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ORIGINAL RESEARCH ARTICLE

Diagnosis of helminth parasites in tissue sections of freshwater fish from Wisconsin, USA

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Abstract. Histological sections of 6 species of parasites infecting 11 species of freshwater fishes and 2 species of turtles from Wisconsin were studied and described. The parasites included metacercariae of *Ornithodiplostomum ptychocheilus* (Faust, 1913) and *Posthodiplostomum minimum* (MacCallum, 1912) (Trematoda), plerocercoids of *Proteocephalus ambloplitis* (Leidy, 1887) (Cestoda), parenteral forms of *Pomphorhynchus bulbocolli* Linkins in Van Cleave, 1919 cysts (Acanthocephala), and 2 species of leeches: *Myzobdella lugubris* Leidy, 1851 and *Placobdella ornata* (Verrill, 1872) (Hirudinea). The hosts included bowfin *Amia calva* Linn. (Amiidae), bluegill *Lepomis macrochirus* Raf., green sunfish, *Lepomis cyanellus* Raf., pumpkinseed *Lepomis gibbosus* (Linn.), smallmouth *Microperus dolomieu* (Lacépède), largemouth bass *Micropterus salmoides* (Lacépède), rock bass *Ambloplitis rupestris* (Raf.) (Centrarchidae), carp *Cyprinus carpio* Linn. (Cyprinidae), lake chubsucker *Erimyzon sucetta* (Lacépède) (Catostomidae), brown bullhead *Ictalurus nebulosus* (LeSueur), channel catfish *Ictalurus punctatus* (Raf.) (Ictaluridae), and 2 species of turtles: midland painted turtle *Chrysemys picta marginata* Agassiz and Blanding turtle *Emydoidea blandingi* Holbrook in Silver and Tichigan lakes and associated channels, Wisconsin. Plerocercoids of *P. ambloplitis* were the most widely distributed among most host species in the present collection, and the matrix of all infected host tissues were documented also to represent the different stratification of host tissue. Organs infected included intestinal wall and mesenteries, ovaries, liver, and epidermal layer in the case of leeches.

Keywords: Immature helminths; Leeches; Fish tissues; Turtles; Wisconsin; Histological sections.

Diagnosticarea paraziților helminți în secțiuni de țesut ale peștilor de apă dulce din Wisconsin, SUA

Rezumat. Au fost studiate și descrise secțiuni histologice a 6 specii de paraziți care infectează 11 specii de pești de apă dulce și 2 specii de țestoase din Wisconsin. Paraziții au inclus metacercarii de *Ornithodiplostomum ptychocheilus* (Faust, 1913) și *Posthodiplostomum minimum* (MacCallum, 1912) (Trematoda), plerocercoizii de

Proteocephalus ambloplitis (Leidy, 1887) (Cestoda, bulbi de Clemente, 1887) (Cestoda, 1912), bulbi de Pomphorenteral, 1887. Acanthocephala), și 2 specii de lipitori: *Myzobdella lugubris* Leidy, 1851 și *Placobdella ornata* (Verrill, 1872) (Hirudinea). Gazdele au inclus bowfin *Amia calva* Linn. (Amiidae), bluegill *Lepomis macrochirus* Raf., pește-soare verde, *Lepomis cyanellus* Raf., semințe de dovleac *Lepomis gibbosus* (Linn.), gura mică *Microperus dolomieu* (Lacépède), chin cu gura mare *Micropterus salmoides* (Lacépède), chin de stâncă *Ambloplitis rupestris* (Raf.) Centrarchidae), crap *Cyprinus carpio* Linn. (Cyprinidae), țestoasa de lac *Erimyzon sucetta* (Lacépède) (Catostomidae), țestoasa brună *Ictalurus nebulosus* (LeSueur), somnul de canal *Ictalurus punctatus* (Raf.) (Ictaluridae) și 2 specii de țestoase: broasca țestoasă pictată din Midland *Chrysemys picta marginata* Agassiz și broasca țestoasă *Emydoidea blandingi* Holbrook în lacurile Silver și Tichigan și canalele asociate, Wisconsin. Plerocercoizii de *P. ambloplitis* au fost cei mai larg răspândiți printre majoritatea speciilor gazdă din colecția prezentă, iar matricea tuturor țesuturilor gazdă infectate a fost documentată, de asemenea, pentru a reprezenta stratificarea diferită a țesutului gazdă. Organele infectate au inclus peretele intestinal și mezenterele, ovarele, ficatul și stratul epidermic în cazul lipitorilor.

Cuvinte cheie: Helminți imaturi; Lipitori; Țesuturi de pește; Țestoase; Wisconsin; Secțiuni histologice.

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Introduction

Few publications dealt with the identification of metazoan parasites in host tissue sections of fish or aquatic animals. Much of the earlier reports are sporadic in nature, dealt with small groups of parasites or hosts, and are usually found in the larger texts on fish diseases and disorders, ex., Woo (1995). The 3 most comprehensive such publications were provided by Chitwood and Lichtenfels (1972), Gardiner and Poynton (1999), and Bruno et al. (2006). Chitwood and Lichtenfels (1972, p. 407) designed their report "to assist veterinarians and medical pathologists in the identification of metazoan parasites found in lesions, cysts, and tissues of vertebrates, mostly of mammals". They covered representatives of Arthropoda, Pentastomida, Trematoda, Cestoda, Acanthocephala, Nematomorpha, and Nematoda and provided a key for the identification to phyla of parasitic Metazoa in sections. Their work was in black/while and did not address helminths of fish or aquatic animals.

Gardiner and Poynton (1999) produced an Atlas divided into sections describing the general appearance of metazoan parasites, with 270 color photos and a key separating the basic metazoan parasite groups. The discussion addresses 6 parasitic groups: nematodes, acanthocephalans, trematodes, cestodes, arthropods, and pentastomes. Bruno et al. (2006) provided a guide to the identification of fish protozoan and metazoan parasites in stained tissue sections prepared for light microscopy with emphasis on protozoans such as Myxosporidia and small metazoan parasites likely to be missed during the gross examination. Their guide included very few colored images of helminths. In addition, Nowak et al. (2002) briefly reviewed the characteristics of representative protozoan and metazoan parasites in sections of fish tissue and provided a simplified key to the major groups of parasites.

Our contributions in this series are based on materials from collections of helminth parasites of many species of freshwater fish from Wisconsin. Our treatment emphasizes aspects of histopathology not before addressed by earlier reports, as comprehensive as some may have been. The descriptive and ecological work has already been published, but tissue sections have been left untouched since. Relevant publications from Wisconsin include Amin (1981) on leeches, Amin (1982) on larval trematodes (Strigeoidea), Amin (1987) on ecology and host relationships of *Pomphorhynchus bulbocolli*, Amin (1990a) on ecology and pathology of *Proteocephalus ambloplitis*, Amin (1990b) on *Proteocephalus* in *Esox* and other fish species, and Amin and Boarini (1992) on plerocercoids of *P. ambloplitis.*

Materials and methods

Collections

Collections of fish were made annually using electrofishing or seins in the spring, summer, and autumn of 1977 to 1981 from 2 eutrophic lakes in southeastern Wisconsin: Silver Lake is a 200-ha land-locked lake in Kenosha County (42°32′58″N 88°10′9″W), and Tichigan Lake is a 107 ha lake in Racine County on the Fox River (42°49′44″N 88°11′51″W), a tributary of the Mississippi River.

In the first year, 1,736 fishes representing 32 species and 10 families (Amiidae, 1 fish species; Catostomidae 7; Centrarchidae, 9; Cyprinidae, 2; Esocisae, 2; Ictaluridae, 4; Lepisosteidae, 1; Percidae, 2; Salmonidae, 2; Serranidae, 2) were captured by electro-shocking. An additional 657 fishes representing 6 species (Cyprinidae, 4; Gastrosteidae, 1; Umbridae, 1) were seined or minnow trapped in a large channel draining the western swamps of Tichigan Lake.

Leeches were recovered from 2 species in Tichigan Lake, and others were recovered from minnow traps and from the underside of a canoe used in the Tichigan Lake drainage channel. Minnow traps were baited with cat food and canned sardines. Comparable numbers of fish were captured and examined for parasites during each of the next 4 years. Fish were brought to the lab, and most were immediately dissected, or occasionally kept on ice for second-day dissections by a team of 4 persons.

Methods

The intestinal tract, body cavity organs (liver, spleen, heart, gonads, peritoneum, mesenteries), and external surfaces were carefully examined for parasites using dissecting scopes. Recovered parasites were refrigerated in distilled water for a day or two then fixed in cold 70% ethanol before processing for microscopy, identification, and subsequent studies. Internal organs clearly exhibiting parasitic infections were targeted for histological studies and were separately fixed in buffered formalin.

Histology

Infected host tissue was fixed in 10% buffered formalin for 24 hours then washed and stored in 70% ethanol. After dehydration and paraffin blocking, the specimens were processed using standard methods comparable to those of Keirnan (2002). The paraffin blocked tissue was sectioned at 10 microns, placed on glass slides, and stained with hematoxylin and eosin (HE). The prepared glass slides were viewed with an LSM laser (Carl Zeiss, Thornwood, New York) equipped compound light microscope with representative pictures taken at varying magnifications with a digital camera. The histopathological sections (figures 1-60) were selected from a much larger collection of sections on 250 glass slides in OMA's collection.

Results and discussion

The collection of histological sections made between 1977 and 1981 is studied. Sectioned parasites interfacing with pathological host tissue included metacercariae of *Posthodiplostomum minimum* (MacCallum, 1921) Dubois, 1936, and metacercariae of *Ornithodiplostomum ptychocheilus* (Faust, 1913) Dubois, 1936 (Trematoda), plerocercoids of *Proteocephalus ambloplitis* (Leidy, 1887) (Cestoda), parenteral forms of *Pomphorhynchus bulbocolli* Linkins in Van Cleave, 1919 cysts (Acanthocephala), and 2 species of leeches: *Myzobdella lugubris* Leidy, 1851 and *Placobdella ornata* (Verrill, 1872) (Hirudinea). The following is a systematic documentation of these parasites in their respective host tissues. Some parasite sections are presented from more than 1 host species in separate plates to document their variations and to emphasize the different host species' tissue organization in which these parasites are embedded.

Trematoda

Metacercariae of *Posthodiplostomum minimum* (MacCallum, 1912) Dubois, 1936

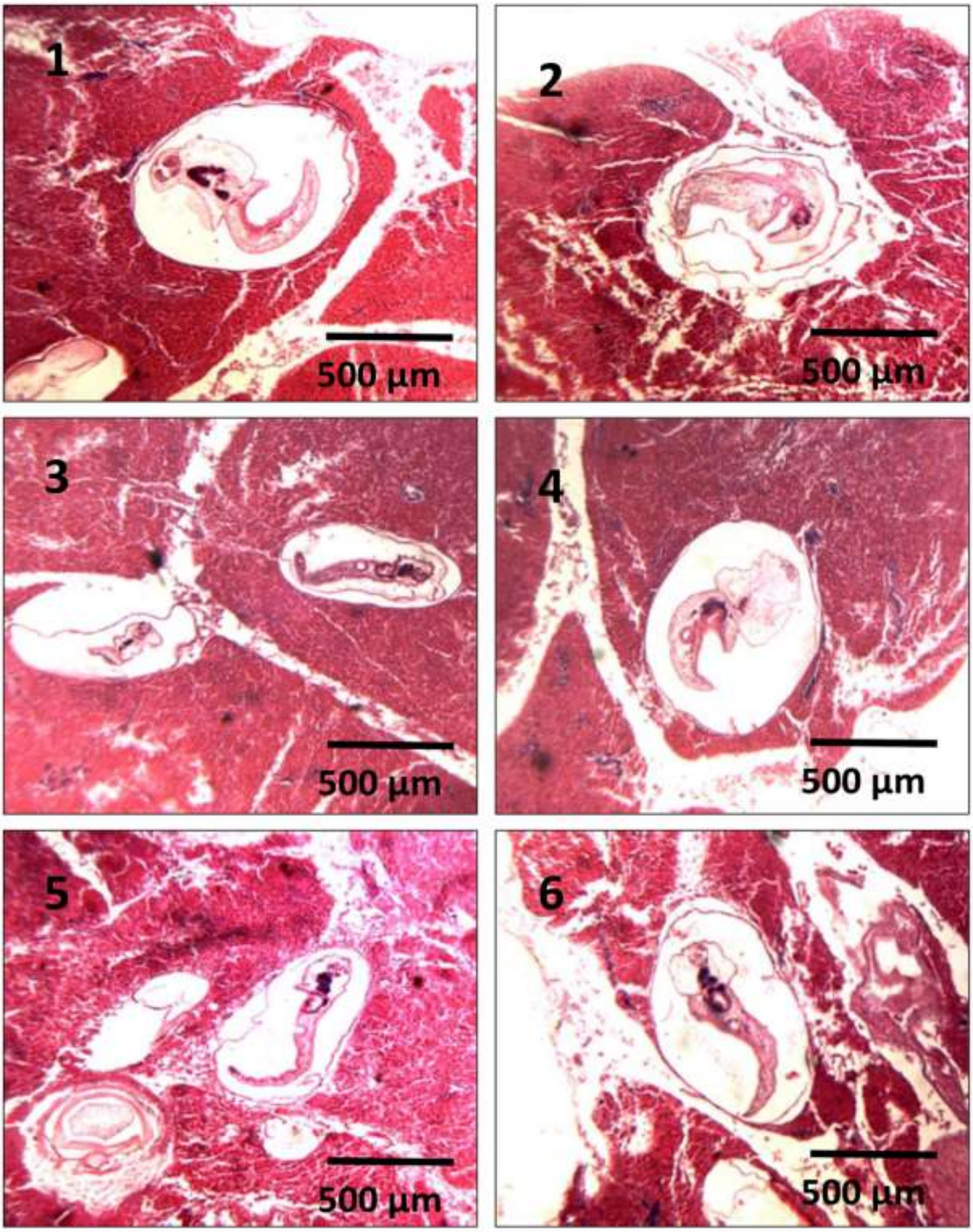
Metacercariae of *P. minimum* were found in 11 species of fish belonging to 5 families (Cyrinidae, 1 fish species; Catostomidae, 1; Ictaluridae, 1; Centrarchidae, 7; Percidae, 1) from both Silver and Tichigan lakes with *L. macrochirus* being the most commonly infected host (Amin, 1982). Hoffman (1999), and many authors quoted therein, reported many aspects of its wide host distribution and pathology from Alabama, Arkansas, British Columbia, California, Colorado, Georgia, Iowa, Kansas, Kentucky, Minnesota, North Dakota, Oklahoma, Ontario, Oregon, Pennsylvania, South Dakota, St. Lawerence watershed, Texas, Virginia, West Virginia, and Wisconsin. Special reports of the chemical nature of the metacercarial cysts include those by Bogitsh, e.g., Bogitsh (1962). An excellent review of North American studies of *P. minimum* can be found in Spall and Summerfelt (1969) who reported as many as 2,041 metacercariae in one bluegill, *Lepomis macrochirus* from an Oklahoma reservoir. We found metacercariae in all visceral organs mostly in the liver, ovaries, intestinal surface, and integument, and note histopathology due to compression or occulation of vital organs as noted by Heckmann and Inchausty (2008).

Eighty-five cysts dissected from bluegills from Tichigan Lake measured 890 (630-1,148) long by 536 (280-840) wide and 85 metacercariae 878 (546-1,484) by 350 (210-560). These measurements were comparable to those Heckmann (2006) reported for metacercariae of largemouth bass *Micropterus salmoides* in Florida. No attempt was made to distinguish between *P. m. minimum* and *P. m. centrarchi* Hoffman, 1958 (Amin, 1982). Many encysted metacercariae sectioned from the liver of bluegill collected in Silver Lake in April 1978 (figures 1-6) show variations in body form and suckers as reported by Ferguson (1937), Palmieri (1975). Metacercariae reported by Heckmann's (2006, figures 1, 2) from *M. salmoides* and by Heckmann and Inchausty's (2008, figure 9) from the viscera of *L. macochirus* were similar to ours (figures 1-6) from the liver of *L. macrochirus*. In one study, “the heart, liver, and kidneys contained approximately 79% of the total metacercariae in bluegill (*Lepomis macrochirus*)” (Heckmann and Inchausty, 2008, p. 36).

The double-walled and occasionally triple-walled cysts were also observed in our sections (figure 2) as reported by other authors and immunologically confirmed by Crider and Meade (1975). Note the compact, dense liver tissue in which the cysts are embedded (figures 1-6). “The occurrence of numerous metacercariae in visceral organs suggest deleterious effects on the well being of the host and implicates *P. minimum* as a cause of mortality or morbidity” Heckmann and Inchausty (2008, p. 37).

Mortality due to stress and trauma from penetration of the cercariae has been observed in the laboratory following exposure of suitable fish host to large numbers of cercariae (Bedinger and Meade, 1967). “There is a zoonotic potential due to some metacercariae in (grubby) fish capable of infecting humans” Heckmann and Inchausty (2008, p. 30).

Specimens: 14 slides in USNM Helm. Coll. Nos. 76661-76669 and 11 slides in MPM (Milwaukee Public Museum) Coll. No. IZ365g. Histological sections (2 slides) in HWML Coll. (Lincoln, Nebraska) No. 216734.



Figures 1-6. Morphological variations in the shape of metacercariae of *Posthodiplostomum minimum* from the liver of *Lepomis macrochirus* collected from Silver Lake in April, 1978. Metacercariae of this very common trematode are also found in the spleen, kidneys, mesenteries, sinus venosus, heart, and ovaries. Cercarial penetration will elicit hemorrhage at the site of invasion, congestion of adjacent venules, local edema, and an aggregation of phagocytic leucocytes at the point of entry. Their pathogenicity is usually due to compression or occlusion of vital organs. Note the double-triple membranes of the cyst wall (Fig. 2), the erosion of hepatic tissue adjacent to cysts (Figs. 2, 6), disruption of outer cyst wall and vacuolation necrosis (Fig. 2), and granulomatous hepatitis (Figs. 1, 3, 4). The large muscular oral sucker and acetabulum in the anterior body are clearly visible in almost all figures. When present in the integument, they can disfigure the fish and render them unsightly and unpalatable for purchase and consumption.

Metacercariae of *Ornithodiplostomum ptychocheilus* (Faust, 1913) Dubois, 1936

Metacercariae of *O. ptychocheilus* were found in 10 species of fish belonging to 4 families (Cyrinidae, 1 fish species; Ictaluridae, 1; Centrarchidae, 7; Percidae, 1) from both Silver and Tichigan lakes, with centrarchids being the most heavily infected hosts. Eighty-five cysts of *O. ptychocheilus* from bluegills from Tichigan Lake measured 843 (672-1,092) long by 468 (350-658) wide and 85 metacercariae 489 (350-616) by 273 (154-378) (Amin, 1982). Many encysted metacercariae sectioned from the liver of bluegill collected in Silver Lake in April, 1978 (figures 7-12) show variations in body form and suckers as reported by Hughes and Piszczek (1928) and as depicted in figures 3-6 in Amin (1982, p. 211) for metacercariae from the liver and mesenteries of *L. macrochirus*. In the USA, Hoffman (1999) and other authors quoted therein reported metacercariae of *O. ptychocheilus* from other sites including the body cavity, brain, and eyes in many species of fish, especially cyprinids, from Alberta, Arkansas, Arizona, Colorado, Minnesota, Montana, North Dakota, Ohio, Ontario, South Carolina, and Texas.

Specimens: 14 slides in USNM Helm. Coll. Nos. 76670-76675, and MPM Cool. No. IZ365h. Histological sections (2 slides) in HWML Coll No. 216734

Acanthocephala

Pomphorhynchus bulbocolli Linkins in Van Cleave, 1919 cysts

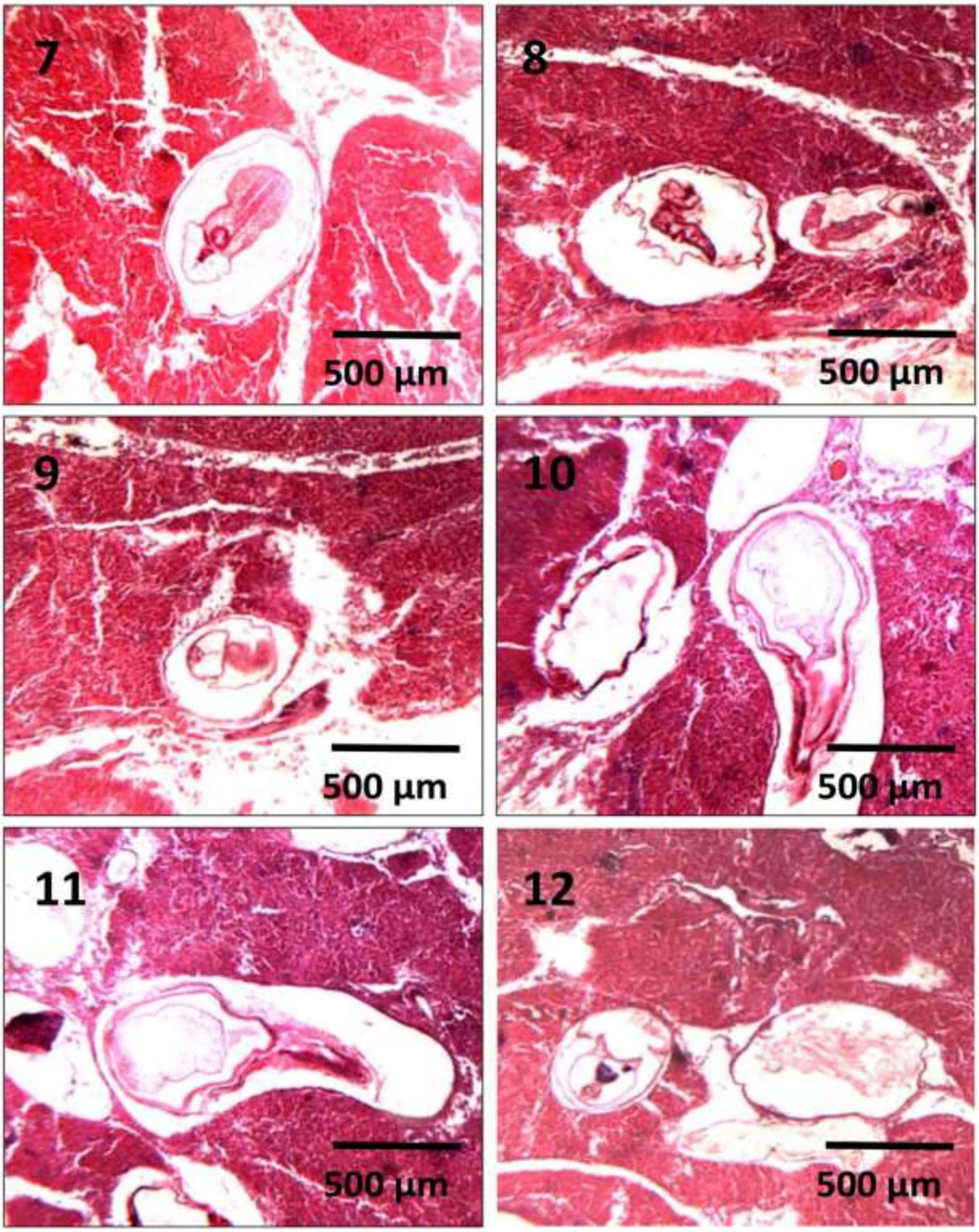
Pomphorhynchus bulbocolli is widely distributed in North American freshwater fishes and has been reported from 81 host species up till about 45 years ago (Samuel et al., 1976), including 31 species in Wisconsin (Fischthal, 1953). In the USA, Hoffman (1999) and other authors quoted therein, reported 39 and 11 genera of definitive and paratenic species of fish hosts of adults and larvae of *P. bulbocolli*, respectively, from 32 states, Canadian provinces, and Mexico. We have collected extra-intestinal forms from a large number of fish species from both Silver and Tichigan lakes. In a sample of 572 fishes examined from Tichigan Lake alone in 1977-1978, 13 species of paratenic hosts were found infected with parenteric forms. The intestinal mesenteries were most heavily and frequently infected (in 126 fishes), followed by 24 (in liver), 23 (in body cavity), 10 (in ovary), and 3 (in spleen) (Amin, 1987).

The serosal surface of the intestine, particularly of carp, was often studded with encysted larvae, mostly young cystacanths. These forms would have been acquired through the ingestion of the infected amphipod intermediate host but dead-ended in situ. *Cyprinus carpio* appears to be the most important paratenic host among these fish, irrespective of season or fish size.

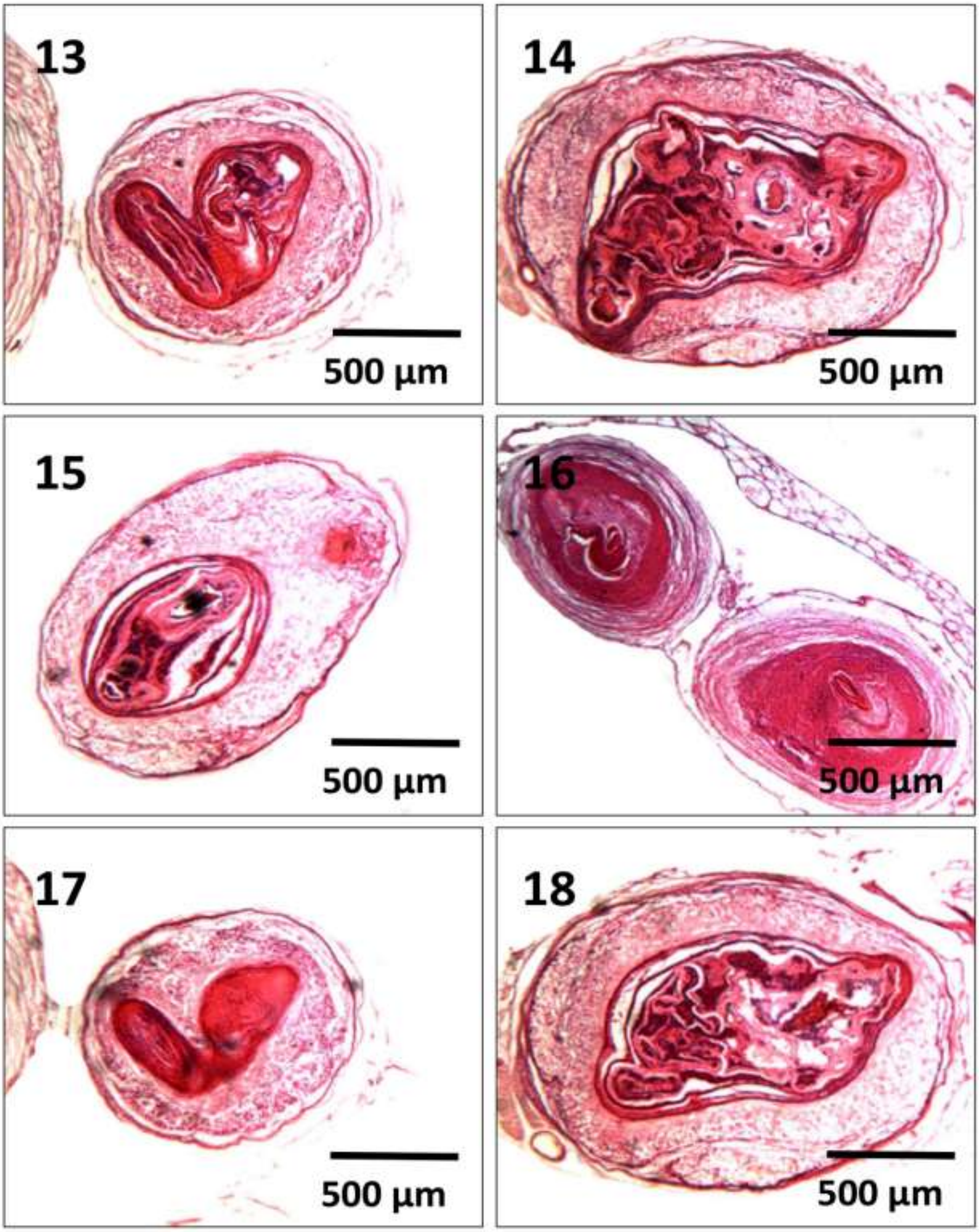
Encysted cystacanths appeared well defined in preparations from carp of all sizes (table III in Amin, 1987), indicating long-term survival. However, similar cysts revealing poorly defined, unidentifiable, or simply homogenized components were also encountered. Considering the prevalence of paratenic hosts, transfer from them to definitive hosts may represent an important amplification cycle in the life history of *P. bulbocolli* (Amin, 1987).

Cysts that do not transfer become dead-end infections. We include images of cysts from the intestinal mesenteries of channel catfish *Ictalurus punctatus* (Raf.) (figures 13-18) and from the body cavity of the more heavily infected carp *Cyprinus carpio* Linn. (figures 19-24) collected in the spring of 1978 in Tichigan Lake. Note differences in the matrix of host tissue in which the cysts are embedded.

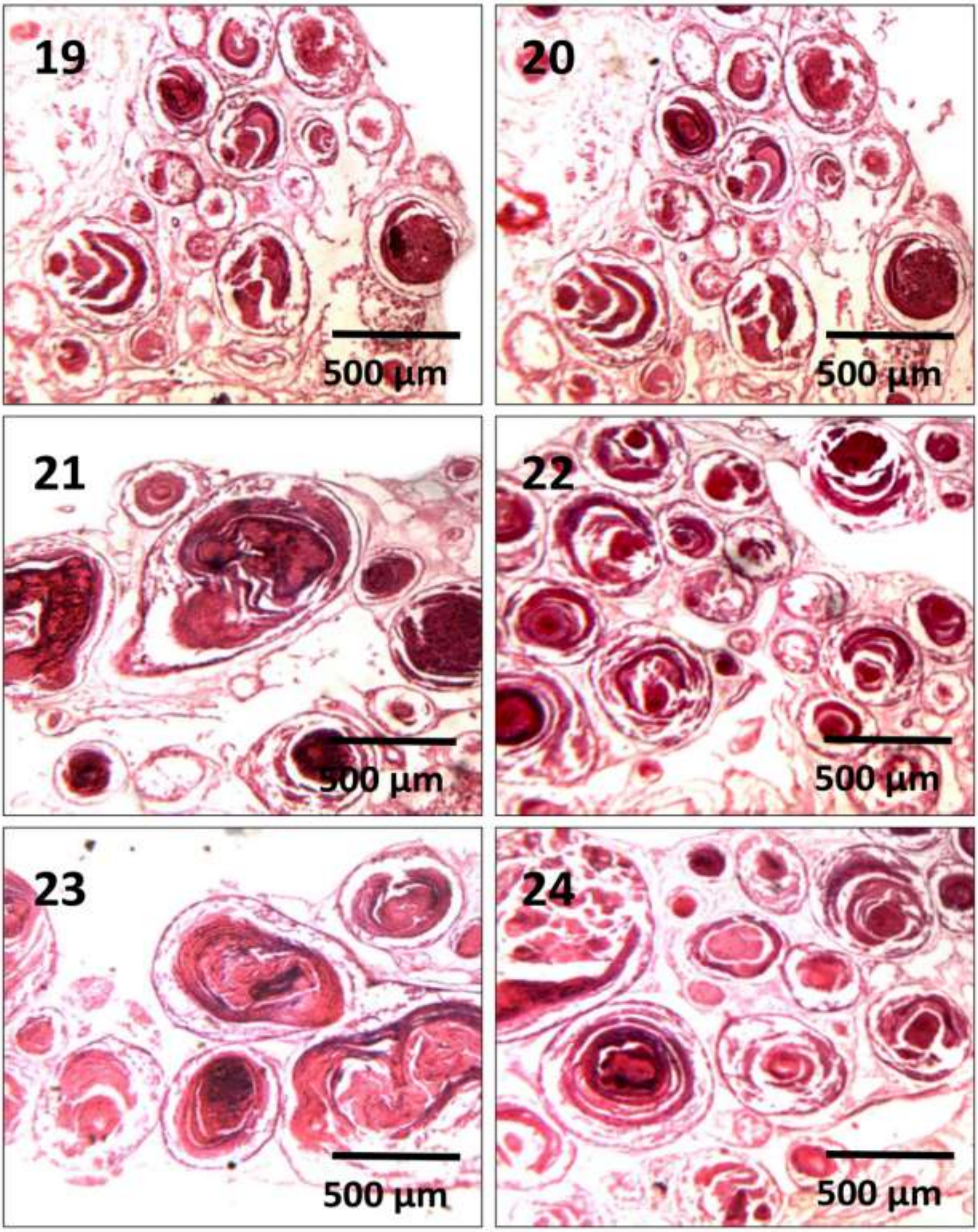
Specimens: USNM Helm. Coll. Nos. 23139-23150. HWML (Harold W. Manter Lab., Lincoln, Nebraska) Coll. Nos. 23139-23150 (body cavity encapsulated larvae) from 11 fish species in both lakes) and Coll. Nos. 216735-216738 (histological sections on 4 slides from *I. punctatus C. carpio*, *E. sucetta*, and *M. dolomieu*.



Figures 7-12. Morphological variations in the shape metacercariae of *Ornithodiplostomum ptychocheilus* from the liver of *Lepomis macrochirus* collected from Silver Lake in April 1978. The most typical form is depicted in Fig. 7 characterized by a near parallel outline and the smaller space occupied within the cyst wall, which distinguish it from the metacercariae of *P. minimum*. Note granulomatous hepatitis (Figs. 8, 9, 10, 11) and erosion of hepatic tissue surrounding cysts (Figs. 9-12). The pathogenicity of metacercariae of this trematode has not been as frequently studied as that of *P. minimum* but appears comparable.



Figures 13-18. Parenteric cysts of *Pomphorhynchus bulbocolli* from the mesenteries of *Ictalurus punctatus* collected from Tichigan Lake in the spring of 1978. The fish serves as second intermediate host in the indirect life cycle of acanthocephalans where the proboscis is inverted (Figs. 13, 15, 17). Note the various parasite forms and the encapsulating cyst walls. The outer collagenous cyst walls are of host origin.



Figures 19-24. Parenteric cysts of *Pomphorhynchus bulbocolli* from the mesenteries of *Cyprinus carpio* were collected from Tichigan Lake in the spring of 1978. Cysts can also be found in the liver and spleen where they can be highly pathogenic especially when present in large numbers as in this case. Note the various parasite forms, the encapsulating cyst walls, and the considerably heavier infection compared to that in *I. punctatus*

Cestoda

Proteocephalus ambloplitis (Leidy, 1887) plerocercoids

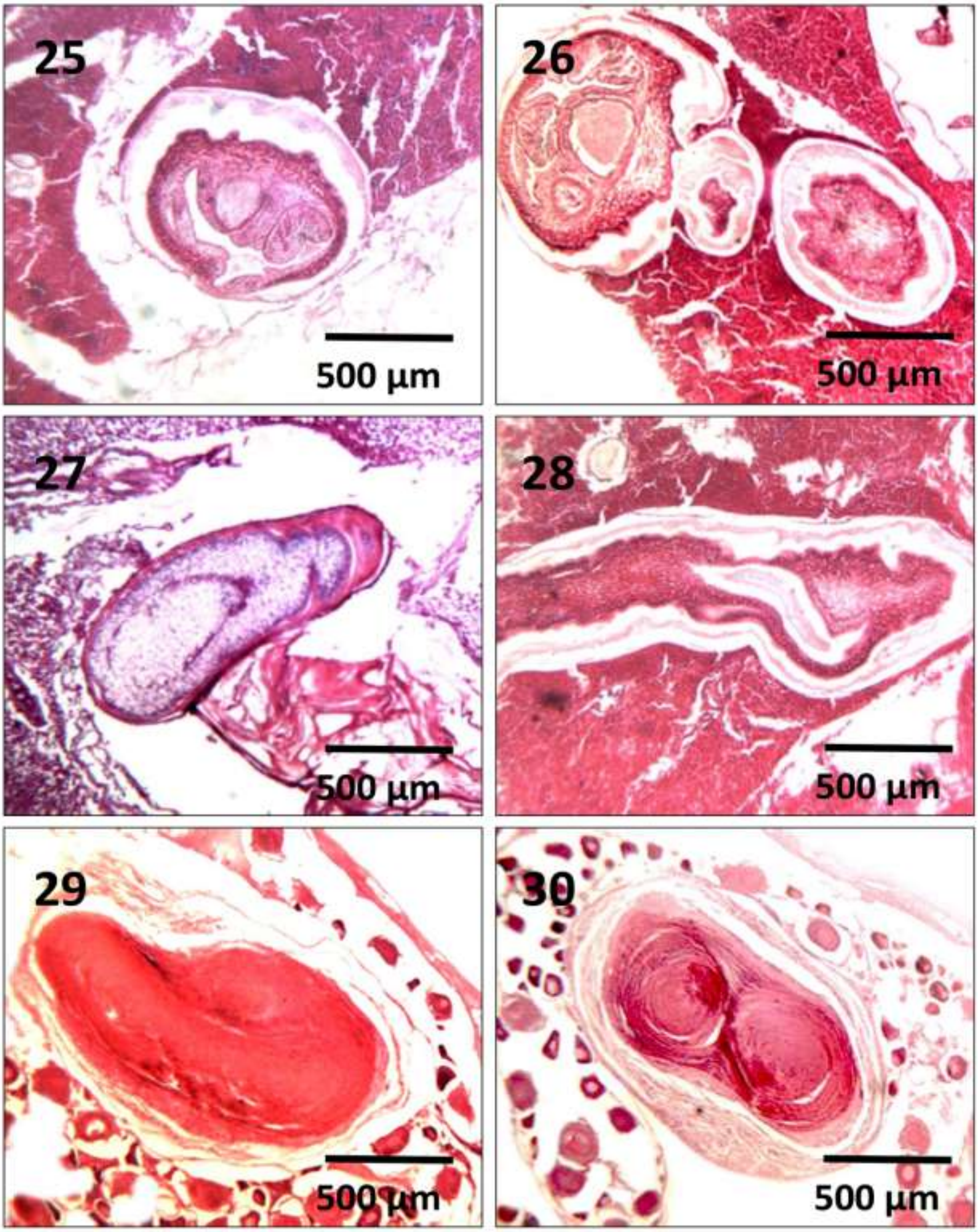
The bass tapeworm has been reported throughout the United States and southern Canada by many authors. We have reported parenteric plerocersoids from 18 species of fish in 6 families (Amiidae, in 1 fish species; Catostomidae, 3; Centrarchidae, 7; Ictaluridae, 3; Lepisosteidae, 1; Percidae, 2) in Silver and Tichigan lakes between 1976 and 1979) (Amin, 1990a, b; Amin and Boarini, 1992; Amin and Cowen, 1990). Heaviest infection with plerocercoids was noted in *Amia Calva* Linn. and *Micropterus salmoides* (Lacépède) compared to *M. dolomieu* Lacépède, in both lakes during all seasons (Amin and Cowen, 1990). Comparable findings of *P. ambloplitis* plerocercoids heavily infecting the hepatic tissue of *M. dolomieu* from Lake Powel between Utah and Arizona were reported by Clothier and Heckmann (2002). Segments from the intestinal wall of individuals of *Lepomis gibbosus* (Linn.) and *ambloplites rupestris* (Raf.) were studded with as many as a few hundred to 1000 encysted plerocercoids (see images figures 1, 2 in Amin, 1990a). Prevalence and intensity of infections were considerably higher in the land-locked Silver Lake than in the river-connected Tichigan Lake, especially in the spring. Parenteric infections were most common in the mesenteries and the gonads than in the liver and spleen of infected fish (Amin, 1990a). Amin and Boarini (1992) classified parenteric stages into initial, middle, and terminal plerocercoids and depicted 10 morphological types (figures 1-10, p. 195), tracing their development in various body cavity cites. The first initial form is cystic in shape with only internal elements of the 4 scolex suckers. Subsequent forms begin to elongate, segment, and ultimately show the anterior displacement of the actively secretory accessory fifth sucker forward, which later on atrophies. All the stages are represented in our histological sections from various host species. Near terminal stages (figures 25-30) are represented from the liver and ovary of bluegills collected from Silver Lake in April 1978. Dense collagenous connective tissue encapsulated the migrating plerocercoids (figures 27-30) then became necrotic. Complete necrosis was observed in older infections. Our hepatic sections (figures 25-28) are comparable to those from the liver of *M. dolomieu* from Lake Powel reported by Clothier and Heckmann (2002, figures 4, 6, 7). Late initial and middle plerocercoids were represented in intestinal wall sections of 4 species of fish with distinct tissue matrixes. (1) from smallmouth bass, *Microperus dolomieu* (Lacépède) collected from in April 1978 in Tichigan Lake (figures 31-36). (2) from brown bullhead, *Ictalurus nebulosus* (LeSueur) collected in the spring of 1978 in Tichigan Lake (figures 37-42). (3) from green sunfish *Lepomis cyanellus* Raf., collected in the spring of 1978 in Tichigan Lake (figures 43-48). (4) from rock bass, *Ambloplitis rupestris* (Raf.) collected in April 1978 in Tichigan Lake (figures 49-54).

Specimens: Eight slides of histological sections from *M. dolomieu*, *I. nebulosus*, *L. cyanellus*, *Lepomis macrochirus*, *A. rupestris* HWML Coll. Nos. 216739-216744.

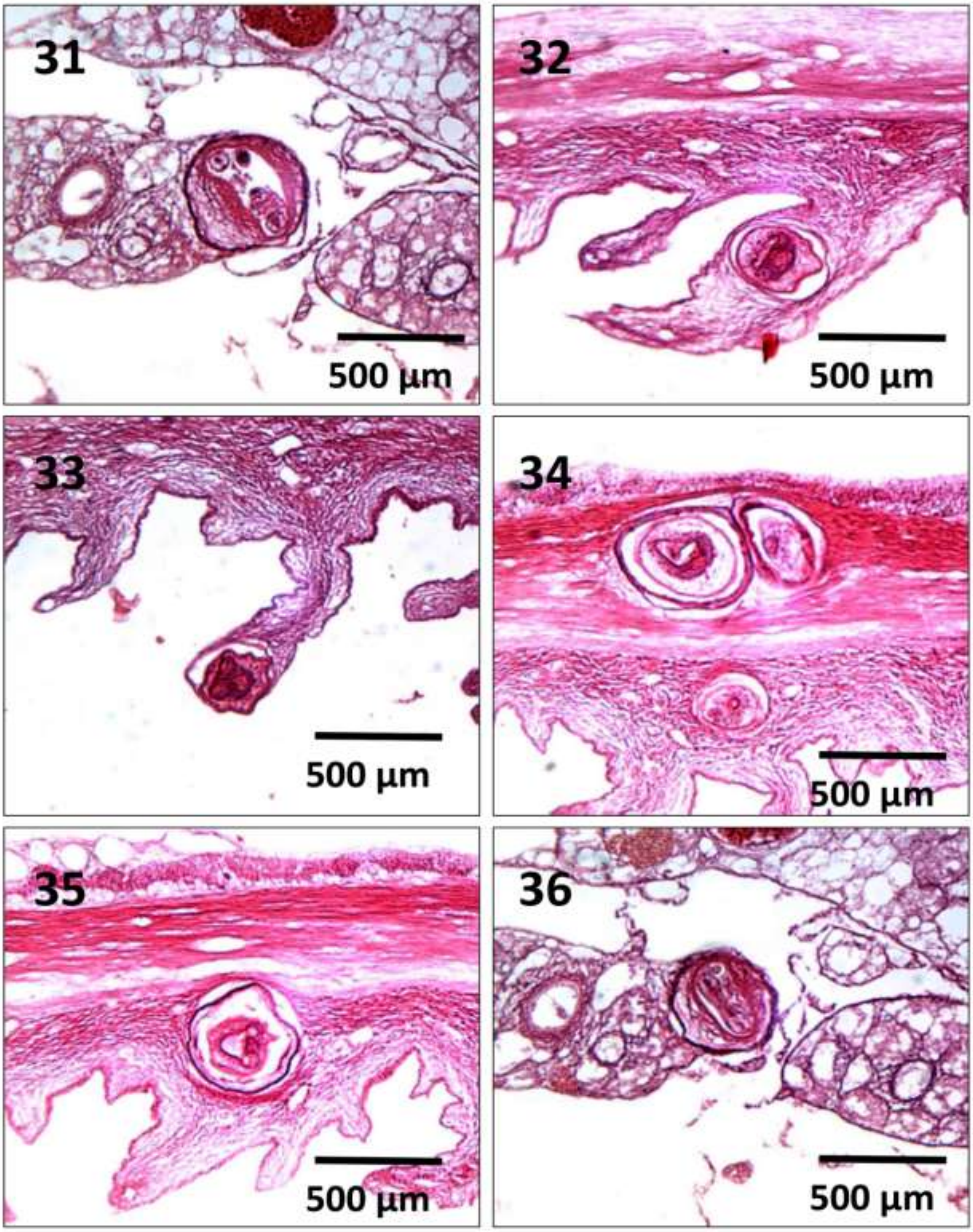
Hirudinea

Myzobdella lugubris Leidy, 1851

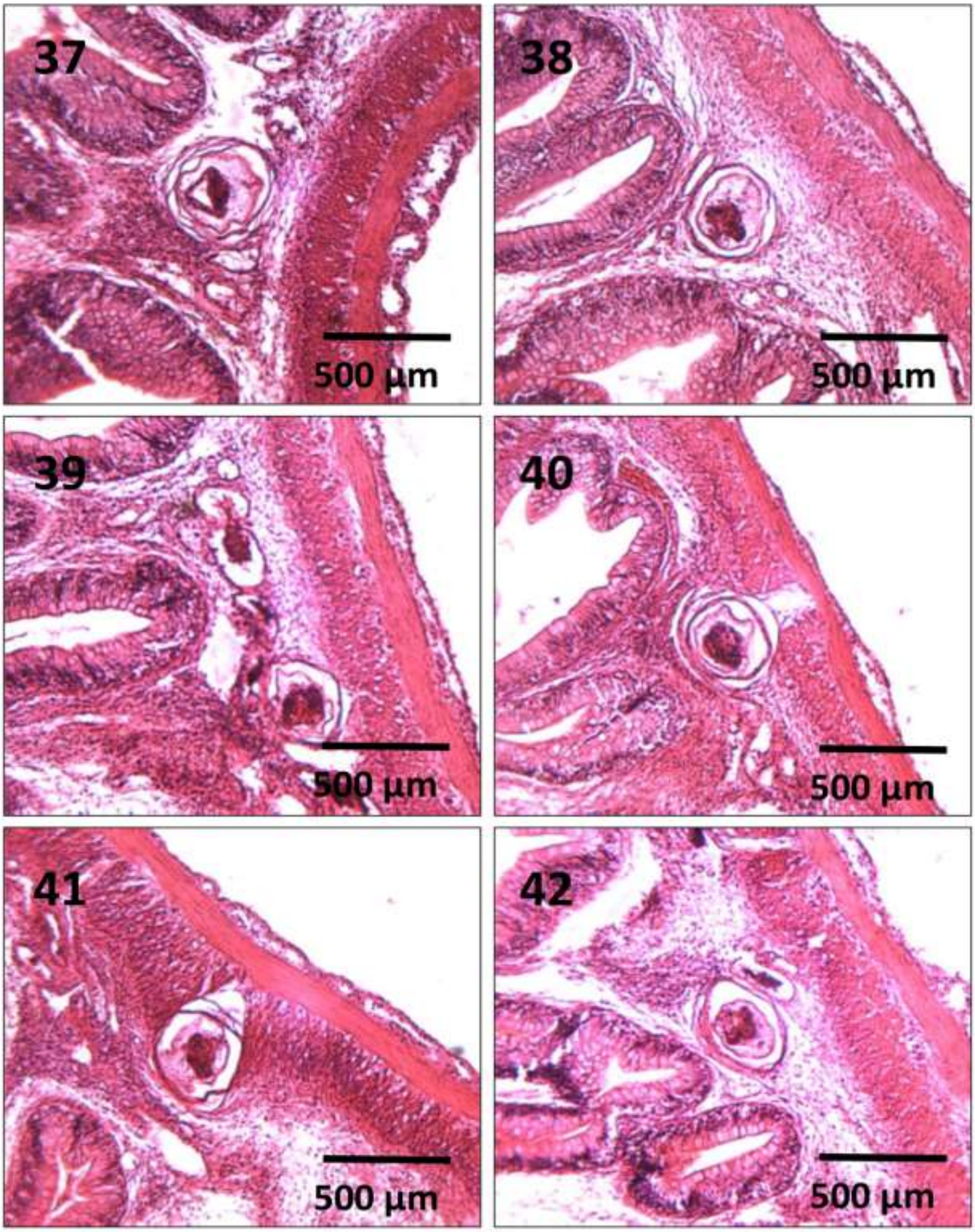
Myzobdella lugubris is widely distributed in North America except for Alaska and Northern Canada where "its color and size varies apparently widely with host and locality" (Hoffmann, 1999, p. 308). In Wisconsin, a total of 453 specimens of this leech were collected from 13 fish species in 4 families (Esocidae, 2 fish species; Cyprinidae, 1; Ictaluridae, 1; Centrarchidae, 9) from Silver and Tichigan lakes between 1976-1978. Centrarchids were most commonly infected on the fins, especially the pectorals, but channel catfish was most heavily infected, usually below the mouth as previously observed by Hoffman (1999) causing lesions that "often become infected with filamentous fungi" (p. 308). One catfish infected with 35 individuals of *M. lugubris* exhibited 150 older leech attachment sites (wells) on its tail (Amin, 1981).



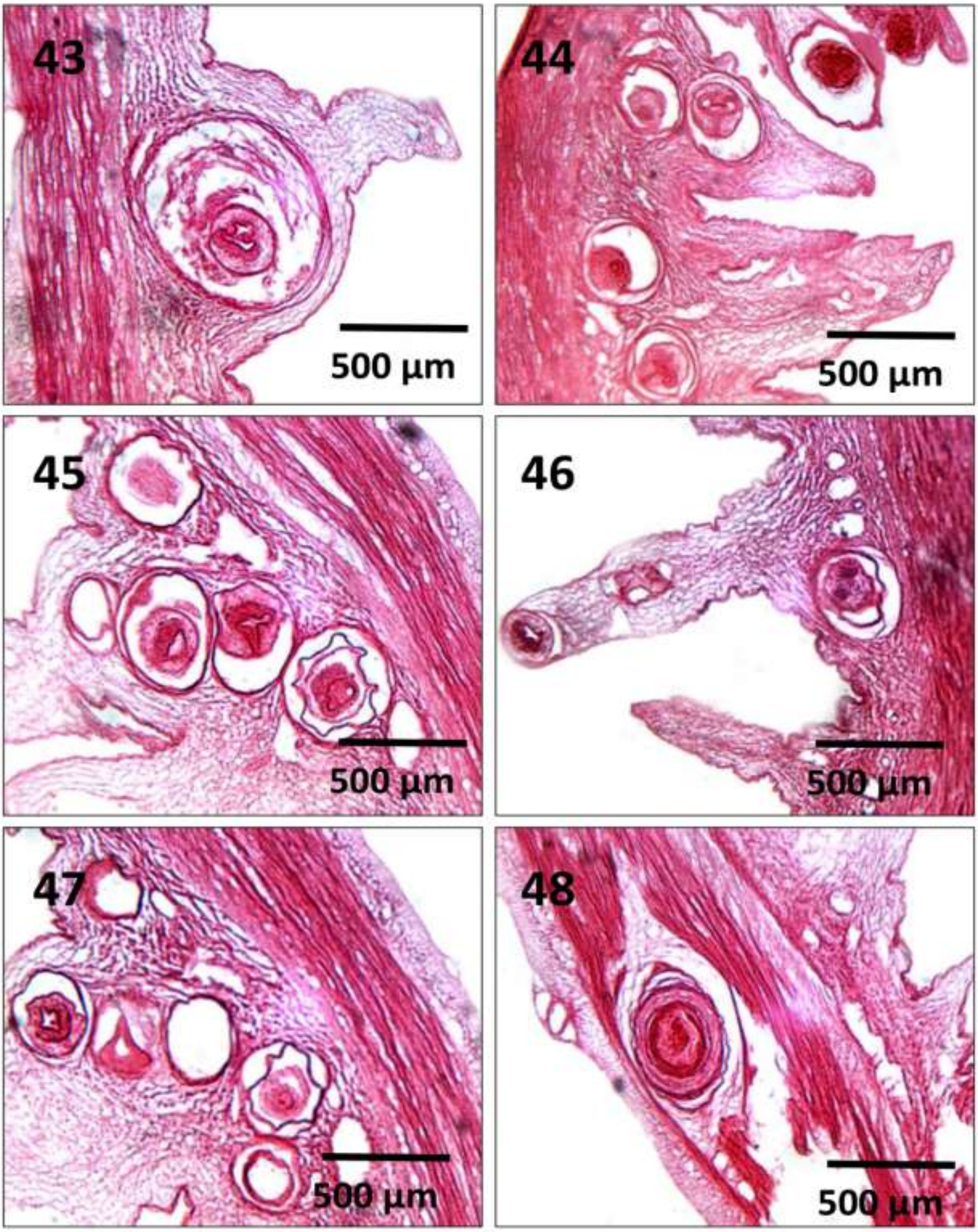
Figures 25-30. Collagen encysted middle plerocercoids of *Proteocephalus ambloplitis* from the liver of *Lepomis macrochirus* showing necrotic and hemorrhagic regions, collected in Silver Lake in April 1978 (Figs. 25-28), and from the ovary of the same host species and the same lake and season (Figs. 29-30). Note the well-developed scolex (Figs. 25, 26) and encapsulation, host tissue vacuolation, and leukocytosis (Fig. 27), hepatocytes and hepatic sinusoids (Figs. 26-28). The section in Fig. 26 shows the rudimentary sucker. Coiled elongated plerocercoid (Fig. 29) is cross-sectioned (Fig. 30) and encapsulated in a multi-layered collagen host cyst wall showing transverse banding pattern. The migration track of plerocercoids is evident in Fig. 28. Migrating plerocercoids can cause death when vital organs are severely injured. Migrating plerocercoids can penetrate the gut wall of bass and develop to the adult intestinal stage in the gut.



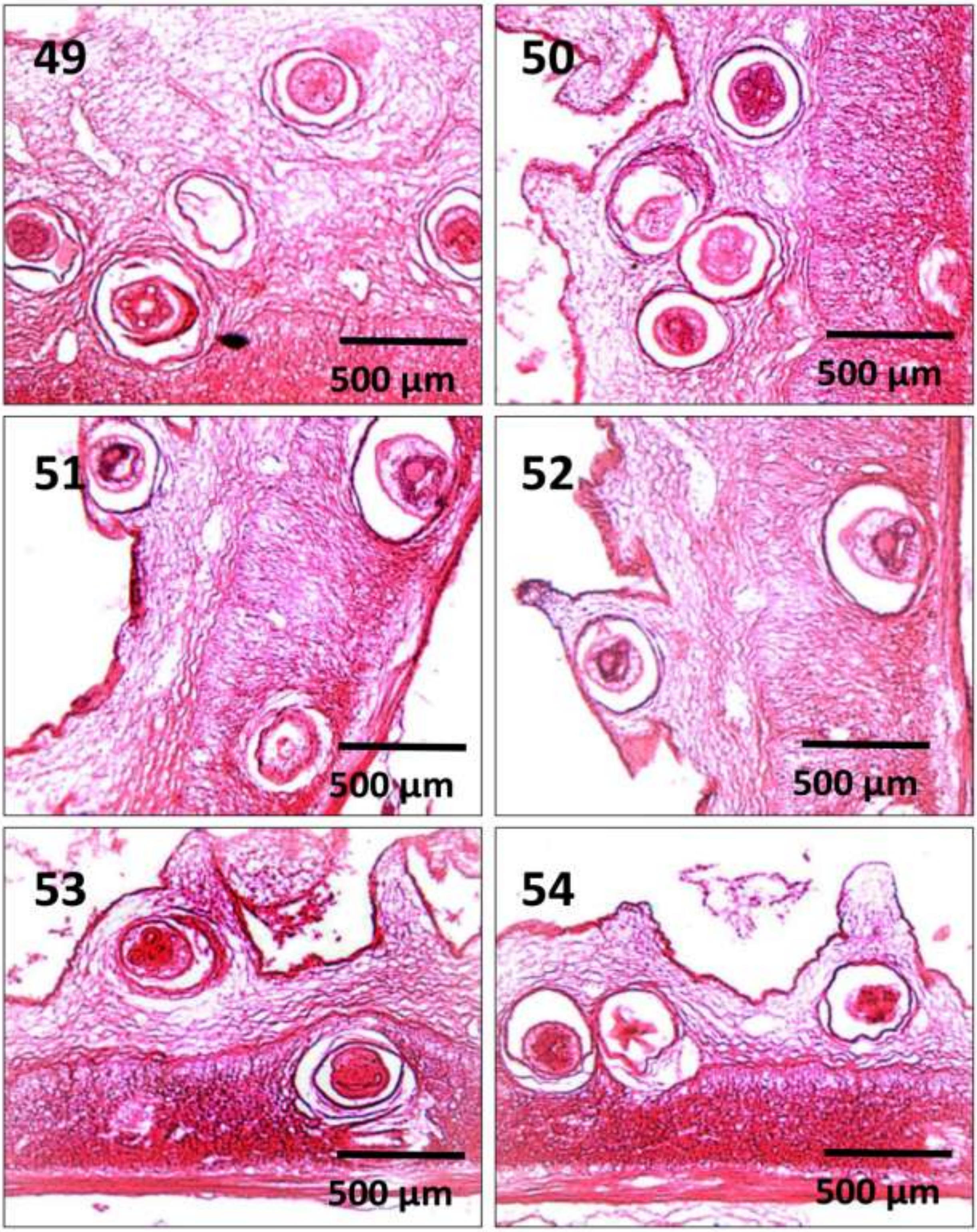
Figures 31-36. Initial plerocercoids of *Proteocephalus ambloplitis* from the intestinal wall of *Microperus dolomieu* were collected in April 1978 from Tichigan Lake. Different stages of development and organization of the scolex and suckers are shown in all cysts throughout the thickness of the gut wall from the villi (Fig. 33) to the serosa (Fig. 34), with at least 2 cysts walls evident (Figs. 34, 35). The outer cyst walls are of host origin and the inner cyst walls of parasite origin. There is vacuolation of host tissue immediately surrounding some cysts especially in the muscle layer (Fig. 34).



Figures 37-42. More developed Initial plerocercoids depicting the scolex and suckers of *Proteocephalus ambloplitis* from the intestinal wall of *Ictalurus nebulosus* collected in the spring of 1978 from Tichigan Lake. Note the denser and stronger stratification of the host gut wall perhaps affecting the lesser density of parasite invasion. Double cyst walls are seen in most sections. Plerocercoids migrating in the visceral cavity across the gut barrier may produce adhesions that are very damaging to the fish.



Figures 43-48. Well-developed scolexes of initial plerocercoids of *Proteocephalus ambloplitis* from the intestinal wall of *Lepomis cyanellus* collected in the spring of 1978 from Tichigan Lake. Many plerocercoids are featured in the gut wall, all of which have double cysts walls clearly compromising the mechanical function and elasticity of the gut wall. Some cysts have penetrated the muscular layer and developed more cyst walls (Fig. 48). The inflammatory reaction noted in most sections exerts mechanical pressure on the gut wall interfering with the digestive process.



Figures 49-54. Well-developed scolexes of initial plerocercoids of *Proteocephalus ambloplitis* from the intestinal wall of *Ambloplitis rupestris* were collected in April 1978 in Tichigan Lake. All layers of the gut wall are infected, including the muscular layer (Figs. 52, 53) affecting the muscular function of the gut wall. WBC infiltration observed around cysts in some sections (Fig. 51).

No differences in infection or seasonal parameters were observed between the two lakes. Figures 55 and 56 depict attachment sites of young and older infections on the pectoral fin of a bluegill and figure 57 show the remaining posterior part of the leech in figure 56.

Specimens: MPM (Milwaukee Public Museum) Coll. No. IZ328h (39 specimens on 5 slides). HWML Coll. No. 216745 (2 slides)

***Placobdella ornata* (Verrill, 1872)**

A total of 164 specimens (49 adults and 115 juveniles) were collected from Tichigan Lake in 1978. Only 3 adults were attached to a single blanding turtle *Emydoidea blandingi* Holbrook. All other specimens were collected from minnow traps in the Tichigan Lake channel. Our observations agree with those of Sawyer (1972) that *P. ornata* is more commonly encountered off the turtle hosts (Amin, 1981). One brooding adult carried as many as 86 juveniles and 12 individuals carried 13 attached spermatophores. Three sections of an adult from anterior to midsections (figures 58-60) show the transition of organs from anterior to middle sections. Descriptions of this leech detail external features such as that provided by Sawyer (1972) but make no reference to internal anatomy such as that presented in this paper (figures 58-60). The most recent report by Moser et al. (2012) also emphasized external features, presented some DNA comparisons with related species, and barely made reference to the alimentary canal and reproductive system.

Specimens: USNM Helm. Coll. No. 64137 and MPM (Milwaukee Public Museum Coll. No. IZ328g (*P. ornate* and *P. parasitica* (Say, 1824). HWML Coll. No. 216746 (2 slides).

Conclusions

The parasites of North American freshwater fishes have regarded as having been neglected with their importance decreasing during the last decades (Scholz and Choudhury, 2014). Our present report attempts to renew interest in this field of research. We offer a new perspective for the visualization and diagnosis of common tissue parasites in familiar fishes from Wisconsin, a midwestern state, for the first time and present an additional dimension not projected by other authors. Much taxonomic and ecological research have been reported from Wisconsin by the first author, but no histological studies were completed. This contribution complements the quoted morphological and ecological studies, among others, and makes good use of materials collected and sectioned at the time. A new perspective of the quoted publications can now be appreciated for the first time. The presentation of certain parasites such as *P. ambloplitis* may appear to be repetitious from different host species, but the degree of developments of parasites varied, and the matrix in which the parasites were embedded is different in each host species making each histological section unique. The histological distinction between *P. minimum* and *O. ptychocheilus* from the same host is made more distinct. The variable distribution of the *P. bulbocolli* cysts in *I. punctatus* and *C. carpio* is evident. Variability in the shape and cyst wall patterns in plerocercoids of *P. ambloplitis* are histologically apparent. The attachment site and anatomy of *M. lugubris* are documented, and *P. ornata* is seen for the first time.

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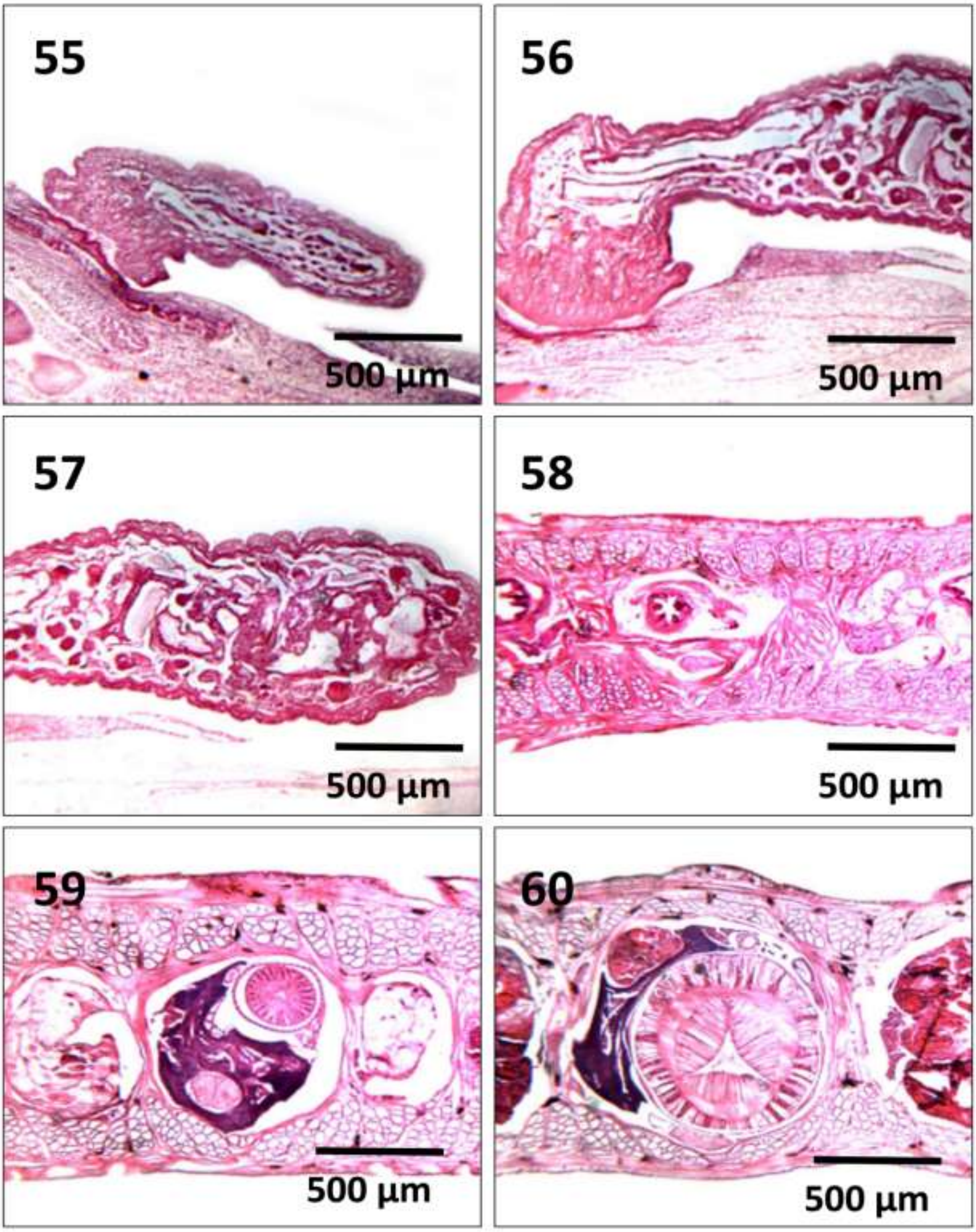
Conflict of interest. None.

Ethical standards. Fieldwork was run according to acceptable relevant ethical standards.

Acknowledgments. We thank our former students at the UW-Parkside research facility for their dedication and hard labor in collecting and processing the reported specimens and many others.



Figures 55-60. Sections of *Myzobdella lugubris* on the pectoral fin of *Lepomis macrochirus* collected from Silver Lake in the spring of 1978 (Figs. 55-57) and of *Placobdella ornata* from *Chrysemys picta marginata* from Tichigan Lake in the spring of 1978 (Figs. 58-60). The attachment site of a small specimen of *M. lugubris* is shown (Fig. 55). Anterior and posterior halves of a larger specimen are shown in Figs. 56 and 57. Note the erosion of the cuticular layer of host pectoral fins at attachment sites. Internal anatomy of *P. ornata* from anterior to posterior sections (Figs. 58-60) show the posterior progression and development of the highly muscular alimentary canal (center) lined with 3 prominent muscular lobes and crop ceca (Figs. 59-60). They reproduce by spermatophore implantation.

References

Amin O.M. 1981. Leeches (Hirudinea) from Wisconsin, and a description of the spermatophore of *Placobdella ornata*. Trans. Am. Microsc. Soc. 100: 42-51.

Amin O.M. 1982. Two larval trematodes (Strigeoidea) of fishes in south eastern Wisconsin. Proc. Helminthol. Soc. Wash. 49: 207-213.

Amin O.M. 1987. Acanthocephala from lake fishes in Wisconsin: Ecology and host relationships of *Pomphorhynchus bulbocolli* (Pomphorhynchidae). J. Parasitol. 73: 278-289.

Amin O.M. 1990a. Cestoda from lake fishes in Wisconsin: The ecology and pathology of *Proteocephalus ambloplitis* plerocercoids in their fish intermediate hosts. J. Helminthol. Soc. Wash. 57: 113-119.

Amin O.M. 1990b. Cestoda from lake fishes in Wisconsin: Occurrence of *Proteocephalus* in *Esox* and other fish species. J. Helminthol. Soc. Wash. 57: 132-139.

Amin O.M. and Boraini M.A. 1992. Cestoda from lake fishes in Wisconsin: The morphological identity of *Proteocephalus ambloplitis*. Trans. Am. Microsc. Soc. 111: 193-198.

Amin O.M., Cowen M. 1990. Cestoda from lake fishes in Wisconsin: the ecology of *Proteocephalus ambloplitis* and *Haplobothrium globuliforme* in bass and bowfin. J. Helminthol. Soc. of Wash. 57: 120-131.

Bedinger C.A., Meade T.G. 1967. Biology of a new cercaria for Posthodiplostomum minimum (Trematoda: Diplostomidae). J. Parasitol. 53: 985-988.

Bogitsh B.J. 1962. The chemical nature of metacercarial cysts. I. Histological and histochemical observations on the cyst of *Posthodiplostomum minimum*. J. Parasitol. 48: 55-60.

Bruno D.W., Nowak B., Elliot D.G., 2006. Guide to the identification of fish protozoan and metazoan parasites in stained tissue sections. Diseases of Aquatic Organisms 70: 1-36.

Chitwood M., Lichtenfels J.R. 1972. Identification of parasitic metazoan in tissue sections. Exp. Parasitol. 32: 407-519.

Clothier B.D., Heckmann R.A. 2002. Plerocercoid infections of *Proteocephalus ambloplitis* (Leidy, 1887) in hepatic tissue of smallmouth bass, *Micropterus dolomieu* Lacepede, a histopathology study. Proc. Parasitol. 33: 1-25.

Crider C.R., Meade T.G. 1975. Immunological studies on the origin of the cyst wall of *Posthodiplostomum minimum* (Trematoda: Diplostomidae). Proc. Helminthol. Soc. Wash. 42: 21-24.

Ferguson, M.S. 1937. Experimental studies on Posthodiplostomum minimum (MacCallum, l92l), a trematode from herons. Ph.D. Thesis. Univ. of Illinois, Urbana. 24E pp.

Fischthal J.H. 1953. Parasites of northwest Wisconsin fishes. IV. Summary and limnological observations. Trans. Wisconsin Acad. Sci., Arts, Letters 42: 83-108.

Gardiner C.H., Poynton S.L. 1999. An Atlas of metazoan parasites in animal tissue. Registry of Veterinary Pathology, Armed Forces Institute of Pathology, Washington, DC

Heckmann R.A. 2006. The efficacy of prazioquentel for the treatment of Posthodiplostomum minimum metacercariae and Proteocephalus ambloplitis (Cestoda) in trophy largemouth bass, Micropterus salmoides from Florida, USA. Varying drug dosage, delivery and exposure time. Proc. Parasitol. 42: 65-79.

Heckmann R.A., Inchausty V.H. 2008. Unique locations and incidence for metacercariae (Trematoda: Digenea) in fish. Proc. Parasitol. 46: 29-47.

Hoffman G.L. 1999. Parasites of North American freshwater fishes. 2nd ed. Cornell Univ. Press, New York, 539 pp.

Hughes R.C., Piszczek F.R. 1928. Studies on the trematode family Strigeidae (Holostomidae). Xl. *Neascus ptychocheilus* (Faust). J. Parasitol. 15:58-62.

Keirnan J.A. 2002. Histological and histochemical methods: theory and practice. Churchill Livingston, Edingburgh, UK, 1–520 p.

Moser W.E., Richardson D.J., Hammond C.I., Lazo-Wasem E.A. 2012. Redescription of *Placobdella ornata* (Verrill, 1872) (Hirudinidae: Glossiphoniidae). Bull. Peabody Mus. Nat. Hist.53: 325-330.

Nowak B., Elliott D.G., Bruno D.W. 2002. The identification of parasites in fish tissue sections. Bull. Eur. Assoc. Fish Pathol. 22: 173-177.

Palmieri, J.R. 1975. Host parasite relationships and intraspecific variation in the strigeoid trematode *Posthodiplostomum minimum* (Trematoda: Diplostomatidae). Ph.D. Thesis, Iowa State Univ., Ames. 166 pp.

Samuel N., Nickol B.B., Mayes M.A. 1976. Acanthocephala of Nebraska fishes. Am. Midland Natural. 96: 391-406.

Sawyer R.T. 1972. North American freshwater leeches, exclusive of Piscicolidae, with a key to all species. Illinois Biol. Monogr. 46: 1-154.

Spall R.D., Summerfelt R.C. 1969. Host-parasite relations of certain endoparasitic helminthes of the channel catfish and white crappie in an Oklahoma reservoir. Bull. Wild. Dis. Assoc. 5: 45-67.

Schloz T., Choudhury A. 2014. Parasites of freshwater fishes in North America: why so neglected? J. Parasitol. 100: 26-45.

Woo P.T.K., Ed. 1995. Fish diseases and disorders. Vol. 1, Ed. 2 Wallingford, UK. Cab International, 808 pp.