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RESEARCH ARTICLE



Prey, populations, and the pleistocene: evidence for low COI variation in a widespread North American leech

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ABSTRACT

Placobdella rugosa has long presented challenges to leech biologists. Its extreme morphological variability and similarity to some congeneric species has confounded classification for over a century. Recent molecular analyses revealed a surprising lack of genetic variation among morphologically disparate, geographically widespread specimens of *P. rugosa*. Given the lack of any obvious mechanism by which this species could disperse between distant habitats, it was expected that widespread populations would be genetically isolated from each other. In the present study, we investigate the relationship between geographic distance and genetic diversity in *P. rugosa* using COI sequences from specimens collected across Canada and the United States. Although we find preliminary evidence for a barrier to gene flow between eastern and western collecting localities, our vastly expanded dataset largely corroborates prior studies, showing minimal phylogeographic signal among the sequences and negligible levels of genetic isolation by distance. A recent range expansion following the last ice age and/or host-mediated dispersal are discussed as potential explanations for this unexpected phylogeographic pattern.

ARTICLE HISTORY

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KEYWORDS

Hirudinea; *Placobdella*;
leech; COI variation;
phylogeography;
North America

Introduction

Placobdella rugosa (Verrill 1874) is a sanguivorous leech common to freshwater ecosystems in the northeastern United States and Canada (Sawyer 1986). It is an ectoparasite that primarily feeds on turtle blood and has been shown to vector blood parasites between its testudine hosts (Siddall and Desser 1992, 2001). Opportunistically, *P. rugosa* will also feed on waterfowl, alligators, fish, and mammals (Sawyer 1972, 1986; Davies 1973; Trauger and Bartonek 1977; Brooks et al. 1990). The species is externally characterized by a dark, dorso-medial stripe interrupted by areas of lighter pigmentation; five dorsal rows of papillae, with heavy secondary papillation, and a distinctive pattern of two rows of three papillae, followed by two pairs of paramedial papillae adjacent to the anus; a repeated marginal alternation of two light-coloured annuli, followed by one dark annulus in mid-body segments; and an unpigmented 'mask' near the two pairs of coalesced eyespots (Moser et al. 2012). Previous observations of *P. rugosa*, however, demonstrate that these characteristics are not universal: instead, papillation and coloration are highly variable among individuals, more so than in other species of *Placobdella* (Klemm 1985; Moore 1905; Moser et al. 2012; de Carle et al. 2017; Langer et al. 2018) (see also Figure 1). Furthermore, there has been over a century of taxonomic confusion between *P. rugosa* and *Placobdella ornata* (Verrill 1872). The identity of the two species has only recently been

formalized (Moser et al. 2012), but the literature is riddled with examples of confusion between them (see Moser et al. 2016 and references therein).

A recent investigation into phylogenetic relationships and species delimitation within the genus *Placobdella* revealed a lack of phylogeographic structure among the sampled specimens of *P. rugosa* (de Carle et al. 2017). The nine specimens included in the study displayed significant variation in patterns of dorsal papillation and pigmentation, characters which are commonly – and reliably – used to diagnose congeneric species (Klemm 1985). These specimens were collected from localities in Ontario, Saskatchewan, Manitoba, Minnesota, North Dakota and Nebraska. In spite of this, phylogenetic analyses revealed that all nine specimens nested together with short branch lengths, and their relationships did not reflect geographic structure. Observed genetic distances in these specimens were also low relative to other leech species: average intra-specific distance at the cytochrome c oxidase subunit I (COI) locus was 0.9% (minimum = 0.000%; maximum = 2.2%) (de Carle et al. 2017). These findings were surprising for a number of reasons. Molecular analyses continue to demonstrate the prevalence of cryptic diversity within leeches (Siddall and Borda 2003; Trontelj and Utevsky 2005; Sket and Trontelj 2008; Saglam et al. 2018). In fact, de Carle et al. (2017) found evidence for cryptic diversity within multiple

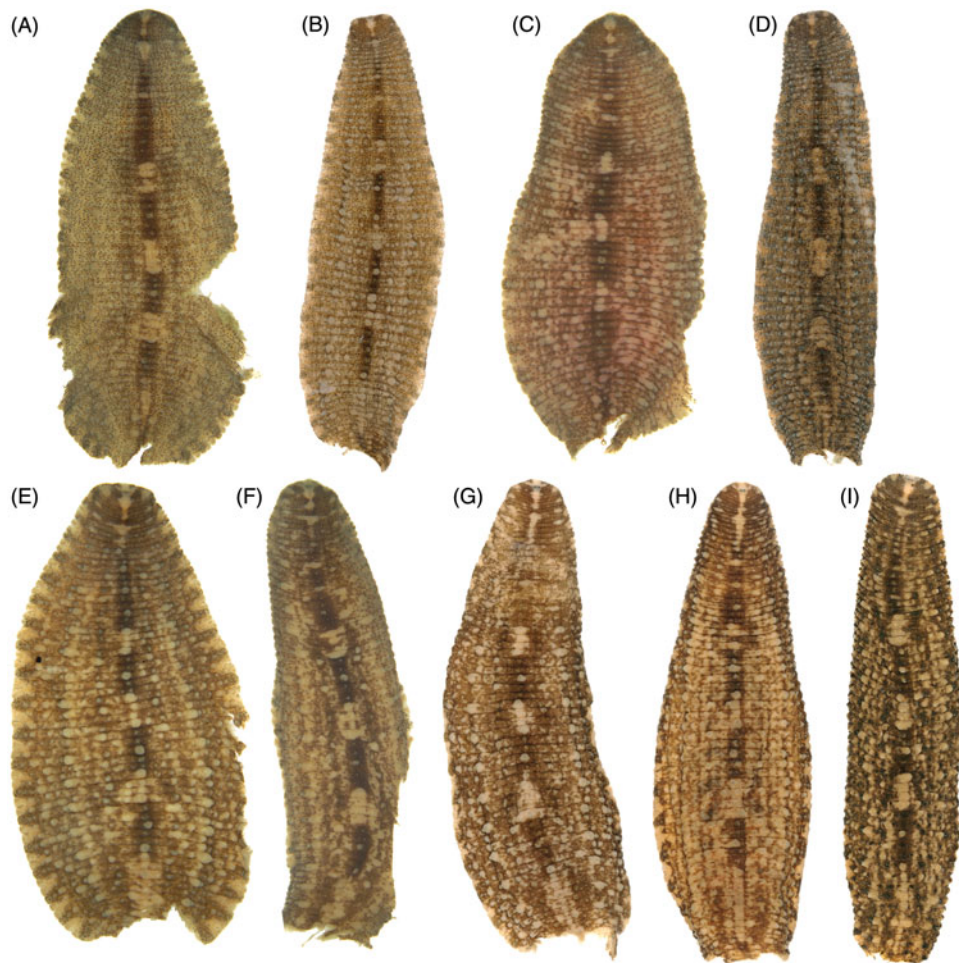


Figure 1. Nine preserved specimens of *P. rugosa* from Québec. Note the variation in mottling and coloration, which is typically more vibrant in living specimens. All have the characteristic dorsomedial stripe, alternating marginal light and dark annuli, and an unpigmented mask around the eyespots. The caudal suckers and some portions of marginal tissue have been removed for sequencing. A = ROMIZ I12379 (MK929750), B = ROMIZ I12384 (MK929755), C = ROMIZ I12461 (MK929768), D = ROMIZ I12383 (MK929754), E = ROMIZ I12459 (MK929766), F = ROMIZ I12457 (MK929764), G = ROMIZ I12447 (MK929761), H = ROMIZ I12378 (MK929749), I = ROMIZ I12377 (MK929748).

species of *Placobdella*. Furthermore, ecological observations of *P. rugosa* reveal no obvious mechanism by which the species could readily disperse between habitats. Like other glossiphoniid leeches, *P. rugosa* engages in extensive parental care, brooding their newly hatched offspring over the summer months and delivering them to their first blood meal before the onset of cold weather. Brooding takes place entirely off of the host, and during this time, neither adults nor juveniles feed (Siddall and Dessler 2001). This behaviour would seem to preclude juvenile dispersal and limit gene flow between populations. Even while not engaged in brooding, *P. rugosa* has been described as 'sedentary' and 'sluggish'. They are not capable swimmers, and they detach from their hosts immediately after feeding (Moore 1901; Siddall and Dessler 2001; de Carle et al. 2017).

To thoroughly investigate the phylogeography of *P. rugosa*, we leverage increased sampling from across a large section of the species' range. We perform phylogenetic and haplotype analyses to examine the relationship between genetic and geographic distance of these specimens and consider the possible influence of post-glacial recolonization and host dispersal in explaining the observed patterns.

Materials and methods

Specimen collection

The present study used leeches sampled during Royal Ontario Museum (ROM) field expeditions to the Canadian provinces of Saskatchewan (SK), Manitoba (MB), Ontario (ON), and Québec (QC) as well as Nebraska (NE) and Minnesota (MN) in the USA (Table 1 and Figure 2). Specimens were collected in shallow ponds and lakes from underneath rocks, aquatic debris, and exposed skin. Leeches were relaxed by the gradual addition of ethanol (maximally 15–20% ethanol) to pond water and subsequently fixed in 95% ethanol. All specimens are deposited in the ROM's invertebrate zoology collection.

DNA amplification, purification and sequencing

Tissue was cut from the caudal suckers to minimize contamination by gut contents and kept at -20°C until processing. DNA was extracted using a DNeasy Blood and Tissue Kit (Qiagen, Valencia, CA). The present study focused on the mitochondrial COI locus, which was amplified using

Table 1. Taxon names, locality information, and accession numbers for the specimens used in the study. Newly sequenced taxa are shown in bold font.

Taxon	Specimen code	Locality	Coordinates	COI GenBank Accession Numbers
Ingroup				
<i>Placobdella rugosa</i>	ROMIZI I10089	Mijinemungshing Lake, Lake Superior Provincial Park, Ontario		MF067106
<i>Placobdella rugosa</i>	ROMIZI I10090	Mijinemungshing Lake, Lake Superior Provincial Park, Ontario	47.702673N 84.730059W	MK929652 MK929653
<i>Placobdella rugosa</i>	ROMIZI I10091	Mijinemungshing Lake, Lake Superior Provincial Park, Ontario	47.702673N 84.730059W	
<i>Placobdella rugosa</i>	ROMIZI I10096	Mijinemungshing Lake, Lake Superior Provincial Park, Ontario	47.702673N 84.730059W	MK929654
<i>Placobdella rugosa</i>	ROMIZI I10100	Mijinemungshing Lake, Lake Superior Provincial Park, Ontario		MF067105
<i>Placobdella rugosa</i>	ROMIZI I10101	Mijinemungshing Lake, Lake Superior Provincial Park, Ontario		MF067104
<i>Placobdella rugosa</i>	ROMIZI I10112	Kenny Lake, Lake Superior Provincial Park, Ontario	47.282636N 84.561766W	MK929655
<i>Placobdella rugosa</i>	ROMIZI I10118	Paquette Lake, Ontario	46.748837N 83.885505W	MK929656
<i>Placobdella rugosa</i>	ROMIZI I10119	Paquette Lake, Ontario		MF067103
<i>Placobdella rugosa</i>	ROMIZI I10120	Paquette Lake, Ontario	46.748837N 83.885505W	MK929657
<i>Placobdella rugosa</i>	ROMIZI I10121	Paquette Lake, Ontario	46.748837N 83.885505W	MK929658
<i>Placobdella rugosa</i>	ROMIZI I10132	Ranger Lake, Ontario	46.879187N 83.569371W	MK929659
<i>Placobdella rugosa</i>	ROMIZI I10133	Ranger Lake, Ontario	46.879187N 83.569371W	MK929660
<i>Placobdella rugosa</i>	ROMIZI I10137	Ranger Lake, Ontario	46.879187N 83.569371W	MK929661
<i>Placobdella rugosa</i>	ROMIZI I10165	Unnamed Pond, Grundy Lake Provincial Park, Ontario	45.931319N 80.568046W	MK929662
<i>Placobdella rugosa</i>	ROMIZI I10166	Unnamed Pond, Grundy Lake Provincial Park, Ontario	45.931319N 80.568046W	MK929663
<i>Placobdella rugosa</i>	ROMIZI I10167	Unnamed Pond, Grundy Lake Provincial Park, Ontario	45.931319N 80.568046W	MK929664
<i>Placobdella rugosa</i>	ROMIZI I10185	Unnamed pond, Grundy Lake Provincial Park, Ontario	45.931319N 80.568046W	MK929665
<i>Placobdella rugosa</i>	ROMIZI I10210	Clear Lake, Grundy Lake Provincial Park, Ontario	45.932639N 80.575461W	MK929666
<i>Placobdella rugosa</i>	ROMIZI I10212	Unnamed pond, Restoule Provincial Park, Ontario	46.060783N 79.763913W	MK929667
<i>Placobdella rugosa</i>	ROMIZI I10213	Unnamed pond, Restoule Provincial Park, Ontario	46.060783N 79.763913W	MK929668
<i>Placobdella rugosa</i>	ROMIZI I10245	Unnamed pond, Restoule Provincial Park, Ontario	46.060783N 79.763913W	MK929669
<i>Placobdella rugosa</i>	ROMIZI I10253	Moore Lake, Samuel de Champlain Provincial Park, Ontario	46.292453N 78.878476W	MK929670
<i>Placobdella rugosa</i>	ROMIZI I10254	Moore Lake, Samuel de Champlain Provincial Park, Ontario	46.292453N 78.878476W	MK929671
<i>Placobdella rugosa</i>	ROMIZI I10255	Moore Lake, Samuel de Champlain Provincial Park, Ontario	46.292453N 78.878476W	MK929672
<i>Placobdella rugosa</i>	ROMIZI I10263	Pacaud Lake, Samuel de Champlain Provincial Park, Ontario	46.290257N 78.89958W	MK929673
<i>Placobdella rugosa</i>	ROMIZI I10264	Pacaud Lake, Samuel de Champlain Provincial Park, Ontario	46.290257N 78.89958W	MK929674
<i>Placobdella rugosa</i>	ROMIZI I10267	Pacaud Lake, Samuel de Champlain Provincial Park, Ontario	46.290257N 78.89958W	MK929675
<i>Placobdella rugosa</i>	ROMIZI I10273	Pacaud Lake, Samuel de Champlain Provincial Park, Ontario	46.290257N 78.89958W	MK929676
<i>Placobdella rugosa</i>	ROMIZI I10275	Pacaud Lake, Samuel de Champlain Provincial Park, Ontario	46.290257N 78.89958W	MK929677
<i>Placobdella rugosa</i>	ROMIZI I10286	Burbot Lake, Ontario	46.258237N 78.901717W	MK929678
<i>Placobdella rugosa</i>	ROMIZI I10287	Burbot Lake, Ontario	46.258237N 78.901717W	MK929679
<i>Placobdella rugosa</i>	ROMIZI I10288	Burbot Lake, Ontario	46.258237N 78.901717W	MK929680
<i>Placobdella rugosa</i>	ROMIZI I10289	Burbot Lake, Ontario	46.258237N 78.901717W	MK929681
<i>Placobdella rugosa</i>	ROMIZI I10325	Unnamed pond, Ontario	46.13038N 78.306168W	MK929682
<i>Placobdella rugosa</i>	ROMIZI I10338	Pearkes Lake, Ontario	44.497839N 76.560586W	MK929683
<i>Placobdella rugosa</i>	ROMIZI I10339	Pearkes Lake, Ontario	44.497839N 76.560586W	MK929684
<i>Placobdella rugosa</i>	ROMIZI I10342	Unnamed pond, Frontenac Provincial Park, Ontario	44.569695N 76.505173W	MK929985
<i>Placobdella rugosa</i>	ROMIZI I10362	Unnamed pond, Frontenac Provincial Park, Ontario	44.569695N 76.505173W	MK929686

(continued)

Table 1. Continued.

Taxon	Specimen code	Locality	Coordinates	COL GenBank Accession Numbers
<i>Placobdella rugosa</i>	ROMIZI I10363	Unnamed pond, Frontenac Provincial Park, Ontario	44.569695N 76.505173W	MK929687
<i>Placobdella rugosa</i>	ROMIZI I10476	Lost Lake, Cook, Minnesota	47.816683N 92.403817W	MK929688
<i>Placobdella rugosa</i>	ROMIZI I10477	Lost Lake, Cook, Minnesota	47.816683N 92.403817W	MK929689
<i>Placobdella rugosa</i>	ROMIZI I10478	Lost Lake, Cook, Minnesota	47.816683N 92.403817W	MK929690
<i>Placobdella rugosa</i>	ROMIZI I10479	Lost Lake, Cook, Minnesota	47.816683N 92.403817W	MK929691
<i>Placobdella rugosa</i>	ROMIZI I10480	Lost Lake, Cook, Minnesota	47.816683N 92.403817W	MK929692
<i>Placobdella rugosa</i>	ROMIZI I10481	Lost Lake, Cook, Minnesota	47.816683N 92.403817W	MK929693
<i>Placobdella rugosa</i>	ROMIZI I10482	Lost Lake, Cook, Minnesota	47.816683N 92.403817W	MK929694
<i>Placobdella rugosa</i>	ROMIZI I10483	Lost Lake, Cook, Minnesota	47.816683N 92.403817W	MK929695
<i>Placobdella rugosa</i>	ROMIZI I10484	Lost Lake, Cook, Minnesota	47.816683N 92.403817W	MK929696
<i>Placobdella rugosa</i>	ROMIZI I10485	Lost Lake, Cook, Minnesota	47.816683N 92.403817W	MK929697
<i>Placobdella rugosa</i>	ROMIZI I10486	Lost Lake, Cook, Minnesota	47.816683N 92.403817W	MK929698
<i>Placobdella rugosa</i>	ROMIZI I10487	Lost Lake, Cook, Minnesota	47.816683N 92.403817W	MK929699
<i>Placobdella rugosa</i>	ROMIZI I10488	Lost Lake, Cook, Minnesota	47.816683N 92.403817W	MK929700
<i>Placobdella rugosa</i>	ROMIZI I10489	Lost Lake, Cook, Minnesota	47.816683N 92.403817W	MK929701
<i>Placobdella rugosa</i>	ROMIZI I10499	Eagle Lake, Minnesota	44.206883N 93.88975W	MK929702
<i>Placobdella rugosa</i>	ROMIZI I10500	Eagle Lake, Minnesota	44.206883N 93.88975W	MK929703
<i>Placobdella rugosa</i>	ROMIZI I10513	Unknown pond, Nevens Ranch, Nebraska	41.192745N 101.303333W	MK929704
<i>Placobdella rugosa</i>	ROMIZI I10514	Unknown pond, Nevens Ranch, Nebraska	41.192745N 101.303333W	MK929705
<i>Placobdella rugosa</i>	ROMIZI I10522	Unknown pond, Nevens Ranch, Nebraska	41.192745N 101.303333W	MK929706
<i>Placobdella rugosa</i>	ROMIZI I10564	Swan Lake, Arthur County, Nebraska	41.737617N 101.4745W	MK929707
<i>Placobdella rugosa</i>	ROMIZI I10565	Swan Lake, Arthur County, Nebraska		MF067102
<i>Placobdella rugosa</i>	ROMIZI I10566	Swan Lake, Arthur County, Nebraska	41.737617N 101.4745W	MK929708
<i>Placobdella rugosa</i>	ROMIZI I10567	Swan Lake, Arthur County, Nebraska	41.737617N 101.4745W	MK929709
<i>Placobdella rugosa</i>	ROMIZI I10568	Swan Lake, Arthur County, Nebraska	41.737617N 101.4745W	MK929710
<i>Placobdella rugosa</i>	ROMIZI I10569	Swan Lake, Arthur County, Nebraska	41.737617N 101.4745W	MK929711
<i>Placobdella rugosa</i>	ROMIZI I10578	Swan Lake, Arthur County, Nebraska	41.7638N 102.43885W	MK929712
<i>Placobdella rugosa</i>	ROMIZI I11411	Pipestone Creek, Moosomin Regional Park, Saskatchewan		MF067101
<i>Placobdella rugosa</i>	ROMIZI I11443	Anglin Lake beach, Saskatchewan	53.742748N 105.895182W	MK929713
<i>Placobdella rugosa</i>	ROMIZI I11553	Adam Lake, Turtle Mountain Provincial Park, Manitoba		MF067100
<i>Placobdella rugosa</i>	ROMIZI I11587	Singuish Lake, Duck Mountain Provincial Park, Manitoba		MF067099
<i>Placobdella rugosa</i>	ROMIZI I11598	Unnamed pond, Duck Mountain Provincial Park, Manitoba		MF067098
<i>Placobdella rugosa</i>	ROMIZI I12327	Unnamed pond, Quebec	45.640243N 75.832892W	MK929714
<i>Placobdella rugosa</i>	ROMIZI I12334	Lac Blair, Quebec	45.668094N 75.796329W	MK929715
<i>Placobdella rugosa</i>	ROMIZI I12340	Lac Jadot, Tee Lake, Timiskaming, Quebec.	46.757492N 79.04616W	MK929716
<i>Placobdella rugosa</i>	ROMIZI I12341	Lac Jadot, Tee Lake, Timiskaming, Quebec.	46.757492N 79.04616W	MK929717
<i>Placobdella rugosa</i>	ROMIZI I12344	Lac Pian, Quebec	47.857443N 79.113793W	MK929718
<i>Placobdella rugosa</i>	ROMIZI I12345	Lac Levéque, Quebec	47.86929N 79.000097W	MK929719
<i>Placobdella rugosa</i>	ROMIZI I12346	Lac Levéque, Quebec	47.86929N 79.000097W	MK929720
<i>Placobdella rugosa</i>	ROMIZI I12347	Lac Levéque, Quebec	47.86929N 79.000097W	MK929721

(continued)

Table 1. Continued.

Taxon	Specimen code	Locality	Coordinates	COI GenBank Accession Numbers
<i>Placobdella rugosa</i>	ROMIZI I12348	Lac Levéque, Quebec	47.86929N 79.000097W	MK929722
<i>Placobdella rugosa</i>	ROMIZI I12349	Lac Levéque, Quebec	47.86929N 79.000097W	MK929723
<i>Placobdella rugosa</i>	ROMIZI I12350	Lac Levéque, Quebec	47.86929N 79.000097W	MK929724
<i>Placobdella rugosa</i>	ROMIZI I12351	Lac Levéque, Quebec	47.86929N 79.000097W	MK929725
<i>Placobdella rugosa</i>	ROMIZI I12352	Lac Levéque, Quebec	47.86929N 79.000097W	MK929726
<i>Placobdella rugosa</i>	ROMIZI I12353	Lac Levéque, Quebec	47.86929N 79.000097W	MK929727
<i>Placobdella rugosa</i>	ROMIZI I12357	Big Cedar Lake, Quebec	46.287888N 76.092519W	MK929728
<i>Placobdella rugosa</i>	ROMIZI I12358	Big Cedar Lake, Quebec	46.287888N 76.092519W	MK929729
<i>Placobdella rugosa</i>	ROMIZI I12359	Big Cedar Lake, Quebec	46.287888N 76.092519W	MK929730
<i>Placobdella rugosa</i>	ROMIZI I12360	Big Cedar Lake, Quebec	46.287888N 76.092519W	MK929731
<i>Placobdella rugosa</i>	ROMIZI I12361	Big Cedar Lake, Quebec	46.287888N 76.092519W	MK929732
<i>Placobdella rugosa</i>	ROMIZI I12362	Big Cedar Lake, Quebec	46.287888N 76.092519W	MK929733
<i>Placobdella rugosa</i>	ROMIZI I12363	Lac Boutin, Quebec	46.279762N 76.092655W	MK929734
<i>Placobdella rugosa</i>	ROMIZI I12364	Lac Boutin, Quebec	46.279762N 76.092655W	MK929735
<i>Placobdella rugosa</i>	ROMIZI I12365	Lac Boutin, Quebec	46.279762N 76.092655W	MK929736
<i>Placobdella rugosa</i>	ROMIZI I12366	Big Cedar Lake, Quebec	46.28826N 76.094493W	MK929737
<i>Placobdella rugosa</i>	ROMIZI I12367	Big Cedar Lake, Quebec	46.28826N 76.094493W	MK929738
<i>Placobdella rugosa</i>	ROMIZI I12368	Big Cedar Lake, Quebec	46.28826N 76.094493W	MK929739
<i>Placobdella rugosa</i>	ROMIZI I12369	Big Cedar Lake, Quebec	46.28826N 76.094493W	MK929740
<i>Placobdella rugosa</i>	ROMIZI I12370	Big Cedar Lake, Quebec	46.28826N 76.094493W	MK929741
<i>Placobdella rugosa</i>	ROMIZI I12371	Big Cedar Lake, Quebec	46.28826N 76.094493W	MK929742
<i>Placobdella rugosa</i>	ROMIZI I12372	Big Cedar Lake, Quebec	46.28826N 76.094493W	MK929743
<i>Placobdella rugosa</i>	ROMIZI I12373	Big Cedar Lake, Quebec	46.28826N 76.094493W	MK929744
<i>Placobdella rugosa</i>	ROMIZI I12374	Lac Edelweiss, Quebec	45.648138N 75.852593W	MK929745
<i>Placobdella rugosa</i>	ROMIZI I12375	Unnamed pond, Quebec	45.640243N 75.832892W	MK929746
<i>Placobdella rugosa</i>	ROMIZI I12376	Unnamed pond, Quebec	45.640243N 75.832892W	MK929747
<i>Placobdella rugosa</i>	ROMIZI I12377	Unnamed pond, Quebec	45.640243N 75.832892W	MK929748
<i>Placobdella rugosa</i>	ROMIZI I12378	Unnamed pond, Quebec	45.640243N 75.832892W	MK929749
<i>Placobdella rugosa</i>	ROMIZI I12379	Unnamed pond, Quebec	45.640243N 75.832892W	MK929750
<i>Placobdella rugosa</i>	ROMIZI I12380	Unnamed pond, Quebec	45.640243N 75.832892W	MK929751
<i>Placobdella rugosa</i>	ROMIZI I12381	Unnamed pond, Quebec	45.640243N 75.832892W	MK929752
<i>Placobdella rugosa</i>	ROMIZI I12382	Unnamed pond, Quebec	45.640243N 75.832892W	MK929753
<i>Placobdella rugosa</i>	ROMIZI I12383	Unnamed pond, Quebec	45.640243N 75.832892W	MK929754
<i>Placobdella rugosa</i>	ROMIZI I12384	Unnamed pond, Quebec	45.640243N 75.832892W	MK929755
<i>Placobdella rugosa</i>	ROMIZI I12385	Unnamed pond, Quebec	45.640243N 75.832892W	MK929756
<i>Placobdella rugosa</i>	ROMIZI I12386	Unnamed pond, Quebec	45.640243N 75.832892W	MK929757
<i>Placobdella rugosa</i>	ROMIZI I12387	Unnamed pond, Quebec	45.640243N 75.832892W	MK929758

(continued)

Table 1. Continued.

Taxon	Specimen code	Locality	Coordinates	COI GenBank Accession Numbers
<i>Placobdella rugosa</i>	ROMIZI I12388	Unnamed pond, Quebec	45.640243N 75.832892W	MK929759
<i>Placobdella rugosa</i>	ROMIZI I12446	Lac Blair, Quebec	45.668094N 75.796329W	MK929760
<i>Placobdella rugosa</i>	ROMIZI I12447	Lac Blair, Quebec	45.668094N 75.796329W	MK929761
<i>Placobdella rugosa</i>	ROMIZI I12451	Lac Blair, Quebec	45.668094N 75.796329W	MK929762
<i>Placobdella rugosa</i>	ROMIZI I12455	Lac Donovan, Quebec	45.769997N 75.728258W	MK929763
<i>Placobdella rugosa</i>	ROMIZI I12457	Lac Donovan, Quebec	45.769997N 75.728258W	MK929764
<i>Placobdella rugosa</i>	ROMIZI I12458	Lac Donovan, Quebec	45.769997N 75.728258W	MK929765
<i>Placobdella rugosa</i>	ROMIZI I12459	Lac Donovan, Quebec	45.769997N 75.728258W	MK929766
<i>Placobdella rugosa</i>	ROMIZI I12460	Lac Donovan, Quebec	45.769997N 75.728258W	MK929767
<i>Placobdella rugosa</i>	ROMIZI I12461	Lac Donovan, Quebec	45.769997N 75.728258W	MK929768
<i>Placobdella rugosa</i>	ROMIZI I12464	Unnamed pond, Frontenac Provincial Park, Ontario	44.569582N 76.505861W	MK929769
<i>Placobdella rugosa</i>	ROMIZI I12465	Unnamed pond, Frontenac Provincial Park, Ontario	44.569582N 76.505861W	MK929770
<i>Placobdella rugosa</i>	ROMIZI I12467	Unnamed pond, Frontenac Provincial Park, Ontario	44.569582N 76.505861W	MK929771
<i>Placobdella rugosa</i>	ROMIZI I12469	Unnamed pond, Frontenac Provincial Park, Ontario	44.569582N 76.505861W	MK929772
<i>Placobdella rugosa</i>	ROMIZI I12470	Unnamed pond, Frontenac Provincial Park, Ontario	44.569582N 76.505861W	MK929773
<i>Placobdella rugosa</i>	ROMIZI I12471	Unnamed pond, Frontenac Provincial Park, Ontario	44.569582N 76.505861W	MK929774
<i>Placobdella rugosa</i>	ROMIZI I12472	Unnamed pond, Frontenac Provincial Park, Ontario	44.569582N 76.505861W	MK929775
<i>Placobdella rugosa</i>	ROMIZI I12473	Unnamed pond, Frontenac Provincial Park, Ontario	44.569582N 76.505861W	MK929776
<i>Placobdella rugosa</i>	ROMIZI I12474	Unnamed pond, Frontenac Provincial Park, Ontario	44.569582N 76.505861W	MK929777
<i>Placobdella rugosa</i>	ROMIZI I12475	Unnamed pond, Frontenac Provincial Park, Ontario	44.569582N 76.505861W	MK929778
<i>Placobdella rugosa</i>	ROMIZI I12476	Unnamed pond, Frontenac Provincial Park, Ontario	44.569582N 76.505861W	MK929779
<i>Placobdella rugosa</i>	ROMIZI I12478	Unnamed pond, Frontenac Provincial Park, Ontario	44.569582N 76.505861W	MK929780
<i>Placobdella rugosa</i>	ROMIZI I12481	Unnamed pond, Frontenac Provincial Park, Ontario	44.569582N 76.505861W	MK929781
<i>Placobdella rugosa</i>	ROMIZI I12519	Unnamed pond, Frontenac Provincial Park, Ontario	44.569582N 76.505861W	MK929782
<i>Placobdella rugosa</i>	ROMIZI I12520	Unnamed pond, Frontenac Provincial Park, Ontario	44.569582N 76.505861W	MK929783
<i>Placobdella rugosa</i>	ROMIZI I12522	Unnamed pond, Frontenac Provincial Park, Ontario	44.569582N 76.505861W	MK929784
<i>Placobdella rugosa</i>	ROMIZI I12523	Unnamed pond, Frontenac Provincial Park, Ontario	44.569582N 76.505861W	MK929785
<i>Placobdella rugosa</i>	ROMIZI I12524	Unnamed pond, Frontenac Provincial Park, Ontario	44.569582N 76.505861W	MK929786
<i>Placobdella rugosa</i>	ROMIZI I12525	Unnamed pond, Frontenac Provincial Park, Ontario	44.569582N 76.505861W	MK929787
<i>Placobdella rugosa</i>	ROMIZI I12526	Unnamed pond, Frontenac Provincial Park, Ontario	44.569582N 76.505861W	MK929788
<i>Placobdella rugosa</i>	ROMIZI I12527	Unnamed pond, Frontenac Provincial Park, Ontario	44.569582N 76.505861W	MK929789
<i>Placobdella rugosa</i>		Belcourt Lake, Turtle Mountain, North Dakota, USA		JX412986
<i>Placobdella rugosa</i>		Belcourt Lake, Turtle Mountain, North Dakota, USA		JX412987
<i>Placobdella rugosa</i>		Belcourt Lake, Turtle Mountain, North Dakota, USA		JX412988
<i>Placobdella rugosa</i>		Belcourt Lake, Turtle Mountain, North Dakota, USA		JX412989
<i>Placobdella rugosa</i>		Belcourt Lake, Turtle Mountain, North Dakota, USA		JX412990
Outgroup				
<i>Placobdella multilineata</i>		Maurepas Swamp, Louisiana, USA		AY962464

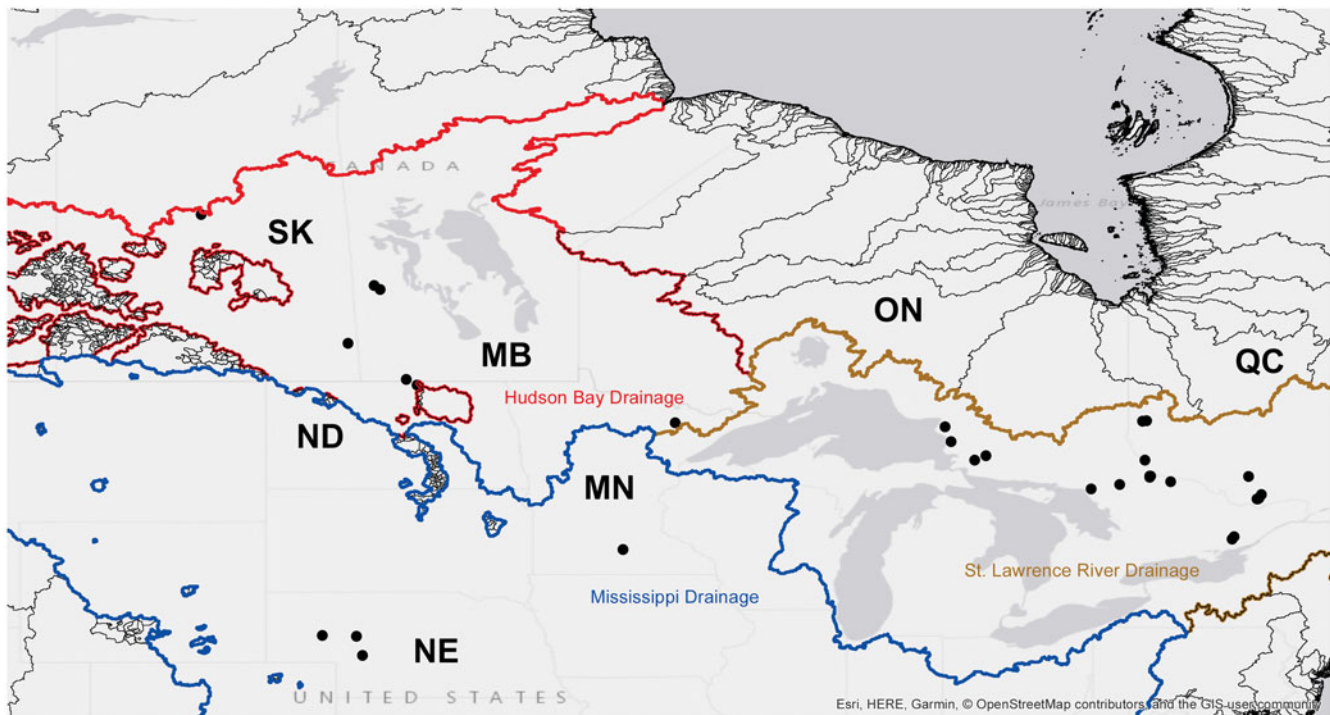


Figure 2. Map of all localities where leeches were sampled with an overlay of drainage basins. Each black dot represents a unique sampling locality. A total of 37 localities are represented.

polymerase chain reaction in 25 μL volumes per sample. Each reaction consisted of 16.34 μL distilled water, 2.5 μL buffer, 2.5 μL MgCl_2 , 1 μL of each primer at 10 μM concentration, 0.56 μL dNTPs at 10 μM concentration, 0.1 μL *Taq* Polymerase (Invitrogen, Carlsbad, CA), and 1 μL DNA. The primers used were LCO1490 (5'-GGTCAACAAATCATAAATATTGG-3') and HCO2198 (5'-TAAACTTCAGGGTGACCAAAAAATCA-3') (Folmer et al. 1994). Amplification used the following thermoprofile: 94 $^{\circ}\text{C}$ (1 min) followed by five cycles of 94 $^{\circ}\text{C}$ (30 s), 40 $^{\circ}\text{C}$ (40 s), 72 $^{\circ}\text{C}$ (1 min), then 35 cycles of 94 $^{\circ}\text{C}$ (30 sec), 46 $^{\circ}\text{C}$ (40 sec), 72 $^{\circ}\text{C}$ (1 min), and final extension at 72 $^{\circ}\text{C}$ (5 min). All PCR products were examined by electrophoresis on a 1.2% agarose gel and samples were purified with a solution of 1.5 μL H_2O :0.5 μL ExoSap-IT (Affymetrix, Santa Clara, CA). Cycle sequencing reactions were carried out using 4 μL of distilled water, 0.5 μL of ABI Big Dye Terminator ver. 3.1, 0.5 μL Big Dye 5X Sequencing Buffer (Applied Biosystems, Carlsbad, CA), 2 μL primer at 10 μM concentration, and 3 μL PCR product. Cycle sequencing was followed by ethanol precipitation, and the samples were then sequenced on an ABI PRISM 3730 (Applied Biosystems, Carlsbad, CA) at the ROM. Raw sequences were edited and assembled using Geneious ver. 11.1.4 (Kearse et al. 2012). To augment the dataset, five COI sequences for *P. rugosa* from its type locality in Belcourt Lake, North Dakota (ND) were downloaded from GenBank and added to our analyses, as were nine *P. rugosa* sequences from the study by de Carle et al. (2017). In total, 152 *P. rugosa* sequences were used, 138 of which were newly generated for the present study.

Alignments were carried out using the online version of MAFFT ver. 7 (Katoh and Standley 2013), applying default settings. All phylogenetic trees were rooted at *Placobdella*

multilineata (Moore 1953), following the phylogenetic hypothesis recovered by de Carle et al. (2017). The final matrix was created in Mesquite ver. 3.5 (Maddison and Maddison 2016). All newly generated sequences are deposited in GenBank under accession numbers MK929652-MK929789.

Gene tree inference, haplotype networks and genetic distances

Two optimality criteria were used to construct the phylogenetic hypotheses: parsimony and maximum likelihood (ML). For parsimony analyses, trees were constructed using a new technology search in TNT ver. 1.5 (Goloboff et al. 2008). The heuristic search consisted of 1000 replications, five rounds of ratcheting and five rounds of tree fusing, stipulating that the minimum length tree be recovered at least 10 times before terminating the search. Nodal support values were estimated by 1,000 rounds of standard bootstrapping, employing default settings. Prior to the ML analysis, PartitionFinder ver. 2.1.1 (Lanfear et al. 2016) was used to simultaneously estimate the optimal model of nucleotide evolution and the best fitting partitioning scheme, testing each codon position as a separate partition under the greedy algorithm. The ML tree was constructed using RAxML ver. 8 (Stamatakis 2014) on the CIPRES platform (Miller et al. 2010). The search used 25 initial GAMMA rate categories that were allowed to fluctuate independently for each partition, and final optimization with four GAMMA shape categories. Support values for clades were estimated via 1,000 iterations of the rapid bootstrap algorithm.

Average genetic distances (\pm standard error) were estimated in MEGA ver. 7.0.26 (Kumar et al. 2016), using the bootstrap method for variance estimation under the uncorrected p -distance model, with uniform rates among sites and complete deletion of missing data (note that no internal gaps were present in the alignment). To examine the relationship between collecting locality and genetic distance, specimens were grouped according to clades recovered by the phylogenetic and haplotype analyses, and according to the drainage basins from which they were collected. Within and between group genetic distances were then calculated for these specimens. To test for a possible correlation between genetic distance and geographic distance among the specimens, a Mantel test was run in Alleles in Space (AIS) applying default settings (Mantel 1967; Miller 2005). Finally, a haplotype network based on statistical parsimony was constructed in TCS (Clement et al. 2000), with a 90% connection limit and gaps treated as missing data. Adobe Illustrator (Adobe Systems, Inc., San Jose, CA) and Inkscape 0.92.3 (Free Software Foundation, Inc., Boston, MA) were used to further polish the haplotype network. The map of our sampling localities was constructed in ArcMap 10.6.1 (Esri, Inc., Redlands, CA) and drainage boundaries were taken from the HydroSHEDS database (Lehner et al. 2008).

Results

Gene trees

The parsimony and ML trees are highly similar – though lacking in resolution – showing only a few recognizable clades, and minimal evidence of phylogeographic structure (Figures 3 and 4). Both topologies resolve a ‘western’ clade, consisting of all but one sequence from specimens collected in Saskatchewan, Manitoba, Nebraska, Minnesota and North Dakota (parsimony bootstrap support [PBS] = 29%; ML bootstrap support [MLBS] = 74%). In the ML tree, this western clade is the sister group to a clade consisting of ROMIZ I10513 (MK929704), and ROMIZ I11443 (MK929713), collected from Nebraska and Saskatchewan, respectively (MLBS = 21%; Figure 3). Although these individuals form a sister pair in the parsimony tree as well (PBS = 51%), their position cannot be further resolved (Figure 4). The remainder of the tree, consisting almost entirely of sequences from Ontario and Québec, is largely unresolved. However, this group contains some clades of uniquely Québec origin, the largest of which includes 15 sequences from various localities in the province (PBS = 64%, MLBS = 52%).

Two anomalies bear mentioning: first, both trees agree on the placement of a sequence from Ontario (ROMIZ I10255 [MK929672]) within the otherwise exclusively western clade (MLBS = 74%, PBS = 29%); second, both trees agree on the placement of a Minnesota sequence (ROMIZ I10500 [MK929703]) within a clade of Ontario and Québec specimens (MLBS = 77%, PBS = 60%). These oddities are further discussed in the context of haplotypes and genetic distances, below.

Haplotype network

There is a total of 57 unique haplotypes in our dataset, 34 of which are unique to a single specimen. The network largely mirrors the results of the phylogenetic analyses, inasmuch as a cluster of haplotypes from western areas of collection (MB, SK, MN, ND and NE) is separated by six substitutions from a larger cluster of eastern (ON and QC) haplotypes (Figure 5). Additionally, much like the gene trees, there are exceptions to the geographic groupings and, unsurprisingly, the haplotype network more closely resembles the most parsimonious phylogenetic hypothesis. The aforementioned sequences from Nebraska and Saskatchewan (ROMIZ I10513 [MK929704] and ROMIZ I11443 [MK929713]) are separated by four and six substitutions, respectively, from the most inclusive Ontario + Québec haplotype. Moreover, the Ontario sequence that places within the western grouping in the phylogenies (ROMIZ I10255 [MK929672]) shares a haplotype with four other Nebraskan sequences and is separated from the nearest Ontario haplotype by 10 substitutions. Similarly, the sequence from Minnesota (ROMIZ I10500 [MK929703]) that clustered with Ontario and Québec specimens in the phylogenies shares its haplotype with 8 sequences from those provinces and is separated from other Minnesota sequences by at least 11 substitutions.

COI distances

The average COI distance among all *P. rugosa* specimens is $0.8\% \pm 0.2$, with the greatest distance reaching 2.6%. This greatest distance occurs between ROMIZ I10567 (NE; MK929709) and ROMIZ I10119 (ON; MF067103), as well as between ROMIZ I10578 (NE; MK929712) and three sequences from Ontario and Québec (ROMIZ I10119 [ON; MF067103], ROMIZ I12367 [QC; MK929738], and ROMIZ I12371 [QC; MK929742]).

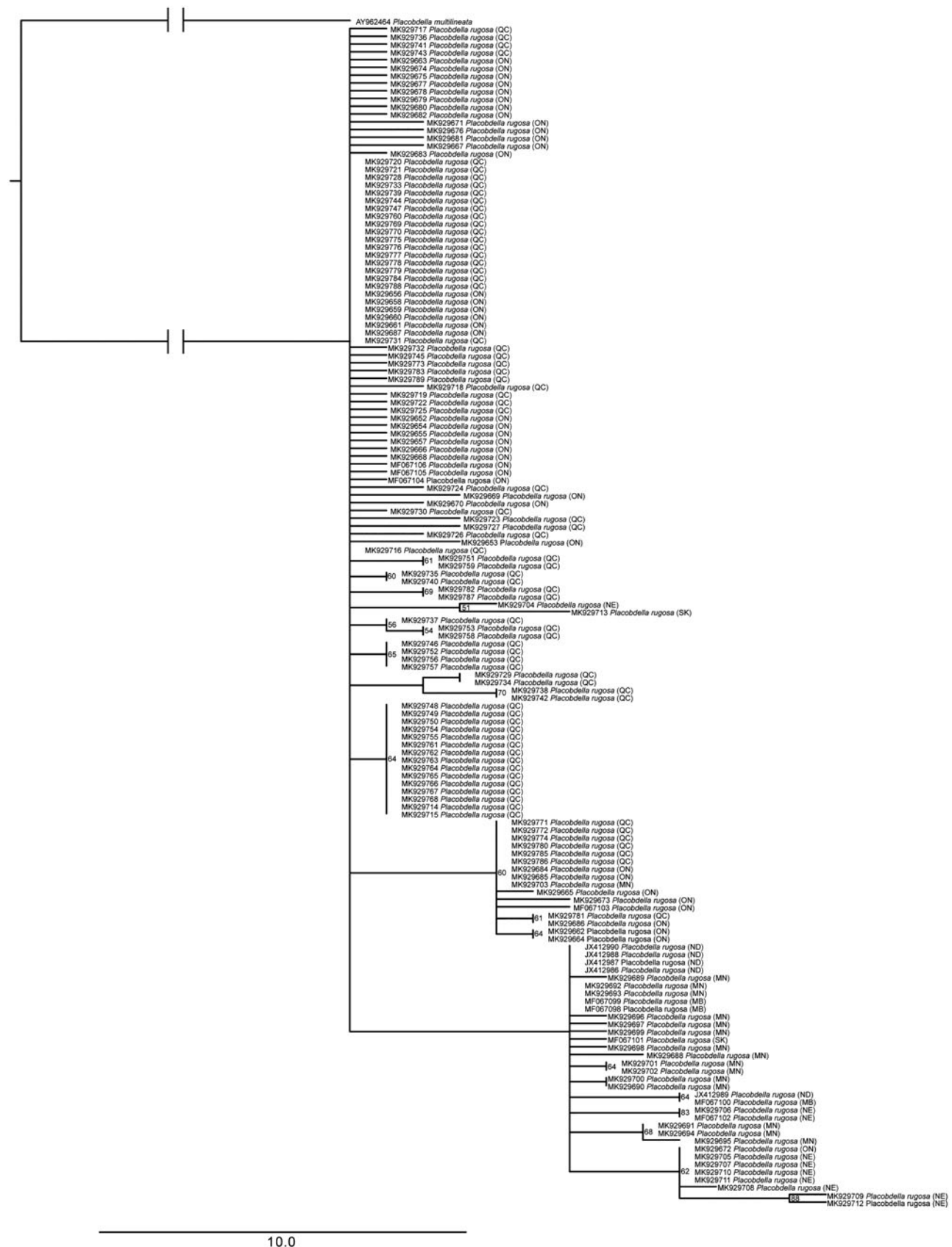
Genetic distances were calculated within and between the eastern and western haplotype groupings. In the phylogenetic and haplotype analyses, ROMIZ I10255 (ON) was included in the western clade, as were ROMIZ I10513 (NE) and ROMIZ I11443 (SK); similarly, ROMIZ I10500 (MN) was included in the eastern grouping of Ontario and Québec specimens. The average distance between the two groups amounted to $1.4\% \pm 0.4$, while the average within-group genetic distance for the western clade was $0.6\% \pm 0.2$ and the average within-group distance for the eastern grouping was $0.5\% \pm 0.1$. We also investigated how the specimens were dispersed across the North American freshwater drainage basins (Figure 2), which, to our knowledge, has not been considered in other population-level studies of leeches. Specimens from Nebraska and Minnesota reside in the Mississippi drainage basin; those from Québec and Ontario reside in the St. Lawrence River drainage basin; while those from Nebraska, Saskatchewan, and Manitoba reside in the Hudson Bay drainage basin. Genetic distances among the drainages were as follows: $0.7\% \pm 0.2$ between Hudson Bay and Mississippi drainages; $1.3\% \pm 0.4$ between St. Lawrence River and Hudson Bay drainages; and $1.4\% \pm 0.4$ between St. Lawrence River and Mississippi drainages. Within group, distances did not



Figure 3. Maximum-likelihood tree ($\ln L = -1846.112147$) resulting from the analysis of the COI dataset. Maximum likelihood bootstrap values above 50% are shown above each node. GenBank accession numbers are noted before each terminal and collection localities are noted after each taxon name. ON = Ontario, QC = Québec, MB = Manitoba, SK = Saskatchewan, ND = North Dakota, MN = Minnesota, NE = Nebraska.

exceed $0.8\% \pm 0.2$. Because the overwhelming majority of specimens from the Mississippi and Hudson Bay drainages grouped together in both the phylogenetic and haplotype analyses, we also grouped specimens from these two regions

and compared genetic distances within this group to specimens from the St. Lawrence drainage basin. The genetic distance within the two drainages was $0.7\% \pm 0.2$, while the distance between groups was $1.4\% \pm 0.3$.



10.0

Figure 4. Strict consensus of 107 most parsimonious trees (182 steps) resulting from the analysis of the COI dataset. Parsimony bootstrap values above 50% are shown above each node. GenBank accession numbers are noted before each terminal and collection localities are noted after each taxon name. ON = Ontario, QC = Québec, MB = Manitoba, SK = Saskatchewan, ND = North Dakota, MN = Minnesota, NE = Nebraska.

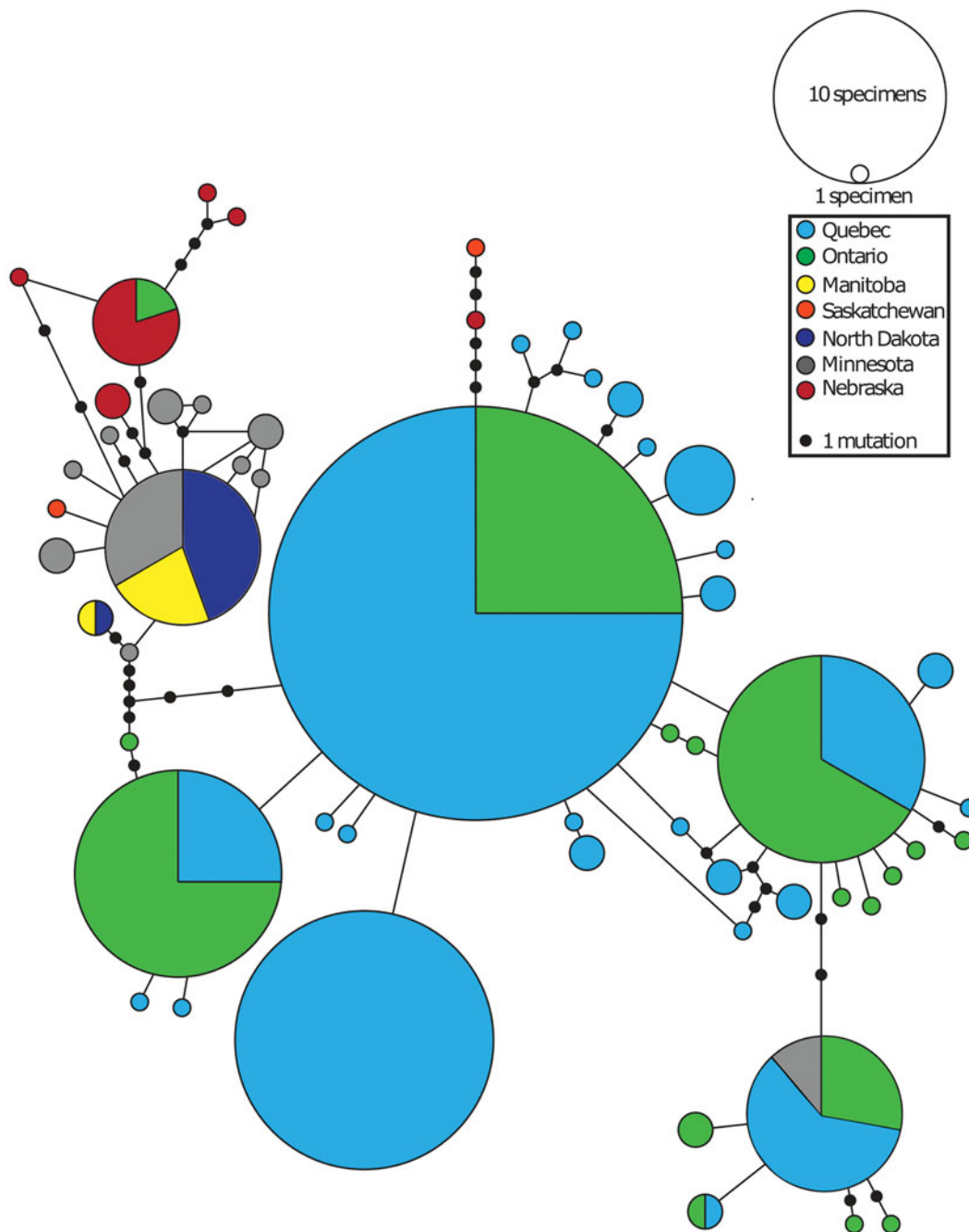


Figure 5. Haplotype network based on statistical parsimony resulting from the analysis of the COI dataset. Colours correspond to different provinces/states and solid black circles represent one transition.

Finally, we considered the genetic distances between specimens from the edges of our sampling range (Table 1 and Figure 2). Distances were computed between the westernmost and northernmost sample, ROMIZ I11443 (SK; MK929713); the southernmost sample, ROMIZ I10513 (NE; MK929704); and the easternmost sample, ROMIZ I12455 (QC; MK929763). The Saskatchewan and Québec samples, separated by 2322 km, differed by $1.1\% \pm 0.4$ in COI; the Québec and Nebraska samples, separated by 2115 km, differed by $0.76\% \pm 0.3$; finally, the Nebraska and Saskatchewan samples, separated by 1432 km, differed by $0.61\% \pm 0.3$. We tested for a correlation between genetic distance and geographic distance using a Mantel test, and obtained a correlation

coefficient of $r = 0.22$, $p < 0.001$, indicating a lack of correlation between the two parameters.

Discussion

Our results suggest that there is no evidence for cryptic diversity, using the COI locus as a proxy, within the *Placobdella rugosa* specimens sampled herein. While the greatest intraspecific distance within our dataset exceeds 2%, it is still within the range of intraspecific variation commonly encountered for leeches (Kvist 2014; Mack and Kvist 2019). Furthermore, the calculated average intraspecific distance of

0.83% is less than the average intraspecific distance for *Placobdella* species – $1.5\% \pm 1.8$ (de Carle et al. 2017). This strongly indicates that populations of *P. rugosa*, from Québec to Saskatchewan to Nebraska, constitute a single species in spite of the observed morphological variation (Figure 1). This is further corroborated by the lack of differentiation in the nuclear loci used by de Carle et al. (2017). Importantly, notwithstanding rigorous sampling efforts, we have yet to record *P. rugosa* west of Saskatchewan. Although *Placobdella rugosa* has been reported from more westerly localities in the United States and Canada (Sawyer 1972; Klemm 1985), these records are likely the result of confusion with a separate, as yet undescribed, species that closely resembles *P. rugosa* in external appearance (*Placobdella* sp.1; de Carle et al. 2017). The two species co-exist in Saskatchewan but, westward, *P. rugosa* appears to be replaced by *Placobdella* sp. 1 (Kvist, personal observation). Although this undescribed species is externally similar to *Placobdella rugosa*, it is more closely related to two other western species: *Placobdella kwetlumye* Ocegüera-Figueroa et al. (2010) and *Placobdella burrensonae* Siddall and Bowerman (2006), known from Washington and Oregon, respectively (de Carle et al. 2017). As it cannot presently be determined whether records ranging as far east as Nova Scotia and as far south as Texas (Klemm 1985) correspond to *P. rugosa sensu stricto*, *Placobdella ornata*, or another cryptic species not yet described, the northern, eastern, and southern geographic boundaries of *P. rugosa*'s range are still unknown.

Our phylogenetic and haplotype analyses recover a 'western' clade containing nearly all individuals from west of the Great Lakes (i.e. those in the Hudson Bay and Mississippi drainages) (SK, MB, MN, ND and NE; Figure 2); this was distinct from an unresolved cluster of haplotypes from areas in the Great Lakes region (i.e. those in the St. Lawrence river drainage) (ON and QC; Figure 2). It is possible that these results represent preliminary evidence for a barrier to gene flow between the Hudson Bay and Mississippi drainages and the St. Lawrence River drainage. This is reflected in the fact that genetic distance among specimens from these two groups is much less than between them. The same pattern is observed for the 'western' and 'eastern' haplotype groups. However, we are reluctant to draw conclusions based solely on the present data. First of all, a single Ontario specimen (ROMIZ I10255 [MK929672]) places within the western clade, and a single Minnesota specimen (ROMIZ I10500 [MK929703]) places within an otherwise eastern clade (Figures 3 and 4). The parsimony analysis also fails to recover two specimens from western localities – ROMIZ I10513 (NE; MK929704) and ROMIZ I11443 (SK; MK929713) – as part of the larger 'western' clade. Most importantly, however, the current study does not include nuclear data, which would be necessary to demonstrate gene flow, or a lack thereof, across drainage basins. Phylogenetic analyses by de Carle et al. (2017) leveraged both mitochondrial and nuclear data but failed to identify geographic structure within *P. rugosa*. Their study, however, included far fewer individuals. It is possible that a dataset encompassing nuclear data for a larger number of specimens may reveal a different pattern. For the time being, the notion that individuals of *P. rugosa* constitute a

continuous population cannot be ruled out. Indeed, our Mantel test showed negligible correlation between genetic and geographic distance. This raises the question: what forces could maintain an apparent lack of geographic structure in such a widespread organism? We propose two possible explanations: the potential for host-mediated dispersal, and the impact of glaciation.

Populations of *Placobdella rugosa* could be panmictic, dispersing with the aid of their vertebrate hosts. The primary hosts for *P. rugosa* are freshwater turtles, such as the snapping turtle, *Chelydra serpentina* (Linnaeus 1758); the painted turtle, *Chrysemys picta* (Schneider 1783); the spotted turtle, *Clemmys guttata*, (Schneider 1792); and Blanding's turtle, *Emydoidea blandingii* (Holbrook 1838) (Davy et al. 2009). It has also been known to feed opportunistically – yet reluctantly – on waterfowl, mammals, and fish (Sawyer 1972, 1986; Davies 1973; Trauger and Bartonek 1977; Brooks et al. 1990; de Carle, personal observation). The spotted turtle is the least vagile of the reported testudine hosts, existing in isolated, structured populations connected by limited gene flow (Davy and Murphy 2014). The species is known to move 1–2 km during active months and has a relatively small home range of approximately 300–1000 m (Joyal et al. 2001; Milam and Melvin 2001; Seburn 2003; Rasmussen and Litzgus 2010). The three remaining turtle hosts are known to travel far overland. The most mobile of these is the snapping turtle *Che. serpentina*, which is a common host of multiple *Placobdella* species, possibly due to its abundance, small plastron, and relatively large area of exposed skin (Stone 1976; Davy et al. 2009). One study on *Che. serpentina* populations across southeastern USA found high phylogeographic uniformity in mitochondrial DNA, which the authors attributed to its propensity for overland travel and broad habitat usage (Phillips et al. 1996; Walker et al. 1998; Gailbraith 2008). For example, gravid females can undertake migrations to nesting sites that last multiple days, and range from less than 1 km to over 11 km (Obbard and Brooks 1981; Congdon et al. 2008). Other studies have also reported mean annual movements of 1 km and have discovered individuals far from the nearest body of water (Barbour 1950; Klimstra 1951; Lovich and Ernst 2009). Interestingly, *Che. serpentina* was among the first turtle species to recolonize northern habitats after the last glacial maximum (Holman and Andrews 2008), followed by the painted turtle, *Chr. picta*. The latter species is also known to disperse across great distances: one study in Saskatchewan showed that male turtles moved, on average, 6 km during the active season, while females moved an average of 2.2 km (MacCulloch and Secoy 1983). The final candidate for testudine dispersal of *P. rugosa* is Blanding's turtle. Unlike the common snapping turtle, this species is highly phylogeographically structured across its range (Lovich and Ernst 2009; Davy et al. 2014; Sethuraman et al. 2014). Nonetheless, it has a fondness for overland travel and will frequently move between wetlands, sometimes over distances greater than 2 km (Joyal et al. 2001; Edge et al. 2010; Millar and Blouin-Demers 2011). A single Blanding's turtle may even occupy up to six wetlands in a summer (Lovich and Ernst 2009).

Although the turtle hosts of *Placobdella rugosa* are known to travel large distances overland, it has been demonstrated that these leeches do not remain on their hosts after feeding (Siddall and Dessler 1992). At present, we do not know how long *P. rugosa* takes to begin feeding after finding a suitable host, nor do we know how long feeding lasts in this species. *Macrobodella decora* (Say 1824), another blood-feeding leech common to the same habitats, is known to feed for an average of 68 minutes, and may take up to 30 minutes to commence feeding after attaching to a host (Munro et al. 1991). Assuming that *P. rugosa* exhibits similar behaviors, we cannot reasonably expect these leeches to disperse over long distances on turtles. This is especially true since *M. decora* is, on average, much larger than *P. rugosa*, and feeding time, as well as the volume of blood ingested, is positively correlated with body size in leeches (Munro et al. 1991). Instead, it is more likely that host-mediated dispersal takes place via repeated instances of short-distance translocation. Whether this would be sufficient to generate the observed patterns of COI variation is not clear, but under this scenario, we might expect to observe greater genetic distance between individuals at the boundaries of the range than those at the centre. Our results, however, do not support any relationship between geographic and genetic distance. Birds present a more likely driver of host-mediated dispersal, as they are able to disperse much faster than turtles, and have been shown to act as agents of dispersal for other leech species (Siddall et al. 2013). Although *P. rugosa* is known to feed opportunistically on waterfowl, the frequency with which it does so is unclear.

Alternatively, the patterns we observe could result from dispersal dynamics following glaciation. The most recent ice age had deep biogeographical and genetic impacts on temperate organisms in North America and Europe (Hewitt 1996, 2000; Dyke 2004; Clark et al. 2009). Glacial advances during the Pleistocene (2.58 Mya to 11,700 years ago) repeatedly forced organisms from northern latitudes into warmer southern, or equatorial, pockets of refuge (Cohen et al. 2018). As the glaciers receded, species recolonized their former ranges (Hewitt 2000). Minimal phylogeographic structure is one of the signatures of a rapid expansion, and has been documented in many temperate species that migrated north after the last glacial maximum (Bernatchez and Wilson 1998; Milá et al. 2000; Hewitt 2004; Canestrelli et al. 2008; Grill et al. 2009; Trontelj and Utevsky 2012; Moore et al. 2015). Typically, this manifests as a lack of phylogenetic resolution, and a reduction in the genetic diversity of northern populations relative to southern populations (Slatkin and Hudson 1991; Hewitt 1996, 2000; Avise 2000). Our newly generated trees show a distinct lack of genetic diversity, in line with patterns that have been ascribed to the signature of repeated glacial expansion and retraction. This same hypothesis has been implicated to explain low phylogeographic signal in other freshwater organisms, like the European medicinal leech *Hirudo medicinalis* Linnaeus 1758, and the Arctic char *Salvelinus alpinus* (Linnaeus 1758) (Trontelj and Utevsky 2012; Moore et al. 2015). Under this scenario, it is possible that gene flow between populations of *Placobdella rugosa* is limited or non-existent, but that there has not been

sufficient time for those populations to amass differences at the COI locus that would be indicative of such genetic isolation. Assuming that leech mitochondrial DNA evolves at a similar rate to that of other animals (Brown et al. 1979; DeSalle et al. 1987; DeSalle and Templeton 1988; Fleischer et al. 1998; Eo and DeWoody 2010), and taking into account the average interspecific distance within *Placobdella* ($16.06\% \pm 1.99$; de Carle et al. 2017), it would stand to reason that the observed intraspecific variation in *P. rugosa* (mean = 0.83%, maximum = 2.6%) reflects standing variation in pre-glacial populations.

Given the broad geographic range recorded for *Placobdella rugosa*, it is somewhat surprising that the COI locus does not provide definitive evidence for population structure across northern North America. Each of the explanations (postglacial recolonization and host-mediated dispersal) invoked here could result in patterns of low geographic structure and COI variation, much like the observed patterns in *Placobdella rugosa*. It is important to note, however, that more data are needed to unequivocally refute the hypothesis that all individuals included in this study represent a single, continuous population. Future studies should focus on increasing gene sampling, including nuclear data, to robustly test for barriers to gene flow across the range of this ecologically important leech.

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Disclosure statement

No potential conflict of interest was reported by the authors.

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