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A new species of *Helobdella* (Hirudinida: Glossiphoniidae) from Oregon, USA

WILLIAM E. MOSER[1,7], STEVEN V. FEND[2], DENNIS J. RICHARDSON[3], CHARLOTTE I. HAMMOND[3], ERIC A. LAZO-WASEM[4], FREDRIC R. GOVEDICH[5] & BETTINA S. GULLO[6]

[1]*Smithsonian Institution, National Museum of Natural History, Department of Invertebrate Zoology, Museum Support Center, MRC 534, 4210 Silver Hill Road, Suitland, Maryland 20746, U.S.A. E-mail: moserw@si.edu*
[2]*U.S. Geological Survey, 345 Middlefield Rd., Mailstop 465, Menlo Park, California, 94025, U.S.A. E-mail: svfend@usgs.gov*
[3]*School of Biological Sciences, Quinnipiac University, 275 Mt. Carmel Avenue, Hamden, Connecticut 06518, U.S.A. E-mail: Dennis.Richardson@quinnipiac.edu and Charlotte.Hammond@quinnipiac.edu*
[4]*Division of Invertebrate Zoology, Peabody Museum of Natural History, Yale University, P.O. Box 208118, New Haven, Connecticut 06520, U.S.A. E-mail: eric.lazo-wasem@yale.edu*
[5]*Department of Biological Sciences, Southern Utah University, Cedar City, Utah 84720, U.S.A. E-mail: govedich@suu.edu*
[6]*Cátedra Zoologia Invertebrados I. Facultad de Ciencias Naturales y Museo, Universidad Nacional de La Plata. Paseo del Bosque s/ n B1900FWA, La Plata, Argentina. E-mail: bgullo@fcnym.unlp.edu.ar*
[7]*Corresponding author*

Abstract

Helobdella bowermani **n. sp.** is described from specimens collected in fine sediment of open water benthos of Upper Klamath Lake, Klamath County, Oregon. The new species has pale yellow/buff coloration with scattered chromatophore blotches throughout the dorsal surface, lateral extensions or papillae only on the a2 annulus, dorsal medial row of papillae with small papilla on a1 and larger papillae on a2 and a3, and a small oval scute (rarely triangular). *Helobdella bowermani* **n. sp.** is morphologically similar to *Helobdella atli* and *Helobdella simplex*. Molecular comparison of CO-I sequence data from *H. bowermani* **n. sp.** revealed differences of 10.6%–10.8% with *Helobdella californica*, differences of 12.2%–13.7% with *H. atli,* and differences of 12.7%–13.2% with *H. simplex*.

Key words: *Helobdella californica*, *Helobdella simplex*, *Helobdella atli*, Pacific Northwest, Oregon, Upper Klamath Lake, Glossiphoniidae, Hirudinea, Rhychobdellida, Clitellata, leech

Introduction

The genus *Helobdella* Blanchard, 1896 is comprised of a group of species in the family Glossiphoniidae that are widely distributed throughout the Americas with 48 nominal species being represented from South America and 8 species recognized in the United States (Siddall & Borda 2003; Christoffersen 2009). Members of the genus *Helobdella* are small predaceous leeches characterized by a pair of well-separated cephalic eye spots. These benthic leeches are often associated with organically enriched waters (Sawyer 1986; Moser *et al.* 2009). Although several comprehensive studies have addressed *Helobdella* spp. (Siddall & Borda 2003; Siddall *et al.* 2005; Christoffersen 2009; Lai *et al.* 2009) relatively little is known about the helobdellid leech fauna of the northwestern United States. In the course of a seasonal distribution survey of benthic invertebrates of the Upper Klamath Lake Ecosystem (Klamath County, Oregon), a previously undescribed species of the genus *Helobdella* was encountered and is described herein.

Materials and methods

Collection of Leeches. Leeches were collected from May 2008–May 2012 in Upper Klamath Lake at Bay Ball (42° 24’ 21”N 122° 01’ 01”W), mid-trench (42° 23’ 05”N 121° 55’ 38’W), mid-north (42° 26’ 22”N 122° 00’ 40”

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W), and Modoc Rim (42° 24' 37"N 121° 51' 52"W) Klamath County, Oregon with an Ekman grab sampler (15×15 centimeter cross section by approximately 10 cm deep) and a 0.5 mm sieve bucket. Specimens of *Helobdella simplex* were collected from Buenos Aires Province, Argentina to provide comparative material. Specimens were bulk preserved in formalin or relaxed, examined, and fixed as described by Moser *et al.* (2006). For internal anatomy investigations, seven whole mount specimens were stained with Semichon's acetocarmine and mounted in Canada balsam for examination by light microscopy. Terminology for plane shapes follows Clopton (2004). Specimens were deposited in the Smithsonian Institution, National Museum of Natural History (USNM), Washington, District of Columbia and the Peabody Museum of Natural History (YPM), Yale University, New Haven, Connecticut.

DNA analyses. Molecular analyses were conducted on newly collected material 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 lysed overnight at 56ºC. 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) as specified by 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: 94ºC for 5 min.; 35 cycles of (94ºC for 30 sec, 50ºC for 30 sec, 72ºC for 45 sec); 72ºC 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 subsequently 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* and neighbor joining (NJ) analysis of COI sequence data was performed using SeaView4 with the Jukes-Cantor (JC) model and bootstrap analysis with 1000 replicates.

Family Glossiphoniidae Vaillant, 1890

Helobdella bowermani n. sp.

Figures 1–3

Material examined. Holotype (USNM 1213041) Ball Bay (42° 24' 21"N 122° 01' 01"W) Upper Klamath Lake, Klamath County, Oregon on 30 May 2012.

Paratypes (USNM 1213042) 8 specimens mid-trench (42° 23' 05"N 121° 55' 38'W), 6 November 2008, 4 whole mount slides (USNM 1225776–1225779); USNM 1213043 3 specimens Ball Bay (42° 24' 21"N 122° 01' 01"W) on 30 May 2012, 2 whole mount slides (USNM 1225780–1225781); USNM 1213044 9 specimens mid-trench (42° 23' 05"N 121° 55' 38'W), 18 June 2008; USNM 1213045 2 specimens mid-trench (42° 23' 05"N 121° 55' 38'W), May 2008; USNM 1213046 8 specimens mid-north (42° 26' 22"N 122° 00' 40" W), 30 May 2012, 1 whole mount slide (USNM 1225782); USNM 1213047 3 specimens Modoc Rim (42° 24' 37"N 121° 51' 52"W), 18 June 2008; YPM IZ 67710 4 specimens mid-trench (42° 23' 05"N 121° 55' 38'W), 18 June 2008; Upper Klamath Lake, Klamath County, Oregon.

Description. External morphology. Body lanceolate; length of preserved specimens 5.2–9.7 mm, mean ± SE 7.2 ± 0.2 mm (n=38), width at widest point 1.7–4.5 mm, mean 3.0 ± 0.1 mm (n=38). Dorsum pale yellow/buff with scattered chromatophores throughout the dorsal surface (Figs. 1A–B). Pair of widely separated eye spots and pair of black longitudinal pigment lines extending posteriad from the eye spots for a few annuli. Small oval-shaped nuchal scute (occasionally triangular) on VIII, raised in preserved specimens (Figs. 1A–B). Dorsal medial row of

papillae with papillae on the a1 (papilla small), a2 (papilla large) and a3 (papilla large) annulus (Figs. 1A–B). Medial dorsal papillae row extends posteriad below the nuchal scute to a couple of annuli anteriad of the anus. Additional row of papillae at the extreme lateral margins on the a2 (neural) annulus, giving the body a serrated or denticulate appearance (Figs. 1–2). Lateral papillae row begins as 2 papillae below the anus and extends anteriad. Anus located 1 annulus anteriad of the caudal sucker. Caudal sucker small to moderate size (half the diameter of mid-body, 0.7–1.3 mm in diameter, mean ± SE, 1.0 ± 0.0 (n=38), with few black chromatophores and no papillae. Ventrum without papillae and unpigmented (Fig. 2). Male gonopore on annulus and female gonopore in furrow (1 ½ annuli between gonopores).

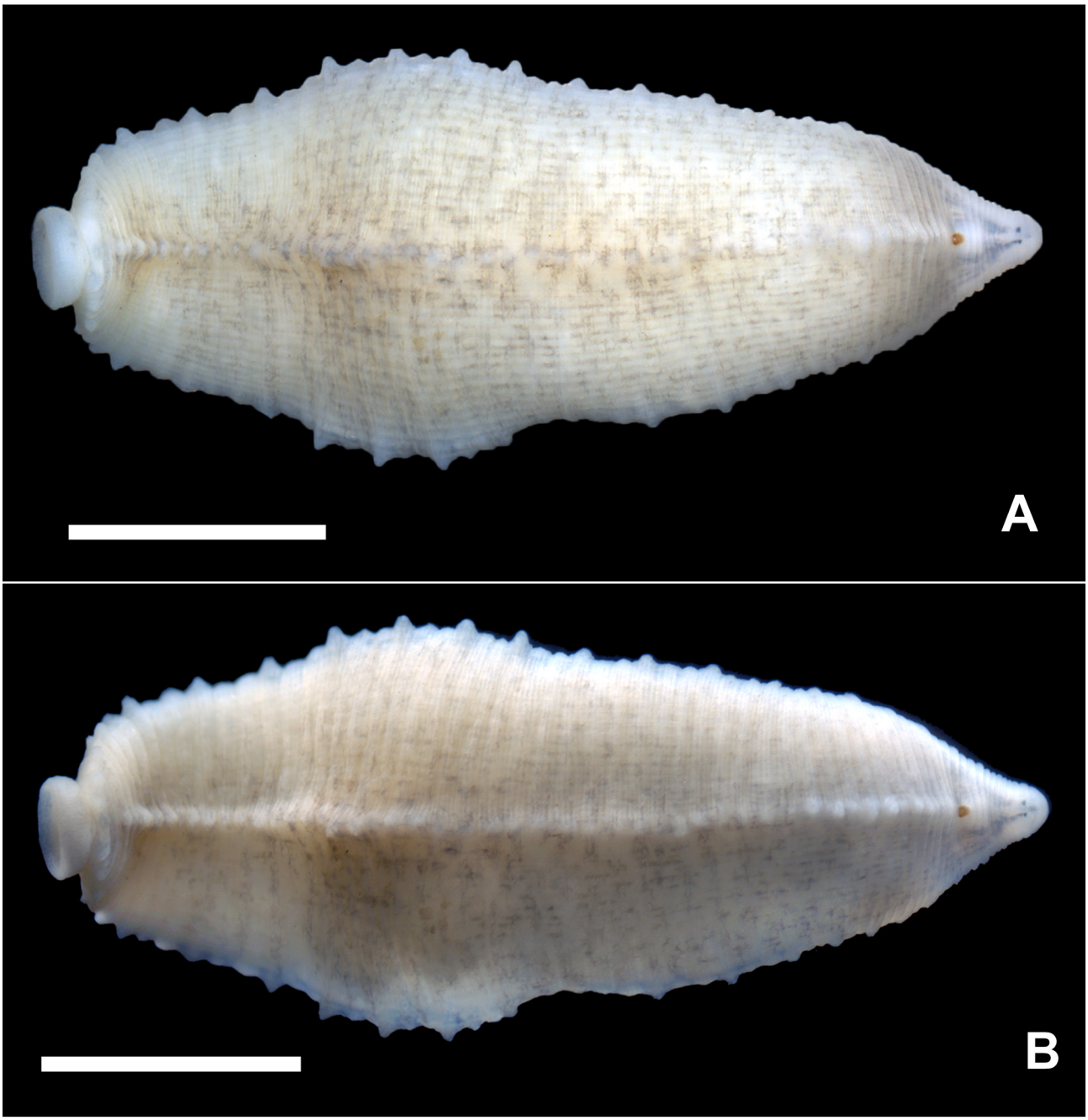

FIGURE 1. Holotype (USNM 1213041) of *Helobdella bowermani* **n. sp.** A. Dorsal surface B. Dorsal surface with shadow to highlight dorsal medial row of papillae. Scale bar equals 2 mm (A, B).

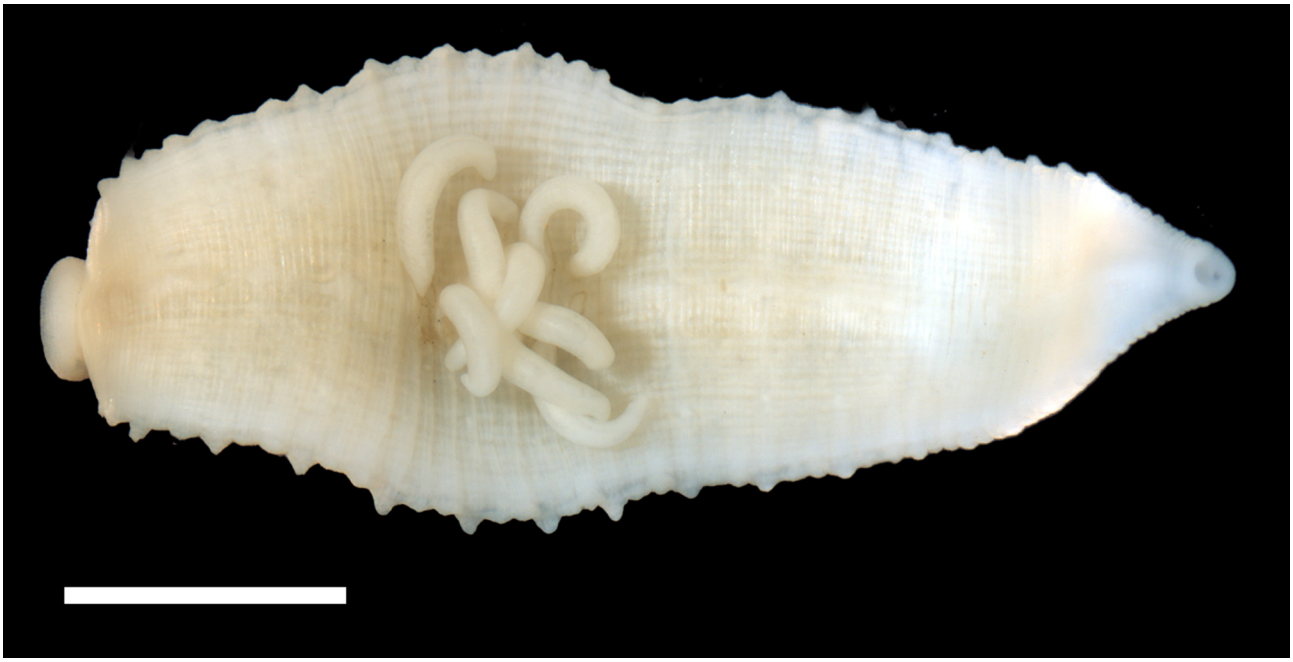

FIGURE 2. Ventral surface of *Helobdella bowermani* **n. sp.**, Holotype USNM 1213041. Scale bar equals 2 mm.

Internal morphology. Digestive system: Proboscis pore at center of anterior sucker. Robust proboscis uniformly cylindrical and in membranous sheath. In the anterior third of body, salivary glands diffusely scattered on either side of the proboscis and salivary ductule bundles attaching at each side of the base of the proboscis (Figs. 3A–B). Short, simple esophagus. Six pair of short, simple unlobed and unbranched crop ceca with no post ceca and four pair of intestinal ceca with the last pair reduced (Figs. 3A–B). Rectum robust, pyriform and recurved in some specimens (Figs. 3A–B).

Reproductive system: Male atrium opening into paired falciform to luniform atrial cornuae that extend laterally and anteriorly into ejaculatory ducts without atrial loops (Figs. 3A–B). Six pair of testisacs (Figs. 3A–B). Female gonopore simple, opening to pair of simple, tubular ovisacs. Length of ovisacs dependent on the reproductive state of the leech.

Taxonomic summary. Type locality. Upper Klamath Lake, Klamath County, Oregon.

Type material. Holotype USNM 1213041, Paratypes USNM 1213042–1213048, USNM 1225776–1225782, and YPM IZ 67710.

Etymology. Named to honor scientist and naturalist Jay Bowerman of the Sunriver Nature Center, Sunriver, Oregon.

DNA analysis. Molecular characterization of 614 nucleotides of CO-I revealed differences of 0.0% to 0.2% (0–1 nucleotide) among three specimens of *Helobdella bowermani* **n. sp.** (GenBank KF683192–KF683194). Differences of 10.6% to 10.8% (65 to 66 nuceotides) were found between *H. bowermani* **n. sp.** and a specimen of *Helobdella californica* (GenBank HQ686307) collected from San Francisco, California. Comparison of CO-I sequence data of three specimens of *H. bowermani* **n. sp.** revealed differences of 12.2% to 13.7% (75 to 84 nucleotides) among three specimens of *Helobdella atli* (GenBank HQ179850–HQ179852), differences of 12.7% to 13.2% (78 to 81 nucleotides) among three specimens of *Helobdella simplex* (GenBank KF683195–KF683197), differences of 14.5% to 14.7% (89 to 90 nucleotides) among two species of *Helobdella modesta* from Washington (GenBank HQ179853–HQ179854), differences of 14.7% to 14.8% (90 to 91 nucleotides) from a specimen of *H. modesta* from Ohio (GenBank AF329040), and differences of 14.7% to 14.8% (90 to 91 nucleotides) from a specimen of *Helobdella stagnalis* from the United Kingdom (GenBank AF329041).

A neighbor joining tree of *Helobdella* spp. based on CO-I sequence data is presented in Figure 4. *Helobdella bowermani* **n. sp.** formed a clade with *H. californica*, and a sister clade with *Helobdella atli* had modest support. The sister clade relationship of *Helobdella simplex* and *Helobdella sorojchi* had robust support.

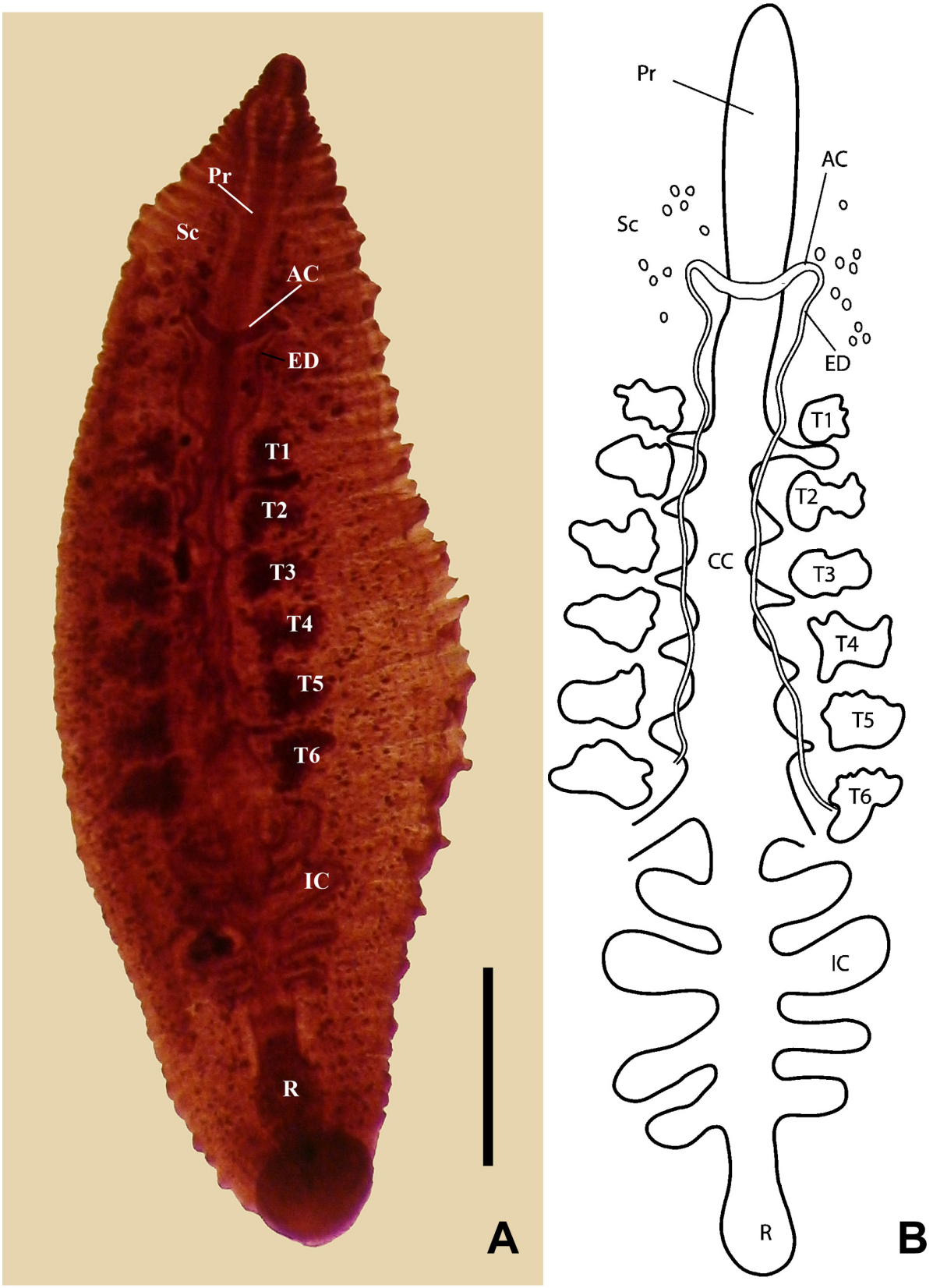

FIGURE 3. Internal morphology of *Helobdella bowermani* **n. sp.** Paratype USNM 1225776, ventral view. A. Cleared and stained specimen, scale bar equals 1 mm B. Schematic drawing.
Figure legends: atrial cornuae (AC), crop ceca (CC), ejaculatory duct (ED), intestinal ceca (IC), proboscis (Pr), rectum (R), salivary cells (Sc), testisac (T1–T6).

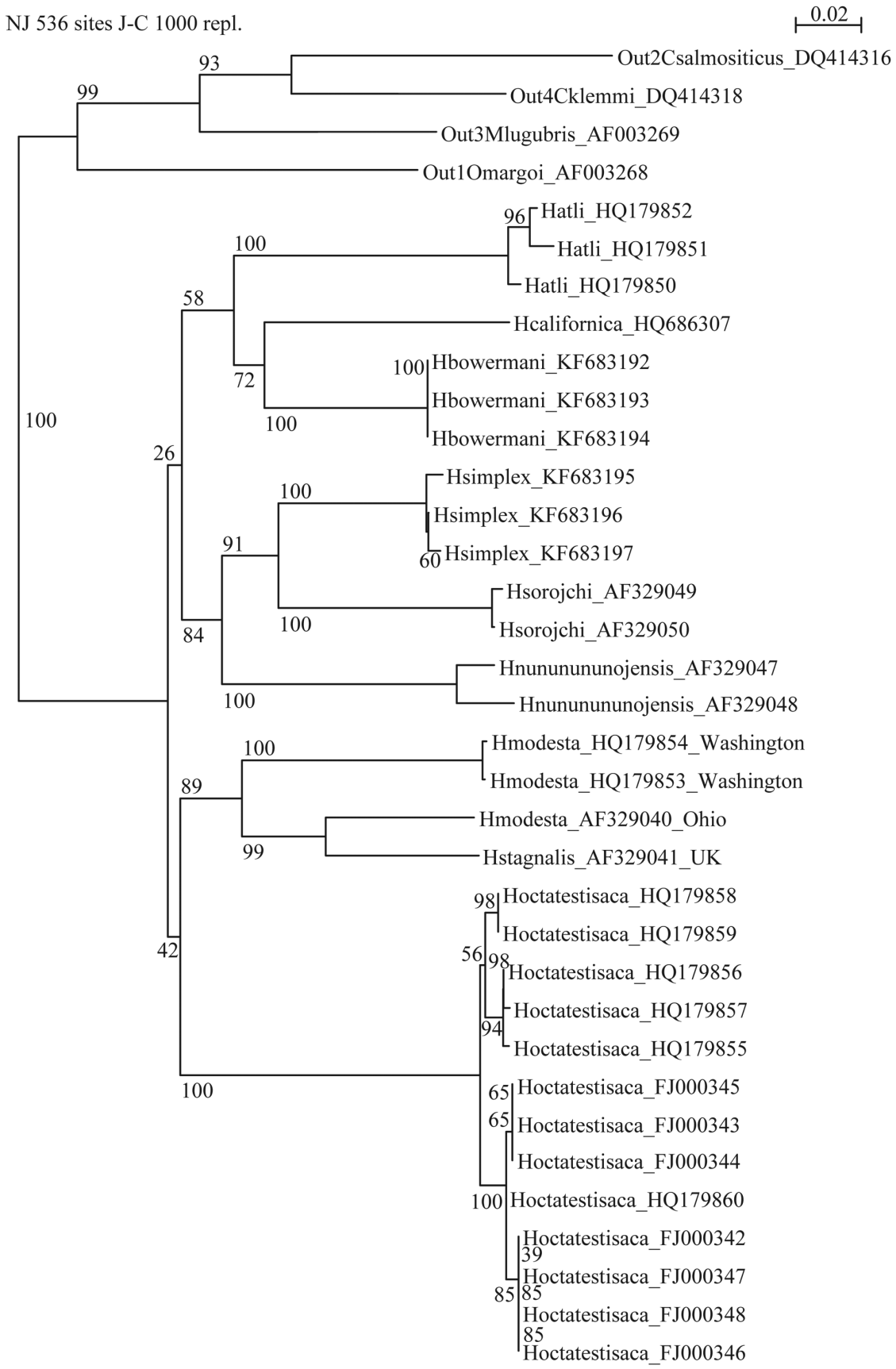

FIGURE 4. Neighbor joining (NJ) tree based on the Jukes-Cantor (JC) model and bootstrap analysis with 1000 replicates of CO-I sequence data of *Helobdella* spp.

Natural history. *Helobdella bowermani* **n. sp.** occurs abundantly in the fine sediment and was collected in the open water benthos with an Ekman grab and a 0.5 mm sieve bucket throughout Upper Klamath Lake.

The reproductive period for *Helobdella bowermani* **n. sp.** was during spring with specimens brooding eggs and hatchlings in May and June, and a second reproductive period was observed in August and September.

Remarks. Three nominal species of *Helobdella* with a nuchal scute occur in the United States: *Helobdella modesta* (Verrill 1872), *Helobdella californica* Kutschera 1988, and *Helobdella bowermani* **n. sp.** Whether a fourth species of *Helobdella* with a nuchal scute, *Helobdella stagnalis* (Linneaus 1758), occurs in the United States is in doubt. At one time, every specimen of *Helobdella* with a nuchal scute was identified as *H. stagnalis*, but differences in genetic distance prompted Siddall *et al.* (2005) to resurrect *Helobdella modesta* (Verrill 1872). In a molecular characterization of *H. modesta* from the type locality (New Haven, Connecticut), Moser *et al.* (2011) suggested *H. stagnalis* is a complex of cryptic species and additional species similar to *H. stagnalis* likely exist in the United States. Although *H. bowermani* **n. sp.** has a nuchal scute, its dorsal medial row of papillae and a2 papillae on the lateral margins easily distinguish it from the non-papillated *H. modesta* and *H. stagnalis*.

Helobdella californica, which formed a clade with *H. bowermani* **n. sp.**, exhibited a 10.6% to 10.8% difference among COI sequence data. Both nuchal scute species occur in the western United States, but *H. californica* has a pair of longitudinal stripes, diverticulated crop ceca at the lateral ends and no papillae which differentiate it from *H. bowermani* **n. sp.** *Helobdella californica* is also only known from Golden Gate Park [Stow Lake (type locality), San Francisco Botanical Garden Gunnera Creek and Mallard Lake], San Francisco, California (Kutschera 1988; 2011).

Helobdella bowermani **n. sp.** is morphologically similar to *H. atli* and *H. simplex*. All three species have a nuchal scute on VIII and dorsal-medial row of papillae. Comparison of *H. bowermani* COI sequence data with the other two species, revealed differences of 12.2% to 13.7% with *H. atli* and differences of 12.7% to 13.2% with *H. simplex*. There are also morphological differences between *H. bowermani* **n. sp.**, *H. atli* and *H. simplex*. *Helobdella simplex* has a pale brownish coloration, a dorsal medial black line, 24 fine longitudinal lines, lateral extensions on every annulus, dorsal medial row of papillae with papillae of the same size on every annulus and it has a nuchal gland, but rarely nuchal scute (Moore 1911; Siddall & Borda 2004). *Helobdella atli* has a white or yellowish base color without any pigmentation, lateral extensions only on the a2 and a3 annuli, dorsal medial row of papillae with papillae only on the a2 and a3 annuli and a small triangular scute (Oceguera-Figueroa & Leon-Regagnon 2005). *Helobdella bowermani* **n. sp.** is differentiated from *H. atli* and *H. simplex* by its pale yellow/buff coloration with scattered chromatophore blotches throughout the dorsal surface, lateral extensions or papillae only on the a2 annulus, dorsal medial row of papillae with small papilla on a1 and larger papillae on a2 and a3, and a small oval scute (rarely triangular).

Helobdella bowermani **n. sp.** is also ecologically distinct, occurring in the fine sediment of open water benthos of an eutrophic lake. Whereas, *H. atli* is attached underneath submerged rocks and on plants in a lake (Oceguera-Figueroa & Leon-Regagnon 2005) and *H. simplex* is attached on submerged substrata and aquatic vegetation in rivers, streams and lakes (Moore 1911; Siddall & Borda 2004; Gullo 1998; 2007; César *et al.* 2009).

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