

RH: Redescription and Molecular Characterization of *Placobdella michiganensis*

Redescription and Molecular Characterization of *Placobdella michiganensis* (Sawyer, 1972) (Hirudinida: Glossiphoniidae)

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Abstract: *Placodella michiganensis* (Sawyer, 1972) was originally described from free-living individuals collected from Mill Creek, St. Joseph County, Michigan, USA. The acquisition of contemporary specimens from its type locality has facilitated redescription of *P. michiganensis*. *Placobdella michiganensis* does not have a confusing taxonomic history like many other members of the genus *Placobdella* as it has only been collected two other times since its description. *Placodella michiganensis* is distinguished from its congeners by its five rows of whitish single-tipped papillae, genital and pre-anal patches, five pre-anal papillae, and absence of a dark dorsal-medial line. *Placobdella michiganensis* is a morphologically distinct species with a 16.0% to 22.0% difference in CO-I sequence data among congeners.

Sawyer (1972) described *Batracobdella michiganensis* based on free-living specimens collected from Mill Creek, St. Joseph County, Michigan. Sawyer (1972) pointed out the striking similarities of *B. michiganensis* to *P. "phalera"* sensu Moore, 1906, with the former differing from the latter in the possession of "five series of slightly raised metameric dots, including a mid-dorsal series." The only report of *B. michiganensis* since its original description was by Klemm (1982) who reported collecting several specimens from Emmet County Michigan. *Batracobdella michiganensis* was subsequently transferred to the genus *Placobdella* by Sawyer (1986), to the genus *Desserobdella* by Barta and Sawyer (1990), and back to the genus *Placobdella* by Siddall et al. (2005). The feeding ecology of *P. michiganensis* is unknown although the species is assumed to blood-feed on amphibians (Sawyer 1974). The present study provides a redescription and molecular characterization of *P. michiganensis* recently collected from its type locality, Mill Creek, St. Joseph County, Michigan.

Materials and Methods

Four specimens conforming to the description of *P. michiganensis* were collected from Mill Creek, St. Joseph County, Michigan (41°53'10.27"N 85°42'56.64"W) on 24 February 2017. Specimens were relaxed, fixed, and examined as described by Moser *et al.* (2006). A specimen was pressed, stained with Semichon's acetocarmine and mounted in Canada Balsam for examination by light microscopy according to techniques outlined by Richardson (2006), as modified by Richardson and Barger (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 (USNM 1615727 – 1615728) and in the Peabody Museum of Natural History (YPM IZ), Yale University, New Haven, Connecticut (YPM IZ 106122 – 106123).

DNA Analyses

Molecular analyses were conducted on newly collected material according to Richardson *et al.* (2010) as follows: DNA was isolated from the caudal sucker of the individual leech 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.

Polymerase chain reactions (PCR) 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 2 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 (Benson *et al.* 2018). Uncorrected p distance was calculated using PAUP*.

Results and Discussion

The following redescription of *P. michiganensis* is based on the type series of *Batracobdella michiganensis* (USNM 51898 Holotype and USNM 51899 Paratype) and newly collected specimens (YPM

IZ 106122 – 106123, USNM 1615727 – 1615728) from the type locality of Mill Creek, St. Joseph County, Michigan, USA.

Placobdella michiganensis
(Sawyer, 1972) Sawyer, 1986

Figures 1–2

External morphology. Body ovoid to deltoid. Dorsum base color brownish-green-gray with numerous dark chromatophores. Two pairs of near-coalesced eyespots on an unpigmented anteriad region with a white/light yellowish pigment patch below the eyespots. Unpigmented nuchal band with white/light yellow pigment, a genital white/light yellowish patch (dorsal to the gonopores), and a pre-anal white/light yellowish patch (Figure 1). Five rows of single-tipped papillae with white/light yellowish pigment. Medial and lateral papillae rows extend to the nuchal band and paralateral papillae rows extend to the genital patch (Figure 1). Lateral margins of the leech with one annulus with dark brownish-green-gray pigment and two annuli unpigmented with white/light yellowish chromatophores – pattern is from nuchal band to the caudal sucker. Beginning adjacent to the anus, just anterior to the anus furrow, and commencing anteriad are two rows of five pre-anal papillae (the last row, most anteriad, papillae are medially indented) (Figure 1). Caudal sucker with brownish-green-gray pigment and two rows of unpigmented papillae. Ventrums unpigmented with male and female gonopores in furrows and separated by 2 annuli.

Alimentary tract. Proboscis pore just posteriad of the rim/lip of the oral sucker. Blunt-tipped proboscis, nearly uniformly cylindrical and in membranous sheath. Salivary cells diffuse and most abundant at the base of the proboscis. Salivary ductule bundles attaching at each side of the base of the proboscis. Slim, flaccid esophagus extends from the base of the proboscis with one pair of saccular mycetomes. Seven pairs of diverticulated crop ceca, last pair extended posteriorly and diverticulated into four sections (Figure 2). Four pairs of simple, saccular intestinal ceca with last pair extending posteriad. Simple rectum opening to anus, located one annulus anteriad of the caudal sucker.

Reproductive system. (Male) Male gonopore slightly raised. Male atrium opening into narrowly elliptoid atrial cornuae extending laterally and anteriorly from male gonopore into coiled, muscular ejaculatory ducts, recurving posteriorly into seminal vesicles and narrow vas deferentia connecting to testisacs. Six pairs of testisacs and each testisac located in the space between a pair of crop ceca (Figure 2). (Female) Female gonopore simple, opening to a 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. In the specimens examined in this study, ovisacs extend posteriad to the second pair of crop ceca.

Molecular comparison of 670 nucleotides of CO-I from one specimen of *P. michiganensis* collected from Mill Creek, St. Joseph County, Michigan (type locality) (GenBank MT579302; USNM 1615728) revealed interspecific differences of 20.7% to 22.0% (139 – 148 nucleotides) from four specimens of *Placobdella appalachiensis* (GenBank KF990590 – KF990593) collected from the type locality (Smyth County, Virginia), 18.1% (121 nucleotides) from one specimen of *Placobdella biannulata* (GenBank AF116021) collected from North Carolina, 16.3% to 16.9% (109 – 113 nucleotides) from seven specimens of *Placobdella cryptobranchii* (GenBank KF601755–KF601761) collected from Missouri, 17.6% to 19.0% (118 – 127 nucleotides) from four specimens of *Placobdella hollensis* (GenBank KF771652–KF771655) collected from the type locality (Barnstable County, Massachusetts), 17.3% (116 nucleotides) from one specimen of *Placobdella montifera* (GenBank AY047323), 17.3% to 18.4% (116 to 123 nucleotides) from two specimens of *Placobdella nuchalis* (GenBank MF535241 - MF535241) collected from the Holotype locality (Orangeburg County, South Carolina) and Paratype locality (Gates County, North Carolina), 16.0% to 16.1% (107 – 108 nucleotides) from five specimens of *Placobdella ornata* (GenBank JQ8128–JQ8132) collected from the type locality (New Haven County, Connecticut), 17.5% to 18.9% (117 – 127 nucleotides) from five specimens of *Placobdella papillifera* (GenBank KC505241–

KC505245) collected from the type locality (New Haven County, Connecticut), 16.6% (112 nucleotides) from one specimen of *Placobdella picta* (GenBank AF116020) collected from Ontario, 18.9% to 19.7% (127 – 132 nucleotides) from five specimens of *Placobdella rugosa* (JX412986-JX412990) collected from the type locality (Rolette County, North Dakota), 19.0% to 19.1% (128 nucleotides) from two specimens of *Placobdella sophiae* (KF990594–KF990595) collected from Oregon, and 16.0% to 18.1% (107 – 121 nucleotides) from two specimens of *Placobdella translucens* (GenBank AY047328, JX1227788) (see Table 1). *Placobdella michiganensis* is a distinct species with a 16.0% to 22.0% difference in CO-I sequence data among congeners.

Placobdella michiganensis does not have a confusing taxonomic history like many other members of the genus *Placobdella* as it has only been collected two other times since its description (Klemm 1982; this study). Although *P. michiganensis* is similar to other species of the genus *Placobdella* with a nuchal band – *P. cryptobranchii*, *P. ornata* (*P. phalera* is a junior synonym – Moser et al. 2012), *P. picta*, and *P. translucens*, *P. michiganensis* are distinguished from their congeners by its five rows of whitish single-tipped papillae, genital and pre-anal patches, five pre-anal papillae, and absence of a dark dorsal-medial line. *Placobdella cryptobranchii*, *P. ornata*, and *P. translucens* have unpigmented genital and anal patches, but *P. cryptobranchii* has two nuchal bands, two lateral rows of dorsal papillae, and two rows of four pre-anal papillae (Moser et al. 2013) and *P. ornata* has three rows of dorsal papillae where the medial row is black-tipped and two rows of five pre-anal papillae (Moser et al. 2012). *Placobdella translucens* has no papillae, two rows of unpigmented spots, and a broken, dark dorsal-medial line (Sawyer and Shelley 1976; Klemm 1982; 1985). *Placobdella picta* is differentiated by having 6-7 rows of papillae, a dark dorsal-medial line, and no genital or anal patches.

Klemm (1991) referred to *P. michiganensis* as an uncommon leech that has only been reported from two counties in Michigan. Although *P. michiganensis* is only known from Michigan, it potentially occurs in Indiana, Southern Ontario, and Wisconsin. Additional investigations would help in elucidating the geographic distribution of *P. michiganensis*. In addition, little is known about the life history of *P. michiganensis*. Sawyer (1974) surmised that the species blood-feeds on amphibians and Sawyer (1972) found individuals with eggs in their ovisacs in late May.

On the basis of examination of the type series and contemporary material from the type locality (Mill Creek, St. Joseph County, Michigan), *P. michiganensis* is redescribed in this study. *Placobdella michiganensis* is a morphologically distinct species with a 16.0% to 22.0% difference in CO-I sequence data among congeners.

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Figure Legends

Figure 1. Living specimen, dorsal view of *Placobdella michiganensis* from the type locality, Mill Creek, St. Joseph County, Michigan, USA (USNM 1615728). Scale bar equals 1 mm.

Figure 2. Schematic drawing of internal morphology of *Placobdella michiganensis*. Figure legend: atrial cornuae (AC), crop ceca (CC), intestinal ceca (IC), ovisac (O), proboscis (PR), salivary cells (SC), testisac (T).

Table 1. CO-I interspecific percent sequence differences of congeners of *Placobdella michiganensis*.

Species	1	2	3	4	5	6	7	8	9	10	11	12	13
<i>P. michiganensis</i> (1)	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>P. appalachiensis</i> (2)	20.7-22.0	-	-	-	-	-	-	-	-	-	-	-	-
<i>P. biannulata</i> (3)	18.1	17.5-19.1	-	-	-	-	-	-	-	-	-	-	-
<i>P. cryptobranchii</i> (4)	16.3-16.9	17.6-19.6	17.1-17.7	-	-	-	-	-	-	-	-	-	-
<i>P. hollensis</i> (5)	17.6-19.0	17.6-18.9	17.9-19.1	15.4-17.4	-	-	-	-	-	-	-	-	-
<i>P. montifera</i> (6)	17.3	19.2-20.3	17.4	18.4-18.7	17.0-18.2	-	-	-	-	-	-	-	-
<i>P. nuchalis</i> (7)	17.3-18.4	17.7-19.1	16.8-17.6	15.5-18.1	16.2-18.1	16.0-17.7	-	-	-	-	-	-	-
<i>P. ornata</i> (8)	16.0-16.1	18.3-19.6	17.2-17.4	5.7-6.6	16.1-16.5	16.5-16.8	14.2-16.1	-	-	-	-	-	-
<i>P. papillifera</i> (9)	17.5-18.9	17.9-19.4	18.2-19.5	14.8-17.2	15.1-16.3	17.1-18.1	17.1-19.5	14.7-14.9	-	-	-	-	-
<i>P. picta</i> (10)	16.6	16.1-17.7	12.6	15.5-16.3	14.9-16.3	16.0	15.9-16.9	14.7-15.1	16.6-17.8	-	-	-	-
<i>P. rugosa</i> (11)	18.9-19.7	19.3-20.6	18.4-19.1	16.5-18.3	16.5-18.4	18.8-20.2	18.2-19.5	14.9-15.9	15.0-17.7	16.4-16.9	-	-	-
<i>P. sophiae</i> (12)	19.0-19.1	18.2-19.6	17.1-17.2	16.8-18.2	14.2-15.6	17.9-18.2	14.5-16.1	17.1-18.0	17.6-19.3	16.1-16.3	16.3-17.7	-	-
<i>P. translucens</i> (13)	16.0-18.1	18.1-19.5	16.3-18.5	11.3-12.8	15.5-17.1	17.9-19.0	15.7-18.0	10.9-11.8	14.0-15.6	16.3-17.8	15.6-16.7	16.7-17.3	-
	1	2	3	4	5	6	7	8	9	10	11	12	13