RH: Redescription and Molecular Characterization of *Placobdella pediculata*

Redescription and Molecular Characterization of *Placobdella pediculata* Hemingway, 1908 (Hirudinida: Glossiphoniidae)

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Abstract: *Placobdella pediculata* Hemingway, 1908 was originally described from individuals collected attached to *Aplodinotus grunniens* (freshwater drum) in Lake Pepin, Minnesota. Apparently, no type material was deposited. The acquisition of contemporary specimens from its type host in the type locality facilitated redescription of *P. pediculata*. *Placobdella pediculata* is different from its congeners in that its caudal sucker is extended from the body by a pedicel (peduncle), bears digitate processes near the rim of the caudal sucker, a smooth body surface, and its anal placement (few annuli anteriad of the caudal sucker). Molecular comparison of CO-I sequence data from *P. pediculata* revealed differences of 13.8% to 17.4% among congeners. *Placobdella pediculata* is a distinct species.

Key Words: Rhychobdellida, Hirudinea, Clitellata, Glossiphoniidae, *Placobdella pediculata*, *Actinobdella*, Minnesota, leech

Placobdella pediculata Hemmingway, 1908 was originally described from individuals collected from the gill chambers of freshwater drum, *Aplodinotus grunniens* Rafinesque 1819 in Lake Pepin, Minnesota. Apparently, no type material was deposited. In the Leeches of Minnesota, Hemmingway (1912) reprinted the description of *P. pediculata* and provided additional information and figures. Since its original description, this leech has been reported from *A. grunniens* throughout the Midwestern United States including Iowa, Illinois, Kentucky, Michigan, Minnesota, Missouri, Ohio, Oklahoma, Tennessee, and Wisconsin, Lake Erie, Lake Ontario, and Lake St. Clair, as well as Maine (Klemm 1985, 1991; Bur 1994; Wolf et al. 2008). *Placobdella pediculata* was thought to be an exclusive parasite of *A. grunniens* (Klemm 1985, 1991) until Schultz et al. (2011) found that 2 of 112 Northern Shorthead Redhorse, *Moxostoma macrolepidotum* were infested with *P. pediculata*. *Placobdella pediculata* is redescribed on the basis of contemporary material collected from freshwater drum in the type locality (Lake Pepin, Minnesota) from which Hemingway (1908) collected specimens. A molecular characterization of *P. pediculata* is also provided.

Materials and Methods

Collection of Leeches

Specimens conforming to the description of *P. pediculata* by Hemingway (1908) were collected from the gill arches of freshwater drum *A. grunniens* from Lake Pepin (type locality) in Goodhue County, Minnesota (44°32’45”N 92°20’24”W) on 6 August 2015 and 29 August 2018. Freshwater drum were collected by Minnesota Department of Natural Resources (MNDNR) staff using a Smith-Root pulsed DC electrofishing boat to sample nearshore habitat. Additional specimens of *P. pediculata* were collected from freshwater drum sampled using a bottom trawl as part of the large lake sampling protocol described by Schlesser (2016). Leech specimens were relaxed, examined, and fixed as described by Moser et al. (2006). Terminology for plane shapes follows Clopton (2004). Specimens were deposited in the Smithsonian Institution, National Museum of Natural History (USNM), Washington, District of Columbia, and the Peabody Museum of Natural History (YPM IZ), Yale University, New Haven, Connecticut.

DNA Analyses

Molecular analyses were conducted on newly collected material according to Richardson et al. (2010) as follows: DNA was isolated from tissue removed from the caudal suckers of individual leeches with the DNeasy Blood & Tissue Kit from Qiagen (Cat. No. 69504), following the protocol given for the purification of total DNA from animal tissues (spin-column). For the proteinase K treatment step, tissue samples were lysed overnight at 56°C. DNA was eluted from the spin columns with 150 µl of buffer.

PCR Reactions were prepared using the Illustra PuRe Taq Ready-To-Go PCR beads from GE Health Care (Cat. No. 27-9559-01). Primers were purchased from Invitrogen and were comprised of two primers each for cytochrome c oxidase subunit I (CO-I) as specified by Folmer et al. (1994) and Light and Siddall (1999). Specifically the CO-I primers were LCO1490 (5’GGTCAACAAATCATAAAGATATTGG 3’) and HCO2198 (5’TAAACTTCAGGGTGACCAAAAAATCA 3’). Final volume of PCR reactions was 25 µl with 2 µl of leech genomic DNA added per reaction. DNA was amplified under the following PCR conditions: 94°C for 5 min.; 35 cycles of (94°C for 30 sec, 50°C for 30 sec, 72°C for 45 sec); 72°C for 7 min. Following PCR, samples were cleaned up using a QIAquick PCR purification kit from Qiagen (Cat. No. 28104).

Purified PCR products were sequenced using the HCO2198 primer and the LCO1490 primer for the Cytochrome c oxidase subunit I products by the W. M. Keck Foundation Biotechnology Resource Laboratory at Yale University. The DNA sequences were aligned using Clustal W version 2 (Larkin et al. 2007) and checked manually using SeaView 4 (Gouy et al. 2010) and then analyzed using PAUP* 4.0b10

(Swofford 2002) and compared to other leech DNA sequences contained within Genbank. Uncorrected p distance was calculated using PAUP*.

Results and Discussion

Specimens collected from *Aplodinotus grunniens* (freshwater drum) in Lake Pepin, Goodhue County, Minnesota, type locality of *P. pediculata*, were consistent with the description of *Placobdella pediculata* by Hemingway (1908) and provided the basis for redescription and molecular characterization of the species (Figure 1). The redescription is based upon USNM 1486915-1486916 and YPM IZ 103394 and 104864.

Placobdella pediculata Hemingway, 1908

Syn. *Actinobdella pediculata* (Hemingway 1908), Sawyer, 1986

Figures 1 - 5

External morphology: Body broadly elliptoid to ovoid with caudal sucker extended from the body by a pedicel (peduncle) (Figure 2). Caudal sucker unpigmented and with digitate processes near the rim (Figure 3). Dorsum base color brownish with marbled unpigmented sections and smooth (lacking papillae) (Figures 1-2). No specific metameric pattern on dorsal surface. Cephalic apical end unpigmented with two pair of eyespots where the posteriad pair are much larger than the anteriad pair and eyespots separated by half the diameter of the larger eyespots. Ventrum base color brownish with unpigmented sections. Male and female gonopores unpigmented, in furrows, and separated by two annuli.

Alimentary tract: Proboscis pore on rim/lip of the anterior sucker, proximal to the eye spots. Short, blunt-tipped proboscis, nearly uniformly cylindrical, slightly enlarged at the base, and in membranous sheath (Figure 4). Salivary cells scattered in the anterior half of the body (diffuse salivary glands) and more abundant near the base of the proboscis. Salivary ductal bundles attach on each side of the base of the proboscis (Figure 4). Slim, flaccid esophagus extends from the base of the proboscis with one pair glandular mycetomes. Seven pair of diverticulated crop ceca, last pair extending posteriorly and diverticulated into four sections. Four pair of intestinal ceca. Simple rectum opening to anus, located a few annuli anteriad of the caudal sucker in XXIII/XXIV.

Reproductive system:
MALE. Male gonopore slightly raised. Paired shallowly elliptoid atrial cornua extending laterally from male gonopore into robust, loosely coiled muscular ejaculatory ducts, recurving posteriorly to seminal vesicles and narrow vas deferentia connecting to testisacs (Figure 5). Six pair of testisacs, each testisac located between pair of crop ceca.
FEMALE. Female gonopore simple, opening to pair of bifurcated ovisacs and located within coelomic space that is attached on the ventral body wall. Ovisac length depends on the reproductive condition of the leech (ovisacs short in YPM IZ 103394; extending to the fourth testisac in mature individuals) (Figure 5).

Molecular comparison of 670 nucleotides of CO-I revealed an intraspecific difference of 0.2% to 0.3% (1 - 2 nucleotides) between one specimen of *P. pediculata* collected from Lake Pepin, Minnesota (type locality)(GenBank MN043903, USNM 1486916) and *P. pediculata* (GenBank AY047327, GenBank MF067120, MF067121). In addition, specimens of *P. pediculata* (GenBank MN043903, USNM 1486916; GenBank AY047327, GenBank MF067120, MF067121) were 0.0% to 0.2% (0 to 1 nucleotide) different from one specimen of *P. pediculata* from Arkansas (GenBank MN043904, USNM 1138863). Interspecific differences of 15.2% (102 nucleotides) were found between a specimen of *P. pediculata* (GenBank

MN043903, USNM 1486916) and a specimen *of Placobdella montifera* (GenBank AY047323); 15.7% to 16.0% differences (105 – 107 nucleotides) between a specimen of *P. pediculata* (GenBank MN043903, USNM 1486916) and two specimens of *Placobdella sophieae* (GenBank KF990594–KF990595) collected from Oregon; 15.2% to 17.0% differences (102 – 114 nucleotides) between a specimen of *P. pediculata* (GenBank MN043903, USNM 1486916) and two specimens of *Placobdella nuchalis* (GenBank MF535240 - MF535241) collected from the locality of the holotype (South Carolina) and paratype locality (North Carolina); 13.8% to 13.9% differences (93 nucleotides) between a specimen of *P. pediculata* (GenBank MN043903, USNM 1486916) and five specimens of *Placobdella ornata* (GenBank JQ8128–JQ8132) collected from the type locality (West River, New Haven County, Connecticut); 15.4 % to 15.7% differences (103 – 105 nucleotides) between a specimen of *P. pediculata* (GenBank MN043903, USNM 1486916) and five specimens of *Placobdella parasitica* collected from the type locality (Minnesota; GenBank KF058895–KF058899); 15.6% to 17.4% differences (104 – 117 nucleotides) between a specimen of *P. pediculata* (GenBank MN043903, USNM 1486916) and five specimens of *Placobdella rugosa* (GenBank JX412986-JX412990) collected from the type locality (North Dakota); 16.0% to 17.0% differences (107 – 114 nucleotides) between a specimen of *P. pediculata* (GenBank MN043903, USNM 1486916) and two specimens of *Placobdella translucens* (GenBank AY047328, JX122778). *Placobdella pediculata* is a distinct species with a 13.8% to 17.4% difference in CO-I sequence data among congeners.

Placobdella pediculata is differentiated from other glossiphoniid species by its caudal sucker which is extended from the body by a pedicel (peduncle); with digitate processes near the rim of the caudal sucker; by its smooth dorsal surface (lacking papillae), and its anal placement (few annuli anteriad of the caudal sucker). Another glossiphoniid leech with digitate processes and a caudal sucker extended from the body by a pedicel is *Actinobdella inequiannulata* Moore 1901. Sawyer (1986) pointed out the similarities between *P. pediculata* and *A. inequiannulata*, *e.g.* diffuse salivary glands, caudal sucker set off from its body by a distinct pedicel, and digitate processes on the outer rim of the caudal sucker. However, the pedicel of *A. inequiannulata* is much shorter than the pedicel (peduncle) of *P. pediculata*, *A. inequiannulata* has 1 to 5 rows of papillae, and the anus of *A. inequiannulata* is at the typical location (one annulus anteriad of the caudal sucker). In some specimens of *A. inequiannulata*, a mid-dorsal ridge is present (Klemm 1985).

Due to the similarities between *P. pediculata* and *A. inequiannulata*, Sawyer (1986) created the combination *Actinobdella pediculata*. Based on molecular data, however, Siddall et al. (2005) and De Carle et al. (2017) clearly show that *P. pediculata* is a member of the genus *Placobdella.* However, the collection locality of the representative specimens of *P. pediculata* in the publication (GenBank AY047327, MF067120, MF067121) are not known (Light and Siddall 1999; Siddall et al. 2005; De Carle et al. 2017). In the present study, sequence data (GenBank MN043903) of *P. pediculata* from its type locality (Lake Pepin, Minnesota) are presented for the first time.

Eight individuals identified as *Actinobdella inequiannulata* by McAllister et al. (2011) (see USNM 1138863), collected from the gill chamber of a single white crappie, *Pomoxis annularis* from the Ouachita River in Dallas County, Arkansas, were reexamined and redetermined to be *P. pediculata*. In the current study, a specimen (formerly identified as A. inequiannulata from Arkansas (GenBank MN043904, USNM 1138863) was 0.0% to 0.2% (0 to 1 nucleotide) different from one specimen of *P. pediculata* collected from the type locality of Lake Pepin, Minnesota (GenBank MN043903, USNM 1486916) and *P. pediculata* (GenBank AY047327, GenBank MF067120, MF067121). The eight specimens reported by McAllister et al. (2011) are *Placobdella pediculata* and these specimens represent a new locality record (Arkansas) and the white crappie (*Pomoxis annularis*) is a new host record.

Placobdella pediculata is a blood-feeding leech on fish, occurring primarily on the freshwater drum, *A. grunniens*, where it typically attaches below the gill chamber, on or near the isthmus or the

inside surface of the opercula (Hemingway 1908, 1912; Sawyer 1972; Klemm 1985, 1991; Bur 1994). Schultz et al. (2011) also found *P. pediculata* on the Northern Shorthead Redhorse (*Moxostoma macrolepidotum*) and McAllister et al. (2011) found *P. pediculata* (re-identification) on a white crappie (*Pomoxis annularis*). At the attachment site, the host tissue becomes inflamed and gradually grows around the caudal sucker up to the anus and leaves a deep pit in the host tissue (Hemingway 1908, 1912; Wolf et al. 2008). Other than its hosts, little is known regarding the life history of *P. pediculata*. Hemingway (1908, 1912) suggested that part of its life history may be spent off the host. However, Bere (1931) is the only report of *P. pediculata* being collected free-living (attached to submerged substrata).

On the basis of examination of contemporary material from the type locality (Lake Pepin, Minnesota), *P. pediculata* is redescribed in this study. *Placobdella pediculata* is a morphologically distinct species with a 13.8% to 17.4% difference in CO-I sequence data among congeners.

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FIGURE CAPTIONS

Figure 1. Living specimen of *Placobdella pediculata* attached on its host *Aplodinotus grunniens* (freshwater drum) from the type locality, Lake Pepin, Goodhue County, Minnesota, USA. Scale bar equals 25 mm.

Figure 2. Lateral surface of preserved specimen of *Placobdella pediculata* from the type locality, Lake Pepin, Goodhue County, Minnesota, USA. USNM 1486915, lateral surface, preserved specimen, caudal sucker pedicel (arrow). Scale bar equals 2 mm.

Figure 3. Ventral surface of preserved specimen of *Placobdella pediculata* (USNM 1486915), showing caudal sucker with digitate processes (arrows). Scale bar equals 1 mm.

Figure 4. Dissected specimen of *Placobdella pediculata* showing alimentary system, YPM IZ 103394. *Abbreviations*: D, diffuse salivary cells; Pr, proboscis. Scale bar equals 1 mm.

Figure 5. Dissected specimen of *Placobdella pediculata* showing reproductive system, YPM IZ 103394. *Abbreviations*: AC, atrial cornuae; O, ovisac. Scale bar equals 1 mm.