Survey of Freshwater Red Algae from the Batrachospermales (Rhodophyta) in South Carolina

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Abstract - Freshwater red algae are important components of the algal flora in streams and rivers with high water quality. The order Batrachospermales is the most species-rich portion of the red algal taxa reported throughout North America. We investigated 30 stream segments in South Carolina for the presence of freshwater red algae classified within the Batrachospermales. We collected a total of 50 specimens representing 7 genera and 9 species. We documented *Batrachospermum gelatinosum*, *B. turfosum*, *Kumanoa skujana*, *Montagnia australis*, *Sheathia americana*, *S. heterocortica*, *Sirodotia suecica, Tuomeya americana*, and *Virescentia viride-americana*. We observed *M. australis* and *T. americana* from the greatest number of streams and in multiple years from the same site. We observed *V. viride-americana* in 3 streams; our specimens represent the only new record for the state. We generated DNA sequence data of the *rbc*L gene or gleaned it from the literature for 8 of the 9 taxa identified in the study and confirmed their morphological identification. We collected stream temperature, pH, and conductivity data from sites where we collected 6 of the taxa (*Batrachospermum gelatinosum, B. turfosum, M. australis, Sheathia americana, T. americana*, and *V. viride-americana*). Our records were within previously reported ranges for these taxa, although water temperatures tended to be higher than those in previous reports. Present data for the diversity of Batrachospermales in South Carolina represent 64% of the generic/infrageneric and 20% of the species diversity known for North America. This diversity may still be an underestimation of what might be detected by future studies that target more specialized habitats; taxa that are known from neighboring states but not yet reported from South Carolina might be discovered.

Introduction

Freshwater red algae occur most often in shallow streams with moderate water flow but are found in a variety of habitats (Sheath and Vis 2015). Although various species have been collected from streams with diverse environmental characteristics, they are most common in streams with pH 6–7, low to moderate nutrient concentrations, and low illumination (Eloranta and Kwandrans 2007, Sheath and Hambrook 1990, Sheath and Vis 2015). As a result, several red algal species have been used as bioindicators of higher water quality in North America and Europe (Stancheva and Sheath 2016). These red algae are also important components of aquatic ecosystems, serving as both food and shelter for macroinvertebrates (Sheath et al. 1995, 1996).

Freshwater taxa are distributed throughout most of the rhodophyte lineage (Kumano 2002, Yoon et al. 2006). However, the order Batrachospermales is the

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most taxon-rich, comprising ~150 species (Sheath 1984). Since the first molecular systematics study of members of this order, the species originally assigned to the genus *Batrachospermum* have been shown to be paraphyletic, with the result that several new genera have been recently described (Chapuis et al. 2017; Entwisle et al. 2009; Necchi et al. 2018, 2019; Salomaki et al. 2014). These latest studies have shown some taxa to be morphologically cryptic, and can be positively identified only by using DNA sequence data. For example, there are multiple species within the genus *Sheathia* that are so similar in appearance that genetic data are needed to distinguish *Sheathia americana* from *S. heterocortica* (Salomaki et al. 2014). This requirement can be problematic in evaluating historic records (based solely on morphology), and some identifications remain ambiguous (Table 1).

The state of South Carolina has 5 distinct ecoregions. In this study, we sampled the 3 largest ecoregions (Piedmont, Southeastern Plains, and the Middle Atlantic Coastal Plain), as prior studies suggested that rhodophytes have been identified only in these ecoregions (Griffith et al. 2002). The Piedmont ecoregion is in the central area of the state and serves as a transition between the mountains and the flat coastal plains. The Southeastern Plains region is primarily an agricultural landscape with crops, farmland, forests, and woodlands. The streams in the Middle Atlantic Coastal Plain tend to be humic and stained due to tannins from the dominant *Pinus* (pine) vegetation and have a primarily sandy substrate (Carey et al. 2007).

In South Carolina, freshwater red algae from the Compsopogonales, Acrochaetiales, and Batrachospermales have been previously reported (Table 1 and the references therein). Similar to the general trend in taxonomic distribution in North America, the majority of taxa identified were from the Batrachospermales, with 11 species representing 7 genera. Many of these collections were recorded prior to the first molecular systematics publications on freshwater red algae (Vis et al. 1998). Therefore, we undertook the present study to re-survey freshwater red algae of the Batrachospermales in the state, with a focus on comparing previous records of Batrachospermales in conjunction with DNA sequence data for identification, and broadening the knowledge base of stream environmental data for these taxa.

Methods

We sampled freshwater red algal specimens from 30 locations in South Carolina primarily during the period May–September from 2008, 2009, 2010, and 2012 (Fig. 1, Appendix 1). Of these, we visited 18 sites once, while we made collections from 12 of them in multiple years (Appendix 1). We categorized each site based on ecoregion: Southeastern Plains (17), Piedmont (12), or Middle Atlantic Coastal Plain (1) (Griffith et al. 2002). Stream habitats varied from open to closed canopy and from slow-moving waters to sites with higher current velocity (Fig. 2). At each location, we measured water temperature, pH, and conductivity in situ using a YSI Professional Plus multiparameter instrument (YSI, Yellow Springs, OH). Due to equipment failure, we did not obtain stream measurements at some sites and on some dates.

We deposited a pressed herbarium voucher for each specimen in the Bartley Herbarium Ohio University (BHO). We dried some samples in silica desiccant for use in DNA extraction. We identified all specimens using morphological characteristics and standard taxonomic literature (Kumano 2002, Necchi and Vis 2012, Salomaki et al. 2014) with taxonomy updated using AlgaeBase (Guiry and Guiry 2018). We ground samples for DNA sequencing in liquid nitrogen with a mortar and pestle. We extracted DNA using the NucleoSpin Plant II DNA kit (Macherey-

Table 1. Freshwater red algae previously reported from South Carolina. Nomenclature updated using Algaebase (Guiry and Guiry 2018).

Taxon	Reference
Compsopogonales	
Boldia erythrosiphon Herndon	Dillard (1967), Howard and Parker (1980), Jacobs (1968)
Compsopogon caeruleus (Balbis ex C.Agardh) Montagne	Jacobs (1968)
Acrochaetiales	
Audouinella hermannii (Roth) Duby (as *A. violacea*)	Dillard (1967), Necchi et al. (1993a)
Batrachospermales	
Batrachospermum section *Batrachospermum*	
B. gelatinosum (L.) De Candolle (as *B. moniliforme*)	House et al. (2010), Sheath and Cole (1993), Vis et al. (1996)
B. trichocontortum Sheath & M.L.Vis	Vis and Sheath (1996)
Batrachospermum section *Turfosa*	
B. turfosum Bory (as *B. vagum*)	Jacobs (1968), Wolle (1882)
K. skujana (Necchi) Necchi & M.L.Vis	Vis et al. (2012)
Lemanea	
L. fucina Bory	Dillard (1967)
Montagnia	
M. australis Montagne (as *Audouinella macrospora*)	Necchi et al. (1993b), Sheath et al. (1994)
Kumanoa	
Paralemanea	
Paralemanea grandis (Wolle) S. Kumano (as *L. emanea australis*)	Atkinson (1931)
Sheathia	
S. americana Salomaki & M.L.Vis	Salomaki et al. (2014)
S. heterocortica (Sheath & K.M.Cole) Salomaki & M.L.Vis	Salomaki et al. (2014), Vis et al. (1996)
[A]*S. americana* or *S. heterocortica*	
Sirodotia	
S. suecica Kylin	Lam et al. (2012), Necchi et al. (1993c)
Tuomeya	
T. americana (Kützing) Papenfuss (as *T. fluviatilis*)	Dillard (1967), Goldstein and Manzi (1976), Jacobs (1968)

[A]Vis et al. (1996) reported *Batrachospermum boryanum*. However, *S. boryana* only occurs in Europe and it is unclear whether the current name would be *S. americana* or *S. heterocortica* because these species can only be distinguished with sequence data (see Salomaki et al. 2014).

Nagel, Düren, DE) according to the manufacturer's protocol. We conducted a polymerase chain reaction (PCR) using an Applied Biosystems 2720 Thermocycler (Applied Biosystems, Foster City, CA) to amplify a 1282-bp fragment of the chloroplast rubisco large subunit gene (*rbc*L). The primers we used to amplify this region were F160 and *rbc*LR (Vis et al. 1998). The PCR reaction mix consisted of 32.75 µL dH_2O, 5.0 µL 10x buffer, 4.0 µL dNTP, 4.0 µL $MgCl_2$, 0.25 µL Ex Taq (Takara Bio Inc., Mountain View, CA), 1.5 µL of each amplification primer, and 1.0 µL of extracted DNA. The PCR thermocycler conditions were as follows: an initial denaturing at 95 °C for 1 h, followed by 35 cycles of 93 °C for 30 min, 50 °C for 30 min, and 72 °C for 1 h. We used the UltraClean® PCR Clean-up DNA purification kit (Mo Bio, Carlsbad, CA) to purify the PCR products and sequenced them at the

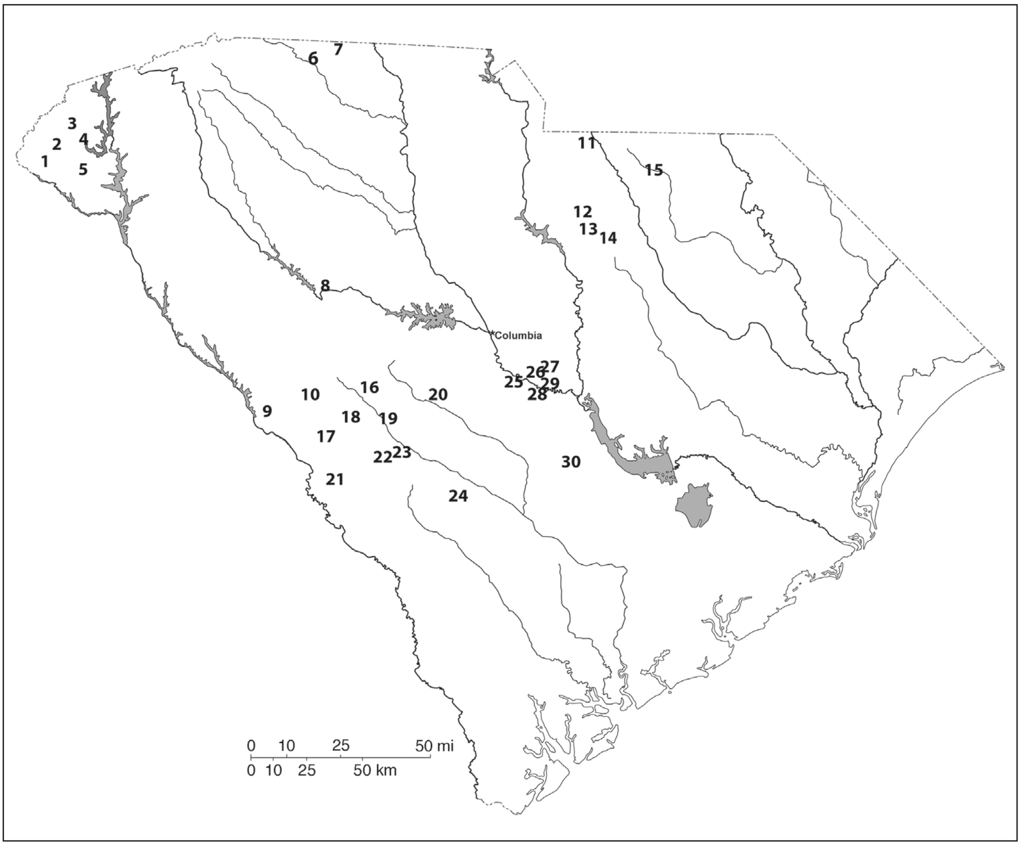

Figure 1. Map of South Carolina showing location of collection sites. More information about ecoregions and collection sites in Appendix 1.

Figure 2 (following page). Representative habitats in which freshwater red algae were collected. (A) A site in the Mid-Atlantic Coastal Plain with shallow, nearly stagnant, waters, sand and muck substrate, and *Taxodium distichum* (L.) Rich. (Bald Cypress) trees in the water and on the bank. (B) A site in the Southeastern Plains with sandy substrate, moderately flowing waters and shade from overhanging vegetation. (C) A site in the Piedmont with swift rapids over boulder and rock substrate and an open canopy.

Figure 2. [Caption on preceding page.]

Ohio University Genomics Facility. We used additional internal primers to fully sequence the 1282-bp in both sense and anti-sense directions (Table 2). We used Sequencher® version 5.2.4 (GeneCodes Corp, Ann Arbor, MI) to assemble DNA fragments and submitted final sequences to GenBank.

We searched GenBank for *rbc*L sequences of the same species as determined from our morphological assessment. We combined the sequences from this study with sequences of the same species and a wide range of Batrachospermales taxa to build a phylogeny. If more than 1 specimen from this study had an identical sequence, we included only 1 representative sequence in subsequent phylogenetic analyses. We subjected a total of 46 sequences to maximum likelihood (ML) using RAxML (Stamatakis 2014) and Bayesian inference (BI) analysis using MrBayes v.3.2 (Ronquist et al. 2012) in Geneious plug-ins V10.2.3 (Kearse et al. 2012). We employed a GTR+G model for both the ML and BI analyses. To determine support values, we conducted 1000 ML bootstrap replicates and, for the BI posterior probabilities, 3×10^6 generations sampling every 100 generations with the first 250 generated trees removed as burn-in.

Results

The 30 streams from which we collected freshwater red algal taxa varied in several key environmental conditions (Appendix 1). Among the 12 Piedmont sites, min–max (and mean) values varied for water temperature from 21 °C to 30 °C (23 °C), pH from 6.2 to 7.3 (7), and specific conductance from 31 µS cm^{-1} to 138 µS cm^{-1} (65 µS cm^{-1}). We sampled the 17 Southeastern Plains sites when the water temperature was relatively warm (19–29 °C [mean = 24 °C)]; pH was acidic to neutral (5.2–7.5)—most streams were acidic (mean pH = 5.3)—and specific conductance was low (min–max = 2–195 µS cm^{-1}, mean = 26). We recorded no chemical stream

Table 2. Internal primers used for sequencing. Herbarium numbers as in Appendix 1.

Internal Primer	Primer Sequence 5’-3’	BHO Number
R472.4	GGAACTATTGTTGAGCGCGAAAG	A-1083, A-1089, A-1094
R897	GCAGGTAACTCAACTTATTCTCG	A-0118, A-0125
R897.1	GCTGGTAATTCAACATACTACG	A-0024, A-0093, A-0149, A-0151, A-0153, A-0157, A-0291, A-0293, A-0295, A-0297, A-0312, A-0313, A-1085
F650	ATTAACTCTCAACCATTTATGCG	A-0024, A-0093, A-0118, A-0125, A-0291, A-0293, A-0295, A-0297, A-0312, A-0313, A-1083, A-1085, A-1089, A-1094
F650.1	ATTAATTCTCAGCCTTTCATGCG	A-0149, A-0151, A-0153, A-0157
F1087.1	ATCATTTAAGTGTTAATCTACCTC	A-0157
F1087.71	GTCATTTAGATGTTAATTTACCTC	A-1094

data at the 1 site in the Middle Atlantic Coastal Plain. For the most part, the streams in the Southeastern Plains had an acidic pH, whereas those in the Piedmont were more neutral (mean = 5.3 and 7.0, respectively). Although there were a few streams in the 2 regions that had specific conductance greater than 100 µS cm^{-1}, these regions were very similar overall (mean = 65 µS cm^{-1} and 26 µS cm^{-1}, respectively). The stream temperatures all reflected the season—summer—in which it was measured (mean = 23 °C and 24 °C, respectively) (Appendix 1).

From the 30 sites, we collected and identified 50 specimens as members of 7 genera and 9 species within the Batrachospermales (Appendix 1). *Montagnia australis* was the most frequently occurring species; specimens were collected from a total of 13 streams and it was present in more than 1 year in 7 of the 8 streams sampled in multiple years (Appendix 1). We documented *Tuomeya americana* in 11 streams and in more than 1 year from 5 of those streams. We observed the remaining taxa in a single year: *Virescentia viride-americana* in 5 streams; *Sheathia heterocortica* in 3 streams; *B. turfosum* in 2 streams; and *Kumanoa skujana, Sheathia americana*, *Sirodotia suecica,* and *B. gelatinosum* in a single stream each. Of the 30 streams sampled, 24 streams contained a single species, 4 streams had 2 species, and there was a single stream with 3 species as well as 1 stream with 4 species (Appendix 1).

We obtained associated water temperature, pH, and specific conductance for 9 of the species collected (Appendix 1). At 1 *B. gelatinosum* location, we collected the following data: water temperature = 22 °C, pH = 6.2, and a specific conductance of 51 µS cm^{-1}. Stream data were available for the 3 *V. viride-americana* specimens, with water temperatures varying from 19 °C to 30 °C, a circumneutral pH (min–max = 6.9–7.5), and specific conductance from 45 µS cm^{-1} to 195 µS cm^{-1}. We collected 15 specimens of *M. australis* in waters with temperatures ranging from 20 °C to 27 °C, acidic pH (min–max = 5.2–6.3), and low specific conductance (min–max = 2–30 µS cm^{-1}). We collected *B. turfosum* from 2 sites that had data water temperatures of 23 °C and 28 °C, acidic pH of 5.6 and 5.8, and low specific conductance 21 µS cm^{-1} and 25 µS cm^{-1}, respectively. We obtained stream data for 3 *Sheathia heterocortica* specimens: water temperature = 22–23 °C, pH = 6.2–6.9, and specific conductance = 45–119 µS cm^{-1}. We collected 10 *T. americana* specimens in streams with temperatures of 21–29 °C, pH from 5.5 to 7.0, and specific conductance of 6–96 µS cm^{-1}. We had no stream data for specimens of *K. skujana*, *Sheathia americana*, or *Sirodotia suecica*.

Among the 3 ecoregions sampled in this study, we collected 5 species in the Piedmont, 6 in the Southeastern Plains, and 1 from the Middle Atlantic Coastal Plain. We collected *V. viride-americana* from streams in all 3 of these ecoregions, whereas *T. americana* was collected in Southeastern Plains and Piedmont, but not Middle Atlantic Coastal Plain. We detected the remaining taxa within a single ecoregion (Appendix 1).

We obtained DNA sequence data for the *rbc*L gene from 25 specimens; 20 sequences were newly generated in this study and 5 previously submitted to GenBank from other studies (Appendix 1). Many of the specimens were from the same species; thus, we did not obtain DNA sequence data from all specimens. *Batrachospermum gelatinosum* was the only species collected for which *rbc*L data could not be generated (Appendix 1). Four taxa had more than 1 DNA sequence generated, and those were highly similar within a species. Among the 10 specimens of *T. americana* sequenced from South Carolina, there were only 8-bp differences (0.6%). For *Sheathia heterocortica*, there were 5-bp differences (0.4%) among the 4 specimens from South Carolina. Lastly, there were 2-bp differences (0.2%) between the 2 *M. australis* specimens and no differences between the 2 *V. viride-americana* specimens.

Phylogenetic analyses using both ML and BI showed similar tree topology, but the relationships among *Kumanoa*, *Virescentia*, and *B. turfosum* were not resolved in the BI analysis. The ML tree is shown with both bootstrap and posterior probability support (Fig. 3). The specimens from 8 taxa identified from South Carolina were placed in a clade with other sequences from the same species and had high support values (>90 bootstrap [bs], >0.9 posterior probability [pp]). Likewise, *K. skujana*, for which there is no other sequence data available, was within a clade of *Kumanoa* species with high support (>90 bs, >0.9 pp). It is notable that the taxa from this study represent a wide distribution throughout the Batrachospermales clade (Fig. 3).

Discussion

Numerous taxa from the order Batrachospermales identified via morphology had been previously recorded from South Carolina. This project and recent studies with DNA sequence information have confirmed the presence of *B. gelatinosum*, *B. turfosum*, *M. australis*, *Sheathia americana*, *S. heterocortica*, *Sirodotia suecica*, and *T. americana* in the state (Lam et al. 2012, Salomaki et al. 2014, Vis et al. 2012). However, we did not document *B. trichocontortum* in the current study even though it was described as a new species from a locality in South Carolina (Vis and Sheath 1996). In the subsequent years, this species has not been reported from other localities and was not recollected in the type location when visited some years later (M.L. Vis, pers. observ.). This species differs from those to which it is closely related in having a twisted trichogyne. It is possible that this morphological characteristic is environmentally induced rather than taxonomically informative; if that were the case, the specimen may represent *B. gelatinosum*. For example, specimens with knobs or other protrusions of the trichogyne/carpogonium from streams in Italy with high nitrate concentrations (20.4–53.6 $mg \cdot l^{-1}$) were identified to be *B. gelatinosum* from DNA sequence data (Abdelahad et al. 2015). Therefore, it is unclear if *B. trichocontortum* represents a distinct species or is an ecotypic variant. Both *Lemanea* and *Paralemanea* have been recorded from a few locations in South Carolina (Atkinson 1931, Dillard 1967); these locations were not resampled in the current study. In the southeastern US, the genus *Paralemanea* seems to be common and there has been at least 1 report of *Lemanea* in the neighboring state of North Carolina (Vis and Sheath 1992). Future research could target these taxa by concentrating sampling efforts on the habitats with high current velocity in which they tend to grow.

Using DNA sequences, we recorded 2 taxa in the present study that had not been previously reported for the state by using morphology alone. *Virescentia viride-americana* is a widespread taxon in the eastern US and has been collected from Maine to Louisiana, including the neighboring state of North Carolina (House et al. 2008, Necchi et al. 2018, Sheath et al. 1994). Therefore, our documentation of this taxon in 3 locations in South Carolina is not outside the range of this species. We

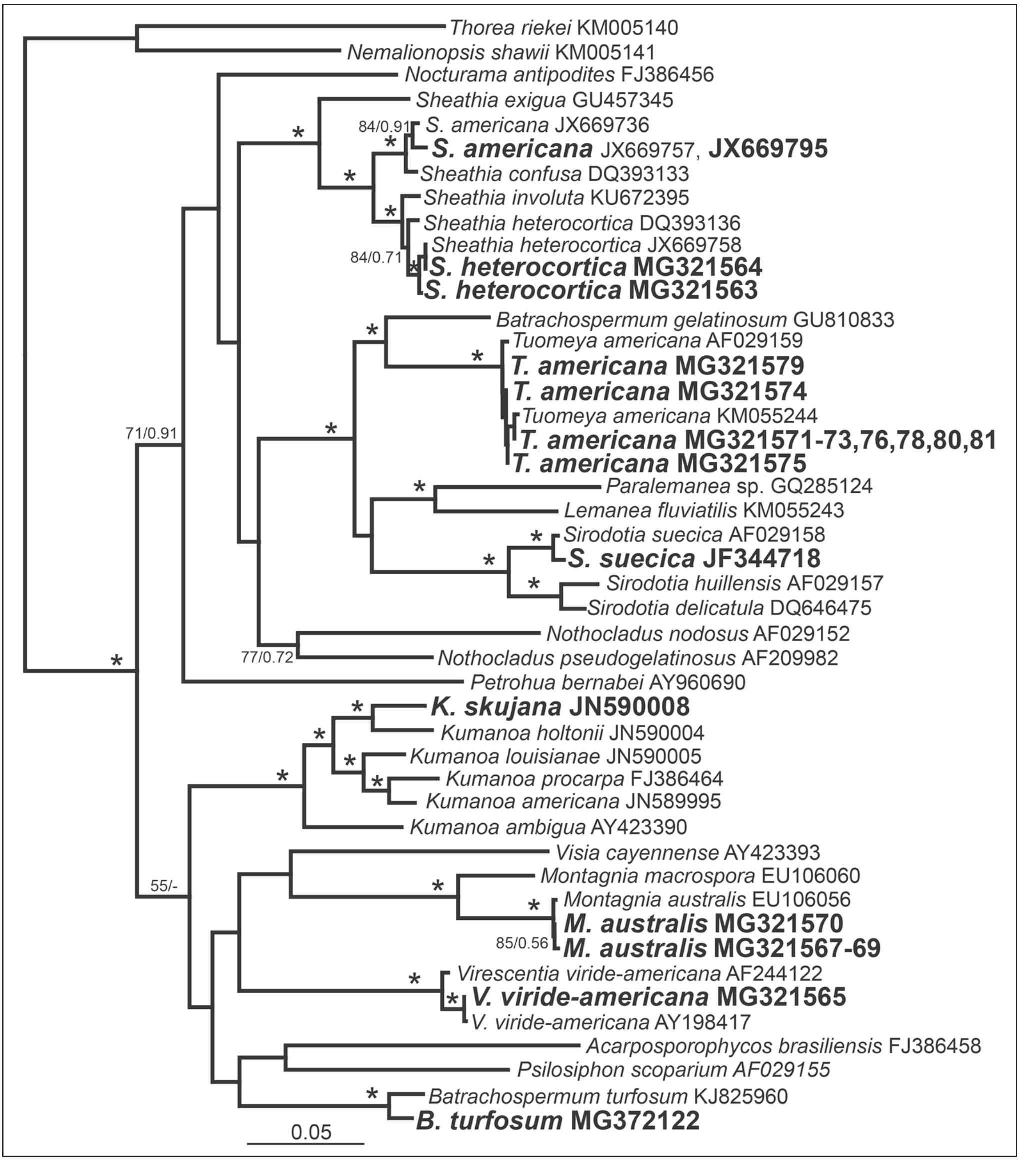

Figure 3. Phylogenetic tree derived from the ML analysis with branch support. Branches with an asterisk (*) = >90 bootstrap (bs) and >0.9 posterior probability (pp) and branches with no numbers had <50 bs and <0.50 pp. Taxon name and GenBank number provided. When more than 1 GenBank number is given, all specimens had the same sequence. South Carolina specimens in bold; GenBank numbers as in Appendix 1.

also found a second newly reported taxon—*K. skujana*. This species was originally described from Brazil and previously was unknown outside of Brazil, but it has been suggested that it migtht occur in other locations in North America (Necchi and Vis 2012). Given that other *Kumanoa* species, such as *K.americana*, that have been reported from the southeastern US, the occurrence of this species is noteworthy but not unusual.

The DNA sequence data produced for this study showed that the specimens of the 9 taxa had few base-pair differences compared with previous sequence data in GenBank. However, it is notable that there appear to be 2 *T. americana* haplotypes differing by 8 bp. Of the 10 specimens from this study, 3 were identical to sequence data for a specimen from North Carolina (AF029159) and 7 were identical to sequence data for a specimen from Georgia (KM055244). Although these genetic differences fit well within intraspecific variation for members of the Batrachospermales (Lam et al. 2012, Necchi et al. 2018, Salomaki et al. 2015), they may prove interesting in future phylogeographic studies.

Data on stream water chemistry were available for 6 species collected in this study. *Batrachospermum gelatinosum* is known from a wide geographic range in North America and Europe, and collection sites have varied in their stream chemistry (Eloranta et al. 2016, House et al. 2010, Keil et al. 2015, Vis et al. 1996). In this study, the water temperature, pH, and specific conductance measurements from streams containing *B. gelatinosum* were all within the span of values previously reported (0–22 °C, pH 6.4–8.3, 45–216 µS cm^{-1}; Eloranta et al. 2016, Vis et al. 1996). *Virescentia viride-americana* is distributed throughout temperate eastern North America (House et al. 2008, Necchi et al. 2018, Sheath et al. 1994). Water chemistry measurements from the current study are within the span of values previously reported for pH (4.9–8.5) and specific conductance (30–320 µS cm^{-1}), but above previous temperatures (8–20 °C) (Sheath et al. 1994), which can be explained by the warmer period of the collections. The range in stream measurements for sites containing *M. australis* in this study encompasses the previously reported ranges (Vis et al. 2004). The stream temperature (28 °C) of the site where we collected *B. turfosum* in this study extends the upper bound from a previous study (0–22 °C), and the pH and specific conductance from this study were within the span of values from the previous study (pH 3.9–8.2, 18–130 µS cm^{-1}) (Sheath et al. 1994). Likewise, the stream temperature at one of the *Tuomeya americana* sites was 29 °C, which is above the values from the previous study (5–26°C), while the pH and specific conductance ranges were within the previous spans reported (pH 5.5–8.1, 10–100 µS cm^{-1}) (Kaczmarczyk et al. 1992). Prior to this study, water parameter measurements for *Sheathia heterocortica* were only recorded at a single location; the previous temperature (22 °C) was within the span of values recorded in the present study, while previous pH and specific conductance values (pH 8.3, 220 µS cm^{-}1, respectively) were greater than this study's (pH 6.2–6.7 and 51–119 µS cm-1) (Sheath and Cole 1990).

Overall, freshwater red algae classified in the Batrachospermales appear to be well represented in the streams of South Carolina. Currently, there are 4 sections of *Batrachospermum* and 10 genera reported from North America (Chapuis et al. 2017, Evans et al. 2017, Necchi et al. 2018, Sheath and Vis 2015). Our study, in South Carolina, reported the 2 *Batrachospermum* sections (*Batrachospermum* and *Turfosa*) and 5 genera (*Kumanoa*, *Montagnia*, *Sheathia*, *Sirodotia, Tuomeya*, and *Virescentia*), which along with 2 older reports of *Lemanea* and *Paralemanea*, is ~64% of the diversity known in North America. The genera *Balliopsis*, *Lympha, Torularia*, and *Volatus* were not collected in previous studies nor by this study from South Carolina. However, all but *Balliopsis* have been reported from nearby states. Sheath and Vis (2015) reported 38 species in the Batrachospermales, and 6 species were added in 2 more recent papers (Chapuis et al. 2017, Evans et al. 2017) for a total of 44 species in North America. Therefore, ~20% of the freshwater red algal species diversity currently known for North America has now been recorded in South Carolina. The number of species recorded in the state relative to the number known on the continent suggests that the state's richness represents high diversity, but we are unaware of other comparable surveys. This richness may still be an underestimation, and more discoveries could be made by targeting more specialized habitats and taxa that are known from neighboring states but not yet reported from South Carolina.

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Appendix 1. Collection information (location, latitude [°N], longitude [°W], herbarium voucher specimens) and data for locations at which we sampled freshwater red algae. Site number as in Figure 1. A dash denotes no data.

Site #	Collection information	Date	Water temp. (°C)	pH	Specific conductance ($\mu S.cm^{-1}$)	Ecoregion (major)	Taxon	rbcL GenBank number	Publication for GenBank number
1	Toxaway Creek at SR 34, Oconee County, SC, 34.65731787, 83.18110147, BHO A-1089	16.viii.2012	22	6.9	65	Piedmont	*T. americana*	MG321580	This study
2	Ramsey Creek, SC, 34.682033167, 83.14631045, BHO A-0061, A-0093 A-0311	25.viii.2009	22	6.2	51	Piedmont	*Sheathia heterocortica*	JX669796	Salomaki et al. 2014
		25.viii.2009	22	6.2	51	Piedmont	*Sheathia heterocortica*	MG321564	This study
		25.viii.2009	22	6.2	51	Piedmont	*B. gelatinosum*[A]	-	
		25.viii.2009	22	6.2	51	Piedmont	*T. americana*	-	
3	Langham Creek, SC, 34.76926, 83.01149, BHO A-0062	11.viii.2009	23	6.7	119	Piedmont	*Sheathia heterocortica*	JX669758	Salomaki et al. 2014
4	Little Cane Creek, SC, 34.7692689, 83.0114982, BHO A-0125, A-0291, A-0024, A-0313	25.viii.2009	23	6.9	45	Piedmont	*T. americana*	MG321566	This study
		25.viii.2009	23	6.9	45	Piedmont	*V. viride-americana*	MG321571	This study
		31.vii.2010	-	-	-	Piedmont	*S. heterocortica*	MG321563	This study
		31.vii.2010	-	-	-	Piedmont	*T. americana*	MG321576	This study
5	Snow Creek at SR 51, Oconee County, SC, 34.62361839, 82.99464525, BHO A-1083	16.viii.2012	21	6.5	51	Piedmont	*T. americana*	MG321578	This study
6	Buck Creek at Peach Shed Road, Spartanburg County, SC, 35.111326, 81.893809, BHO A-0312, A-1092	1.ix.2009	22	6.5	31	Piedmont	*T. americana*	MG321575	This study
		17.viii.2012	-	-	-	Piedmont	*T. americana*	-	
7	Suck Creek, SC, 35.17904594, 81.77741736, BHO A-0232	21.viii.2009	-	-	-	Piedmont	*K. skujana*	JN5890008	Vis et al. (2012)
8	Simmons Creek, SC, 34.35548275, 81.8863821, BHO A-0317a, A-0317b	23.vi.2009				Piedmont	*Sheathia americana*	JX669795	Salomaki et al. 2014
		23.vi.2009	-	-	-	Piedmont	*V. viride-americana*	MG321577	This study

Site #	Collection information	Date	Water temp. (°C)	pH	Specific conductance ($\mu S.cm^{-1}$)	Ecoregion (major)	Taxon	rbcL GenBank number	Publication for GenBank number
9	Steven's Creek at SC 23, SC, 33.7294532, 82.1822998, BHO A-0101	23.vi.2010	30	7.3	138	Piedmont	*V. viride-americana*	-	
10	Oconee Creek at SR 129, Oconee County, SC, 33.84058078, 81.98948165, BHO A-1094	16.viii.2012	23	7.0	40	Piedmont	*T. americana*	MG321581	This study
11	Wildcat Creek at SR 39, Lancaster County, SC, 34.74425328, 80.54156474, BHO A-1085	16.viii.2012	25	6.7	96	Piedmont	*T. americana*	MG321579	This study
12	Grannie's Quarter, CW-078, SC, 34.402453, 80.641947, BHO A-0297	1.ix.2009	-	-	-	Piedmont	*T. americana*	MG321574	This study
13	Sanders Creek at SC 97, Kershaw County, SC, 34.316325, 80.641708, BH0 A-1077	18.vii.2012	27	5.5	21	SE Plains	*M. australis*	-	
14	Little Pine Creek at SR 132, Kershaw County, SC, 34.271461, 80.587686,BHO A-0151, A-1086	13.viii.2008	-	-	-	SE Plains	*M. australis*	MG321568	This study
		18.vii.2012	24	5.5	20	SE Plains	*M. australis*	-	
15	Skipper Creek at SC 145, SC, 34.6227847, 80.1901289, BHO A-135	30.vi.2010	26	5.3	15	SE Plains	*M. australis*	-	
16	McTier Creek, SC, 33.7536023, 81.6016588, BHO A-0306, A-0295	21.vii.2009	22	5.8	6	SE Plains	*T. americana*	-	
		7.ix.2010	-	-	-	SE Plains	*T. americana*	MG321573	This study
17	Little Horse Creek at SR 104, Aiken County, SC, 33.5639, 81.874213, BHO A-0298, A-1093	15.ix.2010	-	-	-	SE Plains	*T. americana*	-	-
		3.vii.2012	29	5.9	22	SE Plains	*T. americana*	-	-

Site #	Collection information	Date	Water temp. (°C)	pH	Specific conductance ($\mu S.cm^{-1}$)	Ecoregion (major)	Taxon	rbcL GenBank number	Publication for GenBank number
18	Shaw's Creek at SR 153, Aiken County, SC, 33.658875, 81.718127, BHO A-0293, A-0156, A-1088	22.vii.2009	21	5.5	7	SE Plains	*T. americana*	MG321572	This study
		7.ix.2010	-	-	-	SE Plains	*M. australis*	-	
		29.vii.2012	24	6.1	37	SE Plains	*M. australis*	-	
19	Rocky Springs Creek at Moore Road, Aiken County, SC, 33.660925, 81.557007, BHO A-1080	3.vii.2012	26	5.4	15	SE Plains	*M. australis*	-	
20	Black Creek at SR 278, Lexington County, SC, 33.7324142, 81.3031812, BHO A-0155, A-0157, A-1081	21.vii.2009	-	-	-	SE Plains	*M. australis*	-	
		4.ix.2010	23	5.6	2	SE Plains	*M. australis*	MG321570	This study
		5.viii.2012	25	5.6	30	SE Plains	*M. australis*	-	
21	Hollow Creek at SR 5, Aiken County, SC, 33.340036, 81.8219917, BHO A-0139, A-1079	18.vi.2010	22	6.3	12	SE Plains	*M. australis*	-	
		3.vii.2012	23	5.7	15	SE Plains	*M. australis*	-	
22	Cedar Creek at SR 79, Aiken County,SC, 33.4535488, 81.6428341, BH0 A-0141, A-1087	18.vi.2010	21	6.1	-	SE Plains	*M. australis*	-	
		2.vii.2012	20	5.4	20	SE Plains	*M. australis*	-	
23	Upper Three Runs Creek at SR 113, Aiken County, SC, 33.4762662, 81.5884466, BHO A-0137, A-1091	18.vi.2010	21	5.4	18	SE Plains	*M. australis*	-	
		3.vii.2012	-	-	-	SE Plains	*M. australis*		
24	Little Salkehatchie River, SC, 33.3109941, 81.1929748, BHO A-0127	2.ix.2009	19	7.5	195	SE Plains	*V. viride-americana*	-	
25	Sandy Run Creek at US 176, Calhoun County, SC, 33.48078, 80.580209, BHO A-1078	3.viii.2012	28	5.8	25	SE Plains	*B. turfosum*	-	

Site #	Collection information	Date	Water temp. (°C)	pH	Specific conductance (μS.cm-1)	Ecoregion (major)	Taxon	rbcL GenBank number	Publication for GenBank number
26	Cedar Creek at SR 734, Richland County, SC, 33.8411981, 80.85997581, BHO A-0153, A-0211, A-1090	28.i.2009	-	-	-	SE Plains	*M. australis*	MG321569	This study
		6.viii.2009	-	-	-	SE Plains	*B. turfosum*	MG372122	This study
		2.viii.2012	23	5.6	21	SE Plains	*M. australis*	-	
27	Cedar Creek, DHEC site RS-09312, SC, 33.927148, 80.819343, BH0 A-0149	10.vi.2009	25	6.1	2	SE Plains	*M. australis*	MG321567	This study
28	Big Beaver Creek at US Highway 176, Calhoun County, SC, 33.759551, 80.914301, BHO A-0267, A-1082	16.v.2008	-	-	-	SE Plains	*Sirodotia suecica*	JF344718	Lam et al. 2012
		5.viii.2012	25	5.3	16	SE Plains	*M. australis*	-	
29	Cedar Creek at SR 66, Richland County, SC, 33.897136, 80.819206, BHO A-1084	3.viii.2012	24	5.2	22	SE Plains	*B. macrosporum*	-	
30	Cow Castle Creek at bridge on S-38-198, SC, 33.41942, 80.74017, BHO A-0118	17.ii.2009	-	-	-	Mid-Atlantic Coastal Plain	*Virescentia viride-americana*	MG321565	This study

[A]No *rbc*L sequence data were generated for BHO A-0093; however, COI-5P data from House et al. (2010) confirm its identity as *Batrachospermum gelatinosum*.