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Monograph of the North American freshwater fish leeches (Oligochaeta: Hirudinida; Piscicolidae) and molecular phylogeny of the family Piscicolidae

Julianne I. Williams

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MONOGRAPH OF THE NORTH AMERICAN FRESHWATER FISH LEECHES
(OLIGOCHAETA: HIRUDINIDA: PISCICOLIDAE) AND MOLECULAR PHYLOGENY OF
THE FAMILY PISCICOLIDAE

A Dissertation
Presented to
The Faculty of the School of Marine Science
The College of William and Mary in Virginia

In partial Fulfillment
Of the Requirements for the Degree of
Doctor of Philosophy

by
Julianne I. Williams
2007
APPROVAL SHEET

This dissertation is submitted in partial fulfillment of
the requirements for the degree of
Doctor of Philosophy

Julianne I. Williams

Approved, February 2007

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Abstract:

The phylogeny of the Piscicolidae was analyzed from combined sequence data of 18S rDNA, cytochrome c oxidase subunit I (CO-I), nicotinamide adenine dinucleotide dehydrogenase subunit I (ND-I), and morphological data using parsimony. A worldwide distribution of Piscicolidae was represented for the first time in a phylogenetic analysis. While the family Piscicolidae was supported as a monophyletic group, the traditional subfamilies based on morphology were not supported. The Platybdeillinae was polyphyletic and formed four distinct clades, and Bathybdella sawyeri did not group with any other platybdeilliids. The Piscicolinae was also polyphyletic, also forming four distinct clades. The pontobdellid genus Stibarobdella was shown to be the basal taxon within the Piscicolidae; however, the Pontobdellinae was found to be paraphyletic if Oxytonostoma was included. The genera Aestabdella, Austrobdella, and Malmiana were found to be paraphyletic; the genera Calliobdella, Cystobranchus, and Platybdella were found to be polyphyletic. The species Myzobdella lugubris was not found to be monophyletic. It is proposed that Oxytonostoma be removed from the Pontobdellinae, that Aestabdella be synonymized with Pterobdella, that Calliobdella vivida, Cystobranchus mammillatus, Cystobranchus meyeri, Cystobranchus salmositicus, and Cystobranchus virginicus be reassigned to Gonimosobdella, and that Piscicolaria be synonymized with Myzobdella. The synonymy of Malmiana and Heptacyclus is confirmed, with Heptacyclus having priority. Piscicola milneri is confirmed to be a separate species from Piscicola geometra.

Eleven species of freshwater leeches from North America are redescribed. The description of Gonimosobdella mammillata is emended to correctly describe the conducting tissue and atrium. The reassignment of Piscicola salmositica to Cystobranchus and now Gonimosobdella is confirmed on the basis of the conducting tissue arrangement and absence of mycetomes. The description of Gonimosobdella virginica is emended to include information on pigmentation, conducting tissue, accessory gland cells, coelomic system, and mycetomes. The reproductive and coelomic systems of Gonimosobdella meyeri, Myzobdella reducta, Piscicola milneri, and Piscicola punctata are reported for the first time.
MONOGRAPH OF THE NORTH AMERICAN FRESHWATER FISH LEECHES (OLIGOCHAETA: HIRUDINIDA: PISCICOLIDAE) AND MOLECULAR PHYLOGENY OF THE FAMILY PISCICOLIDAE
Chapter 1: Overview

The current taxonomic state of many lesser-known organisms is complicated by a confusing history of name changes, a lack of collections, and brief and vague original descriptions. The classification of the family Piscicolidae, the fish leeches, is riddled with all of these problems. Works published before the mid 1900s often relied solely on external characteristics and did not consider internal characters currently used for classification. Few internal features were described because the histologic methods used now were not yet available to all scientists or their importance was not yet realized. Without knowledge of the internal features of a leech it is nearly impossible to identify, as genus descriptions are now heavily dependent upon the structure of the reproductive, digestive, and coelomic systems. Some species descriptions are insufficient with little more than a paragraph of text describing characters that could apply to many different leech species. Synonymy is another problem that arises with the earlier works. Descriptions and redescriptions were sometimes published in obscure journals and were not available to all of the taxonomists at the time. The most recent attempts to publish all of the name changes for the Piscicolidae were by Soos (1965) and Sawyer (1986) and both are in need of updating. Prior monographs were performed by Johansson (1896) and Selensky (1915) and both concentrated mainly on the European species. Much has changed in the knowledge of piscicolid leeches and in the methods of taxonomy. Thus, a detailed study of the entire family is warranted.

Freshwater piscicolid leeches in North America, in particular, have received little attention. While there was a surge in research of this discipline in the 1940s and 1950s (Meyer, 1940; 1946a; 1946b; Richardson, 1948; Moore and Meyer, 1951; Meyer and Moore, 1954), it was short lived and little has been done since that time. North American freshwater piscicolid leeches are reported mainly from the Great Lakes region (Klemm, 1982), which is the location of the most intensive sampling. Many states do not have a single piscicolid leech reported, most likely
due to a lack of sampling effort. Currently there are 11 valid species of freshwater piscicolid leech in North America representing 4 genera. A single species has been reported from Mexico (Caballero, 1940), 6 from Canada, and 9 from the United States (Klemm, 1982). Recent work has led to the realization that there may be many undescribed and erroneously identified specimens throughout the continent.

Biology

Piscicolid leeches are found in all of the world's oceans and in freshwater on all continents except Antarctica. Most are parasitic on demersal fishes, including sharks, skates, and rays. Many Piscicolidae are generalists and will attach to a number of different hosts, while some exhibit a greater degree of host specificity by attaching only to a certain species of fish. They often attach to the gills, mouth, or fins of the host and feed on the host blood with a proboscis. When done feeding, they will detach from the host and reattach to another host when the next meal is required. Most piscicolid leeches have the ability to swim with undulating motions. Those leeches that show greater host specificity may remain attached to the same host even when not feeding.

Mating usually occurs when detached from the host. During mating one leech produces a spermatophore that is injected into the reproductive area of another leech. The sperm migrates to the ovisacs, possibly carried there in a specialized conducting tissue (Mann, 1962). A cocoon is secreted from the clitellum of the leech, fertilized eggs are deposited into it, and the cocoon is cemented to a hard surface such as rocks or the exoskeleton of a crustacean (Selensky, 1923; Daniels and Sawyer, 1975).

Leeches are parasites because they feed on the blood of the host. Rarely does this actually cause damage to the host, except in rare cases of high infections. The true threat is that many leeches serve as vectors of blood-borne pathogens such as trypanosomes, trypanoplasmas, and haemogregarines (Khan, 1978; Burreson and Zwerner, 1982; Siddall and Desser, 2001).
Leeches provide the means of transport for the pathogens between fish hosts, which allows for the outbreak of disease in fish populations. Leeches detaching from their host can facilitate yet another pathogenic effect. The wound left by the leech's sucker and proboscis may provide a portal of entry for bacteria and other pathogens that can establish an infection.

Fish leeches can show a varying degree of seasonality. Their reproductive cycles are often closely tied to the lifecycle of their host or to temperature cues. *Cystobranchus mammillatus* (Malm 1863) in Norway has never been collected in the summer months. It appears to hatch out of the cocoons in the winter then begins to grow in the spring as temperatures rise (Halvorsen, 1971). *Mysidobdella borealis* (Johansson 1898) in New Jersey was never found when water temperatures were above 20°C (Allen and Allen, 1981). The lifecycle of *Cystobranchus virginicus* Hoffman 1964, which feeds on fish eggs, is thought to be closely tied to the spawning of the fish host with young leeches hatching out of their cocoons as the host eggs are laid (Light *et al.*, 2005)

**Anatomy of piscicold leeches**

Piscicolid leeches are characterized by a constant number of segments (34), obvious oral and caudal suckers, and a coelomic system that is reduced to a series of tubes and canals. The bodies of piscicolid leeches are divided into three main regions, the trachelosome, clitellar region, and urosome (Fig. 1). The trachelosome, containing somites I to X, is the anterior one third of the leech. This is where the oral sucker and proboscis are found. Rudimentary eyes composed of chromatophore cells may be present on the oral sucker (Sawyer, 1986a). The clitellar region, somites XI and XII, consists of the terminal portion of the male and female reproductive systems, including the male and female gonopores. The urosome, consisting of somites XIII to XXXIV, is the posterior two thirds of the leech and contains the testisacs, ovisacs, crop, postceca, intestine, rectum, anus, and caudal sucker (Selensky, 1915). Chromatophore cells can be located along the length of the body, which are termed segmental ocelli, or on the caudal sucker, which are termed...
Figure 1

Diagrammatic representation of the position of ganglia and the division of the body into regions.
caudal ocelli (Sawyer, 1986a). The caudal sucker is the main attachment organ, though leeches can move around on the host using both suckers with inchworm-like movements (Mann, 1962; Sawyer, 1986a).

**Nervous system:** The ganglia of the first 6 segments are fused into an anterior brain. A ventral nerve cord runs the entire length of the body with a single ganglion for 21 of the segments (Fig. 1). The last 7 segments are again fused, forming a posterior brain (Sawyer, 1986a). Annuli are external divisions of the segments and can range from 3 to 14 in number.

**Digestive system:** Piscicolid leeches feed with a proboscis that protrudes through a mouthpore located on the oral sucker. Salivary gland cells secrete peptides into the proboscis to prevent coagulation of blood during feeding (Sawyer, 1986b). The digestive system then continues dorso-medially through the leech. The proboscis opens into an esophagus that often has associated paired mycetomes. Mycetomes are attached through paired ducts and house bacteria that are believed to aid in the digestion of blood (Sawyer, 1986b). The esophagus leads to the crop in the testicular segments and often expands between the testisacs (Fig. 2), forming crop ceca. Posterior to the testisacs the crop splits into a dorsal intestine and paired ventral postceca. The postceca are blood storage organs that may have evolved to allow the leech to consume a larger bloodmeal and therefore subsist longer between meals. The intestine lies dorsal to the postceca and often contains intestinal ceca (Fig. 2). The intestine leads to the rectum that opens through a dorsal anus.

**Reproductive system:** Leeches are hermaphrodites but are not known to be capable of self-fertilization. The female system consists of a female gonopore in the posterior portion of segment XII that opens into a vagina then a common oviduct. The common oviduct splits into 2 oviducts, which lead to paired ovisacs (Selensky, 1915) (Fig. 2, 3A, 3B). The male system can contain 4, 5, 6, or 13 pairs of testisacs (Sawyer, 1986b; Williams and Burreson, 2005), one pair per segment (Fig. 2), which are connected with a pair of thin vas efferens. The vas efferens travels anteriorly and becomes thicker and is termed the vas deferens. From the vas deferens...
Figure 2

Diagrammatic representation of the reproductive and digestive systems. C, crop; F, fenestrae; I, intestine; MR, terminal portion of the male reproductive system; O, ovisac; P, proboscis; PC, postceca; R, rectum; T1, first pair of testisacs; T6, sixth pair of testisacs.
Figure 3

A. Diagrammatic view of the terminal reproductive systems, ventral view. B. Diagrammatic view of the terminal reproductive systems, lateral view. C. Diagrammatic view of coelomic system, left side intersegmental, right side segmental. AG, accessory gland cells; A, atrium; B, bursa; CT, conducting tissue; D, dorsal sinus; E, epipdidymus; ED, ejaculatory duct; FG, female gonopore; L, thick-walled lateral sinus; LT, thin-walled lateral sinus; M, male gonopore; O, ovisac; PV, pulsatile vesicle; T, testisac; V, ventral sinus; VD, vas deferens.
continues the ejaculatory bulbs, which usually turn ventrally to enter paired atrial cornua. Atrial cornua form the spermatophore. These cornua will join to become the common atrium, which then enters the bursa that opens through a male gonopore (Fig. 3A, 3B). The male gonopore in the anterior portion of segment XII is always anterior to and often larger than the female gonopore. The ejaculatory bulbs, atrium, and bursa of the male reproductive system can be surrounded by accessory gland cells (Selensky, 1915), which aid in the production of spermatophores that are injected into mates. Cords or masses of conducting tissue can be present between the atrium or bursa and the oviducts or ovisacs (Fig. 3A, 3B). This conducting tissue is believed to transfer sperm to the ovisacs (Mann, 1962).

Coelomic system: The coelomic system of leeches is reduced to a series of interconnected sinuses (Sawyer, 1986a). A dorsal and ventral sinus is present in almost all piscicolid leeches. The dorsal sinus contains the dorsal blood vessel while the ventral sinus contains the ventral blood vessel and the nerve cord. Lateral sinuses can be present and when present may connect either with the dorsal, ventral, or both sinuses (Fig. 3C). There is also a sinus that surrounds the testisacs in most species. Segmental extensions of the coelomic system can also occur in the epithelium outside the muscular body wall of the leech. These extensions are called pulsatile vesicles and connect to the lateral sinus (Fig. 3C). Piscicolid leeches can have no pulsatile vesicles, 1 pair per segment, 2 pairs per segment, or a continuous canal with connections to the lateral sinus (Sawyer, 1986b). This characteristic has heretofore determined in which subfamily the leech belongs. The subfamily Platybdellinae has no pulsatile vesicles, while the subfamilies Piscicolinae and Pontobdellinae have 1 and 2 pairs per segment, respectively. Those leeches with a continuous canal, which includes only 2 of the 61 genera, are placed into a subfamily based on the number of connections to the lateral sinus per segment.
North America taxonomy

Few works have been performed that focus on the North American freshwater piscicolid leeches. Verrill was one of the first researchers of freshwater piscicolid leeches in North America. He described *Piscicola punctata* (Verrill 1871) (=*Ichthyobdella punctata*) and *Piscicola milneri* (Verrill 1874), but did so with very short descriptions of the external morphology (Verrill, 1871; Verrill, 1874). His original descriptions do not provide enough information to distinguish these two species from other species. Meyer (1940) redescribed 5 previously known species (*P. punctata, P. milneri, Piscicola geometra* (Linnaeus 1758), *Piscicola zebra* Moore 1898, and *Piscicola rectangulata*, now *Beringobdella rectangulata* (Levinsen 1881). Seven new species were described, 5 of which were in a new genus *Illinobdella* Meyer 1940 and were all later synonymized with *Myzobdella lugubris* Leidy 1851 (Sawyer et al., 1975). Meyer (1940) provided drawings of the general internal anatomy of the new species. Some information was given regarding the internal anatomy and body shape of the previously described species, but unfortunately no pigmentation was described as most specimens he examined were from museum collections and had lost their pigmentation during fixation. The key to freshwater leeches of Canada by Davies (1971) is suitable for fresh material as many of the dichotomies for the species of *Piscicola* Blainville 1818 rely on pigmentation of the caudal sucker. The species that have since been synonymized with *M. lugubris* are also included in the key, along with *Cystobranchus verrilli* Meyer 1940, a species distinguished solely on body shape. Klemm (1982) also provided a review of all the North American freshwater leeches north of Mexico. Distribution maps based on the previous literature, drawings of all the species (most based on museum specimens), a key to the species, and lists of synonymies are included, but basic descriptions of the leeches and their internal anatomy are lacking. The remainder of publications on these leeches consists of reports of occurrences. A comprehensive study has yet to be done on this group of leeches in North America.
There are 11 valid species (Klemm, 1982; Caballero, 1940) reported from North America representing the following 4 genera: *Cystobranchus* Diesing 1859, *Myzobdella* Leidy 1851, *Piscicola*, and *Piscicolaria* Whitman 1889. *Cystobranchus* and *Piscicola* possess 1 pair of pulsatile vesicles per segment and are therefore members of the subfamily Piscicolinae. *Myzobdella* and *Piscicolaria* both lack pulsatile vesicles and belong to the subfamily *Platybdellinae*. There are no representatives of the subfamily Pontobdellinae as they are found exclusively on marine fishes.

*Cystobranchus* is defined as having large pulsatile vesicles, seven annuli per segment, and a copulatory area posterior to the female pore with vector tissue leading to the ovisacs (Sawyer, 1986b). *Piscicola* differs in that the pulsatile vesicles are not as conspicuous; there are 14 annuli per segment, and by having a copulatory area with vector tissue where the vector tissue may be entered by the ovisacs (Sawyer, 1986b).

*Cystobranchus verrilli* was reported from the Illinois and Rock Rivers, Illinois on *Sander vitreum* (Percidae) (=Stizostedion vitreus), *Micropterus dolomieu* (Centrarchidae), and *Pylodictis olivaris* (Ictaluridae) and was later reported from *Lepomis macrochirus* (Centrarchidae) in New Jersey (Meyer, 1946a), *Lota lota* (Lotidae) on Monitoulin Island, Ontario, Canada (Meyer and Moore, 1954), and free-living at the Ste-Anne Rapids, Quebec (Ricciardi and Lewis, 1991). The species is described as having 2 pairs of eyes and brownish-black stellate chromatophores (Meyer, 1940) and lacking caudal ocelli (Meyer, 1946a).

*Cystobranchus meyeri* Hayunga and Grey 1976 was described from *Catostomus commersonii* (Catostomidae) in the Mohawk River drainage, New York (Hayunga and Grey, 1976). It has since been found free-living at the Ste-Anne Rapids in Quebec (Ricciardi and Lewis, 1991) and parasitizing *C. commersonii* in Lake Ontario (Klemm, 1982). This distinctive species possesses 2 pairs of eyes, 8 caudal ocelli, and segmental ocelli. It is also distinguished by transverse bands of stellate chromatophores (Hayunga and Grey, 1976).
Lota lota is the only reported host for Cystobranchus mammillatus (Malm, 1863). Meyer and Roberts (1977) first reported C. mammillatus in North America from the Mackenzie River, Northwest Territories. It was first described by Malm (1863) from the Norde River in Sweden as Platybdella mammillata. Blanchard (1894) synonymized C. mammillatus with Cystobranchus respirans (Troschel 1850), but they were later recognized as 2 separate species (Johansson, 1896). Cystobranchus mammillatus lacks eyes, although there are 2 darker spots in a transverse pigment band on the oral sucker that may be referred to as rudimentary eyes, and possesses pigment bands on the oral sucker and trachelosome (Malm, 1863).

A unique leech that does not feed on blood, but rather fish eggs, is Cystobranchus virginicus. It was first reported by Richardson (1948) in Brome Lake, Quebec, Canada feeding on the eggs of Semotilus (=Leucosomus) corporalis (Cyprinidae) but was misidentified as Piscicolaria reducta Meyer 1940, another freshwater piscicolid leech. Characteristics described by Richardson (rapidly fading eyes, feeding on eggs, flaccid body) confirm that the leech was actually C. virginicus. Hoffman (1964) described the leech from the Roanoke River, Virginia. It was reported from Opequon Creek, West Virginia feeding on longnose dace (Putz, 1972a, b), although recent collections at the same location found only C. meyeri attached to longnose dace. Cystobranchus meyeri was not described until four years after Putz's study. Cystobranchus virginicus was recently reported from fish nests in North Carolina and South Carolina and was never observed feeding on a fish host (Light et al., 2005).

Four species of Piscicola are found in North America. Piscicola punctata was originally described as Ichthyobdella punctata from Lake Superior. Moore (1912) then reassigned it to Piscicola, but it was later discovered by Meyer (1940) that Moore was not actually describing I. punctata but rather another species. Meyer named this new species Illinobdella moorei and P. punctata continued to be the name used for the species described by Verrill. It has since been reported from many hosts, including Ictiobus cyprinellus (Catostomidae) (=Megastomatobus cyprinella), Moxostoma erythrurum (Catostomidae), C. commersonii, Ambloplites rupestris.
(Centrachidae), *Osmerus mordax* (Osmeridae), *Onchorhynchus mykiss* (Salmonidae) (= *Trutta iridea*), *Coregonus clupeaformis* (Salmonidae), *Salvelinus fontinalis* (Salmonidae), *Perca flavescens* (Percidae), *Percopsis omiscomaycus* (Percopsidae), *Cottus Bairdii* (Cottidae), and *Acipenser fulvescens* (Acipenseridae) and in many locations, including New York (Meyer, 1940), Illinois (Wetzel, 1982), Michigan (Klemm, 1972), Lake Superior (Dechtiar and Lawrie, 1988), Lake Huron (Dechtiar, Collins, and Reckahn, 1988), and Ontario (Davies, 1971). Many specimens have been erroneously identified as *P. punctata* (Meyer, 1940) and the true range is not known. The species is described as having 2 pairs of eyes and no caudal ocelli (Meyer, 1940).

*Icthyobdella milneri* was first described from Thunder Bay, Michigan with no host data (Verrill, 1874). Ryerson (1915) observed specimens collected from Georgian Bay, Canada on *Salvelinus namaycush* (Salmonidae) (= *Cristivomer namaycush*) and reassigned the species to *Piscicola*. *Piscicola milneri* was later reported from Lake Nipigon, Ontario on *C. clupeaformis*; from St. Joseph, Michigan and Lake Michigan on *C. clupeaformis* (Meyer, 1940); from Wisconsin and Lake Huron also on *C. clupeaformis* (Meyer, 1946a); from Great Bear Lake and Great Slave Lake, Canada on *S. namaycush* (Moore and Meyer, 1951); from Lac Larouche, Ontario, Canada on *S. namaycush* (Meyer and Moore, 1954); from Washtenaw County, Michigan on *C. commersonii* (Klemm, 1972); and from Lake Superior on *Prosopium cylindraceum* (Salmonidae) (Dechtiar and Lawrie, 1988). *Piscicola milneri* is distinguished from *P. punctata* by the presence of caudal ocelli (Meyer, 1940).

*Piscicola geometra* has been reported from the District of Columbia on German carp (Moore, 1898) and trout from lakes in Wisconsin (Bere, 1929). Klemm (1972) later discovered the leech in Michigan parasitizing *Lepomis gibbosus* (Centrarchidae). *Piscicola geometra* is also reported from Canada (Oliver, 1958). This species is very common in European countries and underwent numerous name changes since its discovery in Europe. It has been known as *P.*
geometra as long as it has been known in North America. The caudal sucker of \textit{P. geometra} has 12 to 14 ocelli with dark rays between the spots (Davies, 1971).

\textit{Piscicola salmositica} Meyer 1946 was described from \textit{O. mykiss} (=\textit{Salmo giardneri gairdneri}) from Soos Creek, Washington and Eel River, California (Meyer, 1946b). It has not been reported from any host other than various species of salmon. The species was reassigned to \textit{Cystobranchus} by Epshtein (1969) but that change has not been accepted in subsequent literature.

There exists a single record of \textit{Piscicola zebra} from Nova Scotia on \textit{Petromyzon marinus} (Petromyzontidae) (Moore, 1898). \textit{Piscicola zebra} is separated from \textit{P. salmositica} by the placement of the caudal ocelli at the end of each dark ray versus in between the rays as seen in \textit{P. salmositica} (Davies, 1971). The types are in poor condition and no other specimens have been reported since the original description, leading Sawyer \textit{et al.} (1975) to designate it a \textit{species inquirendae}.

\textit{Myzobdella} and \textit{Piscicolaria} differ on many basic anatomical and behavioral features. \textit{Myzobdella} is found both in brackish and in fresh waters while \textit{Piscicolaria} is found strictly in freshwater. \textit{Piscicolaria} has 3 annuli per segment and a large mouthpore that occupies most of the oral sucker. \textit{Myzobdella} has 12 to 14 annuli per segment and a typical centrally located mouthpore (Sawyer, 1986b).

\textit{Myzobdella lugubris} was described by Leidy (1851) from the blue crab, \textit{Callinectes sapidus} (Portunidae), with no location information (Leidy, 1851) and has since been reported from numerous fish and crustacean hosts in both freshwater and estuarine locations (Appy and Cone, 1982; Daniels and Sawyer, 1975; Hutton and Sogandares-Bernal, 1959; Schramm \textit{et al.}, 1981; Woods \textit{et al.}, 1990). \textit{Ichthyobdella funduli} and all species of \textit{Illinobdella} (\textit{Illinobdella alba}, \textit{Illinobdella elongata}, \textit{Illinobdella moorei}, and \textit{Illinobdella richardsoni}) have been synonymized with \textit{M. lugubris} (Sawyer \textit{et al.}, 1975). \textit{Myzobdella lugubris} is now considered widely distributed throughout the central and eastern US and Canada.
Another species in the genus *Myzobdella* is *Myzobdella patzcuarensis* Caballero 1940, a leech found in Mexico. It has been reported from *Chirostoma estor* (Atherinopsidae), *Micropterus salmoides* (Centrarchidae), and *Alganea lacustris* (Cyprinidae) in Lago de Patzcuaro, Michoacan on the Yucatan Peninsula (Jimenez, 1985).

The genus *Piscicolaria* contains a single species, *P. reducta*, which was described by Meyer (1940) from the Sangamon River on *Percina phoxocephala* (Percidae) (= *Hadropterus phoxocephalus*). It has since been reported from various cypriniform, siluriform, and perciform fish throughout the Great Lakes region and south central US. All reports of hosts and locations are summarized in Price and Nadolny, 1993. *Piscicolaria reducta* has a unique pattern of longitudinal stripes that are unlike any of the other freshwater fish leeches (Meyer, 1940).

**Higher level taxonomy**

Leeches are worms belonging to the Phylum Annelida (Siddall *et al.*, 2001). The hypothesis that leeches are members of the Oligochaeta was explored through a series of recent papers. Studies include morphological, combined morphological and molecular, and strictly molecular approaches. Relationships between the Euohirudinea (leeches), Acanthobdellida (arctic salmon worm), and Branchiobdellida (crayfish worms) were tested using 651 bp of mitochondrial cytochrome *c* oxidase subunit 1 (CO-I) gene (Siddall and Burreson, 1998). Seven of the 10 euhirudinean families were represented along with *Acanthobdella peledina* Grube 1851 (a monotypic genus comprising the order Acanthobdellida), a branchiobdellid, 4 oligochaetes, and 2 polychaetes. The main reason molecular data were used is because of a lack of overlapping morphological characters between the ingroup and outgroup. The reduced coelomic system occurring in the leeches is not present in the outgroup and therefore the internal morphology is sufficiently different enough that character states cannot be assigned that represent all conditions (Siddall and Burreson, 1998). The generally accepted relationship of *A. peledina* as sister group to the Euhirudinea was contradicted by the results and *A. peledina* was shown to be sister to the
Branchiobdellida + Euhirudinea clade, therefore more distantly related to leeches than previously thought. This relationship was corroborated with a combined morphological and molecular study, which utilized the CO-I gene sequence and 1.8kb of the nuclear gene 18S ribosomal DNA (18S rDNA) along with 30 morphological characters (Apakupakul et al., 1999). In 2001 a study was performed that again confirmed these results using solely molecular data to avoid the possibility that similar morphology was a result of a similar parasitic lifestyle (Siddall et al., 2001). The Euhirudinea, Acanthobdellida, and Branchiobdellida families were all found to lie within the Oligochaeta clade (Siddall et al., 2001). A new classification scheme was proposed to reflect this hypothesis. The Class Clitellata was synonomized with the Class Oligochaeta without a decision made as to which name should be used. Acanthobdellida, Branchiobdellida, and Hirudinida (leeches) were proposed as orders within the Class Oligochaeta/Clitellata along with traditional oligochaete orders. Synapomorphies for the Acanthobdellida + Branchiobdellida + Hirudinida clade are established as a constant number of body somites, the reduction or loss of chaetae, the reduction of the coelom, and the possession of a caudal sucker for attachment (Siddall et al., 2001).

Relationships among the leeches were also considered in these studies. A morphological analysis resulted in 2 equally parsimonious trees, with differences seen in the positions of the Piscicolidae species (Siddall and Burreson, 1995). One tree formed a clade containing the family Ozobranchidae and the subfamilies Pontobdellinae and Piscicolinae in the family Piscicolidae, which shared a common ancestor with a clade containing the subfamily Platybdellinae in the family Piscicolidae and the family Glossiphiaciidae (Fig. 4A). The alternate tree grouped (Ozobranchus (Pontobdella (Piscicola (Platybdella + Glossiphiaciidae)))) (Fig. 4B). Regardless of which tree is considered, the family Piscicolidae was found to be paraphyletic. Therefore, it was proposed that the 3 subfamilies of Piscicolidae be elevated to the family level. The first molecular study (Siddall and Burreson, 1998) included 4 species of "Piscicolidae". Three of the species were in the subfamily Piscicolinae (P. geometra, Calliobdella vivida (Verrill 1872), and
Figure 4

Phylogenetic trees adapted from previous studies. A. Siddall and Burreson, 1995, Fig. 13; B. Siddall and Burreson, 1995, Fig. 11; C. Siddall and Burreson, 1998, Fig. 2; D. Apakupakul et al., 1999, Fig. 3; E. Utevsky and Trontelj, 2004, Fig. 3, Platybdellinae Group A = Trulliobdella capitis and Myzobdella lugubris, Platybdellinae Group B = Crangonobdella sp., Oceanobdella sp., Heptacyclus virgatus, Notostomum cyclostomum, Nototheniobdella cyclostomum, Nototheniobdella sawyeri, and Platybdellinae sp. n.
Branchellion torpedinis (Savigny 1822)). The remaining species, M. lugubris, belonged in the subfamily Platybdellinae. No representatives of the subfamily Pontobdellinae were available for the study. This study found the Piscicolidae to be monophyletic and sister to the Ozobranchidae (Fig. 4C), which is contradictory to the previous morphological study. The Glossiphoniids were found to be sister to the larger clade of Ozobranchidae + Piscicolidae + Arhynchobdellida (leeches possessing jaws). The 3 species of the subfamily Piscicolinae were supported as a clade, but this relationship is cautioned, as there were no Pontobdellinae species in the analysis.

The family Piscicolidae was again found to be monophyletic in the morphological and molecular study (Apakupakal et al., 1999). Ozobranchidae was also found to be sister to the Piscicolidae, as was found in the 1998 study. This study included a species in the subfamily Pontobdellinae, Stibarobdella macrothela (Schmarda 1861), and allowed for relationships to be determined among the 3 Piscicolidae subfamilies. The subfamily Piscicolinae was found to be monophyletic within the Piscicolidae. The Pontobdellinae are sister to the Piscicolinae with the Platybdellinae sister to the Pontobdellinae + Piscicolinae (Fig. 4D).

Recently a study was completed that explored the relationships within the family Piscicolidae in greater detail (Utevsky and Trontelj, 2004). Twenty-three species were compared using COI and 12SrDNA data along with morphological data. The results supported the division of the Piscicolidae into the historical 3 subfamilies. The Pontobdellinae were found to be sister to the Piscicolinae + Platybdellinae. Maximum parsimony analysis resulted in a paraphyletic Platybdellinae (Fig. 4E), while the subfamily was found to be monophyletic in both the maximum likelihood and Bayesian analyses. Conclusions were made regarding the evolution of the coelomic system. The presence of 2 pulsatile vesicles per segment is taken to be the ancestral state with 2 separate reductions resulting in the 2 clades forming the subfamily Platybdellinae.
Chapter 2: Molecular and morphological phylogeny of the family Piscicolidae

Materials and methods

Taxa:

Thirty-seven species of piscicolid leeches, representing 22 of the 60 known genera in the Piscicolidae, were used in the phylogenetic analysis. Nineteen outgroup taxa were used for the molecular analysis; 5 Arhynchobdellida (1 Cylicobdellidae, 1 Haemopidae, and 3 Hirudinidae), and 14 Rhynchobdellida (13 Glossiphoniidae and 1 Ozobranchidae). Table 1 lists all taxa included in this study, the collection locality, GenBank accession numbers for the nucleotide sequences, and catalog numbers for voucher specimens that were deposited in the United States National Museum.

The following specimens identified as *Piscicola geometra* (Linnaeus 1761) from the Canadian Museum of Nature (NMCA or NMCIC) were examined: NMCIC1977-0407, NMCA1985-0379, NMCIC1982-1504, NMCA1984-0231, NMCA1984-0226, NMCA1984-0206, NMCIC1984-0218, NMCA1984-0227, NMCIC1984-0205, NMCA1990-0020, and NMCA1990-0018. The following specimens identified as *Piscicola milneri* (Verrill 1874) from the Canadian Museum of Nature were examined: NMCIC1978-0340, NMCIC1977-0406, NMCIC1984-0754, and NMCIC1984-0322. Newly collected specimens of *P. geometra* from Ukraine and *P. milneri* from Heart Lake, Quebec (the same location as NMCA1985-0379, which was identified as *P. geometra* in the Canadian Museum of Nature collection) were also examined and subsequently processed using histological methods to determine internal anatomy.

Morphology:

Twenty-five morphological characters were used in the analysis. A matrix was assembled for the ingroup taxa only (Table 2), outgroup taxa were not included due to inapplicability of many of the characters. The following characters were used:

1. Mouthpore location in oral sucker: (0) central, (1) terminal
Table 1. Taxa used in the analysis along with collection location, GenBank accession numbers for the nucleotide sequences, and catalog number for a voucher specimen, when available. All voucher specimens are deposited in the United States National Museum.

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**Subfamily Piscicolinae**

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a Siddall and Burreson, 1998  
b Apakupakul et al., 1999  
c Light and Siddall, 1999  
d Trontelj et al., 1999  
e Siddall, 2002  
f Siddall and Borda, 2003  
g Borda and Siddall, 2004  
h Siddall et al., 2005  
i Borda and Siddall, unpublished
Table 2. Morphological matrix used in parsimony analysis. Characters and character states listed in Materials and Methods. "?" indicates an unknown state. "-" indicates a non-applicable character.

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<td>1</td>
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<td>1 1</td>
</tr>
</tbody>
</table>
2. Eyes on oral sucker and trachelosome: (0) none, (1) 1 pair, (2) 2 pairs, (3) 3 pairs
3. Caudal sucker attachment: (0) subterminal, (1) terminal
4. Caudal ocelli: (0) absent, (1) present
5. Annuli per segment: (1) 3, (2) 6, (3) 7, (4) 12, (5) 14
6. Body ornamentation: (0) none, (1) papillae, (2) tubercles
7. Branchiae: (0) none, (1) 31 pairs, (2) 33 pairs
8. Crop cecae per segment: (0) none, (1) 1, (2) 2, (3) 3, (4) 4, (5) 6
9. Postcecae: (0) absent, (1) present
10. Fusion of postcecae: (0) absent, (1) present
11. Conducting tissue: (0) absent, (1) present
12. Conducting tissue morphology: (0) ducts, (1) mass, (2) mass with ducts
13. Conducting tissue originates at bursa: (0) no, (1) yes
14. Testisacs: (0) 5 pairs, (1) 6 pairs, (2) 13 pairs
15. Accessory gland cells: (0) absent, (1) present
16. External coelomic space: (0) absent, (1) present
17. Continuous external canal: (0) absent, (1) present
18. Pulsatile vesicles per mid-body segment: (0) none, (1) 1, (2) 2
19. Segments with pulsatile vesicles: (0) 10, (1) 11
20. Lateral sinus: (0) absent, (1) present
21. Dorsal sinus: (0) absent, (1) present
22. Testicular sinus: (0) absent, (1) present
23. Connection from dorsal sinus to any other sinus at the ganglia: (0) absent, (1) present
24. Connection from dorsal sinus to any other sinus intersegmentally: (0) absent, (1) present
25. Connection from ventral sinus to any other sinus at the ganglia: (0) absent, (1) present
DNA extraction, purification, and amplification:

Leeches were preserved and stored in 95% ethanol at -20°C until used for DNA extraction. Tissue lysis and purification of DNA was accomplished using QIAamp DNA Mini Kit (Qiagen Inc. Valencia, CA), following the tissue protocol.

PCR amplification was accomplished using the primers listed in Table 3. Each primer pair for the 18S rDNA gene produced a fragment of approximately 600 base pairs (bp) that overlapped to form the 18S rDNA fragment of approximately 1680 bp used in this study. The nicotinamide adenine dinucleotide dehydrogenase subunit I (ND-I) gene and cytochrome c oxidase subunit I (CO-I) gene amplification resulted in fragments of approximately 600 bp and 660 bp each, respectively.

Amplification reaction mixtures used Ready-To-Go PCR Beads (Amersham-Pharmacia Biotech, Piscataway, NY), 0.5 μl of each 10 μM primer, 1 μl template DNA, and 23 μl RNAse-free H₂O or 2.5 μl 10X PE II buffer, 2.5 μl 25 mM MgCl₂, 2 μl 0.2 mM dNTPs, 1 μl BSA (10mg/ml), 0.15 μl of each 10 μM primer, 0.12 μl Amplitaq (Perkin-Elmer Corp., Foster City, CA), 4 μl 50 ng/μl template DNA, and 12.58 μl dH₂O. Amplification reactions were performed in a GeneAmp PCR System 9700 (PE Applied Biosystems), a GeneAmp PCR System 9600 (PE Applied Biosystems), or a TGradient (Biometra). Amplification protocols were as follows: 18S rDNA – initial denaturation for 5 min at 94°C; 35 cycles of 94°C (15 s), 44°C (20 s), 70°C (90 s); followed by a final extension for 7 min at 72°C. CO-I – initial denaturation for 4 min at 94°C; 35 cycles of 94°C (30 s), 44°C (30 s), 70°C (90 s); followed by a final extension for 5 min at 70°C. ND-I – initial denaturation for 4 min at 94°C; 39 cycles of 94°C (30 s), 50°C (30 s), 72°C (90 s); followed by a final extension for 7 min at 72°C.

PCR products were purified using the QIAquick PCR Purification Kit protocol (Qiagen Inc., Valencia, CA) or by adding 0.5 μl exonuclease I (USB Bioscience), 0.5 μl shrimp alkaline

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Table 3. List of primers used in genetic analyses.

<table>
<thead>
<tr>
<th>Gene</th>
<th>Primer name</th>
<th>Primer sequence</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nuclear</td>
<td>5R</td>
<td>5'-CTTGCCAAATGCTTTTCGC-3'</td>
<td>Giribet et al., 1996</td>
</tr>
<tr>
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<td>1F</td>
<td>5'-TACCTGGTTGATCCTGCCAGTAG-3'</td>
<td>Giribet et al., 1996</td>
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<tr>
<td></td>
<td>8R</td>
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<td>3F</td>
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<td>Giribet et al., 1996</td>
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<td>A2.0</td>
<td>5'-ATGGTTGCAAAAGCTGAAAC-3'</td>
<td>Giribet et al., 1999</td>
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<tr>
<td>Mitochondrial</td>
<td>LND300</td>
<td>5'-TGGCAGAGTATCATTAGG-3'</td>
<td>Light and Siddall, 1999</td>
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<td>HND1932</td>
<td>5'-CCTCAGAAAAATCAAGATGG-3'</td>
<td>Light and Siddall, 1999</td>
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<tr>
<td>COI</td>
<td>LCO1490</td>
<td>5'-GGTCAACAAAATCATAAGATATTGG-3'</td>
<td>Folmer et al., 1994</td>
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<tr>
<td></td>
<td>HCO2198</td>
<td>5'-TAAACTTCAAGGGTGACCAAAAATCA-3'</td>
<td>Folmer et al., 1994</td>
</tr>
</tbody>
</table>
phosphatase (USB Bioscience), and 1 μl shrimp alkaline phosphatase dilution buffer to 3 μl amplification product and incubating at 37°C (30 min), 80°C (15 min), and then held at 4°C.

**DNA sequencing:**

Sequencing reactions contained 1 μl primer, 3 μl purified amplification product, and 1 μl Big Dye (Applied Biosystems, Perkin Elmer Corporation) and were cycled at 96°C (70 s), 44°C (5 s), and 60°C (4 min) for 35 cycles. Sequencing products were purified using 70% isopropanol/70% ethanol precipitation or 3M sodium acetate/95% ethanol precipitations. Sequencing products were electrophoresed in an ABI Prism 3000 or 3700 (Applied Biosystems).

**DNA sequence alignment:**

Complimentary strands were reconciled using Sequencher 4.5 (Gene Codes Corporation). Alignment of 18S rDNA sequences was performed using Clustal W with default parameters of 10:5 for the pairwise alignment and 10:5 for the multiple alignment in MacVector 8.0.2 (Accelrys). ND-I and CO-I protein sequences were aligned by eye using the alignment of the protein sequences to confirm the nucleotide alignment. Only the sequence data was used for the analyses.

**Phylogenetic analyses:**

The data were explored using parsimony methods, as parsimony does not rely on the use of models that are derived from the data. Parsimony analyses were performed using PAUP* 4.0b10 (Swofford 2000). Parsimony analyses were run using 100 random taxon addition replicates and tree-bisection-reconnection branch swapping. All characters were equally weighted and non-additive. Gaps were treated as missing data. Retention index (RI) and consistency index (CI) values were calculated in PAUP* (Swofford, 2000). Jackknife (jac)
values were calculated using TNT (Goloboff et al., 2000). Bremer support values were obtained using TreeRot.v2c (Sorenson, 1999).

A parsimony analysis was run combining data presented here and CO-I data from Utevsky and Trontelj (2004), GenBank accession numbers AY336010 – AY336030. This analysis was conducted because Utevsky and Trontelj (2004) had access to species not included in our analyses, mainly other species of *Piscicola* that provided information on the relationship of *Piscicola geometra* and *Piscicola milneri*, and *Pontobdella muricata* (Linnaeus 1758), a member of the type genus of the subfamily Pontobdellinae, that provided information on the monophyly of the Pontobdellinae.

Results

Sequences of 18S rDNA, CO-I, and ND-I were generated from the taxa shown in Table 1. Molecular and morphological data combined resulted in a dataset with a total of 3185 characters, 1089 of which were parsimony-informative. Parsimony informative characters were distributed with 367 in the 18S rDNA, 324 in CO-I, and 374 in ND-I. Parsimony analysis of the combined dataset yielded 2 most-parsimonious trees with 7351 steps (RI=0.692; CI=0.294). One of the 2 most parsimonious trees, with jackknife and Bremer support values, is shown in Fig. 5. Analysis of the morphological data alone and of each gene separately failed to resolve the higher-level relationships; however, the species-level relationships seen in majority rule trees for each gene were similar to the relationships obtained from the combined dataset. The exceptions were that *Johanssonia arctica* (Johansson 1898) grouped with *Myzobdella* Leidy 1851 instead of with *Bathydella sawyeri* Burreson 1981 and *Calliobdella lophii* Beneden and Hesse 1863 in the CO-I analysis, and that *Oxytonostoma typica* Malm 1863 was inside of the *Austrobdella* Badham 1916 clade in the ND-I analysis. The morphology dataset resulted in little resolution. Relationships among the species of *Branchellion* Savigny 1822 were resolved. This *Branchellion* clade grouped sister to a clade containing the traditional Pontobdellinae and *J. arctica*. The species of
**Figure 5**

One of 2 most parsimonious trees resulting from a combined analysis of 18S rDNA, CO-I, ND-I, and morphological data. Tree length is 7351 steps with an RI of 0.692 and a CI of 0.294. Jackknife values shown above branches. Bremer support values shown below branches. Dotted branches indicate branches that are not resolved in a strict consensus tree. Traditional Pontobdellinae shaded grey, names in traditional Platybdellinae are in bold type, and names in traditional Piscicolinae have no shading or bold type. Thin branches within the Piscicolidae indicate 5 pairs of testisacs, thick branches within the Piscicolidae indicate 6 pairs of testisacs, and grey branch within the Piscicolidae indicates 13 pairs of testisacs. * indicates a name change has been proposed from this analysis.

Abbreviations: BER, Bering Sea; BI, Bouvet Island; BOR, Borneo; EST, estuarine; FW, freshwater; HER, Heron Island; HI, Hawaii; HS, host *Hippoglossina stomata*; NOR, Norway; PV, host *Pleuronichthys verticalis*; SG, host *Scorpaena guttata*; SGI, South Georgia Island; SSI, South Sandwich Island; VA, Virginia.
Cystobranchus Diesing 1859, Calliobdella Beneden and Hesse 1863, and Piscicola Blainville 1818 were basal to the Branchellion and traditional Pontobdellinae clades. Austrobdella bilobata Ingram 1957 and Austrobdella californiana Burreson 1977 grouped together and basal to the Cystobranchus clade. Notobdella Benham 1909 grouped with Oceanobdella Caballero 1956 among the traditional Platybdellinae, which formed a mostly unresolved bush basal to the traditional Piscicolinae and Pontobdellinae. Bathybdella Burreson 1981, Notostomum Levinsen 1881, and Platybdella Malm 1863 also formed a clade among the Platybdellinae.

In the analysis of all data combined, the Rhynchobdellida was found to be paraphyletic because the Glossiphoniidae was sister to the Arhynchobdellida. The family Piscicolidae was found to be monophyletic (jac=100, Fig. 5). The traditional subfamily divisions of the Piscicolidae failed to be resolved (Fig. 5). However, support values on the basal nodes were low with the exception of the node defining the Piscicolidae.

Members traditionally classified in the Pontobdellinae were not found to be monophyletic. The members of the genus Stibarobdella Leigh-Sharpe 1925 were monophyletic and were basal to the remaining Piscicolidae. The other Pontobdellinae species, O. typica, was basal to the Platybdellinae clade containing Notobdella.

The Platybdellinae was also not found to be monophyletic. The genera traditionally classified in the Platybdellinae were found in four distinct groups, with the exception of B. sawyeri, which grouped apart from all other Platybdellinae (Fig. 5). Bathybdella sawyeri was found nested within a clade with members of the traditional “Piscicolinae”, sister to C. lophii and J. arctica (jac=94, Fig. 5). One of the “Platybdellinae” clades consisted of Aestabdella Burreson 1976, Pterobdella Kaburaki 1921, and Zeylanicobdella de Silva 1963. Zeylanicobdella was considered as a member of the “Platybdellinae” in this analysis after serial sectioning of specimens showed that there were no pulsatile vesicles present. The genus Aestabdella was paraphyletic, with Pterobdella amara Kaburaki 1921 sister to Aestabdella leistomi Burreson 1991 (jac=92, Fig. 5) and Aestabdella abditovesiculata (Moore 1952) sister to those species.
Of the remaining “Platybdellinae” clades, one was exclusively Notobdella, a second included members of the genera Myzobdella, Piscicolaria Whitman 1889, and Austrobdella, and the third included the remaining Platybdellinae genera: Beringobdella Caballero 1974, Heptacyclus Vasiliev 1939, Malmiana Strand 1942, Notostomum, Oceanobdella, and Platybdella. Austrobdella was paraphyletic, with Myzobdella sister to the Austrobdella species from California (jac=100, Fig. 5), and the remaining species of Austrobdella, from Australia, sister to that clade (jac=12, Fig. 5). Malmiana was also a paraphyletic genus because Heptacyclus virgatus Vasiliev 1939 was nested within the Malmiana species (jac=88, Fig. 5). Myzobdella lugubris Leidy 1851 was found not to be monophyletic because the genus Piscicolaria nested within it (jac=100, Fig. 5). Two specimens identified as Platybdella anarrhichae (Diesing 1859) collected from Norway and the Bering Sea were found not to be monophyletic in that sequences from specimens from the different locations fell into different clades.

The traditional subfamily Piscicolinae was found not to be monophyletic. Branchellion was found to be monophyletic forming a clade (jac=99, Fig. 5), as was Piscicola (jac=100, Fig. 5). A third “Piscicolinae” clade was comprised of Calliobdella vivida (Verrill 1872), Gonimosobdella klemmi Williams and Burreson 2005, and the species of Cystobranchus (jac=100, Fig. 5). The final “Piscicolinae” clade was comprised of C. lophii and J. arctica (jac=51, Fig. 5). Calliobdella vivida did not group with the type species of Calliobdella, C. lophii, making the genus Calliobdella polyphyletic. The genus Cystobranchus was paraphyletic because C. vivida and G. klemmi grouped among the species of Cystobranchus.

Six pairs of testisacs is shown to be the plesiomorphic state. Reductions to 5 pairs of testisacs occurred 5 times and an increase from 6 to 13 pairs of testisacs occurred once (Fig. 5).

CO-I data were aligned with the CO-I sequences from Utevsky and Trontelj (2004) and combined with the 18S rDNA and ND-I data from this study. Parsimony analysis resulted in 6 most parsimonious trees of 7803 steps (CI = 0.279, RI = 0.647). One of the 6 most parsimonious
trees, with jackknife and Bremer support values, is shown in Fig. 6. There was low support at the higher taxonomic levels, however some generic level relationships were well supported. *Pontobdella muricata*, the type species of *Pontobdella* Leach 1815, was sister to the species of *Stibarobdella*, supporting the monophyly of the Pontobdellinae (jac=76, Fig. 6). *Piscicola geometra* was found to be sister to a clade formed by *Piscicola cf. annae* and *Piscicola* sp. (jac=63, Fig. 6), and *P. milneri* was sister to all other *Piscicola* species (jac=80, Fig. 6).

No differences were seen externally or internally between the morphology of *P. geometra* and *P. milneri* examined for the present study. Both species were found to have a similar pigmentation pattern with 2 pairs of eyes, between 10 and 14 caudal ocelli, and a transverse banding pattern. The internal anatomy also was similar with ejaculatory ducts entering the atrial cornua from the ventral surface, atrial cornua that are long and thin, extensive accessory gland cells, conducting tissue forming a mass posterior to the female pore and connecting via ducts to the ovisacs, a coelomic system as described for *P. geometra* by Sawyer (1986b), and a digestive system as described for *P. geometra* by Bielecki (1997).

Discussion

These results are consistent with those of Utevsky and Trontelj (2004) in terms of the basal position of the Pontobdellinae within the Piscicolidae. However, the traditional subfamilies Piscicolinae and Platybdellinae were not supported because both were polyphyletic in these analyses. Most of the 18 tribes of Epshtein *et al.* (1994) also were not supported in these analyses. For instance, *Aestabdella*, *Pterobdella*, and *Zeylanicobdella* were placed in the tribes Hemibdellini, Austrobdellini, and Piscicolini, respectively (Epshtein *et al.*, 1994), but they formed a well-supported monophyletic group in our analysis. According to Epshtein *et al.* (1994), the tribe Platybdellini consists of *Platybdella*, *Cryobdella* Harding 1922, *Heptacyclus*, *Malmiana*, *Oceanobdella*, *Sanguinothus* de Silva and Burdon-Jones 1961, and *Arctobdella* de Silva and Kabata 1961, but in this analysis *Platybdella*, *Heptacyclus*, *Malmiana*, and...
Figure 6

One of 6 most parsimonious trees resulting from a combined analysis of 18S rDNA, CO-I, ND-I, morphological data, and CO-I data from Utevsky and Trontelj (2004). Tree length is 7803 steps with an RI of 0.647 and a CI of 0.279. Jackknife values shown above branches. Bremer support values shown below branches. Dotted branches indicated branches that are not resolved in a strict consensus tree. Traditional Pontobdellinae shaded grey, names in traditional Platybdellinae are in bold type, and names in traditional Piscicolinae have no shading or bold type. * indicates a name change has been proposed from this study. † indicates samples from Utevsky and Trontelj (2004), GenBank accession numbers AY336010 – AY336030.

Abbreviations: BER, Bering Sea; BI, Bouvet Island; BOR, Borneo; EST, estuarine; FW, freshwater; GER, Germany; HER, Heron Island; HI, Hawaii; HS, host *Hippoglossina stomata*; NOR, Norway; PV, host *Pleuronichthys verticalis*; RUS, Russia; SG, host *Scorpaena guttata*; SGI, South Georgia Island; SSI, South Sandwich Island; UKR, Ukarine; VA, Virginia.
Oceanobdella formed a monophyletic group with Beringobdella, which Epshtein et al. (1994) placed in the tribe Crangonobdellini with Crangonobdella Selensky 1914, Trulliobdella (=Notobdella), Cryobdellina (=Notobdella), and Antarctobdella (=Notobdella).

The only distinction that has become apparent is that the Pontobdellinae, if Oxytonostoma Malm 1863 is removed, is sister to all other Piscicolidae. Combining the previously known CO-I sequence (Utevsky and Trontelj, 2004) for P. muricata, the type species of Pontobdella, with the sequences from 2 species of Stibarobdella supported the monophyly of the Pontobdellinae. Based on these results, removal of O. typica from the Pontobdellinae may be warranted, although its position as sister taxon to Notobdella nototheniae Benham 1909 is not well supported (jac=17, Fig.1; jac=27, Fig. 6). Removal of O. typica from the Pontobdellinae is also justified on morphological grounds. Oxytonostoma typica has small papillae and 14 annuli per segment as opposed to large tubercles and 3–5 annuli per segment in all other members of the Pontobdellinae. Oxytonostoma is 1 of 2 genera in the Piscicolidae with a continuous coelomic canal external to the musculature, the other being Austrobdella (Sawyer, 1986b). Oxytonostoma has traditionally been placed in the Pontobdellinae because there are 2 connections per somite from the continuous canal through the muscle layer to the main coelomic system, which was considered to be homologous to the 2 pulsatile vesicles per somite seen in the Pontobdellinae. Based on our combined analysis, however, they do not appear to be homologous characters and Oxytonostoma should no longer be considered a member of the Pontobdellinae.

The presence of tubercles and a small number of annuli per segment (i.e. 3, 4, or 5) are the characters that distinguish the members of the Pontobdellinae from other members of the Piscicolidae. Tubercles alone cannot be used as the diagnostic character since there is 1 genus not traditionally placed in the Pontobdellinae that possesses tubercles, Orientobdella Epshtein 1962, which has 14 annuli per segment and was traditionally placed in the Piscicolinae because of the presence of 1 pair of pulsatile vesicles per urosome segment (Sawyer, 1986b). Megaliobdella Meyer and Burreson 1990 has provisionally been placed in the Pontobdellinae but has 14 annuli.
per segment (Meyer and Burreson, 1990) versus the small number typically seen in the Pontobdellinae. Unfortunately specimens of Orientobdella and Megaliobdella were not available for this study and it would be interesting to see if they group with the Pontobdellinae or the remaining Piscicolidae.

Because of the large amount of morphological diversity in the non-pontobdellins, and the low support values for many basal clades, it does not seem appropriate to formally propose subfamilies for this large group of genera. There are no morphological or geographical features that can be used to further subdivide the non-pontobdellins. The monophyly of piscicolins based on number of testisacs, an important generic character, was not supported. Nonetheless, the possession of 6 pairs of testisacs appears to be the pleisiomorphic condition with the reduction of the number of testisacs occurring multiple times among fish leeches. Since there are no other morphological characters that show any large-scale patterns, it is not possible to predict where species of Piscicolidae that were not included in this analysis would be placed based on morphological data alone.

A few generic level changes can be proposed from this analysis and are summarized in Table 4. Heptacyclus virgatus, the type and only species in the genus, grouped within the genus Malmiana, rendering Malmiana paraphyletic. The monotypic Heptacyclus has priority; therefore all species of Malmiana should be reassigned to the genus Heptacyclus. This synonymy has been proposed by Epsthein (1967), but was done so in a footnote without explanation, and has not been widely accepted. The synonymy was also suggested by Sawyer (1986b) who stated that Heptacyclus is “inadequately distinguished from Malmiana.” It was again suggested in the recent description of a new species of Malmiana (Burreson and Kalman, 2006) where the only difference stated between the genera is the overall larger size of H. virgatus compared with species of Malmiana.

The validity of Oceanobdella as a separate genus from Malmiana and Heptacyclus has also been questioned (Burreson and Kalman, 2006), as the primary difference is the smaller oral
Table 4. Proposed name changes resulting from the parsimony analysis

<table>
<thead>
<tr>
<th>Current name</th>
<th>Proposed name</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aestabdella Burreson</td>
<td>Pterobdella Kaburaki</td>
</tr>
<tr>
<td>Calliobdella vivida (Verrill 1872)</td>
<td>Gonimosobdella vivida (Verrill 1872)</td>
</tr>
<tr>
<td>Cystobranchus meyeri Hayunga and Grey 1976</td>
<td>Gonimosobdella meyeri (Hayunga and Grey 1976)</td>
</tr>
<tr>
<td>Cystobranchus salmositicus (Meyer 1946)</td>
<td>Gonimosobdella salmositica (Meyer 1946)</td>
</tr>
<tr>
<td>Cystobranchus virginicus Hoffman 1964</td>
<td>Gonimosobdella virginica (Hoffman 1964)</td>
</tr>
<tr>
<td>Malmiana Strand</td>
<td>Heptacyclus Vasiliev</td>
</tr>
<tr>
<td>Piscicolaria reducta Meyer 1940</td>
<td>Myzobdella reducta n. comb. (Meyer 1940)</td>
</tr>
</tbody>
</table>
sucker seen in *Oceanobdella*. However, based on these analyses *Oceanobdella* appears to be a valid genus (jac=100, Fig. 5) despite the very similar morphology to *Malmiana* and *Heptacyclus*.

It appears that a species tentatively identified as *Playbdella cf. anarrhichae* from the Bering Sea (Siddall and Burreson, 1994) and *P. anarrhichae* from Norway are not the same species and do not even belong in the same genus. Since *P. anarrhichae* is the type species in the genus, and Norway is the type locality, the species from the Bering Sea should be reassigned to a different genus when a full morphological study can be performed on the specimens.

*Austrobdella* was not found to be monophyletic. *Austrobdella translucens* Badham 1916 (the type species) and *A. bilobata*, both from Australia, are sister taxa and should remain as *Austrobdella* species. *Austrobdella californiana* should be placed in another genus. Given that *Austrobdella* and *Oxytonostoma* are the only genera with an external continuous canal (Sawyer, 1986b) and *A. californiana*, which has this feature, does not group with the type species of either genus, a new genus should be created for *A. californiana*. *Austrobdella* has been poorly studied and a detailed morphological study needs to be conducted on the genus to determine generic level differences between *A. translucens* (the type species) and *A. californiana*.

Specimens identified as *M. lugubris*, the type species of *Myzobdella*, form a paraphyletic group with *Piscicolaria reducta* Meyer 1940, clearly indicating that *P. reducta* should be placed in *Myzobdella* and making *Piscicolaria* a junior synonym of *Myzobdella*. *Piscicolaria reducta* can be easily distinguished externally from *M. lugubris* by the annulation and pigmentation (Klemm, 1982). *Myzobdella lugubris* was originally described by Leidy (1851) from the blue crab and is therefore from an estuarine habitat, but no type specimens were deposited. Meyer (1940) described 4 species of *Illinobdella* Meyer 1940 and the monotypic *P. reducta* from freshwater. A complete redescription of *Myzobdella* was provided by Moore (1946), in which he states that there is little doubt that the species of *Illinobdella* should be reassigned to *Myzobdella*, but suggests a study of the coelomic system before such a change is made. Freshwater tolerance experiments were performed on *M. lugubris* from estuarine environments and it was shown that
M. lugubris is freshwater tolerant (Sawyer et al., 1975). Based on this, all Illinobdella species were synonymized with M. lugubris (Sawyer et al., 1975). Myzobdella lugubris from estuarine environments, and from freshwater on the U. S. mainland and in Hawaii are morphologically similar externally, while Myzobdella (=Piscicolaria) reducta is distinct in pigmentation and annulation. This suggests that M. reducta is a distinct species from M. lugubris, which, if valid, renders M. lugubris paraphyletic in our analyses. This result suggests that the Myzobdella specimens from estuarine environments, from freshwater on the U. S. mainland, and from Hawaii are actually distinct species. A more thorough population study should be conducted, both genetically and using information from the internal morphology, to resolve these issues.

A well-supported relationship between B. sawyeri, C. lophii, and J. arctica was hypothesized in our analyses (jac=94, Fig. 5). Bathybdella sawyeri is 1 of 2 species from the “Platybdellinae” used in this study that has 6 pairs of testisacs, an unusual feature for a platybdellin leech. Therefore its placement with the 2 “Pisciciolinae” species that also have 6 pairs of testisacs is not surprising, although B. sawyeri lacks pulsatile vesicles, which are present in C. lophii and J. arctica.

Cystobranchus is a paraphyletic genus because the type species, Cystobranchus respirans (Troschel 1850), did not group with the other Cystobranchus species. It has been proposed that C. respirans is in fact a member of the genus Piscicola (Epshtein, 1969), however, it does not group with Piscicola in this study or in the study performed by Utevsky and Trontelj (2004). As C. respirans is the type species, it must remain in Cystobranchus but the other species should be removed and placed in a different genus. Gonimosobdella klemmi should also be a member of this genus as it groups sister to C. salmositicus. Gonimosobdella Williams and Burreson 2005 has priority for this grouping and results in the new combinations: Gonimosobdella meyeri (Hayunga and Grey 1976), Gonimosobdella salmositicus (Meyer 1946), and Gonimosobdella virginica (Hoffman 1964). Gonimosobdella was described as a new genus because of the unique number of testisacs—13 pairs versus the usual 5 or 6 pairs in Piscicolidae. Gonimosobdella was
specifically separated from *Cystobranchus* by the number of testisacs as well as the annulation and the coelomic system connections (Williams and Burreson, 2005). Annulation has been shown to vary among species in the same genus (Bielecki, 1997) and the characteristics of the coelomic system were known for only 2 of the 5 species of *Cystobranchus*. Internal features seen in *G. klemmi*, *G. meyeri*, *G. salmositica*, and *G. virginica* are the lack of mycetomes, the presence of conducting tissue, and large intestinal ceca (Williams and Burreson, 2005).

*Calliobdella vivida* is clearly a member of the genus *Gonimosobdella* resulting in the new combination *Gonimosobdella vivida* (Verrill 1872). In our study and the study performed by Utevsky and Trontelj (2004), *Gonimosobdella vivida* did not group with *Calliobdella lophii*, the type species of *Calliobdella*. *Gonimosobdella vivida* was originally described by Verrill (1872) as *Cystobranchus vividus* and was reassigned to *Calliobdella* by Sawyer *et al.* (1975) when it was synonymized with *Calliobdella carolinensis*. The original description of *Cystobranchus vividus* was brief with no internal anatomy depicted (Verrill, 1872) and no type specimens are known. However, the description of *C. carolinensis* is complete in providing all relevant details of the internal anatomy (Sawyer and Chamberlain, 1972). Sawyer and Chamberlain (1972) suggested that *C. carolinensis* was similar to members of *Piscicola*, *Cystobranchus*, *Calliobdella*, and *Trachelobdella* Diesing 1850, but placed it in the genus *Calliobdella* based on similarity to the seminal receptacle found in *C. lophii* and *Calliobdella nodulifera* Malm 1863. The seminal receptacle in both those *Calliobdella* species appears to be nothing more than strands of conducting tissue attached to the posterior portion of the bursa (Leigh-Sharpe, 1914; Selensky, 1915) and a seminal receptacle should not be considered a generic character for *Calliobdella*. Strands of conducting tissue are present in *G. vivida* connecting the posterior portion of the seminal receptacle to the ovisacs (Sawyer and Chamberlain, 1972), as typically seen in *Calliobdella* species. However, the same arrangement of conducting tissue has also been noted in *G. salmositica* and *G. virginica* (Burreson *et al.*, 2005). The seminal receptacle of *G. vivida* seems to be a unique condition within *Gonimosobdella*.
Removing *G. meyeri*, *G. salmositica*, and *G. virginica* from *Cystobranchus* leaves only *C. reparans*, the type species, *C. mammillatus*, and *C. verrilli* in the genus. Although specimens were not available for genetic analysis, it is proposed that *C. mammillatus* also be reassigned to *Gonimosobdella* resulting in the new combination *Gonimosobdella mammillata* (Malm 1863). *Gonimosobdella mammillata* lacks mycetomes (see Chapter 3), as do all other species of *Gonimosobdella* except for *G. vivida*. There is also a similar arrangement of conducting tissue seen in *G. mammillata* as is present in the other *Gonimosobdella* species. Too little is known of *C. verrilli* to determine in which genus it belongs, therefore it should remain in *Cystobranchus* until further studies can be conducted. *Cystobranchus respirans* has mycetomes (Jaschke, 1933) and has a conducting tissue and vector tissue arrangement similar to species of *Piscicola* (Epshtein, 1969).

The paraphyly of *Aestabdella* indicates that it should be synonymized with *Pterobdella*. *Pterobdella* has priority and *Aestabdella* becomes a junior synonym. While species of the 2 genera are distinctly different externally, especially in general body shape, they share important internal features such as a spacious, ramifying coelomic system and 2 pairs of mycetomes (Burreson, 2006).

Collections were made from Heart Lake, Quebec of a leech identified by me as *P. milneri*. In addition, specimens identified as *P. geometra* from the Canadian Museum of Nature (NMCA1985-0379) from the same location, were examined. Taxonomic keys have used inconsistent characters for both species making it difficult to provide a proper identification (Davies, 1971; Klemm, 1982; Sawyer, 1986b). Sawyer (1986b), listed *P. milneri* as a distinct species, but also stated that it is “inadequately distinguished from *Piscicola geometra*.” *Pisciola geometra* has been considered to have a Palaearctic distribution and *P. milneri* is restricted to the Great Lakes and Canada (Sawyer, 1986b; Klemm, 1982). Using data from this study and the sequences made available by Utevsky and Trontelj (2004), *P. milneri* was found to be sister to a clade containing *P. geometra* from Ukraine, Germany, and France, *Piscicola cf. annae*, and
Piscicola sp. (jac=80, Fig. 6). *Piscicola geometra* and *P. milneri* did not form an exclusive monophyletic group and are therefore not the same species. All specimens in North America should be considered as *P. milneri*, with the exception of 3 specimens of *P. geometra* collected from German carp, *Cyprinus carpio carpio* (Cyprinidae), that may have been introduced with the fish (Moore, 1898). A wild population of *P. geometra* is doubtful in North America.

While this has been the most comprehensive study to date of piscicolid leech phylogeny, there are still many questions remaining. The lack of clear divisions within the non-pontobdellins and inability to place taxa that were not sampled into the non-pontobdellin clades based on morphology is disconcerting. Perhaps adding more genes will provide increased support and reveal which morphological characters are important in the taxonomy of the Pisciciolidae.
Chapter 3: Monograph of the North American freshwater fish leeches

Materials and methods

Collections:

Attempts were made to collect all known freshwater fish leeches in North America. Fish were collected using seines and electrofishing equipment, when available. Specimens collected were placed in either 95% ethanol for DNA analysis or a weak ethanol solution for relaxation and subsequent histological and morphological analyses. Once relaxed, specimens were fixed in formalin or Bouin’s solution. Those placed in formalin were washed with 70% ethanol after one day and then stored in 70% ethanol for subsequent paraffin histology. Those placed in Bouin’s solution were washed with 50% ethanol, changed once a day, until a yellow color was no longer observed. Specimens were then stored in 70% ethanol. Suitable museum specimens were used for determination of external characteristics when fresh material was not available. Observations of all available type specimens were made to confirm identifications of newly collected material.

Descriptions:

Species were redescribed when new specimens were collected and significant new information could be determined. One new genus was found while studying museum collections and was described (Williams and Burreson, 2005). Observing both external and internal characteristics is necessary to describe a species. Preliminary drawings of external characteristics of species were made using a camera lucida; final drawings were produced in Adobe Illustrator 10 (Adobe Systems Incorporated). In order to observe the internal characteristics, histologic methods were used. Preserved leeches were infiltrated with paraffin on an automated tissue processor and embedded in paraffin on end in stainless steel tissue molds. Serial transverse and longitudinal sections were cut at a five to ten micron thickness. Sections were mounted on glass slides by floating ribbons of sections on a puddle of 2% formalin on slides coated with Szombathy’s affixative. Slides were air dried in an oven at 42°C overnight. Slides were deparaffinized and stained with hematoxylin and eosin on an automated slide stainer. Cell types
and structures were identified and the internal anatomy was reconstructed. The species were described on the basis of pigmentation, number of eyes, size of suckers, overall body shape, and the architecture of the male and female reproductive systems, the coelomic system, and the digestive system.
Genus *Cystobranchus* Diesing 1859

Type species: *Cystobranchus respirans* (Troschel 1850)

Type locality: Europe

Diagnosis

One pair of pulsatile vesicles per urosomal segment. Six pairs of testiscas. Two pairs of eyes on oral sucker.

Discussion

The genus diagnosis for *Cystobranchus* is left broad because of the lack of information regarding *Cystobranchus verrilli* Meyer 1940. Genera diagnoses are now based mostly on the internal anatomy, little of which is known for *C. verrilli*. Once more is known about *C. verrilli* the generic diagnosis may become more specific. There has been much debate regarding generic characters able to distinguish *Cystobranchus* and *Piscicola* Blainville 1818, however those debates focused on the species of *Cystobranchus* that have subsequently been reassigned to the genus *Gonimosobdella* Williams and Burreson 2005 (Chapter 2, this dissertation). The remaining species of *Cystobranchus* are morphologically indistinguishable from *Piscicola*, however they are genetically distinct (Utevksy and Trontelj, 2004; Chapter 2, this dissertation). *Cystobranchus* should remain a valid genus until specimens of both *C. respirans* and *C. verrilli* can be examined.
Cystobranchus verrilli Meyer 1940

Diagnosis

Description

External morphology: Average length is 10.2 mm. Body divided into trachelosome and urosome by constriction around clitellar region. Urosome dorsoventrally flattened. Oral sucker eccentrically attached with 2 pairs of eyes. Caudal sucker large, wider than body at widest point (Fig. 7). Pigmentation consisting of scattered brownish-black stellate chromatophores or no pigmentation. Caudal ocelli and segmental ocelli not present.

Digestive system: Five crop ceca present, alternating with testisacs (Fig. 7). Intestine with 3 ceca. Postceca fused, number of fenestrae unknown.

Coelomic system: Eleven pairs of pulsatile vesicles.

Reproductive system: Six pairs of testisacs (Fig. 7). Ovisacs reach to second pair of testisacs.

Holotype: Illinois Natural History Survey (INHS) SI.13578
Paratype: USNM 20539; Rock River, near Rockford, Illinois; 15 March 1926; slide.

Remarks
Specimens could not be collected for examination. Material from the USNM (USNM 42598) had previously dried out. Transverse sections were cut, but no information could be
Figure 7

*Cystobranchus verrilli*. Reconstruction of reproductive and digestive systems, drawing adapted from Meyer (1940). B, bursa; C, crop; I, intestine; O, ovisac; PC, postceca; R, rectum; T, testisac.
ascertained given the poor state of the specimens. The placement of this species in the genus *Cystobranchus* may not be correct. Given that the genera *Cystobranchus, Gonimosobdella,* and *Piscicola* are differentiated by internal morphology and very little is known of the internal anatomy of this leech, *C. verrilli* could belong in any of these 3 genera. Specimens need to be collected and sectioned in order to determine what genus this species should be placed in.

It is not known if the pigmentation is described from preserved specimens or live specimens. It is most likely from preserved specimens as it is described as either with no color or with scattered stellate chromatophores, which are what many preserved specimens look like after the pigment has faded.

There is very little known of this species, but it is still possible to differentiate it from the other species of *Cystobranchus. Cystobranchus respirans* has 10 ocelli on the caudal sucker and 4 interstinal ceca (Bielecki, 1997) while *C. verrilli* has no caudal ocelli and 3 intestinal ceca.

**Biology**

Found exclusively in freshwater. Nothing is known regarding seasonality or habitat preference.

**Known hosts and ranges (Fig. 8)**


USNM 20539; Rock River, near Rockford, Illinois; 15 March 1926; slide; reported by Meyer (1940).

USNM 42598; Cedar River, Cedar Falls, Iowa; 20 June 1946.

Figure 8

Distribution map of *Cystobranchus verrilli*. 
Genus *Gonimosobdella* Williams and Burreson 2005

= *Cystobranchus*, in part

Type species: *Gonimosobdella klemmi* Williams and Burreson 2005

Type locality: Arkansas, USA

Emended Diagnosis

Eleven pairs of pulsatile vesicles, one pair per urosomal segment. Six or more pairs of testisacs. Accessory gland cells present or absent. Conducting tissue present connecting bursa and ovisacs. Postceca fused.

Discussion

The diagnosis of *Gonimosobdella* is emended to allow for the variations seen in the 5 species being reassigned to this genus (see Chapter 2, this dissertation). The number of eyes, presence of accessory gland cells, coelomic system connections, and annulation are now variable.

Epshtein *et al.* (1994) include 4 species in *Cystobranchus*: *Cystobranchus mammillatus* (Mlam 1863), *C. verrilli*, *Cystobranchus salmositicus* (Meyer 1946), and *Cystobranchus meyeri* Hayunga and Grey 1976. They were apparently unaware of the existence of *Cystobranchus virginicus* Hoffman 1964 and did not list it in their analyses. All of these species, with the exception of *C. verrilli* are being reassigned to the genus *Gonimosobdella*. Little is known about *C. verrilli*, therefore it is assumed that when Epshtein *et al.* (1994) referred to *Cystobranchus*, they were referring to this group that is being reassigned to *Gonimosobdella*. Epshtein *et al.* (1994) listed 6 species in *Piscicola*: *Piscicola geometra* (Linnaeus 1761), *Piscicola fasciata* Kollar 1842, *Piscicola respirans* (=*Cystobranchus respirans*), *Piscicola punctata* (Verrill 1871), *Piscicola milneri* (Verrill 1874), and *Piscicola pojmanskae* Bielecki 1994. Epshtein (1969) and Epshtein *et al.* (1994) defined the species reassigned to *Gonimosobdella* as having a long bursa, well-developed conducting tissue, and a copulatory area located on the bursa, but lacking
accessory gland cells on the atrium. *Piscicola* were defined as having a long bursa, well-developed conducting tissue, accessory gland cells on the atrium, and a copulatory area located on the clitellum (Epshtein et al., 1994). The only difference between the species reassigned to *Gonimosobdella* and *Piscicola*, as interpreted by Epshtein et al. (1994), is the presence or absence of accessory gland cells on the atrium and the location of the copulatory area.

Bielecki (1997) provided a thorough discussion of the characters used to define the species reassigned to *Gonimosobdella* and *Piscicola*. He differentiated conducting tissue and vector tissue by restricting conducting tissue to the long strands or cords of tissue that lie in close proximity to the ovisacs and vector tissue as a ventral mass of tissue associated with the copulatory area. According to Bielecki (1997) and confirmed in this study, the species reassigned to *Gonimosobdella* have conducting tissue connecting the ovisacs with the atrium (=bursa), whereas *Piscicola* has conducting tissue connecting the ovisacs with a ventral mass of vector tissue. However, Bielecki (1997) also states that accessory gland cells are absent in the species reassigned to *Gonimosobdella*, which is not correct. He also states that *Piscicola* is characterized by mycetomes, which are not present in the species reassigned to *Gonimosobdella*. While this is true for the freshwater *Gonimosobdella* species, the estuarine *Gonimosobdella vivida* (Verrill 1872) possesses mycetomes. Lack of mycetomes can no longer be considered a generic feature for *Gonimosobdella*.

Key to the North American freshwater species of *Gonimosobdella*

1. a. Two pairs of eyes on oral sucker . . . 2
   b. No eyes on oral sucker . . . *Gonimosobdella mammillata*

2. a. Ocelli present on caudal sucker . . . 3
   b. Ocelli absent . . . *Gonimosobdella klemmi*

3. a. Segmental ocelli present . . . *Gonimosobdella meyeri*
   b. Segmental ocelli absent . . . 4
4. a. Body black . . . *Gonimosobdella salmositica*

   b. Body translucent . . . *Gonimosobdella virginica*
Gonimosobdella klemmi Williams and Burreson 2005

Diagnosis

Length up to 12 mm. Body flattened and wide, generally cream colored with transverse bands of stellate chromatophores and small orange-pigment flecks. Suckers well developed. Two pairs of eyes present on oral sucker, ocelli absent on caudal sucker. Urosomal segments 3(5) annulate. Mycetomes absent. Crop ceca and large intestinal ceca present. Postceca fused with 4 fenestrae. Coelomic system with ventral, dorsal, lateral, and testicular sinuses, and 11 pairs of pulsatile vesicles. No coelomic connections intersegmentally. Thirteen pairs of testisacs. Accessory gland cells present on atrium. Ovisacs long, often to XIX then turning anteriorly, becoming oviscas, and ending at level of female pore. Conducting tissue present in the form of a single strand originating from the posterior portion of the bursa that splits into 2 strands around the common oviduct and then anastomoses into a single mass posterior to female pore that surrounds oviducts and ovisacs.

Description

External morphology: Length up to 12 mm; width up to 4.5 mm. Dorsoventrally flattened; unusually wide and flat throughout the urosome. Live specimens cream colored with black stellate chromatophores over almost entire body (Fig. 9A). Unpigmented longitudinal band medially on dorsal surface. Seventeen evenly spaced transverse unpigmented bands on dorsal and ventral surfaces. Oral sucker with 3 patches of black chromatophores, 2 pairs of eyes concealed in posterior patch. Caudal sucker with diffuse black chromatophores. Entire body covered with uniformly distributed orange flecks. Most preserved specimens translucent with little pigmentation although some have diffuse brown spots present on entire body. Two pairs of eyes present on oral sucker of some preserved specimens, most have only 1 obvious pair. No segmental ocelli or caudal sucker ocelli. Annulation 3(5) with the first and third primary annuli
Figure 9

*Gonimosobdella klemmi.* A. External morphology. B. Reconstruction of reproductive and digestive systems. C, crop; I, intestine; O, ovisac; P, proboscis; PC, postceca; R, rectum; T, testisac.
divided in some segments. Oral sucker strongly eccentric and of moderate size. Caudal sucker subterminal and oblong.

**Digestive system:** Mouthpore located in posterior half of oral sucker. Mycetomes absent. Proboscis extends nearly to ganglion X. Crop expands at each urosonic ganglia from XIII to XVIII, alternating with testisacs (Fig. 9B). Intestine and postceca begin at ganglion XIX. Intestine with 4 pairs of large, lateral ceca; postceca fused with 4 fenestrae at the level of ganglia XX-XXIII.

**Reproductive system:** Thirteen pairs of testisacs intersegmentally from XIII to XXVI (Fig. 9B). Vasa deferentia enlarge to ejaculatory bulbs at XI. Ejaculatory ducts enter atrial cornua that fuse to become common atrium. Atrium muscular with a small lumen that opens into bursa. Bursa extends slightly posterior to male pore. Accessory gland cells cover dorsal and lateral surfaces of common atrium. Female gonopore occurs just posterior to ganglion XII. Short common oviduct divides into paired oviducts that typically extend posteriorly to XV but can extend as far as ganglion XIX; in either case, at their posterior extent they become coiled and then turn anteriorly and become ovisacs, continuing anteriorly to level of female gonopore (Figs. 10B, 10C).

Conducting tissue present as a single strand originating in epithelial lining of bursa lumen. Conducting tissue strand runs posteriorly, moving to outer surface of bursa then detaching and becoming a free strand. After detaching from bursa, conducting tissue splits into 2 strands around common oviduct and then anastomoses into a single mass posterior to female pore, surrounding oviducts and ovisacs (Figs. 10B, 10C).

**Coelomic system:** Eleven pairs of pulsatile vesicles (Fig. 10A) Ventral, dorsal, lateral, and testicular sinuses present. Ventral sinus expands at ganglia to connect to anterior portion of pulsatile vesicle and then to expanded dorsal sinus. Posterior portion of pulsatile vesicle connects to lateral sinus. No coelomic connections intersegmentally.
Figure 10

*Gonimosobdella klemmi.* A. Diagrammatic view of coelomic system, left side segmental, right side intersegmental. B. Reconstruction of the terminal reproductive systems, ventral view. C. Reconstruction of the terminal reproductive systems, lateral view. AG, accessory gland cells; CT, conducting tissue; D, dorsal sinus; ED, ejaculatory duct; FG, female gonopore; L, lateral sinus; M, male gonopore; O, ovisac; PV, pulsatile vesicle; T, testisac; V, ventral sinus.
Holotype: United States National Museum (USNM) 1073199; Middle Fork, Little Red River, Searcy County, Arkansas; 35°48'42.9" N; 92°33'16.3" W; 31 March 2004; host - *Campostoma pullum* (Cyprinidae).

Paratypes: USNM 1073200; Middle Fork, Little Red River, Searcy County, Arkansas; 35°48'42.9" N; 92°33'16.3" W; 31 March 2004; host - *C. pullum*.

Remarks

The combination of characteristics that separates this species from all other piscicolid leeches is the presence of 13 pairs of testisacs, the ovisac morphology, and the conducting tissue arrangement. The number of testisacs is the most unusual characteristic and can stand alone as the distinguishing character of *G. klemmi*. All the other 150 described species of piscicolid leeches have 4, 5, or 6 pairs of testisacs (Sawyer, 1986b).

The oviduct/ovisac morphology in *G. klemmi* is unusual. The portion of the female reproductive system between the female gonopore and the posterior coil is interpreted as paired oviducts because of the narrow, tubular nature of this portion of the system. The enlarged, more saccular, portion that extends anteriorly is interpreted as ovisacs. Most piscicolid leeches have very short oviducts that reach the first or second pair of testisacs, whereas *G. klemmi* can have oviducts that reach the level of the seventh pair of testisacs. It is also unusual that the oviducts coil at their posterior ends and then enlarge as ovisacs and continue anteriorly to the level of the female pore.

Conducting tissue is a character that recently has been given increased importance in the classification of piscicolid leeches. *Gonimosobdella mammillata* (Malm 1863) and *Gonimosobdella salmositica* (Meyer 1946) both have 2 strands of conducting tissue connecting the bursa to the ovisacs that remain separate for the entire length of the conducting tissue (Meyer and Roberts, 1977; Burreson *et al.*, 2005). *Gonimosobdella meyeri* (Hayunga and Grey 1976) has
the same arrangement as *G. klemmi* except that the mass remains confined to the space between the 2 ovisacs in *G. meyeri* while it surrounds the ovisacs in *G. klemmi*. *Gonimosobdella virginica* (Hoffman 1964) has an arrangement of conducting tissue similar to that of *G. klemmi* except that the mass forming posterior to the female pore in *G. virginica* has extensions that continue posteriorly along the medial surface of the ovisacs (Burreson *et al.*, 2005), whereas *G. klemmi* does not have these posterior extensions.

**Biology**

Two infected specimens of *Campostoma pullum* were collected and observed alive. One fish had a leech on a pectoral fin and another on the caudal fin; the second fish had 2 leeches on the caudal fin and 2 on an anal fin. The caudal sucker of all leeches was firmly attached medially to a fin ray, whereas the oral sucker was often unattached. On removal from the fish, the leeches did not attach to the container they were placed in for some time and initially seemed unable to attach to a flat surface. Typical "inch worm-like" movement was attempted but not successfully because of the inability to attach the caudal sucker. Little other movement was observed with the exception of slight constriction and elongation. The prevalence ranged from 19% to 100% and was 100% for 5 of 9 samples. Average infection intensity ranged from 1 to 3 leeches per fish host and was greater than 1 for 6 of 11 samples. The leeches appear to be abundant during the months of March and April, but further information on the seasonality of the leech is not available.

**Known hosts and ranges (Fig. 11)**

USNM 1073199; Middle Fork, Little Red River, Searcy County, Arkansas; 35°48'42.9" N; 92°33'16.3" W; 31 March 2004; host - *C. pullum*.
Figure 11

Distribution map of *Gonimosobdella klemmi*.
USNM 1073200; Middle Fork, Little Red River, Searcy County, Arkansas; 35°48'42.9" N;
92°33'16.3" W; 31 March 2004; host - C. pullum.

USNM 1075116; Little Red River, Searcy County, Arkansas; 23 March 1997; host –
Campostoma oligolepis Cyprinidae.

USNM 1075117; Green Creek, Union County, Illinois; 24 April 1997; host – Lythrurus
umbratilis Cyprinidae.

USNM 1075118; Mill Creek, Union County, Illinois; 37°21'04"N; 89°19'38"W; 30 April 1997;
host – Cyprinella lutrensis Cyprinidae.

USNM 1075119; Little Red River, Searcy County, Arkansas; 23 March 1997; host – C. pullum.

USNM 1075120; Castor River, Bollinger County, Missouri; 12 March 1996; host – C. oligolepis.

USNM 1075121; Apple Creek, Perry County, Missouri; 22 March 1996; host – C. pullum.

USNM 1075122; St. Francis River, Wayne County, Missouri; 12 March 1996; host – C. pullum.

USNM 1075123; Juden Creek, Cape Girardeau County, Missouri; 16 April 1978; host – C.
pullum.

USNM 1075124; Big River, Washington County, Missouri; 20 April 1996; host – Campostoma
hybrid.

USNM 1075125; Big River, Washington County, Missouri; 20 April 1996; host – C. oligolepis.

USNM 1088447; Middle Fork, Little Red River, Searcy County, Arkansas; 35°48'42.9" N;
92°33'16.3" W; 31 March 2004; host - C. pullum.
Gonimosobdella mammillata (Malm 1863) n. comb.
= Cystobranchus mammillatus (Malm 1863)
= Platybdella mammillata Malm 1863

Diagnosis

Length up to 35 mm. Body dorsoventrally flattened and wide with brownish grey transverse bands on oral sucker, 6 bands on trachelosome and brownish gray shading on lateral edges of urosome. No eyes, segmental ocelli, or caudal ocelli. Oral sucker as wide as trachelosome. Caudal sucker large, subterminal. Mycetomes absent. Postceca fused with 5 fenestrae. Coelomic system with ventral, dorsal, thin-walled lateral, thick-walled lateral, and testicular sinuses. Eleven pairs of pulsatile vesicles. Coelomic connections segmentally and intersegmentally. Six pairs of testisacs. Very small common atrium. Conducting tissue present as a single cord off the posterior surface of the bursa, dividing around the female gonopore and remaining separate and closely associated with the ovisacs. Ovisacs reach to second pair of testisacs.

Description

*External morphology:* Length up to 35 mm. Body dorsoventrally flattened and wide. Oral sucker as wide as trachelosome, eccentrically attached. Caudal sucker large, subterminal. No eyes, segmental ocelli, or caudal ocelli. Pulsatile vesicles visible externally. Brownish gray pigment band on oral sucker. Six transverse brownish gray bands on the trachelosome. Lateral edges of urosome with brownish gray shading (Fig. 12A). Seven annulate.

*Digestive system:* Mouthpore located just posterior to center of oral sucker. Proboscis reaches ganglion IX. Crop expands between testisacs (Fig. 12B). Intestine with 3 pairs of diverticula. Postceca fused with 5 fenestrae. Mycetomes absent.
Figure 12

*Gonimosobdella mammillata.* A. External morphology, pigmentation based on descriptions by Malm (1863) and Bielecki (1997). B. Reconstruction of reproductive and digestive systems. B, bursa; C, crop; F, fenestrae; I, intestine; O, ovisac; P, proboscis; PC, postceca; R, rectum; T, testisac.
Coelomic system: Eleven pairs of pulsatile vesicles. Ventral, dorsal, thin-walled lateral, thick-walled lateral, and testicular sinuses present. Ventral sinus expands at ganglia to connect to thin-walled lateral sinus and anterior portion of pulsatile vesicle (Fig. 13C). Posterior portion of pulsatile vesicle connects to thick-walled lateral sinus. Dorsal sinus expands intersegmentally to connect to testicular sinus and thin-walled lateral sinus.

Reproductive system: Six pairs of testisacs intersegmentally from XIII/XIV to XVIII/XIX (Fig. 12B). Vasa deferentia loop then expand to form the atrial cornua (Fig. 13A, 13B). Atrial cornua remain separate for most of their length then fuse to form a small common atrium that immediately enters the bursa. Ovisacs reach to the second pair of testisacs. Conducting tissue present as a single cord off the posterior surface of the bursa, dividing around the female gonopore and remaining separate and closely associated with the ovisacs. No accessory gland cells.

Holotype: none deposited
Paratype: none deposited
A neotype should be designated from the type locality in Europe when suitable material is available.

Remarks

Newly collected specimens were not available for this study, however, specimens reported by Meyer and Roberts (1977) and deposited in the USNM (USNM 52717) were available for examination and sectioning. Transverse serial sections were cut to confirm the identification of the leech and some minor differences were seen when compared with the description given by Meyer and Roberts (1977) and Bielecki (1997). Nonetheless, these specimens are believed to be G. mammillata and remain the only specimens of G. mammillata reported from North America. Meyer and Roberts (1977) prepared frontal sections, which are not
Figure 13

*Goninosobdella mammillata.* A. Reconstruction of the terminal reproductive systems, dorsal view. B. Reconstruction of the terminal reproductive systems, lateral view. C. Diagrammatic view of coelomic system, left side segmental, right side intersegmental. A, atrium; B, bursa; C, crop; CT, conducting tissue; D, dorsal sinus; ED, ejaculatory ducts; FP, female gonopore; G, ganglion; L, thick-walled lateral sinus; LT, thin-walled lateral sinus; M, male gonopore; O, ovisac; PV, pulsatile vesicle; T, testisac; V, ventral sinus.

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ideal for examining the reproductive systems. The conducting tissue arrangement is described as paired strands connecting each ovisac with the posterior wall of the bursa (Meyer and Roberts, 1977; Bielecki, 1997). When the frontal sections (USNM 52718) deposited by Meyer and Roberts (1977) were examined, no conducting tissue could be seen. The transverse sections revealed conducting tissue that begins as a single cord on the dorsal surface of the bursa, then splits around the female gonopore and becomes closely associated with the ovisacs. Whether or not this is a difference between the North American and Eurasian populations cannot be determined until specimens from the Eurasian population can be examined.

Meyer and Roberts (1977) also reported that the vasa deferentia expand in size and join independently with the bursa. This did appear to be the case in the frontal sections (USNM 52718), but the transverse sections showed that the vasa deferentia expand in size, forming the atrial cornua, then fuse just before fusing with the bursa. Given the extremely small size of the common atrium, it would be nearly impossible to see the common atrium in frontal sections.

*Gonimosobdella mammals* differs from all other species of *Gonimosobdella* in terms of pigmentation, number of fenestrae, and presence or absence of eyes. *Gonimosobdella klemmi*, *G. meyeri*, and *G. virginica* all have transverse bands on the entire body and *G. salmositica* has a black body. *Gonimosobdella mammals* has transverse bands but they are restricted to the trachelosome with some pigmentation on the lateral edges but no banding on the urosome. *Gonimosobdella klemmi*, *G. meyeri*, and *G. salmositica* all have 4 fenestrae in the postceca while *G. mammals* has 5 fenestrae. The number of fenestrae is not known in *G. virginica*.

*Gonimosobdella mammals* has no eyes on the oral sucker and all other species of *Gonimosobdella* have 2 pairs of eyes. However, Malm (1863) does note dark spots that resemble eyes, but he states they should not be considered as eyes. Bielecki (1997) states that eyes are usually not present and when they are present there are 2 poorly developed pairs of eyes.

*Gonimosobdella mammals* appears to be most similar to *G. virginica* as both share the same coelomic connections and the same arrangement of conducting tissue. *Gonimosobdella*
salmositica shares the same coelomic connections with the dorsal sinus connecting to the
testicular and lateral sinuses intersegmentally but has 2 strands of conducting tissue that arise
independently from the posterior portion of the bursa. Gonimosbdella mammillata, G. klemmi,
G. meyeri, and G. virginica all have a single strand or mass off the posterior portion of the bursa
that splits around the female gonopore. Gonimosobdella klemmi has no intersegmental coelomic
connections and G. meyeri has all sinuses connecting intersegmentally.

Biology

Found exclusively in freshwater. Gonimosobdella mammillata is a specific parasite of
Lota lota. This species has only been reported from 1 location in North America. However, the
host fish has a circumartic freshwater distribution (Froese and Pauly, 2006) and G. mammillata
most likely has a similar distribution given the fish host's wide range in U.S.S.R., Poland,
Germany, France, Sweden, and Norway (Meyer and Roberts, 1977). A seasonality study
performed in Norway found that G. mammillata parasitizes L. lota from early winter to late
spring. During this time the host is generally found in shallow water. Leeches were not seen in
the summer when the fish were in deeper waters (Halvorsen, 1971). The leeches were always
found in the gill cavity, either on the inside of the operculum or attached to a gill arch (Halvorsen,
1971).

Known North American hosts and ranges (Fig. 14)
USNM 52717; Mackenzie River, near Aklavik, Northwest Territories, Canada; 15 June 1959;
host – Lota lota; reported by Meyer and Roberts (1977).
USNM 52718; Mackenzie River, near Aklavik, Northwest Territories, Canada; 15 June 1959;
host – L. lota; reported by Meyer and Roberts (1977).
Figure 14

Distribution map of *Gonimobdella mammillata*.
Goninosobdella meyeri (Hayunga and Grey 1976) n. comb.
=Cystobranchus meyeri Hayunga and Grey 1976

Diagnosis
Length up to 6 mm. Body slightly dorsoventrally flattened with 17 transverse orange pigment bands. Oral sucker eccentrically attached with 2 pairs of eyes. Caudal sucker subterminal with 8 ocelli. Urosomal segments 7 annulate. Two pairs of segmental ocelli in urosomal segments. Mycetomes absent. Postceca fused with 4 fenestrae. Coelomic system with dorsal, ventral, lateral, and testicular sinuses, and 11 pairs of pulsatile vesicles. Six pairs of testisacs. Accessory gland cells present. Conducting tissue present in the form of a mass on the bursa that splits into 2 strands around the common oviduct then anastomoses into a mass posterior to the female pore between the ovisacs.

Description
External morphology: Length up to 6 mm. Width up to 0.5 mm. Slightly dorsoventrally flattened. Body not distinctly divided into trachelosome and urosome in well-relaxed specimens, but more distinct in contracted specimens. Oral sucker eccentrically attached and of moderate size. Two pairs of eyes present on the oral sucker, no eyes on the trachelosome (Fig. 15C). Caudal sucker subterminal and equal to or wider than widest part of body. Urosomal segments 7 annulate. Eight ocelli present on the caudal sucker, 4 on each lateral margin and none at the posteriormost part of the sucker (Fig. 15D). Two pairs of segmental ocelli present in urosomal segments. Body with 17 transverse orange pigments bands with small stellate chromatophores (Fig. 15A). Oral sucker with 3 distinct pigment bands, 1 on the anteriormost portion of the sucker, 1 on the middle sucker, and the darkest encompassing the eyes. Caudal sucker with diffuse pigmentation and no distinct rays.
Figure 15

*Gonimosobdella meyeri.* A. External morphology. B. Reconstruction of reproductive and digestive systems. C. Oral sucker, lateral view. D. Caudal sucker, lateral view. E. Reconstruction of the terminal reproductive systems, ventral view. F. Reconstruction of the terminal reproductive systems, lateral view. G. Diagrammatic view of coelomic system, left side intersegmental, right side segmental. A, atrium; AG, accessory gland cells; B, bursa; C, crop; CT, conducting tissue; D, dorsal sinus; F, fenestrae; FG, female gonopore; I, intestine; L, lateral sinus; M, male gonopore; O, ovisac; P, proboscis; PC, postceca; PV, pulsatile vesicle; R, rectum; T, testisac; V, ventral sinus.
Digestive system: Mouthpore located centrally in oral sucker. Proboscis extends past VIII. Crop ceca alternate with testisacs (Fig. 15B). Postceca and intestine begin at XIX. Intestine with 4 ceca. Postceca fused with 4 fenestrae. Rectum short, wide, begins at XXIV. Mycetomes absent.

Coelomic system: Eleven pairs of pulsatile vesicles. Ventral, dorsal, lateral, and testicular sinuses present (Fig. 15G). All sinuses connect intersegmentally. Pulsatile vesicle connects to lateral sinus at ganglia.

Reproductive system: Six pairs testisacs intersegmentally from XIII/XIV to XVIII/XIX (Fig. 15B). Vasa deferentia enlarge to ejaculatory ducts at XI, immediately turning ventrally entering atrial cornua. Atrial cornua shortly fuse forming wide common atrium with narrow opening into wide bursa. Bursa extends slightly posterior to male gonopore. Thin layer of accessory gland cells surrounds ejaculatory ducts with larger mass on ventral surface of bursa. Female gonopore occurs at ganglion XII. Short common oviduct divides into paired short ovisacs. Conducting tissue present as mass on posterior surface of bursa. Conducting tissue splits into two strands around common oviduct and then anastomoses into a single mass between ovisacs (Figs. 15E, 15F).

Holotype: USNM 52719: April 1975, Lisha Kill, Mohawk River, Nikayuna Township, Schenectady County, NY on Catostomus commersonii.

Paratypes: USNM 52720: April 1975, Lisha Kill, Mohawk River, Nikayuna Township, Schenectady County, NY on C. commersoni; British Museum (Natural History) BM(NH) 1975.21.2; AMNH 4016; H. W. Manter 20275.

Remarks

The original description is emended to include information regarding the conducting tissue and coelomic system and corrected to indicate that there are 4 and not 5 fenestrae in the postceca. The figures also show that the crop ceca are larger than drawn by Hayunga and Grey (1976, Fig. 1), although that may be dependent on when the last blood meal was taken. Hayunga
and Grey (1976, Fig. 3) draw the pigmentation on the oral sucker as a dark sucker with unpigmented edges. Specimens collected for this study had 3 distinct pigment bands on the oral sucker.

This species is easily recognized by the small size. It is the smallest of the *Gonimosobdella* species with the maximum length reaching 6 mm. The segmental ocelli are also a distinguishing feature as *G. meyeri* is the only freshwater species of *Gonimosobdella* with segmental ocelli. *Gonimosobdella meyeri* most closely resembles *G. virginica*. Externally they show differences in body size and shape, presence or absence of segmental ocelli, and shape of caudal ocelli. The pigmentation patterns are similar throughout the trachelosome and beginning of the urosome, posterior to this region *G. virginica* exhibits no pigmentation until the caudal sucker. Both species have nearly identical conducting tissue arrangements and similar accessory gland cell distributions. However, *G. meyeri* has only short extensions of the conducting tissue after it fuses posterior to the female pore and *G. virginica* has long extensions. Differences in the coelomic system are also seen between these 2 species. *Gonimosobdella meyeri* has an intersegmental connection between the ventral and dorsal sinuses and no segmental connections while *G. virginica* has an intersegmental connection between the lateral and dorsal sinuses and a segmental connection between the ventral and lateral sinuses.

**Biology**

Found exclusively in freshwater environments. The range of this species may be far greater than shown by the current collections and reports. Piscicolid leeches are rarely collected except by researchers specifically targeting them and given the extremely small size of this species, it can be easily missed, even by those looking for it. Vector studies performed using *C. meyeri* found that it was present in the Opequon Creek, West Virginia from late March through early May with the highest abundances in April (Putz, 1972b). Some specimens collected for this
study were collected in late May from Spivey Creek, Tennessee (USNM 1088444), indicating there may be a slight regional variability in the seasonality of G. meyeri.

Known hosts and ranges (Fig. 16)
USNM 52719; Lisha Kill, Mohawk River, Nikayuna Township, Schenectady County, New York; April 1975; host – C. commersoni; reported by Hayunga and Grey (1976)
USNM 52720; Lisha Kill, Mohawk River, Nikayuna Township, Schenectady County, New York; April 1975; host – C. commersoni; reported by Hayunga and Grey (1976).
USNM 1088444; Spivey Creek, Tennessee; 36°04.001'N; 82°30.135'W; 20 May 2004; new report.
British Museum (Natural History) BM(NH) 1975.21.2; Lisha Kill, Mohawk River, Nikayuna Township, Schenectady County, New York; April 1975; host – C. commersoni; reported by Hayunga and Grey (1976).
AMNH 4016; Lisha Kill, Mohawk River, Nikayuna Township, Schenectady County, New York; April 1975; host – C. commersoni; reported by Hayunga and Grey (1976).
H. W. Manter 20275; Lisha Kill, Mohawk River, Nikayuna Township, Schenectady County, New York; April 1975; host – C. commersoni; reported by Hayunga and Grey (1976).
Opequon Creek, Leetown, West Virginia; 39°22'30"N; 77°57'30"W; host - Rhinichthys cataractae (Cyprinidae); reported as C. virginicus by Putz (1972a) – see C. virginicus remarks.
Craig's Creek, Virginia; 37°30.712'N; 80°05.359'W; 22 April 2004; host – Campostoma anomalum (Cyprinidae); new report.
Little River, Virginia; 37°00.309'N; 80°22.903'W; 24 April 2004; host - C. anomalum, Nocomis sp.; new report.
Opequon Creek, Leetown, West Virginia; 39°22.025'N; 77°57.463'W; 29 April 2004; host – R. cataractae; new report.
Figure 16

Distribution map of *Gonimosobdella meyeri*. 
Caney Creek, Tennessee; 35°48.693’N; 83°14.873’W; 20 May 2004; host – *Campostoma* sp.

Little River, Virginia; 37°00.309’N 80°22.903’W; 24 April 2004; hosts – *C. anomalum, Nocomis* sp., *Percina* sp.; new report.
Gonimosobdella salmositica (Meyer 1946) n. comb.
= Cystobranchus salmositicus (Meyer 1946)
= Piscicola salmositica Meyer 1946

Diagnosis

Description
External morphology: Length up to 31 mm. Width up to 6.0 mm. Body smooth, lacking tubercles, or papillae. Eleven pairs of large pulsatile vesicles obvious on lateral margins of urosome (Fig. 17A). Oral sucker up to 2.5 mm in diameter, 2 pairs of eyes (Fig. 17C). Pigmentation on oral sucker variable, stellate black chromatophores cover sucker with variable unpigmented area anterior to eyes. Caudal sucker up to 4.0 mm in diameter with 8 to 10 large ocelli on lateral and posterior margin. Most ocelli rod-shaped, but ocelli on lateral margins appear crescent shaped. Caudal sucker evenly pigmented with stellate black chromatophores except for unpigmented areas outside of ocelli (Fig. 17D). Pigmentation consists of stellate black chromatophores distributed evenly on urosome giving leech an overall dark gray to black coloration.
Reproductive system: Six pairs of large testisacs intersegmentally from XIII/XIV to XVIII/XIX. Ejaculatory ducts convoluted entering large atrial cornua. Bursa large. Well-developed masses of accessory gland cells dorsally and laterally on atrial cornua and on common atrium (Figs. 18A, 18B). Two cords of conducting tissue arising independently from posterior portion of bursa.
Figure 17

Figure 18

Gonimosobdella salmositica. A. Reconstruction of the terminal reproductive systems, dorsal view. B. Reconstruction of the terminal reproductive systems, lateral view. C. Diagrammatic view of coelomic system, left side intersegmental, right side segmental. AG, accessory gland cells; B, bursa; CT, conducting tissue; D, dorsal sinus; E, epididymus; FG, female gonopore; L, thin-walled lateral sinus; LT, thick-walled lateral sinus; M, male gonopore; O, ovisac; PV, pulsatile vesicle; T, testisac; V, ventral sinus.
Cords continue posteriorly, separated for their entire length, and become closely associated with paired ovisacs (Figs. 18A, 18B).

**Coelomic system:** Eleven pairs of large pulsatile vesicles. Dorsal and ventral sinuses, and both thin-walled and thick-walled lateral sinuses present. Ventral sinus connects to thin-walled lateral sinus and pulsatile vesicle segmentally. Posterior portion pulsatile vesicle connects with thick-walled lateral sinus. Testicular sinus connects with thin-walled lateral sinus and dorsal sinus intersegmentally (Fig. 18C).

**Digestive system:** Mouthpore located centrally in oral sucker. Proboscis extends posterior to IX. Crop ceca alternate with testisacs (Fig. 17B). Postceca and intestine begin at XIX. Intestine with 5 ceca, last pair very small. Postceca fused with 4 fenestrae. Moderate size rectum. Mycetomes absent.

**Holotype:** USNM 20803; Soos Creek Hatchery, Washington.

**Paratype:** USNM 42696; Eel River, California; 5 February 1941.

**Remarks**

Comparison of specimens collected from Vancouver Island, British Columbia, Canada, with the holotype and paratypes of *Piscicola salmositica* confirm that the specimens from Canada are the same species. The original description of *P. salmositica* by Meyer (1946b), based on preserved specimens from California and Washington, U.S.A., was complete and accurate in almost all respects, omitting only information on the presence or absence of accessory gland cells on the atrial cornu, the presence or absence of mycetomes, and erroneously reporting the absence of conducting tissue. Bielecki (1997) also interpreted the absence of accessory gland cells on the atrium in the figure of Meyer (1946b) as true absence of these glands, but Meyer (1946b) made no mention of accessory gland cells; much less state their absence. Bielecki (1997) stated that plate III, figure 3 in Meyer (1946b) illustrates conducting tissue connecting the ovaries with the
atrium, but this interpretation is incorrect. Nonetheless, *G. salmositica* does possess conducting tissue.

Live specimens were examined from Vancouver Island and had 8 ocelli on the caudal sucker, and all had the body and suckers covered by stellate-shaped black chromatophores. Meyer (1946b) noted between 8 and 10 ocelli on the caudal sucker, with most specimens from California having 10 ocelli and most specimens from Washington have 8 ocelli. Meyer (1946b) also noted that most specimens from California had stellate chromatophores, whereas most specimens from Washington lacked such pigmentation; but Meyer (1946b) only observed preserved specimens, and his observations on the absence of pigmentation on most Washington specimens may have been a fixation artifact. None of the 33 paratypes examined comprising USNM 42696, all of which were collected in California, displayed pigmentation.

*Gonimosobdella salmositica* has cords of conducting tissue connecting the ovisacs to the bursa. This supports the inclusion of *G. salmositica* in *Gonimosobdella* (=Cystobranchus, in part) as suggested by both Epshtein *et al.* (1994) and Bielecki (1997). *Gonimosobdella salmositica* also has accessory gland cells on the atrium, a character of *Piscicola* sensu Epshtein *et al.* (1994) and Bielecki (1997). However, those authors developed criteria primarily on the basis of European species because the internal anatomy of North American species is inadequately known. It is now clear that presence or absence of accessory gland cells is not a good criterion for separating the 2 genera. The best criterion is the presence of cords of conducting tissue connecting the ovisac and the bursa in *Gonimosobdella* in contrast to cords of conducting tissue connecting ovisacs with a ventral mass of vector tissue in *Piscicola*. In addition, *Piscicola* has an external ventral copulatory area, whereas *Gonimosobdella* has a copulatory area on the internal wall of the bursa. Given the sense of *Gonimosobdella* and *Piscicola*, *Piscicola salmositica* is placed within *Gonimosobdella* as suggested by Epshtein *et al.* (1994) and Bielecki (1997), and the combination *Gonimosobdella salmositica* (Meyer 1946) is proposed.
Biology

Specimens for this study were collected from the Nitnat River, Vancouver Island, Canada. The number of individuals of *G. salmositica* in the Nitnat River, and perhaps in many other Pacific Northwest salmon rivers, is one of the highest concentrations of piscicolid leeches anywhere. At the hatchery, the returning fish are anesthetized slightly with clove oil and lifted to sorting troughs with a large lift auger. The water from the auger is diverted into a large round tank, and the fish slide down aluminum sorting troughs. The round tank held thousands of leeches that had become dislodged from the fish during the lifting process from the previous day's operations. On 14 October 2003, only 1 raceway of returning fish was processed; but by the end of the operation, many thousands more leeches had been added to the effluent tank, and the sorting trough also contained an abundance of leeches that had detached as the fish slid down the trough.

In a study performed along the western coast of British Columbia, Washington, Oregon, and California, specimens of *G. salmositica* were collected in the fall, winter, and early spring with highest abundances reported in November and December. They were never encountered in the summer (Becker and Katz, 1965). During the fall the host fish, usually a species of salmon, migrates into the streams from the Pacific Ocean and the leeches begin to appear. Specimens were found in streams with swift currents, low water temperature, high dissolved oxygen content, and gravelled beds (Becker and Katz, 1965). The abundance of leeches was tightly correlated with the abundance of migrating salmon. It is believed that most leeches die after the migration of the salmon, however some smaller leeches may survive through the summer (Becker and Katz, 1965).

Known hosts and ranges (Fig. 19)

USNM 20803; Soos Creek Hatchery, King County, Washington.

USNM 42696; Eel River, Mendocino County, California; 5 February 1941.
Figure 19

Distribution map of *Gonimosobdella salmositica.*
USNM 50139; Fall Creek, Siskiyou County, California; 30 January 1937.

USNM 60126; Crystal Lake, near Cassel, Shasta County, California; 27 May 1974.

USNM 84500; Seattle, Lake Washington, Washington; 16 May 1899.

USNM 84511; Eel River, Mendocino County, California; 5 February 1941.

USNM 84512; Fall Creek, Klamath River Drainage, Siskiyou County; 14 April 1938.

USNM 1070012; Nitnat River, Vancouver Island, British Columbia, Canada; 14 October 2003;
host – *Oncorynchus* spp.; new report.

USNM 1088445; Nitnat River, Vancouver Island, British Columbia, Canada; 14 October 2003;
host – *Oncorynchus* spp.; new report.

North Santiam River, Oregon; summer 2006; host – *O. mykiss*; new report.
Gonimosobdella virginica (Hoffman 1964) n. comb.

= Cystobranchus virginicus Hoffman 1964

Diagnosis

Length up to 35 mm. Body flaccid, with 9 to 10 faint brownish-orange pigment bands. Oral sucker eccentrically attached with 2 pairs of rapidly fading eyes. Caudal sucker subterminal with rapidly fading ocelli. Mycetomes absent. Postceca fused. Coelomic system with dorsal, ventral, thin-walled lateral, thick-walled lateral, and testicular sinuses and 11 pairs of pulsatile vesicles. Six pairs of testisacs. Accessory gland cells present. Conducting tissue present as single cord emerging from bursa, bifurcating around common oviduct, fusing posterior to common oviduct, bifurcating again, and becoming closely associated with ovisacs.

Description

External morphology: Length up to 35.0 mm. Width up to 4.0 mm. Body smooth, thin-walled, transparent, extremely flaccid, almost entirely lacking in musculature. Eleven pairs of small pulsatile vesicles apparent on lateral margins of urosome (Fig. 20A). Suckers small, oral up to 1.0 mm in diameter, caudal up to 1.5 mm in diameter. Oral sucker eccentrically attached with faint brownish-orange pigment band and 2 pairs of black pigment concentration resembling eyes (Fig. 20C). Eyes fade rapidly after death, even in absence of fixatives. Caudal sucker subterminal with brownish-orange pigment bands radiating from urosome/sucker juncture to sucker margin, terminating in ten black pigment concentrations resembling ocelli (Fig. 20D). Ocelli fade rapidly on death. Pigmentation consists of faint brownish-orange transverse bands, 5 bands on trachelosome, 3 on posterior portion of clitellum, 1 or 2 on anterior portion of urosome (Fig. 20A).

Reproductive system: Six pairs of testisacs. Large looping ejaculatory ducts. Large bursa. Accessory gland cells weakly developed on dorsal surface of atrial cornu. Conducting tissue
Figure 20

present as a single cord emerging dorsally from posterior portion of bursa in an area where interior bursa consists of highly folded columnar epithelium. Conducting tissue cord continues posteriorly, bifurcating around common oviduct and fusing into single cord posterior to common oviduct (Fig. 21A, 21B). Conducting tissue bifurcates again and cords are closely associated with ovisacs for their length. External, ventral copulatory area absent.

Coelomic system: Eleven pairs of pulsatile vesicles. Dorsal and ventral sinus and thin-walled and thick-walled lateral sinuses present. Ventral sinus connects to thin-walled lateral sinus and pulsatile vesicle segmentally. Posterior portion of pulsatile vesicle connects to thick-walled lateral sinus. Testicular sinus connects to thin-walled lateral sinus and dorsal sinus intersegmentally (Fig. 21C).

Digestive system: Mouthpore located centrally in oral sucker. Proboscis extends posterior to VIII. Crop ceca large (Fig. 20B). Postceca and intestine begin at XIX. Intestine with 5 ceca, last pair very small. Postceca fused, presence of fenestrae could not be determined. Moderate size rectum. Mycetomes absent.

Holotype: USNM 30843; South Fork Roanoke River, Virginia, about 3 miles south of Shawsville; 2 May 1963.

Paratype: USNM 30844; South Fork Roanoke River, Virginia, about 3 miles south of Shawsville; 2 May 1963.

Remarks

Eyes on the oral sucker and ocelli on the caudal sucker are much more ephemeral than those typically observed in the Piscicolidae, which often persist even after fixation. Nonetheless, *G. virginica* should be considered to have 2 pairs of eyes on the oral sucker and 10 ocelli on the caudal sucker.
Figure 21

Gonimosobdella virginica. **A.** Reconstruction of the terminal reproductive systems, ventral view. **B.** Reconstruction of the terminal reproductive systems, lateral view. **C.** Diagrammatic view of coelomic system, left side intersegmental, right side segmental. A, atrium; AG, accessory gland cells; B, bursa; CT, conducting tissue; D, dorsal sinus; E, epididymus; ED, ejaculatory duct; FG, female gonopore; L, thin-walled lateral sinus; LT, thick-walled lateral sinus; M, male gonopore; O, ovisac; PV, pulsatile vesicle; T, testisac; V, ventral sinus.

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Comparison of our specimens from the Valley River, North Carolina, U. S. A., with the holotype and paratype of *G. virginica* clearly indicates that the specimens from the Valley River are *G. virginica*. The original description of *G. virginica* by Hoffman (1964) is accurate in most respects and is adequate to allow identification of the species. His drawing of the entire leech (Hoffman, 1964, Fig 1) is excellent and, with the exception of the pigmentation, shows most of the important features of the gut and external morphology. The emended description presented here adds information regarding the pigmentation, provides details of the coelomic system, and documents the presence of conducting tissue and accessory gland cells on the male atrium, which were not mentioned by Hoffman (1964). Serial sections also document the absence of mycetomes associated with the esophagus. Hoffman (1964) mentioned the presence of esophageal glands, referring to what are now called salivary gland cells associated with the proboscis.

This leech has an unusual feeding behavior, apparently feeding entirely on the yolk of fish eggs (Light et al., 2005). Richardson (1948) described an identical situation from nests of fallfish, *S. corporalis*, in Fisher Creek, Brome Lake, Quebec, Canada, where leeches were “obviously engorged on eggs.” Richardson (1948) identified the leeches as *P. punctata* although he noted their resemblance to *P. milneri*, an observation also noted by Hoffman (1964) in his description of *G. virginica*. The feeding behavior noted by Richardson (1948) and his description of the morphology, including the transparent body and rapidly fading eyes and ocelli, match in all respects the morphology of *G. virginica* on the basis of observations during the study reported herein. Thus, the leech examined by Richardson (1948) was likely *G. virginica*, and his report extends the range of the species to Quebec, Canada.

The report of a heavy infestation of *G. virginica* on white catfish, *Ameiurus catus* Ictaluridae, in the York River estuary in Virginia (Paperna and Zwerner, 1974) is in error. The leech was later determined by Sawyer (1986b) and confirmed by Burreson (1995) to be *Myzobdella lugubris* Leidy 1851, on the basis of specimens deposited in the USNM (USNM 52722).
The report by Putz (1972a, b) of *G. virginica* feeding on longnose dace, *Rhinichthys cataractae* and as a vector for *Cryptobia cataractae* (Cryptobiidae) should be viewed with caution. No morphological description of the leech was reported, and no specimens were deposited in museums, so it is impossible to confirm the identity of the leech. However, the fish blood feeding behavior and the long-term maintenance of the leeches in the laboratory reported by Putz (1972a, b) suggest that it was not *G. virginica*. All *G. virginica* collected during this research for feeding experiments died within a few hours of collection. In addition, leeches were not observed on any of the fish collected in the vicinity of *Moxostoma* spp. nests, where leeches were present (Light *et al.*, 2005). Collections in April 2004 at Providence Forge, West Virginia, U.S.A., using the same electroshocking fish-collecting method in the exact location and time of year that Putz (1972a, b) sampled, yielded only *G. meyeri*, on longnose dace. These collections suggest that the leeches on longnose dace examined by Putz (1972a, b) and the vector for *C. cataractae* were *G. meyeri*, which had not been described when Putz (1972a, b) conducted his research.

**Biology**

Specimens collected for this study in the Valley River, North Carolina were obtained by digging up "nests" of *Moxostoma carinatum* (Catostomidae), and the leeches were clearly engorged with egg material, rather than fish blood. The gut contents were visible through the transparent body of the leech, and the yellow-orange color was the same as that of the fish eggs. In fact, the leeches were at first mistaken for fish eggs because the gut was so engorged with egg material and the body of the leech is so transparent and flaccid that, in the dip net, they looked like bags of egg material. There has been no evidence of blood feeding in this species.

Specimens of *G. virginica* collected for a recent study were also collected from fish nests and were never seen attached to a fish. This study states that *G. virginica* may be an opportunistic or obligate egg-feeder. Collections were attempted in January, April, May, and
August. Specimens were only collected during the spawning season, April and May, of the fish that make the nests. No specimens were found in January and August (Light et al., 2005). There appears to be a strong relationship between the spawning season of the fish and the life cycle of G. virginica.

Known hosts and ranges (Fig. 22)

USNM 30843; South Fork Roanoke River, Virginia, about 3 miles south of Shawsville; 2 May 1963; free-living; reported by Hoffman (1964).

USNM 30844; South Fork Roanoke River, Virginia, about 3 miles south of Shawsville; 2 May 1963; free-living; reported by Hoffman (1964).

USNM 1024419; Valley River, North Carolina; 35°10.669’N 83°54.453’W; free-living; new report.

USNM 1088446; Valley River, North Carolina; 35°10.669’N 83°54.453’W; free-living; new report.

Brome Lake, Quebec, Canada; May 1934; free-living; reported by Richardson (1948).

Spivey Creek, Tennessee; 36°04.001N 82°30.135W; free-living; new report.

Valley River, upstream of Murphy, North Carolina; between 35°10’39”N 83°53’34”W and 35°5’34”N 83°25’45”W; 23 April 2000, 14 May 2000, and 24 May 2000; free-living; reported by Light et al. (2005).

Savannah River System (Meyers Branch), just south of Aiken, South Carolina; between 33°10’59”N 81°34’54”W and 33°10’05”N 81°36’29”W; 2 to 14 June 2000 and 8 May to 26 June 2001; free-living; reported by Light et al. (2005).
Distribution map of *Gonimosobdella virginica*.
Genus *Myzobdella* Leidy 1851

Type species: *Myzobdella lugubris* Leidy 1851

Type locality: estuary

**Diagnosis**


**Discussion**

The genus *Myzobdella* is the most widely distributed of the North American freshwater genera due largely to the species *M. lugubris*. *Myzobdella* is the most clearly recognized of the genera because of the very small, terminal caudal sucker. There are currently 5 species in the genus *Myzobdella*. All occur in the western hemisphere with *M. lugubris* widely distributed throughout North America (Daniels and Sawyer, 1975), *Myzobdella reducta* (Meyer 1940) occurring throughout the eastern U. S. and Canada (Price and Nadolny, 1993), *Myzobdella patcuarensis* (Caballero 1940) reported from a single locale in Mexico (Jimenez, 1985), *Myzobdella platensis* (Cordero 1933) reported from South America (Cordero, 1933), and *Myzobdella uruguayensis* Mañé-Garzón and Montero 1977 reported from Uruguay (Mañé-Garzón and Montero, 1977).

The characters defining *Myzobdella* have been fairly constant and there should have been little confusion as to what constitutes this genus in the literature. However, researchers were not aware of the genus *Myzobdella* and a new genus, *Illinobdella*, was erected. Many of the collections still have specimens labeled as *Illinobdella* sp. even though *Illinobdella* was made a junior synonym of *Myzobdella* in 1975 (Sawyer et al., 1975).

**Key to the North American species of *Myzobdella***

1. a. Body without stripes and with 14 annuli per segment . . . 2
b. Body with longitudinal stripes and 3 annuli per segment. . . *Myzobdella reducta*

2. a. Body uniformly brown . . . *Myzobdella lugubris*

   b. Body uniformly white or white with green flecks . . . *Myzobdella patzuarensis*
Myzobdella lugubris Leidy 1851
=Ichthyobdella funduli Verrill 1872
=Piscicola punctata Moore 1912
=Illinobdella alba Meyer 1940
=Illinobdella elongata Meyer 1940
=Illinobdella moorei Meyer 1940
=Illinobdella richardsoni Meyer 1940
=Myzobdella funduli (Verrill 1872)
=Myzobdella moorei (Meyer 1940)

Diagnosis
Postceca fused with no fenestrae. Accessory gland cells present. Conducting tissue absent.

Description

External morphology: Length up to 26 mm, width up to 5 mm. Body fusiform. Pigmentation uniformly brown on dorsal and ventral surfaces (Fig. 23A). Body can have green appearance due to gut contents. Oral sucker eccentrically attached with a single pair of eyes (Fig. 23C). Caudal sucker terminal (Fig. 23D). No segmental or caudal ocelli. 14 annulate.

Digestive system: Mouthpore located in center of oral sucker. Proboscis extends almost to ganglion X. Mycetomes present in X/XI (Fig. 23B). Crop expands slightly between testisacs. Intestine and postceca begin at XIX. Intestine with a single pair of anteriorly directed ceca. Postceca present with no fenestrae.

Reproductive system: Five pairs of testisacs intersegmentally from XIV/XV to XVIII/XIX (Fig. 23B). Vasa deferentia enlarge to ejaculatory bulbs at XII. Epididymus present in XI/XII (Fig. 24A, 24B). Ejaculatory bulbs turn ventrally at XI and enter very small, thin atrial cornua lumen.
Figure 23

Figure 24

*Myzobdella lugubris.* A. Reconstruction of the terminal reproductive systems, dorsal view. B. Reconstruction of the terminal reproductive systems, lateral view. C. Diagrammatic view of coelomic system, left side intersegmental, right side segmental. AG, accessory gland cells; B, bursa; C, crop; D, dorsal sinus; E, epididymus; ED, ejaculatory duct; FG, female gonopore; M, male gonopore; O, ovisac; T, testisac; V, ventral sinus.

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Atrial cornua fuse to form common atrium. Common atrium small and shortly opens into bursa. Bursa immediately opens to male gonopore with small posterior projection. Accessory gland cells present as a mass anterior to ejaculatory ducts, surrounding ejaculatory ducts and atrial cornua. Female gonopore opening to long common oviduct (Fig. 24A, 24B). Common oviduct leads to wide vagina that splits into 2 oviducts. Oviducts fuse with ovisacs in XIII/XIV. Ovisacs reach just posterior to XIV. Conducting tissue absent.

Coelomic system: No pulsatile vesicles present. Ventral, dorsal, and testicular sinuses present. Ventral, dorsal, and testicular sinuses connect intersegmentally (Fig. 24C). No connections at the ganglia.

Holotype: none deposited
Paratype: none deposited
A neotype should be designated from an estuarine location when suitable material is available.

Remarks

Myzobdella lugubris was described by Leidy (1851) from the estuarine blue crab, C. sapidus, with no location information. Moore (1912) mistakenly described specimens of M. lugubris under the name Piscicola punctata. This has lead to numerous misidentifications and many specimens that are actually M. lugubris are identified in collections as P. punctata. Sawyer et al. (1975) synonymized most of the freshwater species described under the genus Illinobdella, with the exception of Illinobdella patzcuarensis (=M. patzcuarensis), with M. lugubris.

Specimens of M. lugubris have been collected from freshwater fish in Hawaii. The specimens are most likely introduced from the U. S. or Canada and do not represent a native population.

Myzobdella lugubris can be easily distinguished from M. reducta, the other species of Myzobdella that occurs in the U. S. and Canada, by the high number of annuli per segment and
the uniformly brown pigmentation. *Myzobdella reducta* has 3 annuli per segment as compared to 14 annuli per segment in *M. lugubris* and *M. reducta* has longitudinal stripes on the dorsal surface that are not present in *M. lugubris*. Internally they are very similar with no differences seen in the digestive and coelomic systems. The only difference seen in the reproductive system is the complexity of the epididymus. In *M. lugubris* the epididymus is highly convoluted while in *M. reducta* it is relatively simple with few twists. *Myzobdella lugubris* differs from *M. patzcuarensis* by the pigmentation. *Myzobdella lugubris* is uniformly brown and *M. patzcuarensis* is unpigmented and appears uniformly white.

Biology

Present in both freshwater and estuarine environments. Found on the fins, in the mouth, or on the body of a variety of hosts. *Myzobdella lugubris* is considered a semi-permanent parasite, remaining attached to a single location on the host for long periods of time (Burreson, 1995). Scars and other tissue damage from the caudal sucker have been noted in numerous studies (Paperna and Zwemer, 1974; Paperna and Overstreet, 1981; Appy and Cone, 1982; Noga et al., 1990).

In estuarine environments, *M. lugubris* can be found year round on both the various fish hosts and on *Callinectes sapidus*, the blue crab. A seasonal cycle was noticed where the *M. lugubris* population increases in size from October through January and decreases in size from February though April. The blue crab is used as a site for cocoon deposition, which occurred most often during the summer months (Daniels and Sawyer, 1975). The seasonal cycles have not been studied in any freshwater populations.

Known hosts and ranges (Fig. 25)

Avalon, New Jersey; summer 1892; host – *Callinectes sapidus*; reported by Moore (1946).
Figure 25

Distribution map of *Myzobdella lugubris*. 

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Ogchoee River, Ossabaw Sound, Georgia; February 1973; host – *A. catus*; reported by Sawyer et al. (1975).

Ossabaw Sound, Georgia; winter 1972; host – *Ancylopsetta ommata* (Paralichthyidae); reported by Sawyer et al. (1975).

Mulatto Bay, Escambia Bay; hosts – *Mugil cephalus* (Mugilidae), *C. sapidus*; reported by Sawyer et al. (1975).

NMCIC 1977-0408; St. Lawrence River, Quebec, Canada; 29 October 1975.

NMCA 1988-0178; Ontario, 48km west of Ottawa; 45°20.00’N 076°17.00’W.


NMCIC 1981-0589; Lake St. Clair, Ontario; 42°18’N 82°31’W; July 1980; host – *P. caprodes*.

NMCA 1988-0140; Rideau Canal, Carleton, Ottawa, Ontario, Canada; 45°26.00’N 075°42.00’W; 23 May 1960; host – *I. nebulosus*.

NMCA 1985-0123; St. Lawrence River, Gentilly, Quebec; 29 October 1975.

USNM 18013; Bethany Beach, Delaware; 4 July 1910.

USNM 30490; Galveston Bay, Texas; 29 April 1960.

USNM 37495; Clear Lake, Lake County, California; 18 April 1940.

USNM 37496; Lower Echo Lake, El Dorado County, California; 20 May 1951.

USNM 38821; Mullet Pond, Beaufort, North Carolina; 26 June 1931; host *C. sapidus*.

USNM 38822; Solomons Island, Maryland; 1942.

USNM 38824; Volusia County, Florida; 1957.

USNM 38825; Boca Ciega Bay, near Tampa Bay, Florida; 18 May 1960.

USNM 39997; Great Pond, Falmouth, Massachusetts; 21 August 1907.
USNM 42538; Chesapeake and Ohio Canal National Historical Park, Chain Bridge, Washington, D. C.; 24 April 1910.

USNM 42597; Little Sioux River, near Lake Okoboji, Iowa; 21 July 1946.

USNM 42658; Lake County, California; September, 1949.

USNM 42742; Point Aux Herbes, Lake Pontchartrain, Louisiana; 30.2°N 89.9°W; 4 June 1955.

USNM 42743; Salt Bayou, Lake Pontchartrain, Louisiana; 30.2°N 89.8°W; 9 June 1955.

USNM 42744; first point West of Liberty Bayou, Lake Pontchartrain, Louisiana; 30.2°N 89.9°W; 9 June 1955.

USNM 42745; Point Aux Herbes, Lake Pontchartrain, Louisiana; 30.2°N 89.9°W; 22 June 1955.

USNM 48501; Big Rock Creek, 1 mile south of Big Rock; 2 September 1971.

USNM 48502; Mississippi River, 3 miles north of Savanna, Illinois; 10 August 1971.

USNM 48503; Mississippi River, 3 miles north of Savanna, Illinois; 8 May 1971.

USNM 49583; Lake Mattamuskeet, North Carolina; 22 January 1900.

USNM 49584; Bluewater Lake, New Mexico; 18 July 1937.

USNM 49686; Minnesota, 1 March 1900.

USNM 49962; Ashley River, 0.25 mile north of Middleton Gardens, Dorchester County, South Carolina; 24 March 1972.

USNM 50906; Carleton Creek, below County Line Bridge, Muskegon County, Michigan; 26 June 1952.

USNM 51388; Ocean Springs, Front Beach, Mississippi; 7 May 1973.

USNM 51389; Ocean Springs, Front Beach, Mississippi; 7 May 1973.

USNM 51390; Ocean Springs, Front Beach, Mississippi; 8 May 1973.

USNM 51528; Ocean Springs, near Davis Bayou, Mississippi.

USNM 51718; Fort Johnson, Charleston Harbor, South Carolina; 5 July 1973.

USNM 51719; Fort Johnson, Charleston Harbor, South Carolina; 5 July 1973.
USNM 51720; Fort Johnson, Charleston Harbor, South Carolina; 8 July 1973.
USNM 51723; Moncks Corner, Berkley County, South Carolina; 1970.
USNM 51753; Praters Creek, Six Mile, South Carolina; July 1968.
USNM 51763; Mile Creek, South Carolina; 26 November 1967. (not mapped, location could not be found)
USNM 51764; Crow Creek, at highway 172, Pickens County, South Carolina; 26 March 1968.
USNM 51766; Sandy Springs, South Carolina; 26 October 1968.
USNM 51767; Wendlee + Ashepoo, Blue Channel, South Carolina; 14 June 1973.
USNM 52722; lower York River, Virginia; 28 June 1972.
USNM 53190; Lopka River, 2.5 miles southwest of Magnolia Springs, Baldwin County, Alabama; 6 April 1968.
USNM 55015; Chestertown, a few yards south of Chester River Bridge, 12 miles upriver from the Chesapeake Bay, Maryland; 1977.
USNM 55412; Bahia de Panama, between Islas Torola and Tortolita, Panama; 8°51'50"N 79°33'53"W; 8 meters depth.
USNM 84502; Charleston, South Carolina.
USNM 84503; Charleston, South Carolina.
USNM 84504; Charleston, South Carolina.
USNM 84505; Charleston, South Carolina.
USNM 84506; Charleston, South Carolina.
USNM 84507; Charleston, South Carolina.
USNM 84510; Kern River, Bloomfield Ranch, east of Onyx, south fork of river, Kern County, California; 9 September 1940.
USNM 84513; Big Wind River, Fremont County, Wyoming; 2 August 1935.
USNM 84514; Cadillac Lake, Michigan; 13 August 1924.
USNM 84515; Foster Spring, 1 mile west of Summer Lake in ditch, Lake County, Oregon; 23 June 1939.

USNM 84517; Cass River, north branch of river, Sanilac County, Michigan; 12 August 1937.

USNM 84545; South Edisto, South Carolina; 4 February 1971.

USNM 132424; Loblockee Creek, Lee County, Alabama; 15 February 1972.

USNM 132425; Loblockee Creek, Lee County, Alabama; 15 February 1972.

USNM 132426; Euphapy Creek, Alabama; 27 March 1972. (not mapped, location could not be found)

USNM 132427; Loblockee Creek, Lee County, Alabama; 15 February 1972.

USNM 132429; Bon Secour River, near mouth, Alabama; 22 January 1971.

USNM 132430; Loblockee Creek, Lee County, Alabama; 15 February 1972.

USNM 132431; Loblockee Creek, Lee County, Alabama; 15 February 1972.

USNM 133119; Lake Winnepeg, north end of lake, Manitoba, Canada; 1900.

USNM 155347; Santa Teresa Lagoon, Humacao, Puerto Rico; 29 May 1992.

USNM 155348; Santa Teresa Lagoon, Humacao, Puerto Rico; 29 May 1992.

USNM 155349; Santa Teresa Lagoon, Humacao, Puerto Rico; 29 May 1992.

USNM 155350; Santa Teresa Lagoon, Humacao, Puerto Rico; 29 May 1992.

USNM 172128; Arecibo, small creek between small boat and large boat marinas, Puerto Rico, 12 November 1995.

USNM 1088426; Rappahannock River, Virginia; 9 August 2004; host – *Fundulus heteroclitus heteroclitus* (Fundulidae); new report.

USNM 1088427; Craig's Creek, Virginia; 37°30.712’N 80°05.359’W; 22 April 2004; hosts – *C. anomalum, Nocomis leptocephalus* (Cyprinidae), *M. dolomieu, Scartomyzon cervinum* (Catostomidae); new report.
Holston River, Virginia; 36°55.468’N 81°37.591’W; 23 April 2004; hosts – *C. anomalum, M. dolomieu*; new report.

Little River, Virginia; 37°00.309’N 80°22.903’W; 24 April 2004; hosts – *C. anomalum, Nocomis sp*; new report.

Roanoke River, near Dixie Caverns, Virginia; 24 April 2004; host – *C. anomalum*; new report.


Spivey Creek, Tennessee; 36°04.001’N; 82°30.135’W; 20 May 2004; new report.
Myzobdella patzcuarensis (Caballero 1940)

= Illinobdella patzcuarensis Caballero 1940

Diagnosis


Description

*External morphology:* Length up to 12 mm, width up to 2 mm. Body fusiform. Pigmentation uniformly white or white with green flecks. Oral sucker eccentrically attached with a single pair of eyes (Fig. 26B). Caudal sucker terminal (Fig. 26C). No segmental or caudal ocelli. 14 annulate.

*Digestive system:* Mouthpore located in center of oral sucker. Proboscis extends to IX or X. One pair of mycetomes present. Crop expands slightly between testisacs (Fig. 26A). Intestine and postceca begin at XIX. Intestine with a single pair of anteriorly directed ceca. Postceca present with no fenestrae.

*Reproductive system:* Five pairs of testisacs intersegmentally from XIV/XV to XVIII/XIX (Fig. 26A). Vasa deferentia enlarge to ejaculatory bulbs at XII. Ejaculatory bulbs turn ventrally at XI and enter atrial cornua (Fig. 26D). Atrial cornua fuse to form common atrium. Common atrium shortly opens into bursa. Bursa quickly opens to male gonopore. Accessory gland cells present as a mass anterior to ejaculatory ducts. Female gonopore opening to common oviduct. Ovisacs reach to first pair of testisacs. No conducting tissue present.

*Coelomic system:* No pulsatile vesicles present. Nothing known of the sinuses and connections.
Figure 26

Holotype: 1815; Colección Helmintológica del Instituto de Biología, Universidad Nacional Autónoma de México (U.N.A.M.); Ihuatzio, Michoacán, Mexico.

Paratypes: 1812; Colección Helmintológica del Instituto de Biología, U.N.A.M.; Ihuatzio, Michoacán, Mexico.

1813; Colección Helmintológica del Instituto de Biología, U.N.A.M.; Ihuatzio, Michoacán, Mexico.

1814; Colección Helmintológica del Instituto de Biología, U.N.A.M.; Ihuatzio, Michoacán, Mexico.

Remarks

*Myzobdella patzcuarensis* was described by Caballero (1940) as *Illinobdella patzcuarensis*. Strong similarities were seen between *I. patzcuarensis* and *Illinobdella alba* (=*M. lugubris*). Differences were noted as being the presence of 14 pairs of nephridia in *I. patzcuarensis*, the structure and form of the atrium, and the structure and physiology of the esophageal gland cells and intestinal gland cells. The structure and form of the atrium is a valid feature to distinguish between 2 species, however the other characters are not considered diagnostic for a species and are variable. The structure of the atrium of *I. alba* was not described in enough detail for a comparison to be made between the 2 species since the only description was “the atrium is especially well developed” (Meyer, 1940).

*Ilinobdella patzcuarensis* was reassigned to *Myzobdella* by Sawyer *et al.* (1975) who noted the similarity between *M. lugubris* and *M. patzcuarensis*. No newly collected specimens were available for study for this research and a complete study could not be performed. However, *M. patzcuarensis* appears to be indistinguishable from *M. lugubris*. The only difference that has been documented is the pigmentation. *Myzobdella patzcuarensis* is described as uniformly white (Caballero, 1940; Jimenez, 1985) but this could not be confirmed and would be a very unusual characteristic for a fish leech. The description provided by Jimenez (1985) is
"Leeches of white color, with transparent skin". This is interpreted to mean leeches lacking pigmentation and appearing white, as leeches with no pigment often do appear white. The coelomic system could also not be studied and differences may exist there. *Myzobdella patzcuarensis* can be easily distinguished from *M. reducta* by the number of annuli per segment. *Myzobdella patzcuarensis* has 14 annuli per segment and *M. reducta* has 3 annuli per segment.

Biology

*Myzobdella patzcuarensis* has only been reported from Lago de Patzcuaro, a freshwater lake. This species attaches in the mouth, on the fins, and on the body of the host fish. Little is known of the seasonality of *M. patzcuarensis*, except that specimens are present in January and reproductively active between August and September (Jimenez, 1985).

Known hosts and ranges (Fig. 27)

Colección Helmintológica del Instituto de Biología, Universidad Nacional Autonoma de Mexico (U.N.A.M.) No. II-177; Lago de Patzcuaro, Michoacán, Mexico.

Colección Helmintológica del Instituto de Biología, U.N.A.M. No. 224-3; Lago de Patzcuaro, Michoacán, Mexico.

Lago de Patzcuaro, Michoacán, Mexico; 19 and 20 January 1980; hosts – *C. estor, M. salmoides, A. lacustris*; reported by Jimenez (1985).
Figure 27

Distribution map of *Myzobdella patzcuarensis.*
Myzobdella reducta (Meyer 1940)
=Piscicolaria reducta Meyer 1940

Diagnosis
Postceca fused with 4 fenestrae. Accessory gland cells present. No conducting tissue present.

Description
External morphology: Length up to 9 mm, width up to 2 mm. Body fusiform. Six longitudinal, brown stripes the length of the body, not present on suckers (Fig. 28A). Oral sucker eccentrically attached with a single pair of eyes (Fig. 28C). Caudal sucker terminal (Fig. 28D). No segmental or caudal ocelli. Three annulate.

Digestive system: Mouthpore located centrally in oral sucker. Proboscis extends just posterior to ganglion VIII. Mycetomes large, connect to esophagus at X (Fig. 28B). Crop expands between testisacs. Postceca and intestine begin at XIX. Intestine with 2 pairs of ceca. First pair large, anteriorly directed, located at XIX. Second pair small, anteriorly directed, located at XX. Postceca fused with 4 fenestrae and posterior ends fused. Rectum long, begins at XXII.

Reproductive system: Five pairs of testisacs intersegmentally from XIV/XV to XVIII/XIX (Fig. 28B). Vasa deferentia enlarge and become loosely coiled epipdidymus at XIII (Fig. 29A, 29B). Ejaculatory ducts turn ventrally and fuse to form common atrium that immediately enters bursa. Bursa narrow, opening to male pore with short posterior projection. Accessory gland cells cover ejaculatory ducts, atrium, and bursa. Female gonopore opening to 2 oviducts. Oviducts fuse with ovisacs shortly posterior to female gonopore. Ovisacs reach to end of first pair of testisacs. No conducting tissue present.
Figure 28

Figure 29

*Myzobdella reducta.* A. Reconstruction of the terminal reproductive systems, dorsal view. B. Reconstruction of the terminal reproductive systems, lateral view. C. Diagrammatic view of coelomic system, left side intersegmental, right side segmental. AG, accessory gland cells; B, bursa; C, crop; D, dorsal sinus; E, epididymus; FG, female gonopore; G, ganglion; M, male gonopore; O, ovisac; T, testisac; V, ventral sinus.
Coelomic system: No pulsatile vesicles present. Dorsal, ventral, testicular sinuses present. Dorsal, ventral, testicular sinuses connect intersegmentally (Fig. 29C). No connections at ganglia. Ventral sinus expansive at ganglia.

Holotype: INHS SI.13583
Paratype: None deposited

Remarks

_Myzobdella reducta_ does not have conducting tissue, however it does have sperm in the ovisacs in what appears to be conducting tissue. It does not actually seem to be conducting tissue since it does not start as strands or a mass. There may just be a swelling of the ovisac wall that is filled with sperm. Only one specimen was available for study of the internal anatomy. Sectioning another specimen that has not mated recently would be helpful in determining what type of tissue is present in this species.

_Myzobdella reducta_ was originally described as _Piscicolaria reducta_ by Meyer (1940). Molecular and morphological analysis has determined that _P. reducta_ forms a monophyletic group with _M. lugubris_ (Chapter 2, this dissertation). _Piscicolaria reducta_ was sister to the freshwater _M. lugubris_ and the 2 species were then sister to _M. lugubris_ from Hawaii. This would normally be interpreted as _P. reducta_ being the same species as _M. lugubris_. Morphological differences exist consistently between the 2 species. _Piscicolaria reducta_ has 3 annuli per segment versus the 14 annuli per segment seen in _M. lugubris_. _Piscicolaria reducta_ also has longitudinal stripes on the dorsal surface that are not present in _M. lugubris_. Internally they are very similar regarding the digestive and coelomic systems and differ only in the complexity of the epididymus. _Piscicolaria reducta_ has a very simple, looping epididymus while _M. lugubris_ has a complex, twisted epididymus. Given these differences, _P. reducta_ is reassigned to the genus _Myzobdella_ and remains a separate species as _M. reducta_.

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Myzobdella reducta differs from *M. patzcuarensis* by pigmentation and number of annuli. *Myzobdella reducta* is brown with longitudinal dark brown stripes and *M. patzcuarensis* is unpigmented appearing uniformly white. The number of annuli per segment is much lower in *M. reducta*, which has 3 annuli per segment, than in *M. patzcuarensis*, which has 14 annuli per segment. Differences in the coelomic system and complexity of the epididymus could not be determined due to a lack of *M. patzcuarensis* specimens.

**Biology**

This species occurs in freshwater environments from the Great Lakes region and to the east of the Great Lakes. *Myzobdella reducta* generally occurs on the fins of the host and can parasitize a wide range of cypriniform, siluriform, and perciform fish hosts. Nothing is known of the seasonality of this leech.

**Known hosts and ranges (Fig. 30)**

Sangamon River, Illinois; host – *P. phoxocephala*; reported by Meyer (1940).

NMCA 1987-0463; Avon River, Perth, Ontario, Canada; 43°18.00'N 081°10.00'W; 27 July 1965.

USNM 4021; Illinois Central Station, Illinois; November 1887.

USNM 39884; Mill River, Tidal Basin, Long Island Sound, Connecticut; January 1942.

USNM 48499; Embarras River, 3 mile southwest of Greenup, Cumberland County, Illinois; 23 August 1971.

USNM 48500; Embarras River, 1 mile north of Greenup, Cumberland County, Illinois; 22 August 1971.

USNM 49812; Perkiomen Creek, tributary to Schuylkill River, southeast Pennsylvania; 17 October 1972.
Figure 30

Distribution map of *Myzobdella reducta.*
USNM 49813; Perkiomen Creek, tributary to Schuylkill River, southeast Pennsylvania; 17 October 1972.

USNM 49814; Perkiomen Creek, tributary to Schuylkill River, southeast Pennsylvania; 8 August 1972.

USNM 49815; Perkiomen Creek, tributary to Schuylkill River, southeast Pennsylvania; 24 August 1972.

USNM 49816; Perkiomen Creek, tributary to Schuylkill River, southeast Pennsylvania; 6 September 1972.

USNM 49817; Perkiomen Creek, tributary to Schuylkill River, southeast Pennsylvania; 21 September 1972.

USNM 49818; Perkiomen Creek, tributary to Schuylkill River, southeast Pennsylvania; 8 August 1972.

USNM 49819; Perkiomen Creek, tributary to Schuylkill River, southeast Pennsylvania; 21 November 1972.

USNM 51751; Sandy Springs, South Carolina; 26 October 1968.

USNM 51752; Crow Creek, Route 157, Pickens County, South Carolina; 26 March 1968.

USNM 132428; Loblockee Creek, Lee County, Alabama; 15 February 1972.

USNM 132432; Loblockee Creek, Lee County, Alabama; 15 February 1972.

USNM 151916; Blackwater Creek, Florida; 28 July 1983.

USNM 186412; Santee Caves, Orangeburg County, South Carolina; 33.5°N 80.5°W; 30 March 1999.

Shawano Lake, Wisconsin; 24 August 2005; host - *L. gibbosus*; new report.

Little River, North Carolina; 35°42.162’N 83°48.926’W; 19 May 2004; host – darter; new report.
Genus *Piscicola* Blainville 1818

Type species: *Piscicola geometra* (Linnaeus 1761)

Type locality: Europe

Diagnosis


Discussion

The genus *Piscicola* currently contains 17 species, 11 were described by Bielecki (1997) from Poland. All species are found exclusively in freshwater environments. Species of *Piscicola* are most similar to the species of *Gonimosobdella*. Externally, species of *Gonimosobdella* are considered to have larger pulsatile vesicles and are 7 annulate while species of *Piscicola* have small pulsatile vesicles and are 14 annulate (Sawyer, 1986b). Internally, there are a number of differences in the reproductive systems. Species of *Piscicola* have vector tissue present posterior to the female pore (Bielecki, 1997) that is lacking in species of *Gonimosobdella*. Species of both genera have cords of conducting tissue. Conducting tissue in species of *Gonimosobdella* connects the bursa and the ovisacs (this dissertation; Burreson *et al.*, 2005) while conducting tissue in species of *Piscicola* connects the vector tissue to the ovisacs (Bielecki, 1997). Species have been switched between the two genera, such as *G. salmositica*, due to inconsistencies in genera descriptions. The defining character, according to this research, appears to be the presence or absence of vector tissue.

Bielecki (1997) notes the presence of a spermatheca in species of *Piscicola*, however this structure was not seen in specimens from North America or in specimens of *P. geometra* from the
Ukraine. The vector tissue did appear to have an open cavity in some specimens, but it was not a consistent character that would warrant being described as a spermatheca. The description of the genus *Piscicola* provided by Sawyer (1986b) has already been questioned (Bielecki, 1997). Sawyer states that the ovaries do not enter the vector tissue and that the vector tissue does not reach anteriorly to the male pore. This research supports Bielecki’s (1997) statements regarding the arrangement of vector and conducting tissue.

Key to the North American species of *Piscicola*

1. a. Ocelli present on caudal sucker . . . *Piscicola milneri*

   b. Ocelli absent on caudal sucker . . . *Piscicola punctata*
Piscicola milneri (Verrill 1874)

= Ichthyobdella milneri Verrill 1874

Diagnosis


Description

External morphology: Length up to 48 mm, width up to 2.5 mm. Body fusiform. Pigmentation variable, generally consisting of 2 brown patches per segment, often fused by a thin connection (Fig. 31A). Posterior end with 1 large brown patch. Pigmentation of urosome and trachelosome consisting of brown patches formed by an unpigmented mid-dorsal longitudinal stripe and segmental transverse unpigmented stripes. Pigmentation on ventral surface light. Stellate chromatophores on oral sucker, unpigmented circles anterior and lateral to eyes, variable unpigmented regions on lateral edge. Stellate chromatophores on caudal sucker, unpigmented regions from ocelli to lateral edge. Oral sucker eccentrically attached on thin peduncle with 2 pairs of eyes (Fig. 31C). Caudal sucker subterminal with up to 14 ocelli (Fig. 31D). Urosomal segments 14 annulate. Eleven pairs of small pulsatile vesicles visible (Fig. 31A).

Digestive system: Mouthpore located centrally in oral sucker. Proboscis extends to IX. Mycetomes connect to esophagus at XI, extending anteriorly to X. Crop expands between
Figure 31

*Piscicola milneri.* A. External morphology. B. Reconstruction of reproductive and digestive systems. C. Oral sucker, lateral view. D. Caudal sucker, lateral view. B, bursa; C, crop; F, fenestrae; I, intestine; O, ovisac; P, proboscis; PC, postceca; R, rectum; T, testisac.
testisacs (Fig. 31B). Postceca and intestine begin at XIX. Intestine with 5 pairs of ceca. Postceca with 4 fenestrae and posterior ends fused. Rectum narrow.

Coelomic system: One pair of pulsatile vesicles per urosomal segment. Ventral, dorsal, thin-walled lateral, thick-walled lateral, and testicular sinuses present. Ventral blood vessel not contained within ventral sinus (Fig. 32C). Pulsatile vesicle connects to thin- and thick-walled lateral sinuses and ventral sinus at ganglia. No connections intersegmentally.

Reproductive system: Six pairs of testisacs intersegmentally from XIII/XIV to XVIII/XIX (Fig. 31B). Vasa deferentia enlarge at XI/XII to become ejaculatory ducts. No epididymus present. Ejaculatory ducts turn ventrally at IX/X then continue posteriorly, becoming smaller in diameter (Fig. 32A, 32B). At X/XI ejaculatory ducts fuse with ventral surface of atrial cornua. Atrial cornua long and thin, fuse at XI/XII to become common atrium. Common atrium fuses with bursa posterior to the male gonopore at XII. Bursa continues anteriorly to male gonopore. Accessory gland cells extensive, cover atrial cornua, common atrium, and bursa. Female gonopore opening to 2 oviducts, with anterior extension (Fig. 32A, 32B). Oviducts fuse with ovisacs shortly after female gonopore. Ventral groove present posterior to female gonopore and extending to XIII (Fig. 32B). Ventral groove surrounded internally by vector tissue. Wide cords of conducting tissue from vector tissue fuse with ovisacs at XII/XIII. Ovisacs reach to second pair of testisacs.

Holotype: None deposited

Paratype: None deposited

A neotype will be designated from collections made at Heart Lake, Quebec, Canada.

Remarks

Piscicola milneri cannot be adequately distinguished morphologically from P. geometra, which occurs in the palearctic region. Specimens of P. geometra from Ukraine were observed.
Figure 32

*Piscicola milneri.* A. Reconstruction of the terminal reproductive systems, dorsal view. B. Reconstruction of the terminal reproductive systems, lateral view. C. Diagrammatic view of coelomic system, left side intersegmental, right side segmental. A, atrium; AG, accessory gland cells; B, bursa; C, crop; D, dorsal sinus; ED, ejaculatory duct; FG, female gonopore; L, thin-walled lateral sinus; LT, thick-walled lateral sinus; M, male gonopore; O, ovisac; PV, pulsatile vesicle; T, testisac; V, ventral sinus; VB, ventral blood vessel; VG, ventral groove; VT, vector tissue.
and sectioned. Externally, no differences were seen in length, width, size and attachment of suckers, pigmentation, and number of eyes and caudal ocelli. There were also no differences internally regarding the digestive, coelomic, and reproductive systems. However, it can be distinguished using molecular methods (Chapter 2, this dissertation). The 2 species were not found to form a monophyletic clade, with *P. milneri* sister to a clade consisting of *Piscicola cf. annae*, *Piscicola* sp., and *P. geometra*. The 2 species do not overlap in geographic range. *Piscicola milneri* occurs in the Great Lakes region and Canada (Klemm, 1982; Sawyer, 1986b). *Piscicola geometra* occurs in the palearctic region, with the exception of a single occurrence in the U. S. (Moore, 1898) where it was found on *Cyprinus carpio carpio* that were most likely introduced. A native population of *P. geometra* in the U. S. or Canada is highly unlikely.

**Biology**

*Piscicola milneri* is found exclusively in freshwater environments. There is nothing known of the seasonality or habitat preference of this leech.

**Known hosts and ranges (Fig. 33)**

Thunder Bay, Michigan; 1873; reported by Verrill (1874).

Georgian Bay, Canada; host – *S. namaycush*; reported by Ryerson (1915).

Lake Nipigon, Canada; 1921, 1922, 1923; host – *C. clupeaformis*; reported by Moore (1924).

Washtenaw County, Michigan; host – *C. commersonii*; reported by Klemm (1972)

Lake Superior; host – *P. cylindraceum*; reported by Dechtiar and Lawrie (1988)

Heart Lake, Quebec, Canada; 45° 46.596' N; 075° 14.073' W; 24 June 2005; host – *S. fontinalis*; new report.

Canadian Museum of Nature

Figure 33

Distribution map of *Pisicola milneri.*
NMCIC 1977-0406; Wabamun Lake, Alberta, Canada; 4 March 1961; host – *C. commersonii*.

NMCIC 1984-0322; Burlington Bay, Lake Ontario, Ontario, Canada; 43° 19' N 79° 48' W; 31 September 1983.

NMCIC 1977-0407; Blue Sea Lake, Quebec; 24 May 1959; host – *S. namayacush*; reported as *P. geometra*.

NMCA 1985-0379; Heart Lake, Quebec; 30 October 1985; reported as *P. geometra*.

NMCIC 1982-1504; Mazinaw Lake, Ontario; 44° 55' N 77° 12' W; 13 May 1982.

NMCA 1984-0231; mouth of Bay, Cree Lake, Lazy Edward Bay, Saskatchewan, Canada; 57° 20.33' N 106° 40.35' W; host – *C. commersonii* - reported as *P. geometra*.

NMCA 1984-0226; North Shore of Bay, Cree Lake, Lazy Edward Bay, Saskatchewan, Canada; 57° 20.25' N 106° 41.12' W; host - *C. clupeaformis* - reported as *P. geometra*.

NMCA 1984-0206; Murphy Islands, Lac La Ronge, Saskatchewan, Canada; 55° 05.50' N 105° 14.00' W; reported as *P. geometra*.

NMCIC 1984-0218; north end of Big Stone Lake, Saskatchewan, Canada; 55° 07' N 105° 21' W; 30 May 1987; reported as *P. geometra*.

NMCA 1984-0227; north shore of bay near island, Cree Lake, Lazy Edward Bay, Saskatchewan, Canada; 57° 19.45' N 106° 42.05' W; host – *C. clupeaformis* - reported as *P. geometra*.

NMCIC 1984-0205; Lac la Ronge, Murphy Islands, Saskatchewan, Canada; 55° 06' N 105° 13.5' W; 25 May 1978; reported as *P. geometra*.

NMCA 1985-0052; Toobally Lake, Yukon, Canada; 23 July 1978.

Smithsonian Institution

USNM 20369; Otterskin lake, near Lake-of-the-Woods, Ontario; 7 July 1935.

USNM 20544; St. Joseph County, Michigan; 15 June 1918.

USNM 22750; from small lake near East Oumalik, about 100 mile SE, Alaska; 20 June 1949.

USNM 39594; Champlain lake, New York; 17 February 1911.
USNM 39595; Lake Nipigon, Ontario; 16 August 1923.

USNM 39596; Vedder Crossing, Calta's Lake, British Columbia, Canada; 18 November 1924.

USNM 39597; Waterton Lakes Park, Waterton Lake, Alberta, Canada; 20 June 1937.

USNM 39598; Amisk Lake, Saskatchewan, Canada; 6 July 1951.

USNM 39599; Great Bear Lake, Whitefish River, Northwest Territories, Canada; 16 August 1945; reported by Moore and Meyer (1951).

USNM 39600; Great Bear Lake, Good Hope Bay, Northwest Territories, Canada; on bottom; 17 August 1945; reported by Moore and Meyer (1951).

USNM 39601; – 21 August 1945, Great Bear Lake, Caribou Nest Bay, Northwest Territories, Canada; 21 August 1945; host – *S. namaycush*; reported by Moore and Meyer (1951).

USNM 39602; Great Bear Lake, Snipe Island, Northwest Territories, Canada; 28 August 1945.

USNM 39603; Great Slave Lake, 6 mile off Round Island, near Fort Resolution; 23 June 1944.

USNM 39604; Great Slave Lake, 1 mile west of Round Island, Northwest Territories, Canada; 24 June 1944.

USNM 39605; Great Slave Lake, Resolution, Northwest Territories, Canada; 22 June 1945.

USNM 39606; Great Slave Lake, Slave Point, Hay River, Northwest Territories, Canada; 29 June 1946.

USNM 39610; Ithaca, Cayuga Lake, New York; 1943.

USNM 39866; Lake Superior; May 1947.

USNM 42635; Quick, California; 15 November 1932 (not on map, could not find location).

USNM 42695; Manitoba, Canada; 1927 (not on map, location not specific enough).

USNM 84501; North end of Lake Winnipeg, Manitoba, Canada; 1900.

USNM 1088449; Heart Lake, Quebec, Canada; 45° 46.596’ N; 075° 14.073’ W; 24 June 2005; host – *S. fontinalis*; new report.
Piscicola punctata (Verrill 1871)

= Icthyobdella punctata Verrill 1871

Diagnosis


Description

External morphology: Length up to 21 mm, width up to 2 mm. Body fusiform. Pigmentation of live specimens unknown. Preserved specimens with stellate chromatophores on dorsal and ventral surfaces. Unpigmented longitudinal stripe mid-dorsal (Fig. 34A). Stellate chromatophores on oral sucker, unpigmented circles anterior and lateral to eyes. Oral sucker eccentrically attached with 2 pairs of eyes (Fig. 34C). Caudal sucker subterminal (Fig. 34D). No caudal or segmental ocelli. Urosomal segments 14 annulate. Eleven pairs of small pulastile vesicles visible (Fig. 34A).

Digestive system: Mouthpore located centrally in oral sucker. Proboscis extends almost to IX. Mycetomes connect to esophagus at XI. Crop expands between testisacs (Fig. 34B). Postceca and intestine begin at XIX. Intestine with 3 pairs of ceca. Postceca with 4 fenestrae and posterior ends fused. Rectum large.

Coelomic system: One pair of pulsatile vesicles per urosomal segment. Ventral, dorsal, thin-walled lateral, thick-walled lateral, and testicular sinuses present. Ventral blood vessel not contained within ventral sinus (Fig. 34G). Pulastile vesicle connects to thin- and thick-walled
Figure 34

*Piscicola punctata.* A. External morphology, pigmentation based on preserved specimens. B. Reconstruction of reproductive and digestive systems. C. Oral sucker, lateral view. D. Caudal sucker, lateral view. E. Reconstruction of the terminal reproductive systems, dorsal view. F. Reconstruction of the terminal reproductive systems, lateral view. G. Diagrammatic view of coelomic system, left side intersegmental, right side segmental. AG, accessory gland cells; B, bursa; C, crop; D, dorsal sinus; ED, ejaculatory duct; F, fenestrae; FG, female gonopore; G, ganglion; I, intestine; L, thin-walled lateral sinus; LT, thick-walled lateral sinus; M, male gonopore; O, ovisac; P, proboscis; PC, postceca; PV, pulsatile vesicle; R, rectum; S, salivary gland cells; T, testisac; V, ventral sinus; VB, ventral blood vessel; VT, vector tissue.
lateral sinuses at ganglia. Ventral sinus expands slightly at ganglia. Dorsal sinus expands slightly intersegmentally.

**Reproductive system:** Six pairs of testisacs intersegmentally from XIII/XIV to XVIII/XIX (Fig. 34B). Vasa deferentia enlarge at XII to become ejaculatory ducts (Fig. 34E, 34F). Epididymus present at XII as simple bends. Ejaculatory ducts turn ventrally at XI and fuse with atrial cornua. Atrial cornua fuse to common atrium at level of male gonopore. Common atrium fuses with bursa posterior to male gonopore, near ganglion XII. Bursa continues anteriorly to male gonopore. Accessory gland cells cover atrial cornua, common atrium, and bursa and project off posterior end of bursa as two cords. Cords end freely and do not connect to female reproductive system. Female gonopore opening to 2 oviducts (Fig. 34E, 34F). Oviducts fuse with ovisacs just posterior to female gonopore. Vector tissue present just posterior to female gonopore. Cords of conducting tissue connect to ovisacs from vector tissue just anterior to XIII. Vector tissue ends at ganglion XIII. Ovisacs reach to first pair of testisacs.

Holotype: None deposited
Paratype: None deposited

A neotype should be designated when suitable material is available.

Remarks

There has been much confusion regarding which specimens truly represent the species *P. punctata*. The species was first described by Verrill (1874) as *Icthyobdella punctata*. In Meyer’s description of *Icthyobdella moorei* he notes that Moore’s description of *P. punctata* in 1912 was actually of *I. moorei* (Meyer, 1940). *Icthyobdella* has since been synonymized with *Myzobdella* (Sawyer et al., 1975). Many specimens have been identified as *P. punctata* even though they are probably *M. lugubris*. *Piscicola punctata* has been reported from as far south in North America as Mississippi. These specimens, along with specimens reported from the eastern U. S., were
examined. The specimens were newly identified as *M. lugubris* (USNM 15180, USNM 15181, USNM 15182, USNM 20673 - partial) and *G. vivida* (USNM 20673 - partial, USNM 39875, USNM 39877). This species is probably restricted to cold, freshwater environments and most likely does not exist in estuarine environments.

The original description of *P. punctata* was brief, containing little detail other than number of eyes and the pigmentation of preserved specimens (Verrill, 1874). A further description provided little more detail than the number of testisacs and the digestive system morphology (Meyer, 1940). Unfortunately, no live specimens could be obtained for this study and pigmentation of live specimens could not be provided. Specimens from the Canadian Museum of Nature were sectioned to provide details regarding the internal anatomy of *P. punctata*.

Biology

Found exclusively in freshwater environments. Nothing is known of the seasonality of *P. punctata*. An outbreak of this leech occurred in the Rock River, Illinois. Leeches infested nearly every bigmouth buffalo, *Ictiobus cyprinellus*, during February and March of 1926 (Thompson, 1927). The scars left by the leeches made the fish difficult to sell and left the fish vulnerable to fungal infections in April. There was no apparent reason for the outbreak (Thompson, 1927).

Known hosts and ranges (Fig. 35)

Naonan Island, Lake Nipigon, Ontario, Canada; 1922; reported by Moore (1924).

Slate Islands, Lake Superior, Ontario, Canada; reported by Verrill (1871).

Rock River, Illinois; hosts – *I. cyrinella*, *M. erythrurum*, *C. commersonii*; reported by Meyer (1940).

Lake Champlain, New York; host – *O. mordax*; reported by Meyer (1940).

Cold Spring Harbor, New York; host - *O. mykiss*; reported by Meyer (1940).
Figure 35

Distribution map of *Piscicola punctata*.
St. Joseph, Michigan; host - *C. clupeaformis*; reported by Meyer (1940).


NMCIC 1984-0754; Pukaskwa National Park, Willow Lake, Ontario, Canada; 4 April 1984; host - *S. fontinalis*.

NMCA 1990-0019; Red Deer River; November, 1989; host - *C. commersonii*.

NMCA 1990-0017; Red Deer River; January, 1990; host - *C. commersonii*.

NMCIC 1982-0705; Windermere Lake, south of Gout Wharf, British Columbia; 50° N 115° W; 2 August 1969.

USNM 10484; Sodus Bay, West side, between Bay Bridge and Third Creek, New York; 3 August 1909.

USNM 20375; Quetico Park, Kahnipiminanikok Lake, Ontario; 5 August 1935.

USNM 20545; Rock River, near Galt, Illinois; 7 December 1926.

USNM 39868; Gray Cloud Island and Slough, Minnesota; 24 August 1899.

USNM 39869; Minnesota; December 1907.

USNM 39870; Ombabika Bay, Lake Nipigon, Ontario, Canada; 8 October 1922; host - *Sander canadensis* (Percidae); reported by Moore (1924).

USNM 39871; Windigo Bay, Lake Nipigon, Ontario, Canada; 26 July 1923, free living; reported by Moore (1924).

USNM 39872; Glacier National Park, Lake Macdonald, Montana; 3 August 1925.

USNM 39873; Rock River, near Rockford, Illinois; 15 March 1926.

USNM 39874; Rock River, near Rockford, Illinois; 18 March 1926.

USNM 39876; Lake Nipissing, near Blueberry Island, Ontario, Canada; 29 August 1934.

USNM 39878; Kalamalka Lake, British Columbia, Canada; 14 August 1935.

USNM 39879; Okanogan Lake, 535 Bay, British Columbia, Canada; 2 August 1935.

USNM 39880; Okanogan Lake, 535 Bay, British Columbia, Canada; 28 August 1935.
USNM 39996; Toronto, Ontario; 20 January 1929.

USNM 42548; Lac La Ronge, Saskatchewan, Canada; 25 May 1948.

USNM 42698; Waskesiu, Saskatchewan, Canada; 7 September 1950.

USNM 53193; Hudson River, opposite Germantown Yacht Club, New York; 3 May 1941.

Manitoulin Island, Ontario, Canada; 18 July 1951; host – *L. lota*; reported by Meyer and Moore (1954).

SI. 13576; Rock River, Illinois; 7 December 1926; host - *I. cyprinellus*.

SI. 13577; Rock River, Illinois; 7 December 1920; host – *I. cyprinellus*.

Discussion

The 2 species of *Piscicola* that occur in North America can be distinguished by the presence or absence of caudal ocelli. *Pisciola milneri* has up to 14 caudal ocelli while ocelli are absent in *P. punctata*. *Pisciola milneri* can also be distinguished by the attachment of the oral sucker on a thin pedicle, while *P. punctata* has a typical thicker attachment. Pigmentation differences are not known since the pigmentation of live *P. punctata* has yet to be reported. Internally there are a number of differences between the 2 species. *Pisciola milneri* has 5 intestinal ceca and a thin, tube-like rectum while *P. punctata* has only 3 intestinal ceca and a wide, bulbous rectum. *Pisciola milneri* has a connection between the pulsatile vesicle and the ventral sinus, which is absent in *P. punctata*. The ejaculatory ducts of *P. milneri* fuse with the atrial cornua on the ventral surface, which is rather unusual, while *P. punctata* has the typical fusion occurring on the anterior surface of the atrial cornua. The female reproductive system of both species consists of vector tissue with cords of conducting tissue that connect to the ovisacs but *P. milneri* has an externally visible ventral groove associated with the vector tissue that is absent in *P. punctata*. The North American species of *Piscicola* share the interesting feature of a ventral blood vessel that is not contained within the ventral sinus, as it is in most of the proboscis-bearing leeches (Sawyer, 1986a).
Bielecki (1997) states that both *P. milneri* and *P. punctata* may be members of his newly erected genus *Pawlowskiella* Bielecki 1997, as they have identical reproductive systems. The generic characters of *Pawlowskiella* fall within the very broad and variable generic characters for *Piscicola* with the exception of the reproductive system. The single species of *Pawlowskiella* has oviducts that pass through the vector tissue and strands of conducting tissue that do not fuse with the ovisacs. Species of *Piscicola* are described as having oviducts that open to the female gonopore anterior to the vector tissue and strands of conducting tissue that fuse with the ovisacs. Both species from North America, *P. milneri* and *P. punctata*, have oviducts that open to the female gonopore anterior to the vector tissue and strands of conducting tissue that fuse with the ovisacs. Therefore, both species should remain in the genus *Piscicola*. 
Chapter 4: Summary

Systematics has never been and will never be an exact science. The end product is the formation of a hypothesis that can be altered as more or different information becomes available, but it will probably never result in a definitive answer. The piscicolid leeches are an excellent example of this. For over 50 years the fish leeches have been divided into subfamilies based on a single morphological character. Then molecular methods became the standard for taxonomic studies and it was discovered that the subfamily divisions are not valid. Piscicolid phylogenetics is still in its developing stages. The findings from Chapter 2 show that the morphological characters used for classifications may not be as relevant as previously thought. Not only were the subfamilies disrupted, but also many generic level relationships and a species level relationship were shown to be invalid given the molecular data. Studies need to be performed to determine just which morphological characters are taxonomically important and should be used to define a subfamily, genus, or species. The genetic data seems to make classification easier when a single tree can be chosen as the phylogeny, but if morphological features cannot be matched to the clades formed, the whole classification system needs to be reconsidered.

While genetic data may be one of the more accurate methods for identifying species, it is costly, not available to most people, and requires a large database of sequences to test against. Morphological methods of identification are still the most widespread and practical techniques used. Chapter 3 represents the first comprehensive study of the North American freshwater fish leeches yet there is still much that needs to be explored to fully understand this group of organisms. In particular, live specimens of *Gonimosobdella mammillata* (Malm 1863), *Cystobranchus verrilli* Meyer 1940, *Myzobdella patzcuarensis* (Caballero 1940) and *Piscicola punctata* (Verrill 1871) would be useful to observe and confirm reported pigmentation patterns. Newly collected specimens of *C. verrilli* are also needed to confirm the placement in *Cystobranchus* Diesing 1859. Little is known of the internal anatomy of this species. Given the close relationship between *Cystobranchus, Gonimosobdella* Williams and Burreson 2005, and
*Piscicola* Blainville 1818 internal anatomy is needed to determine to which genus *C. verrilli* belongs.

Widespread sampling should be performed throughout North America to determine the ranges and host preferences of these species. Sampling in the middle United States, northern Canada, and most of Mexico has been sparse. A new species was discovered in Arkansas and there may be more undescribed species in North America. Throughout this study sampling was a limiting factor. Collections were attempted for almost all species but various problems arose. One major problem was the ability to capture the fish host. Electroshocking with a backpack electroshocking device was the most effective method for collecting freshwater fish but could not be performed in the deeper rivers and in lakes. Invasive fish species are also a nuisance as many of the populations of originally reported fish hosts are dwindling and becoming harder to find. Species-specific leeches may find it difficult to adjust as the fish species compositions are changing.

Our understanding of the relationships among piscicolid leeches is just beginning to take shape. This work has demonstrated how much more there is left to explore.
DISCLAIMER

Name changes presented solely in this dissertation are not intended to establish new names.
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