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ARTICLE

Leeches (Annelida: Hirudinea) from the far north of Ontario: distribution, diversity, and diagnostics

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Abstract: Leeches have a worldwide distribution, yet numerous geographical regions remain to be adequately surveyed. Here, we present leech species records for one of these regions: the far north of Ontario, Canada. This region is primarily wetland habitat and includes two of Ontario's three ecozones. Morphological examinations, as well as a single instance of successful DNA amplification with subsequent molecular identification, allowed us to identify representatives of 12 species from two predatory families (Erpobdellidae and Haemopidae) and one parasitic family (Glossiphoniidae) among samples of 130 individuals. To provide a more inclusive list of species records for this remote region, our data were also augmented by 25 largely unpublished collection records (for 102 individuals) from the Canadian Museum of Nature, which revealed the presence of an additional species. We comment on finds of particular interest in our sampling with comparison to relevant literature and provide new distribution data for these species.

Key words: Hirudinea, leeches, northern Ontario, species distribution, specimen records, checklist.

Résumé : Si la répartition des sangsues est planétaire, de nombreuses régions géographiques n'ont pas été adéquatement étudiées. Nous documentons des registres d'espèces de sangsues pour une de ces régions, l'extrême-nord de l'Ontario (Canada). Cette région consiste principalement en des habitats de milieu humide et comprend deux des trois écozones de l'Ontario. Des examens morphologiques et un seul cas réussi d'amplification d'ADN avec identification moléculaire subséquente nous permettent d'identifier des représentants de 12 espèces de deux familles de sangsues prédatrices (erpobdellidés et hémopidés) et d'une famille de sangsues parasitiques (glossiphoniidés) parmi des échantillons de 130 individus. Afin d'en arriver à une liste plus inclusive de registres d'espèces pour cette région éloignée, nos données ont également été complétées par 25 registres de collections (pour 102 individus) principalement non publiés du Musée canadien de la nature, qui révèlent la présence d'une autre espèce. Nous discutons de découvertes d'intérêt particulier dans notre échantillonnage au vu de publications pertinentes et présentons de nouvelles données relatives à la répartition pour ces espèces. [Traduit par la Rédaction]

Mots-clés : hirudinés, sangsues, nord de l'Ontario, répartition des espèces, registres de spécimens, liste de vérification.

Introduction

Leeches (Annelida: Hirudinea) are a globally distributed, sparsely diverse, but highly abundant group of annelids. They have been recorded from every ocean and every continent, and inhabit freshwater, marine, and terrestrial ecosystems alike. They are defined by the absence of parapodia and chetae, unpaired genital openings on the clitellum, and suckers on both ends of their elongate bodies (Sket and Trontelj 2008). Only half of the roughly 680 known species represent parasitic forms, which feed either on the blood of vertebrates or haemolymph of invertebrates; others are predaceous, scavenging, or macrophagous, feeding on smaller invertebrates (Sket and Trontelj 2008; Bielecki et al. 2011). Leeches are a primary food source for numerous freshwater and marine fishes within their habitats (Clady 1974; Davies and Kasserra 1989; Koperski 2006), making them important components of aquatic food webs (Sawyer 1986; Adamiak-Brud et al. 2016). Because predaceous leeches are often found in abundance across ecosystems, they may act as useful gauges of water pollution (Bendell and McNicol 1991; Macova et al. 2009). Beyond their use as bioindicators, leeches are also frequently used in modern authoritative medicine, as relievers of venous congestion following flap or digit replantation surgery (e.g., Dabb et al. 1992; Soucacos et al. 1994). One of the key innovations granting them medical utility is their production of highly potent anticoagulants that keep the blood flowing in and around the incision site during the extended feeding period (Kvist et al. 2013, 2014, 2016; Siddall et al. 2016).

Approximately 480 of the 680 described leech species are associated with freshwater, with the remaining species classified as marine or terrestrial (Sket and Trontelj 2008). About 80 freshwater and terrestrial species have been recorded from North America (Moser et al. 2006; Sket and Trontelj 2008; Kubova et al. 2013), and Davies (1973) acknowledged 42 of these species as inhabiting Canada. Much like other cosmopolitan taxa, leech populations can shift in the face of ecological changes in climate, biota, or landscape. Variance in substrate, for example, can have a large impact on leech communities (Kubova et al. 2013): many species, like those in the family Glossiphoniidae, use inchworm-type locomotion, such that their movement is impeded by softer substrates (Adamiak-Brud et al. 2016). Environmental features such as rocks and submerged logs assist with this type of movement and act as shelter, protecting them from daylight, predators, and wave

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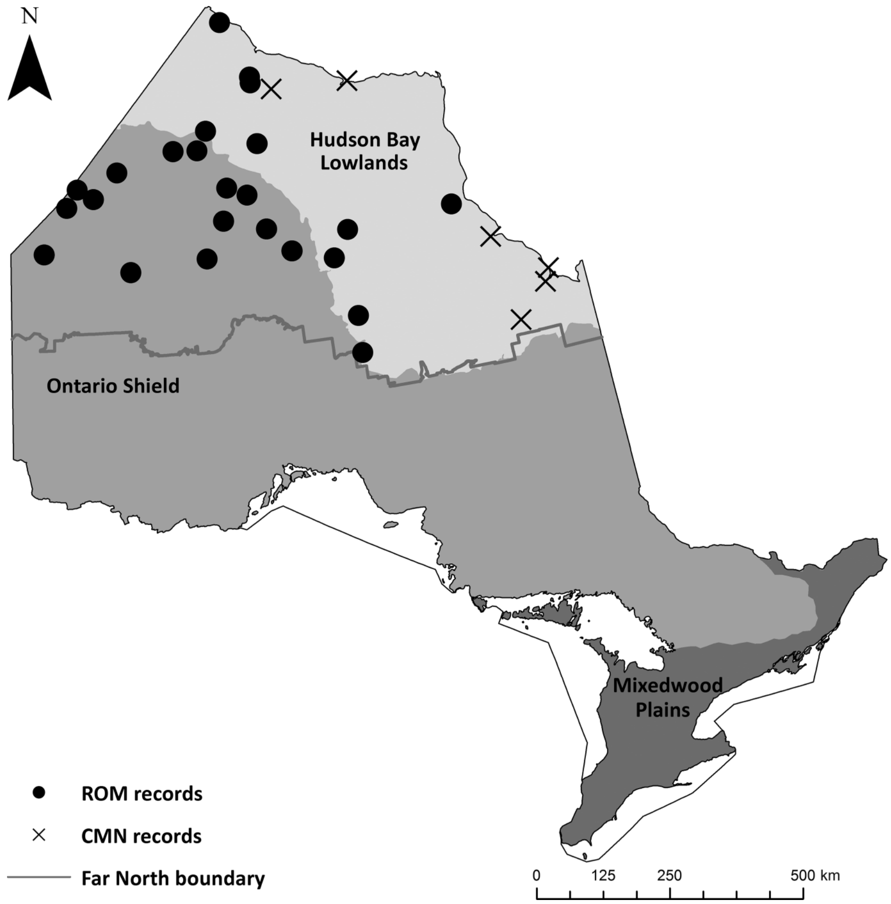
Fig. 1. Distribution of leech samples of the family Erpobdellidae that were collected for the present study across the far north of Ontario's two ecozones: Hudson Bay Lowlands and Ontario Shield. Projection: Canadian Lambert Conformal Conic. Datum: NAD 1983. ROM, Royal Ontario Museum; CMN, Canadian Museum of Nature.



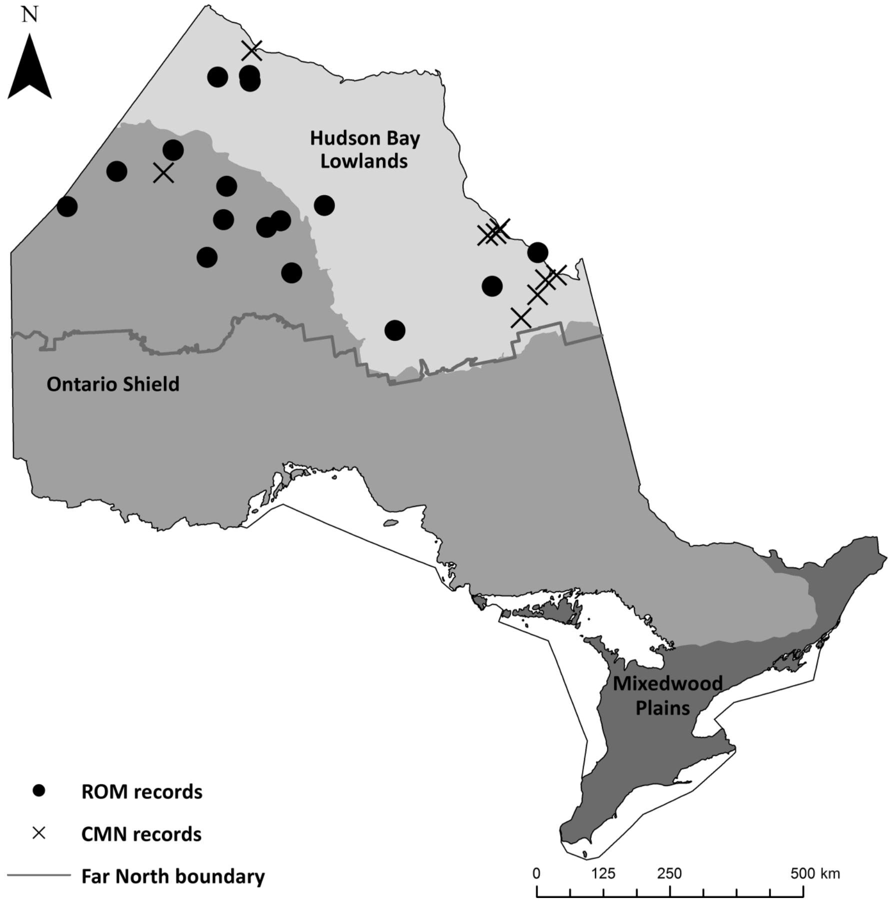
action (Adamiak-Brud et al. 2016); however, several leeches, e.g., members of Hirudinidae and Macrobdellidae, are capable swimmers. Tracking changes that might occur at a community level could be indicative of large-scale landscape alterations. Such endeavours are contingent on comprehensive distribution records covering extended periods of time. Hirudinea is just one of many taxa that have large gaps in knowledge regarding its detailed geographical distribution.

One such gap in our knowledge is the far north of Ontario, which covers 451 808 km^2 (42% of the province; Marshall and Jones 2011) as defined in Ontario's *Far North Act* of 2010 (S.O. 2010, c.18 s.2). This area includes two of Ontario's three ecozones: the Hudson Bay Lowlands and the Ontario Shield (Crins et al. 2009). Ecozones are characterized by physical processes that can be used to explain differences in the flora and fauna present (Crins et al. 2009). The Hudson Bay Lowlands ecozone contains the third largest wetland in the world, is the only area in Ontario influenced by oceanic currents, and has an area of continuous permafrost that extends along the Hudson Bay coastline (Crins et al. 2009). The Ontario Shield ecozone contains tens of thousands of lakes that range in size, depth, transparency, and shoreline complexity (Marshall and Jones 2011). The larger lakes have been surveyed for fish since the Patricia inventory in 1959 (Marshall and Jones 2011). Since then, extensive fieldwork has been conducted in Ontario's far north through the Ontario Ministry of Natural Resources and Forestry (OMNRF) Far North Biodiversity Project (FNBP). From 2009 to 2014, selected areas were surveyed using a variety of methods aimed at recording a diverse inventory of flora and fauna. The present study is based on these collection efforts and aims to provide insight into the composition of northern Ontario's hirudofauna. The first of its kind, our research may serve as a starting point for future studies investigating changes to population distributions as a result of biotic or abiotic influences.

Materials and methods

Leech specimens were collected in the far north of Ontario by the FNBP between 2010 and 2014. Importantly, leeches were not necessarily the target taxa for these collection efforts, which likely resulted in fewer organisms collected, compared with more leech-centric, targeted approaches. Individuals were most often captured using aquatic net and trap surveys. Aquatic net surveys used D-nets to sample freshwater habitats at scheduled times throughout the day. Aquatic traps were galvanized steel minnow traps baited with dog food and a ball of tinfoil — intended to attract minnows — and left in the water for 24 h before recovery. Four traps were placed at each locality. For detailed aquatic sampling methods from this project see Goertz and Phoenix (2015). Two individual leeches were also caught in pitfall and pan trap surveys. Pitfall traps were constructed from 250 mL plastic cups, inserted into the ground, and left for 3 days before recovery. Pan traps of blue, white, and yellow bowls were dug into shallow holes arranged in a cross and were checked daily. Additionally, leeches were collected when observed outside of scheduled trapping methods, here referred to as incidental. Baited traps are often employed in leech-specific surveys to target sanguivorous and scavenging leech species, but this may bias catches based on feeding habits (Bendell and McNicol 1991). The surveys conducted during this project

Fig. 2. Distribution of leech samples of the family Haemopidae that were collected for the present study across the far north of Ontario's two ecozones: Hudson Bay Lowlands and Ontario Shield. Projection: Canadian Lambert Conformal Conic. Datum: NAD 1983. ROM, Royal Ontario Museum; CMN, Canadian Museum of Nature.



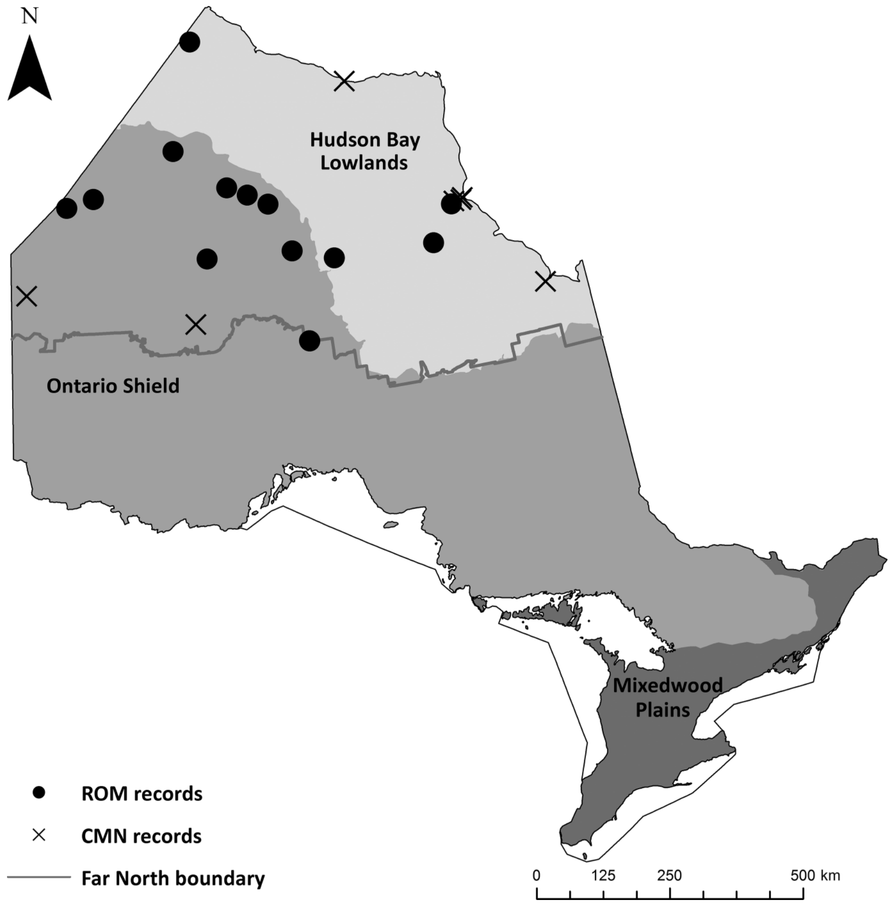
were not intended to be leech-specific, allowing us the opportunity to sample leeches using a number of methods, but potentially limiting our catches for some species. For example, no large fish specimens were collected during the current project, which resulted in a complete absence of fish leeches (Piscicolidae) — it is likely that extended fishing efforts would reveal several piscicolid species in the region. All collected specimens were preserved in 70% denatured ethanol. Leeches were sorted from these samples and brought to the Royal Ontario Museum (ROM) for identification and long-term curation. Identification was done according to specialized literature (e.g., Davies 1971, 1973; Klemm 1985; Sawyer 1986) and examination of specimens was conducted using a Leica Wild M10 dissecting microscope (Leica Microsystems) fitted with a Spot Flex 15.2 (64 Megapixel) camera (Spot Imaging). Several juvenile individuals proved refractory to morphological identification and, for five of these, molecular identification via DNA barcoding (see Hebert et al. 2003) using cytochrome *c* oxidase subunit I (COI) was attempted; for this purpose, DNA extraction, amplification, and sequencing methods followed Phillips et al. (2010). Molecular identification relied both on a BLAST approach (Altschul et al. 1997) and on a phylogenetic approach using the parsimony criterion. All COI sequences pertaining to the genus *Haemopis* Savigny, 1822 were downloaded from GenBank on 21 August 2017 and these were then jointly aligned using the online version of MAFFT version 7 (Katoh and Standley 2013). A phylogenetic tree was then constructed using TNT (Goloboff et al. 2008); the analysis employed 100 iterations, 5 rounds of ratcheting, and 3 rounds of fusing. Because the tree is intended to infer specimen identifications rather than relationships, the tree was mid-point rooted.

Our ROM-curated records were augmented by largely unpublished records in the online database of the Canadian Museum of Nature (CMN). To increase the comprehensiveness of the present study, 102 individuals collected at 25 localities, representing 10 different species, were included from the CMN database, only 4 species of which have been previously published (see Hovingh 2006). Maps were produced with ArcMap version 10.2 (ESRI 2011) using open-source outlines from the OMNRF and an ecozone layer (Commission for Environmental Cooperation 2009).

Results

A total of 130 individual leeches were collected between 2010 and 2014, representing three families: Erpobdellidae (n = 51) (Fig. 1), Haemopidae (n = 33) (Fig. 2), and Glossiphoniidae (n = 46) (Fig. 3). Among these, 12 species were recorded, representing both predaceous and parasitic taxa. Locations (including GPS coordinates), dates, and methods of capture for all specimens are listed in Table 1. Several specimens were damaged or juvenile, precluding successful morphological identification. DNA extraction and amplification was attempted for five individuals whose specific identities were unknown; however, likely due to long-term preservation in relatively low concentrations of ethanol (70%), four of these attempts were unsuccessful. As a result, these four individuals are only identified to the genus level, as are juvenile individuals and individuals in which the diagnostic features were obscured owing to preservation methods. Each species is briefly presented below, with location of capture summarized by ecodistrict; this method of classifying the far north is described by Crins et al. (2009). CMN

Fig. 3. Distribution of leech samples of the family Glossiphoniidae that were collected for the present study across the far north of Ontario's two ecozones: Hudson Bay Lowlands and Ontario Shield. Projection: Canadian Lambert Conformal Conic. Datum: NAD 1983. ROM, Royal Ontario Museum; CMN, Canadian Museum of Nature.



records are accompanied by the location descriptions from the museum's database.

Family Erpobdellidae

Erpobdella obscura (Verrill, 1872)
(Fig. 4A)

REMARKS: Specimens possess 4 pairs of eyes (2 anterior pairs and 2 posterior pairs); variable pigmentation, most often with relatively small, scattered black blotches on a lighter background; and 2 annuli between gonopores (in furrows between annuli). One specimen was opened to reveal a spirally coiled atrial cornua. All of these characters suggest that the specimens belong to *Erpobdella obscura*. This species was one of the most abundant among the samples and individuals were collected from the ecodistricts Sandy Lake, Wunnummin Lake, Albany River, Winisk River, Dickey River, and Lower Sachigo River. In addition, three records (five individuals) of this species were available in the CMN database; these were collected from "between Mattic and Opazatika River", Moose Factory, and "North Point, 29 km north of Moosonee", respectively (Table 1).

Erpobdella punctata (Leidy, 1870)
(Fig. 4B)

REMARKS: Members of *Erpobdella punctata* exhibit remarkable variation in dorsal pigmentation patterns, but all possess 2 or 4 longitudinal rows of darkly pigmented dots; these dots are sometimes fused into laterally oriented black bars, which can occasionally be interpreted as continuous stripes of pigmentation. This species is moderate to large in size (up to 100 mm), has gonopores separated by 2 annuli (with large male pore), and 3 pairs of eyes. *Erpobdella punctata* is abundant throughout southern Canada (Davies 1973; Klemm 1985), but the northernmost limit of its distribution is still unclear. The three specimens were collected from three different ecodistricts: Lower Sachigo River, Wunnumin Lake, and Winisk River. Two additional records from south of Albany River and Moose Factory were present in the CMN database (Table 1).

Erpobdella parva (Moore, 1912)
(Fig. 4C)

REMARKS: Specimens possess gonopores separated by 3½ annuli, with the male gonopore elevated on a ring, 4 pairs of eyes (2 posterior pairs of labial eyes behind the anterior 2 pairs), uniform dorsal colouration, and absence of a mid-dorsal stripe. Collectively, these characters identify *Erpobdella parva*. Only a single individual of this species was collected from the Wunnummin Lake ecodistrict and a single record from the Winisk tributary existed in the CMN database (Table 1).

Erpobdella dubia (Moore and Meyer, 1951)
(Fig. 4D)

REMARKS: Members of *Erpobdella dubia* possess a dark, mid-dorsal, longitudinal stripe that immediately sets them apart from most other species of *Erpobdella*. In addition, the dorsum is often greenish in colour and heavily mottled, gonopores are normally separated by 3½ annuli (2½ and 3 annuli have also been reported; Klemm 1985), and 4 pairs of eyespots are present (2 posterior pairs of

Table 1. Metadata for leeches collected from the far north of Ontario and deposited at the Royal Ontario Museum (ROM) or the Canadian Museum of Nature (CMN).

Species	Survey	Date	Latitude (°N)	Longitude (°W)	Number of specimens	Catalogue number
Family Erpobdellidae						
Erpobdella dubia	Aquatic net	20 June 2013	53.592	88.364	1	ROMIZ I12101
	Unknown	24 July 1965	55.333	87.283	1	CMNA 1987-0646
Erpobdella obscura	Aquatic trap	6 June 2010	52.981	87.926	1	ROMIZ I12119
		13 June 2010	52.560	87.300	5	ROMIZ I10631, I10632, I10633, I10634, I10636
		15 June 2010	52.560	87.298	2	ROMIZ I12110, I12111
		23 June 2010	52.340	86.163	2	ROMIZ I12105, I12106
		30 June 2011	52.826	94.215	1	ROMIZ I12002
		30 June 2011	52.827	94.217	2	ROMIZ I12011, I12012
		16 July 2011	53.907	93.214	1	ROMIZ I10630
		20 June 2014	54.752	89.363	1	ROMIZ I12091
		22 June 2014	54.752	89.364	1	ROMIZ I12092
		23 June 2014	54.441	87.902	1	ROMIZ I12081
		23 June 2014	54.441	87.901	1	ROMIZ I12080
		25 June 2014	55.482	87.880	1	ROMIZ I12086
	Aquatic net	5 June 2010	52.983	87.932	1	ROMIZ I12112
		5 June 2011	53.198	89.112	1	ROMIZ I12067
		17 June 2011	54.456	90.365	1	ROMIZ I12051
		21 June 2011	54.456	90.365	1	ROMIZ I12055
		11 July 2011	52.456	91.820	1	ROMIZ I12071
		11 July 2011	53.602	93.538	1	ROMIZ I12022
		23 June 2014	54.443	87.898	1	ROMIZ I12083
	Incidental	9 July 2011	53.602	93.537	1	ROMIZ I12006
	Unknown	24 June 1920	50.733	81.483	1	CMNA 1900-5992
		14–15 July 1920	51.267	80.617	3	CMNA 1900-5993
		22 August 1979	51.483	80.45	1	CMNA 1988-0132
Erpobdella parva	Aquatic net	7 July 2011	52.462	91.820	1	ROMIZ I12072
	Unknown	27 July 1965	55.283	85	1	CMNA 1978-0329
Erpobdella punctata	Aquatic net	9 June 2011	54.164	92.044	1	ROMIZ I12062
		28 June 2014	55.482	87.881	1	ROMIZ I12087
	Incidental	11 June 2011	53.752	88.919	1	ROMIZ I12013
	Unknown	14–15 July 1920	51.267	80.617	1	CMNA 1900-5968
		12 July 1965	52.217	81.8	1	CMNA-1978-0328
Erpobdella sp.	Aquatic trap	26 June 2014	55.582	87.877	1	ROMIZ I12075
	Aquatic net	5 June 2010	52.782	85.679	1	ROMIZ I12116
		2 June 2011	53.196	89.108	2	ROMIZ I12064, I12065
		5 June 2011	54.431	89.667	1	ROMIZ I12063
		17 June 2011	54.455	90.366	1	ROMIZ I12121
		11 July 2011	53.602	93.538	1	ROMIZ I12024
		15 July 2011	53.733	92.762	6	ROMIZ I12042, I12043, I12044, I12045, I12046, I12047
		23 June 2012	52.893	82.684	1	ROMIZ I12069
		11 June 2013	52.586	89.675	1	ROMIZ I12096
		1 July 2013	50.686	85.790	1	ROMIZ I12094
		20 July 2013	51.314	85.748	1	ROMIZ I12103
		22 July 2013	51.314	85.748	1	ROMIZ I12104
		4 July 2014	56.566	88.592	1	ROMIZ I12089
Family Haemopidae						
Haemopis grandis	Aquatic trap	4 June 2011	53.196	89.112	1	ROMIZ I12001
		21 June 2011	54.449	90.359	1	ROMIZ I12003
	Aquatic net	12 July 2013	50.937	84.852	1	ROMIZ I12098
	Incidental	12 July 2011	53.606	93.522	1	ROMIZ I12016
	Unknown	10 July 1965	52.217	81.883	1	CMNA 1987-0667
		11 July 1965	52.217	81.883	1	CMNA 1987-0668
Haemopis lateromaculata	Incidental	20 June 2010	52.163	87.394	1	ROMIZ I12115
	Unknown	1920	51.05	80.917	1	CMNA 1985-0259
		24 June 1920	50.733	81.483	1	CMNA 1985-0262
		12 July 1965	52.217	81.65	1	CMNA 1985-0279
		24 July 1965	54.083	90.7	1	CMNA 1987-0670
Haemopis marmorata	Aquatic trap	4 June 2010	52.981	87.926	1	ROMIZ I12113
		6 June 2010	52.981	87.926	1	ROMIZ I12120
		14 July 2010	53.219	86.225	1	ROMIZ I12118
		18 June 2011	54.456	90.365	1	ROMIZ I12014
		18 June 2011	54.457	90.363	1	ROMIZ I12017

Table 1 (*concluded*).

Species	Survey	Date	Latitude (°N)	Longitude (°W)	Number of specimens	Catalogue number
		21 June 2011	54.449	90.359	1	ROMIZ I12004
		15 June 2012	51.743	80.650	5	ROMIZ I12057, I12058, I12059, I12060, I12061
		25 June 2014	55.582	87.877	2	ROMIZ I12084, I12085
		26 June 2014	55.582	87.877	5	ROMIZ I12074, I12076, I12077, I12078, I12079
		28 June 2014	55.621	88.838	1	ROMIZ I12093
	Aquatic net	7 July 2010	53.064	87.515	1	ROMIZ I12117
		5 June 2011	53.196	89.108	1	ROMIZ I12066
		28 June 2014	55.482	87.881	1	ROMIZ I12088
	Incidental	11 June 2011	54.164	92.043	1	ROMIZ I12005
		11 June 2011	53.753	88.919	1	ROMIZ I12015
	Unknown	14–15 July 1920	51.267	80.617	1	CMNA 1977-0404
		16 August 1920	52.283	81.517	1	CMNA 1985-0281
		11 June 1975	51.3	80.3	1	CMNA 1986-0143
		2 August 1980	56	87.717	1	CMNA 1981-0062
Haemopis sp.	Aquatic trap	15 June 2012	51.743	80.650	1	ROMIZ I12122
	Incidental	20 June 2010	52.163	87.394	1	ROMIZ I12114
	Pitfall trap	13 July 2012	51.358	82.057	1	ROMIZ I12073
	Pan trap (blue)	8 June 2013	52.586	89.677	1	ROMIZ I12082
Family Glossiphoniidae						
Glossiphonia elegans	Aquatic trap	23 June 2010	52.340	86.163	2	ROMIZ I12107, I12108
	Aquatic net	7 July 2011	53.602	93.537	1	ROMIZ I12040
		12 July 2012	52.306	83.384	1	ROMIZ I12070
		10 June 2013	52.586	89.677	1	ROMIZ I12095
		20 June 2013	53.592	88.362	1	ROMIZ I12102
		6 July 2013	51.008	87.146	1	ROMIZ I12100
		10 July 2014	56.289	89.567	1	ROMIZ I12090
	Unknown	14 July 1920	51.267	80.617	1	CMNA 1900-8243
		15 July 1965	52.933	82.6	1	CMNA 1978-0327
		22 July 1965	55.283	85.083	1	CMNA 1978-0326
		11 August 1967	52.133	94.733	1	CMNA 1987-0671
Glossiphonia sp.	Incidental	9 July 2011	53.602	93.537	1	ROMIZ I12007
Helobdella stagnalis	Aquatic net	7 July 2011	53.603	93.536	1	ROMIZ I12041
		11 July 2011	53.602	93.537	5	ROMIZ I12030, I12031, I12032, I12033, I12148
		11 July 2011	53.602	93.538	8	ROMIZ I12019, I12020, I12021, I12023, I12025, I12026, I12027, I12028
		15 July 2011	53.733	92.765	2	ROMIZ I12048, I12049
		17 June 2011	54.456	90.365	3	ROMIZ I12052, I12053, I12054
		13 June 2011	53.753	88.920	1	ROMIZ I12056
		21 June 2012	52.893	82.683	1	ROMIZ I12068
		25 June 2013	53.397	87.800	1	ROMIZ I12097
		6 July 2013	51.012	87.154	1	ROMIZ I12099
	Unknown	15 July 1965	52.967	82.35	56	CMNA 1987-0669
		16 August 1965	52.933	82.4	21	CMNA 1987-0666
Helobdella sp.	Unknown	26 July 1963	51.267	80.617	1	CMNA 1987-0662
Placobdella montifera	Unknown	5 August 1961	51.5	90.15	1	CMNA 1987-0659
Placobdella rugosa	Aquatic net	11 July 2011	53.602	93.537	1	ROMIZ I12037
Theromyzon rude	Aquatic net	11 July 2011	53.602	93.538	1	ROMIZ I12018
Theromyzon tessulatum	Aquatic trap	13 June 2010	52.560	87.298	4	ROMIZ I12109
Theromyzon sp.	Aquatic trap	11 July 2011	53.603	93.536	1	ROMIZ I12038
	Aquatic net	7 July 2011	53.603	93.536	1	ROMIZ I12147
		11 July 2011	53.602	93.537	4	ROMIZ I12029, I12034, I12035, I12036
		11 July 2011	53.603	93.536	1	ROMIZ I12039
		15 July 2011	53.737	92.763	1	ROMIZ I12050

labial eyes behind the anterior 2 pairs). A single specimen of *Erpobdella dubia* was collected from the Lower Sachigo River ecodistrict; one record was also available from the CMN database, collected from "narrows at source of Shell Brook" (Table 1).

Family Haemopidae

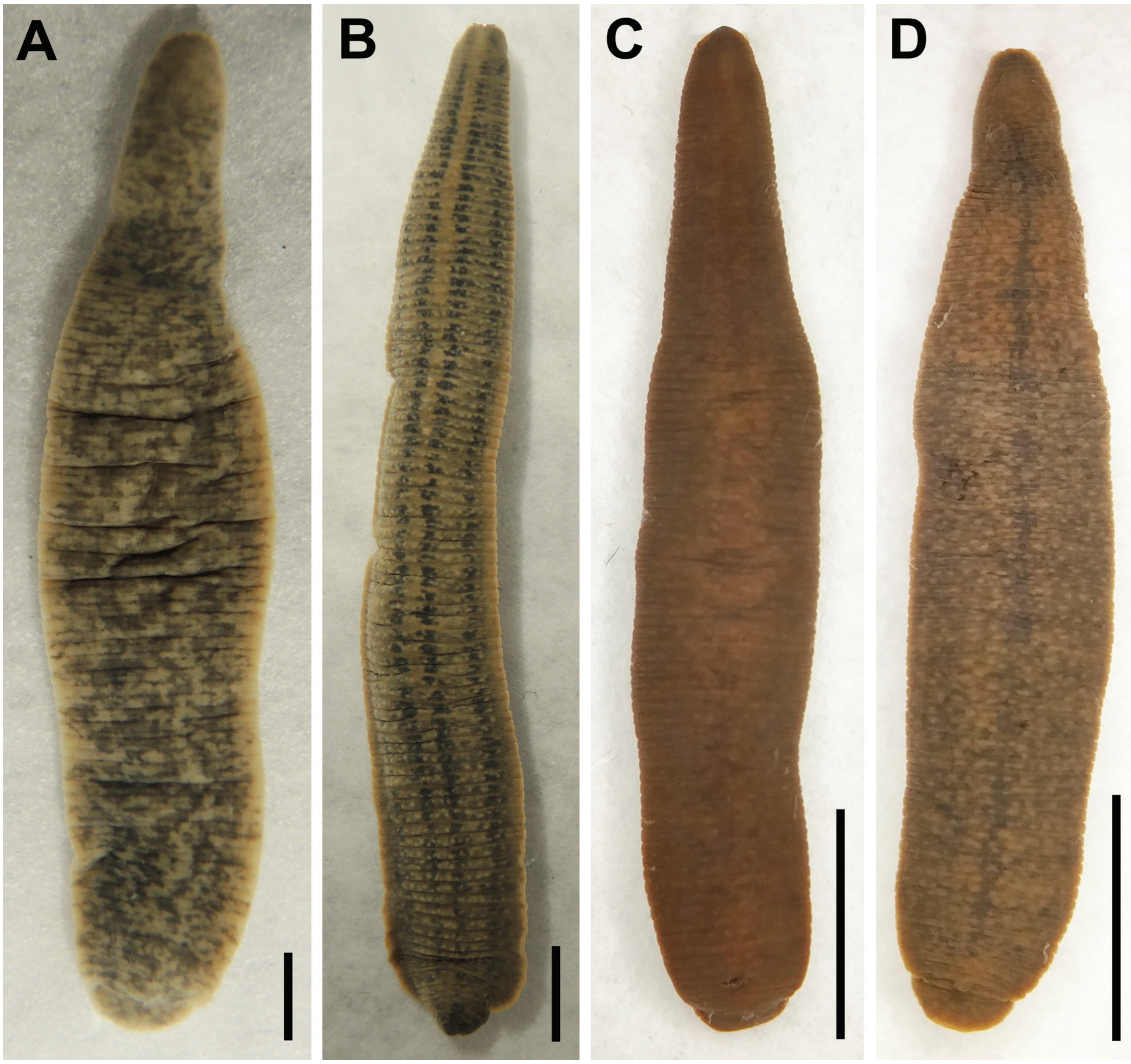
Haemopis grandis (Verrill, 1874)
(Fig. 5A)

REMARKS: Specimens of *Haemopis grandis* are relatively large in size and exhibit a thin margin of the oral sucker, no jaws, 12 pharyngeal folds, and black blotches of dorsal pigmentation (or entirely black background with beige or light green blotches), as is diagnostic for the species (Klemm 1985). A total of four individuals were collected from three different ecodistricts: Wunnummin Lake, Sandy Lake, and Lower Kenogami Lake. Two individuals were recorded from the CMN database, both collected from Fort Albany, 1.5 km west of Albany River (Table 1).

Haemopis marmorata (Say, 1824)
(Fig. 5B)

REMARKS: Specimens of *Haemopis marmorata* are diagnosed by the relatively large body size, the presence of jaws with 12–16 teeth, a

Fig. 4. Dorsal views of select representative specimens of Erpobdellidae from the newly collected material; scale bars represent 0.5 cm. (A) *Erpobdella obscura* (ROMIZ I12051), (B) *Erpobdella punctata* (ROMIZ I12062), (C) *Erpobdella parva* (ROMIZ I12072), and (D) *Erpobdella dubia* (ROMIZ I12101). Colour version online.



caudal sucker that is about half the width of the widest part of the body, and a uniformly grey dorsal colouration with moderate to heavy black, irregular blotching. Superficially, this species resembles *H. grandis* but is distinguished therefrom by the presence of jaws, as well as by the general size of the adult forms: *H. grandis* grows to be almost twice as long as *H. marmorata* (Klemm 1985). *Haemopis marmorata* was the most abundant species among our samples and was represented by 24 individuals from five different ecodistricts: Wunnummin Lake, Lower Sachigo River, Albany River, Winisk River, and Dickey River. Another four records for this species existed in the CMN database and these were collected from the Albany River, Moosonee District 3 km from Partridge River, west of Fort Severn and Moose Factory (see Table 1).

Haemopis cf. *lateromaculata* Mathers, 1963
(Fig. 5C)

REMARKS: A single individual presents morphological characters that suggest an affinity with *Haemopis lateromaculata*. The specimen possesses a green dorsum with heavy dark blotching and few scattered yellow blotches, a darker venter with few indistinct dark or yellow pigmentation blotches, a caudal sucker broadly attached by a pedicel, and jaws with 10–12 teeth. This individual was collected from the Wunnummin Lake ecodistrict (Table 1). In addition, four records exist in the CMN database (identity adopted from Hovingh 2006; the database lists one record without a species identification and the others as *H. marmorata*). These were collected from "North-northeast of east end of Anderson Island in Albany River", "between Mattis and Opazatika River Missinabi River", "North end of lake near outlet Severn Lake", and "between new post M.B.C. and Moose River Abitibi River" (see Table 1). Because of this species' resemblance to *H. marmorata*, this specimen was subjected to DNA sequencing to enable robust molecular identification via DNA barcoding of the COI locus (GenBank accession No. MF706261). The resulting sequence was BLASTed against GenBank's nonredundant (nr.) nucleotide sequence database using a BLASTn protocol. The closest match recovered was to *Haemopis terrestris* (Forbes, 1890), but these sequences still showed 14% difference. When constraining the BLAST search against *H. lateromaculata* (only a single COI sequence available on GenBank), the sequences showed 18% dissimilarity. Moreover, in the tree

Fig. 5. Dorsal views of select representative specimens of Haemopidae from the newly collected material; scale bars represent 1 cm. (A) *Haemopis grandis* (ROMIZ I12001), (B) *Haemopis marmorata* (ROMIZ I12015), and (C) *Haemopis lateromaculata* (ROMIZ I12115). Colour version online.

resulting from the phylogenetic analysis (Supplementary Fig. S1)[1] of the COI region, the new specimen was located on a long branch and did not nest closely with other species, suggesting that this taxon is not otherwise represented in the data set. The identification of our specimen as *Haemopis* cf. *lateromaculata* should therefore be viewed with appropriate caution — it could be that this specimen represents undescribed diversity or, alternatively, that the COI sequence for *H. lateromaculata* in GenBank is mislabelled.

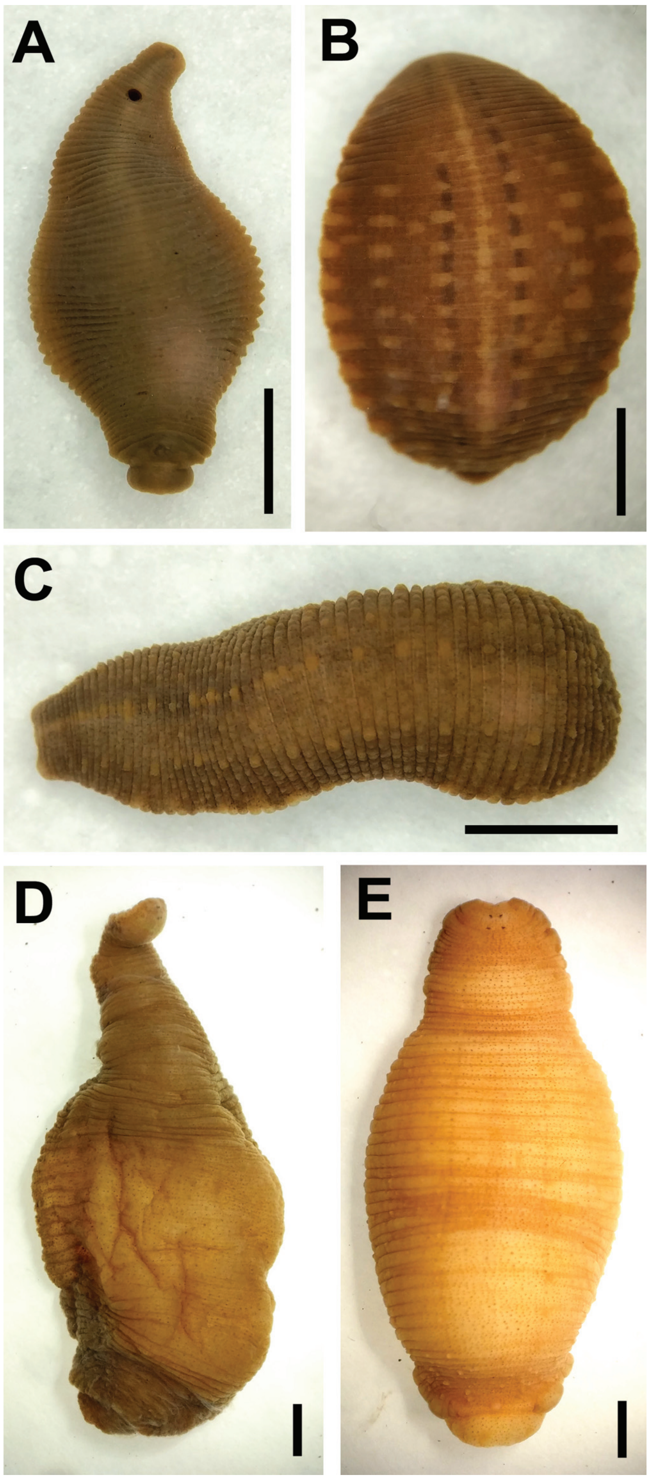
Family Glossiphoniidae

Helobdella stagnalis (Linnaeus, 1758)
(Fig. 6A)

REMARKS: Specimens are small (often <1 cm); possess a dorsal, nuchal, chitinous scute on somite VIII; and are light grey or beige in colour. Much like *Haemopis marmorata*, the abundance of *Helobdella stagnalis* was relatively high compared with that of most other species: 23 individuals were collected from four different ecodistricts (Wunnumin Lake, Sandy Lake, Albany River, and Lower Sachigo River); two records (6 adults and 71 juveniles) for this species are present in the CMN database, both of which were collected from the Attawapiskat River (Table 1). The taxonomic history of this species and its constituents is rather muddled. Historically, individuals bearing a distinct nuchal scute have all been identified as *H. stagnalis* but, concurrently with our growing knowledge of species boundaries, several other nuchal scute-bearing species have been described (e.g., Siddall 2001; Oceguera-Figueroa et al. 2010; Salas-Montiel et al. 2014; Beresic-Perrins et al. 2017), some with geographic distributions overlapping that of *H. stagnalis*. Indeed, the boundaries between

[1]Supplementary Fig. S1 is available with the article through the journal Web site at http://nrcresearchpress.com/doi/suppl/10.1139/cjz-2017-0078.

Fig. 6. Dorsal views of select representative specimens of Glossiphoniidae from the newly collected material; scale bars represent 0.25 cm. (A) *Helobdella stagnalis* (ROMIZ I12030), (B) *Glossiphonia elegans* (ROMIZ I12095), (C) *Placobdella rugosa* (ROMIZ I12037), (D) *Theromyzon rude* (ROMIZ I12018), and (E) *Theromyzon tessulatum* (ROMIZ I12109). Colour version online.



nuchal scute-bearing species are obscured; a thorough revision of the genus is direly needed. All of the specimens examined here show morphological characters that are compatible with those of *H. stagnalis*.

Glossiphonia elegans (Verrill, 1872) Castle, 1900
(Fig. 6B)

REMARKS: Our specimens of *Glossiphonia elegans* possess 3 pairs of eyes arranged in parallel (i.e., not in an arch-like manner); a pair of dark paramedial stripes dorsally and ventrally, interrupted by metameric white spots (sometimes lacking from juvenile individuals); and an opaque body with beige to green or gray colouration. Molecular data have suggested that the North American form of the species commonly identified as *Glossiphonia complanata* (Linnaeus, 1758) may not be conspecific with its European counterpart (Siddall et al. 2005). As a result, Siddall et al. (2005) resurrected the specific epithet *Glossiphonia elegans* to represent the North American form. The specimens were collected from the ecodistricts of Sandy Lake, Albany River, Dickey River, Wunnummin Lake, and Lower Sachigo River; in addition, four records for this species exist in the CMN database, which were collected from Moose Factory, "Winisk 100 metres from east bank of river, 14 km upstream from village", "10 km west of Attawapiskat Monument Channel", and at outlet of Stout Lake (Table 1).

Placobdella rugosa (Verrill, 1874)
(Fig. 6C)

REMARKS: The single specimen of *Placobdella rugosa* possesses heavy dorsal papillation with 5 rows of more prominent papillae, lateral margins with alternating light and dark pigmentation (in the order of light, light, dark, light, light, dark, etc.), and scattered dark chromatophores on the ventral and anterodorsal surfaces. The identification of members of *Placobdella*, particularly the papillated forms, is fraught with difficulty, and the taxonomy of *Placobdella rugosa* is no exception. The species was originally described as a subspecies of *Placobdella ornata* (Verrill, 1872), subsequently elevated to full species status, reassigned to *P. ornata*, and finally resurrected in 2012 (Moser et al. 2012 and references therein). The species is abundant across Canada and is associated with varying climates and faunas (Klemm 1982; de Carle et al. 2017). This particular specimen was collected from the Sandy Lake ecodistrict (Table 1).

Placobdella montifera (Moore, 1906)

REMARKS: Whereas the newly collected material did not include individuals pertaining to *Placobdella montifera*, the collections from CMN do include one record. This species occurs in Ontario and westward across Canada (Davies 1973) and can be distinguished readily by its 3 prominent pointed keels or ridges on the dorsal surface — no other species of *Placobdella* possess this character — and its constricted neck region, resulting in a discoid head. This specimen was collected from Central Patricia Crow River (Table 1).

Theromyzon rude (Baird, 1869)
(Fig. 6D)

REMARKS: A single specimen of *Theromyzon rude* was collected from the Sandy Lake ecodistrict (no species records were available from the CMN database; Table 1). It possesses 2 annuli between gonopores, a gelatinous and slightly transparent body with 2 paramedial rows of brown spots on the dorsum. Members of this genus are typically distinguished from congeners by the number of annuli between gonopores, but Baird (1869) failed to note the number of annuli in his original description of *T. rude*. Based on material collected from the type locality, Moore and Meyer (1951) and Meyer and Moore (1954) re-described the species and noted 3 annuli between gonopores. Later, the original type material was re-examined by Oosthuizen and Davies (1992) who noted that only 2 annuli separated the gonopores; the confused species, with 3 annuli between gonopores, was subsequently described by Davies and Oosthuizen (1993). As a result, much uncertainty has surrounded the taxonomy of the species. The problem was exacerbated by the resulting difficulties in separation of *T. rude* sensu lato and *Theromyzon biannulatum*

Klemm, 1977, both of which possess 2 annuli between gonopores. Notwithstanding that our specimen is compatible with the morphology of *T. rude*, we concede that a taxonomic revision of the group, including molecular techniques, is needed before robustly inferring the identity of any specimen of *Theromyzon* with 2 annuli between gonopores.

Theromyzon tessulatum (O.F. Müller, 1774)
(Fig. 6E)

REMARKS: Specimens display 4 annuli between gonopores and gelatinous bodies with 2 paramedial lines on the dorsum. Four leech individuals (one adult and a brood of three juveniles) were collected in the Lower Sachigo River ecodistrict (no specimen records were available in the CMN database; Table 1). As opposed to the congeneric *T. rude*, this species is distinct and therefore more easily identified by virtue of the 4 annuli separating the gonopores. This species was thought to be confined to Europe, but it is now understood that its distribution is transatlantic (Pawlowski 1948; Herrmann 1970; Davies 1973; Klemm 1982). The origin of this distribution is indeed intriguing, seeing as this species is often encountered attached to, and feeding from, waterfowl, which may explain its spread and current range.

Discussion

Of the 42 species of leeches that were recorded in the last comprehensive study of Canada's hirudofauna (Davies 1973), 33 are nonpiscicolid taxa. Members of Piscicolidae are almost exclusively encountered attached to their fish hosts, but the remaining species can perhaps be more easily collected from rocks and debris. Of these 33 species, 25 species have previously been recorded from Ontario (Davies 1973). In total, 12 species were recovered from our newly collected samples and 1 additional species was recorded in the database of CMN. It is remarkable that, despite the harsh winter climates in northern Ontario (including the area of continuous permafrost on the northern coast), 48% of Ontario's known nonpiscicolid leech diversity is also recovered from the far north of the province. Taken together, the collection sites cover a substantial part of this region and, as far as the authors can tell, 8 of the 12 species records are first reported in the present study (see Hovingh 2006). Some of the species recovered herein are more or less ubiquitous across Canada, but some represent taxa that are only infrequently recovered. Below, we discuss records of particular interest and provide a general discussion on species distributions across North America in general, and Ontario in particular.

Members of Erpobdellidae are considered widespread and some species, e.g., *Erpobdella punctata*, have been described as the most commonly encountered and widely distributed leeches in North America (Sawyer 1970; Klemm 1985; Kutschera and Wirtz 2001), though only three individuals were present among the newly collected samples. In contrast, other species within the genus, such as *Erpobdella parva*, have been recorded relatively infrequently (Klemm 1982); this species is represented by a single specimen among our samples. Given their abundance throughout North America, it is likely that species of *Erpobdella* play important roles in the ecosystems that they inhabit, and it is already known that they represent a significant source of food for several freshwater fish species (e.g., Sinha and Jones 1967; Clady 1974; Davies and Kasserra 1989; Koperski 2006).

Of the haemopid species recovered, *Haemopis grandis* and *H. marmorata* are considered common and widely distributed in North America, with *H. grandis* being especially well represented in the Great Lakes region (Klemm 1982). *Haemopis lateromaculata* has more recently been described as widely distributed in North America (Hovingh 2006). Members of the genus are known to be amphibious (Madill and Hovingh 2007), yet only *Haemopis terrestris* is described as fully terrestrial (Forbes 1890). It is worth noting, however, that the distinction between amphibious and terrestrial is sometimes difficult to accurately identify: many primarily aquatic leeches of the order Arhynchobdellida are known to spend time foraging and depositing cocoons on land immediately surrounding freshwater habitats (Siddall and Burreson 1996). Interestingly, two specimens of an unknown species of *Haemopis* were collected in terrestrial traps, but these specimens do not conform to the morphological description of *Haemopis terrestris.* The specimens lack a median longitudinal stripe and possess jaws with distichous teeth and a relatively narrow caudal sucker, suggesting that they belong to *Haemopis marmorata.* However, because of the terrestrial nature of these collecting events, we have refrained from providing a specific epithet for these individuals. Freshly collected material from which DNA can be extracted and successfully amplified will likely shed light on the identity of these unknown leeches.

Both *Erpobdella* and *Haemopis* are predaceous, with prey items ranging from oligochaetes and insect larvae, to snails, small crustaceans, and dead fish (Sawyer 1972; Barton and Metcalfe 1986; Kutschera and Wirtz 2001; Moser et al. 2006). Moreover, previous expeditions have successfully collected representatives of both genera using aquatic traps baited with beef liver and ground beef (de Carle et al. 2017). Predaceous leeches swallow their prey either whole or in pieces (Kutschera and Wirtz 2001; Pfeiffer et al. 2005; Sket and Trontelj 2008). For the present study, one specimen of a *Haemopis* species was caught in an aquatic trap while feeding on an adult predaceous diving beetle (Dytiscidae: *Dytiscus dauricus* Gebler, 1832) (Fig. 7). A similar observation was made by Bielecki et al. (2011), wherein *Haemopis sanguisuga* (Linnaeus, 1758) was collected in the act of feeding on a predaceous diving beetle (*Dytiscus marginalis* Linnaeus, 1758) larva. Species of *Haemopis* are opportunistic feeders and are frequently found with exoskeletons of larval aquatic insects in their digestive tracts (Moser et al. 2006).

All of the newly collected glossiphoniid species are considered common and widespread (Klemm 1982). *Glossiphonia complanata* (the European counterpart to the North American *G. elegans*; Siddall et al. 2005) and *Helobdella stagnalis* are considered some of the most widely distributed species in Europe (Kutschera and Wirtz 2001) and are also commonly encountered across North America (Klemm 1985). Although *Theromyzon* is considered Holarctic (Mason et al. 2005), members of this genus are less frequently encountered than other glossiphoniids (Klemm 1982). This may be a function of their particular host affinity: members of *Theromyzon* have a propensity to feed on birds (Mason et al. 2005), an association which may also explain why they are so widely distributed across the landscape (Sket and Trontelj 2008).

Glossiphoniids are unique among leeches in that they exhibit a high degree of parental care (Siddall et al. 2005). Leeches of this family produce membranous cocoons — unlike the hardened or spongy cocoons of other leeches — that are attached to the venter of the parent and are carried until the offspring hatch. After hatching, the juvenile leeches remain attached to their parent until being transported to their first meal (Siddall et al. 2005). Members of Glossiphoniidae mainly employ a parasitic lifestyle, feeding on the blood of vertebrates or the haemolymph of invertebrates (Siddall et al. 2005), though some may also eat small prey (Sket and Trontelj 2008). Species of *Placobdella* are primarily ectoparasitic on aquatic reptiles and amphibians, though some are known to feed on fish, and many will feed opportunistically on other vertebrates such as birds and humans (Sawyer 1972; Jones and Woo 1990; Siddall and Gaffney 2004; Siddall and Bowerman 2006; Moser et al. 2014). Oddly, only a single specimen of *Placobdella rugosa* was newly collected and one additional record of *Placobdella montifera* was available in the CMN database. This paucity of *Placobdella* diversity and abundance starkly contrasts that of the southern parts of the province. Indeed, eight species of *Placobdella* are currently known from Ontario (Davies 1973; Klemm 1985; de Carle et al. 2017), some of which are commonly encountered in high densities (de Carle et al. 2017).

Fig. 7. Preserved *Haemopis* sp. feeding on a predaceous diving beetle, *Dytiscus dauricus* (ROMIZ I12122), recovered from an aquatic trap in the Albany River ecodistrict. Colour version online.

Each of the collected families shows widespread distribution in the far north and is seemingly not limited by ecozone, including the area of continuous permafrost on Ontario's northern coast. By and large, the diversity of leeches recorded from the far north of Ontario is lower than the diversity recovered in the southern regions (possibly due to more leech-directed research in southern Ontario), and the species composition of natural populations seems to be somewhat different between the two regions. This may reflect biotic or abiotic barriers to dispersal that are difficult to conquer. Future studies of leech species distributions across Canada that incorporate diversity from northern provinces and territories, as well as northern regions of southern provinces, will likely evince the mechanisms that have produced distributions among these economically and ecologically influential organisms.

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