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New Host and Distribution Records of the Leech *Placobdella multilineata* Moore, 1953 (Hirudinida: Glossiphoniidae)

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Running Title: New Host and Distribution Records of *Placobdella multilineata*

Placobdella multilineata was described by Moore (1953) based on free-living specimens collected from New Orleans, Louisiana and Norman, Oklahoma (Meyer 1968). *Placobdella multilineata* is a blood-feeding leech with relatively low host specificity, being reported from alligators, amphiuma, and turtles (Sawyer and Shelley 1976, Forrester and Sawyer 1974, Saumure and Doody 1998). The geographic range of *P. multilineata* includes the southeastern United States and extends northward through the Mississippi Valley as far north as Illinois and Iowa (Klemm 1982, 1985). Although, it is a relatively common species, it was only recently reported from Arkansas (Moser et al. 2006, McAllister and Moser 2012).

Between 2007–2014, leeches were collected as follows: a single individual of *P. multilineata* was collected from a broad-banded watersnake (*Nerodia fasciata confluens*) from Big Cane Conservation Area, Butler County, Missouri (36°29'56"N 90°29'40"W) on 6 June 2007; a single free-living individual of *P. multilineata* was collected from Jonesboro, Craighead County, Arkansas (35°45'20.94"N 90°42'43.93"W) on 12 February 2012; ten free-living individuals of *P. multilineata* were collected from Lukfata Creek, McCurtain County, Oklahoma (33°58'05.51"N, 94°45'57.06"W) on 8 October 2011; single individuals of *P. multilineata* were collected from a red-eared slider (*Trachemys scripta elegans*) and a northern diamond-backed water snake (*Nerodia rhombifer*) from a cattle tank in Broken Bow at Lukfata, McCurtain County, Oklahoma (34°00'22.03"N, 94°45'53.81"W) on 11 June and 13 June 2012, respectively; a single individual of *P. multilineata* was collected from a red-eared slider (*T. scripta elegans*) from 7 km east of Harrell on Highway 278, Calhoun County, Arkansas (33°32'09.4"N 92°19'49.5"W) on

11 January 2012; a single free-living individual of *P. multilineata* was collected from Spring Mill off US Highway 69, Independence County, Arkansas (35°49'42"N 91°43'24"W) on 25 July 2013; a single individual of *P. multilineata* was collected from an eastern musk turtle (*Sternotherus odoratus*) from intersection of county road 407 and county road 409, Jonesboro, Craighead County, Arkansas (35°46'08"N 90°42'51"W) on 8 March 2014. Specimens were prepared as described by Moser et al. (2006).

Molecular analyses were conducted on newly collected material 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) and 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*.

Leeches were identified with the assistance of taxonomic keys (Klemm 1982, 1985) and examination of the type series of *P. multilineata* (USNM 36383–36484, USNM 36413, USNM 36428, USNM 36435). Voucher specimens of leeches were deposited in the Invertebrate Zoology Collections of the Department of Invertebrate Zoology, National Museum of Natural History (USNM), Smithsonian Institution, Washington, D. C. (USNM 1253384–1253390) and the Peabody Museum of Natural History at Yale University (YPM IZ 58313–58315, 58392 and 67729).

Molecular comparison of 637 nucleotides of CO-I revealed an intraspecific difference of 1.1% (7 nucleotides) between two specimens of *P. multilineata* collected from Lukfata Creek, Oklahoma (GenBank KM396760 & KM396761). An intraspecific difference of 1.3% (8 nucleotides) was found between *P. multilineata* collected from Lukfata Creek, Oklahoma and a specimen of *P. multilineata* (GenBank AY962464) collected from Maurepas Swamp, Louisiana. Comparison of CO-I sequence data of three specimens of *P. multilineata* (GenBank KM396760, KM396761 and AY962464) revealed differences of 13.6 % to 14.0% (86 to 89 nucleotides) from five specimens of *Placobdella parasitica* collected from its type locality (Minnesota; GenBank KF058895 – KF058899), differences of 16.7% to 17.8% (106 to 113 nucleotides) from five specimens of *Placobdella papillifera* from Connecticut (GenBank KC505241–KC505245), differences of 16.4% to 18.0% (104 to 115 nucleotides) from three specimens of *Placobdella ali* from Connecticut and New York (GenBank HM347040–HM347042), and differences of 15.5% to 16.6% (99 to 105 nucleotides) from five specimens of *Placobdella rugosa* from North Dakota (GenBank JX412986–JX412990).

Placobdella multilineata is a relatively large and sharply dorsoventrally flattened species. It is characterized by its five precise longitudinal rows of papillae, narrow, uninterrupted (sometimes interrupted)

dorsal-medial line, and stripes on the ventral surface. Examination of the type series *P. multilineata* (USNM 36383-36484, USNM 36413, USNM 36428, USNM 36435) and specimens collected in this study revealed a pattern of two rows of three pre-anal papillae, followed by two pairs of prominent paramedial papillae (Fig. 1). This distinct pre-anal papillae pattern also occurs in *P. ali* and *P. rugosa* (Hughes and Siddall 2007, Moser et al. 2012).

In summary, *P. multilineata* is reported from Missouri for the first time. Recorded hosts for *P. multilineata* are presented in Table 1. New host records in this study include broad-banded watersnake (*N. fasciata confluens*), northern diamond-backed water snake (*N. rhombifer*), red-eared slider (*T. scripta elegans*), and eastern musk turtle (*S. odoratus*). *Placobdella multilineata* has now been reported from 17 species and subspecies of alligators, amphiumas, crocodiles, snakes and turtles.

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Figure 1. Posterior end of *Placobdella multilineata* showing papillar pattern of pre-anal region.

A) USNM 36384. Paratype, scale bar equals 2 mm. B) USNM 1253387, specimen collected from Lukfata Creek, McCurtain County, Oklahoma in present study. Scale bar equals 2 mm.

New Host and Distribution Records of *Placobdella multilineata*Table 1. Reported Hosts of *Placobdella multilineata* Moore 1953

Host	Common name	Reference
<i>Alligator mississippiensis</i>	American alligator	Forrester and Sawyer 1974, Glassman et al. 1979, Khan et al. 1980, Cherry and Ager 1982, Brantley and Platt 1991
<i>Crocodylus porosus</i> *	estuarine crocodile	Yang and Davies 1985a,b
<i>Amphiuma tridactylum</i>	three-toed amphiuma	Saumure and Doody 1998
<i>Chelydra serpentina</i>	snapping turtle	Stone 1976, Sawyer and Shelley 1976, Readell et al. 2008
<i>Clemmys muhlenbergii</i>	bog turtle	Saumure and Carter 1998, Saumure and Beane 2001
<i>Chrysemys picta</i>	painted turtle	Readell et al. 2008
<i>Kinosternon leucostomum</i>	white-lipped mud turtle	Rossow et al. 2013
<i>Kinosternon scorpioides</i>	scorpion mud turtle	Rossow et al. 2013
<i>Macrochelys temminckii</i>	alligator snapping turtle	Forrester and Sawyer 1974
<i>Rhinoclemmys funereal</i>	black river turtle	Rossow et al. 2013
<i>Sternotherus carinatus</i>	razor-backed musk turtle	McAllister and Moser 2012
<i>Sternotherus odoratus</i>	eastern musk turtle	this study
<i>Trachemys scripta</i>	pond slider	Readell et al. 2008
<i>Trachemys scripta elegans</i>	red-eared slider	this study
<i>Trachemys scripta scripta</i>	yellow-bellied slider	Sawyer and Shelley 1976
<i>Nerodia rhombifer</i>	n. diamond-backed watersnake	this study
<i>Nerodia fasciata confluens</i>	broad-banded watersnake	this study

*Accidental infestation at the Beijing Zoo, People's Republic of China

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