



Molecular Characterization of *Glossiphonia elegans* (Verrill, 1872) (Glossiphoniidae: Hirudinida) from its type locality, West River, New Haven County, Connecticut, USA

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Clepsine elegans Verrill, 1872 was described based on material collected from the West River, New Haven, New Haven County, Connecticut, USA (Verrill, 1872). Moore (1901) synonymized *C. elegans* with the European congener *Glossiphonia complanata* (Linnaeus, 1758) in view of the morphological similarities of the two species. Subsequently, all North American leeches bearing 6 eyespots and a pair of paramedial lines were considered *Glossiphonia complanata*. Using specimens collected from Ontario and Michigan, Siddall et al. (2005) resurrected *Glossiphonia elgans* (Verrill, 1872), because specimens collected from Ontario, Canada and Michigan, USA showed considerable genetic distinction from, and did not form monophyletic group with, European specimens of *G. complanata*.

In the present study, a molecular characterization of specimens conforming to the original description of *G. elegans* collected from the West River, New Haven, New Haven County, Connecticut is provided along with a comparison of this material with other populations of *Glossiphonia* spp.

Collection of Leeches. In the course of a survey of the leech fauna of south-central Connecticut, individuals conforming to the original description of *G. elegans* by Verrill (1872) were collected by hand from submerged substrate in the West River, New Haven, New Haven County, Connecticut, the type locality of *G. elegans*. Specifically, collections 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 and Hegel (1990) and Konolds Pond (impoundment of West River, 41°20'52.1N 72°58'41.6W) between May 2008 and September 2009. Comparative material was collected from Lake Bemidji, Beltrami County, Minnesota (47°29'N 94°52'W) and Deschutes River, near Little Lava Lake, Deschutes County, Oregon (43°54'30.9N 121°45'53.58W). 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 284). Specimens were deposited in the Peabody Museum of Natural History (YPM 55694–55698, 55939, 55941), Yale University, New Haven, Connecticut and the Smithsonian Institution, National Museum of Natural History (USNM 1162070 - 1162075), 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 (5 specimens of *G. elegans* from West River, Connecticut; 9 specimens from Lake Bemidji, Minnesota; and 4 specimens from Deschutes River, Oregon) 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 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 2 primers each for cytochrome c oxidase subunit I (CO-I) and nicotinamide adenine dinucleotide dehydrogenase subunit 1 (ND-1) as specified by Light and Siddall (1999). Specifically the CO-I primers were LCO1490 (5'GGTCAACAAATCATAAAGATATTGG 3') and