

MOLECULAR SYSTEMATICS OF THE FLORIDEOPHYCEAE (RHODOPHYTA) USING NUCLEAR LARGE AND SMALL SUBUNIT rDNA SEQUENCE DATA¹

James T. Harper² and Gary W. Saunders

Centre for Environmental and Molecular Algal Research, Department of Biology, University of New Brunswick, Fredericton, NB,
Canada, E3B 6E1

Sequence data are presented for approximately 85% of the nuclear large subunit (LSU) rDNA gene for one member of the Bangiophyceae and 47 members of the Florideophyceae, the latter representing all but one of the currently recognized florideophyte orders. Distance, parsimony, and maximum likelihood analyses of these data were used to generate phylogenetic trees, and bootstrap resampling was implemented to infer robustness for distance and parsimony results. LSU phylogenies were congruent with published nuclear small subunit (SSU) rDNA results in that four higher level florideophyte lineages were resolved: lineage 1, containing the order Hildenbrandiales; lineage 2, recovered only under distance analysis, composed of the orders Acrochaetiales, Balliales, Batrachospermales, Corallinales, Nemaliales, Palmariales, and Rhodogorgonales; lineage 3, containing the Ahnfeltiales; and lineage 4, composed of the orders Bonnemaisoniales, Ceramiales, Gelidiales, Gigartinales, Gracilariales, Halymeniales, Plocamiales, and Rhodymeniales. Analyses were also performed on a combined LSU–SSU data set and an SSU-only data set to account for differences in taxon sampling relative to published studies using this latter gene. Combined LSU–SSU analyses resulted in phylogenetic trees of similar topology and support to those obtained from LSU-only analyses. Phylogenetic trees produced from SSU-only analyses differed somewhat in particulars of branching within lineages 2 and 4 but overall were congruent with the LSU-only and combined LSU–SSU results. We close with a discussion of the phylogenetic potential that the LSU has displayed thus far for resolving relationships within the Florideophyceae.

Key index words: Florideophyceae; group I intron; large subunit rDNA; molecular phylogeny; red algae; Rhodophyta; small subunit rDNA; systematics; taxonomy

Abbreviations: LSU, nuclear large subunit rDNA; rbcL, large subunit of ribulose biphosphate carboxylase; SSU, nuclear small subunit rDNA

The Rhodophyta is an assemblage of marine and freshwater photosynthetic eukaryotes distinct among algal lineages in lacking flagella, storing food reserves as floridean starch, possessing a combination of unique photosynthetic pigments, and having chloroplasts with

nonaggregated thylakoids and lacking external endoplasmic reticulum (Woelkerling 1990). The Rhodophyta is currently considered as consisting of two classes: Bangiophyceae and Florideophyceae.

The most widely followed classifications for the red algae (Kylin 1923, 1925, 1932, 1956) were based almost exclusively on characteristics of female reproductive anatomy and postfertilization development. Novel interpretations of life history patterns (Guiry 1974, 1978, Fredericq and Hommersand 1989, Maggs and Pueschel 1989) and the advent of ultrastructural observations (Pueschel and Cole 1982), notably the fine structure of pit plugs (proteinaceous structures in the connections between cells), led to a considerable restructuring of red algal classification at the ordinal level (cf. Saunders and Kraft 1997). The introduction of molecular tools for inferring phylogenetic relationships among organisms afforded a new suite of characters upon which to examine red algal systematics. The most widely used systems have been sequences of the nuclear small subunit (SSU) rDNA and the large subunit of ribulose biphosphate carboxylase (*rbcL*). Ragan et al. (1994) and Freshwater et al. (1994) presented the first extensive molecular phylogenies of the florideophyte orders based on these two genes. Although these phylogenies showed some congruency with those based on morphological and ultrastructural characters, there were some shortcomings. These studies suffered the inevitable consequences of being first: low taxon sampling with some critical lineages completely excluded and uncertainty as to the appropriateness of the gene systems being used relative to the taxonomic level of the question being asked. Because of the more conservative nature of the SSU, for example, phylogenies generated from this gene generally provided resolution at earlier nodes. Conversely, the more variable *rbcL* data provided increased resolution at more recent nodes but failed to resolve earlier ones (cf. Freshwater and Bailey 1998). Subsequent molecular investigations using SSU data, however, have been integral in the establishment of several new orders (Saunders and Kraft 1994, 1996, Sheath and Müller 1999, Choi et al. 2000), have provided support for published phylogenetic hypotheses on pit plug evolution (Pueschel 1994, Saunders and Bailey 1997), and have resolved four distinct higher level lineages within the Florideophyceae (Saunders and Bailey 1997, Saunders and Kraft 1997).

Sequences of the large subunit (LSU) rDNA have been used considerably less for phylogenetic inference. The more conservative regions of the LSU have been used to infer evolutionary relationships among Archaeobacteria (Leffers et al. 1987, Gouy and Li 1989a),

¹Received 25 October 2000. Accepted 24 July 2001.

²Author for correspondence: e-mail jharper@unb.ca.

eukaryotic kingdoms (Cedergren et al. 1988, Gouy and Li 1989b, Lenaers et al. 1989), helminths (Qu et al. 1986, Gill et al. 1988), protists (Baroin et al. 1988, Preparata et al. 1989), and higher plants (Kuzoff et al. 1998). The more divergent regions, on the other hand, have proven useful for assessing phylogenetic relationships among more closely related taxa (Baroin et al. 1988, Qu et al. 1988, Lenaers et al. 1991). In red algal systematics, LSU sequences have been used sparingly. In the only published accounts to our knowledge, LSU sequences were used in a multigene phylogeny of the order Gelidiales (Freshwater and Bailey 1998, Freshwater et al. 1999) to examine the origin of red algae and cryptomonad nucleomorphs (Van der Auwera et al. 1998) and to assess the potential utility of this gene for red algal systematics (Freshwater et al. 1999, Harper and Saunders 2001).

Until now an LSU-based molecular systematic investigation of red algae on the scale of those published for the SSU and *rbcL* (Freshwater et al. 1994, Ragan et al. 1994) has not been undertaken. Because of the greater size of the LSU, its regions of higher sequence variation, and preliminary evidence that suggests this gene has considerable potential for use in red algal systematics (Freshwater and Bailey 1998, Freshwater et al. 1999, Harper and Saunders 2001), we present herein LSU phylogenies for comparison with published SSU-based phylogenies within the Florideophyceae.

MATERIALS AND METHODS

Species included in this study are presented in Table 1. Samples were processed, and genomic DNA was isolated as previously described (Saunders 1993). Approximately 85% of the LSU was PCR amplified, cleaned, and sequenced following the protocols of Harper and Saunders (2001). PCR and sequencing primers are listed in Table 2. Secondary structure information was used to align sequence data from this study with two previously published LSU sequences (Van der Auwera et al. 1998). The resulting multiple alignment was modified by eye, and ambiguous regions were removed, including an intron in *Hildenbrandia rubra*.

Phylogenetic analyses were performed on three data sets. The first included only LSU sequence data for all 50 included taxa (2481 sites; accessible via all GenBank Accession numbers; Table 1). The second data set included combined LSU and SSU sequence data for 45 of the included taxa (4288 sites; SSU sequences have not been determined for *Ahnfeltia fastigiata*, *Gastroclonium clavatum*, *Melobesia mediacris*, *Sarcodia antarctica*, and *Schizymenia pacifica*). The third data set included SSU data for 44 of the included taxa (1653 sites; the SSU sequence for *Batrachospermum macrosporum* is unusually divergent from the remainder of the Batrachospermales [see Vis et al. 1998] and was additionally removed). For all three data sets, *Bangia atropurpurea* (Bangiophyceae) was selected as the outgroup taxon (Freshwater et al. 1994, Ragan et al. 1994).

Distance, parsimony, and maximum likelihood analyses were performed using PAUP, version 4 (Swofford 2000). For distance analyses, alignments were converted into distance matrices using the Kimura two-parameter correction model, and phylogenetic trees were constructed using the neighbor-joining algorithm. Unweighted and transversion-weighted (transversion/transition ratio of 2:1) parsimony analyses were completed (50 random sequence addition replicates, gaps treated as missing data) using a heuristic search with stepwise addition and tree bisection-reconnection branch swapping (Swofford 2000). The LSU-only and LSU-SSU data sets were subjected to both unweighted and weighted parsimony, but the SSU-only data set was only subjected

to unweighted parsimony. To estimate the robustness of internal nodes in distance and parsimony analyses, bootstrap resampling (2000 replicates) was completed (Felsenstein 1985) (10 random sequence addition replicates, gaps treated as missing data under parsimony). Maximum likelihood analyses were performed (20 random sequence addition replicates) using a heuristic search with stepwise addition and tree bisection-reconnection branch swapping, empirical base frequencies, and a transversion/transition ratio of 2. Because of the computationally intensive nature of maximum likelihood, bootstrap resampling was not performed for this analysis.

RESULTS

LSU-only phylogeny. Figure 1 presents the maximum likelihood tree (with superimposed distance and parsimony bootstrap values) for the LSU-only alignment ($-\ln$ likelihood = 27777.47). Distance and parsimony analyses produced trees of similar topology. Unweighted parsimony resulted in three most parsimonious trees, with a length of 4568 (consistency index [CI] = 0.403, retention index [RI] = 0.639), whereas weighted parsimony analysis produced four most parsimonious trees with a length of 6376 (CI = 0.402, RI = 0.660). Three of the four florideophyte lineages of Saunders and Bailey (1997)—lineage 1 (Hildenbrandiales), lineage 3 (Ahnfeltiales), and lineage 4 (Bonnemaisoniales, Ceramiales, Gelidiales, Gigartinales, Gracilariales, Halymeniales, Plocamiales, and Rhodymeniales)—were monophyletic with full bootstrap support under distance and parsimony. Lineage 1 was positioned as the most basal florideophyte lineage with the remaining lineages forming a fully supported monophyletic clade. Lineage 2 was divided into two separate groups: group A (Coralinales and Rhodogorgonales) and group B (Acrochaetiales 1, Acrochaetiales 2, Balliales, Batrachospermales, Nemaliales, Palmariales, and Thorealess). Parsimony, like maximum likelihood, resolved group A as a monophyletic sister to lineages 3 and 4 (55% unweighted and 53% weighted), whereas distance joined groups A and B to give a monophyletic lineage 2 (a result not supported with bootstrap resampling). Group B received full support from distance analysis but somewhat weaker support (79% unweighted and 78% weighted) from parsimony. All orders (recognizing the two groups of Acrochaetiales as distinct) within lineage 2 were resolved as monophyletic under distance and parsimony, usually with strong to full support. Exceptions included low support under distance (67%) and moderate support under parsimony (83% unweighted and 86% weighted), for monophyly of the Palmariales and Nemaliales, respectively. The Balliales was resolved as the earliest divergence in group B, and a larger grouping of Acrochaetiales 1 and 2, Nemaliales, and Palmariales was strongly supported in distance and parsimony analyses. Lineage 3 (Ahnfeltiales) was unequivocally placed as sister to lineage 4 in all analyses. Most of the included orders within lineage 4 were resolved as monophyletic with moderate to full support, although relationships among these orders were equivocal. Species of the Gigartinales were scattered throughout lineage 4: *Peyssonnelia* was weakly positioned as the most basal lineage (55% dis-

TABLE 1. List of species used in this study and GenBank accession numbers for LSU and SSU data.

Taxon and authority	LSU	SSU*
Acrochaetiales		
<i>Audouinella dasyae</i> (Collins) Woelkerling	AF419100	L26181
<i>Audouinella endophytica</i> (Batters) Dixon	AF419101	AF079789
<i>Audouinella hermannii</i> (Roth) Duby	AF419102	AF026040
<i>Rhodochorton purpureum</i> (Lightfoot) Rosenvinge	AF419103	U23816
Ahnfeltiales		
<i>Ahnfeltia fastigiata</i> (Postels <i>et</i> Ruprecht) Makienko	AF419104	No data
<i>Ahnfeltia plicata</i> (Hudson) Fries	AF419105	Z14139
Balliales		
<i>Ballia callitricha</i> (C. Agardh) Kützing	AF419106	AF236790
Bangiales		
<i>Bangia atropurpurea</i> (Roth) C. Agardh	AF419107	L36066
Batrachospermales		
<i>Batrachospermum boryanum</i> Sirodot	AF419108	AF026044
<i>Batrachospermum macrosporum</i> Montagne	AF419109	AF026048
<i>Lemanea fluviatilis</i> (Linnaeus) C. Agardh	AF419110	AF026051
Bonnemaisoniales		
<i>Atractophora hypnoides</i> Crouan	AF419111	Unpubl.
<i>Bonnemaisonia hamifera</i> Hariot	AF419112	L26182
Ceramiales		
<i>Centroceras clavulatum</i> (C. Agardh) Montagne	AF419113	Unpubl.
<i>Pterothamnion villosum</i> (Kyllin) Athanasiadis <i>et</i> Kraft	AF419114	Unpubl.
<i>Spyridia dasyoides</i> Sonder	AF419115	Unpubl.
Corallinales		
<i>Corallina officinalis</i> Linnaeus	AF419116	L26184
<i>Melobesia mediocris</i> (Foslie) Setchell <i>et</i> Mason	AF419117	No data
Gelidiales		
<i>Pterocladia lucida</i> (Brown <i>ex</i> Turner) J. Agardh	AF419118	Y11958
<i>Suhria vittata</i> (Linnaeus) Endlicher	AF419119	Unpubl.
Gigartinales		
<i>Chondrus crispus</i> Stackhouse	AF419120	Z14140
<i>Dilsea californica</i> (J. Agardh) Kuntze	AF419121	U33126
<i>Dumontia alaskana</i> Tai, Lindstrom <i>et</i> G. W. Saunders	AF419122	AF317101
<i>Erythrophyllum delesseriodes</i> J. Agardh	AF419123	AF317105
<i>Euthoria cristata</i> (C. Agardh) J. Agardh	AF419124	Unpubl.
<i>Mastocarpus stellatus</i> (Stackhouse in Withering) Guiry	AF427518	L26195
<i>Peyssonnelia</i> sp.	AF419125	Unpubl.
<i>Predaea</i> sp.	AF419126	Unpubl.
<i>Sarcodia antarctica</i> Hariot <i>nom. int.</i>	AF419127	No data
<i>Schizymenia dubyi</i> (Chauvin <i>ex</i> Duby) J. Agardh	AF419128	U33136
<i>Schizymenia pacifica</i> (Kyllin) Kylin	AF419129	No data
<i>Schimmelmannia schousboei</i> (J. Agardh) J. Agardh	AF419130	Unpubl.
<i>Schmitzia</i> sp.	AF419131	Unpubl.
Gracilariales		
<i>Gracilaria verrucosa</i> (Hudson) Papenfuss	Y11508*	M33638
<i>Gracilariopsis lemaneiformis</i> (Bory de Saint-Vincent) Dawson, Acleto <i>et</i> Foldvik	AF419132	L26214
Halymeniales		
<i>Cryptonemia undulata</i> Sonder	AF419133	U33125
<i>Sebdenia flabellata</i> (J. Agardh) P. G. Parkinson	AF419134	U33138
Hildenbrandiales		
<i>Apophlaea lyallii</i> Hooker f. <i>et</i> Harvey	AF419135	AF076996
<i>Hildenbrandia rubra</i> (Sommerfelt) Meneghini	AF419136	AF076995
Nemaliales		
<i>Cumagloia andersonii</i> (Farlow) Setchell <i>et</i> Gardner	AF419137	Unpubl.
<i>Galaxaura marginata</i> (Ellis <i>et</i> Solander) Lamoroux	AF419138	AF006090
Palmariales		
<i>Camontagnea oxyclada</i> (Montagne) Pujals	AF419139	AF079794
<i>Palmaria palmata</i> (Linnaeus) Kuntze	Y11506*	Z14142
<i>Rhodophyllum elegans</i> (P. <i>et</i> H. Crouan <i>ex</i> J. Agardh) Dixon	AF419140	U23817
Plocamiales		
<i>Plocamium cartilagineum</i> (Linnaeus) Dixon	AF419141	U09619
Rhodogorgonales		
<i>Rhodogorgon carriebowensis</i> J. Norris <i>et</i> Bucher	AF419142	AF006089
Rhodymeniales		
<i>Faucheia repens</i> (C. Agardh) Montagne <i>et</i> Bory de Saint-Vincent	AF419143	AF085267
<i>Gastroclonium clavatum</i> (Roth) Ardissonne	AF419144	No data
Thoreales		
<i>Thorea</i> sp.	AF419145	Unpubl.
<i>Thorea violacea</i> Bory de Saint-Vincent	AF419146	AF026042

*The SSU sequences, as well as those LSU accession numbers marked with an asterisk, were not determined in this study. Collection details and isolate numbers are available from the authors upon request.

TABLE 2. Nucleotide sequences for PCR and sequencing primers of the LSU used in this study (see Harper and Saunders 2001 for primer diagram).

Primer name	Primer sequence	Strand complement
T01	TAAGCATATCAGTAAGCGGAG	Coding
T01N ^a	GATGACCCGCTGAATTTAAG	Coding
T04	GCAGGACGGTGGCCATGGAAGT	Coding
T05	GCAACGGGCAAAGGGAATCCG	Coding
T08 ^b	CAGAGCACTGGGCAGAAATCAC	Noncoding
T10 ^b	CACCTTGGAGACCTGCTGCGG	Noncoding
T13	GCAGGTGAGTTGTTACACTC	Noncoding
T14 ^b	CGTCCGGCTCGCCTTCGACGG	Coding
T15	TGATAGGAAGAGCCGACATCGA	Noncoding
T16	GAGACCGATAGCGAAACAAGTAC	Coding
T19	TTATCCTACACCTCTCAAGTAC	Noncoding
T19N ^a	AAGCTCAACAGGGTCTTCTTTC	Noncoding
T22	GGAAAGAAGACCCTGTTGAGCTT	Coding
T24	GCACTAATCATTGCTTTTACC	Noncoding
T25	GAAAGATGGTGAACATATGCC	Coding
T30	TGTTAGACTCCTTGGTCCGTG	Noncoding
T33 ^a	TCCGCTTGTCGGGAGAAACACG	Coding

^a LSU primers not included in Harper and Saunders (2001): T01N replaces T01, T19N replaces T19, and T33 replaces T14.

^b LSU primers based on sequences kindly provided by C. Bailey and W. Freshwater.

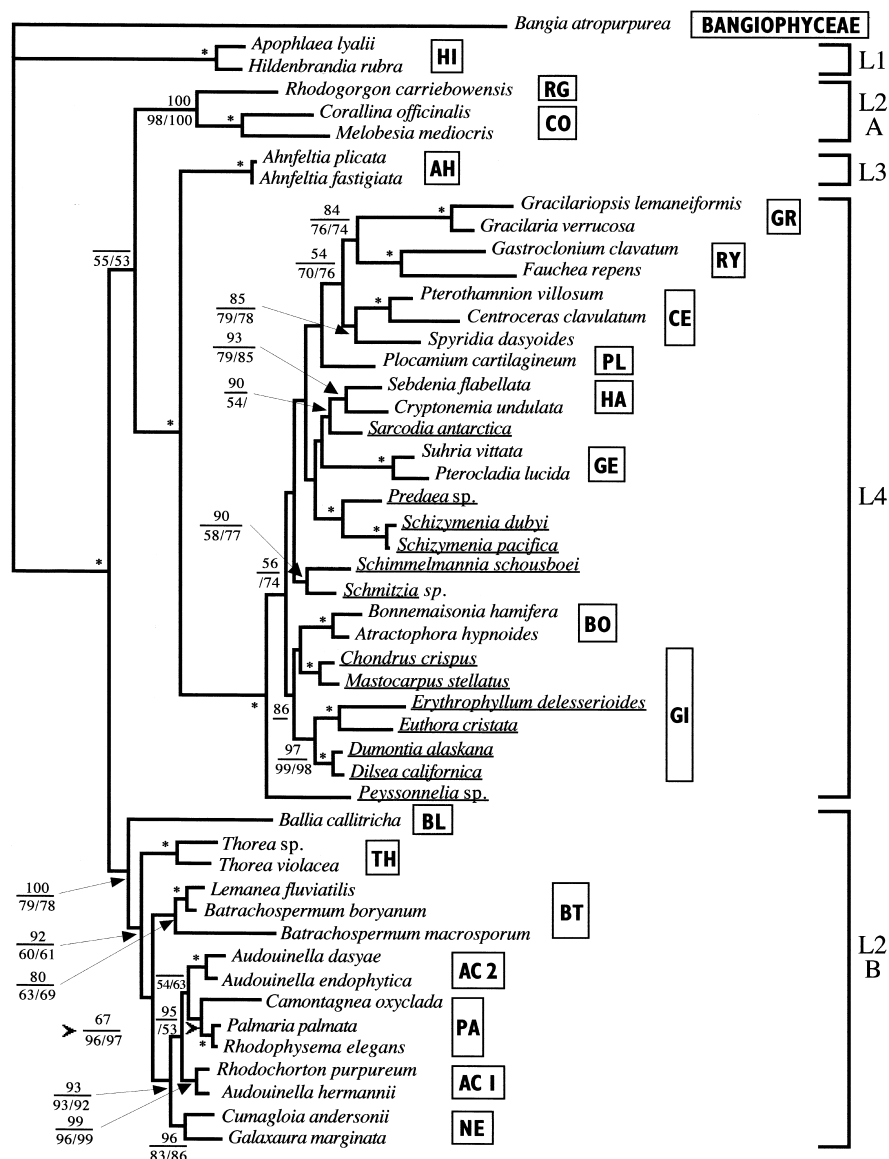


FIG. 1. Phylogenetic tree resulting from maximum likelihood analysis for LSU sequences only. Numbers above branches indicate bootstrap values for distance analysis and numbers below branches indicate bootstrap values for unweighted (left) and transversion-weighted (right) parsimony analyses (% of 2000 replicates). Internal branches lacking values had <50% bootstrap support. Internal nodes marked with an asterisk received 100% bootstrap support in distance and both parsimony analyses. Scale bar, 0.01 substitutions. L1, lineage 1; L2, lineage 2; L3, lineage 3; L4, lineage 4. Underlined taxa are currently considered members of the order Gigartinales. AC 1, Acrochaetiales group 1; AC 2, Acrochaetiales group 2; AH, Ahnfeltiales; BL, Balliales; BO, Bonnemaisoniales; BT, Batrachospermales; CE, Ceramiales; CO, Corallinales; GE, Gelidiales; GI, Gigartinales *sensu stricto*; GR, Gracilariaceae; HA, Halymeniales; HI, Hildenbrandiales; NE, Nemaliales; PA, Palmariales; PL, Plocamiales; RG, Rhodogorgonales; RY, Rhodymeniales.

tance and 74% weighted parsimony), *Predaea* and *Schizymenia* grouped together with full support but were not allied to any order, *Chondrus* (Gigartinae) and *Mastocarpus* (Petrocelidaceae) grouped together with full support but weakly associated with the Bonnemaisoniales, and the Dumontiaceae and Kallymeniaceae grouped together with nearly full support as sister to the Bonnemaisoniales/Gigartinae and Petrocelidaceae clade.

Combined LSU and SSU phylogeny. The maximum likelihood result (with superimposed distance and parsimony bootstrap values) for the LSU-SSU alignment is presented in Figure 2 ($-\ln$ likelihood = 39924.94016). Again, distance and parsimony analyses produced trees of similar topology to the maximum likelihood result. Unweighted parsimony resulted in a single most parsimonious tree, with a length of 6352 (CI = 0.435, RI = 0.647), whereas weighted parsimony also produced a single tree with a length of 8828 (CI = 0.436, RI = 0.670). Similar to the LSU-only results, three of the

four florideophyte lineages (lineages 1, 3, and 4) were resolved with full support. Also congruent with the LSU-only results was the positioning of lineage 1 as the earliest florideophyte lineage and lineage 3 as sister to lineage 4. Interestingly, in LSU-SSU combined distance analysis moderate bootstrap support (85%) for a monophyletic lineage 2 was obtained, whereas parsimony provided weak support consistent with the maximum likelihood result (Fig. 2) for these groups being independent. Within groups A and B of lineage 2, relationships were resolved as for the LSU-only results. The relationships among and support for orders within lineage 4 were similar to the LSU-only results. One significant difference was that the Gigartinales *sensu stricto* (including *Chondrus*, *Dilsea*, *Dumontia*, *Erythrophyllum*, *Euthora*, and *Mastocarpus*) was moderately resolved as monophyletic by distance and parsimony analyses, with the Bonnemaisoniales weakly resolved as sister to this assemblage (Fig. 2).

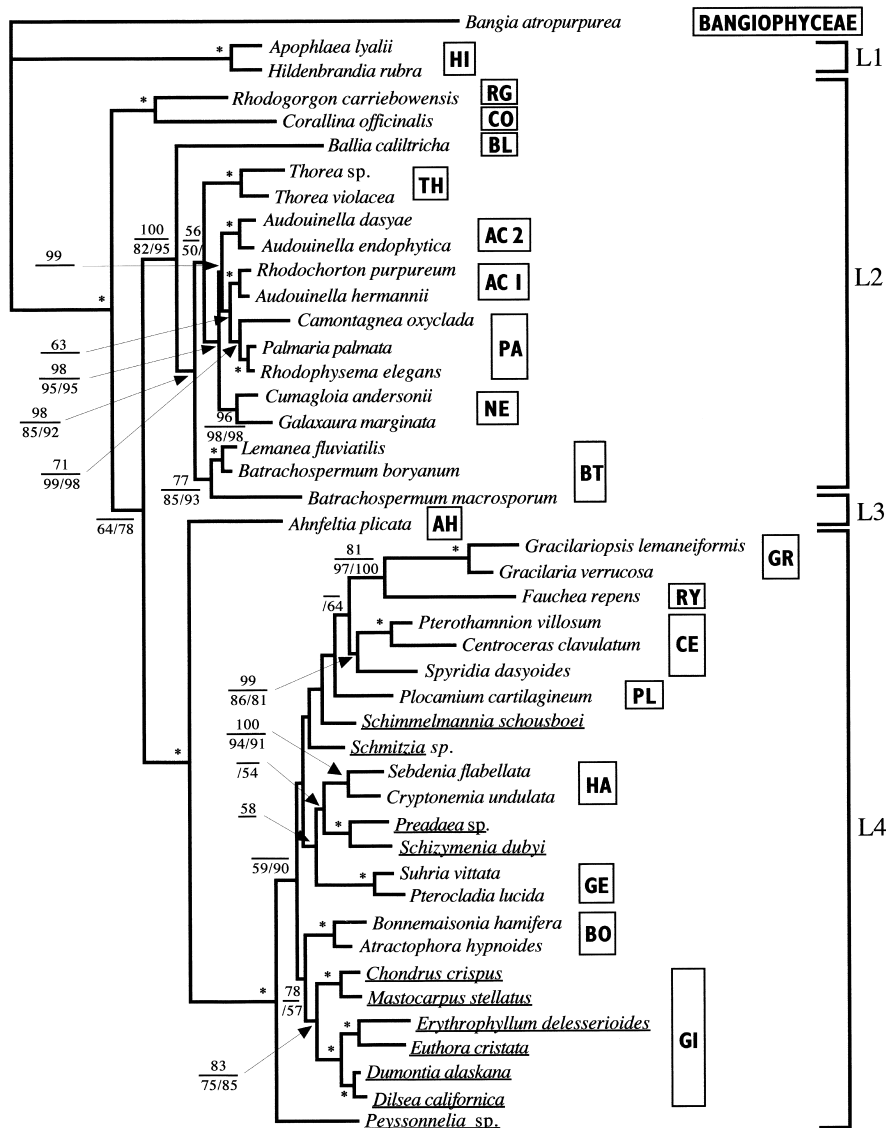


FIG. 2. Phylogenetic tree resulting from maximum likelihood analysis for combined LSU and SSU sequences. Numbers, abbreviations, and symbols as for Figure 1. Scale bar, 0.01 substitutions.

SSU-only phylogeny. The maximum likelihood result (with superimposed distance and parsimony bootstrap values) for the SSU-only alignment is presented in Figure 3 ($-\ln$ likelihood = 13218.92883). Again, distance and parsimony analyses produced trees of similar topology to the maximum likelihood result and were similar to those recovered in the LSU-only and LSU-SSU analyses. Unweighted parsimony resulted in six most parsimonious trees (length = 2004, CI = 0.482, RI = 0.69). Again, lineages 1, 3, and 4 were resolved for all trees in the SSU-only analyses. The distance result gave 93% support for a monophyletic lineage 2, which was not supported under maximum likelihood or parsimony. Bootstrap support within each of the lineage 2 groups was again similar to that obtained for the LSU-only and LSU-SSU trees. Within group B the constituent

orders and groups were well supported in distance and parsimony analyses, but the relationships between these groups remained equivocal. A similar situation was noted within lineage 4: Strong bootstrap support was recovered for monophyly of most of included orders, but relationships among these same assemblages were equivocal. The Gigartinales *sensu stricto* (including *Chondrus*, *Dilsea*, *Dumontia*, *Erythrophyllum*, *Euthora*, and *Mastocarpus*) was weakly resolved as monophyletic (58% distance and 53% parsimony) and a grouping of *Predaea* and *Schizymenia* received moderate to strong support (93% distance and 77% parsimony).

DISCUSSION

The first extensive phylogenies based on SSU (Ragan et al. 1994) and *rbcL* sequence data (Freshwater et al.

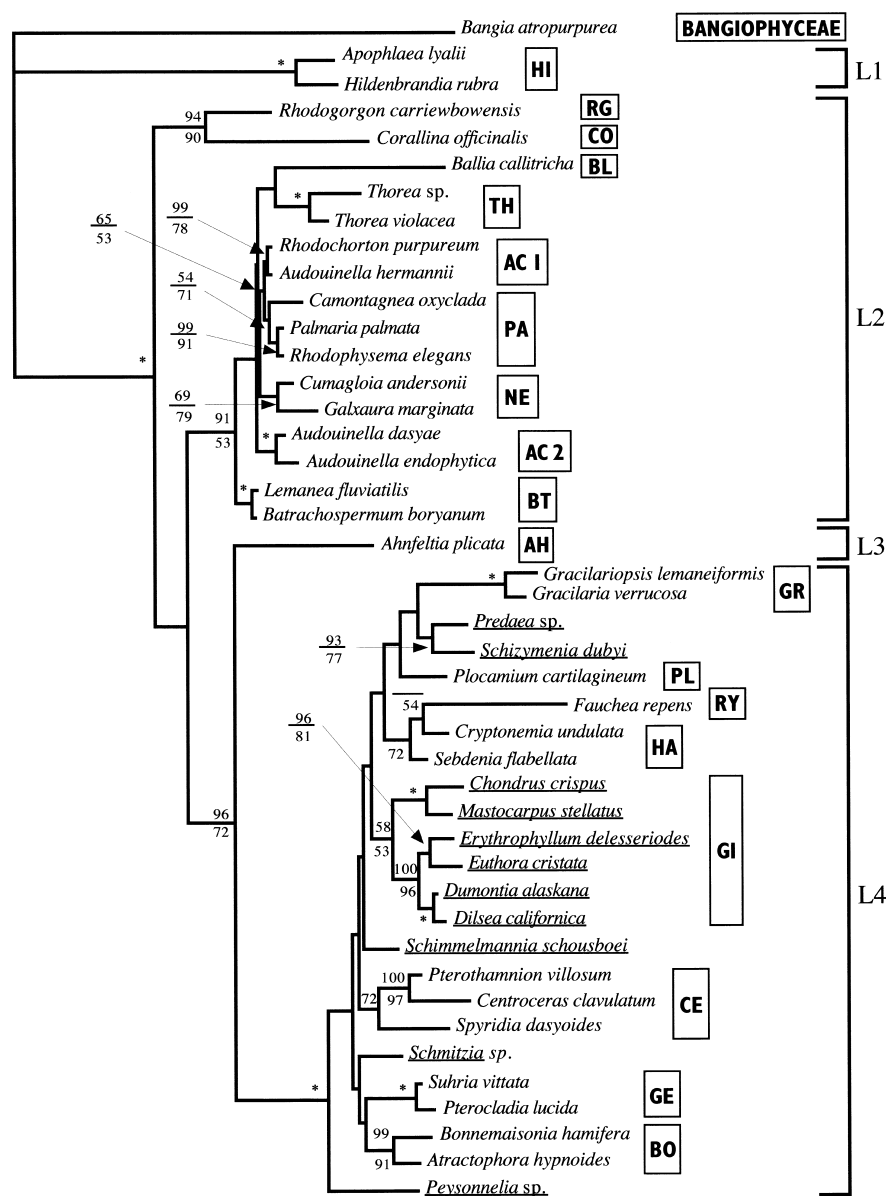


FIG. 3. Phylogenetic tree resulting from maximum likelihood analysis for SSU sequences only. Numbers, abbreviations, and symbols at branches as for Figure 1. Scale bar, 0.01 substitutions.

1994) were essential in establishing a foundation for future molecular systematic research on red algae. The strengths and weaknesses of both these systems for inferring phylogenies within the Florideophyceae are better appreciated today (Saunders and Kraft 1997) and explain some of the discrepancies between the 1994 publications with respect to one another and to current SSU-based phylogenetic perspectives at the supraordinal level in red algae (Saunders and Bailey 1997, 1999). Our results for LSU-only, LSU-SSU, and SSU-only alignments are generally congruent with the published SSU phylogenies of Saunders and Bailey (1997, 1999) in that three of the four higher level florideophyte lineages (1, 3, and 4) were resolved with all methods of analyses, and our distance results for LSU-only, LSU-SSU, and SSU-only data sets resolved a monophyletic lineage 2. Monophyly for lineage 2 has consistently harnessed strong bootstrap support under distance analyses, whereas parsimony and maximum likelihood are generally equivocal on an association for the Corallinales/Rhodogorgonales clade with the other lineage 2 taxa (Saunders and Bailey 1997, 1999). This anomaly is most likely the result of taxon sampling in combination with divergent rates of evolution in the different lines (notably those that are poorly represented). As an example, if the *Ahnfeltia* spp., which have a relatively short branch in the LSU phylogenies, are removed from the LSU-only and combined data sets under parsimony and maximum likelihood, a monophyletic lineage 2 is resolved. For the SSU-only analyses, removing *Ballia*, which has a relatively long branch, results in a monophyletic lineage 2 under parsimony. Unfortunately, both the Ahnfeltiales and Balliales are each represented by only a few species in a single genus, and expanding taxon sampling for these groups is not possible. It is noteworthy that Choi et al. (2000) with their more extensive sampling of the Corallinales, as well as a second sequence for *Ballia*, resolved the best overall support for lineage 2 to date (99% distance, 79% parsimony).

Despite the variable molecular support for lineage 2, the included taxa are united by the presence of two cap layers associated with their pit plugs, attributes associated with these structures having substantial phylogenetic significance (Saunders and Bailey 1997). Despite our confidence in recognizing these four florideophyte lineages, our phylogenies were weak at resolving many of the relationships within these same groups, a result consistent with published SSU-based phylogenies (Saunders and Bailey 1997, 1999, Saunders and Kraft 1997).

Lineage 1. The Hildenbrandiales is considered to be the only order in this lineage (Saunders and Kraft 1997), and molecular results position it as the earliest divergence within the Florideophyceae (Ragan et al. 1994, Saunders and Bailey 1997). Our analyses of all data sets consistently recovered the same result with full bootstrap support. All members of this lineage are characterized by pit plugs with a single cap layer and a membrane (Pueschel and Cole 1982). The LSU se-

quence determined herein had a putative group I intron of 540 base pairs at position 844. This same isolate also had a group I intron in the SSU (Saunders and Bailey 1999). (Note: Because of the increased size of the PCR fragment that included this intron, portions of the intron are based on single strand sequence data.)

Lineage 2. Within lineage 2, analyses of LSU-only and LSU-SSU data did not provide increased support for previously unresolved nodes relative to published SSU phylogenies, although topologies and support were congruent in all cases (Saunders and Bailey 1997, Harper and Saunders 1998, Vis et al. 1998, Sheath and Müller 1999, Choi et al. 2000). For those species for which ultrastructural data are available, pit plugs in this lineage are characterized by having two cap layers (Pueschel and Cole 1982). A recent SSU investigation (Choi et al. 2000) led to the proposal of a new order, Balliales, in lineage 2 for species previously classified in the Ceramiales (lineage 4). This order received unequivocal support as sister to a complex containing the Acrochaetiales, Batrachospermales, Nemaliales, and Palmariales for all analyses of LSU-only and LSU-SSU data and for the distance analysis of the SSU-only data. Maximum likelihood and parsimony were equivocal in the placement of the Balliales among the groups of lineage 2 for the SSU alignment, a result generally consistent with Choi et al. (2000).

The inter- and infraordinal relationships of the Batrachospermales have recently been examined using SSU and *rbcL* data (Vis et al. 1998, Pueschel et al. 2000). Our results are congruent with those findings in that the Thoreaceae was positioned as a distinct clade within lineage 2 and the family Batrachospermaceae and genus *Batrachospermum* were resolved as paraphyletic within a monophyletic Batrachospermales (excluding Thoreaceae). Recently, Sheath et al. (2000) proposed that the Thoreaceae should be elevated to ordinal rank. Our results do not position this taxon within the Batrachospermales, and given the unique combination of morphological, anatomical, and ultrastructural characteristics that the Thoreaceae possesses, ordinal recognition appears justified.

A number of SSU-based studies have illustrated the close relationship between the florideophyte orders Acrochaetiales, Nemaliales, and Palmariales but have failed to resolve the relationships within this complex (Ragan et al. 1994, Saunders et al. 1995, Saunders and Bailey 1997, Harper and Saunders 1998). Harper and Saunders (1998) reported that the rather morphologically homogenous Acrochaetiales in actuality is comprised of two divergent groups and that the order as it currently stands may be polyphyletic. Our analyses were consistent with the results of Harper and Saunders (1998), and the LSU and LSU-SSU combined analyses additionally supported a monophyletic grouping of the Acrochaetiales and Palmariales, with the Nemaliales sister to this assemblage. Our alignments included representatives of three palmariacean families—Palmariaceae (*Palmaria*), Rhodophysemales (*Rhodophysemataceae* (*Rhodophysemataceae*),

and Rhodothamniellaceae (*Camontagnea*)—each of these having been previously classified in other florideophyte orders (Rhodymeniales, Gigartinales, and Acrochaetales/Ceramiales, respectively). All analyses variously supported a monophyletic Palmariales, a result that is consistent with previously published SSU phylogenies (Saunders et al. 1995, Saunders and Kraft 1996, Harper and Saunders 1998, Saunders 2001).

Lineage 3. The Ahnfeltiales has been considered distinct since its removal from the Gigartinales by Maggs and Pueschel (1989), and the order remains monogeneric with only three recognized species (Maggs et al. 1989). Members of the Ahnfeltiales are unique among the florideophyte lineages in that their pit plugs are naked. Previously published SSU-based analyses (Ragan et al. 1994, Saunders and Bailey 1997) were unable to resolve the affinities of the Ahnfeltiales, and it was considered the sole member of lineage 3 (Saunders and Bailey 1997). More recently, Saunders and Bailey (1999) found increased support for the Ahnfeltiales as sister to lineage 4, and all our analyses have echoed this result.

Lineage 4. This large grouping of florideophyte taxa was supported in all our analyses. With the exception of the Gelidiales, whose members' pit plugs possess a cap, the pit plugs of lineage 4 members lack caps and possess a membrane. Previously published SSU-based phylogenies for lineage 4 taxa have established that nearly all recognized orders are monophyletic but have provided little resolution among the included orders (Saunders and Kraft 1994, 1996, Saunders et al. 1999). Analyses of all our data sets have provided similar results. With the exception of the Gigartinales, the monophyly of each of the other lineage 4 orders was supported by the analyses of LSU-only and LSU-SSU combined data. Analyses of the SSU-only data, however, failed to resolve a monophyletic Halymeniales. In the case of the Halymeniales, the lack of support probably resulted from reduced taxon sampling relative to the published SSU investigations that resolved this order as monophyletic (Saunders and Kraft 1996, 1997).

As indicated, members of the Gigartinales were scattered about lineage 4, showing various affiliations in all analyses. *Peyssonnelia*, *Predaea*, *Sarcodia*, *Schimmelmannia*, *Schmitzia*, and *Schizymenia* failed to resolve within a monophyletic Gigartinales, which is consistent with recent SSU-only phylogenies (Saunders and Kraft, unpublished data). The Gigartinales have long been recognized as a heterogeneous group within lineage 4, and molecular results are now uncovering exactly how much diversity exists within this group. Further detailed examinations including representatives of all gigartinean families will hopefully elucidate the relationships among these taxa.

The LSU-only and combined LSU-SSU analyses resolved an interesting relationship within lineage 4—a monophyletic clade containing the Ceramiales, Gracilariales, and Rhodymeniales, the latter two receiving moderate to strong bootstrap support. This novel relationship has not been elucidated using any other traditional or molecular data and is in contrast with the affinities

suggested between the Halymeniales and the Rhodymeniales by our SSU phylogenies and those of Saunders and Bailey (1997) and Saunders and Kraft (1996, 1997). This association in the current analyses is probably an artifact of low taxon sampling and the relatively long branches leading to the taxa in the Gracilariales and Rhodymeniales.

The multitude of unresolved relationships within lineage 4 is not entirely unexpected. Almost all extensive molecular phylogenies for members of this lineage have failed to resolve relationships between the included orders (Freshwater et al. 1994, Ragan et al. 1994). Thus, as in lineage 2, the higher level relationships within lineage 4 remain equivocal. Perhaps acquiring additional LSU sequence data for members within these lineages will improve resolution. Alternatively, the relationships among these orders may prove unsolvable using molecular data, a situation that could have arisen because of periods of rapid evolutionary radiation.

Our results indicate that the LSU is generally consistent with the SSU for resolving supraordinal and some ordinal relationships within the Florideophyceae. As such, the LSU provides confirmation for earlier results generated with the SSU. At the intraordinal and intrafamilial level, the SSU has generally provided little phylogenetic signal (Vis et al. 1998, although see Saunders et al. 1999 for an exception). On the other hand, the *rbcL* has worked reasonably well at this level, particularly among more closely related species (Fredericq and Ramírez 1996, Fredericq et al. 1996). Our results (Harper and Saunders 2000, 2001) and those of our peers (Vis et al. 1999, Dalen and Saunders 2000, Nicki and Saunders 2000) indicate that the LSU will provide a bridge between the phylogenetic resolution of the SSU and *rbcL* systems, and this may prove to be the LSU's most significant contribution to red algal systematics.

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