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Assessment of red macroalgal (Rhodophyta) diversity in Michigan, USA[1]

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Abstract. The state of Michigan is 253,800 km^2 in size with abundant freshwater habitats, including lakes, streams, ponds, and rivers that are ideal for freshwater red algae from the order Batrachospermales. In addition, there are numerous herbarium records, but approximately 60% are more than 20 years old. Recent collections have been from a small geographic area and only a handful of specimens have associated DNA sequences that are often needed for accurate species identification. We surveyed broadly in the Upper and Lower Peninsula, revisiting previously sampled sites and exploring new localities. Freshwater red algae were collected from 19 sites that varied from small streams, rivers, and lake inlet/outlets with a corresponding range in water temperature, pH, and conductivity. Four genera were collected, *Batrachospermum*, *Paludicola*, *Sheathia,* and *Virescentia*, with species identifications verified with *rbc*L gene sequencing. The genus *Sheathia* was the most abundant (15 sites) and represented by two species, *S. grandis* and *S. involuta*. At seven sites, we found *Batrachospermum gelatinosum*, whereas *Paludicola communis* and *Virescentia viride-americana* were each collected once. Although these four genera have been previously known from the state, this study provided the first *rbc*L sequence for *B. gelatinosum* and *P. communis* in Michigan. At one location, we confirmed the presence of *Virescentia viride-americana* 30 years after it had first been collected, and likewise, we confirmed *B. gelatinosum* at another site 27 years later. *Sheathia grandis* was recently described from only two locations in nearby states. This study was the first report in Michigan and added considerably to the number of known locations. This research has yielded new records, species identification with DNA sequence, and expanded habitat information.

Key words: Batrachospermales, biodiversity, freshwater, herbarium specimens, *rbc*L, river, systematics

Freshwater red algae comprise ~3% of the Rhodophyta (Guiry and Guiry 2024). Although freshwater taxa are present in many lineages, two-thirds of the diversity is concentrated in the order Batrachospermales (Vis and Necchi 2021). Most of the 22 genera and ~160 described species have macroscopic gametophytes that are typically 1–10 cm in length (Sheath and Vis 2015). These conspicuous macroalgae are attached to hard substratum in a variety of freshwater habitats including wadable streams, large rivers, ponds, and lakes (Necchi 2016). Ecological and physiological studies suggest that they are primarily shade-adapted and are negatively impacted by nutrient pollution (Abdelahad *et al*. 2015).

Michigan is well-known for being surrounded by the Great Lakes. Within the Great Lakes, the

[1] We thank Bob Anderson and Mike Gretz for providing sampling locations in the Upper Peninsula and Mike Wynne for securing access to the Fleming Creek site within the Matthaei Botanical Garden, University of Michigan. Algae were sampled under permits from the Michigan Department of Natural Resources, the State of Wisconsin (permit numbers SNA22-8 and SNA22-10), the Hiawatha National Forest, and the Forest Preserves of Cook County and Illinois Nature Preserves Commission. This project was funded by a Phycological Society of America Grant in Aid of Research to RMC, Ohio University (OU) Honors Tutorial College Dean's Funding grant to GAL, Huron Mountain Wildlife Foundation award to SJSC and SAKH, startup funds from the University of Alabama at Birmingham (UAB) College of Arts and Sciences to SAKH, and the National Science Foundation (NSF OIA-1946412) to SAKH. SJSC was supported by the NSF Graduate Research Fellowship (GRFP-202095779). SAKH was supported in part by NSF (DEB-2113745) and the Norma J. Lang Early Career Fellowship from the Phycological Society of America. Logistical support was also provided by the Department of Biology at UAB.

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Table 1. Previous literature records of freshwater red macroalgae in Michigan exclusive of the Great Lakes.

Taxon	Reference(s)	*rbc*L GenBank accession numbers
Acrochaetiales		
Audouinella hermannii (as *A. violacea*)	Necchi *et al.* 1993	—
Batrachospermales		
Batrachospermum gelatinosum (as *B. moniliforme*)	Prescott 1962, Vis *et al.* 1996a	—
Lemanea borealis	Vis and Necchi 2021	MZ043869
Lemanea condensata	Vis and Necchi 2021	MZ043872
Lemanea sp. (as *L. fluviatilis*)	Vis and Sheath 1992	—
Paludicola communis	Vis *et al.* 2020a	—
Paludicola turfosa	Vis *et al.* 2020a	MN943943
Paludicola sp. (as *Batrachospermum vagum*)	Prescott 1962	—
Sheathia involuta	Salomaki *et al.* 2014	JX669748
Sheathia sp. (as *Batrachospermum boryanum*, *B. ectocarpum*)	Prescott 1962, Vis *et al.* 1996b	
Virescentia viride-americana (as *Batrachospermum helminthosum*)	Vis *et al.* 2001	AF244109, AF244110

macroscopic red alga *Bangia atropurpurea* (Mertens ex Roth) C. Agardh forms a conspicuous reddish-brown band at the water line on boulders and jetties (Shea *et al.* 2014). In addition, a microscopic red alga, *Chroodactylon ramosum* (Thwaites) Hansgirg, has been frequently reported as part of the epiphytic flora on *Cladophora glomerata* (Linnaeus) Kützing (Vis and Sheath 1993). However, Michigan has abundant other freshwater habitats including lakes, streams, rivers, and marshes from which freshwater red macroalgae are likely to be found.

Although it is probable to encounter freshwater red algae from inland habitats of Michigan, species identification can be challenging. With the most recent revisions of freshwater macroalgae in the Batrachospermales, most genera are distinct and can be readily identified using morphological characteristics (Vis and Necchi 2021). However, species and sometimes genera require DNA sequence data for positive identification because of morphological similarity. For example, *Batrachospermum* species and those of *Sheathia* without heterocortication cannot be distinguished morphologically (Vis *et al.* 2020b). Collecting DNA sequence data for identification can require more effort but can potentially provide biogeographic data and potentially elucidate cryptic species (Chapuis *et al.* 2017).

A review of the literature yielded only a few reports of red macroalgae for Michigan from freshwater habitats, exclusive of the Great Lakes (Table 1). The six genera previously reported, *Batrachospermum*, *Lemanea*, *Paludicola*, *Sheathia,* and *Virescentia* in the Batrachospermales and *Audouinella* in the Acrochaetiales, were documented as part of primarily systematics studies that also provided some *rbc*L sequence data. Additionally, there were collections of "chantransia"—the microscopic sporophytic phase of numerous batrachospermalean species (Chiasson *et al.* 2007).

Michigan has abundant habitats for freshwater red algae as evidenced from the literature and historical collections. New sampling would allow for DNA sequence data to be generated for positive species identification and addition to our knowledge of these species' distributions. Therefore, we initiated the current study with the goal to provide a modern assessment of the freshwater red algal diversity in this region as well as collecting for *Batrachospermum gelatinosum*. We visited previously collected sites, surveyed new potential locations, and evaluated herbarium records as part of the research.

Materials and Methods. We sampled macroscopic freshwater red algae from 19 locations in Michigan during May 2022 (Fig. 1). At each location, we measured water temperature, pH, and conductivity using handheld Oakton meters (Cole-Palmer, Vernon Hills, IL) and current velocity with a General Oceanics Mechanical Flowmeter with 3-inch diameter rotor (General Oceanics, Miami, FL) (Table 2). The stream width and depth were measured as well as the accessible stream length with freshwater red algae. We noted percent canopy cover, water color, and substrate types. We made preliminary identifications of the genera and species in the

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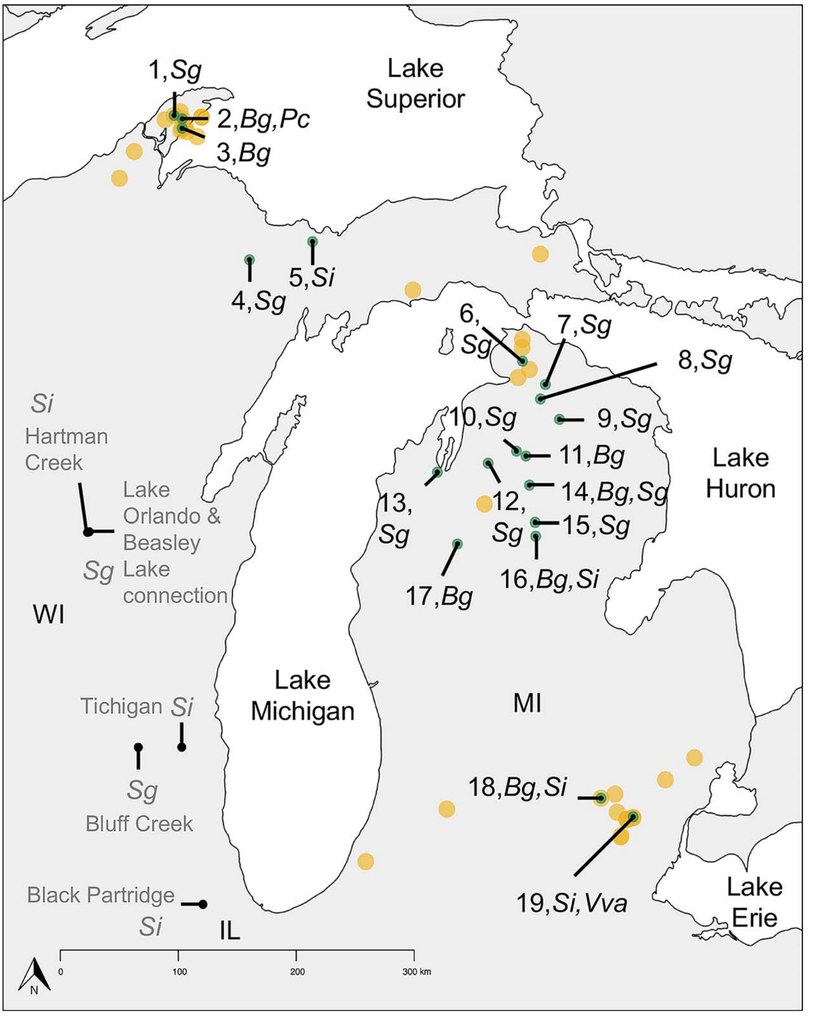

Fig. 1. Map showing sites sampled in Michigan, USA, from which the following species were sampled: *Si, Sheathia involuta*; *Sg*, *Sheathia grandis*; *Bg*, *Batrachospermum gelatinosum*; *Pc*, *Paludicola communis*; *Vva*, *Virescentia viride-americana*. The numbers correspond to the site numbers given in Table 2. Sites with names in gray are from Wisconsin and Illinois, USA, in Appendix 1. Circles without numbers are locations from the herbarium record in Table 3.

field and collected specimens of each taxon. We placed a portion of the thallus in silica desiccant (Flower Drying Art® Silica Gel, Actíva, Marshall, TX) for subsequent DNA extraction and mounted the remaining portion on herbarium paper. Herbarium vouchers were deposited in the Floyd Bartley Herbarium at Ohio University (BHO). In addition to the Michigan sites, we visited and sampled in the same manner four locations in Wisconsin and one location in Illinois, with those data reported as well (Table S1).

For DNA extraction and PCR, two protocols were used. In the first protocol, we ground silica desiccated samples in liquid nitrogen prior to using Nucleospin II Plant Mini kit (Macherey-Nagel, Düren, Germany). The protocol was modified with a longer lysis period of 40 min and elution in 50 μl of molecular grade water instead of elution buffer. PCRs were performed on a total volume of 50 μl, using 2 μl DNA template with 300nM of each primer, 1X buffer, 1.5 mM $MgCl_2$, 200 μM dNTPs, and 1 U Invitrogen *taq* DNA polymerase (ThermoFisher Scientific, Waltham, MA). In the second protocol, we ground the sample with 2.8-mm ceramic beads using BeadMill 24 (Fisher Scientific, Waltham, MA) at a speed of 4.0 m/s for 30-

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Table 2. Collection information and water chemistry data for sites with freshwater red algae. Site numbers correspond to Figure 1. Sites 1–5, 13 collected by SJSC, APO, BMT, and SAKH; 6–19 by RCM, GL, MLV. Ecoregions as defined by Albert (1995).

Site[1]	Ecoregion	Location name	Site type	Latitude, Longitude	Date (2022)	Water temp. (°C)	pH	Specific conductance (μS·cm^{-1})
Upper Peninsula								
1*	IX	Trap Rock River	Stream	47°17′13.5024″N, 88°19′14.0304″W	11 May	7.5	7.0	32.5
2*	IX	Traverse River, Mohawk Gay Road	Stream	47°15′45.306″N, 88°14′13.6428″W	11 May	7.6	5.5	19.2
3*	IX	Traverse River, Big Traverse Road	Stream	47°11′42.5616″N, 88°14′21.21″W	10 May	9.1	7.1	30.9
4	VIII	Chandler Brook	Stream	46°15′37.9044″N, 87°33′20.574″W	13 May	22.1	8.2	258
5	VIII	Rock River	Stream	46°23′23.4492″N, 86°54′53.9712″W	14 May	12.3	8.3	247
Lower Peninsula								
6	VII	West Branch Maple River	Stream	45°32′25.0404″N, 84°47′0.5028″W	13 May	19.0	8.3	340
7	VII	Crumley Creek	Stream	45°22′29.0204″N, 84°33′3.0888″W	12 May	16.8	7.9	370
8	VII	West Branch Sturgeon River	Stream	45°16′17.868″N, 84°36′5.544″W	13 May	14.8	8.5	420
9	VII	Black River	Stream	45°7′36.3″N, 84°24′27.72″W	12 May	19.7	8.4	420
10	VII	Manistee River	Stream	44°54′4.932″N, 84°50′42.576″W	14 May	14.3	8.2	290
11	VII	Kolkee Creek	Lake outflow	44°51′59.364″N, 84°44′54.168″W	14 May	19.1	7.9	300
12	VII	Rapid River	Stream	44°48′55.044″N, 85°7′57.216″W	14 May	16.0	8.6	330
13	VII	Stream crossing Garfield Trail	Stream	44°45′6.4224″N, 85°38′50.2368″W	15 May	10.4	8.3	733
14	VII	Cut River	Stream	44°39′34.992″N, 84°42′47.124″W	12 May	20.8	8.2	320
15*	VII	Au Sable River	Stream	44°23′45.1896″N, 84°39′17.3124″W	12 May	19.4	7.9	250
16	VII	Knappen Creek	Lake Inflow	44°17′54.3732″N, 84°38′57.6348″W	12 May	18.0	7.7	190
17	VII	Cadillac Lake	Lake/Lake Inflow	44°14′37.032″N, 85°26′39.984″W	14 May	24.3	7.1	110
18*	VI	Portage Creek near Hell	Stream	42°26′2.7204″N, 83°59′11.9472″W	11 May	19.5	8.3	500
19*	VI	Fleming Creek	Stream	42°18′7.2″N, 83°39′36.36″W	10 May	20.0	8.2	740

[1] Sites with "*" had previous herbarium records with freshwater red algae; see Table 1.
[2] R = rock, C = cobble, L = logs, S/G = sand/gravel, G = gravel, B = boulder, V = aquatic vegetation, P = pebble, Se = sediment, W = wood.
[3] *S.* = *Sheathia*, *B.* = *Batrachospermum*, *P.* = *Paludicola*, *V.* = *Virescentia*.
[4] BHO = Floyd Bartley Herbarium of Ohio University.

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Table 2. Extended.

Current Velocity ($m \cdot s^{-1}$)	Stream width (m)	Substrate type[2] (%)	Water clarity, color	Water depth (cm)	Canopy cover (%)	Taxon[3]	BHO[4] herbarium number	GenBank accession number
0.4	6.64	B: 75, C: 20, Se: 5	Clear, light brown color	81.5	0	*S. grandis*	A-1890	PP235039, PP235040
0.3	3.32	C: 80, Se: 10, P: 10	Clear, dark brown color	66	0	*P. communis*, *B. gelatinosum*	A-1886 A-1887	PP235028, PP235029, PP235030, PP235031
0.5	7.74	C: 50 Se: 50	Clear, dark brown color	54	5	*B. gelatinosum*	A-1889	PP235038
0.1	5.7	S: 30, C: 30, P: 30, Se: 10	Clear, colorless	28	28	*S. grandis*	A-1892	PP235047
0.4	6.77	S: 10, R: 50, C: 40	Clear, colorless	43.5		*S. involuta*	A-1894	PP235050, PP235051
1.0	9.6	W: 1, R: 70, S/G: 30	Clear, colorless	28.5	0	*S. grandis*	A-1831	PP235023
0.2	2.4 to pipe, 5.4 total	R: 40, S/G: 40, W: 20	Clear, colorless	26	0	*S. grandis*	A-1829	PP235021
1.05	8	B: 5, R: 60, C: 19, S: 15, W: 1	Clear, colorless	20	<5	*S. grandis*	A-1822	PP235015
0.6	8.5	C/R: 75, S/G: 19, B:5, W:1	Clear, colorless	17	0	*S. grandis*	A-1830	PP235022
0.4	6	Upstream R: 60, S/G: 30, W: 10 Downstream G: 60, S: 30, C: 5, W: 5	Clear, colorless	90	0	*S. grandis*	A-1836	PP235026
0.56	3	R: 40, C:3 0, S: 10	Clear, colorless	32	0	*B. gelatinosum*	A-1833	PP235025
0.46	6	R:50, C: 49, W: 1	Clear, colorless	80	0	*S. grandis*	A-1837	PP235027
—	—	—	Clear, colorless	—	—	*S. grandis*	A-1895	PP235052
0.11	12-15	S/G: 98, R: 1, W: 1	Clear, colorless	95	0	*B. gelatinosum*, *S. grandis*	A-1823, A-1824	PP235016, PP235017
Only surface flow	12	R: 40, S/G: 50, C: 10	Clear, colorless	54	0	*S. grandis*	A-1825	PP235018
Below detectable	3.4	S/G: 90, W: 1, R: 1, V: 8	Clear, light brown color			*B. gelatinosum*, *S. involuta*	A-1826, A-1827	PP235019, PP235020
Waves	2.1-3.6	S/G: 97, B:3	Clear, light brown color	44	30	*B. gelatinosum*	A-1832	PP235024
0.85	8-10	C: 20, G/S: 80	Clear, colorless	57	10	*B. gelatinosum*, *S. involuta*	A-1819, A-1818	PP235013, PP235014
0.75	6	R: 5, C: 50, G:40, L: 5	Clear, colorless	37	0	*S. involuta*, *V. viride-americana*	A-1816, A-1817	PP235011, PP235012

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sec pulses until homogenized. The Nucleospin II Plant Mini kit protocol was modified with a lysis period of 1 hr at room temperature. PCRs were performed on a total volume of 20 µl, using 2 µl DNA template with 300nM of each primer, 1X buffer, 1.5 mM $MgCl_2$, 200 µM dNTPs, and 1 U Promega GoTaq DNA polymerase (Promega, Madison, WI). For all samples, a 1282 bp fragment of the *rbc*L gene was amplified using the primers F160 (5′-CCT CAA CCA GGA GTA GAT CC-3′) and *rbc*LR (5′-ACA TTT GCT GTT GGA GTC TC-3′). The PCR cycle included an initial denaturation step at 95 °C for 1 min, then 35 cycles of 93 °C for 30 sec, 50 °C for 30 sec, and 72 °C for 1 min, and a final extension of 72 °C for 10 min. In the first protocol, PCR products were purified using the PureLink™ Quick PCR Purification kit (ThermoFisher Cat #: K310001). In the second protocol, PCR products were purified using ExoSAP-IT (ThermoFisher Cat #: A35005). For sequencing, a combination of two internal primers were used so that the entire 1282 bp fragment was obtained. The forward primer F650 (5′-ATT AAC TCT CAA CCA TTT ATG CG-3′) was used for *Batrachospermum*, *Sheathia,* and *Virescentia,* and F650.wbc1 (5′-ATTAATTCACAGCCATTTATGCG-3′) was used for *Paludicola*. The reverse primer R897 (5′-CGT GAG TAT GTT GAA TTA CCA GC-3′) was used for *Virescentia* and *Paludicola,* R897.1 (5′-CGT GAG TAT GTT GAA TTA CCA GC-3′) for *Sheathia*, and R897.3 (5′-CGT GAA TAT GTA GAG TTA CCT GC-3′) for *Batrachospermum*. Commercial sequencing was conducted at Eton Bioscience (Union, NJ) and Eurofins Genomics (Louisville, KY). Sequences were visualized and edited using Geneious Prime 2022.1 (https://www.geneious.com). Sequences obtained were compared to previously published sequences on GenBank using a BLAST, and these new sequences are available from GenBank (Benson *et al.* 2017; Table 2).

To place the new sequence data for species in context, they were compared to the previous data in GenBank. For *B. gelatinosum*, *Paludicola communis*, and *V. viride-americana*, all *rbc*L sequence data in GenBank were downloaded and genetic differences among sequence data assessed. For *Sheathia grandis* and *S. involuta*, the same procedure was followed, and in addition, a phylogenetic tree was produced to visualize the relationships among specimens. Only sequences that were 1282 bp or greater for *S. grandis*, *S. involuta,* and closely related species *S. americana*, *S. boryana*, *S. confusa,* and *S. heterocortica* were utilized for alignments. All duplicate sequences were removed before conducting phylogenetic analyses in Geneious. The final alignment of 38 *Sheathia* sequences was analyzed using the maximum likelihood program RaxML with the nucleotide model of GTR GAMMA and bootstrap support determined by 1000 replicates as implemented in Geneious.

To place the finding from this study in a broader context, we searched for records in the literature as well as for herbarium specimens. For the literature search, we examined recent literature and the references therein as well as a search of Google Scholar® with the terms: Michigan, flora, and algae (Table 1). For herbarium specimens, we queried the Macroalgal Portal (macroalgae.org/portal/) using freshwater macroalgal genera names as key words. After downloading the results into an Excel spreadsheet, all results were sorted by location, date, and collector to determine duplicate specimens for a location (Table 3). When latitude and longitude data were available, they were plotted on a map (Fig. 1). All digital specimens were examined and identified to genus or species, when possible. Taxonomy was updated to reflect currently recognized names. We also included all specimens from BHO.

Results. A search of herbarium records yielded six genera, *Batrachospermum*, *Lemanea*, *Paludicola*, *Sheathia,* and *Virescentia* in the Batrachospermales, and *Audouinella* in the Acrochaetiales. In addition, there was one specimen that could be identified only to genus and with the morphological characters present could represent either *Kumanoa* or *Volatus* (Table 3). For many of the records, herbarium-supplied geographic coordinates were mapped for visualization (Fig. 1). Most records were clustered in the southeastern part of the state, at the apex of the Lower Peninsula and in the Upper Peninsula's Keweenawan region.

The 19 sites we sampled included five in the Upper Peninsula and 12 in the lower (Table 2). These sites represent all four ecoregions numbered six through nine as defined by Albert (1995). Most sites were streams (16 sites), but three were associated with lakes (Figs. 2–4). The streams ranged from 2.1 to 15.0 m in width with a similar wide variation in depth (20–90 cm) and current velocity (< 0.10–$1.05\ m \cdot s^{-1}$). Water temperature varied greatly with most sites in the Upper Peninsula ~ 7

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Table 3. Herbarium records of freshwater red macroalgae in Michigan exclusive of the Great Lakes. Procedure for compiling records in Materials and Methods section. Locations revisited in May 2022 are in bold.

Taxon[1]	Name on herbarium label	Location	Ecoregion	Latitude, Longitude[2]	Collection date	Herbarium voucher information[3]
Audouinella hermannii	*A. violacea*	Benton Harbor	VI	41°59′4.7724″N, 86°22′26.4822″W	17 April 1963	BUT 503013
A. hermannii	*A. violacea*	Oden Fish Hatchery	VII	45°25′25.0392″N, 84°49′41.1672″W	4 Aug. 1941	OSC-A-008434
A. hermannii	*A. violacea*	Carp River	VII	45°41′40.545″N, 84°47′6.864″W	14 Aug. 1941	OSC-A-008436
Batrachospermum gelatinosum	*B. moniliforme*	Tobin Harbor, Moose Lake	IX	48°8′34.0614″N, 88°30′45.7272″W	16 July 1930, 22 July 1930	MICH 642837, 642838, 642867, 642869
B. gelatinosum	*B. moniliforme*	Pickerel Cove, Moose Lake	IX	48°7′43.554″N, 88°38′16.2564″W	9 Aug. 1930	MICH 642839, 642868
B. gelatinosum		**Traverse River at Big Traverse Road**	IX	**47°11′44.4984″N, 88°14′23.3592″W**	7 Aug. 2010	BHO A-0066, 0067, 0068
B. gelatinosum		**Traverse River at Mohawk Gay Road**	IX	**47°15′45″N, 88°14′13.599″W**	7 Aug. 2010	BHO A-0069
B. gelatinosum		**Trap Rock River**	IX	**47°17′13.3002″N, 88°19′14.001″W**	7 Aug. 2010	BHO A-0070, 0071
B. gelatinosum		Snow Mobile Trail	IX	47°18′42.9978″N, 88°15′24.9984″W	16 May 2021	BHO A-1732
B. gelatinosum	*B. gelatinosum*	**Hell Creek**	VI	42°26′2.7204″N, 83°59′11.9472″W	15 April 1995	MICH 642895
B. gelatinosum	*B. pyramidale*	Lakeland	VI	42°27′48.1248″N, 83°50′38.799″W	28 May 1922	MICH 642926
B. gelatinosum	*B. moniliforme* or *B. ectocarpum*	East of Levering	VII	45°38′9.0378″N, 84°47′13.1784″W	8 July 1940, 14 July 1942	F C0504326F, C0504431F; OSC-A-008435, 008438; NY 03559834
B. gelatinosum	*B. moniliforme*	Trout Pond	VII	—	May 1964	NY 02143535
B. gelatinosum	*Batrachospermum* sp.	Chippewa	VIII	46°18′1.9542″N, 84°36′8.7474″W	16 Aug. 1941	OSC-A-000951
Batrachospermum sp. or *Sheathia* sp.[4]	*Batrachospermum* sp.	Maple River	VII	45°28′52.0386″N, 84°42′44.154″W	22 July 1941	OSC-A-000950
Batrachospermum sp. or *Sheathia* sp.	*Batrachospermum* sp.	Near Douglas Lake	VII	—	21 July 1947	TENN-A-002903

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Table 3. Continued.

Taxon[1]	Name on herbarium label	Location	Ecoregion	Latitude, Longitude[2]	Collection date	Herbarium voucher information[3]
Chantransia (sporophyte stage)	*B. boryanum*	Huron River	VI	42°17′1.8996″N, 83°43′26.1834″W	Nov. 1893	MICH 642770, 642791; ABRU00002805, UC1836668, MU 210368, YU.106453, NY 02341299, 02266397, 02266402; MSC0184693
Chantransia		Ann Arbor	VI	42°17′1.8996″N, 83°43′26.1834″W	20 Oct. 1892, 21 Oct. 1892	F C0504609F-C0504611F; OSC-A-001241, 001242; NY 03596812-03596814
Kumanoa sp. or *Volatus* sp.[5]		Big Manistee River	VII	44°31′33.5562″N, 85°10′10.8264″W	1 Sept. 2013	BHO A-1076
Lemanea borealis		Tobacco River	IX	47°8′7.0692″N, 88°5′8.0226″W	6 June 2020	BHO A-1686
L. condensata		Silver River	IX	47°16′28.8222″N, 88°2′31.6746″W	1 June 2020	BHO A-1685
Lemanea sp.		Gratiot River	IX	47°12′11.7966″N, 88°12′12.6282″W	21 May 2020, 31 May 2020, 1 June 2020,	BHO A-1684, 1687-1689
Lemanea sp.		Tahquamenon River	VIII	—	27 Aug. 1956	PH00219163, 00219221
Paludicola communis		Horseshoe Lake	IX	46°50′22.9992″N, 88°52′28.9992″W	5 Aug. 2010	BHO A-182, 183
P. communis		Deer Lake	IX	47°10′43.3992″N, 88°15′11.0016″W	7 Aug. 2010	BHO A-196, 197
Paludicola sp.		Scale Creek	IX	47°15′20.0988″N, 88°24′50.1984″W	7 Aug. 2010	BHO A-0193
P. turfosa		Perrault Bog	IX	47°1′48.1008″N, 88°43′30.4998″W	5 Aug. 2010	BHO A-194, 195
Sheathia involuta		Silver River	IX	47°16′8.8222″N, 88°2′31.6746″W	1 June 2020	BHO A-1693, 1694
S. involuta		**Fleming Creek**	VI	**42°17′45.5202″N, 83°39′39.942″W**	12 Feb 2011, 27 March 2011	BHO A-0400-b, 438, 444; MICH 642812
Sheathia sp.		Trap Rock River Site 2	IX	47°10′16.9494″N, 88°11′29.04″W	6 June 2020	BHO A-1691
Sheathia sp.	*B. boryanum*	Walnut Lake	VI	42°33′53.7978″N, 83°20′0.1386″W	1906	NY 02266439
Sheathia sp.	*B. boryanum*	Macomb County	VI	42°43′28.1136″N, 83°2′9.7506″W	12 April 1932	NY 02266427

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Table 3. Continued.

Taxon[1]	Name on herbarium label	Location	Ecoregion	Latitude, Longitude[2]	Collection date	Herbarium voucher information[3]
Sheathia sp.	*B. anatinum*	Huron River	VI	42°16′58.242″N, 83°43′28.6566″W	22 April 1932	MICH 642619-642621, 642625
Sheathia sp.	*B. boryanum* or *Batrachospermum* sp.	Ann Arbor	VI	—	3 March 1893	MICH 642753; UC95492; MSC0181318; NY 02266438
Sheathia sp.	*B. anatinum*	Huron River	VI	—	21 May 1932	MICH 642617, 642623
Sheathia sp.	*B. anatinum*	Loch Alpine	VI	42°20′5.7186″N, 83°49′23.1708″W	22 April 1932	MICH 642618, 642622
Sheathia sp.	*B. boryanum*	**Saline River**	VI	**42°9′31.4352″N, 83°47′17.7354″W**	16 April 1953	MICH 642810, 642813
Sheathia sp.	*B. moniliforme*	Near Saline	VI	42°10′0.012″N, 83°46′54.012″W	3 May	LSU00169328, UVMVT305268
Sheathia sp.		Vermillion point	VIII	46°2′44.6748″N, 85°53′45.6″W	1 July 2013	BHO A-1413
Virescentia viride-americana	*B. helminthosum*	Spring Brook	VI	42°21′23.9″N, 85°33′04.0″W	22 May 1998	MICH 642688
V. viride-americana		Sycamore Creek	VI	—	12 July 2009	BHO A-0121
V. viride-americana	*B. helminthosum*	**Fleming Creek**	VI	**42°17′45.5202″N, 83°39′39.942″W**	12 Feb. 2011, 27 Mar. 2011, 30 May 2000, 17 Oct 1992	BHO A-0400-a, 0439, 0445, 1473; MICH 642684-642686,

[1] When possible, specimen was identified to species based on examination of herbarium scan or DNA sequence data available.

[2] Latitude and longitude may be approximate as location data often has to be estimated from descriptions on older herbarium sheets.

[3] Herbarium abbreviations as follows: ABRU = Brown University – Algae; BHO = Floyd Bartley Herbarium of Ohio University; BUT = Butler University; F = Field Museum of Natural History; MICH = University of Michigan; MSC = Michigan State University Algae; MU = Miami University – Algae; NY = New York Botanical Garden; OSC = Oregon State University; PH = Drexel University, Academy of Natural Sciences; TENN = University of Tennessee Herbarium; UC = University Herbarium, University of California, Berkeley; UVMVT = University of Vermont, Pringle Herbarium, Algae Collection; YU = Yale University Herbarium.

[4] Potentially either *Batrachospermum* or *Sheathia* based on examination of herbarium scan.

[5] Potentially either *Kumanoa* or *Volatus* based on morphology; species might be *V. ulterior* or *K. faroensis*; DNA sequence data needed for positive identification.

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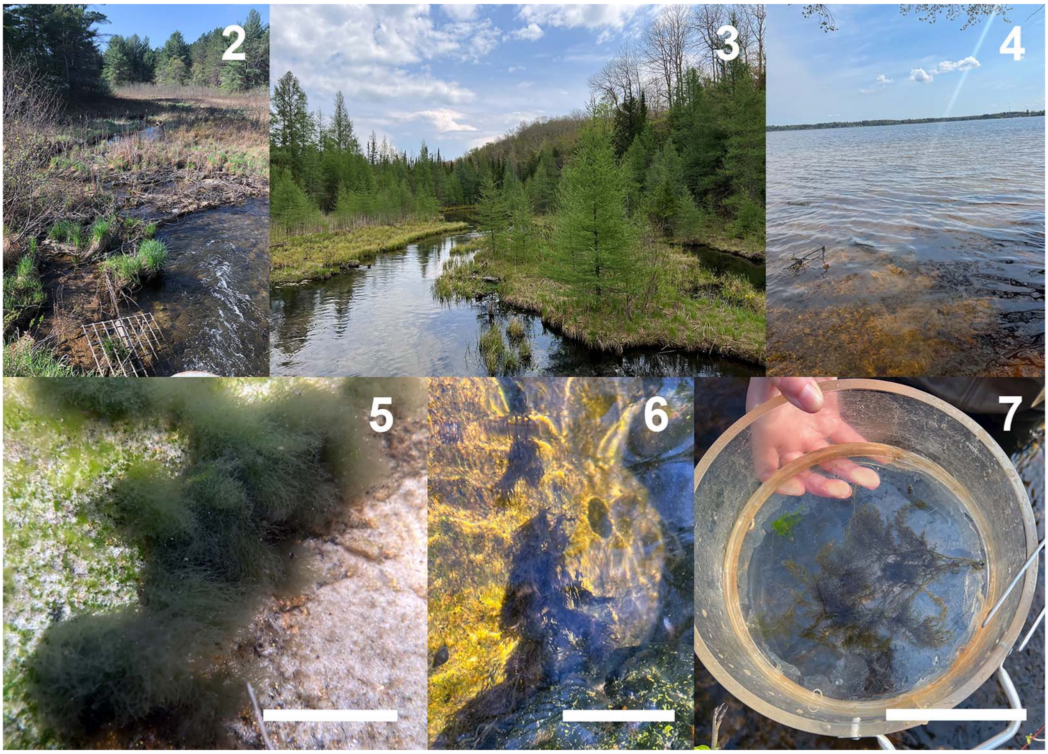

PLATE 1. Representative sites from which freshwater red algae were collected and in situ photographs of three commonly encountered taxa. Site numbers as in Figure 1 and Table 3.

FIG. 2. Kolkee Creek (Site 11), a lake outflow that meandered through primarily wetland vegetation. Photo by RMC.

FIG. 3. Rapid River (Site 12), a large open canopy stream with moderate flow. Photo by GAL.

FIG. 4. Cadillac Lake (Site 17), a lake edge with moderate water motion from waves and a few isolated boulders. Photo by RMC.

FIG. 5. *Batrachospermum gelatinosum in situ* showing crowded dark green gametophytes adhering to large rock. Scale bar = 3 cm. Photo by RMC.

FIG. 6. *Sheathia grandis in situ* showing uncrowded brownish-red gametophytes attached to large rock. Scale bar = 5 cm. Photo by GAL.

FIG. 7. *Sheathia involuta* individual brownish-red gametophytes in a view box. Scale bar = 10 cm. Photo by GAL.

°C to a shallow lake shore at 24 °C (Table 2). All sites had neutral to basic pH (7.0–8.6), except stream 2, which was acidic (5.5). Specific conductance ranged from 19.2 to 733 $\mu S \cdot cm^{-1}$, but most streams were between 110 and 500 $\mu S \cdot cm^{-1}$ with the three from ecoregion IX being uniformly low (19.2–32.5 $\mu S.cm^{-1}$) (Table 2). Most sites had clear, colorless water but some were light or dark brown colored; none were turbid. The stream beds were composed of a variety of substrates, but all had hard substrate (Table 2). All sites had little to no canopy cover (0–30%).

In total, we made 24 collections of macroscopic freshwater red algae from the 19 sites (Table 2). We identified these collections using morphology as representing four genera: *Batrachospermum*, *Paludicola*, *Sheathia* and *Virescentia*. Using the DNA sequence data, we confirmed that all specimens belonged to these genera and determined that five species were collected as follows: *B. gelatinosum* (Fig. 5), *P. communis*, *S. grandis* (Fig. 6), *S. involuta* (Fig. 7), and *Virescentia viride-americana*. *Paludicola communis* was observed in a single location in the Upper Peninsula, and likewise *V. viride-americana* in a single location in the Lower Peninsula. *Sheathia* was collected in 15 of the 19 sites, with *S. grandis* in 11 sites and *S. involuta* in four. Likewise,

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we encountered *B. gelatinosum* in seven sites. In addition, these three species were in both the Upper and Lower Peninsulas (Table 2). At five sites, we collected two species that were growing together. *Batrachospermum gelatinosum* was in four of those sites but growing with either *P. communis*, *S. grandis,* or *S. involuta*. In the fifth site, we collected *S. involuta* and *V. viride-americana*.

We generated DNA sequence data for all taxa encountered and for each taxon at a location. The nine sequences of *B. gelatinosum*, three from one site and one each from the remaining six locations, were identical to each other. There were six other sequences in GenBank that could be traced to locations in North America (Appendix 2). These sequences from Kentucky and Massachusetts, USA, as well as Alberta and Nova Scotia, Canada, were identical or 1 bp different from the sequences in this study. In addition, the Michigan and other North American sequences had high identity (> 98%) to numerous previously published sequences of specimens from Europe and Japan (Appendix 2). A single sequence of *P. communis* was generated. Of the 10 previously published sequences on GenBank, eight were from North America, ranging latitudinally from Maine, USA, to Costa Rica, and two were from French Guiana in northern South America (Appendix 2). Among the 11 sequences, there were few bp differences (0–4 bp) with the new sequence being identical to six of the previous ones from throughout the range (Minnesota, New York, New Hampshire, South Carolina, USA, and French Guiana). We generated a sequence for *V. viride-americana* from the single site at which we collected this species. The sequence was identical to a previously published one from the same site collected in 1999 and 1 bp different from another Michigan site (Appendix 2). Within species variation ranges from 0 to 18 bp and other studies have shown a clade of sequences from Michigan and Tennessee that differ from the other sequences throughout the species range in eastern North America (Vis *et al.* 2001) (Appendix 2).

For the *Sheathia* spp., a total of 20 sequences of *S. grandis* and 31 sequences of *S. involuta* were generated in this study (Table 2, Appendix 1). For *S. grandis*, there were a total of 12 sequences, one each from 10 sites and two from one site in Michigan; there were two Wisconsin sites, one with two sequences and one with six, for a total of eight sequences. These sequences were partitioned into three haplotypes. For *S. involuta*, there were five sequences from four Michigan sites, 24 sequences from two Wisconsin sites, and two sequences from one Illinois site (Table 2, Appendix 1). Of the 31 sequences, 30 were identical to each other and one represented a second haplotype. Interestingly, the two haplotypes were represented from a single site in Michigan, Rock River (Table 2).

Given that multiple haplotypes were observed for the two *Sheathia* spp., a phylogeny was produced to examine geographic patterns (Fig. 8). There were five *S. grandis* haplotypes in total from Michigan, Ohio, New York, and Wisconsin, which were variously related, but there was no statical support for the relationships among the haplotypes (Table 2, Appendix 1, 3). For *S. involuta*, there were 10 haplotypes that formed a well-supported clade (Fig. 8). There was high bootstrap support (100) for two haplotypes from Texas and moderate support (88) for four haplotypes from Alabama, Tennessee, and Virginia (Appendix 3). Our newly generated sequences from Michigan, Wisconsin, and Illinois were associated with samples from the Midwest, including Ohio, Minnesota, Indiana, but additionally more western sites in Oklahoma and Arizona (Fig. 8).

Discussion. We sampled freshwater red algae with a focus on Michigan as well as sampling several other sites in Illinois and Wisconsin. We revisited some locations with previous herbarium records. The three locations in the Upper Peninsula were more recent records (2010) and we found gametophytes again at these sites. However, we noted that there were fewer, smaller specimens and attribute these observations potentially to seasonality, as we collected in May and previous collections were made in August. We collected *V. viride-americana* at Fleming Creek and *B. gelatinosum* at Hell Creek in the Lower Peninsula for which the herbarium records dated back to 1992 and 1995, respectively. Both sites had large numbers of gametophytes and indicate the habitat after ~ 30 years is still suitable even though the streams are adjacent to developed areas. There were two herbarium specimens collected in April 1953 from a park through which the Saline River runs, but no red algae were observed when we visited in May. The lack of gametophytes may have been because of timing of the visit but may be more likely because of changes in habitat quality,

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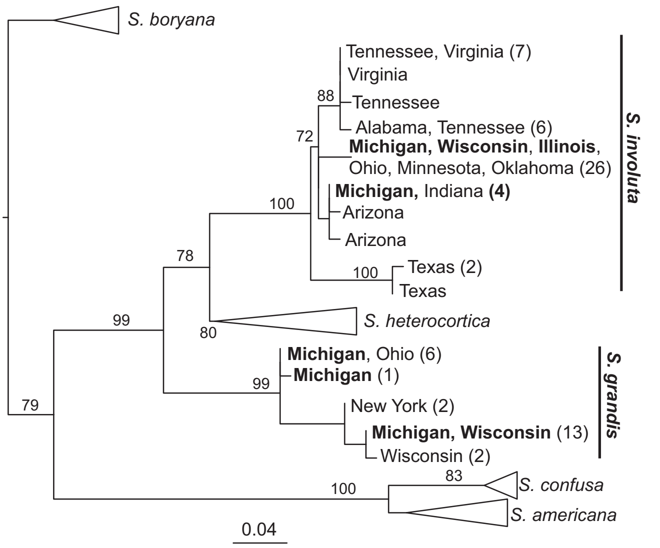

FIG. 8. Maximum likelihood phylogeny showing the relationship of the haplotypes for *Sheathia grandis* and *S. involuta* in relation to each other and the other heterocorticate *Sheathia* species. Bootstrap support values of > 70 shown. Numbers in parentheses are the number of identical sequences represented for the branch (Appendix 3).

as the current was slow and much of the substrate was covered in cyanobacterial mat. Freshwater red algae can often persist at a location if the habitat quality remains consistent but may be extirpated from a location if physical or chemical parameters change (M. L. Vis, pers. ob.).

Based on the previous literature and herbarium records, the genus *Sheathia* sp. has been widely collected in Michigan. The same was true for our current survey. However, we were able to identify two species of *Sheathia* using DNA sequence data. *Sheathia involuta* has a wide known geographic distribution from Arizona and Texas in the Southwest and numerous states in the midwestern and southern US, including Minnesota, Michigan, Indiana, Ohio, Tennessee, Virginia, and Alabama (Salomaki *et al.* 2014). Even though this species has been reported from the state, it had only been verified using DNA data from one location such that we have now added more locations. It is widespread in the state and present in both the Lower and Upper Peninsulas. In addition, we recorded *S. involuta* from new locations in Wisconsin and Illinois, both of which were previously unknown but likely to occur given the range of this species. *Sheathia grandis* was only recently described and was represented by specimens from a location in Wisconsin and one in Ohio (Salomaki *et al.* 2014). In the present research, we showed it has a wider distribution, including more sites in Wisconsin and 11 sites in the Upper and Lower Peninsulas. Moreover, sequence data paired with herbarium specimens from upper New York state expand the overall distribution of *S. grandis* to Wisconsin, Michigan, Ohio, and New York. In the present study, we discovered a recording error for *S. grandis* at Tichigan Creek, Wisconsin. The specimens labeled as WI0201 and WI0202 in Salomaki *et al*. (2014) were from a nearby location, Scuppernong River, and not Tichigan Creek as originally reported. Therefore, the current finding of *S. involuta* at this location as well as another Wisconsin stream confirms both *Sheathia* species in Wisconsin as well as Michigan.

Batrachospermum gelatinosum, like *Sheathia*, has been widely collected in the state based on previous records and the present survey. This finding is in accord with it being the most widespread freshwater red alga in North America (Sheath and Cole 1992). It has been recorded from streams with a wide range of physical and chemical parameters, including pH 4.8–8.5, specific conductivity 10–490 $\mu S \cdot cm^{-1}$, and water temperature 0–27 °C (Vis *et al.* 1996a). Michigan is geographically in the middle of its range in North America, and the sites we sampled had water chemistry well within the previously reported numbers.

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We collected *P. communis* from one location in the Upper Peninsula growing with *B. gelatinosum*. This locale was one of the previous sites from herbarium data, but only *B. gelatinosum* had been collected. Potentially, this taxon was overlooked in the earlier collection. The genus *Paludicola*, including the species *P. communis* and *P. turfosa*, has been recorded before in nearby locations (Vis *et al.* 2020a). This genus tends to inhabit edges of bogs and lakes as well as streams with acidic to slightly acid waters, low conductivity, and dark brown waters (Vis and Necchi 2021 and references therein). The site we collected has those attributes and was the only one in the survey with acidic pH (5.5) such that locations with the specific habitat for the genus are potentially less common in the state. The species *P. communis*, based on sequence data, is known from a wide geographic range that includes eastern North America and northern South America (Vis and Necchi 2021).

We report *V. viride-americana* from a site in which it was previously collected. There were only two other herbarium records from streams in southern Michigan. This is a widespread species in eastern North America, known from streams in the Southeast to the northern streams of Ohio, Tennessee, and Virginia, as well as New England, including Connecticut, Massachusetts, and Rhode Island (Vis and Necchi 2021). It is often quite abundant during the spring and early summer based on literature reports and herbarium records. It was somewhat surprising that it was not encountered more often, but perhaps Michigan is at the northern edge of its range.

The red macroalgal flora of Michigan, including the historical records and new survey, includes seven genera with nine confirmed species. Although few similar studies are available, we can compare our data with a survey of the bordering province of Ontario, Canada (Sheath and Hymes 1980). With updated taxonomy, *Audouinella*, *B. gelatinosum*, *Sheathia* sp., and *Lemanea* sp. are in common, but the macroalgae *Boldia* (Compsopogonales) and *Tuomeya* and *Sirodotia* (Batrachospermales) are unique to Ontario. These three taxa have a more eastern distribution, which may explain their absence from Michigan (Vis and Necchi 2021). Likewise, the Michigan flora contain two taxa absent in Ontario, *Paludicola* and *Virescentia*, with the potential for a third depending on the identity of the herbarium specimen that was either *Kumanoa* or *Volatus* based on morphology. The North American flora of Batrachospermales includes 13 genera, of which six have now been reported from Michigan. It is likely that the generic diversity of Michigan will not grow, as the other genera are unlikely to occur there because of their geographic distributions. However, sequence data has uncovered multiple species within genera, and with more sequencing that number will potentially grow.

Macroalgal surveys coupled with a search of herbarium records, such as this study, are critical to documenting algal diversity in a changing climate. With the recent focus on freshwater red algal systematics, we have made progress in determining the biogeography of genera and species. However, our knowledge of freshwater red algal distributions is still not as well developed as that of their marine counterparts, rendering it more challenging to explore the effects of increasing temperatures and changing phenological patterns.

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Appendices

Appendix 1. Collection information and water chemistry data for sites with *Sheathia* from Illinois (IL) and Wisconsin (WI). Site collected by SJSC, APO, BMT, and SAKH.

Location name	Site type	Latitude, longitude	Date (2022)	Water temp. (°C)	pH	Specific conductance ($\mu S \cdot cm^{-1}$)	Current velocity ($m \cdot s^{-1}$)
Black Partridge, IL	Stream	41°40′52.0212″N, 88°1′40.0218″W	8 May	8.4	8.5	1540	0.5
Tichigan Creek, WI	Stream	42°48′0.8382″N, 88°14′34.116″W	8 May	10.4	8.2	870	0.1
Bluff Creek, WI	Stream	42°47′56.331″N, 88°41′3.0042″W	8 May	10.1	8.4	818	0.1
Hartmann Creek, WI	Stream	44°19′34.41″N, 89°12′1.0116″W	9 May	13.8	9.1	379	0.2
Between Lake Orlando & Beasley Lake, WI	Strait	44°19′48.7914″N, 89°10′57.3456″W	9 May	12.0	8.8	405	0.1

[1] R = rock, C = cobble, L = logs, S/G = sand/gravel, G = gravel, B = boulder, V = aquatic vegetation, P = pebble, Se = sediment.

[2] BHO = Floyd Bartley Herbarium of Ohio University.

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Appendix 1. Extended.

Stream width (m)	Substrate type[1] (%)	Water clarity, color	Water depth (cm)	Canopy cover (%)	Species	BHO[2] herbarium number	GenBank accession number
5.86	C: 60, S: 40	Clear, colorless	17	18	*S. involuta*	A-1896	PP235054, PP235055
3.15	S: 90, V: 10	Clear, colorless	36	0	*S. involuta*	A-1891	PP235041-PP235046, PP766200-PP766215
7.73	V: 40, P: 30, S: 20, C: 10	Clear, light brown-colored	54	0	*S. grandis*	A-1888	PP235032-PP235037
4.95	Se: 50, P: 30, C: 15, B: 05	Clear, colorless	14	18	*S. involuta*	A-1893	PP235048, PP235049
15.15	Se: 90, P: 10	Clear, colorless	9	36	*S. grandis*	A-1897	PP235056, PP235057

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Appendix 2. Sequence data of *rbc*L from GenBank used for comparison with data generated in this study. "*" denotes sequence data from same location as in the present study.

Taxon	Genbank number	Location	Citation
Batrachospermum gelatinosum	DQ393135	Kentucky, USA	Stewart and Vis 2007
	GU810833	Massachusetts, USA	House *et al.* 2010
	GU810835	Massachusetts, USA	House *et al.* 2010
	AF029141	Alberta, Canada	Vis *et al.* 1998
	DQ393134	Nova Scotia, Canada	Stewart and Vis 2007
	GU810836	Nova Scotia, Canada	House *et al.* 2010
	EF375888	United Kingdom	Stewart and Vis 2007
	KM077030	Italy	Abdelahad *et al.* 2015
	KM077034	Italy	Abdelahad *et al.* 2015
	LC626340	Japan	Suzuki and Kitayama 2021
Paludicola communis	MN943922	Minnesota, USA	Vis *et al.* 2020a
	MN943926	New York, USA	Vis *et al.* 2020a
	MN943923	New Hampshire, USA	Vis *et al.* 2020a
	MN943924	New Hampshire, USA	Vis *et al.* 2020a
	MN943925	New Hampshire, USA	Vis *et al.* 2020a
	DQ449028	Maine, USA	Vis *et al.* 2007
	MG372122	South Carolina, USA	Redmond *et al.* 2019
	MN943921	Costa Rica	Vis *et al.* 2020a
	AY423407	French Guiana	Vis *et al.* 2005
	AY423408	French Guiana	Vis *et al.* 2005
Virescentia viride-americana	AF244110*	Michigan, USA	Vis *et al.* 2001
	AF244109	Michigan, USA	Vis *et al.* 2001
	AF029142	Rhode Island, USA	Vis *et al.* 1998
	AF244111	Ohio, USA	Vis *et al.* 2001
	AF244112	Ohio, USA	Vis *et al.* 2001
	AF244113	Connecticut, USA	Vis *et al.* 2001
	AF244114	Louisiana, USA	Vis *et al.* 2001
	AF244115	Massachusetts, USA	Vis *et al.* 2001
	AF244116	North Carolina, USA	Vis *et al.* 2001
	AF244117	Tennessee, USA	Vis *et al.* 2001
	AF244118	Ohio, USA	Vis *et al.* 2001
	AF244119	Rhode Island, USA	Vis *et al.* 2001
	AF244120	Ohio, USA	Vis *et al.* 2001
	AY198417	Tennessee, USA	Chiasson *et al.* 2003
	AY198419	Tennessee, USA	Chiasson *et al.* 2003
	MF940843	Virginia, USA	direct GB submission
	MG321565	South Carolina, USA	Redmond *et al.* 2019
	MG321566	South Carolina, USA	Redmond *et al.* 2019
	MG321577	South Carolina, USA	Redmond *et al.* 2019
	MZ604592	Kentucky, USA	direct GB submission
	MZ604594	Tennessee, USA	direct GB submission
	OR860044	Connecticut, USA	direct GB submission
	OR860045	Connecticut, USA	direct GB submission
	OR860046	Rhode Island, USA	direct GB submission
	OR860048	Massachusetts, USA	direct GB submission
	OR860050	Connecticut, USA	direct GB submission
	OR860051	Connecticut, USA	direct GB submission

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Appendix 3. Sequence data of *rbc*L for heterocorticate *Sheathia* species used for comparison and in the phylogeny (Fig. 8).

Species	Location	GenBank number(s)	Number of identical sequences	Reference(s)
S. grandis	Ohio, Michigan	JX669761, MZ604593, PP235015, PP235017, PP235022, PP235026	6	Salomaki *et al*. 2014, Direct GB submission, this study
S. grandis	Michigan	PP235023	1	This study
S. grandis	New York	PP139962, PP139963	2[1]	Direct GB submission
S. grandis	Michigan, Wisconsin	PP235021, PP235027, PP235039, PP235040, PP235047, PP235052, PP235053, PP235032, PP235033, PP235034, PP235035, PP235036, PP235037	13[1]	This study
S. grandis	Wisconsin	JX669803, JX669762	2	Salomaki *et al.* 2014, Vis *et al.* 2010
S. involuta	Virginia, Tennessee	JX669742, JX669743, JX669744, JX669766, JX669782, JX669783, JX669784	7	Salomaki *et al.* 2014
S. involuta	Virginia	GU457343	1	
S. involuta	Tennessee	JX669801	1	Salomaki *et al.* 2014
S. involuta	Tennessee, Alabama	JX669745, JX669799, JX669800, KU672395	4	Salomaki *et al.* 2014, direct GB submission
S. involuta	Michigan, Wisconsin, Illinois, Ohio, Minnesota, Oklahoma, Poland	JX669746, JX669747, JX669748, JX669749, JX669753, JX669760, JX669768, JX669771, JX669785, JX669797, MF940845, MW354954, MZ437980, PP235011, PP235013, PP235041, PP235042, PP235043, PP235044, PP235045, PP235046, PP235048, PP235049, PP235050, PP235054, PP235055, PP766200, PP766201, PP766202, PP766203, PP766204, PP766205, PP766206, PP766207, PP766208, PP766209, PP766210, PP766211, PP766212, PP766213, PP766214, PP766215	26[2]	Salomaki *et al.* 2014, direct GB submission, this study
S. involuta	Michigan, Indiana	JX669754, JX669755, JX669791, PP235051	4	Salomaki *et al.* 2014, this study
S. involuta	Arizona	OL744080	1	direct GB submission
S. involuta	Arizona	JX669767	1	Salomaki *et al.* 2014
S. involuta	Texas	AF029143, JX669786	2	Vis et al. 1998, Salomaki *et al.* 2014
S. involuta	Texas	JX669787	1	Salomaki et al. 2014
S. americana		OR860049		

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Appendix 3. Continued.

Species	Location	GenBank number(s)	Number of identical sequences	Reference(s)
S. americana		AF029140	9	
S. americana		JX669736		
S. americana		DQ393132	2	
S. boryana		KM593826	2	
S. boryana		KM593805	2	
S. boryana		KM593819		
S. boryana		KM593816		
S. boryana		KM593823		
S. boryana		KM593817	2	
S. boryana		KM077043		
S. boryana		KM593808	2	
S. boryana		JX669770	4	
S. confusa		KM593846		
S. confusa		JX669737	4	
S. confusa		KM593835	2	
S. confusa		KM593836		
S. confusa		DQ393128	9	
S. heterocortica		DQ393136	2	
S. heterocortica		JX669758		
S. heterocortica		JX669763	4	
S. heterocortica		MG321563		
S. heterocortica		JX669750	6	

[1] PP235018 (1018 bp) to both haplotypes.
[2] PP235020 (1083 bp) identical to this haplotype.

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