Research Note

Distribution of *Placobdella hollensis* (Whitman, 1892) (Hirudinida: Glossiphoniidae) WILLIAM E. MOSER^{1, 5}, DENNIS J. RICHARDSON², CHARLOTTE I. HAMMOND², STEVE W. GOTTE³, and ERIC LAZO-WASEM⁴

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ABSTRACT: Confusion regarding the identification of *Placobdella hollensis* (Whitman, 1892) (Hirudinida: Glossiphoniidae), has led to an unclear understanding of the distribution of the species. Two specimens of *P. hollensis* were collected from Merchants Millpond State Park, Gates County, North Carolina, U.S.A. representing a new geographic distribution record. Specimens were confirmed as *P. hollensis* by morphological and molecular study. Specimens of

P. hollensis from North Carolina, U.S.A. had accessory eyes, two thin paramedial dark lines, 3 pair of pre-anal papillae, and molecular comparison of CO-I sequence data revealed a 99.0% to 99.7% similarity to specimens of *P. hollensis* collected from its type locality (Barnstable County, Massachusetts, U.S.A.). From confirmed specimens of *P. hollensis*, this report supports the assertion that *P. hollensis* has an Atlantic coastal distribution.

KEY WORDS: Rhychobdellida, Hirudinea, Clitellata, Glossiphoniidae, *Placobdella hollensis*, Clepsine, North Carolina, leech

Placobdella hollensis (Whitman, 1892) is distinct among its congeners in its possession of accessory eye spots. Nevertheless, there has been confusion regarding the distribution of *P. hollensis. Placobdella hollensis* was described by Whitman (1892) based on material collected from Woods Hole, Massachusetts, U.S.A. Moore (1906) transferred the species to the genus *Placobdella* based upon a specimen collected from Rondeau, Ontario, Canada. Despite the etymology of the specific epithet *hollensis* (meaning from Holl in reference to Woods Hole, Massachusetts. U.S.A.) the scientific literature had applied a Great Lakes origin to the species (see Moser et al., 2014). Sawyer (1972) had included only Iowa, Michigan, Minnesota, U.S.A. and Ontario, Canada in a list of published distribution records for *P. hollensis*. Klemm (1991) called *P. hollensis* an uncommon, cold-water species usually occurring in permanent ponds in the Great Lakes region. After contemporary leeches collected from the type locality of Coonamessett Pond, Barnstable County, Massachusetts, U.S.A. were determined to be conspecific with *P. hollensis*, Moser et al. (2014) redescribed *P. hollensis*, designated a neotype (MCZ 20404), and suggested the species had an Atlantic coastal distribution.

In the course of faunistic studies on the leeches of the eastern United States, 2 specimens of P. hollensis were collected on 8 August, 2014 from underneath submerged substrata in Merchants Millpond State Park, Gates County, North Carolina, U.S.A. (36°25'56"N 076°41'52"W) representing a new geographic distribution record. Voucher specimens were prepared as described by Moser et al. (2006) and deposited in the Smithsonian Institution, National Museum of Natural History, Washington, D.C. (USNM 1422201) and the Peabody Museum of Natural History at Yale University, New Haven, Connecticut (YPM IZ 90153). Molecular analyses were conducted according to Richardson et al. (2010). 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), checked manually using SeaView 4 (Gouy et al., 2010), and then analyzed using PAUP* 4.0b10 (Swofford, 2002), deposited in GenBank (http://www.ncbi.nlm.nih.gov/genbank/), and compared to other leech DNA sequences contained within Genbank. Uncorrected p distance was calculated using PAUP*.

Table 1 and Figure 1 summarize all previous confirmed reports and the new North Carolina, U.S.A. locality record of *P. hollensis*. The 2 specimens were morphologically and molecularly confirmed to be *P. hollensis*. The North Carolina, U.S.A. *P. hollensis* specimens had accessory eyes, two thin paramedial dark lines, and 3 pair of pre-anal papillae (Figure 2). Molecular comparison of 626 nucleotides of CO-I revealed intraspecific differences of 0.3% – 1.0% (2 – 6 nucleotides) between two specimens of *P. hollensis* collected from Merchant's Millpond State Park, North Carolina, U.S.A. (GenBank KY741539 - KY741540) and four specimens of *P. hollensis* collected from the type locality (Massachusetts, U.S.A.; GenBank

KF771652–KF771655). The specimens of *P. hollensis* from Merchant's Millpond State Park, North Carolina, U.S.A. are 99.0% to 99.7% similar to *P. hollensis* specimens collected from its type locality (Barnstable County, Massachusetts, U.S.A.). The identifications of legacy museum (MCZ, USNM and YPM) specimens of *P. hollensis* were confirmed by morphological analysis. This report supports the assertion of Moser et al. (2014) that *P. hollensis* has an Atlantic coastal distribution.

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FIGURE LEGENDS

Table 1. Previous Confirmed Reports and New Locality Record of *Placobdella hollensis* (Whitman, 1892) (Hirudinida: Glossiphoniidae).

Figure 1. Distribution of *Placobdella hollensis* (Whitman, 1892) (Hirudinida: Glossiphoniidae) (circle: new records, triangle: published localities, star: type locality), inset New England at higher magnification.

Figure 2. Dorsal surface of preserved specimen of *Placobdella hollensis* (Whitman, 1892) (Hirudinida: Glossiphoniidae) illustrating the accessory eyes (arrows) and two thin paramedial dark lines (arrowheads). USNM 1422201. Scale bar equals 3 mm.

Table 1. Previous Confirmed Reports and New Locality Record of *Placobdella hollensis* (Whitman, 1892) (Hirudinida: Glossiphoniidae).

Catalog number	* State	County	Locality	Date Collected	Latitude	Longitude	Notes
MCZ 20404	Massachusetts	Barnstable County	Coonamessett Pond, Falmoth	30 May 2013	41°37'10.25	'N 070°34'20.42"W	Neotype; Moser et al., 2014
MCZ 56653	Massachusetts	Nantucket County	Reclamation Pond, Nantucket	11 Jun 2001	41°17'33"N	069°59'38"W	Smith and Klemm, 2003
USNM 40222	Massachusetts	Dukes County	Nonamesset Island	22 Jul 1904	41°30'30"N	070°41'15"W	Klemm, 1985
USNM 1225112	Massachusetts	Barnstable County	Coonamessett Pond, Falmoth	4 May 2013	41°37'10.25	'N 070°34'20.42"W	Moser et al., 2014; GenBank KF771653
USNM 1225113	Massachusetts	Barnstable County	Coonamessett Pond, Falmoth	30 May 2013	41°37'10.25	'N 070°34'20.42"W	Moser et al., 2014
YPM 67547	Massachusetts	Barnstable County	Coonamessett Pond, Falmoth	4 May 2013	41°37'10.25	'N 070°34'20.42"W	Moser et al., 2014; GenBank KF771654
YPM 67540	Massachusetts	Barnstable County	Coonamessett Pond, Falmoth	30 May 2013	41°37'10.25	'N 070°34'20.42"W	Moser et al., 2014
YPM 67560	Massachusetts	Barnstable County	Coonamessett Pond, Falmoth	30 May 2013	41°37'10.25	'N 070°34'20.42"W	Moser et al., 2014; GenBank KF771655
YPM 58264	Massachusetts	Barnstable County	Coonamessett Pond, Falmoth	22 Aug 2010	41°37'11"N	070°34'07''W	Moser et al., 2014; GenBank KF771652
YPM 58425	Massachusetts	Barnstable County	Coonamessett Pond, Falmoth	22 Jul 2013	41°37'11"N	070°34'07''W	Richardson et al., 2015; GenBank KP176605
YPM 67807	Massachusetts	Barnstable County	Coonamessett Pond, Falmoth	22 Jul 2013	41°37'11"N	070°34'07''W	Richardson et al., 2015; GenBank KP176606
MCZ 56650	Rhode Island	Washington County	South Long Pond, New Shoreham, Block Island	15 Sep 2001	41°09'15"N	071°35'31"W	Smith and Klemm, 2003
MCZ 56651	Rhode Island	Washington County	Nevas Pond	23 Jun 2001	41°09'49"N	071°35'06"W	Smith and Klemm, 2003
MCZ 56651	Rhode Island	Washington County	Nathan Mott II Pond	23 Jun 2001	41°09'58"N	071°35'15"W	Smith and Klemm, 2003
YPM 58404	Rhode Island	Washington County	Tucker Pond, South Kingston	24 Jun 2013	41°25'33"N	071°32'59"W	Richardson et al., 2015; GenBank KP176604
USNM 1422201	North Carolina	Gates County	Merchants Millpond, Merchants Millpond State Park	8 Aug 2014	36°25'56"N	076°41'52"W	this study; GenBank KY741539
YPM 90153	North Carolina	Gates County	Merchants Millpond, Merchants Millpond State Park	8 Aug 2014	36°25'56"N	076°41'52"W	this study: GenBank KY741540
USNM 44005	Florida	Polk County	Withlacoochee River	27 Jul 1966	28°18'00"N	082°00'00"W	Klemm, 1985
USNM 62828	Florida	Sumter County	Little Withlacoochee River, Near US 301 Bridge	14 Aug 1972	28°34'22"N	082°09'19"W	Klemm, 1985

^{*}MCZ = Harvard University Museum of Comparative Zoology

USNM = Smithsonian Institution, National Museum of Natural History

YPM = Peabody Museum of Natural History, Yale University



