraterm; uterine seminal receptacle indistinct. Metraterm 450 long or 20% of body length, 75 wide, confluence with uterus posterior to medial axis of ventral sucker, sinistral, extending anteriorly lateral to ventral sucker, muscular and thick-walled, becoming confluent with ejaculatory duct to form a common duct (=herein a 'hermaphroditic duct') within sinus organ (Figs. 1–3). Vitellarium follicular, ventral to ceca, distributing in 2 bilaterally symmetrical fields, distance between fields 870 wide or 70% of body width, extending from level of pharynx to mid-testes; dextral vitelline field 1100 long or 50% of body length, terminating 40% of body length from anterior end of body, terminating 11% of body length from posterior end of body, 88% of dextral ceaca length; sinistral vitelline field 1220 long or 56% of body length, terminating 37% of body

Table 5
Key to *Proterometra* spp. (cercaria).

1a. Tail stem length exceeding 10 mm	2
1b. Tail stem length not exceeding 10 mm	3
2a. Furcae lanceolate, 1–3 mm long; mamillae aspinous	*P. sagittaria*
2b. Furcae ovate, 1–1.3 mm long; 10–20 spines/mamillae	*P. catenaria*
3a. Furcae obcordate, ends rounded, with medial notch	4
3b. Furcae ovate, ends incurled, lacking medial notch	*P. septimae*
4a. Many uterine eggs	5
4b. Few uterine eggs	6
5a. Distome extruded; tail cavity absent; tail stem <3.5 mm long; mamillae aspinous	*P. dickermani*
5b. Distome extruded or withdrawn; tail cavity present; tail stem <4 mm long; mamillae aspinous or with up to 8 spines/mamillae	*P. autraini*
6a. Mamillae arranged in transverse bands throughout tail stem; cercaria does not swim; tail stem ≤3.4 mm long; tail stem ≤0.6 mm wide	*P. edneyi*
6b. Mamillae in a lateral column per body margin posterior to the tail cavity (Fig. 12); cercaria swims; tail stem ≥3.5 mm long; tail stem ≥1 mm wide	7
7a. Tail stem medially constricted; mamillae >0.1 mm, 5–6 per lateral column, aspinous or with up to 6 spines/mamillae; cercaria amber in color; furcae >1 mm long, ≥1.4 mm wide; distome >1.3 mm long	*P. epholkos* sp. n.
7b. Tail stem not medially constricted; mamillae minute <0.05 mm, 8–10 per lateral column, aspinous or with up to 5 spines/mamillae, cercaria white in color; furcae <1 mm long, <1.4 mm wide; distome <1.3 mm long	*P. albacauda*

length from anterior body end, terminating 7% of body length from posterior body end, 91% of sinistral ceacum length; primary vitelline collecting ducts symmetrical, coursing between ovary and testes, postuterine, extending posteromediad from respective vitelline field before becoming confluent medially and forming vitelline reservoir; dextral vitelline collecting duct 650 long or 30% of body length, 15 wide near vitelline reservoir, proximal end branches from vitellarium at 47% of dextral vitelline field length; sinistral vitelline collecting duct 555 long or 25% of body length, 8 wide near vitelline reservoir, proximal end branches from vitellarium at 65% of sinistral vitelline field length; vitelline reservoir medial, transverse, post-ovarian, pre-testicular (Fig. 1). Uterine eggs ovoid or pyriform, enlarging from approximately 40 × 25 in proximal portion of uterus to approximately 70 × 40 in distal portion of uterus, with one polar surface of each egg bearing minute fimbria or papilla-like projections (Figs. 1, 3, 4).

Diagnosis of cercaria based on whole-mounted paratype (USNPC No. 61230). Mamillae (=mound like tegumental protuberances, which are located in the anterior portion of the tail stem) 35 in maximum length, 100 in maximum width or 3.1× wider than long (Fig. 5, 6). Tail stem cavity at anterior end of cercaria, thick walled, muscular. Distome residing within tail stem cavity, 1200 long, 760 wide or 1.6× longer than wide. Eggs absent in paratype.

3.1.1. Taxonomic summary

Type host: Pumpkinseed sunfish, *Lepomis gibbosus* (experimental) (Linnaeus, 1758) Brag, 1949 (Perciformes: Centrarchidae).

Intermediate host: Gravel elimia, *Elimia catenaria* (as *Goniobasis catenaria*) Say, 1822 (Cerithioidea: Pleuroceridae).

Other hosts: see Tables 1 and 2.

Site of infection: Cardiac stomach (fish) and gonoducts (prosobranch snail).

Type locality: Blue Springs (GPS N30°47′23.18″; W85°08′33.57″), Marianna, Jackson County, Florida.

Other localities: see Tables 1 and 2.

Specimens examined: USNPC Nos. 61229 (holotype) and 61230 (paratype).

3.1.2. Remarks

The original description of *P. albacauda* by Anderson and Anderson [22] comprised a general account of an unspecified number of stained, whole-mounted adult specimens as well as live and stained cercariae. That description was incomplete, omitting or inaccurately depicting anatomical details of the proximal and distal portions of the genitalia in the adult and largely omitting internal anatomical details of the distome. Details of the genitalia provided in the original description comprised an illustration of the adult that located the gonads, uterus, hermaphroditic pore (as "genital pore"), and a structure we confirmed as the metraterm. However, it did not provide high magnification views or fine details of their connections, orientations, and relative positions. In light of the fact that this is not atypical for descriptions comprising species of *Proterometra*, we are puzzled by previous authors who have dismissed adult morphology as taxonomically useful, given that no such comparisons have been published to our knowledge. Indeed, features associated with the genitalia of adult digeneans typically differentiate species. As such, documenting the presence/absence, dimensions, and relative proportions and positions of the genitalia among *Proterometra* spp. could likewise be helpful in reducing uncertainty about the identities of species, especially those having suspiciously wide geographic distributions and low host specificity, e.g., "*P. macrostoma*." We accept that many of these features have yet to be proved as diagnostic for species of *Proterometra* but as more detailed descriptions and re-descriptions are entered into the literature such assessments can be made; whereas, now they are impossible due to a lack of comparative data. For example, the redescription provided herein (based on the holotype [intact adult] and a paratype [partial cercaria, including only the anterior portion of the tail stem plus the

Table 6
Key to species of *Proterometra* (adults).

1a. Diameter of oral sucker/ventral sucker ≥2:1	2
1b. Diameter of oral sucker/ventral sucker <2:1	*P. dickermani*
2a. Vitellarium not or slightly extending posteriad beyond margin of ventral sucker; posterior uterus looping anterior or lateral to ventral sucker	3
2b. Vitellarium extending posteriad beyond ventral sucker; uterus looping between ovary and ventral sucker	4
3a. Uterus intercecal or dorsal to ceca; vitellarium not extending beyond level of mid ventral sucker; egg >60 mm in diameter; testes nearly equal in size	*P. septimae*
3b. Uterus extracecal; vitellarium extending to level of or slightly posterior to ventral sucker; egg ≤60 mm long; testes unequal	*P. catenaria*
5a. Body ≤1100 μm long; body ≤650 μm wide	*P. edneyi*
5b. Body >1100 μm long; body >700 μm wide	6
6a. Vitellarium longer than intestinal ceca	7
6b. Vitellarium shorter than intestinal ceca	8
7a. Ventral sucker >270 μm long; oral sucker typically >600 μm long; testes ovoid, not intercecal, typically 0.3 × 0.16 mm	*P. epholkos* sp. n.
7b. Ventral sucker <270 μm long; oral sucker ≤600 μm long; testes elongate pyriform, intercecal, typically 200 μm × 80 μm	*P. sagittaria*
8a. Anterior uterine loops not extending beyond pharynx; vitelline reservoir pretesticular; vitellarium not extending beyond anterior margin of testes	*P. autraini*
8b. Anterior uterine loops extending to posterior third of oral sucker; vitelline reservoir midtesticular; vitellarium extending beyond anterior margin of testes	*P. albacauda*

distome]) provides the first fine-scale anatomical details of the male and female genitalia of *P. albacauda*. We emphasize that a complementary study of live cercariae and adults could help elucidate features of the genitalia as well as the excretory system and nervous system, which have yet to be adequately detailed in *Proterometra* spp.

No information was available previously on the vasa efferentia, verschlussapparat, ejaculatory duct, sinus organ, genital atrium, germarium, oviduct, Laurer's canal, and ootype of *P. albacauda*. We determined that the vasa efferentia extend anteriad from the testes, flanking the ventral sucker, before becoming confluent anterior to the ventral sucker at level of the prostatic sac (Fig. 2). The ejaculatory duct (external to the prostatic sac) extends from the pars prostatica and becomes confluent with the metraterm (Fig. 2), forming a short common hermaphroditic duct that connects with the hermaphroditic pore. We observed a hermaphroditic pore that opens into a large genital atrium (Fig. 2), which in the holotype contains two eggs. The genital atrium communicates with the genital pore, which opens anterior to the ventral sucker (Fig. 2). We infer from Fig. 1 of Anderson and Anderson [22] that they misinterpreted the hermaphroditic pore, considering it the genital pore while not providing detail of another pore associated with the genitalia. The ovary has an obvious germarium, the lumen of which communicates with the oviduct wherein there is a muscular sphincter (Fig. 3). Although not detailed in the narrative of Anderson and Anderson [22], we infer from their Fig. 1 that they interpreted the oviduct as emanating from the sinistral margin of the ovary. However, we confirm that the oviduct courses dorsal to the ovary, where it is extensively convoluted, i.e., no portion of the holotype's oviduct was illustrated by Anderson and Anderson [22]. We also document that the Laurer's canal is present, that the ootype is dorsal to the ovary, and that the metraterm is present and illustrated as a hollow chamber arching anteromediad from the distal portion of the uterus (Fig. 1).

Details of additional genital structures need clarification and correction based on a comparison between the holotype and the original description by Anderson and Anderson [22]. Because no explanation of the following features was provided in the narrative portion of the original description, the following information is sourced from Fig. 1 of Anderson and Anderson [22]. Therein, the seminal vesicle appears as a comma-shaped structure that lacks convolutions within the prostatic sac; however, in the holotype we studied the seminal vesicle is extensively convoluted within the prostatic sac and comprises a swollen proximal portion and a narrow distal portion (Fig. 2). The narrow duct extending from the seminal vesicle and within the prostatic sac is undoubtedly the pars prostatica (Fig. 2); however, they did not identify it as such and their drawing of it shows a uniformly narrow and thin-walled tube. The pars prostatica of the holotype is proximally expanded and thick walled, becoming thin walled and narrow distally before connecting with the ejaculatory duct (Fig. 2). The vitelline reservoir and collecting ducts were not described in detail originally but Fig. 1 of Anderson and Anderson [22] indicates the location of the reservoir between the ovary and testes. Our observations of the holotype confirm that arrangement (Fig. 3).

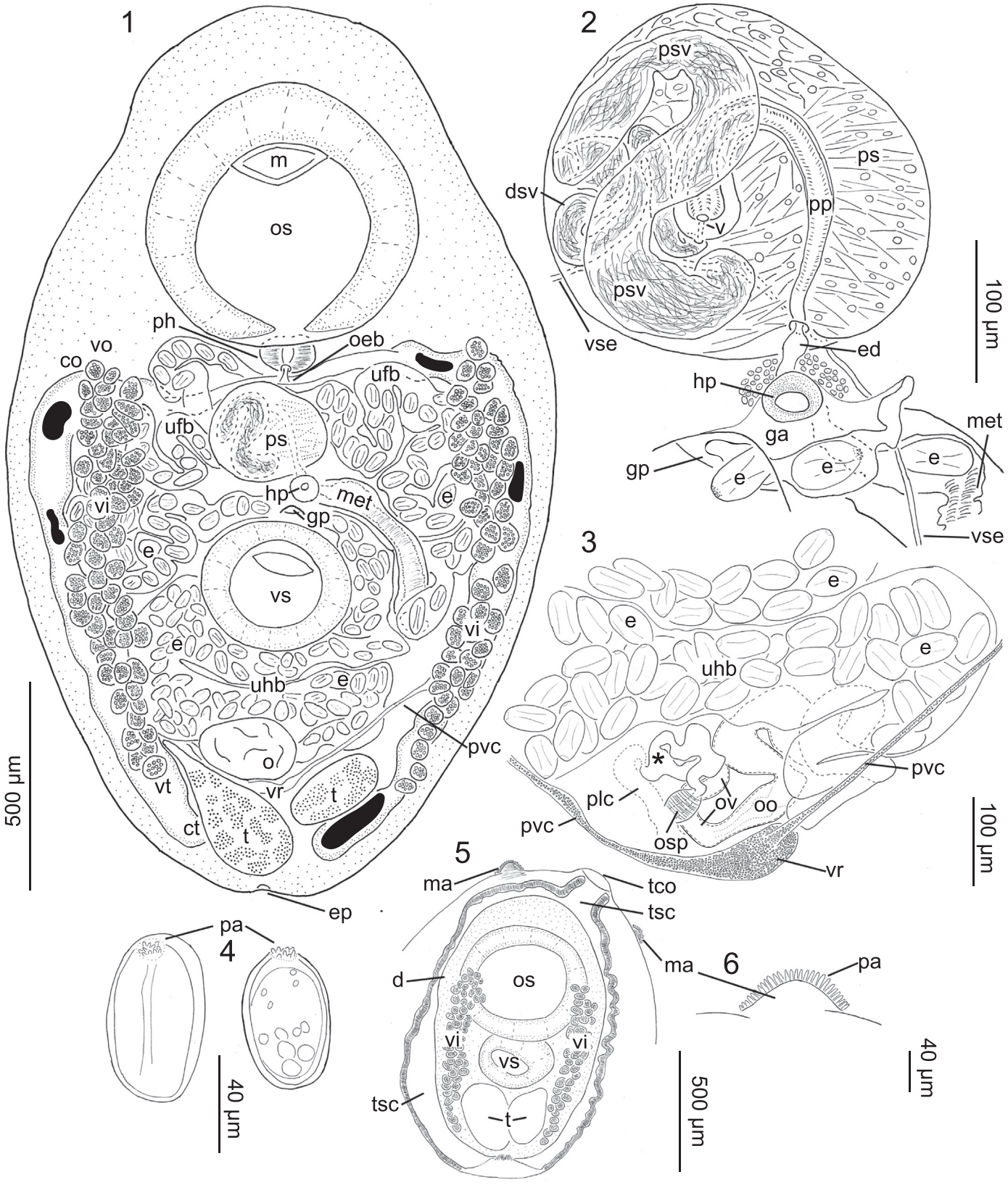
Regarding the cercaria, the original description of *P. albacauda* included an illustration of the distome within the tail stem plus a mamilla (as "papilla") bearing spines. This arrangement of mamillae is evidently characteristic of *P. albacauda*. The partial specimen comprising the paratype has three aspinous mamillae only (Fig. 5). Anderson and Anderson [22] reported that the mamillae of the tail stem were distributed in "*four lateral rows of eight to ten* (mamillae) *each*, *bearing three to five radially arranged spines*"; however, we describe these "rows" as columns, since they are depicted in Fig. 1 of Anderson and Anderson [22] as longitudinal and extending posteriad in parallel with the body margin. The only type material comprising a cercaria is that of the damaged paratype we studied, making impossible a confirmation of that arrangement of mamillae. The paratype lacks eggs and its tail stem cavity, containing the distome, markedly differs from other described cercariae in that the cavity is thick-walled and muscular (Fig. 5), perhaps functioning to forcibly extrude the distome upon contraction.

3.2. Proterometra epholkos sp. n.

(Figs. 7–14)

Diagnosis of adult based on light microscopy of 7 stained whole mounted specimens. Body of adult orange (live coloration), 1980–2300 (2189, 7) long, 820–1280 (1169, 7) or 1.7–2.7 (1.9, 7)× longer than wide, ventrally concave; tegument approximately 10–20 (14, 7) thick; papillae loosely studding rim of the mouth, tightly encircling the outside rim of the ventral sucker, pored, lacking sensory cilium; tegumental projections (not illustrated) distributed evenly from anterior to posterior end (Fig. 7). Excretory system difficult to trace, unites anterior to oral sucker, extends posteriad lateral to ventral sucker, unites with excretory bladder near ovary; excretory pore medial, terminal. Nervous system indistinct in fixed whole-mounted specimens. Oral sucker subterminal, 70–200 (146, 7) or 3–10% (7%, 7) of body length from anterior body end, 600–710 (669, 7) long or 25–36% (31%, 7) of body length, 550–750 (675, 7) wide or 53–67% (58%, 7) of body width, posterior margin 320–680 (441, 7) from anterior margin of ventral sucker (Fig. 7). Ventral sucker in posterior half of body, with anterior margin 1140–1360 (1263, 7) or 52–62% (58%, 7) of body length from anterior body end, 310–360 (339, 7) in length or 14–18% (16%, 7) of body length, 320–400 (357, 7) in width or 27–39% (31%, 7) of body width, consistently wider than longer, 44–58% (51%, 7) of oral sucker length, 49–59% (53%, 7) of oral sucker width. Mouth opening ventrally or anteroventrally, 125–160 (130, 7) long, 135–380 (259, 7) wide, 1.1–2.4 (1.8, 7)× wider than longer (Fig. 7). Pharynx ovoid, posterior to the oral sucker, 120–150 (131, 7) long or 5–7% (6%, 7) of body length 125–150 (142, 7) wide or 1–1.2 (1.1, 7)× wider than longer. Esophagus extending posteriad from mouth 360–720 (540, 7) before bifurcating 15–40 (26, 7) posterior to pharynx, with esophageal branches arching posterolaterad before joining with intestinal ceca; dextral esophageal branch 180–300 (225, 7) long; sinistral esophageal branch 150–265 (181, 7) long; intestinal ceca confluent with esophageal branches, appearing inverse U-shaped inclusive of esophageal branches, comprising paired dextral and sinistral ceca; dextral cecum 870–1260 (1125, 6) or 44–60% (52%, 6) of body length, beginning 810–1080 (932, 6) or 38–49% (43%, 6) of body length from anterior end of body, post-cecal space, 70–320 (157, 6) or 3–14% (7%, 6) of body length from posterior end of body; sinistral cecum 950–1280 (1097, 6) or 44–61% (51%, 6) of body length, beginning 720–1000 (882, 6) or 36–46% (41%, 6) of body length from anterior end of body, post-cecal space, 120–330 (205, 6) or 6–15% (9%, 6) of body length from posterior end of body (Fig. 7).

Testes 2 in number, transverse, abreast, oblique, oval to elliptical in shape; dextral testis 250–335 (290, 7) or 11–17% (13%, 7) of body length, 130–215 (160, 12) or 11–26% (14%, 7) of body width, anterior margin 1660–2120 (1871, 7) from anterior end of body, post testis space, 50–200 (101, 7) from posterior end; sinistral testis 260–380 (301, 7) or 11–19% (14%, 7) of body length, 100–215 (161, 7) or 8–26% (14%, 7) of body width, 1620–1900 (1777, 7) from anterior end, post testis space, 35–245 (140, 7) from posterior end (Fig. 7). Vasa efferentia approximately 8–10 (9, 7) wide, coalescing into 2 main collecting ducts that extend anteriad from testes lateral to ventral sucker before connecting dorsally and in posterior half of prostatic sac with seminal vesicle (=a robust vas deferens enveloped by prostatic sac) (Figs. 7–9). Prostatic sac dextrad or sinistrad, occupying space between oral sucker and ventral sucker, anterior margin 15–365 (15, 7) from posterior margin of oral sucker, posterior margin 55–130 (81, 7) from anterior margin of ventral sucker, 200–250 (227, 7) long, 218–305 (260, 7) wide (Figs. 8, 9). Seminal vesicle thin walled, highly convoluted, having swollen proximal region and narrow distal region; proximal region of seminal vesicle 305–710 (396, 5) long, 58–100 (91, 5) wide; distal region 95–260 (177, 5) long, 15–28 (24, 5) wide, connected to pars prostatica via a short minute duct. Minute duct (=possibly "verschlussapparat" of Looss [58] in



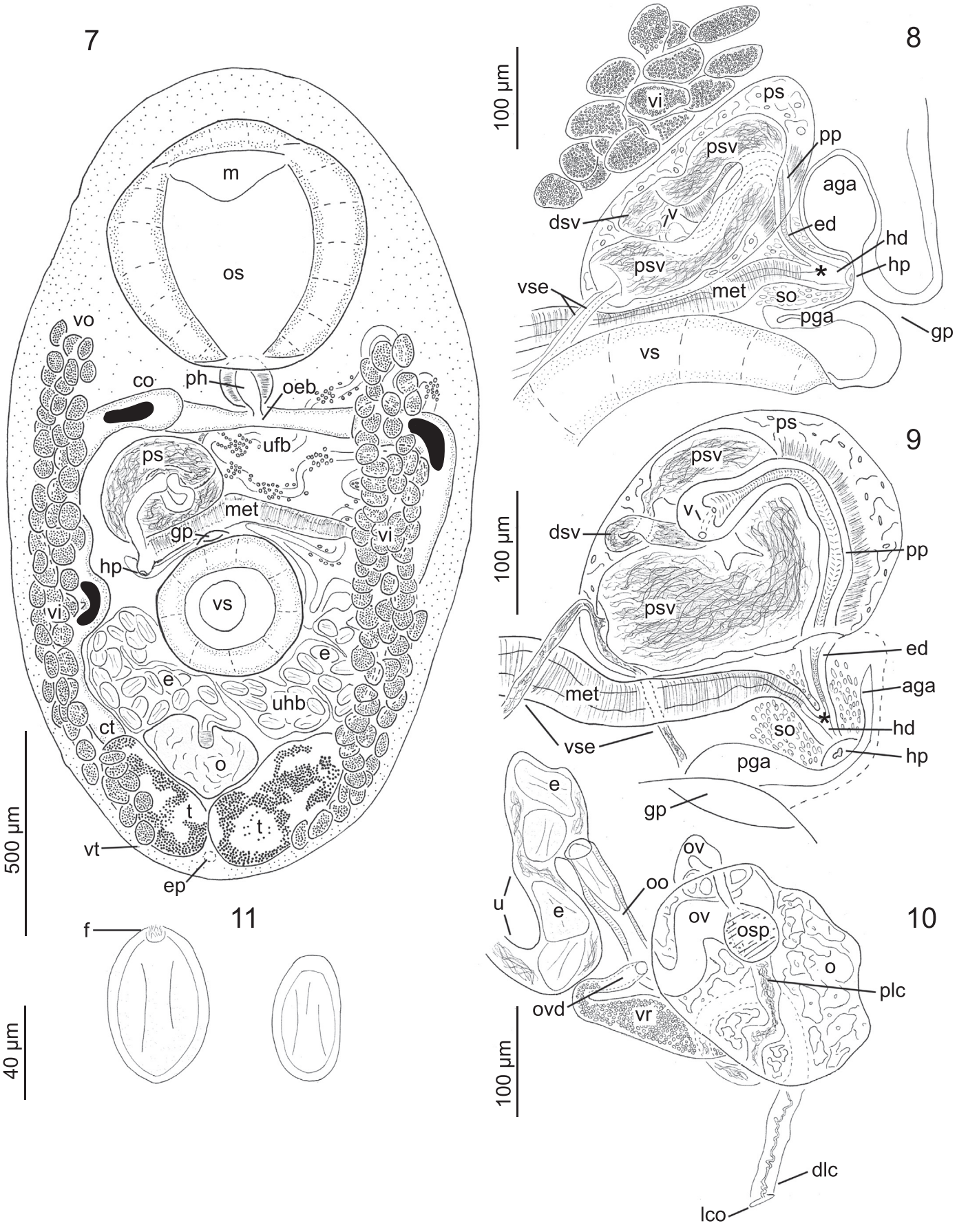
Horsfall [6], page 321) 10–30 (19, 7) long, thin-walled, appearing to pierce proximal surface of ejaculatory duct (=thick walled pars prostatica) (Figs. 8, 9). Pars prostatica 190–262 (219, 7) long, 48–58 (53, 7) wide distally 18–21 (19, 7) wide proximally, arched, lined by prostatic gland cells, thick walled for entire length, extending posteriad before exiting prostatic sac posteriorly. Ejaculatory duct (=continuation of pars prostatica external to prostatic sac) extending posteriad from prostatic sac and becoming confluent with hermaphroditic duct, thick

walled for entire length (lacking gland-like cells or muscle in wall), ventral to metraterm, 25–75 (44, 7) long or 11–34% (20%, 7) of pars prostatica length, 10–20 (15, 7) wide (Figs. 8, 9). Confluence of terminal male and female genitalia occurring within sinus organ. Sinus organ conical, medial, dorsal to genital atrium; papillae in probable region of sinus organ absent. Hermaphroditic pore posterior to prostatic sac, anterior or level with ventral sucker, 49–57% (52%, 7) of body length from anterior body end, directing ventrally before opening into genital atrium (Figs. 8, 9). Genital Atrium connecting hermaphroditic pore and genital pore, thick walled, comprised of a large anterior lobe and small posterior lobe; anterior atrium lobe 30–22 (26, 2) in length; posterior atrium lobe 16–13 (15, 2) in length (Figs. 8, 9). Genital pore immediately anterior to ventral sucker, medial, posterior to prostatic sac, opening ventrally in posterior half of body. Ventro-cervical groove transverse depression surrounding genital pore, extending horizontally then curving posteriorly around ventral sucker, length and width variable (Figs. 8, 9).

Ovary medial, intercecal for entire length, anterior margin anterior to posterior margin of testes, 155–200 (172, 7) long or 7–9% (8%, 7) of body length, 190–290 (218, 11) wide or 15–25% (19%, 7) of body width or 1–1.8 (1.2, 7)× wider than long, post-ovary space 200–440 (275, 7) long or 10–19% (12%, 7) of body length; germarium present, a chamber occupying center portion of ovary, becoming confluent with proximal portion of oviduct where a muscular sphincter is present (Figs. 7, 10); sphincter 40–48 (44, 7) wide; oviduct thin-walled, dorsal and sometimes anterior to ovary, immediately extensively convoluted and extending sinuously anteriad, briefly lined by cuboidal epithelium proximally before becoming confluent with Laurer's canal, extending 160–173 (167, 2) from commissure with Laurer's canal to ootype (Figs. 7, 10). Laurer's canal wide proximally at commissure with oviduct, swollen medially with sperm (=perhaps functioning as a "rudimentary seminal receptacle" [2]), narrow distally, 45–106 (75, 2) long, 35–13 (24, 2) wide including thick glandular wall, opening dorsally and posterior to ventral sucker (Figs. 7, 10). Ovovitelline duct short, arched after emanation from yolk reservoir, becomes confluent with oviduct at distal end immediately prior to ootype. Ootype dorsal or anterior to ovary, directing anteriad, anterior to testes 70–115 (91, 3) long, 30–45 (38, 3) in maximum width, Mehlis gland indistinct. Uterus extensively convoluted, intercecal posterior to ventral sucker, may extend lateral to ceca anterior to ventral sucker, occupying space between posterior third of oral sucker and ovary, comprising a field 1020–1500 (1191, 7) long or 46–65% (54%, 7) of body length and 460–1040 (890, 7) wide or 56–85% (75%, 7) of body width, passing ventral sucker dextrally or sinistrally, thin-walled for entire length 45–100 (76, 7) in maximum width typically with hundreds of eggs, with proximal portion comprising a uterine seminal receptacle and distal portion comprising a metraterm; uterine seminal receptacle with sperm; metraterm 315–635 (471, 5) or 14–32% (22%, 5) of body length, 45–60 (54, 5) wide, confluence with uterus anterior to the medial axis of the ventral sucker, transverse, sinistral or dextral, extending slightly anteriad and transverse from distal end of uterus, becoming confluent with ejaculatory duct to form a common duct (=herein a 'hermaphroditic duct') within sinus organ (Figs. 9, 10). Vitellarium follicular, ventral to ceca, distributing in 2 bilaterally symmetrical fields, distance between fields 510–820 (656, 7) or 43–68% (57%, 7) of body width, extending from near posterior margin of oral sucker to near posterior margin of body, dextral vitelline field, 1060–1520 (1369, 7) long or 54–66% (62%, 7) of body length, terminating anteriorly at 32–38% (34%, 7) of body length, terminating posteriorly at 92–98% (95%, 7) of body length, 1.1–1.3 (1.2, 6)× longer than dextral cecum; sinistral vitelline field 1070–1450 (1297, 7) long or 48–64% (59%, 7) of body length, terminating anteriorly at 28–39% (33%, 7) of body length, terminating posteriorly at 88–98% (92%, 7) of body length, 1–1.3 (1.2, 6)× longer than sinistral cecum; primary vitelline collecting ducts asymmetrical, dextral vitelline collecting duct 2.8 (2.8, 2)× longer than sinistral vitelline collecting duct, extending posteromediad from respective vitelline field before becoming confluent dextrally and forming vitelline reservoir; dextral vitelline collecting duct 145–285 (192, 4) long or 6–12% (9%, 4) of body length, 10–20 (17, 4) wide near yolk reservoir, proximal end branches from vitellarium at 71–78% (75%, 3) of dextral vitelline field length; sinistral vitelline collecting duct, 463–500 (481, 2) long or 21–25% (23%, 2) of body length, 10–20 (14, 4) wide near yolk reservoir, proximal end branches from vitellarium at 64–82% (74%, 3) of sinistral vitelline field length (Fig. 7). Vitelline reservoir slightly dextral, T-shaped, pre- or post-ovarian, pre-testicular. Uterine eggs typically filling entire lumen of uterus, ovoid to pyriform, enlarging from approximately 40–55 (51, 7) × 20–30 (26, 7) in proximal portion of uterus to approximately 68–95 (78, 7) × 40–50 (44, 7) in distal portion of uterus; well-developed eggs having minute fimbria or papillae at one pole (Figs. 7, 10, 11).

Diagnosis of cercaria based on light microscopy of 10 whole-mounted, naturally shed cercariae with withdrawn distome. Furcocystocercous, khaki in color, 6300–8550 (7234, 10) long, 1600–2640 (2040, 10) wide or 2.5–4.6 (3.6, 10)× longer than wide, comprising a tail stem and paired furcae (Figs. 12–14). Tail stem spindle shaped, laterally expanded at midbody, 4980–7300 (5950, 10) long or 76–86% (82%, 10) of cercaria length; comprised of a laterally compressed anterior region and dorsoventrally compressed posterior region (Fig. 13); anterior tail stem region, 3400–4940 (4070, 10) long or 52–62% (56%, 10) of cercaria length, maximum width same as reported for cercaria, cone shaped, containing distome, bearing mamillae, weakly muscular; posterior tail stem region, 1280–2400 (1880, 10) long or 20–30% (26%, 10) of cercaria length, 1000–1350 (1194, 10) wide or 2–4% (3%, 10)× longer than wide, lacking mamillae, strongly muscular. Furcae obocordate (=ends broadly rounded with slight medial notch), amber in color, dorsoventrally compressed, margin smooth to serrulate, paired; dorsal furcae, 1020–1340 (1215, 8) or 15–19%, (17%, 8) of cercaria length, 1420–1840 (1671, 9) or 1.1–1.6 (1.4, 8)× wider than longer (Fig. 13); ventral furcae 1100–1340 (1250, 10) or 14–20% (17%, 10) of cercaria length, 1420–1840 (1636, 10) or 1.2–1.5 (1.3, 10)× wider than longer. Tail stem cavity positioned in anterior portion of tail stem, thin walled, not strongly muscular. Withdrawn distome (=cercarial body) 1340–2040 (1604, 10) long or 17–28% (22%, 10) of cercaria length, 660–880 (760, 10) wide or 2–3 (2, 10)× longer than wider, specimens with 2–42 (20, 10) stage 1 eggs (see [35]) in proximal end of uterus near ootype. Mamillae usually bearing spines, maximum length 115–185 (147, 10), maximum width 185–290 (241, 10) or 1.2–2.5× wider than longer, cercaria length with mamillae 3000–4600 (3748, 10) or 45–56% (52%, 10) of cercaria length, cercaria length without mamillae 2700–4000 (3217, 10) (Figs. 12, 13); mamillae restricted to anterior tail stem region, completely encircling tail at level of tail cavity,

Plate 1. Figs. 1–6: *Proterometra albacauda* Anderson and Anderson, 1967 (Digenea: Azygiidae). Figs. 1–4, adult (holotype, USNPC No. 61229) from cardiac stomach of pumpkinseed sunfish, *L. gibbosus* (Linnaeus, 1758) Berg 1949 (Perciformes: Centrarchidae). Scale values aside each bar. (1) Ventral view of body of adult showing the mouth (m), oral sucker (os), pharynx (ph), esophagus bifurcation (oeb), ceca (co) near origin, vitelline follicles (vo) near origin of vitellarium, uterus convoluted in forebody (ufb), prostatic sac (ps), eggs (e), hermaphroditic pore (hp), metraterm (met), genital pore (gp), ventral sucker (vs), vitelline follicles (vi), uterus in hindbody (uhb) looping between ventral sucker and ovary (o), vitelline reservoir (vr), primary vitelline collecting ducts (pvc), vitelline follicles (vt) near termination of vitellarium, testes (t), ceca termination (ct), and excretory pore (ep). (2) Ventral view of the male genitalia showing the prostatic sac (ps), swollen proximal region of seminal vesicle (psv), distal tubular region of seminal vesicle (dsv), minute pore, possibly verschlussapparat (v) (see Section 3.1), pars prostatica (pp), ejaculatory duct (ed), metraterm (met), vasa efferentia (vse), hermaphroditic pore (hp), genital atrium (ga), eggs (e), and genital pore (gp). (3) Ventral view of the female genitalia with ovary omitted from image, showing the uterus in hindbody (uhb) looping between ovary and ventral sucker, eggs (e), confluence of the oviduct and Laurer's canal (*), proximal end of Laurer's canal (plc), oviduct (ov), ootype (oo), oviduct sphincter (osp), vitelline reservoir (vr), and primary vitelline collecting ducts (pvc). (4) Egg from distal portion of the uterus (left) and proximal portion of the uterus (right) showing papilla-like projections (pa). Figs. 5–6, cercaria (paratype, USNPC No. 61230) from gonoducts of gravel elimia, *E. catenaria* (as *G. catenaria*) Say, 1822 (Cerithioidea: Pleuroceridae). (5) Tail cavity opening (tco), muscular tail stem cavity (tsc), tail stem mamilla (ma), distome (d), and positions of the oral sucker (os), vitelline follicles (vi), ventral sucker (vs), and testes (t) in distome. (6) Tail stem mamilla (ma) showing papilla-like projections (pa).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restricted to 1 lateral column (4 total), of 5–6 mamillae, per body margin at midbody, ending at synthesis of posterior tail stem region; mamilla spines numbering 0–6 per mamilla, erect, proximally expanded, distally sharply pointed. Excretory system with 2 paired primary excretory canals, extending posteriad along the medial axis, from the anterior tail stem region, through the posterior tail stem region, extending independently through furcae, opening via excretory pore in the medial notch of each furcae (Fig. 13).

Diagnosis of germinal sacs based on 4 whole mounted sporocysts collected from cracked snails (see Section 2). Sporocyst 2200–4600 (3145, 4) long, 900–1580 (1155, 4) wide, birth pore at one end, with 4–8 (6, 4) cercariae of varying sizes, 0–5 (2, 4) germ balls present per sporocyst, no pharynx, gut, or mouth observed (Fig. 15).

3.2.1. Taxonomic summary

Type host: Spotted bass, Micropterus *punctulatus* Rafinesque, 1819 (Perciformes: Centrarchidae).

Intermediate host: *Elimia* cf. *modesta* Lea, 1845 (Cerithioidea: Pleuroceridae).

Site of infection: Esophagus (fish); indeterminate site (prosobranch snail).

Type locality: Terrapin Creek (South Fork) (33°51′36.56″N, 85°31′28.15″W), Cleburne County, Alabama.

Prevalence of infection: 2 of 2 (100%) spotted bass had 75 and 17 flukes respectively (mean intensity = 46).

Specimens Deposited: Syntypes USNM Nos. 1251729, 1251730, 1251731 (cercariae), 1251732, 1251733, and 1251734 (adults); intermediate host voucher USNM No. 1251735.

GenBank accession numbers: KM503118 (ITS2, adult) and KM503119 (ITS2, cercaria).

Etymology: The Greek specific epithet *epholkos* (epholkos, alluring) refers to the alluring appearance and presumptive luring behavior of the cercariae while swimming.

3.2.2. Remarks

Naturally shed, fully-developed cercariae of *P. epholkos* sp. n. are most easily distinguished from comparable cercariae of its congeners by the combination of having i) a medially constricted tail stem not exceeding 10 mm long, ii) mamillae of tail stem >0.1 mm in diameter, iii) mamillae distributed in 2 lateral columns on each tail stem surface and each comprising 5–6 mamillae per each body margin (i.e., 4 total columns, 20–24 total mamillae) (not arranged in transverse rows across surface of tail stem), iv) furcae >1 mm long and ≥1.4 mm wide, v) distome withdrawn in swimming cercariae, vi) withdrawn distome (hence also, tail stem cavity) positioned in anterior portion of tail stem (not medial portion of tail stem), and vii) uterine eggs in distome typically numbering <200. In addition, cercariae of *P. epholkos* swim upon shedding.

Adults of *P. epholkos* sp. n. can be distinguished from congeners by the combination of having i) an oral sucker diameter equal to at least twice that of the ventral sucker, ii) a uterine field that extends dorsal to or lateral to the ceca anterior to level of the ventral sucker (not intercecal for entire length), iii) a vitellarium that extends posteriad beyond the posterior margin of the ventral sucker and to near the posterior body end (vitellarium longer than ceca), and iv) a uterus that loops extensively in the space between the ovary and ventral sucker. *P. epholkos* sp. n. most closely resembles *P. albacauda* but can be distinguished from it by the combination of having cercariae with a medially constricted tail stem, long mamillae (>100 μm) that encircle the tail stem anteriorly, that are restricted to 1 lateral column of 5–6 mamillae per body surface and margin, and that are absent from the medial surface of the tail stem as well as by having adults with a vitellarium longer than the ceca, a vitellarium that extends anteriad to the oral sucker, and a uterus that extends dorsal or laterad to the ceca (=extercecal uterus). Although inadequate justification for differentiating it from the new species, *P. albacauda* reportedly infects *E. catenaria* in the Apalachicola River (Gulf of Mexico Basin) and Ogeechee River (Atlantic Basin) as well as *Lepomis* spp., *Noturus gyrinus*, and *Pomoxis annularis* in the Ogeechee River [22,27]. No report of *E. catenaria* in an Alabama river exists to our knowledge.

Alabama rivers harbor 3 species of *Proterometra*: *P. melanophora*, *P. hodgesiana*, and *P. catenaria* (see Smith [15,20,21]). Based on published descriptions of these species, the new species is most easily differentiated from these congeners by having (i) a vitellarium extending beyond anterior margin of testes, (ii) an oral sucker twice as wide as ventral sucker, (iii) obcordate furcae, (iv) a spindle shaped tail stem >4.9 mm long and >1.6 mm wide, and (iv) a swimming cercaria (*P. edneyi* and *P. hodgesiana* do not reportedly swim [15,28], Table 3).

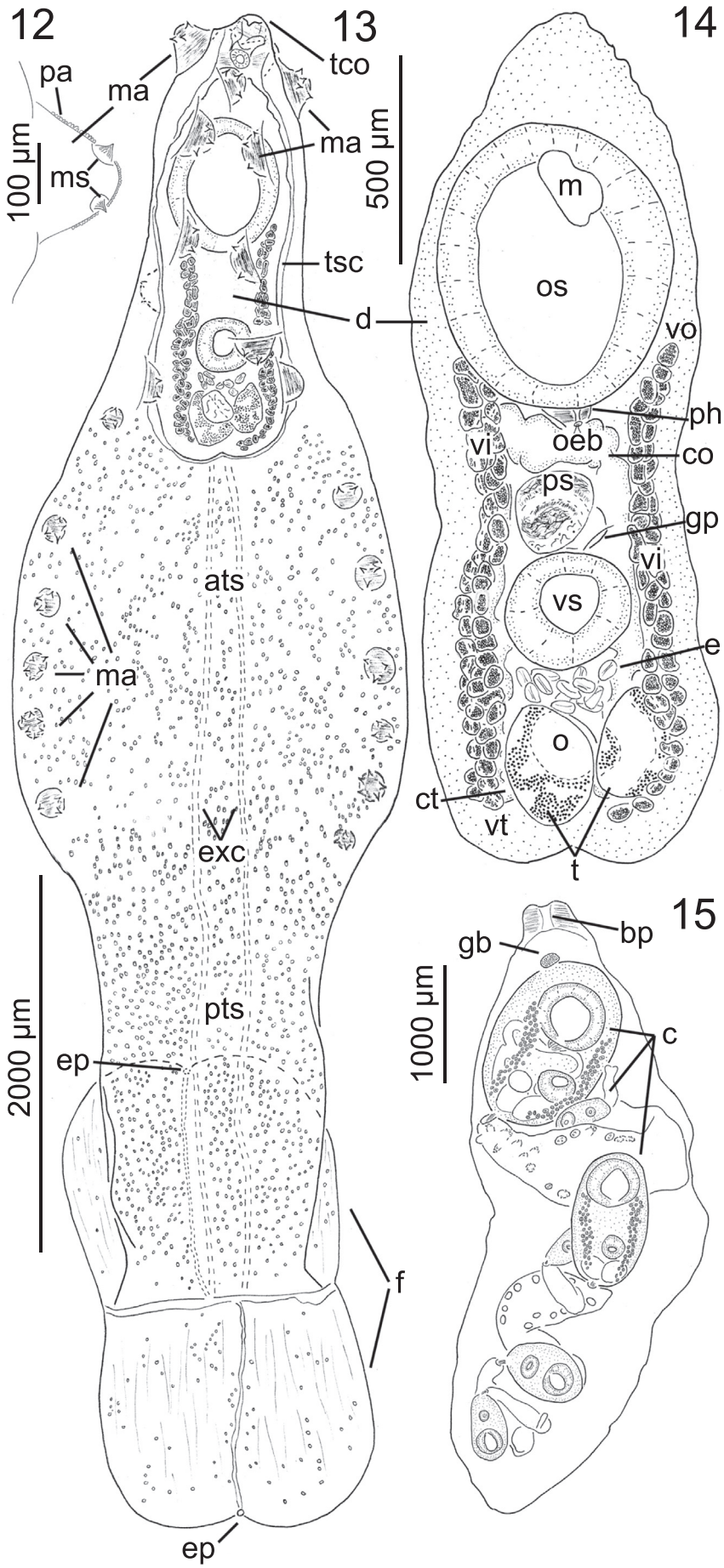
After primer trimming, the sequence data obtained for 2 cercariae from *Elimia* cf. *modesta* and 1 adult from *M. punctulatus* presented 49 bp of the 3′end of the 5.8S ribosomal RNA gene followed by the complete internal transcribed spacer 2 (ITS2) of 251 bp, and ending with 49 bp of the 5′ end of 28S ribosomal RNA gene. All sequences were identical aside from an intra-individual, single-site polymorphism in position '128'; which showed overlapping double peaks of Adenosine and Cytosine. ITS2 sequence data has been routinely employed as a species-level barcode for digeneans [59], and we interpret the 100% sequence match obtained herein to reflect that all specimens were conspecific. This result comprises the first application of molecular sequence data to elucidate a life cycle for a species of *Proterometra*.

4. Discussion

4.1. Diversity and distribution

Comprising the first description of a new species of the genus since 1995, the present study brings the total number of species of *Proterometra* to 10 (Tables 1, 2). *Proterometra* presently accommodates flukes that mature in primary division freshwater fishes of Centrarchidae (species of *Lepomis*, *Micropterus*, and *Pomoxis*) [1,35], Cottidae (*Cottus* spp.) [28,29], Percidae (*Etheostoma* spp. and *Perca flavescens*) [28,29], Lotidae (*Lota lota*) [29], Ictaluridae (Noturus*gyrinus*) [27], and Characidae (*Astyanax mexicanus*) [30] (Table 2). Infections in snails are reported from *Elimia* spp. (earlier records as *Goniobasis*), *Pleurocera* spp. [1,6,13,18], *L. obovata* [4] (see [39] for synonymy), and *C. subsolidum* [3]. Infections have been documented in these hosts ranging in rivers, streams, and lakes in 12 states and 6 water resources regions [60] in North America. To date, no record of an accepted species of *Proterometra* exists from beyond North America, and only one species

Plate 2. Figs. 7–11: *Proterometra epholkos* sp. n. (Digenea: Azygiidae), adults from esophagus of spotted bass, *punctulatus* (Rafinesque, 1819) (Perciformes: Centrarchidae). Scale value aside each bar. (7) Ventral view of body of adult showing mouth (m), oral sucker (os), pharynx (ph), esophagus bifurcation (oeb), ceca (co) near origin, vitelline follicles (vo) near origin of vitellarium, uterus convoluted in forebody (ufb), prostatic sac (ps), hermaphroditic pore (hp), metraterm (met), genital pore (gp), ventral sucker (vs), vitelline follicles (vi), uterus in hindbody (uhb) looping between ventral sucker and ovary (o), eggs (e), ceca termination (ct), testes (t), vitelline follicles (vt) near termination of vitellarium, and excretory pore (ep). (8) Lateral view of male genitalia showing locations of vitelline follicles (vi), prostatic sac (ps), vasa efferentia (vse), swollen proximal region of seminal vesicle (psv), distal tubular region of seminal vesicle (dsv), minute pore, possibly verschlussapparat (v) (see Section 3.1), pars prostatica (pp), ejaculatory duct (ed), metraterm (met), sinus organ (so), confluence of male and female terminal genitalia (*), hermaphroditic duct (hd), hermaphroditic pore (hp), anterior genital atrium lobe (aga), posterior genital atrium lobe (pga), genital pore (gp), and ventral sucker (vs). (9) Ventral view of male genitalia showing locations of the prostatic sac (ps), vasa efferentia (vse), swollen proximal region of seminal vesicle (psv), distal tubular region of seminal vesicle (dsv), minute pore, possibly verschlussapparat (v) (see Section 3.1), pars prostatica (pp), ejaculatory duct (ed), metraterm (met), sinus organ (so), confluence of male and female terminal genitalia (*), hermaphroditic duct (hd), hermaphroditic pore (hp), anterior genital atrium lobe (aga), posterior genital atrium lobe (pga), and genital pore (gp). (10) Ventral view of the female genitalia showing ovary (o), oviduct sphincter (osp), oviduct (ov), proximal portion of laurers canal (plc), distal end of Laurer's canal (dlc), laurers canal opening (lco), vitelline reservoir (vr), ovovitelline duct (ovd), ootype (oo), and proximal end of uterus (u) with eggs (e) and sperm. (11) Eggs from distal (left) and proximal (right) portions of uterus showing fimbria (f) or papilla-like projections.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1000 µm

ranges to the west of the main stem of the Mississippi River (Tables 1, 2). Noteworthy is that members of Centrarchidae are endemic to North American rivers, and, similarly, species of *Elimia* are endemic to eastern North American freshwater environments; many of which are highly endemic to the southeastern United States [52]. Taken together, the flukes, fishes, and snails are all North American endemics, making this parasite-host system rather interesting from the perspective of biodiversity and conservation science in the southeastern United States. The exception to high regional endemism is "*P. macrostoma*" (herein regarded as *species inquirenda*), which reportedly infects 8 snail species and 15 fish species in myriad river basins (Tables 1, 2). Typically, a morphological diagnosis of "*P. macrostoma*" does not accompany these locality reports, and, as previously stated, no molecular data have been sourced from any specimen from which these records are based. As such, we suspect this broad host and geographic distribution may reflect poor taxonomic resolution rather than the true geographic and ecological distribution of the named species. If *P. macrostoma* is a species complex, additional work is needed (see Sections 4.2, 4.3).

P. guangzhouensis, which was described by Lu [61] from rice eels, *Monopterus albus*, of the Zhujiang River (Southeast China) is not an accepted species of *Proterometra*. Based on Lu's [61] figures, this species has several features that would exclude it from *Proterometra*: (i) the vitellarium and the uterus are posterior to the prostatic sac and (ii) the testes are level with the ovary. Moreover, the site of infection reported by Lu [61] was "intestine," not buccal cavity or esophagus, which are the typical sites of infection for adults of species of *Proterometra* infecting fishes. Of interest is that the rice eel is a highly invasive species throughout South Florida [62]. We examined several rice eels from South Florida canals but none was infected by an azygiid. We agree with Gibson [38] that *P. sillagae*, *P. brachyura*, and *P. lamellorchis*, which were described by Wu et al. [63] and collected from sillagos (*Sillago* spp.) captured in the Guangdong Province of China, are not species of *Proterometra*. Based on our observations of Figs. 1–3 of Wu et al. [63], these species have features that exclude them from *Proterometra*: (i) the testes are tandem, and, at least in one species, lobed, (ii) an external seminal vesicle is present in all three species, and (iii) the female genitalia is medial or completely restricted to the forebody.

4.2. Type specimens of "P. macrostoma" et al.

The holotype of *P. macrostoma* (as *Cercaria macrostoma*) has probably been lost or was never designated. This species, the type species of *Proterometra*, was described from a single cercarial specimen taken from "*an aquarium in the Zoological Laboratory of the University of Illinois*" [3]. The collection locality for the cercaria was an aquarium, and no original collection locality for the snails in the aquarium was given; hence, no wild type locality was specified. Because multiple species of snails (i.e., *C. subsolidum* and *Elimia semicarinata* [as *Goniobasis pulchella*]) were present in the single aquarium from where the cercaria was collected, no type host was specified. Because no fish were examined by Faust [3], no adult specimen was described. Finally, no holotype was specified by Faust [3]. Later, however, a holotype was mentioned [6] but not by Faust.

Horsfall [1] reported *C. macrostoma* in snails identified as *Goniobasis livescens* from Salt Fork Branch Vermillion River, Illinois, and in *P. acuta* from Oconomowoc River, WI. She fed the cercariae to several fishes but only centrarchids became infected with adult flukes, which were used to propose the new genus *Proterometra* (*P. macrostoma*, type species). A year later, she [6] studied (i) whole-mounted specimens of *Cercaria fusca* of Pratt [17]; sent to her by HB Ward, (ii) mounted and vialed specimens of the forked tailed cercariae of Cahn [18], (iii) the "*holotype of C. macrostoma which Faust kindly loaned*," which remains the only literary mention of a holotype of *P. macrostoma* (the present disposition of this specimen is indeterminate), (iv) cercariae shed in her laboratory from snails (*Elimia livescens* and *P. acuta*; perhaps sourced from the Des Plaines River) that were sent to her by Dickerman in Summer 1933, and (v) photomicrographs, along with whole-mounted, and vialed specimens of *Cercaria melanophora* (of Smith [20]) from *Elimia opaca* in the Cahaba River, Alabama. After studying the aforementioned material, she considered them as conspecific with *C. macrostoma*, therein assigning them to the collective group name *Cercaria*. This was an odd taxonomic and nomenclatural decision since she had previously made available the genus group name *Proterometra* for the type species *P. macrostoma* (Faust, 1918) Horsfall, 1933. Horsfall [6] reported that "*[t]he typical adults of P. macrostoma are deposited in the United States National Museum, number 8767, 8768 and in the collection of Dr. Henry B. Ward, number 34.10.*" According to the USNPC database USNPC Nos. 8767 and 8768 are extant but listed as vouchers, not types. At the time of submission of this manuscript, specimen number 34.10 from Ward's collection was a part of the USNPC under accession no. 51817 and was labeled as a "cotype." "Cotype" is a term not recognized by the ICZN but formerly was used for either a syntype or paratype but not a holotype (see Recommendation 73E, ICZN).

Like that for the type species of *Proterometra*, a perusal of the literature on *Proterometra* reveals that a lack of type material or uncertainty regarding the holotype is not unusual. Pratt [17] described *C. fusca* from a snail identified as *G. livescens* in the Oneida River, New York, without deposition of type materials. Cahn [18] described "*a new forked tailed cercaria*" from *P. acuta* in the Oconomowoc River, Wisconsin. Adults were reported from "*the young of fish belonging to the family Centrarchidae*." As with Pratt [17], no type or voucher materials reportedly were deposited. Dickerman [19] reported "*a large number of cystocercous cercariae in the mirabilis group*" from snails identified as *G. livescens correcta* in the Des Plaines River, Illinois. No type or voucher materials were deposited. Smith [20], in an abstract, described *C. melanophora* from *Elimia* spp. from Alabama without mentioning type materials. The USNPC specimens of "*Proterometra pinguis*" from *Esox lucius* reportedly deposited by JF Mueller are apparently unaccompanied by a published description, and we herein consider it a nomen nudum. Also, The USNPC database indicates that the type materials for *P. albacauda* (USNPC Nos. 61229-30) and *P. septimae* (USNPC Nos. 61231-32) are crazed; however, we have studied the type materials for *P. albacauda* and they are in good condition. Regarding *P. hodgesiana*, we cannot locate type materials for this species, and none was reportedly accessioned nor resides in the USNPC.

4.3. Is P. macrostoma a species complex?

Dickerman [33] studied cercariae from *E. livescens* (as *G. livescens correcta*) in the Des Plains River, Illinois, and adults from *Pomoxis nigromaculatus* (as *Pomoxis spiroides*), *Micropterus salmoides* (as *Helioperca incisor*), and *L. gibbosus* (as *Eupomotis gibbosus*). He considered these specimens conspecific with *P. macrostoma* but, again, no voucher materials reportedly were deposited. Smith [15] revisited the taxonomic treatment of *C. melanophora*, based on specimens collected from a prosobranch (as *Goniobasis opaca*), and determined that adults recovered from *M. salmoides* (as *Huro floridana*) from Cooley Creek, Alabama, (Cahaba River) were conspecific with the adults of *P. macrostoma*, agreeing with Horsfall [6]. By doing so Smith [15] established a novel combination as *P. melanophora*. Since then,

Plate 3. Figs. 12–15: *P. epholkos* sp. n. (Digenea: Azygiidae), naturally shed cercariae and sporocysts from *Elimia* cf *modesta* (Lea, 1845) (Cerithioidea: Pleuroceridae). Scale values aside each bar. (12) Tail stem mamilla (ma) showing minute papilla-like projections (pa) and mamilla spines (ms) (13) Cercaria showing tail cavity opening (tco), mamillae (ma), tail stem cavity (tsc), distome (d), anterior tail stem region (ats), excretory canals (exc), posterior tail stem region (pts), furcae (f), and excretory pores (ep). (14) View of distome showing mouth (m), oral sucker (os), vitelline follicles (vo) near origin of vitellarium, pharynx (ph), esophagus bifurcation (oeb), ceca (co) near origin, prostatic sac (ps), genital pore (gp), vitelline follicles (vi), ventral sucker (vs), eggs (e), ovary (o), ceca termination (ct), vitelline follicles (vt) near termination of vitellarium, and testes (t). (15) View of sporocyst showing the location of the birth pore (bp), cercariae (c), and a germ ball (gb).

P. melanophora has been typically considered a junior subjective synonym of *P. macrostoma*. Unfortunately, neither author, Smith nor Horsfall, provided what we consider to be a robust taxonomic justification for the synonymy of *P. macrostoma* and *P. melanophora*. Dickerman [13] studied cercariae shed from unspecified species of *Elimia* (as *Goniobasis*) and *Pleurocera* in waters adjacent to the "*Great Lakes Region*." Therein, he initiated the concept of 3 types (="kinds") of *P. macrostoma* (later indicated by Riley and Uglem [8] as "strains," which they further separated into 8 "morphotypes") based on the morphology of cercariae collected from unspecified species of *Goniobasis* (= *Elimia*) in the "*Bass Island region of Lake Erie*." This work, although providing a novel perspective on morphological variation among cercariae assigned to *Proterometra*, added a layer of intrigue to the taxonomy of the genus since it made precedent the use of morphological features of cercariae as diagnostic for strains. Since 1945, no author has described a species later to be considered a junior subjective synonym of *P. macrostoma*. One species, *P. autraini*, was initially identified as *P. dickermani* [37] and later as *P. macrostoma* [26] before being described as a new species [29].

Aside from the nomenclatural and morphological cloud surrounding "*P. macrostoma*," based on the myriad snail and fish species reported as hosts for "*P. macrostoma*" as well as the fact that its geographic distribution encompasses that of all other named species of *Proterometra* (Tables 1, 2), it seems probable that *P. macrostoma* has become a repository for conspicuous, furcocystocercous cercariae shed from snails of *Elimia* in eastern North American rivers and streams. Noteworthy also is that the taxonomy of both the intermediate and definitive hosts have changed considerably since 1918, i.e., species of *Elimia* were previously assigned to *Goniobasis* and *Melania* (see Section 4.8) [39,54,55], many new species of those gastropod genera have been described since the early 20th century, and *Micropterus* now includes several cryptic species typically misidentified as or considered as "*M. salmoides*" [64,65]. We think that continued morphological studies of cercariae and adults plus careful taxonomic identification of the snail hosts (specimens vouchered in curated invertebrate collections) and fish hosts coupled with molecular sequence data can help further resolve these taxonomic uncertainties.

4.4. Proterometra spp. from Alabama

The present study brings the total number of species of *Proterometra* documented from Alabama rivers to four: *P. epholkos*, *P. melanophora*, *P. hodgesiana*, and *P. catenaria*. The latter three species were treated initially by Smith [15,12,21] and all of them require further taxonomic study considering the type materials and based on naturally shed cercariae as well as adults collected from infected, wild-caught fish hosts. Fundamentally problematic is that we could not locate or find reference to an extant type specimen for any species of *Proterometra* described from Alabama. In an abstract of a demonstration at the 8th Annual Meeting of the American Society of Parasitologists, Smith [20] provided scant details for "*[t]wo new cystocercous cercariae*," *P. melanophora* (as *C. melanophora*) and *P. hodgesiana* (as *C. hodgesiana*), based on cercariae shed from *Elimia* spp. *P. melanophora* was subsequently treated by Horsfall [6] and Smith [15] as conspecific with *P. macrostoma* (see above). Smith gave Horsfall [6] "*information concerning host, locality, and distinguishing characteristics*" and cercariae of *P. hodgesiana* shed from a prosobranch (as *Goniobasis* spp.) "*collected in the Warrior River, Alabama*." Smith differentiated the cercariae of *P. hodgesiana* from that of *P. macrostoma* based on the following cercarial characteristics (i) presence of presumed "*functioning genital organs*," (ii) tail stem "*globular anteriorly*" (i.e., circular vs. laterally compressed), (iii) mamillae aspinous, (iv) "*furcae small proportional to tail stem*," and (v) "*cercaria small*." Four years after naming the species in the abstract, Smith [15] revisited the taxonomy of *P. melanophora* and *P. hodgesiana* by providing additional information on the cercariae of *P. hodgesiana* from a prosobranch (as *Goniobasis vicina*) in Big Sandy Creek (Black Warrior River) and Miller Springs (Cahaba River), Alabama. She emphasized that the time of emergence of cercariae occurred in the morning hours rather than at night (as in *P. macrostoma*) and that the cercariae were unable to swim (as opposed to swimming cercariae of *P. macrostoma*). Cercariae of *P. hodgesiana* made "*lashing movements*" that she speculated made for a "*conspicuously attractive object to fish*." Adult flukes were recovered from pumpkinseed sunfish (*L. gibbosus*; as Eupomotis *gibbosus*) that were fed cercariae. No work [6,20,21] listed an accession number for a type specimen. Further noteworthy regarding nomenclature is that Smith [20,21] made available the names *melanophora* and *hodgesiana* in abstracts. Article 9 (pg. 8) of the ICZN defines forms of communication that do not constitute a "published work." Specifically Article 9.9 (pg. 8) of the ICZN states that "*abstracts of articles, papers, posters, texts of lectures, and similar material when issued primarily to participants at meetings, symposia, colloquia or congresses*" do not meet the criteria of published work. However, because these abstracts were published in volumes of their respective journals (i.e., The Journal of the Alabama Academy of Sciences and The Journal of Parasitology), we regard *melanophora* and *hodgesiana* as available names.

Smith [21] described *P. catenaria* based on cercariae from *E. catenaria* (as *G. catenaria*) in the Apalachicola, St. Johns, and Suwannee Rivers of Florida as well as from *Elimia doolyensis* from the Choctawhatchee River (Mobile River), Alabama. She demonstrated that it experimentally infected various centrarchids. She did not list an accession number for a type specimen nor state that any type specimen was deposited. Anderson and Anderson [22] provided supplemental observations on *P. catenaria* based on cercariae taken from *E. catenaria* from Blue Springs, Florida, that were experimentally fed to *Lepomis cyanellus* (sourced from Blue Springs) and *L. gibbosus* (sourced from Douglas Lake, Michigan). Their report on the anatomy of *P. catenaria* lacked a specimen from Alabama, which unambiguously included Smith's [21] type locality. In addition, Anderson and Anderson's [22] work added few additional morphological features of the genitalia, and cercarial features were limited to color, surface features, and swimming behavior. That work is somewhat problematic also in that no diagnosis for any species treated therein was provided, including for that of *P. catenaria*. Hence, from that work it is unclear what diagnostic features made the Florida specimens of Anderson and Anderson [22] conspecific with the Alabama specimens of *P. catenaria* in Smith [21]. In comparing the illustrations in both works, the adults of *P. catenaria* seem indistinct; however, the depictions of the cercariae seem in general agreement, i.e., the distome resides at midbody, the tail stem is massive, and the furcae are somewhat lanceolate.

4.5. Molecular data

In addition to issues related to type materials, another obstacle for taxonomy, systematics, and life history studies of *Proterometra* spp. is that molecular sequence data are lacking for all but one confirmed species (present study). This makes the assessment of operational taxonomic units based on cercarial morphology alone challenging, especially given the low number of reported diagnostic characteristics. Various workers have treated morphology, behavior, and host-parasite relationships of the cercariae [13,6–12,33,66–72], development and morphology of the eggs [35,73] and adults [1,6,15,21–25,28,33,35], and general attributes of the intra-molluscan stages, sporocyst and redia [11,14,74]. Moreover, 8 cercarial strains of *P. macrostoma* have been morphologically characterized from Lake Erie [13], Kentucky [8, 75], Michigan [8], and Ohio [8]. Including the present study, molecular sequence data exist only for *Proterometra* sp. [43] and *P. epholkos*, despite marked advances in molecular systematics of trematodes [76]. Sequence data for the commonly applied markers (ITS2, 18S, 28S) can help throw light on the identity of species that have been reported in the literature. For instance, although low intra-individual and intra-specific variation is assumed because of the action of concerted

evolution on rDNA arrays [77], some studies have shown that this phenomenon might be incomplete in some digeneans [78].

In the present study, sequences from the adult and cercarial specimens each presented divergent intra-genomic ITS2 copies comprising a double chromatogram peak in position 128. Noteworthy is that hybrids having different ITS2 variants from different species have been reported from species of *Schistosoma* [50,79] and *Fasciola* [80]. While our sample size constraints further discussion of the matter, we consider Azygiidae as an interesting group to explore concerted evolution. Moreover, multiple ITS2 copies within individuals might have been overlooked in other digeneans, raising concerns about the use of this locus in phylogenetic analyses because the employment of different variants could mislead such estimations. Also important is that our own results demonstrate that universal primers for flukes are effective with species of *Proterometra*. Molecular approaches are especially needed in determining the level of intraspecific variation in cercariae towards testing strain hypotheses, matching cercariae and adult flukes towards documenting life cycles, and sequencing snail tissues to help confirm their taxonomic identities.

Scant sequence data exist for Azygiidae. Before the present study, GenBank held sequence data for 3 markers (CO1 [1 taxon], 18S [2 taxa], 28S [3 taxa]) representing 3 genera and 5 species: *Azygia angusticauda* (CO1 [526 bp]) [81], *Azygia longa* (28 s [1406 bp]) [43], *Otodistomum veliporum* (18 s [302 bp]) [82], *Otodistomum cestoides* (18 s [1932 bp]) [83], *O. cestoides* (28 s [1303 bp]) [84], and *Proterometra* sp. (28 s [1399 bp]) [43]. As additional studies incorporate and publish azygiid molecular sequence data, a better understanding of interrelationships of azygiid genera will likely emerge; however, at present, impactful phylogenetics is out of reach, simply for lack of available sequence data.

4.6. Differential morphological features

Proterometra spp. are principally differentiated based on cercarial morphology and behavior [1,15,21,22,24,25,28,29] (Table 4). Based on direct observations of cercarial specimens and a review of the published literature, the taxonomic validity of several cercarial characters is uncertain. Taxonomic descriptions of the cercaria of a species of *Proterometra* should derive from naturally shed cercariae only, not from cercariae taken from sporocysts, rediae, or "crushed" snails. Specimens sourced by the latter method are likely immature. Basing taxonomic decisions on immature cercariae has a high probably of causing taxonomic confusion. In addition, naturally shed cercariae should be heat killed (see Section 2) so as to ensure reliable and repeatable morphometric comparisons across species. We have noted that most published descriptions make ambiguous the providence of and fixation method associated with the studied specimens and/or type materials (when designated). Moreover, the distome can be extruded or withdrawn, and, therefore, the size of the cercaria as a whole can vary intraspecifically depending on the state of the specimen when fixed. Perhaps as a result of some of these different approaches, several morphological features of species of *Proterometra* need further clarification. Some seem inconsistently reported; others are likely reliably diagnostic; and some seem dubious.

4.6.1. Tail stem

Tail stem length is the distance from the anterior end of the tail stem, i.e., the opening through which the distome extrudes, to the confluence of the furcae [22]. Tail stem maximum length and width have been used as diagnostic features for species of *Proterometra*, and, given the likelihood that swimming behavior of cercariae is under strong selection pressure, this feature is likely a useful taxonomic feature. However, several species reportedly have inconsistent ranges, wide ranges, or lack morphometric data altogether (e.g., *P. catenaria*, *P. macrostoma*, *P. hodgesiana*; respectively). For example, reported tail stem length and width measurements for *P. macrostoma* are 2–9 mm × 0.59–1.7 mm [6,8]. These ranges encompass those measurements for all but one congener. As another example, Smith [15], for *P. catenaria*, did not report tail stem length but instead reported cercarial length (tail stem + furcae) as 9–16 mm. Supplemental observations of that species reported tail stem length as 9–16 mm and 5.2–8.2 mm in living and fixed specimens, respectively [22]. Thus, for example, clarification is needed regarding the actual tail stem dimensions for *P. macrostoma* and *P. catenaria*. Furthermore, based on our observations of living and fixed cercariae of *P. epholkos* and the published works treating cercariae of *Proterometra* spp., the cercarial tail stem is divided into a laterally expanded anterior portion, which seems less muscular, has mamillae, and is laterally compressed, and a relatively narrow posterior portion, which seems more muscular, lacks mamillae, and is dorso-ventrally flattened (Fig. 13). These features may be diagnostic for species of *Proterometra*, and histological study of the tail stem tegument would be helpful in further characterizing these attributes. In addition to more consistency being applied to how the tail stem is characterized, correlating the shape of the tail stem with swimming behavior and definitive host food habits could reveal an interesting story about cercarial mimicry in stream ecosystems. That is, tail shape relates to swimming behavior, which relates to the type of definitive host that attacks the putatively luring cercaria.

4.6.2. Mamillae and spines

Smith [20] coined the term "*mammilations*" for the mound-like tegumental protuberances (spinous or not) of the cercarial tail stem (Figs. 6,12). Others have referred to these tail stem protuberances as "*papillae*" [22] or "*wartose structures*" [33]. Herein, we refer to Lawrence [85] and call these protuberances on the tail stem 'mamillae'. We think that the application of "cercarial papillae" is ambiguous and potentially confusing because small papillae occur on the tegument of the cercarial distome as well as about the rim of the oral and ventral suckers. Hence, as we define them, mamillae are the protuberances associated with the tail stem, not the distome. These mamillae are not likely homologous to tegumental papillae of the distome, which for the new species are pored but lack a sensory cilium. The distribution, appearance, and number of mamillae in *P. epholkos* are useful as diagnostic features (see Description) but comparable information from congeners is predominantly indeterminate or lacking [1,15,21,22,24,25,28,29]. In some instances, and without the benefit of molecular sequence data, a high degree of variation seems present; leading some authors to diagnose subspecific taxa, i.e., "strains" or "types" [8,13,75]. In addition to the pattern of mamillae, the presence/absence, appearance, length, and number of spines ornamenting each mamilla are intriguing as potential taxonomic features. At least specimens identified as *P. macrostoma*, *P. catenaria*, *P. albacauda*, *P. septimae*, *P. edneyi*, *P. autraini*, and *P. epholkos* possess spinous mamillae in portions of the tail stem (Table 3).

To our knowledge, the function of these spined mamillae of the tail stem is indeterminate. Based on the site of infection in the definitive host (i.e., buccal cavity) and the ability of the distome to withdraw inside the tail stem, we speculate that the mamillae and their spines may act as cleat-like contact surfaces that facilitate affixing the tail stem, along with its withdrawn cercarial distome, to the soft tissues of the buccal cavity epithelium and esophagus upon being engulfed and taken into the buccal cavity by the fish host. Or, simply, these spines could increase the likelihood that engulfed cercaria entangle in the rugose esophageal sphincter of the fish host. This could permit the distome to emerge from the tail stem, attach to the apposed epithelial surface of the buccal cavity, and detach from the tail stem with low risk of being separated from the host surface.

4.6.3. Tail stem cavity

The distome of *Proterometra* spp. can be withdrawn inside the tail stem cavity or extruded. Published observations indicate that whether or not the distome is withdrawn or extruded upon being shed from the snail is species-specific. Aside for *P. dickermani*, the distome of all

species of *Proterometra* is withdrawn upon shedding from the snail host [26]. The relative position of the tail stem cavity may also be diagnostic for some species [22,24,25]. However, this feature should only be described from and compared among naturally shed cercariae because the distome in cercariae excised from crushed snails is typically extruded (MRW, personal observations), suggesting that the distome withdraws immediately before shedding.

4.6.4. Cercarial behavior

As previously discussed, the cercariae of *Proterometra* spp. are unique among most digeneans because of their swimming behavior, which purportedly mimics a fish prey item that lures the definitive fish host to consume it. However, this unique behavioral attribute differs among species of *Proterometra*. *P. hodgesiana* and *P. edneyi* reportedly lack the ability for coordinating swimming (Table 3) and, instead, lure the fish definitive host by rapidly lashing and contracting the tail stem [15,28]. Three studies [28,66,68] have addressed the elements involved and mechanisms responsible for the unique swimming behavior displayed by cercariae of *Proterometra* spp. Consistency of swimming patterns in *Proterometra* spp. is the result of "*a pattern generating mechanism that is centrally, rather than reflexly, controlled*" [66], and inter-specific variation in swimming behavior purportedly could result from the number of myoneural junctions present in the tail stem [28,68]. The results of these studies support the fact that swimming behavior, and likely swimming duration, may reliably differentiate species. Swimming behavior may also reflect the diet of the fish definitive host, e.g., benthic fishes (darters, Percidae) that graze on invertebrates and algae [45] may have more opportunity to consume sedentary cercariae than swimming cercariae that remain higher in the water column. If cercariae are luring, then we assume that cercarial behavior is at least partly determined by host dietary preference and foraging ecology.

4.6.5. Progenesis

"Progenesis" is defined herein as the maturation of gametes before completion of body growth [85,86]. The literature indicates that a specimen typically is regarded as "progenetic" if there is sufficient evidence of precocious egg development, i.e., morphologically viable/embryonated eggs (those enveloping ciliated, active miracidia) present in the uterus of the not yet fully developed individual (larva, cercaria). This evidence supports the classical definition of progenesis since it indicates that the individual fluke indeed has produced mature gametes requisite for fertilization and subsequent embryonic development. The matter can be made more complex when considering that not all fluke eggs necessarily are embryonated ('viable'), and, hence, presence of a uterine egg does not necessarily prove that the individual produced a sperm and an ovum that preceded fertilization. The literature on *Proterometra* spp. seemingly defines a species as progenetic if an egg, of any stage of development, is observed to be present in the uterus of the cercaria: "*P. macrostoma*," *P. catenaria*, *P. sagittaria*, *P. dickermani*, *P. albacauda*, *P. edneyi*, *P. autraini*, and *P. epholkos* sp. n. fit that definition [1,21,22,24,25,28,29]. Interestingly, *P. dickermani* has yet to be collected from a wild fish, and, therefore, may have a single host life cycle [25,26,36]. Caution is needed here to differentiate the apparent lack of infections in a fish host as support for a single host life cycle, however. Perhaps giving support to the single host life cycle, *P. dickermani* is further unique in that it reportedly lacks a tail cavity [25,26]. Yet, cercariae of other species, e.g., *P. sagittaria* and *P. autraini*, have yet to be observed without uterine eggs but infect fishes [24,29] (see Table 2).

4.7. Genitalia as diagnostic

The seminal works regarding *P. macrostoma* by Horsfall [1,6] and Dickerman [33] provided the template for describing the genitalia for species of *Proterometra*. Since these works, however, little attention has been given to the genitalia of adults or cercariae. As such, several features of the genitalia require clarification. Previous descriptions refer to the structure that surrounds the seminal vesicle and pars prostatica as the cirrus sac [1,6,24,29,33]. Herein, this structure is described as the prostatic sac (Figs. 1, 7) (sensu [87]). The portion of the male duct distal to the seminal vesicle is comprised of two structures, the pars prostatica and the ejaculatory duct (Figs. 2, 8) [24,33]. Previous descriptions described the former as a "*bulbous cavity of the prostate region*," and the latter as the "*ductus ejaculatorius*" [1,6]. Light microscopy suggests that the thickness of the wall of these structures differs between *Proterometra* spp. The metraterm (distal, muscular portion of uterus) becomes confluent with the ejaculatory duct within the copulatory organ forming a hermaphroditic duct (Figs. 8, 9). Only one author [1] has described and noted a metraterm. Dickerman [24,33] called the metraterm a "vagina" in *P. macrostoma* and *P. sagittaria*. The copulatory structure, or sinus-organ (sensu [2]), has been described as a "*genital cone*" [33] or "*genital papilla*" [24,25,28,29,33]. We regard species of *Proterometra* as having a permanent sinus organ (Figs. 8, 9), and scant observations of this organ indicate it may or may not be papillate [29]. At the distal end of the sinus organ is a hermaphroditic pore that opens into a large, seemingly bi-lobed, genital atrium (Fig. 8) [2,22,25]. Previous accounts have described this area as a genital sinus [1,24,33]. We think that the anterior lobe is typically larger than the posterior lobe and that the atrium contains eggs in some specimens [24]. Because of the limited number of specimens examined, and since many of these features have not been previously described or detailed, additional observations and molecular data of congeneric species are needed for confirmation. Doing so, along with applying equal precedence to cercariae and adults, will help inform future taxonomic works.

4.8. Snail (*Elimia* spp.) identification is critical

Species-level identification of snail hosts, especially those of *Elimia*, is a major concern regarding logistics of accurately documenting the host and geographic distribution of species of *Proterometra* as well as to testing hypotheses concerning the specificity of these digeneans to their molluscan first intermediate hosts. The chronic lack of taxonomic resolution within Pleuroceridae, specifically regarding *Elimia*, is especially problematic because (i) most snail hosts of *Proterometra* spp. are presently assigned to *Elimia* and (ii) each nominal species of *Proterometra* has been reported to infect at least one species of *Elimia* as well as *P. acuta*, *L. obovata*, and *C. subsolidum* (Table 1). Illustrated field guides that treat species of *Elimia* are equivocal regarding differentiating species without geographic locality data, and, to our knowledge, no modern dichotomous key to *Elimia* spp. has been published in the peer-reviewed literature or exists as an unpublished field guide (Dr. Paul Johnson, Alabama Department of Conservation and Natural Resources, Marion, Alabama; personal communication). Because previous authors have not (i) given characters for which they used to identify snails, (ii) cited resources or personal communication with malacologists regarding snail identification, or (iii) co-deposited snail vouchers with the parasite type/voucher material, we doubt that most of the snail hosts for *Proterometra* spp. have been identified correctly. For example, *P. catenaria*, *P. albacauda* and *P. septimae* have been reported and described from the same snail host, *E. catenaria* (as *G. catenaria*), in the same river, Apalachicola River, Blue Springs, Florida. However, according to Johnson et al. [52], *E. catenaria* does not range in Florida nor any Gulf of Mexico drainage but rather is restricted to Atlantic drainages in Georgia, South Carolina, North Carolina, and Virginia. Issues such as this are problematic regarding progenetic digeneans sensu lato that may have evolved a higher degree of host specificity (fidelity) to the host in which they mature sexually, i.e., the molluscan first intermediate host, as opposed to non-progenetic digeneans that seemingly exhibit a higher level of specificity to the craniate definitive host. In addition, we recommend that molecular markers be applied to assist in identifying the snail hosts as well as per Fukuda et al. [88] or Galindo et al. [89].

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