

***Placobdella akahkway* n. sp. (Clitellata: Hirudinea: Glossiphoniidae), a new leech species from central Canada**

SOPHIA L. FAN^{1,2,3}, DANIELLE DE CARLE^{1,2,4} & SEBASTIAN KVIST^{1,2*}

¹Department of Natural History, Royal Ontario Museum, 100 Queen's Park, Toronto, ON M5S 2C6, Canada

²Department of Ecology and Evolutionary Biology, University of Toronto, 25 Willcocks Street, Toronto, ON M5S 2B4, Canada

³✉ sophia.fan@alum.utoronto.ca; <https://orcid.org/0000-0002-4415-4664>

⁴✉ danielle.decarle@mail.utoronto.ca; <https://orcid.org/0000-0002-6467-5221>

*Corresponding author. ✉ skvist@rom.on.ca; <https://orcid.org/0000-0003-3200-6207>

Abstract

The leech genus *Placobdella* harbours 24 currently-recognized species, and only a handful of new species has been described in the past decade. *Placobdella akahkway* n. sp., a new species of ectoparasitic glossiphoniid leech is herein described from sites in the provinces of Alberta, Saskatchewan, and Manitoba, Canada. Beyond morphological description of the species, a total of 21 specimens (including the five specimens from the type series) were also employed in a phylogenetic analysis using the COI locus. The new species can be distinguished from its congeners by its possession of both compact and diffuse salivary glands. The included specimens of *Placobdella akahkway* n. sp. form a monophyletic group with high support, and place as the sister taxon to *Placobdella kwetlumye*.

Key words: *Placobdella*, Glossiphoniidae, species description, phylogenetic analysis

Introduction

Members of the genus *Placobdella* (Annelida: Clitellata: Glossiphoniiformes: Glossiphoniidae) are sanguivorous, feeding mainly on amphibian and reptile blood, but some species are also known to feed on fish, birds, and mammals (e.g. Sawyer, 1986; Siddall & Gaffney, 2004; de Carle *et al.* 2017). Species of *Placobdella* are distributed broadly throughout North America, with a few species invading Central America (Oceguera-Figueroa & Pacheco-Chaves, 2012; Kvist *et al.*, 2018). The only exception to this distribution is *Placobdella costata* (Fr. Müller, 1846), which is present across large swaths of the Palearctic region (Bielecki *et al.*, 2012 and references therein). The genus currently encompasses 24 nominal taxa, although this is likely an underestimate of the true species diversity within the genus.

In line with the circumscription of *Placobdella* offered by Siddall *et al.* (2005), all species possess one pair of cecate bacteriomes, bilobed ovaries, and two pairs of fused eyespots in placobdelloid arrangement (*i.e.*, one larger posterior pair partially, or fully fused with the smaller anterior pair). Despite these seemingly clear-cut synapomorphies, taxonomic delineations within the genus have remained murky and imprecise. This is partially due to an apparent lack of phylogenetically-informative, identifying characters and partially due to inconsistent or inadequate species descriptions (de Carle *et al.*, 2017). In ameliorating these shortcomings, Moser *et al.* (2012a; 2012b; 2013a; 2013b; 2013c; 2014; 2017; 2019; 2020) commendably performed many re-descriptions of species within *Placobdella*, with much effort afforded to the collection of specimens from their various type localities. These treatises shed light on valuable morphological characters and the distributional patterns of the species.

Using publicly available sequence data, and adding both mitochondrial and nuclear sequence data, de Carle *et al.* (2017) performed a comprehensive phylogenetic analysis of *Placobdella*, including all but one of the recognized species. Beyond evincing the general evolutionary histories of the species, the study also provided evidence of undescribed diversity; four species that neither conformed to known species descriptions, nor nested within clades of well-circumscribed species, were detected. Of interest to the present paper, de Carle *et al.* (2017) recovered *Placobdella* sp. 1 as phylogenetically separate from other species. Herein, we formally describe this new species based on morphological and phylogenetic evidence, and add additional genetic data for some newly sequenced specimens.

Material and methods

Specimen collection and dissection. Numerous leech specimens, including members of the new species of *Placobdella* were collected from ten separate localities in the three Canadian provinces of Alberta, Saskatchewan, and Manitoba during a Royal Ontario Museum (ROM) expedition in 2016 (Table 1 and Fig. 1). Leeches were collected from shallow ponds and lakes on the undersides of rocks, wood, and debris, and from the collectors' exposed skin. Specimens were relaxed in a graded series of ethanol solutions (up to about 15% ethanol) and then fixed permanently in 95% ethanol. Voucher specimens were stored at 4°C, and tissue samples were stored at -8°C until processing. All vouchers are lodged in the Department of Invertebrate Zoology at the Royal Ontario Museum (ROM), Toronto, Canada. Dissections were performed dorsally while the specimens were submerged in 95% ethanol using a Leica Wild M10 dissecting microscope with a scope-mounted Spot Flex 15.2 64-megapixel camera.

TABLE 1. Specimen information for all *Placobdella* sequences used in phylogenetic analysis, including Royal Ontario Museum specimen codes, collection localities, as well as exact coordinates and GenBank accession numbers where applicable. For *Placobdella akahkway n. sp.* sequences, bold font indicates type series, asterisk indicates holotype specimen.

Taxon	Specimen code	Locality	Coordinates	GenBank accession numbers
<i>Placobdella akahkway n. sp.</i>	ROMIZI 11386	Cherry Lake, Saskatchewan, Canada	50°23.640'N, 103°39.571'W	OL743145
<i>Placobdella akahkway n. sp.</i>	ROMIZI 11387	Cherry Lake, Saskatchewan, Canada	50°23.640'N, 103°39.571'W	OL743146
<i>Placobdella akahkway n. sp.</i>	ROMIZI 11356	Muir Lake, Alberta, Canada	53°37.680'N, 113°57.393'W	OL743147
<i>Placobdella akahkway n. sp.</i>	ROMIZI 11358	Muir Lake, Alberta, Canada	53°37.680'N, 113°57.393'W	OL743148
<i>Placobdella akahkway n. sp.</i>	ROMIZI 11464	Unnamed pond, Great Blue Heron Provincial Park, Saskatchewan, Canada	53°44.903'N, 105°53.075'W	OL743149
<i>Placobdella akahkway n. sp.</i>	ROMIZI 11494	Adams Lake, Cypress Hills Provincial Park, Saskatchewan, Canada	49°38.551'N, 109°50.231'W	MF067094
<i>Placobdella akahkway n. sp.</i>	ROMIZI 11279	Half Moon Lake, Alberta, Canada	53°27.351'N, 113°05.010'W	OL743150
<i>Placobdella akahkway n. sp.</i>	ROMIZI 11278	Half Moon Lake, Alberta, Canada	53°27.351'N, 113°05.010'W	OL743151
<i>Placobdella akahkway n. sp.</i>	ROMIZI 11355	Muir Lake, Alberta, Canada	53°37.680'N, 113°57.393'W	OL743152
<i>Placobdella akahkway n. sp.</i>	ROMIZI 11482	Namekus Lake, Prince Albert National Park, Saskatchewan, Canada	53°50.320'N, 106°02.826'W	OL743153
<i>Placobdella akahkway n. sp.</i>	ROMIZI 11357	Muir Lake, Alberta, Canada	53°37.680'N, 113°57.393'W	OL743154
* <i>Placobdella akahkway n. sp.</i>	ROMIZI 11495	Adams Lake, Cypress Hills Provincial Park, Saskatchewan, Canada	49°38.551'N, 109°50.231'W	OL743155
<i>Placobdella akahkway n. sp.</i>	ROMIZI 11541	Unnamed pond, east of Lake Winnipeg, Manitoba, Canada	50°43.371'N, 96°32.349'W	OL743156
<i>Placobdella akahkway n. sp.</i>	ROMIZI 11543	Unnamed pond, east of Lake Winnipeg, Manitoba, Canada	50°43.371'N, 96°32.349'W	OL743157
<i>Placobdella akahkway n. sp.</i>	ROMIZI 11542	Unnamed pond, east of Lake Winnipeg, Manitoba, Canada	50°43.371'N, 96°32.349'W	OL743158

.....continued on the next page

TABLE 1. (Continued)

Taxon	Specimen code	Locality	Coordinates	GenBank accession numbers
<i>Placobdella akahkway n. sp.</i>	ROMIZI 11493	Adams Lake, Cypress Hills Provincial Park, Saskatchewan, Canada	49°38.551'N, 109°50.231'W	OL743159
<i>Placobdella akahkway n. sp.</i>	ROMIZI 11277	Half Moon Lake, Alberta, Canada	53°27.351'N, 113°05.010'W	MF067095
<i>Placobdella akahkway n. sp.</i>	ROMIZI 11247	Big Island Lake, Alberta, Canada	53°29.623'N, 113°11.831'W	OL743160
<i>Placobdella akahkway n. sp.</i>	ROMIZI 11554	Adam Lake, Turtle Mountain Provincial Park, Manitoba, Canada	49°02.607'N, 100°03.631'W	OL743161
<i>Placobdella akahkway n. sp.</i>	ROMIZI 11297	Beaver Lake, Lakelands Provincial Park, Alberta, Canada	54°45.400'N, 111°55.287'W	OL743162
<i>Placobdella akahkway n. sp.</i>	ROMIZI 11551	Adam Lake, Turtle Mountain Provincial Park, Manitoba, Canada	49°02.607'N, 100°03.631'W	OL743163
<i>Haementeria ghilianii</i>	—	BioPharm, French Guiana		AF329035
<i>Placobdella ali</i>	ROMIZI 12965	New York, USA		MF067146
<i>Placobdella ali</i>	ROMIZI 12966	New York, USA		MF067145
<i>Placobdella biannulata</i>	—	North Carolina, USA		AF116021
<i>Placobdella burresonae</i>	ROMIZI 12967	USA		MF067144
<i>Placobdella costata</i>	ROMIZI 12968	Portugal		MF067143
<i>Placobdella costata</i>	ROMIZI 12969	Portugal		MF067142
<i>Placobdella cryptobranchii</i>	—	Missouri, USA		KF601755
<i>Placobdella cryptobranchii</i>	—	Missouri, USA		KF601761
<i>Placobdella hollensis</i>	—	Coonamessett Pond, Barnstable County, Massachusetts, USA		KF771652
<i>Placobdella hollensis</i>	ROMIZI 10341	Frontenac Provincial Park, Ontario, Canada		MF067141
<i>Placobdella hollensis</i>	ROMIZI 10356	Frontenac Provincial Park, Ontario, Canada		MF067140
<i>Placobdella kwetlumye</i>	ROMIZI 12970	Idaho, USA		MF067139
<i>Placobdella kwetlumye</i>	ROMIZI 12971	Squires Lake, Washington, USA		MF067138
<i>Placobdella lamothei</i>	ROMIZI 12973	Tonatico, Estado de México, Mexico		MF067136
<i>Placobdella mexicana</i>	ROMIZI 12976	Mexico		MF067133
<i>Placobdella mexicana</i>	ROMIZI 12977	El Vergel, Mexico		MF067132
<i>Placobdella michiganensis</i>	—	Mill Creek, St. Joseph County, Michigan, USA		MT579302
<i>Placobdella montifera</i>	ROMIZI 12980	Friends Landing, Washington, USA		MF067129
<i>Placobdella montifera</i>	ROMIZI 12979	Friends Landing, Washington, USA		MF067130
<i>Placobdella multilineata</i>	—	Maurepas Swamp, Louisiana, USA		AY962464
<i>Placobdella multilineata</i>	—	Lukfata Creek, Oklahoma, USA		KM396760
<i>Placobdella nuchalis</i>	—	Four Hole Swamp, Orangeburg County, South Carolina, USA		MF535240
<i>Placobdella nuchalis</i>	ROMIZI 12981	Mohonk Camp, New York, USA		MF067128
<i>Placobdella nuchalis</i>	ROMIZI 12983	John Allen Pond, New York, USA		MF067126
<i>Placobdella ornata</i>	—	Shivericks Pond, Massachusetts, USA		JQ812135

.....continued on the next page

TABLE 1. (Continued)

Taxon	Specimen code	Locality	Coordinates	GenBank accession numbers
<i>Placobdella ornata</i>	—	The Donkmeer, East Flanders Province, Belgium		KP176597
<i>Placobdella papillifera</i>	ROMIZI 12984	John Allen Pond, New York, USA		MF067125
<i>Placobdella papillifera</i>	—	West River, Connecticut, USA		KC505241
<i>Placobdella papillifera</i>	ROMIZI 12985	John Allen Pond, New York, USA		MF067124
<i>Placobdella parasitica</i>	—	Lily Lake, Waseka County, Minnesota, USA		KF058895
<i>Placobdella parasitica</i>	ROMIZI 10340	Pearkes Lake, Ontario, Canada		MF067122
<i>Placobdella parasitica</i>	ROMIZI 10311	North of Algonquin Provincial Park, Ontario, Canada		MF067123
<i>Placobdella pediculata</i>	ROMIZI 12986	—		MF067121
<i>Placobdella pediculata</i>	—	Lake Pepin, Goodhue County, Minnesota, USA		MN043903
<i>Placobdella pediculata</i>	ROMIZI 12987	—		MF067120
<i>Placobdella phalera</i>	ROMIZI 12988	Canopus Ramp, New York, USA		MF067117
<i>Placobdella phalera</i>	ROMIZI 12989	—		MF067116
<i>Placobdella phalera</i>	ROMIZI 12990	Tennessee, USA		MF067115
<i>Placobdella picta</i>	ROMIZI 11395	Moose Mountain Provincial Park, Saskatchewan, Canada		MF067109
<i>Placobdella picta</i>	ROMIZI 10111	Kenny Lake, Ontario, Canada		MF067113
<i>Placobdella ringueleti</i>	ROMIZI 12993	Catemaco, Veracruz, Mexico		MF067107
<i>Placobdella ringueleti</i>	ROMIZI 12992	Chiapas, Mexico		MF067108
<i>Placobdella rugosa</i>	ROMIZI 10089	Mijinemungshing Lake, Ontario, Canada		MF067106
<i>Placobdella rugosa</i>	—	Belcourt Lake, Turtle Mountain Indian Reservation, Rolette County, North Dakota, USA		JX412986
<i>Placobdella rugosa</i>	ROMIZI 11411	Pipestone Creek, Moosomin Regional Park, Saskatchewan, Canada		MF067101
<i>Placobdella siddalli</i>	—	Davis Eddy, George County, Mississippi, USA		KY780962
<i>Placobdella sophiaeae</i>	ROMIZI 12994	Washington, USA		MF067097
<i>Placobdella sophiaeae</i>	ROMIZI 12995	Washington, USA		MF067096
<i>Placobdella</i> sp.2 (de Carle <i>et al.</i> , 2017)	ROMIZI 12997	Canopus Ramp, New York, USA		MF067092
<i>Placobdella</i> sp.2 (de Carle <i>et al.</i> , 2017)	ROMIZI 12996	Canopus Ramp, New York, USA		MF067093
<i>Placobdella</i> sp.3 (de Carle <i>et al.</i> , 2017)	ROMIZI 12998	Maryland, USA		MF067091
<i>Placobdella</i> sp.4 (de Carle <i>et al.</i> , 2017)	ROMIZI 12999	Maryland, USA		MF067090
<i>Placobdella transluscens</i>	—	Michigan, USA		AY047328

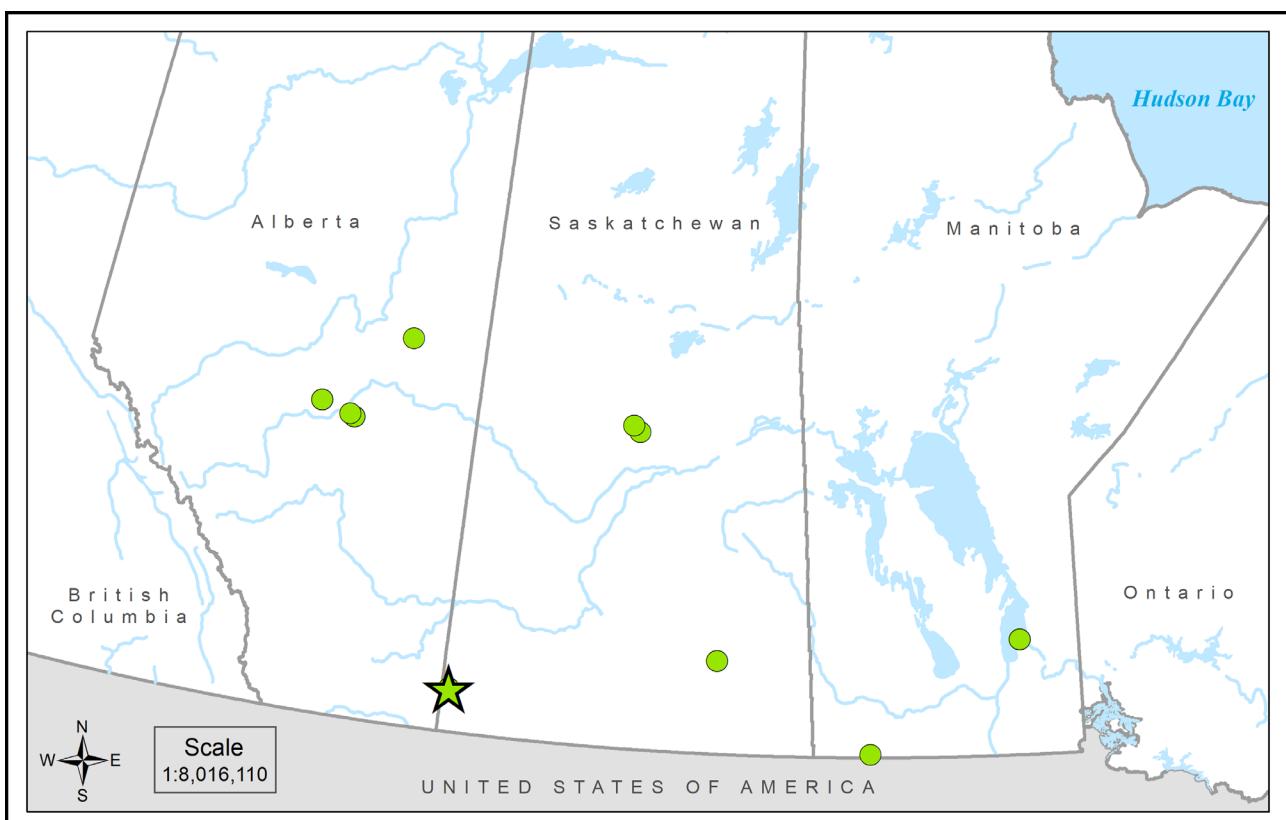


FIGURE 1. Map of Canada showing the collection sites for specimens of *Placobdella akahkway* n. sp. in solid green circles. The star indicates the type locality.

DNA extraction and sequencing. Tissue samples destined for DNA sequencing were obtained from the caudal suckers of the specimens in order to avoid potential contamination by gut contents. DNA was extracted using a DNeasy Tissue Kit (Qiagen, Valencia, CA, USA) according to the manufacturer's protocol. Thereafter, partial sequences for the mitochondrial cytochrome *c* oxidase subunit I (COI) locus were amplified using the universal primers LCO1490 and HCO2198 (Folmer *et al.*, 1994). Polymerase Chain Reactions (PCRs) were carried out in 25 µl reactions consisting of 16.39 µl ddH₂O; 2.5 µl buffer; 2.5 µl MgCl₂; 1 µl of each primer; 0.56 µl dNTPs at 10 mM concentration; 0.05 µl Platinum Taq polymerase (Invitrogen, Carlsbad, CA); and 1 µl total genomic DNA. The thermocycler profile was as follows: 94°C (5 min) followed by 30 cycles of 94°C (45 s), 40°C (45 s), 72°C (45 s) and final extension at 72°C (7 min). PCR products were checked on a 1% agarose gel and amplicons were purified using ExoSAP-IT (Affymetrix, Santa Clara, CA, USA), according to the manufacturer's protocols. Cycle sequencing reactions were performed in 10 µl reactions, consisting of 0.5 µl ABI Big Dye Terminator ver. 3.1, 0.5 µl Big Dye 5x sequencing buffer, 2 µl primer at 10 µM concentration, 2.5 µl purified PCR product, and 4.5 µl ddH₂O. Reaction mixtures were heated to 96°C for 1 min followed by 30 cycles of 96°C (10 s), 50°C (5 s) and 60°C (4 min). The samples were then sequenced on an ABI PRISM 3730 (Applied Biosystems, Carlsbad, CA) at the ROM.

Phylogenetic and genetic variation analyses. Prior to phylogenetic analyses, sequences were BLASTed (using BLASTn) against the non-redundant (nr) database on GenBank to confirm species affinity and to search for potential contamination. In addition to sequences for the new species, a modified version of the COI dataset used by de Carle *et al.* (2017) with the addition of a recently published (Moser *et al.*, 2020) sequence for *Placobdella michiganensis* (Sawyer, 1972) was used for downstream phylogenetic analyses. The final dataset consisted of 75 sequences for all nominal species of *Placobdella*, with 21 sequences originating from the new species (Table 1). Sequences were aligned using the online platform for MAFFT ver. 7 (Katoh *et al.*, 2019), applying default settings. Mesquite ver. 3.11 (Maddison & Maddison, 2016) and ALTER (Glez-Peña *et al.*, 2010) were used to reformat the dataset for phylogenetic analyses and the alignment was trimmed to cover only the “Folmer-region” of the COI locus.

We performed phylogenetic analyses under two optimality criteria: parsimony and maximum likelihood. The parsimony analysis was conducted in TNT ver. 1.5 (Goloboff *et al.*, 2008) applying a New Technology search using 1000 replications with 5 rounds of ratcheting and stipulating that the search be terminated early only if the best scoring tree was hit 10 times (“hits 10”). Support values were calculated using 1000 rounds of standard bootstrapping employing default settings.

Prior to maximum likelihood analysis, the best fitting model of nucleotide evolution, as well as the optimal partitioning scheme, were estimated using PartitionFinder ver. 1.1.1 (Lanfear *et al.*, 2012). For this purpose, each codon position was evaluated separately. The maximum likelihood analyses were conducted in IQ-TREE ver. 1.6.12 (Nguyen *et al.* 2015), using the best-fitting model suggested by the same software (GTR+F+I+G4 in accordance with the Akaike Information Criterion) and running the heuristic search for 1000 replications. Support values were estimated via 1000 bootstrap replicates applying default settings. All trees were rooted with *Haementeria ghilianii* (de Filippi, 1849) following previous phylogenetic hypotheses (de Carle *et al.*, 2017). Note that the monophyletic status of *Placobdella* has been robustly inferred previously (de Carle *et al.*, 2017), negating the need for further outgroup taxa in the present study.

All pairwise COI distances were calculated in PAUP ver. 4b10 (Swofford, 2002) using the function of minimum evolution, uncorrected *p* distances, constraining branches to be non-negative, and ignoring missing/ambiguous sites for affected pairwise comparisons. Average distances within and between each species, as well as standard deviations where appropriate, were calculated. Because this study focuses on circumscribing the new species, we report only COI distances for the new species plus the three most closely related species within the resulting phylogenetic trees.

Taxonomy

Placobdella akahkway n. sp.

Figures 2–5

Placobdella sp. 1; de Carle *et al.*, 2017

Diagnosis. This species can be distinguished from all of its congeners on the basis of its possession of both compact and diffuse salivary tissues. Moreover, the large size of this species, the dorsal pigmentation patterning in combination with the papillation pattern, and its geographic distribution might all lend themselves well to separating this species from other members of the genus. See Figure 2 for imagery of the external morphology for type specimens.

Holotype. ROMIZI11495, whole specimen, undissected, in 95% EtOH; from type locality, collected on June 23, 2016, by SK and Charlotte Calmerfalk Kvist; holotype COI GenBank accession number OL743155.

Type locality. Adams Lake, Cypress Hills Provincial Park, Saskatchewan, Canada (49°38.551'N, 109°50.231'W). From rocks in shallow water on east side of lake.

Paratypes. ROMIZI11494, whole specimen, undissected, all in 95% EtOH; from type locality, collected on June 23, 2016, by SK and Charlotte Calmerfalk Kvist. Three remaining paratypes dissected: ROMIZI11247 from Big Island Lake, Alberta. (53°29.623'N, 113°11.831'W), collected on June 25, 2016, by SK and Charlotte Calmerfalk Kvist; ROMIZI11464, with brood, from unnamed pond, Blue Heron Provincial Park, Saskatchewan. (53°44.903'N, 105°53.075'W), collected on June 19, 2016, by SK and Charlotte Calmerfalk Kvist; ROMIZI11542 from unnamed pond east of Lake Winnipeg, Manitoba. (50°43.371'N, 96°32.349'W), collected on June 8, 2016 by SK and Charlotte Calmerfalk Kvist. All specimens with small part of posterior sucker removed for DNA sequencing.

Other material examined. In addition to the type series, several specimens collected across Alberta, Saskatchewan and Manitoba were examined (Table 1). As part of a previous study of the bacterial symbionts of these leeches (*i.e.*, Manglicmot *et al.*, 2020), the bacteriomes for all of these specimens were removed, such that none of the specimens is fully intact.

Etymology: “*akahkway*” is the Plains Cree word for “leech” in honour of the Indigenous language group spoken at this species’ collection sites (including the type locality) in eastern Alberta and western Saskatchewan, Canada.

Description. Description based on five specimens (two fully intact and three dissected specimens). Body dor-

soventrally flattened, oval to lanceolate with anterior and posterior tapering and widest at midsection. Anterior end markedly narrower than posterior end. Rather variable colouration, ranging from olive brown, reddish/pinkish brown, to darker greyish brown (Fig. 2). Mid-dorsal stripe dark brown and irregularly interrupted; continues past mid-body and ends well before posterior end. Venter beige and without pigmentation or patterning. Average body length 5.27 cm (holotype 5.30 cm), with minimum length 1.0 cm (exception being juvenile specimens at 0.4–0.5 cm) and maximum length 5.5 cm. Average body width at male gonopore 2.10 cm (holotype width 3.10 cm). Complete somites triannulate with partially subdivided annuli throughout (Fig. 3). Somites I and II uniannulate; III and IV biannulate; V–XXIII triannulate; XXIV–XXV biannulate; XXVI–XXVII uniannulate. Two pairs of eyespots on III first annulus (a1) in placobdelloid arrangement (Fig. 4A). Anus dorsal on XXVII (Fig. 4B). Oral sucker 0.5 cm in diameter with mouth pore on anterior border (Fig. 4C). Posterior sucker circular with dorsal papillae (Fig. 4B). Five main rows of papillae: 1 medial, 2 paramedial, and 2 lateral. Smaller papillae interspersed outside of main rows, becoming more conspicuous towards posterior end. Throughout body, dorsal papillae punctiform with most prominent papilla on a2. Male gonopore at XI/XII (Fig. 4D). Female gonopore at XII a2/a3 (2 annuli between gonopores) (Fig. 4D).

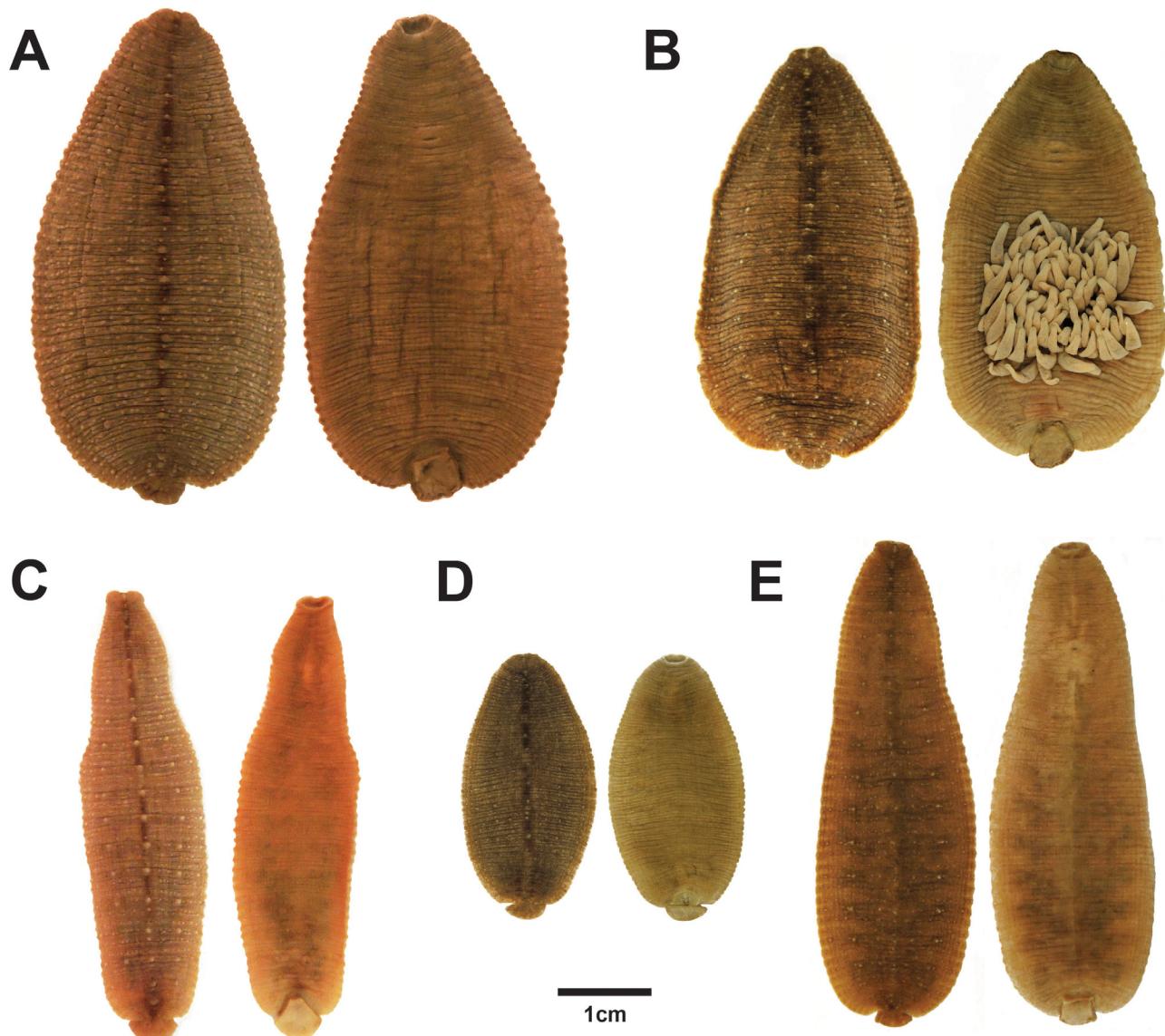


FIGURE 2. *Placobdella akahkway*, n. sp. dorsal (left) and ventral (right) views. (A) Holotype, ROMIZI11495; (B) Paratype, ROMIZI11464, with young attached to the venter; (C) Paratype, ROMIZI11494; (D) Paratype, ROMIZI11542; (E) Paratype, ROMIZI11247.

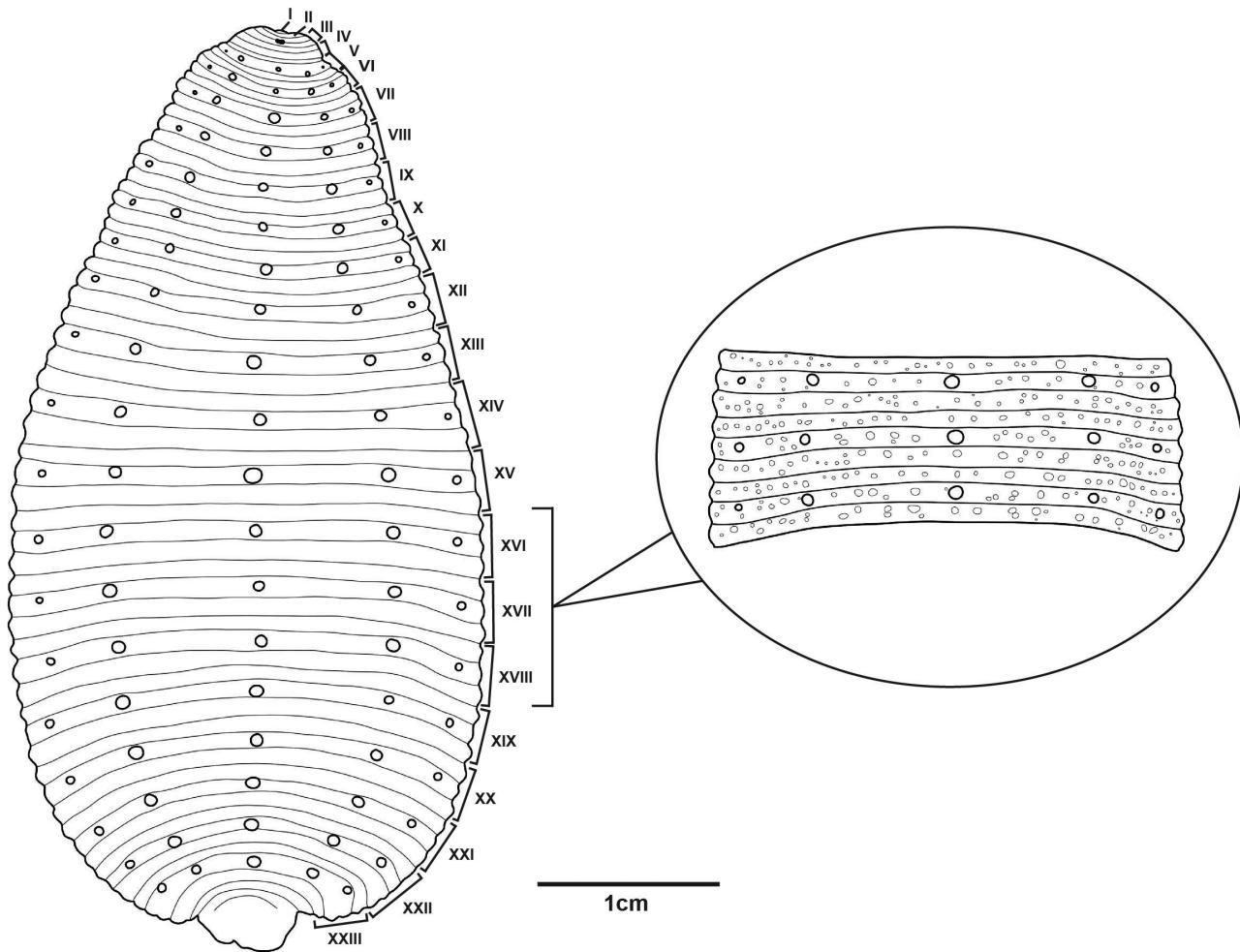


FIGURE 3. *Placobdella akahkway*, n. sp. Diagram showing segmentation and gross papillation pattern of the holotype specimen. Detailed papillation pattern from somites XVI–XVIII shown as an inset to the right.

Internal body cavity with abundance of dark chromatophores scattered randomly. Relatively large proboscis in membranous sheath, extending linearly and posteriorly to XI a1 (Fig. 5). Salivary glands arranged in both a single pair of compact salivary glands and diffuse salivary tissue (Fig. 5). Compact salivary glands (posterior pair) in distinct lobes, extending from IX a3–XI a3 and arranged on either side of proboscis. Diffuse salivary glands (anterior pair) interspersed in tissue, concentrated anteriorly and extending from VIII–XI, less concentrated posteriad. Salivary ductules extending from diffuse glands attached to base of proboscis. Esophagus short and folded (Fig. 5). Bacteriomes arranged as blind sacs, extending anteriorly from esophagus and folded behind proboscis at X a2 in one paratype (Fig. 5). Crop with seven pairs caeca, last pair extending posteriorly to form well-developed post caeca. Intestine with four pairs simple caeca. Male atrial cornuae well-developed with highly coiled ejaculatory ducts (Fig. 6). Testisacs in six pairs, intersegmental, from XIII/XIV– XVIII/XIX. Ovaries curled; ovisacs anteriorly bilobed and without common oviduct (Fig. 6). Anterior ovisac bifurcation at XII/XIII, anterior lobe extending anteriorly to XII and posteriorly to XIV (Fig. 6).

Geographic distribution. The species is known from the following lakes and ponds (Fig. 1): Muir Lake, Alberta ($53^{\circ}37.680'N$, $113^{\circ}57.393'W$); Half Moon Lake, Alberta ($53^{\circ}27.351'N$, $113^{\circ}05.010'W$); Big Island Lake, Alberta ($53^{\circ}29.623'N$, $113^{\circ}11.831'W$); Beaver Lake, Alberta ($54^{\circ}45.400'N$, $111^{\circ}55.287'W$); Adams Lake, Saskatchewan ($49^{\circ}38.551'N$, $109^{\circ}50.231'W$); Cherry Lake, Saskatchewan ($50^{\circ}23.640'N$, $103^{\circ}39.571'W$); unnamed pond in Blue Heron Provincial Park, Saskatchewan ($53^{\circ}44.903'N$, $105^{\circ}53.075'W$); Namekus Lake, Saskatchewan ($53^{\circ}50.320'N$, $106^{\circ}02.826'W$); Adam Lake, Manitoba ($49^{\circ}02.607'N$, $100^{\circ}03.631'W$); unnamed pond East of Lake Winnipeg, Manitoba ($50^{\circ}43.371'N$, $96^{\circ}32.349'W$). The distance between the western-most and eastern-most collecting localities (as the crow flies) is roughly 1,230 km.

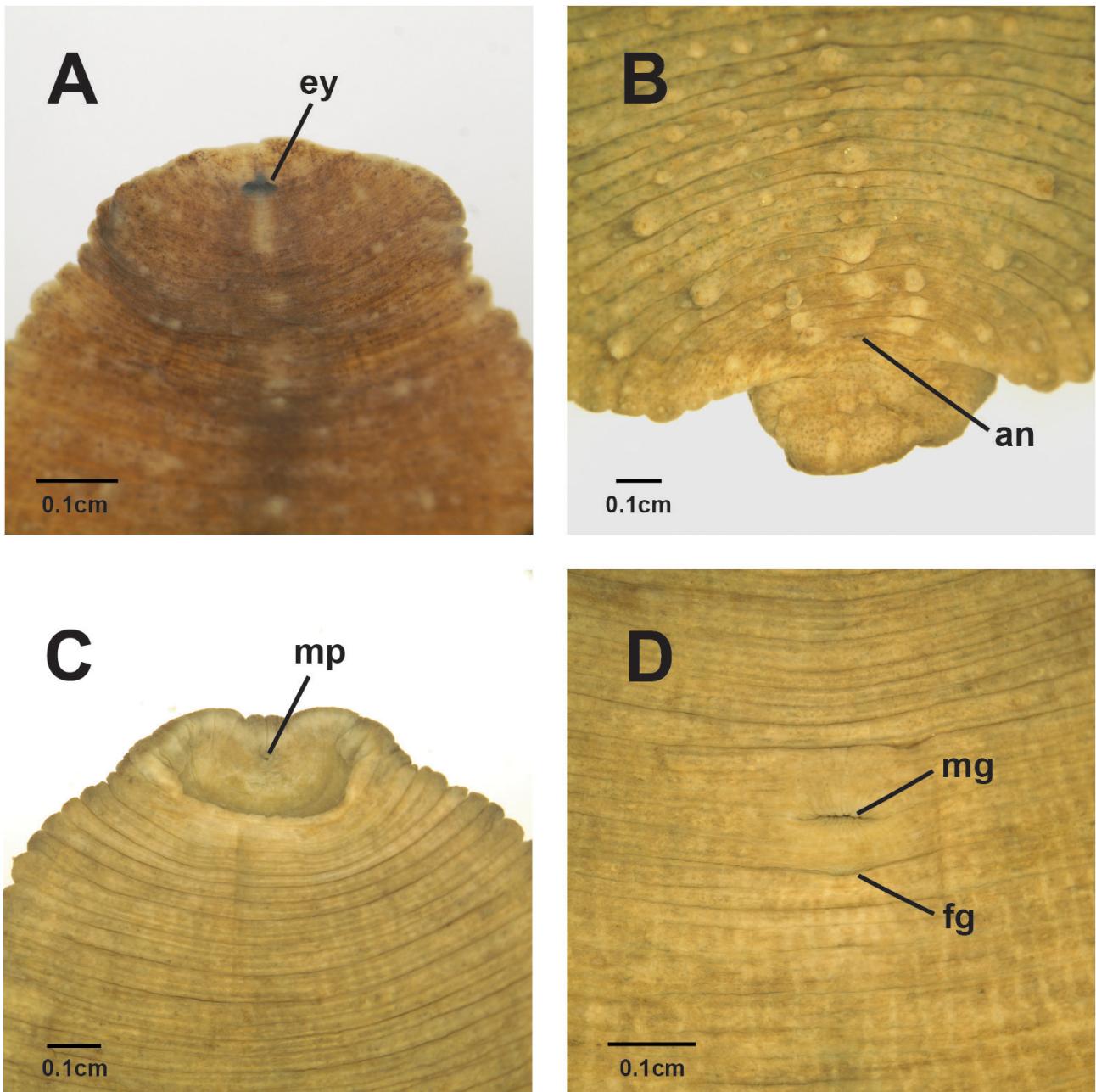


FIGURE 4. *Placobdella akahkway*, n. sp. external features. (A) eyespots (ey); (B) anus (an) and pre-anal papillae; (C) mouth pore (mp); (D) male (mg) and female (fg) gonopores.

Remarks. Despite its relatively non-ornate external appearance compared to other select members of this genus [see e.g. *Placobdella parasitica* (Say, 1824)], this species displays rather variable sizes and colourations across examined specimens. The three dissected specimens show varying amounts of development of reproductive organs, despite similar overall body sizes.

Placobdella akahkway closely resembles *Placobdella kwetlumye* Oceguera-Figueroa, Kvist, Watson, Sankar, Overstreet & Siddall, 2010 in overall external appearance but can be separated from this species by the presence of both compact and diffuse salivary tissue (*P. kwetlumye* does not possess diffuse salivary tissues). In addition, *P. akahkway* is considerably larger than *P. kwetlumye* (average body length 5.27 cm versus 1.77 cm). Finally, the geographic distributions of the species do not seem to overlap, and the known ranges of each species are separated by a substantial geographic barrier (the North American Cordillera). Distribution might therefore aid in future identifications of specimens.

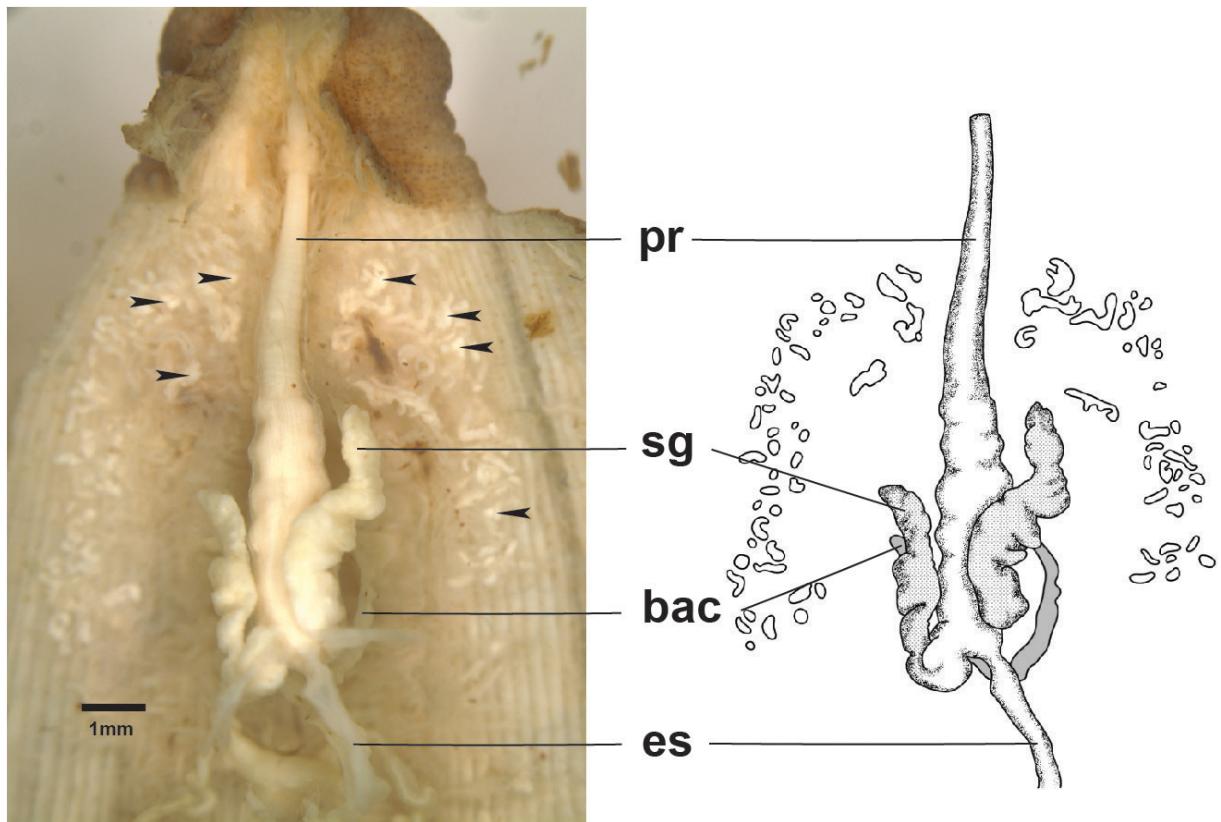


FIGURE 5. *Placobdella akahkway*, n. sp. internal features showing the proboscis (pr), compact salivary glands (sg), bacteriomes (bac) and the esophagus (es). Arrowheads point to some of the diffuse salivary tissues, which are outlined in the schematic drawing and discussed further in the text.

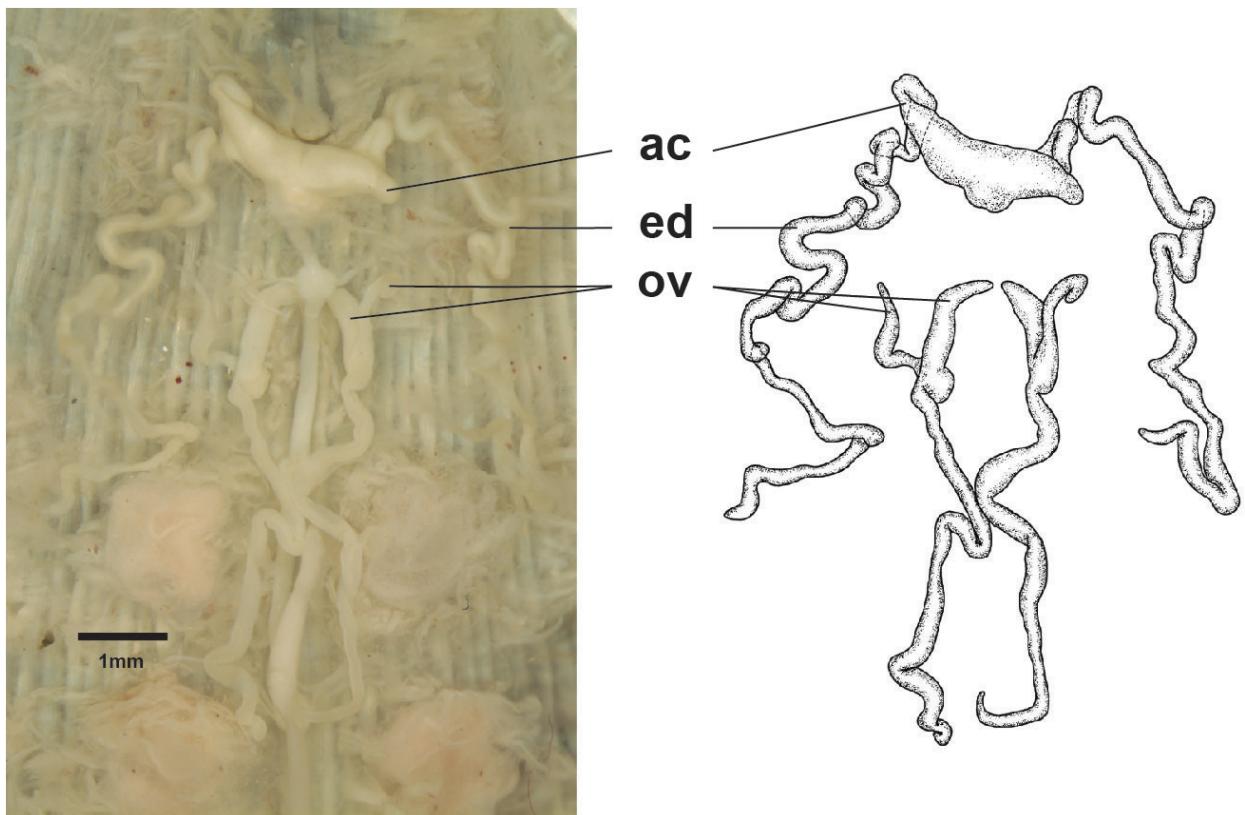


FIGURE 6. *Placobdella akahkway*, n. sp. reproductive systems showing the atrial cornuae (ac) and ejaculatory ducts (ed) of the male reproductive system, as well as the ovaries (ov) of the female reproductive system.

Phylogenetic analyses

Insofar as the phylogenetic hypothesis presented by de Carle *et al.* (2017)—based on both mitochondrial and nuclear data—separates *P. akahkway* (therein named *Placobdella* sp. 1) from congeners, we opted to only sequence COI for the new specimens, assuming that nuclear data from these would mirror previous results. The resulting topologies from the two different optimality criteria are largely congruent, with a few exceptions (Fig. 7; Appendix). Because the present paper concerns itself only with the placement of *Placobdella akahkway*, we will refrain from discussing the discrepancies in other parts of the tree. Unsurprisingly, the included specimens of *Placobdella akahkway* form a monophyletic group with high support (parsimony bootstrap support [PBS] = 100%; maximum likelihood bootstrap support [LBS] = 100%). This clade places as the sister taxon to *Placobdella kwetlumye* (PBS = 87%; LBS = 97%). In turn, *P. akahkway* + *P. kwetlumye* place as the sister group to *Placobdella burresonae* Siddall & Bowerman, 2006 but with somewhat weaker support (PBS = 50%; LBS = 98%). There is disagreement (but also negligible support) between the trees regarding the placement of *Placobdella michiganensis*; parsimony places it in a disparate part of the tree, as sister to *Placobdella ali* Hughes & Siddall, 2007 (PBS <75%), whereas likelihood places it as sister to the clade formed by *P. akahkway*, *P. kwetlumye* and *P. burresonae* (LBS = 86%).

COI distances within and between *Placobdella akahkway* and its closest allies are shown in Table 2. The average intraspecific COI distance within *Placobdella akahkway* was $0.29\% \pm 0.21$. For reference, the intraspecific variation found within *P. kwetlumye* was 2.63% (only a single comparison was possible). The average interspecific COI distance between *P. akahkway* and *P. kwetlumye* was $7.57\% \pm 0.55$. Average interspecific COI distances between *P. akahkway* and *P. burresonae* was $11.45\% \pm 0.14$ and the distance between *P. akahkway* and *P. michiganensis* was $16.13\% \pm 0.19$.

TABLE 2. Pairwise COI distances within and between *Placobdella akahkway* n. sp., *Placobdella kwetlumye*, *Placobdella burresonae*, and *Placobdella michiganensis*. The bottom right triangle represents average COI distances, whereas the top right triangle represents standard deviations (where applicable). The diagonal (in bold) represents average intraspecific COI distances.

	1.	2.	3.	4.
1. <i>Placobdella akahkway</i> n. sp.	$0.29\% \pm 0.21$	± 0.55	± 0.14	± 0.19
2. <i>Placobdella kwetlumye</i>	7.57%	2.63%	± 0.54	± 0.12
3. <i>Placobdella burresonae</i>	11.45%	11.02%	N/A	N/A
4. <i>Placobdella michiganensis</i>	16.13%	16.36%	15.21%	N/A

Discussion

Placobdella akahkway, described herein, represents one of only a handful of species within the genus that has been described in the past decade. Rather than reflecting low diversity, this is likely due to the paucity of collection efforts and taxonomic expertise for this predominantly North American genus. This notion is underscored by genetic data, insofar as undescribed—and potentially cryptic—diversity seems to be more prevalent than previously thought (de Carle *et al.*, 2017).

The geographic distribution, in conjunction with the phylogenetic placement, of the new species warrants some further discussion. Based on sampling efforts (during the expedition in which these specimens were obtained, as well as other ROM collecting trips), *P. akahkway* does not seem to be present west of Alberta or east of Manitoba. Its closest phylogenetic relative, *Placobdella kwetlumye*, was described from Whatcom County, Washington, USA, and has only otherwise been recorded from Lake County, Oregon, USA (Oceguera-Figueroa *et al.*, 2010), and an unknown location in Idaho (de Carle *et al.*, 2017). Only two other species of *Placobdella* are known from western North America: *Placobdella burresonae* and *Placobdella sophiaeae* Oceguera-Figueroa, Kvist, Watson, Sankar, Overstreet & Siddall, 2010. The former is sister to *P. akahkway* and *P. kwetlumye*, and was originally collected from Deschutes County, Oregon (Siddall and Bowerman, 2006). The latter—also described from Whatcom County, WA—is only distantly related to *Placobdella akahkway* (Fig. 7). Interestingly, the known range of *Placobdella akahkway* is separated from *P. kwetlumye* by the entire North American Cordillera, and from the more easterly range of *P. burresonae* by the Rocky Mountains. These substantial geographic features likely constitute a formidable barrier for

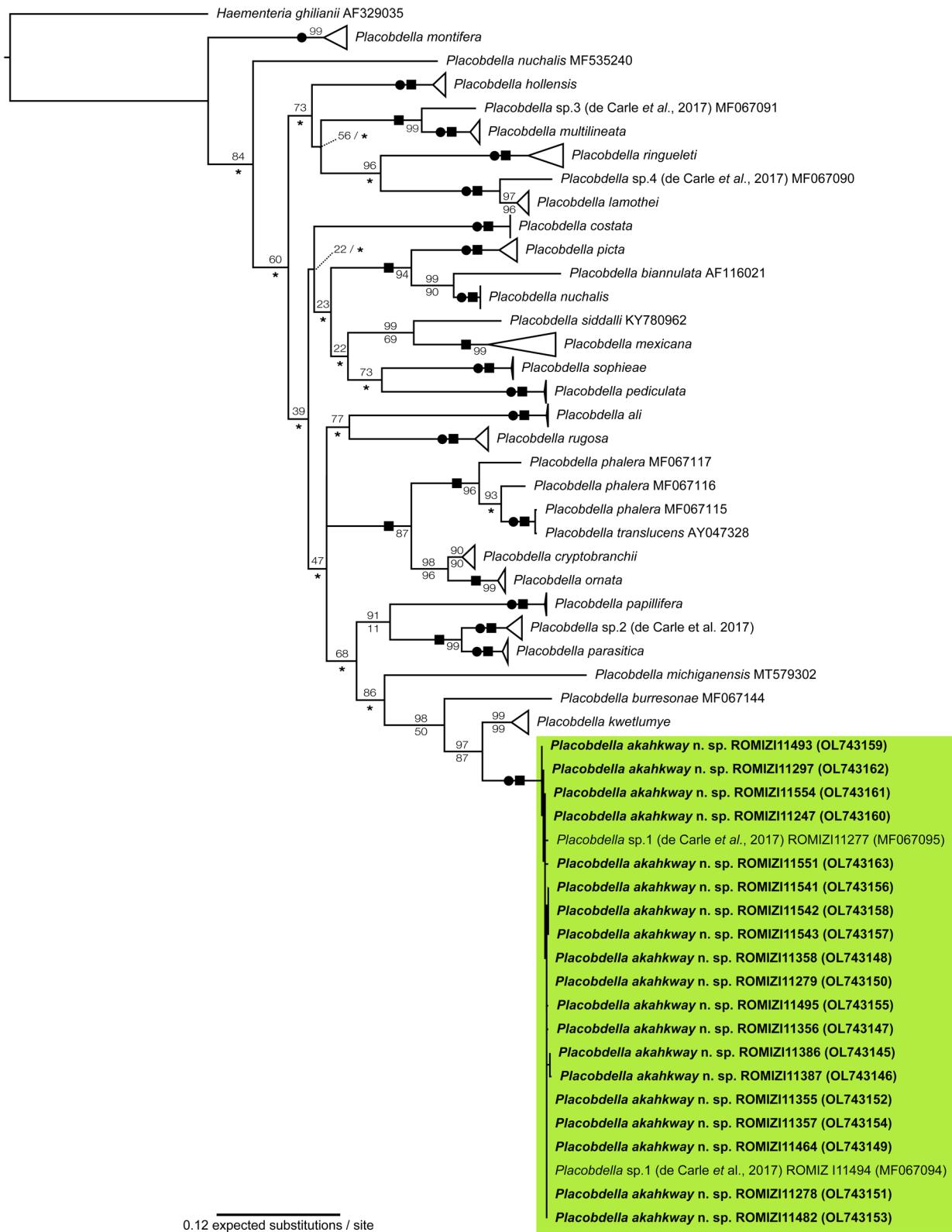


FIGURE 7. Maximum likelihood tree based on the newly derived and GenBank COI sequences of *Placobdella* ($\ln L = -7982.3955$). All non-focal species-level taxa are collapsed when possible, with lone or non-collapsible tips showing GenBank accession numbers. *Placobdella akahkway n. sp.* is indicated within the green box, with new sequences indicated in bold. Maximum likelihood bootstrap support values are shown above each node and parsimony bootstrap support values below each node. 100% support values are indicated by squares for maximum likelihood and circles for parsimony. Asterisks indicate nodes not represented in the maximum likelihood tree.

the dispersal of these organisms, begging the question of how their current distributions came to be. Host-mediated dispersal is a possible mechanism—Oceguera-Figueroa *et al.* (2010) noted that *P. kwetlumye* feeds on waterfowl, which may aid in reaching far-flung habitats—however other, non-blood-feeding genera with similar habitat preferences (*e.g.* *Helobdella*) are also distributed on both sides of these mountain ranges. Other processes, or a combination thereof, are also plausible. For example, the three species could have spread to their current locations from more southerly, less montane regions, or been isolated during the uplift of the west coast mountain ranges. More detailed information about the limits of each species' distribution, as well as their behaviour and host preferences are needed to more rigorously discriminate between historical hypotheses.

Our collection efforts also suggest that *P. rugosa*, a very abundant species in eastern Canada and the USA (Moser *et al.*, 2012b; Mack *et al.*, 2019), is replaced by *Placobdella akahkway* in western portions of the continent: in spite of concerted efforts, the two species have never been recovered from the same locality. There are striking similarities in the ecology of the two species, in that both are relatively sessile, typically recovered from the shallow edges of lakes and ponds and on the underside of rocks and wood. In addition, at most of the collection sites for *P. akahkway*, turtles were also present, suggesting that these could be the main host for the species. The feeding preferences and behavior of the new species have yet to be formally examined (*e.g.*, through examination of gut contents), but it seems possible that the species would compete with *P. rugosa* for resources and that this competition has shaped the geographic distributions we see today.

Placobdella akahkway is visually similar to several other congeners, including *Placobdella rugosa* (Verrill, 1874), *Placobdella ornata* (Verrill, 1872), *Placobdella hollensis* (Whitman, 1892), and *Placobdella multilineata* (Moore, 1953). *P. ornata* possesses diffuse salivary tissue, *P. rugosa* and *P. hollensis* both possess 2 pairs compact salivary glands, while *P. multilineata*'s salivary glands are weakly developed; the unique combination of both compact and salivary tissue in *P. akahkway* can be used as a distinguishing characteristic. All 4 species also have distinct dorsal and marginal patterns that *P. akahkway* lacks.

The morphology of the salivary complex is unique for the new species: no other species of *Placobdella* possesses both compact and diffuse salivary tissues. The majority of *Placobdella* species possess compact salivary glands (either one or two pairs), but one third of species have only diffuse salivary tissue, and some species have salivary glands that are only weakly developed (de Carle *et al.*, 2017). Previous taxonomic accounts have afforded some weight to the presence or absence of compact salivary glands, reaching a pinnacle in the erection of the genus *Desserobdella* to include members possessing only diffuse salivary tissue (Barta & Sawyer, 1990; Jones & Woo, 1990). Siddall *et al.* (2005) found *Desserobdella* species to be non-monophyletic, and suggested that it should be subsumed by *Placobdella*, which has priority by seniority. Each of *Placobdella pediculata* Hemingway, 1908, *Placobdella cryptobranchii* (Johnson & Klemm, 1977), *Placobdella sophieae*, *Placobdella phalera* (Graff, 1899), *Placobdella michiganensis*, and *Placobdella picta* (Verrill, 1872) possesses diffuse salivary tissues, and these species place in disparate parts of the phylogenetic trees presented here, as well as that of de Carle *et al.* (2017). Regardless of lack of phylogenetic signal in this character, the arrangement of salivary tissue seems to be consistent within species. The combination of compact and diffuse salivary tissue in *P. akahkway* is unique among members of *Placobdella*, and is therefore a reliable diagnostic character and a solid synapomorphy for the species.

Acknowledgements

We thank Charlotte Calmerfalk Kvist for her generous aid in collecting specimens. We also thank Brad Muir for allowing us to collect on his property. Parks Canada provided much appreciated help with permitting for provincial parks. We thank Mrinmayee Sengupta and Anne F. Yolland (University of Toronto Department of Geography & Planning) for creating the map of collection sites. We thank Dr. Dorothy Thunder (University of Alberta) and Dr. Robert A. Innes (University of Saskatchewan) for their guidance in verifying the Plains Cree word for “leech.” This study was funded by a Royal Ontario Museum Peer Review grant, an NSERC Discovery Grant to SK, and an NSERC Postgraduate Doctoral Scholarship (PGSD2-518435-2018) awarded to DdC.

References

- Barta, J.R. & Sawyer, R.T. (1990) Definition of a new genus of glossiphoniid leech and a redescription of the type species, *Clepsine picta* Verrill, 1872. *Canadian Journal of Zoology*, 68 (9), 1942–1950.
<https://doi.org/10.1139/z90-273>
- Bielecki, A., Cichocka, J.M., Jabłoński, A., Jeleń, I., Ropelewska, E., Biedunkiewicz, A., Terlecki, J., Nowakowski, J.J., Pakulnicka, J. & Szlachciak, J. (2012) Coexistence of *Placobdella costata* (Fr. Müller, 1846) (Hirudinida: Glossiphoniidae) and mud turtle *Emys orbicularis*. *Biologia*, 67 (4), 731–738.
<https://doi.org/10.2478/s11756-012-0069-y>
- de Carle, D., Oceguera-Figueroa, A., Tessler, M., Siddall, M.E. & Kvist, S. (2017) Phylogenetic analysis of *Placobdella* (Hirudinea: Rhynchobdellida: Glossiphoniidae) with consideration of COI variation. *Molecular Phylogenetics and Evolution*, 114, 234–248.
<https://doi.org/10.1016/j.ympev.2017.06.017>
- Folmer, O., Black, M., Hoeh, W., Lutz, R. & Vrijenhoek, R. (1994) DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. *Molecular Marine Biology and Biotechnology*, 3 (5), 294–299.
- Glez-Peña, D., Gómez-Blanco, D., Reboiro-Jato, M., Fdez-Riverola, F. & Posada, D. (2010) ALTER: program-oriented conversion of DNA and protein alignments. *Nucleic Acids Research*, 38, W14–W18.
<https://doi.org/10.1093/nar/gkq321>
- Goloboff, P.A., Farris, S. & Nixon, K. (2008) TNT, a free program for phylogenetic analysis. *Cladistics*, 24, 774–786.
<https://doi.org/10.1111/j.1096-0031.2008.00217.x>
- Graf, A. (1899) Hirudineenstudien. *Nova Acta Academiae Caesareae Leopoldino-Carolinae Germanicae Naturae Curiosorum*, 72 (2), 215–404.
- Hemingway, E.E. (1908) *Placobdella pediculata* n. sp. *The American Naturalist*, 42 (500), 527–532.
- Hughes, J.L. & Siddall, M.E. (2007) A new species of leech from the New York metropolitan area. *American Museum Novitates*, 2007 (3578), 1–6.
- Johnson, G.M. & Klemm, D.J. (1977) A new species of leech, *Batracobdella cryptobranchii* n. sp. (Annelida: Hirudinea), parasitic on the Ozark hellbender. *Transactions of the American Microscopical Society*, 1977, 327–331.
- Jones, S.R.M. & Woo, P.T.K. (1990) Redescription of the leech *Desserobdella phalera* (Graf, 1899) n. comb. (Rhynchobdellida: Glossiphoniidae), with notes on its biology and occurrence on fishes. *Canadian Journal of Zoology*, 68 (9), 1951–1955.
<https://doi.org/10.1139/z90-274>
- Katoh, K. & Standley, D.M. (2013) MAFFT multiple sequence alignment software version 7: Improvements in performance and usability. *Molecular Biology and Evolution*, 30, 772–780.
<https://doi.org/10.1093/molbev/mst010>
- Kvist, S., de Carle, D., Cornejo, A. & Oceguera-Figueroa, A. (2018) Biological introductions or native ranges: Two curious cases of new distributional records in the Panama Canal. *BioInvasions Records*, 7 (3), 237–244.
<https://doi.org/10.3391/bir.2018.7.3.04>
- Lanfear, R., Calcott, B., Ho, S.Y. & Guindon, S. (2012) PartitionFinder: combined selection of partitioning schemes and substitution models for phylogenetic analyses. *Molecular Biology and Evolution*, 29 (6), 1695–1701.
- Mack, J., de Carle, D. & Kvist, S. (2019) Prey, populations, and the pleistocene: evidence for low COI variation in a widespread North American leech. *Mitochondrial DNA Part A*, 30 (6), 749–763.
<https://doi.org/10.1080/24701394.2019.1634698>
- Maddison, W.P. & Maddison, D.R. (2016) Mesquite: a modular system for evolutionary analysis. Version 3.11. Available from: <http://mesquiteproject.org> (accessed 14 June 2022)
- Manglicmot, C., Oceguera-Figueroa, A. & Kvist, S. (2020) Bacterial endosymbionts of *Placobdella* (Annelida: Hirudinea: Glossiphoniidae): phylogeny, genetic distance, and vertical transmission. *Hydrobiologia*, 847 (4), 1177–1194.
- Moore, J.P. (1953) Three undescribed North American leeches (Hirudinea). *Notulae Naturae of the Academy of Natural Sciences of Philadelphia*, 250, 1–13.
- Moser, W.E., Richardson, D.J., Hammond, C.I. & Lazo-Wasem, E.A. (2012a) Redescription of *Placobdella ornata* (Verrill, 1872) (Hirudinida: Glossiphoniidae). *Bulletin of the Peabody Museum of Natural History*, 53 (1), 325–330.
<https://doi.org/10.3374/014.053.0103>
- Moser, W.E., Richardson, D.J., Hammond, C.I., Govedich, F.R. & Lazo-Wasem, E. (2012b) Resurrection and redescription of *Placobdella rugosa* (Verrill, 1874) (Hirudinida: Glossiphoniidae). *Bulletin of the Peabody Museum of Natural History*, 53 (2), 375–381.
<https://doi.org/10.3374/014.053.0203>
- Moser, W.E., Richardson, D.J., Hammond, C.I. & Lazo-Wasem, E.A. (2013a) Redescription of *Placobdella papillifera* Verrill, 1872 (Hirudinida: Glossiphoniidae). *Bulletin of the Peabody Museum of Natural History*, 54 (1), 125–131.
<https://doi.org/10.3374/014.054.0105>
- Moser, B., Briggler, J.T., Richardson, D.J., Schuette, C.D., Hammond, C.I., Hopkins, W.A. & Lazo-Wasem, E.A. (2013b) Redescription and molecular characterization of *Placobdella cryptobranchii* (Johnson & Klemm, 1977) (Glossiphoniidae, Hirudinida). *ZooKeys*, 338, 1–10.
<https://doi.org/10.3897/zookeys.338.5995>

- Moser, W.E., Richardson, D.J., Hammond, C.I. & Lazo-Wasem, E.A. (2013c) Redescription of *Placobdella parasitica* (Say, 1824) Moore, 1901 (Hirudinida: Glossiphoniidae). *Bulletin of the Peabody Museum of Natural History*, 54 (2), 255–262.
<https://doi.org/10.3374/014.054.0203>
- Moser, W.E., Richardson, D.J., Hammond, C.I. & Lazo-Wasem, E. (2014) Redescription and molecular characterization of *Placobdella hollensis* (Whitman, 1892) (Hirudinida: Glossiphoniidae). *Bulletin of the Peabody Museum of Natural History*, 55 (1), 49–54.
<https://doi.org/10.3374/014.055.0104>
- Moser, W.E., Richardson, D.J., Hammond, C.I., Lazo-Wasem, E.A. & Phillips, A.J. (2017) Redescription and Molecular Characterization of *Placobdella nuchalis* Sawyer and Shelley, 1976 (Hirudinida: Glossiphoniidae). *Bulletin of the Peabody Museum of Natural History*, 58 (2), 311–316.
<https://doi.org/10.3374/014.058.0204>
- Moser, W.E., Richardson, D.J., Schlesser, N.J., Hammond, C.I. & Lazo-Wasem, E.A. (2019) Redescription and Molecular Characterization of *Placobdella pediculata* Hemingway, 1908 (Hirudinida: Glossiphoniidae). *Bulletin of the Peabody Museum of Natural History*, 60 (2), 121–127.
<https://doi.org/10.3374/014.060.0203>
- Moser, W.E., Richardson, D.J., Hammond, C.I. & Lazo-Wasem, E.A. (2020) Redescription and molecular characterization of *Placobdella michiganensis* (Sawyer, 1972) (Hirudinida: Glossiphoniidae). *Bulletin of the Peabody Museum of Natural History*, 61 (2), 97–102.
<https://doi.org/10.3374/014.061.0202>
- Müller, F. (1846) *Clepsine costata*, neue Art. *Archiv für Naturgeschichte*, 12, 82–85.
- Nguyen, L.T., Schmidt, H.A., von Haeseler, A. & Minh, B.Q. (2015) IQ-TREE: A fast and effective stochastic algorithm for estimating maximum likelihood phylogenies. *Molecular Biology and Evolution*, 32, 268–274.
<https://doi.org/10.1093/molbev/msu300>
- Oceguera-Figueroa, A., Kvist, S., Watson, S.C., Sankar, D.F., Overstreet, R.M. & Siddall, M.E. (2010) Leech collections from Washington State, with the description of two new species of *Placobdella* (Annelida: Glossiphoniidae). *American Museum Novitates*, 3701, 1–14.
<https://doi.org/10.1206/3701.2>
- Oceguera-Figueroa, A. & Pacheco-Chaves, B. (2012) Registros de sanguijuelas de Costa Rica y clave para la identificación de las especies con redescrición de *Cylicobdella costaricensis*. *Revista Mexicana de Biodiversidad*, 83, 946–957.
- Sawyer, R.T. (1972) *North American freshwater leeches, exclusive of the Piscicolidae, with a key to all species* 46. University of Illinois Press, Urbana, Illinois, 154 pp.
- Sawyer, R.T. (1986) *Leech Biology and Behaviour*. Oxford University Press, Oxford, 1065 pp.
- Siddall, M.E., Budinoff, R.B. & Borda, E. (2005) Phylogenetic evaluation of systematics and biogeography of the leech family Glossiphoniidae. *Invertebrate Systematics*, 19 (2), 105–112.
<https://doi.org/10.1071/IS04034>
- Siddall, M.E. & Bowerman, J. (2006) A new species of glossiphoniid leech from *Rana pretiosa* (Amphibia: Ranidae) in Oregon. *The Journal of Parasitology*, 92 (4), 855–857.
<https://doi.org/10.1645/GE-778R.1>
- Siddall, M.E. & Gaffney, E.S. (2004) Observations on the leech *Placobdella ornata* feeding from bony tissues of turtles. *The Journal of Parasitology*, 90 (5), 1186–1188.
<https://doi.org/10.1645/GE-277R>
- Swofford, D.L. (2002) PAUP*. Phylogenetic Analysis Using PAUP. Version 4. Sinauer Associates, Sunderland, Massachusetts. [program]
- Verrill, A.E. (1872) Brief contributions to zoology from the Museum of Yale College; No. XVII, Descriptions of North American fresh-water leeches. *American Journal of Science*, 3 (14), 126–139.
- Verrill, A.E. (1874) *Report upon the invertebrate animals of Vineyard Sound and adjacent waters, with an account of the physical features of the region*. Washington Government Printing Office, Washington, 628 pp.
<https://doi.org/10.5962/bhl.title.11688>
- Whitman, C.O. (1892) Metamerism of Clepsine. In: *Festschrift zum siebenzigsten geburtstage Rudolf Leuckart's*. Englemann, Leipzig, pp. 385–395.

APPENDIX. Maximum parsimony tree based the newly derived and GenBank COI sequences of *Placobdella* (length = 1737; consistency index = 0.274; retention index = 0.729). All non-focal species-level taxa are collapsed when possible, with lone or non-collapsible tips showing GenBank accession numbers. *Placobdella akahkway n. sp.* is indicated within the green box, with new sequences indicated in bold. Parsimony bootstrap support values are shown below each node. 100% support values are indicated by circles.

