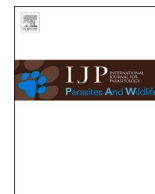


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# A young parasite in an old fish host: A new genus for proteocephalid tapeworms (Cestoda) of bowfin (*Amia calva*) (Holostei: Amiiformes), and a revised list of its cestodes

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## ABSTRACT

A new genus, *Laruella* n. gen., is proposed for the proteocephalid cestode *L. perplexa* (La Rue, 1911) n. comb. (syn. *Proteocephalus perplexus* La Rue, 1911), a parasite of a ‘living fossil’, the bowfin (*Amia calva*), in North America. The new genus is differentiated from other proteocephalid genera by having a massive four-lobed scolex without an apical organ and bearing suckers possessing tear-shaped sphincters on their inner rim, vitelline follicles forming L-shaped lateral fields, with the vitellarium turned inwards (medially) ventrally alongside the posterior margin of the ovary, a ring-like vaginal sphincter situated at a considerable distance from the genital atrium, and ellipsoid eggs resembling those of bothriocephalid and diphyllbothriid tapeworms, except for the absence of an operculum. Phylogenetic relationships of the new genus are not resolved, but it belongs to the so-called Neotropical clade of the Proteocephalidae, which is composed mainly of Neotropical tapeworms of siluriforms and other teleosts, but also Nearctic and Palaearctic species of *Ophiotaenia* La Rue, 1911 from snakes and amphibians. A morphologically similar species, *Proteocephalus ambloplitis* (Leidy, 1887) from bass (*Micropterus* spp.) in North America, is provisionally retained in *Proteocephalus* Weinland, 1858 because its relationships to *L. perplexa* are not yet clear. The former species differs from *L. perplexa* by the presence of a large apical organ, large, elongate vaginal sphincter situated near the genital atrium, vitelline follicles limited to lateral longitudinal fields, strongly coiled vas deferens within the cirrus sac, and a convoluted vaginal canal anterior to the ovarian isthmus. *Laruella perplexa* reportedly has a broad spectrum of hosts but most are likely postcyclic or accidental hosts. A list of cestode parasites reported from bowfin is provided; it includes eight species and three taxa not identified to the species level. However, only three adult cestodes, *L. perplexa* and two species of *Haplobothrium* Cooper, 1914, are typical tapeworm parasites of bowfin, but previous molecular studies indicate possible existence of a putative new species in bowfin.

## 1. Introduction

North America has an extraordinarily rich fauna of freshwater actinopterygian fishes (Warren and Burr, 2014) which are hosts of numerous parasites, including tapeworms (Cestoda) (Hoffman, 1999). A total of 35 species of *Proteocephalus* Weinland, 1858 (Onchoproteocephalidea: Proteocephalidae) have been reported from North American freshwater fishes (Schmidt, 1986; Hoffman, 1999), including 29 species of the *Proteocephalus*-species aggregate (= *Proteocephalus* sensu stricto). This species aggregate was proposed by de Chambrier et al. (2004) to accommodate closely related and morphologically similar Nearctic and

Palaearctic species of the monophyletic clade that contains the type-species of the genus, *Proteocephalus ambiguus* (Dujardin, 1845) (Scholz et al., 2007). Recent taxonomic revisions based on morphological and molecular evaluation of museum and newly collected material of fish tapeworms have shown that the actual number of valid species of this aggregate is considerably lower and only 15 species are currently recognised as valid in North America (Scholz et al., 2019; 2020a, 2021).

However, five North American species, namely *Proteocephalus ambloplitis* (Leidy, 1887) from centrarchids, mainly *Micropterus* spp., *P. australis* Chandler, 1935, *P. elongatus* Chandler, 1935 and *P. singularis* La Rue, 1911 from gars, *Lepisosteus* spp. (Lepisosteiformes),

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*P. chamelensis* Pérez-Ponce de León, Brooks & Berman, 1995 from Pacific sleeper, *Gobiomorus maculatus* (Günther) (Gobiiformes: Eleotridae), and *P. perplexus* La Rue, 1911 from bowfin, *Amia calva* Linnaeus (Amiiformes), differ considerably from species of this aggregate (*Proteocephalus* sensu stricto) in their morphology (Freze, 1965; Pérez-Ponce de León et al., 1995). Molecular data are available only for four taxa, namely *P. ambloplitis*, *P. chamelensis*, *P. perplexus*, and *P. singularis*. They show that these species are not closely related to those of the *Proteocephalus*-aggregate with Holarctic distribution. In contrast, they seem to be more closely related to Neotropical proteocephalids, but their interrelationships remain unresolved (Hypša et al., 2005; de Chambrier et al., 2015, 2017).

*Proteocephalus perplexus* is a common intestinal parasite of bowfin, which are actinopterygians related to gars (Lepisosteiformes) in the infraclass Holostei (Grande 2005, 2010). They are regarded as relicts, being the sole surviving species of the order Amiiformes, which dates from the Jurassic to the present (Grande and Bemis, 1998; Near et al., 2012). Fossil deposits indicate the Amiiformes were once widespread in both freshwater and marine environments with a range that spanned across North and South America, Europe, Asia and Africa (Grande and Bemis, 1998). At present, their range is limited by the bowfin to the eastern United States and adjacent southern Canada, including the drainage basins of the Mississippi River and Great Lakes (Burr and Bennett, 2014). Bowfin are demersal freshwater piscivores, living in lowland rivers and lakes, swamps and backwater areas (Page and Burr, 2011; Burr and Bennett, 2014). They are voracious and opportunist feeders, subsisting on fishes including other sport fishes, frogs, a wide range of macroinvertebrates such as crayfish, insects, and shrimp, and have even been known to consume aquatic birds (Scott and Crossman, 1973; Becker, 1983; Murdy and Musick, 2013; Burr and Bennett, 2014).

Since *P. perplexus* is not closely related to the species of the *Proteocephalus* species-aggregate of de Chambrier et al. (2004) and possesses unique morphological characteristics, a new genus is proposed to accommodate this tapeworm that parasitises an early branching lineage of actinopterygians, the Amiidae, recently represented only by the bowfin, *A. calva* (see Hughes et al., 2018). In addition, this cestode is redescribed, including its first SEM micrographs, based on new, properly fixed material, and a list of cestode parasites of bowfin compiled from the literature and supplemented by the authors' own unpublished data is provided.

## 2. Materials and methods

The present study is based on the examination of the type material of *Proteocephalus perplexus*, and specimens recently collected by the present authors and others. Newly collected tapeworms were rinsed in 0.9% NaCl solution after removing them from the host intestine and fixed in hot 4% formaldehyde solution. Tapeworms were stained with Mayer's carmine, dehydrated in an ascending series of ethanol, cleared in eugenol (clove oil) and mounted in Canada balsam on slides. For scanning electron microscopy (SEM), scoleces of four specimens from Wisconsin, Mississippi and Oklahoma, fixed in hot formalin, were dehydrated through a graded ethanol series, dried in hexamethyldisilazane, coated with gold (thickness of 10–20 nm) and examined in a JEOL JSM-740 1F scanning electron microscope at the Institute of Parasitology, Biology Centre of the Czech Academy of Sciences.

The following museum abbreviations were used in this paper: HWML – Harold W. Manter Laboratory, Lincoln, Nebraska, USA; IPCAS – Institute of Parasitology, Biology Centre of the Czech Academy of Sciences, České Budějovice, Czech Republic; LRP – Lawrence R. Penner Parasitology Collection (LRP) at the University of Connecticut, Storrs, Connecticut, USA; MHNG-PLAT – Natural History Museum, Geneva, Switzerland; USNM – Smithsonian National Museum of Natural History, Washington, D.C., USA. Common and scientific names of fish follow Froese and Pauly (2022).

## 3. Results

### 3.1. *Laruella new genus*

Generic diagnosis: Onchoproteocephalidea, Proteocephalidae. Testes, ovary, vitellarium and uterus medullary. Large worms. Strobila robust, acraspedote, with immature proglottids conspicuously wider than long. Scolex globular, with four prominent lobes bearing uniloculate suckers; tear-shaped inner rim of suckers bearing sphincter. Apex of scolex without apical organ. Inner longitudinal musculature well-developed, formed by single circle of small bundles of muscle fibres. Ventral osmoregulatory thin-walled, wide, situated ventrolateral to vitelline follicles. Dorsal canals thick-walled, narrow, situated mediadorsal to vitelline follicles. Testes medullary, in two or three incomplete layers, in one large field between lateral bands of vitelline follicles, with some testes anterodorsal to ovary. Cirrus-sac large, thin-walled, containing few loops of vas deferens and long cirrus. Ovary medullary, bilobed, with narrow lateral wings. Vagina anterior to cirrus-sac, with circular vaginal sphincter at distance from genital atrium, at level of proximal third of cirrus-sac; terminal (distal) part of vagina thick-walled, lined with chromophilic cells. Gonopores irregularly alternating, pre-equatorial. Vitelline follicles forming two L-shaped lateral bands from anterior to posterior margins of proglottids, with posterior field of follicles turned inwards (medially) ventrally. Uterus medullary, with numerous lateral diverticula, with Type 2 of uterine development of de Chambrier et al. (2004). Uterine pore large, elongate. Eggs ellipsoid. Parasites of bowfin, rarely (incidentally?) in other predatory fish.

Type and only species: *Laruella perplexa* (La Rue, 1911) new combination.

Etymology: The generic name honours George Roger La Rue (1882–1967), American parasitologist, whose monograph on proteocephalid tapeworms published in 1914 represents a milestone in the systematics of this cestode group. The name is treated as feminine.

#### 3.1.1. Differential diagnosis

The new genus differs from other proteocephalid genera by a combination of the following characteristics: a robust, large body (length up to 15 cm) with immature proglottids conspicuously shorter than long, robust, globular scolex with four prominent lobes bearing uniloculate suckers with a tear-shaped sphincter on their inner rim, but lacking any apical organ, vitelline follicles forming L-shaped fields, with posterior follicles bent medially alongside the posterior margin of the proglottid, large, thin-walled cirrus-sac, and a ring-like vaginal sphincter situated at a long distance from the genital atrium.

The type and single species of the genus is redescribed below based on newly collected material from the type host.

### 3.2. *Laruella perplexa* (La Rue, 1911) new combination

#### 3.2.1. Synonym: *Proteocephalus perplexus* La Rue, 1911

*Material studied*: holotype (USNM 1347286) and paratypes (USNM 1348673–1348680) from *Amia calva*, Illinois River, Illinois, USA, collected by H.B. Ward in June and July 1910; vouchers: one specimen from *A. calva* (host code US 029/701), Pascagoula River, Mississippi, USA, collected by V.V. Tkach in March 2009 (MHNG-PLAT-0063348); numerous specimens, including two scoleces for SEM, from *A. calva* (host codes US 610a–i, 611b, 612a–g, 620a, 621c, 622a,b), Green Bay, Lake Michigan, Wisconsin, USA, T. Scholz and A. Choudhury, 15 and 16 May 2017; numerous specimens from *A. calva* (629d–g, 635a, 636a), Lake Poygan, Wisconsin, T. Scholz and A. Choudhury, 17 May 2017; 14 specimens, their cross and longitudinal (frontal) sections and one scolex for SEM from *A. calva* (US 841a, g, 854a), Pascagoula River, Mississippi, USA, T. Scholz, R. Kuchta and M. Oros, 20 and 22 June 2019 (IPCAS C-774/1); two mounted specimens and one scolex for SEM from *A. calva* (US 925a), Turner Pond, McCurtain County, Oklahoma, USA, C.T. McAllister, 1 June 2019 (IPCAS C-774/1); 1 specimen from *A. calva*

(FR19.173.2), Lake Erie, Presque Isle, Pennsylvania, USA, F. Reyda, 15 May 2019.

**Type host:** Bowfin, *Amia calva* Linnaeus (Amiiformes: Amiidae).

**Additional reported hosts (unconfirmed – see Remarks):** *Ambloplites rupestris* (Rafinesque) (Centrarchiformes: Centrarchidae); *Ameiurus melas* (Rafinesque), *A. nebulosus* (Lasueur), *Ictalurus punctatus* (Rafinesque) (Siluriformes: Ictaluridae); *Esox* sp. (Esociformes: Esocidae); *Lepisosteus osseus* (Linnaeus), *L. platostomus* Günther (Lepisosteiformes: Lepisosteidae).

**Type locality:** Illinois River in Havana (40.297067, –90.060004), Mason County, Illinois, USA (Mississippi River basin).

**Type specimens:** holotype – a contracted, overstained immature specimen (USNM 1347286), paratypes – three slides with fragments of the strobila and ten slides with cross, longitudinal and sagittal sections of scoleces and gravid proglottids (USNM 1348673–1348680).

**Distribution (new geographical records marked with asterisk):** Canada (Ontario – Erie, Huron and Ontario Lakes), USA (Illinois, Kentucky, Michigan, Minnesota, Mississippi\*, New York, Oklahoma\* (southwesternmost distribution in North America), Pennsylvania\*, South Carolina, Tennessee, Wisconsin) – La Rue (1911, 1914), Pearse (1924), Hunter (1929), Van Cleave and Mueller (1934), Bangham and Venard (1942), Bangham (1944, 1955), Fischthal (1950), Dechtiar (1972), Bauer and Harley (1973), Anthony (1984), Dechtiar and Christie (1988), Dechtiar et al. (1988), Amin (1990), Aho et al. (1991), Olson and Caira (1999), de Chambrier et al. (2009).

McDonald and Margolis (1995) cited Jarecka et al. (1990) for the record of this tapeworm from New Brunswick, Canada, but that study does not provide any information about source of the bowfin hosts. *Amia calva* does not occur in New Brunswick (Scott and Crossman, 1973), so the fish hosts must have been collected elsewhere. Contemporaneous studies on another bowfin tapeworm, *Haplobothrium globuliforme* Cooper, 1914 by two of Jarecka's coauthors (see MacKinnon and Burt, 1985 a–c, MacKinnon et al., 1985) state Lake Ontario as the source of bowfin in those studies.

**Life cycle:** Not elucidated completely. In an abstract from a conference, Jarecka et al. (1990) reported calanoid copepods as intermediate hosts of *L. perplexa*, but did not provide any details. Plerocercoids presumably conspecific with *L. perplexa* were found in *Ictalurus* sp. (Siluriformes: Ictaluridae), *Morone chrysops* (Rafinesque) (Perciformes: Moronidae), *Pimephales notatus* (Rafinesque) (Cypriniformes: Leuciscidae) (Hoffman, 1999); these fish may serve as second intermediate or paratenic hosts.

**Representative DNA sequences:** (1) 28S rRNA gene (D1–D3): adult from *A. calva*, Hay Bay, Lake Ontario, Ontario, Canada (1,314 bp; AF286940 – Olson and Caira, 1999); adult from *A. calva*, Hay Bay, Lake Ontario, Ontario, Canada, 17 July 1995, MHNG-INVE-0025658 (1,009 bp; AJ275228 – de Chambrier et al., 2004); two adults from *A. calva*, Reelfoot Lake, Tennessee, USA, 30 June 2002, MHNG-INVE-0035366, 0035321 (1,010 bp; FM956089 – de Chambrier et al., 2009); adult from *A. calva*, Reelfoot Lake, Tennessee, USA, 30 June 2002, MHNG-INVE-36139 (1,005 bp; FM956090 – de Chambrier et al., 2009); adult from *A. calva*, Hay Bay, Lake Ontario, Ontario, Canada, LRP 8299 (1,396 bp; KF685873 – Caira et al., 2004); adult from *Ictalurus punctatus*, Reelfoot Lake, Tennessee, USA, 20 June 2002, MHNG-INVE-0036277, sequence identical to MHNG-INVE-0035366 (FM956089). (2) 18S rRNA gene: adult from *A. calva*, Hay Bay, Lake Ontario, Ontario, Canada (2,037 bp; AF124472 – Olson and Caira, 1999); adult from *A. calva*, Hay Bay, Lake Ontario, Ontario, Canada, LRP 8299 (1,978 bp; KF685833 – Caira et al., 2004). (3) Mitochondrial 16S rRNA gene: adult from *A. calva*, Lake Ontario, Ontario, Canada (427 bp; AJ275216 – M. Zehnder, unpublished). (4) Elongation factor-1 $\alpha$ : adult from *A. calva*, Hay Bay, Lake Ontario, Ontario, Canada (987 bp; AF124805 – Olson and Caira, 1999).

**Phylogenetic relationships:** *Laruela perplexa* is a member of the “Neotropical fish” superclade of de Chambrier et al. (2015), but its relationships to members of this species-rich clade are not resolved (de

Chambrier et al., 2004, 2015).

### 3.3. Redescription (Figs. 1–4)

(Based on specimens from *A. calva*, Mississippi, USA, IPCAS C-774/1; measurements in micrometres unless otherwise stated, with the number of measurements (n) in parentheses; measurements from the original description by La Rue, 1911, 1914 in brackets).

Proteocephalidae. Large worms. Total body length up to 152 mm [155 mm], maximum width 1.74 mm [1.7 mm]. Strobila slightly craspedote and trapeziform (Fig. 3), anapolytic, slightly, but continuously widening towards posterior end, consisting of high number (>435) proglottids, especially immature (up to appearance of spermatozoa in vas deferens) > 324 (Fig. 1A), relatively few mature (up to appearance of eggs in uterus; 24) and pregravid (up to appearance of hooks in oncospheres; >87). Immature proglottids much wider than long to wider than long, 45–620 × 510–1,320 (length: width ratio 1 : 2.0–12.1; n = 36), mature proglottids wider than long, 370–770 × 990–1,475 [595 × 1,700] (length: width ratio 1 : 1.3–3.6; n = 24; Fig. 3A), pregravid and gravid proglottids wider than long to slightly longer than wide in terminal proglottids, 525–1,095 × 1,090–1,740 (length: width ratio 1 : 1.4–2.9; n = 26; Fig. 3B).

Scolex covered with capiliform filitriches (Fig. 2E). Four suckers subspherical to almost spherical, 230–340 × 220–315 (n = 36) [340–459 × 255–272], directed almost laterally (Fig. 1C, E, 2). Apical sucker absent; small cells with granular content accumulated in apical part of scolex (Fig. 1E). Neck long, 320–430 (n = 8) [500] wide at narrowest site at short distance posterior to scolex (Fig. 1A, C).

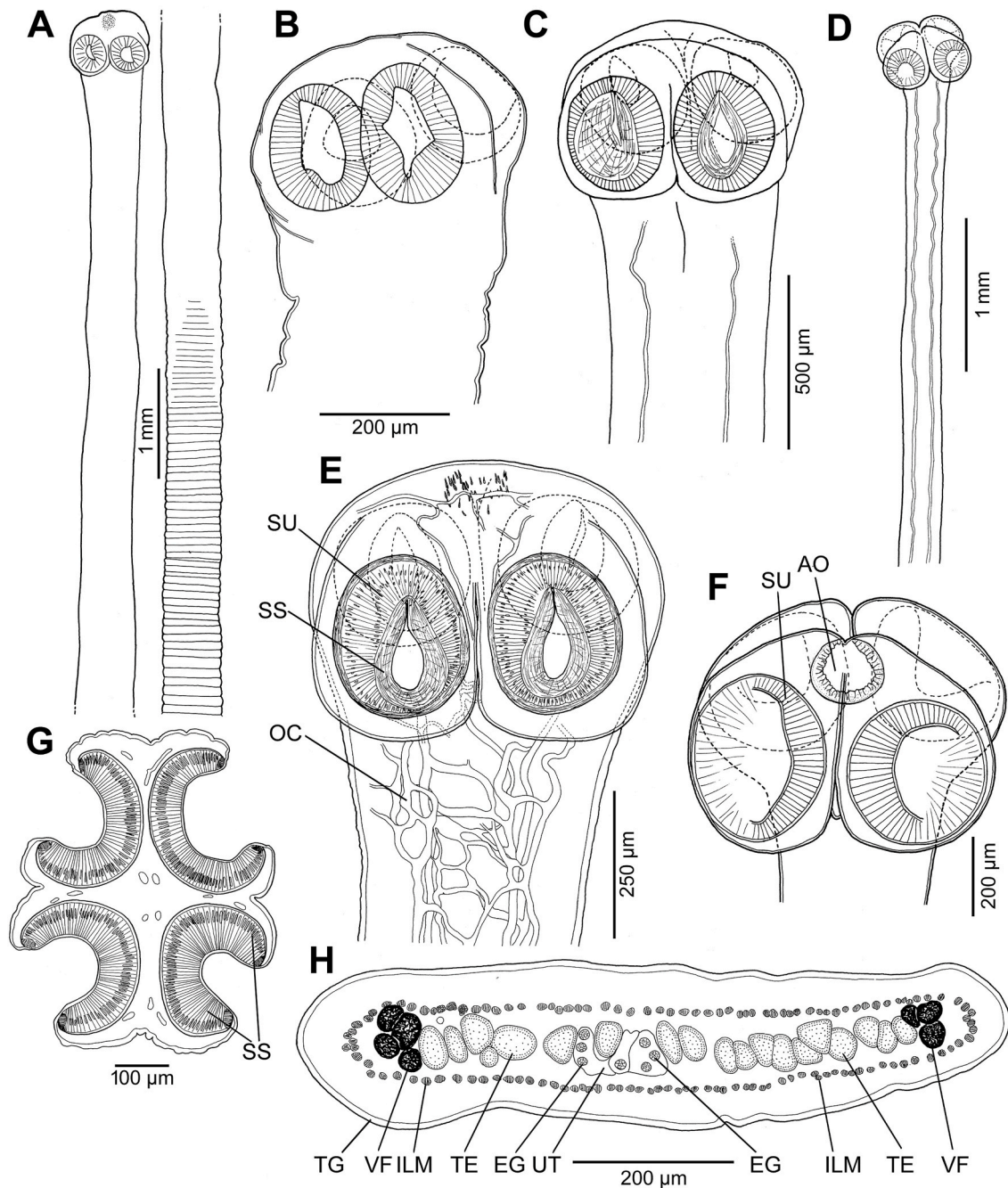
Inner longitudinal musculature well developed, formed by one layer of large bundles of muscle fibres (Fig. 1H). Two pairs of almost straight osmoregulatory canals, with few narrow lateral canals; ventral canals thin-walled, wider, 11–32 in diameter, at level of lateralmost vitelline follicles or slightly lateral to them (Fig. 3); dorsal canals thick-walled, narrower, 5–10 in diameter, dilated between individual proglottids up to width of 13–15, situated more medially, usually at level of inner-most (medial) vitelline follicles (Fig. 3). At level of neck (proliferation zone), osmoregulatory canals widely anastomosed, with secondary canals (Fig. 1E).

Testes medullary, ovoid, subspherical or irregularly shaped, 40–105 × 30–80 (n = 25) [37–69 in diameter] in mature proglottids, in 2–3 irregular layers, difficult to count reliably; 151–248 [135–155] in number (n = 6). Testes form almost uninterrupted field through proglottids (Fig. 3B), including pregravid ones (Fig. 3A), with testes missing only at level of vas deferens, terminal genitalia and ovary; laterally, testes reach only to longitudinal bands of vitelline follicles, rarely overlapping them partly (Fig. 3). Testes also numerous in pregravid and gravid proglottids (Figs. 3B and 4A).

Vas deferens strongly coiled, voluminous, filling large space between cirrus sac and mid-line of proglottids, sometimes slightly overlapping it (Figs. 3A, 4A–C). Cirrus sac elongate, pyriform, thin-walled (Figs. 3, 4A–C), 280–340 × 65–115 in mature proglottids (n = 13) [300–344 long]; cirrus sac length: width ratio 2.7–4.2 (n = 13); length of cirrus sac represents 21–25% (n = 13) of proglottid width. Internal sperm duct forms only few coils (Figs. 3 and 4A, C). Cirrus long, strongly muscular, representing 81–96% of length of cirrus sac. Common genital atrium narrow, relatively deep (Fig. 3), alternating irregularly, markedly pre-equatorial, at 31–39% (n = 20) [25–50%] of length of last mature and first pregravid proglottid from its anterior margin (Fig. 3) and at 26–34% (n = 7) of length of last pregravid proglottid from its anterior margin (Fig. 3B).

Ovary medullary, bilobed, with narrow isthmus and narrow and long lateral wings with numerous lobes (Fig. 3), not surpassing osmoregulatory canals laterally (Figs. 3 and 4D). Length of ovary, i.e., width of ovarian lobes, 85–150, i.e., 22–29% of proglottid length (n = 10); total width of ovary (horizontal) 775–960, i.e., 57–66% of proglottid width (n = 10). Mehlis' gland spherical, small, 53–68 in diameter (n = 10),





**Fig. 1.** *Laruella perplexa* (La Rue, 1911) n. comb. from *Amia calva* (A, B, C, E, G, H) and *Proteocephalus ambloplitis* (Leidy, 1887) from *Micropterus dolomieu* (D, F). A, D – anterior part of body with first proglottids. B, C, E, F – scolex, frontal view; B – holotype (USNM 1347286); note narrow sphincters around sucker opening in C, E). G – cross section through scolex; note sphincters on margin of sucker openings; paratype (USNM 1348679).

Abbreviations: AO - apical organ; EG - egg; ILM - inner longitudinal musculature; OC - osmoregulatory canals; SS - sucker sphincter; SU - sucker; TE - testes; TG - tegument; UT - uterus; VF - vitelline follicles.

representing 3.6–4.6% of proglottid width (Fig. 4D). Relative size of ovary, i.e., ratio of surface of ovary to surface of proglottid (see de Chambrier et al., 2012) 10.3–13.9% (n = 2).

Vaginal canal enlarged to form ovoid, thick-walled seminal receptacle anterodorsal to ovarian isthmus (Figs. 3 and 4D); vaginal canal directed anteriorly in middle part, then turned laterally, almost straight and perpendicular, always anterior to cirrus sac (Figs. 3, 4A–C). Terminal (distal) part of vaginal canal (*pars copulatrix vaginae*) with fine microtriches (Fig. 4B), surrounded by thick layer of chromophilic cells (Figs. 3 and 4A, B); circular vaginal sphincter well-developed, 30–35 × 41–52, at long distance from genital atrium (at level of proximal third of

cirrus sac – Figs. 3, 4A–C).

Vitelline follicles medullary, forming two narrow lateral bands between anterior and posterior margins of proglottids, absent at level of cirrus sac and vagina on ventral side (Fig. 3). Posteriorly, vitellarium turned inwards (medially) ventrally alongside posterior margin of proglottid, reaching up to one third of proglottid width on each side (Figs. 3 and 4D).

Uterus medullary, with type 2 development (see de Chambrier et al., 2004). Uterine stem lined with chromophilic cells appearing in immature proglottids; in mature proglottids, uterine lumen gradually extends from base to apex into digitate diverticula lined with chromophilic cells



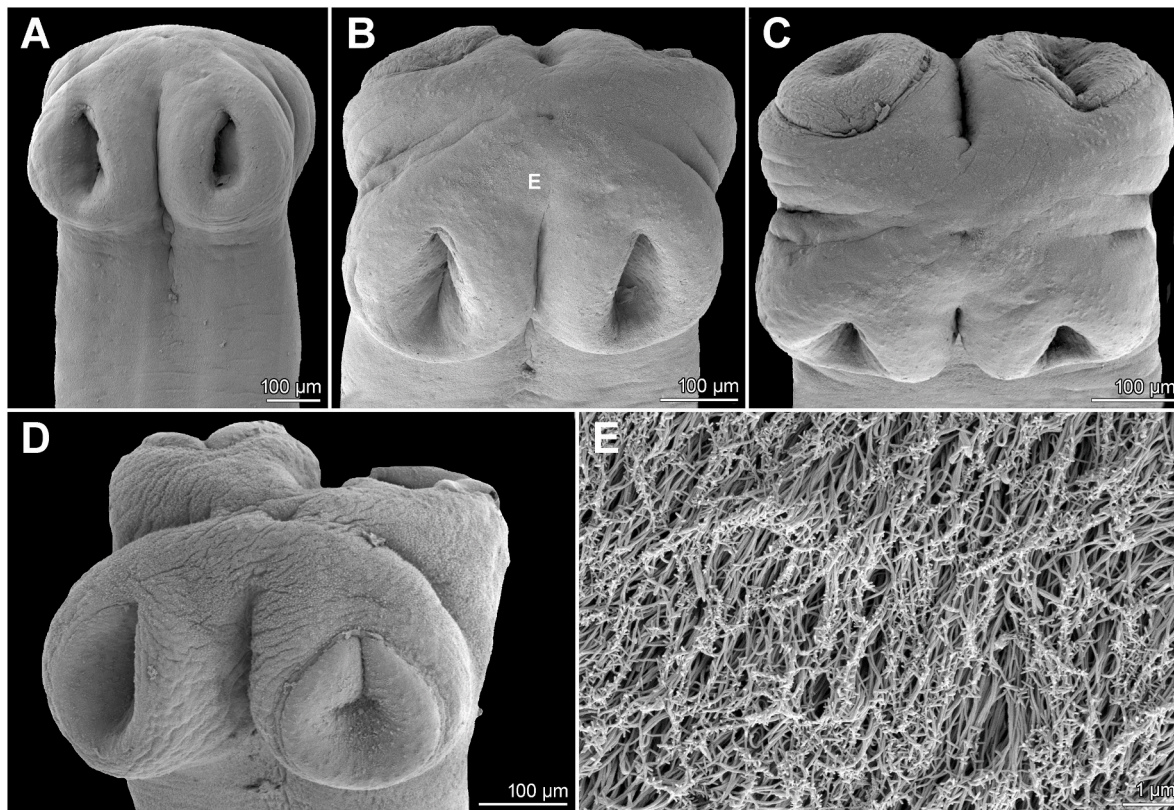


Fig. 2. *Laruella perplexa* (La Rue, 1911) n. comb. from *Amia calva*. SEM micrographs. A – frontal view of scolex. B, D – subapical view of scolex; note tear-shaped inner rim of suckers. C – apical view of scolex; note apical pit. E – capilliform filitriches on scolex (apical region anterior to suckers – indicated by letter E in Fig. 2B).

as in pregravid and gravid proglottids. In pregravid and gravid proglottids, uterus with 16–21 and 15–21 lateral diverticula on poral and aporal sides [20–25], respectively (Fig. 3); width of uterus represents up to 88% of proglottid width. In terminal proglottids, uterus opens by elongate, wide uterine pore situated in posterior half of proglottid length; additional, smaller pore may be present in anterior half of proglottid.

Eggs ellipsoid, resembling those of bothriocephalid and diphyllbothriid tapeworms, except missing operculum. External envelope subspherical to widely oval, 25–30 × 19–22 (n = 24) in eggs *in utero* of mounted specimens and 27–31 × 19–24 (n = 26) in immature eggs from unstained worms in water [24–36 in diameter]; oncosphere subspherical to widely oval, 17–20 × 14–17 (n = 14) in eggs *in utero* of mounted specimens and 17–20 × 14–17 (n = 19) in immature eggs from unstained worms in water [14–16 × 13–14]; embryonic hooks 5–6 long (n = 7).

### 3.4. Remarks

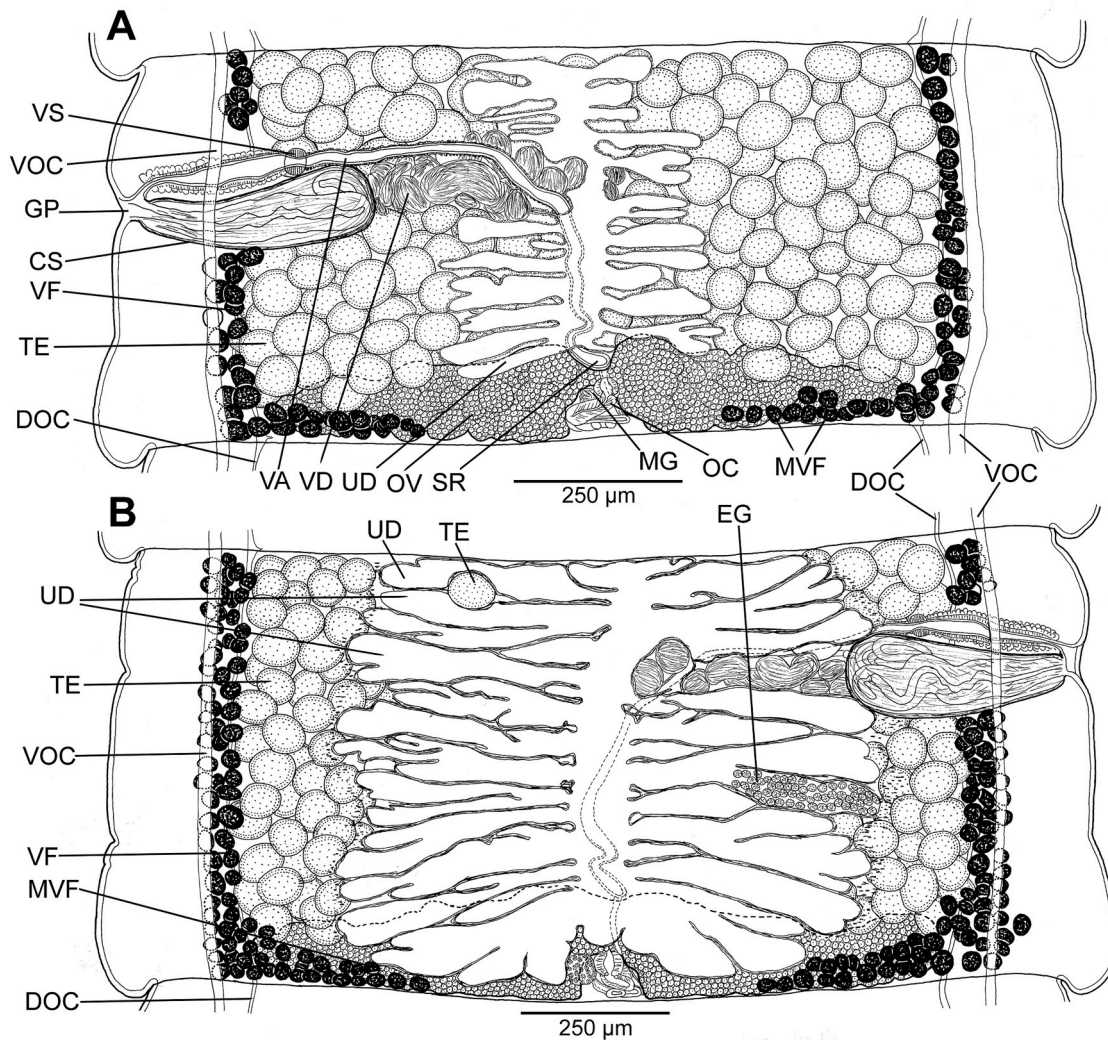
The species was briefly described by La Rue (1911) and later characterised in more detail by the same author (La Rue, 1914), based on tapeworms found in *A. calva* and *L. platostomus* (misspelled as *L. platystomus*) from Illinois. La Rue (1914) pointed out that *L. perplexa* differed from all European species of *Proteocephalus* (= *Proteocephalus*-species aggregate) in the position of its vaginal sphincter and L-shaped fields (see description above) of vitelline follicles, and peculiar shape of the eggs, which resemble those of bothriocephalid and diphyllbothriid tapeworms. In fact, this species is different in its morphology from all proteocephalids and deserves to be placed in a separate, new genus, as confirmed by the present redescription based on specimens from southern USA, which were of the best quality and made it possibly to provide necessary details in illustrations (holotype is a contracted, overstained immature specimen, and paratypes consist of

fragments of the strobila and slides with histological sections of scoleces and gravid proglottids only).

*Laruella perplexa* cestode is the dominant endoparasite of bowfin, as indicated by its wide distribution in North America, high prevalence and high intensity of infection. The present authors found this tapeworm in all 14 bowfin examined: nine fish from Lake Michigan (6) and Poygan Lake (3) in Wisconsin examined in May 2017, three fish from the Pascagoula River in Mississippi in June 2019 and both fish from Turner Pond near Idabel, Oklahoma, in June 2019. Intensity of infection ranged from 1 to about 100 specimens, usually with tens (25–56) of tapeworms present in a single host. Seven of these 14 bowfin were simultaneously, but much less heavily infected with *Haplobothrium globuliforme* Cooper, 1914 (see the list of cestode parasites of bowfin below).

Records of *L. perplexa* from *Ambloplites rupestris* (Rafinesque) (Centrarchiformes: Centrarchidae) and *Esox* sp. (Esociformes: Esocidae) in Wisconsin by Pearse (1924) are doubtful and are considered to be misidentifications, most likely of *P. ambloplitis*, which is a parasite commonly found in these hosts.

*Laruella perplexa* somewhat resembles *P. ambloplitis*, a parasite of bass (*Micropterus* spp.), which has also been reported from gars and ictalurids (La Rue, 1914; Freze, 1965; de Chambrier et al., 2009). Even the species name *perplexus* was proposed by La Rue (1911, 1914) because it was difficult to differentiate it from *P. ambloplitis*. Both species are large, with a robust body with numerous immature proglottids conspicuously wider than long, large, four-lobed scolex, numerous testes (more than 150) and uterine diverticula (>15 on each side), large, thin-walled cirrus-sac, and thick-walled terminal (distal) part of the vaginal canal. However, both species can be easily distinguished from each other by the following characteristics: (i) the scolex of *L. perplexa* is only slightly wider than a wide neck region, and is devoid of any apical structure (Fig. 1B, C, E), whereas the scolex of *P. ambloplitis* is more prominent, much wider than a narrow neck, and contains a large apical organ (Fig. 1D, F); (ii) suckers of *L. perplexa* possess a tear-shaped



**Fig. 3.** *Laruella perplexa* (La Rue, 1911) n. comb. from *Amia calva*, Mississippi, USA (MHNG-PLAT-0063348). A – mature proglottid, ventral view. B – pregravid proglottid, ventral view.

Abbreviations: CS – cirrus sac; DOC – dorsal osmoregulatory canal; EG – egg; GP – genital pore; MG – Mehlis' gland; MVF – median vitelline follicles; OC – oocyst; OV – ovary; SR – seminal receptacle; SU – sucker; TE – testes; UD – uterine diverticula; VA – vagina; VD – vas deferens; VF – vitelline follicles; VOC – ventral osmoregulatory canal; VS – vaginal sphincter.

sphincter on their inner rim (Fig. 1C, E), whereas suckers of *P. ambloplitis* are devoid of any sphincter (Fig. 1F); (iii) vitelline follicles form L-shaped fields (bands) in *L. perplexa* (Figs. 3 and 4D), whereas *P. ambloplitis* has vitelline follicles in two longitudinal bands, which are not bent inwards posteriorly; (iv) the vaginal canal of *L. perplexa* is surrounded by a ring-like sphincter situated at a distance from the genital atrium (Fig. 4A–C), whereas the vaginal sphincter of *P. ambloplitis* is large, thick and it is situated in the terminal (distal-most) portion of the vaginal canal near the genital atrium (Fig. 4F); (v) the vaginal canal of *L. perplexa* is slightly sinuous (Fig. 4D) whereas that of the latter taxon forms numerous coils anterior to the ovarian isthmus (Fig. 4E); (vi) the cirrus-sac of *L. perplexa* contains a few coils of the vas deferens (Fig. 4C, B) rather than numerous loops present within the cirrus sac of *P. ambloplitis* (Fig. 4F).

Molecular data place *L. perplexa* and *P. ambloplitis* in the large unresolved clade, first called 'Neotropical' by Hyspa et al. (2005) and then as a "Neotropical fish superclade" by de Chambrier et al. (2015), mainly composed of tapeworms parasitising Neotropical siluriforms, especially pimelodids (de Chambrier et al., 2004, 2015; Alves et al., 2017). However, relationships of taxa of this large clade are not sufficiently resolved, including interrelationships of *L. perplexa* and

*P. ambloplitis*. For example, the earlier results of de Chambrier et al. (2004) did not indicate that these two species from the Nearctic region are closely related. Therefore, *P. ambloplitis* is provisionally retained in *Proteocephalus* and its generic assignment will be discussed elsewhere, after more robust phylogenetic information is available.

### 3.5. A commented list of tapeworms (Cestoda) reported from bowfin, *Amia calva*

Information on the cestode parasites of *A. calva* is summarised below as an annotated list. The records are based on a critical review of the literature, especially records listed by Margolis and Arthur (1979), McDonald and Margolis (1995), Hoffman (1999) and Gibson et al. (2005). In addition, unpublished data based on authors' examination of bowfin are presented. Tapeworms considered as typical parasites of *A. calva* are marked with asterisk.

Order Bothriocephalidea Kuchta, Scholz, Brabec et Bray, 2008

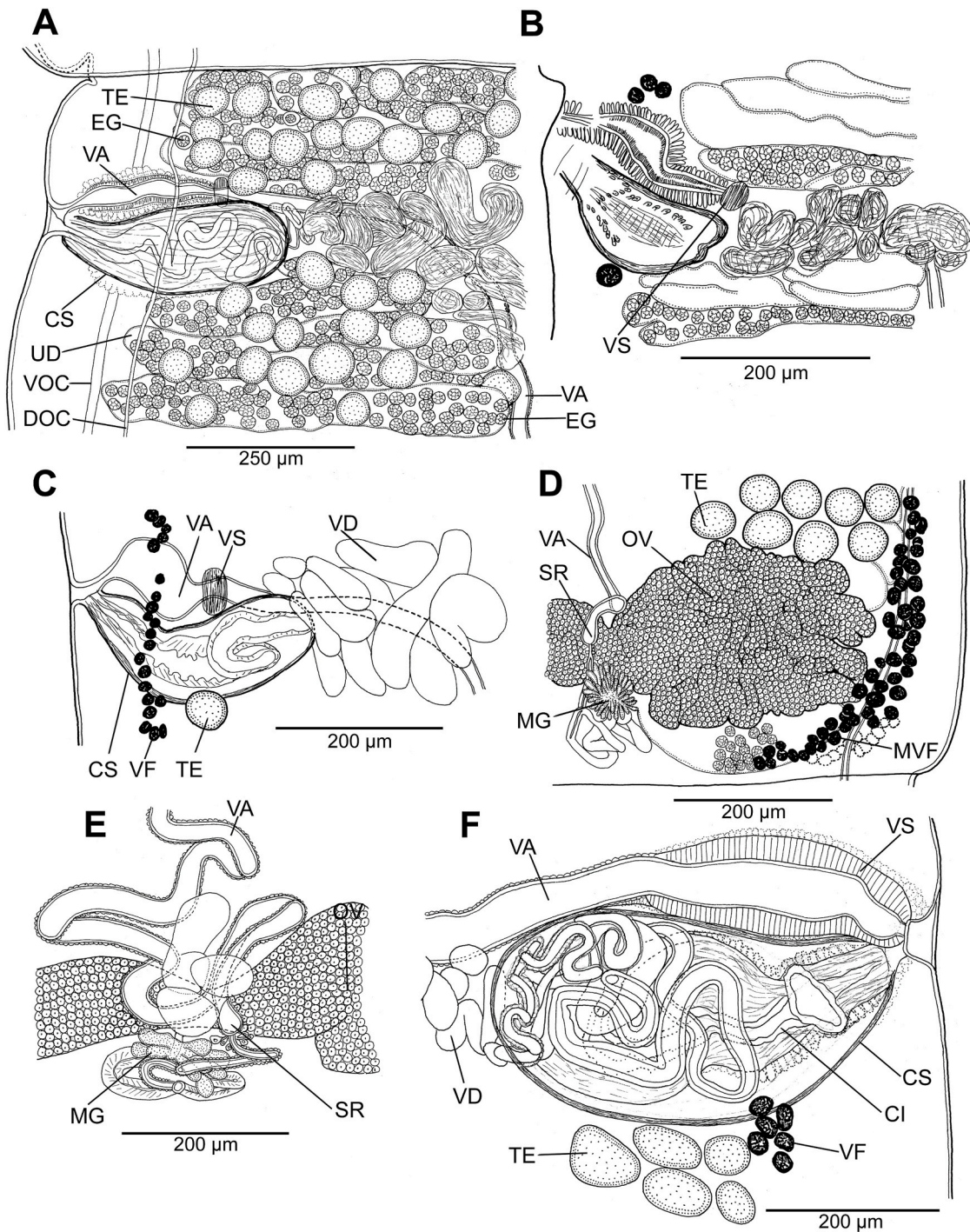
1. *Bothriocephalus* sp. – accidental record of juvenile tapeworms

Records: Wisconsin (Lake Michigan).

Reference: Unpublished data.

Remarks





**Fig. 4.** *Laruella perplexa* (La Rue, 1911) n. comb. from *Amia calva*, Illinois and Mississippi (A–D) and *Proteocephalus ambloplitis* (Leidy, 1887) from *Micropterus dolomieu* (E, F). A – terminal genitalia with uterine diverticula near anterior part of proglottids (MHNG-PLAT-0063348), dorsal view; vitelline follicles are not illustrated. B, C – terminal genitalia, frontal section and ventral view of paratype (USNM 1348679). D – posterolateral end of proglottid (MHNG-PLAT-0063348); note band of posterior (median) vitelline follicles bent inwards. E – proximal part of vaginal canal, dorsal view; note numerous loops. F – cirrus-sac, dorsal view; note large, thick-walled vaginal sphincter and strongly convoluted internal sperm duct.

Abbreviations: CI - cirrus; CS - cirrus sac; DOC - dorsal osmoregulatory canal; EG - egg; MG - Mehlis' gland; MVF - median vitelline follicles; OV - ovary; SR - seminal receptacle; TE - testes; UD - uterine diverticula; VA - vagina; VD - vas deferens; VF - vitelline follicles; VOC - ventral osmoregulatory canal; VS - vaginal sphincter.

Two juvenile tapeworms of *Bothriocephalus* Rudolphi, 1808 were found in one of six bowfin (US 621d) from Lake Michigan in Wisconsin examined by the present authors (TS and AC) in May 2017, but they were fixed in formalin and could not be characterised genetically. Bowfin apparently represents only an accidental host of these cestodes, which may belong to *B. cuspidatus* Cooper, 1917, because this tapeworm

occurs commonly in walleye, *Sander vitreus* (Mitchill), in the Great Lakes basin (Choudhury and Scholz, 2020). Hoffman (1999) also listed juveniles of *Bothriocephalus* sp. in the list of parasites of *A. calva*, but did not provide any specific information about this record.



2. *Triaenophorus nodulosus* (Pallas, 1781) plerocercoids – record requiring confirmation

Records: Canada (Ontario).

Reference: [Dechtiar et al. \(1988\)](#).

Remarks

This tapeworm is a common intestinal parasite of pike (*Esox* spp., Esocidae) and occurs throughout the Holarctic region. Its life cycle includes copepods as first and fish as second intermediate hosts ([Kuperman, 1973](#)). The spectrum of second intermediate hosts of *T. nodulosus* is extremely broad. [Michajlow \(1962\)](#) reported plerocercoids of *T. nodulosus* from 57 species of fishes. From the former Soviet Union, [Kuperman \(1973\)](#) reported almost 50 species of teleosts of 14 families and six orders. In North America, plerocercoids were found in 24 fish species ([Lawler and Scott, 1954](#)). [Dechtiar et al. \(1988\)](#) reported plerocercoids of *T. nodulosus* from the liver of two of 13 bowfin examined from Ontario Lake. It is the only record of this tapeworm from bowfin.

Order Haplobothriidea Joyeux et Baer, 1961

3. *\*Haplobothrium bistrobilae* Premvati, 1969 – specific, extremely rare parasite of bowfin

Records: USA (Florida).

Reference: [Premvati \(1969\)](#).

Remarks

The species was described from tapeworms found in *A. calva* from Florida but has never been found since the original description ([Premvati, 1969](#)). The validity of the species should be confirmed ([Kuchta and Scholz, 2017](#)). [Hoffman \(1999\)](#) erroneously listed report of *H. bistrobilae* from *Ictalurus punctatus* from Lake Erie, Ontario, Canada by [Dechtiar \(1972\)](#). However, this author found *H. globuliforme* (see below).

4. *\*Haplobothrium globuliforme* Cooper, 1914 – specific and common parasite of bowfin

Records: Canada (Ontario), USA (Illinois, Iowa, Louisiana, Michigan, Minnesota, Mississippi – new geographical record, New York, South Carolina, Tennessee, West Virginia, Wisconsin).

References: [Cooper \(1914a, b, 1917, 1919\)](#), [Essex \(1929\)](#), [Thomas \(1930\)](#), [Van Cleave and Mueller \(1934\)](#), [Bangham and Hunter \(1939\)](#), [Fischthal \(1950\)](#), [Bangham \(1955\)](#), [Sogandares-Bernal \(1955\)](#), [Dechtiar \(1972\)](#), [Robinson and Jahn \(1980\)](#), [MacKinnon and Burt \(1985a–c\)](#), [MacKinnon et al. \(1985\)](#), [Dechtiar and Christie \(1988\)](#), [Amin and Cowen \(1990\)](#), [Aho et al. \(1991\)](#), [Olson and Caira \(1999\)](#), [Olson et al. \(2001\)](#), [Joy \(2008\)](#), [Joy et al. \(2009\)](#).

Remarks

This tapeworm was described by [Cooper \(1914a\)](#) and later characterised in more detail by [Cooper \(1919\)](#). The taxonomic position of *H. globuliforme* was a matter of continuous discussion (see [Jones, 1994](#) and [Kuchta and Scholz, 2017](#) for review). It is a specific and widely distributed parasite of *A. calva*. Its life cycle includes copepods (*Cyclops* spp., *Macrocyclus albidus* (Jurine)) harbouring proceroids, fishes such as *Lepomis gibbosus* (Linnaeus) and *Ameiurus nebulosus* (Lesueur) with plerocercoids in the liver, and bowfin as the only definitive host ([Essex, 1929](#); [Thomas, 1930](#); [Van Cleave and Mueller, 1934](#); [Meinkoth, 1947](#); [MacKinnon and Burt, 1985b](#)). The records of *H. globuliforme* in *Anguilla rostrata* (Lesueur) by [Sogandares-Bernal \(1955\)](#) and in *Ictalurus punctatus* (site of infection not specified) by [Dechtiar \(1972\)](#) most likely represent postcyclic or incidental infection. [Thomas \(1983\)](#) and [MacKinnon and Burt \(1985a–c\)](#) and [MacKinnon et al. \(1985\)](#) studied the ultrastructure of different ontogenetic stages of *H. globuliforme* found in bowfin from Paradis and Ruddock, Louisiana, USA, and from Hay Bay, Lake Ontario, Ontario, Canada, respectively.

The present authors found this tapeworm in seven of 14 bowfin examined: six of nine fish infected with a total of 31 tapeworms (mean intensity of 5.2, range 1–14) from Wisconsin and one of three fish

infected with a single tapeworm from Mississippi (vouchers deposited as IPCAS C-223/1). In two bowfin from Oklahoma, no tapeworms were found. These data indicate heavier infection of bowfin from the northern part of the USA, but the number of fish examined was too low for any robust conclusion.

Order Onchoproteocephalidea Caira, Jensen, Waeschenbach, Olson et Littlewood, 2014

5. *\*Laruela perplexa* (La Rue, 1911) n. comb. – specific, probably most common cestode of bowfin (see above)

6. *Megathylacoides giganteum* (Essex, 1928) Jones, Kerley, and Sneed, 1956 – probably accidental record

Record: USA (Tennessee).

References: [de Chambrier et al. \(2009\)](#), [Scholz et al. \(2020b\)](#).

Remarks

[de Chambrier et al. \(2009\)](#) found tapeworms identified as *Megathylacoides* sp. (Proteocephalidae: Essexiellinae) in two bowfin from Reelfoot Lake, Tennessee, USA, in June 2002 (vouchers Nos. MHNG-INVE-0035373, 0035384; 28S DNA sequences Nos. FM956086, FM956087). [Scholz et al. \(2020\)](#) provided molecular evidence these tapeworms belonged to *M. giganteum*, a typical parasite of the channel catfish, *Ictalurus punctatus* (Rafinesque). Bowfin is considered as an accidental host of *M. giganteum*.

7. *Proteocephalus ambloplitis* (Leidy, 1887) – probably accidental records

Records: USA (Wisconsin).

References: [Bangham and Hunter \(1939\)](#), [Amin \(1989\)](#), [Amin and Cowen \(1990\)](#); present study.

Remarks

As discussed above, this is a typical intestinal parasite of large- and smallmouth bass (*Micropterus* spp.). The record of plerocercoids of *P. ambloplitis* encysted in the viscera of *A. calva* is found only in the parasite-host list of [Bangham and Hunter \(1939\)](#) (see [Margolis and Arthur, 1979](#)). The same authors ([Bangham and Hunter, 1939](#)) reported adults from the intestine of *A. calva*. [Amin \(1989\)](#) and [Amin and Cowen \(1990\)](#) also reported *P. ambloplitis* from bowfin in Wisconsin. The present authors (TS and AC) found a juvenile proteocephalid tapeworm in bowfin from Poygan Lake, Wisconsin, examined on 18 May 2017. Since this tapeworm possessed a large fifth (apical) sucker, it was identified as *P. ambloplitis*, which was confirmed by DNA data (28S rDNA and *cox1*; O. Kudlai – unpublished data).

Bowfin is a top predator that also consumes bass and other fish species. Therefore, it likely serves as an accidental and postcyclic host of *P. ambloplitis*.

8. *Proteocephalus pearsei* La Rue, 1919 – doubtful record

Record: Unspecified ([Hoffman, 1999](#)).

Remarks

This cestode is a specific parasite of American yellow perch, *Perca flavescens* (Mitchill) in North America ([Scholz et al., 2019](#)). [Hoffman \(1999\)](#) reported *P. pearsei* from *A. calva*, but only in the host-parasite list, not in the list of hosts of *P. pearsei*. The occurrence of this cestode typical of yellow perch in bowfin is considered doubtful.

9. *Proteocephalus* sp. – accidental records of juvenile tapeworms

Records: Canada (Ontario?), USA (Wisconsin).

Remarks

[Hoffman \(1999\)](#) reported immature *Proteocephalus* tapeworms from *A. calva*, but only in his host-parasite list, and without any detailed

information. Gibson et al. (2005) reported juvenile *Proteocephalus* tapeworms unidentified to the species level from bowfin in Canada, but did not provide a corresponding reference, stating their source as “Sperm ultrastructure in three previously unexamined tetraphyllidean, lecanicephalidean and proteocephalidean cestode species,” but without authors. This record may have referred to ultrastructural studies by MacKinnon and Burt (1985a–c) in Canada, who examined bowfin from Hay Bay of Lake Ontario in Canada.

10. *Testudotaenia testudo* (Magath, 1924) Freze, 1965 – accidental record

Record: USA (Tennessee).

Reference: de Chambrier et al. (2009).

Remarks

de Chambrier et al. (2009) found this parasite of eastern spiny soft-shell turtle, *Apalone spinifer* (Le Sueur) (Testudines: Trionychidae), in two of four bowfin from Reelfoot Lake, Tennessee, USA, examined in June 2002. These authors assumed that the presence of this parasite of turtles in *A. calva* represented an isolated local capture phenomenon, as both hosts were collected in the same place and habitat. Both are opportunist feeders and have a similar diet consisting of fishes, frogs, crayfishes, insects, and shrimps (de Chambrier et al., 2009).

11. ‘New species’ 1 of de Chambrier et al. (2009).

Record: Reelfoot Lake, Tennessee, USA.

Remarks

de Chambrier et al. (2009) found a probably new, adult proteocephalidean cestode morphologically similar to members of the polyphyletic genus *Proteocephalus* in bowfin from Reelfoot Lake on 30 June 2002 (voucher MHNG-PLAT-0035548). Based on its 28S rDNA sequences (FM956088), this tapeworm may represent a new species (de Chambrier et al., 2009). The morphological description of this putative new species was intended in a separate publication, but it has not yet been published because of limited material suitable for morphological description (A. de Chambrier – pers. comm.).

#### 4. Discussion

Parasites of freshwater fishes in North America have not been studied intensively for the past several decades (Scholz and Choudhury, 2014). These include tapeworms, which are mainly represented by species of three cestode orders, namely the Caryophyllidea (predominantly parasites of catostomids, which represent as many as 57 of the total of 102 North American species of fish cestodes), followed by the freshwater Onchoprotocephalidea (Proteocephalidae – 27 spp.) and the Bothriocephalidea (11 spp.) (Scholz and Kuchta, 2017), with additional species detected using molecular systematics (Brabec et al., 2015) and integrative taxonomy (Choudhury and Scholz, 2020). *Laruella* is the fifth genus of proteocephalids parasitising freshwater fishes in the Nearctic region, together with *Proteocephalus* (19 spp., including the recently described *P. culaeae* Scholz, Choudhury & Nelson, 2020), *Corallotaenia* Freze, 1965 (3 spp.), *Megathylacoides* Jones, Kerley & Sneed, 1956 (5 spp.), and *Essexiella* Scholz, de Chambrier, Mariaux & Kuchta, 2011 (monotypic).

*Laruella perplexa* has been reported from a broad spectrum of definitive hosts from different fish families and orders. However, it seems that it is a specific parasite of *A. calva*, whereas other hosts, usually predatory fishes, represent only accidental or postcyclic hosts, in which the prevalence and intensity of infection are very low, especially when compared with the high infection rate of *L. perplexa* in bowfin.

Eight species of tapeworms were listed from bowfin by Hoffman (1999) and Gibson et al. (2005), and de Chambrier et al. (2009) found adults of as many as five proteocephalid species, including three species not reported in previous surveys (*Megathylacoides giganteum*,

*Testudotaenia testudo* and a putative new species). However, a critical review of the literature, together with results of the authors’ own examination of bowfin from three U.S. states, made it possible to reduce the list of true (actual, i.e., non-accidental) cestode parasites of *A. calva* to only three: *L. perplexa* and two species of *Haplobothrium*, even though the validity of *H. bistrobilae* is doubtful. In contrast, de Chambrier et al. (2009), using molecular data, were able to identify a putative new proteocephalid in bowfin from Tennessee. It is possible, if not likely, that future studies of this ancient and fascinating relict in drainages across its range will discover a greater diversity of bowfin tapeworms than is currently known. The high number of apparently non-specific tapeworms (and possibly other parasites) reported from bowfin is related to the fact that it is a top predator that also consumes other fish species and becomes a postcyclic or accidental host. Similarly, pike (*Esox* spp.) are infected with their specific parasite, *Proteocephalus pinguis* La Rue, 1911, but also serve as postcyclic hosts of several species of the *Proteocephalus*-aggregate typical of other fish, such as bass, perch, and whitefish, namely *P. fluviatilis* Bangham, 1925, *P. pearsei* La Rue, 1919 and *P. longicollis* (Zeder, 1800) (see Scholz et al., 2021).

#### Declaration of competing interest

There is no conflict of interest in the submitted manuscript entitled “A young parasite in an old fish host: A new genus for proteocephalid tapeworms (Cestoda) of bowfin (*Amia calva*) (Holostei: Amiiiformes), and a revised list of its cestodes.”

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