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# Molecular Characterization of *Helobdella modesta* (Verrill, 1872) (Hirudinida: Glossiphoniidae) from its type locality, West River and Whitneyville Lake, New Haven County, Connecticut, USA

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*Clepsine modesta* was described by Verrill (1872) based on specimens collected in the West River and Whitneyville Lake, New Haven County, Connecticut, USA. Consistent with Article 73.2.3 of the International Code of Zoological Nomenclature (Anonymous 1999), the Syntype specimens of *C. modesta* originated from two localities and thus the type locality encompasses all of the places of origin. Moore (1898) synonomized *C. modesta* with the European *Helobdella stagnalis* (Linnaeus 1758) based on similarities in morphology. Subsequently, all North American leeches with a nuchal scute were considered as *H. stagnalis* with the exception of *Helobdella californica* Kutschera 1988, known only from Stow Lake, Golden Gate Park, San Francisco, California, USA. In addition to Europe and North America, *Helobdella stagnalis* has been reported from South America, Africa, and Asia (Sawyer 1986).

In a molecular comparison, Siddall & Borda (2003) found that *H. stagnalis* from Columbus, Ohio, USA differed from European *H. stagnalis* in 53 (8%) and 64 (10.3%) nucleotides for regions of the mitochondrial genes, cytochrome c oxidase subunit I (CO-I) and nicotinamide adenine dinucleotide dehydrogenase subunit 1 (ND-1), respectively. Based on the considerable genetic distinctions reported by Siddall & Borda (2003), Siddall *et al.* (2005) resurrected *Helobdella modesta* (Verrill 1872) for North American specimens of *H. stagnalis* even though European and North American specimens are considered morphologically indistinguishable (Moore 1952; Sawyer 1986; Siddall & Borda 2003).
The present study provides a molecular characterization of specimens of *H. modesta* collected from its type locality, West River and Whitneyville Lake, New Haven County, Connecticut, USA.

**Collection and identification of Leeches.** In the course of a survey of the leech fauna of south-central Connecticut, individuals of *Helobdella modesta* were collected by hand from submerged substrate in the West River and Whitneyville Lake, New Haven County, Connecticut, the type locality of *H. modesta*. Specifically, collections from the West River were made from the Whalley Avenue Bridge (41°19’30.13N 72°57’26.76W) south to the “Duck Pond” (41°18’51.30N 72°57’21.75W) and in a slough lying west of the river (41°19’01.00N 72°57’23.83W to 41°19’02.68N 72°57’25.60W), connecting with the river just north of the Edgewood Avenue bridge (41°18’51.30N 72°57’21.75W) as illustrated on pg. 12 of Shumway & Hegel (1990) and Whitneyville Lake (41°20’30.6N 72°54’42.6W) between May 2008 and September 2009. Specimens were relaxed, examined, and fixed as described by Moser *et al.* (2006). Identification was made according to the description of Verrill (1872) and by comparison to the Syntype material (YPM 252, 255, 289). Specimens were deposited in the Peabody Museum of Natural History (YPM 43281, 43284, 43404, 43405, 43417, 43421, 43424, 43429, 43433, 43444, 43453), Yale University, New Haven, Connecticut and the Smithsonian Institution, National Museum of Natural History (USNM 1135389-1135392), Washington, District of Columbia.

**DNA analysis.** Molecular analyses were conducted according to Richardson *et al.* (2010) as follows: DNA was isolated 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 digested overnight at 56°C. DNA was eluted from the spin columns with 150 ul 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 2 primers each for cytochrome c oxidase

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