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The molecular systematics of some agar- and carrageenan-containing marine red algae based on *rbc*L sequence analysis

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Abstract

A hypothesis of phylogenetic relationships inferred by parsimony analysis of plastid-encoded *rbc*L sequences is presented for red algae containing agar- and carrageenan-like phycocolloids; *rbc*L encodes the large subunit of ribulose 1,5 bisphosphate carboxylase/oxygenase. Previous studies have shown that Floridean families that contain sulfated galactans as cell wall components are resolved as a monophyletic clade sister to the agarophyte order Ahnfeltiales. Families that have been identified as containing kappa-type carrageenans, often in addition to lambda-type carrageenans, are resolved in three clades: (1) a complex containing the families Solieriaceae, Cystocloniaceae, Hypneaceae, Caulacanthaceae, Tichocarpaceae, Furcellariaceae, and the genera *Turnerella* and *Opuntiella*; (2) the Gigartinaceae and Phyllophoraceae, and (3) the genus *Endocladia.* Except for *Tichocarpus* and *Endocladia*, these are all members of the Gigartinales *sensu* Kylin (1956). Most of the families previously placed in the Cryptonemiales by Kylin appear to contain only lambda-type carrageenans. These fall into two groups, one that clusters with typical carrageenophyte- and the other with typical agarophyte-taxa. The first of these includes the families Polyideaceae, Kallymeniaceae, Dumontiaceae, and Rhizophyllidaceae. The second includes the type family of the Cryptonemiales, the Halymeniaceae, which is divisible into two well-supported clades, one of which possesses special lambda-like carrageenans, the aeodans, and the Schizymeniaceae, a recently created family containing two former gigartinalean genera. The agarophyte orders Gelidiales, Gracilariales and Ceramiales are well resolved as monophyletic clades, but their topological positions are poorly resolved. The Gelidiales and Ceramiales are associated, but the Gracilariales is included in a clade that contains families belonging to the Rhodymeniales, a possible agarophyte order. Finally, two groups that may contain lambda-like carrageenans, the Plocamiales and the Sarcodiaceae, are resolved as separate clades.

Introduction

The cell walls of red algae consist of skeletal fibers composed primarily of β-linked glycans embedded in a matrix of nonfibrillar materials, the phycocolloids. The most abundant of the cell wall matrix substances are the sulfated galactans, referred to commonly either as agars (agarans) or carrageenans (Craigie, 1990). Except for the Bangiales, sulfated galactans occur primarily in the order Ahnfeltiales and in Florideophycidae having pit plugs that possess cap membranes but lack typical cap layers, with the exception of the Gelidiales, which has one, inner cap layer (Pueschel, 1989). The latter group, somtimes referred to as the higher Florideophycidae, forms a monophyletic clade sister to the Ahnfeltiales in molecular phylogenies based on nuclear-encoded small-subunit rRNA (Ragan et al., 1994) and plastid-encoded *rbc*L (Freshwater et al., 1994). The agaran group of galactans, defined by the presence of a backbone of alternating (1 →

4) linked α-L-galactopyranose and (1 → 3) linked β-D-galactopyranose residues, is recorded from the families Bangiaceae (Bangiales), Gelidiaceae (Gelidiales), Gracilariaceae (Gracilariales), and Ceramiaceae, Delesseriaceae and Rhodomelaceae (Ceramiales; Craigie, 1990). *Gloiopeltis* is an example of an agarophyte genus that is presently placed in a carrageenophyte family, the Endocladiaceae. The carrageenan group, defined as containing a backbone of repeating (1 → 4) linked α-D-galactopyranose and (1 → 3) linked β-D-galactopyranose residues, has been found primarily in families traditionally placed in the Gigartinales (Craigie, 1990). All the genera and species formerly placed in the Rhodymeniales in which the cell wall matrix was characterized as possessing linear polysaccharides composed of β-D-xylopyranose residues having (1 → 4) and (1 → 3) linkages have since been transferred to the order Palmariales. The phycocolloids of typical Rhodymeniales and traditional Cryptonemiales are not so well known. They have sometimes been characterized as agars, sometimes as carrageenans, and sometimes as mixed or hybrid-agar/carrageenan types (Usov, 1992; Usov & Klochkova, 1992; Chopin et al., 1994).

In the present study we investigate the phylogeny of Florideophycideae that contain sulfated galactans in their cell walls using a parsimony analysis of plastid encoded *rbc*L sequences. Particular attention is paid to taxa that have recently been shown to contain λ-carrageenans.

Material and methods

Methods of sample preparation, DNA extraction and *rbc*L sequencing were as previously described (Freshwater & Rueness, 1994; Hommersand et al., 1994; Fredericq & Ramírez, 1995). The percent of the 1467 base pair (bp) *rbc*L coding region produced for the species analyzed is given in Table 1. Because nearly all sequences used in this study were incomplete at the 5′ coding region of the gene, analyses were restricted to the last 1332 bp of the *rbc*L coding region. Sequences were manually aligned and analyzed for phylogenetic reconstruction with the maximum parsimony method using the computer programs PAUP 3.1.1 (Swofford, 1993) and MacClade 3.0 (Maddison & Maddison, 1992).

Table 1. Distribution of informative characters (percent of total), steps (percent of total), and consistency index (CI) across the 1332 base pairs of *rbc*L sequence data for 57 red algal species.

Nucelotide sites	Informative characters (%)	Steps (%)	CI
1–111	7.9	6.9	0.23
112–222	9.0	8.8	0.22
223–333	8.0	7.9	0.24
334–444	9.0	9.5	0.21
445–555	7.7	8.1	0.16
556–666	10.3	10.6	0.22
667–777	8.8	8.8	0.19
778–888	7.3	7.6	0.20
889–999	10.4	11.9	0.21
1000–1110	7.1	8.2	0.19
1111–1221	9.3	7.3	0.28
1221–1332	5.2	4.4	0.22

Global analysis

Fifty-seven species were analyzed using a multiple heuristic search strategy done under the Fitch criterion of equal weights for all substitutions (Fitch, 1971). Initial searches consisted of 500 random sequence additions, MULPARS (but holding only five trees at each step), STEEPEST DESCENT and nearest-neighbor interchange (NNI) branch swapping algorithm to increase the likelihood of swapping within the 'island' of trees leading to the most parsimonious solution (Maddison, 1991). Trees found in these initial searches were then used as starting points for further searches with MULPARS and tree bisection-reconnection (TBR) algorithm until swapping was complete. Final searches were done by swapping to completion with MULPARS, STEEPEST DESCENT and TBR on the minimal trees resulting from the previous searches and on all trees less than or equal to two steps less parsimonious than minimal. As measures of internal support, both bootstrap resampling (Felsenstein, 1985) and decay index (Bremer, 1988) analyses were done. Bootstrap values were determined by performing 120 bootstrap resampling iterations with heuristic searches using simple sequence additions, MULPARS and TBR. Decay indices representing the number of steps less parsimonious than minimal at which branches were no longer resolved were determined based on strict consensus analysis of cladograms found by relaxing parsimony sequentially, one step at a time, up to five steps.

Halymeniaceae/Schizymeniaceae and Plocamiales/Sarcodiaceae analyses

Seventeen (Halymeniaceae/Schizymeniaceae) and sixteen (Plocamiales/Sarcodiaceae) species were analyzed using a multiple heuristic search strategy as described for the global analysis except that initial searches consisted of 500 random sequence additions, MULPARS (but holding only 10 trees at each step), STEEPEST DESCENT and TBR. Bootstrap values were determined based on 320 resampling iterations with heuristic searches using simple sequence additions, MULPARS and TBR. Decay indices were based on strict analysis of cladograms found by relaxing parsimony sequentially up to five steps.

Results

The global analysis data set included 57 species representing 31 families and eight currently recognized orders. It contained 535 informative sites (40%). Based on phylogenetic hypotheses derived from earlier analyses, the Ahnfeltiales was chosen as the outgroup (Freshwater et al., 1994; unpublished results). Tree-lengths of 100 000 randomly generated trees from these data had a skewed distribution ($g_1 = -0.431$, $p < 0.001$) indicating the presence of nonrandom structure (Hillis & Huelsenbeck, 1992; Hillis et al., 1993). Examination of this data set shows that the number of informative characters is evenly distributed throughout the analyzed sequence (Table 1). Likewise, homoplastic nucleotide site mutations are uniformly distributed with CI values for ten of twelve 111 bp segments ranging from 0.19 to 0.24 with two extremes of 0.16 and 0.28 (Table 1). The distribution of homoplasy among codon positions is also very uniform, CI = 0.19 to 0.23, even though the majority of steps (77.9%) occur at third positions (Table 2).

Heuristic searches resulted in a single minimal tree of length = 4315, consistency index (CI) = 0.213 and retention index (RI) = 0.384, excluding uninformative characters (Figure 1). Bootstrap and decay values support some of the resolved relationships but are in conflict for others. Orders and families represented by multiple species are generally resolved as monophyletic assemblages, although the Phyllophoraceae, Dumontiaceae and Rhodymeniales are not. Families currently placed in the Gigartinales *sensu lato* (Kraft & Robins, 1985) are not a monophyletic assemblage nor are the families formerly placed in the Cryptonemiales (Kylin, 1956).

Table 2. Percent of total steps and consistency index (CI) for codon positions of the 1332 base pairs of *rbc*L sequence data for 57 red algal species.

Codo position	Steps (%)	CI
1	17.9	0.19
2	4.3	0.23
3	77.9	0.22

Two smaller searches were done in order to expand portions of the global tree. One of these analyses included sequence data from six Sarcodiaceae species and eight species of the newly established Plocamiales (Saunders & Kraft, 1994). One species each from the Gelidiales and Gracilariales were chosen for the outgroup. This data set contained 290 informative sites and tree length distributions of randomly generated trees indicated the presence of nonrandom structure (100 000 trees, $g_1 = -0.688$, $p < 0.01$). Heuristic searches resulted in a single minimal tree of length = 671, CI = 0.577 and RI = 686 (Figure 2). Both the Plocamiales (d > 5; bootstrap = 100%) and Sarcodiaceae (d > 5; bootstrap = 95%) are well supported monophyletic assemblages. The relationships of species within the Sarcodiaceae clade are very well supported while resolution within the Plocamiales clade is minimal.

An analysis was also done that included nine species representing five Halymeniaceae genera along with four *Schizymenia* and one *Titanophora* species. Based on the global tree topology, three Ceramiales species were chosen for the outgroup. This data set contained 321 informative sites and the distribution of lengths of randomly generated trees from these data indicated the presence of nonrandom structure (100 000 trees, $g_1 = -0.709$, $p < 0.01$). Heuristic searches resulted in a single minimal tree of length = 803, CI = 0.540 and RI = 0.583 (Figure 3). The Halymeniaceae is resolved as a monophyletic group with two lineages. The species of *Schizymenia* and *Titanophora* are also resolved within a single clade. Monophyly of both the Halymeniaceae and Schizymeniaceae is supported in this analysis (decay = d5 or greater; bootstrap = 71% and 88%, respectively).

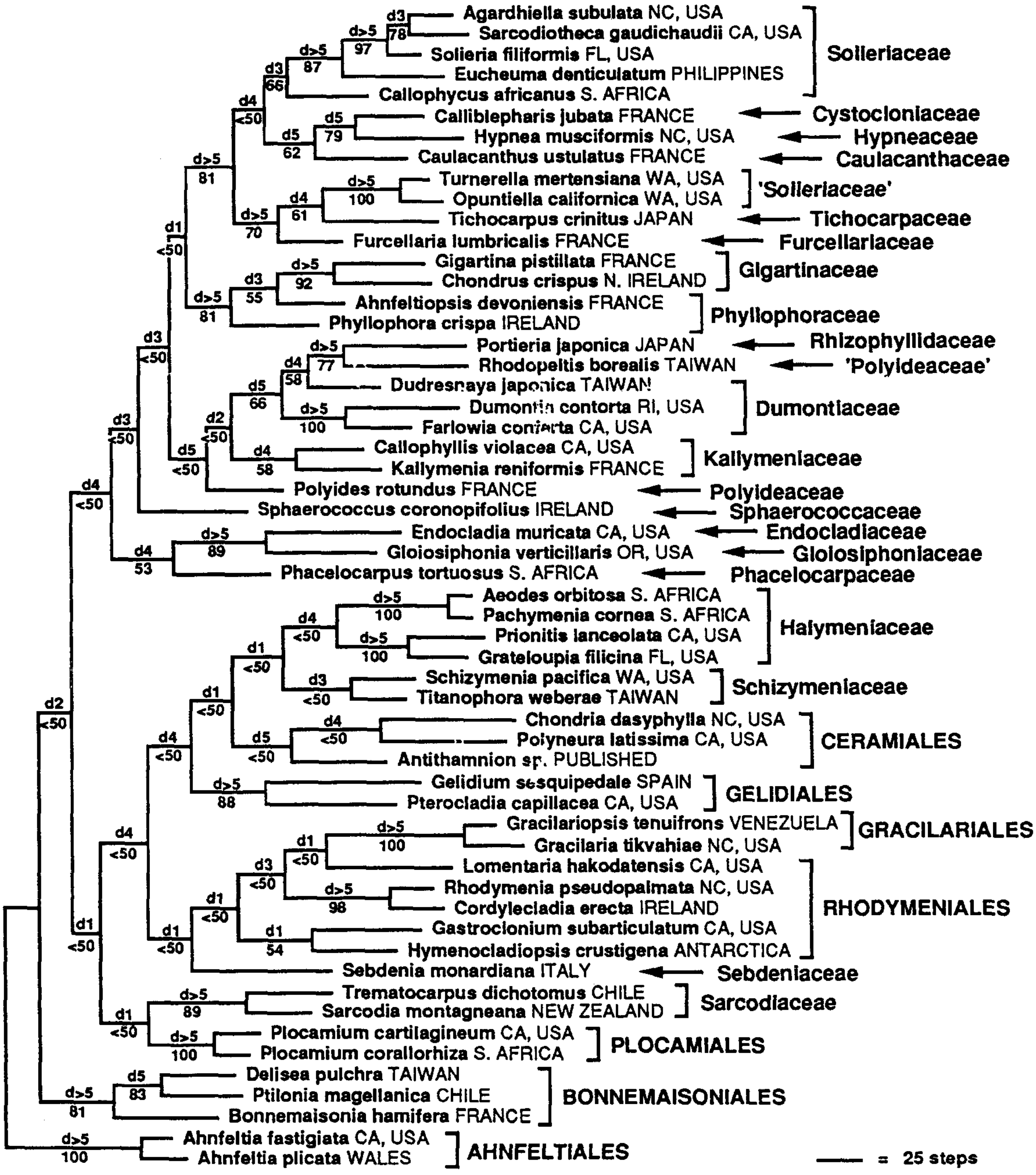

Figure 1. Single maximum parsimony tree of length = 4315, CI = 0.213 and RI = 0.384, resulting from a multiple heuristic search analysis of *rbc*L sequence data from 57 red algal species. Decay indices are indicated above and bootstrap values (%) below branches. Branch lengths are proportional to estimated mutational steps.

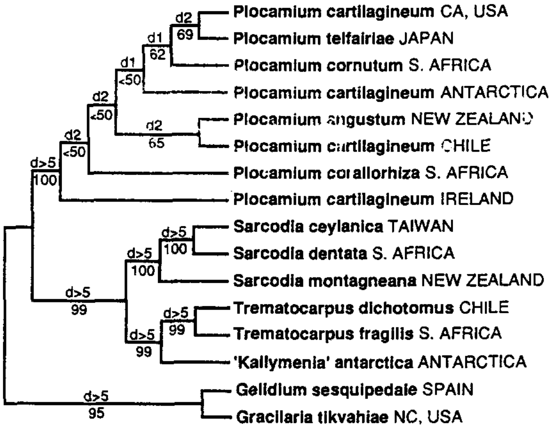

Figure 2. Single maximum parsimony tree of length = 671, CI = 0.577 and RI = 0.686, resulting from a multiple heuristic search analysis of *rbc*L sequence data from eight Plocamiales, six Sarcodiaceae and two outgroup species. Decay indices are indicated above and bootstrap values (%) below branches.

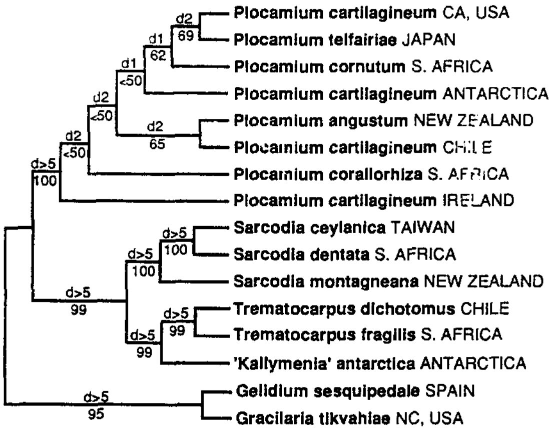

Figure 3. Single maximum parsimony tree of length = 803, CI = 0.540 and RI = 0.583, resulting from a multiple heuristic search analysis of *rbc*L sequence data from nine Halymeniaceae, five *Schizymeniaceae* and three Ceramiales (as outgroup) species. Decay indices are indicated above and bootstrap values (%) below branches.

Discussion

The *rbc*L tree shown in Figure 1 contained 57 taxa selected, wherever possible, to include at least two representatives of each family. Orders and families represented by multiple species were generally resolved as monophyletic clades; however, the topological position of some families, family clusters and orders received little support. The Bonnemaisoniales occupies a basal position next to the outgroup order Ahnfeltiales. Each of the agarophyte orders Gracilariales, Gelidiales, and Ceramiales are well resolved as monophyletic clades, but their topological positions in the overall tree are poorly resolved. The Gelidiales and Ceramiales are associated, and the Gracilariales is included in a clade that contains families belonging to the Rhodymeniales. Families that have traditionally been reported to contain carrageenans (Craigie, 1990) are resolved in two clades, one centered around the Gigartinaceae and the other around the Furcellariaceae and Solieriaceae in Figure 1. All except *Tichocarpus* were placed in the order Gigartinales by Kylin (1956). The remaining families, some of which were placed in the Gigartinales and others in the Cryptonemiales by Kylin, are clustered either with the agarophytes or next to the carrageenophytes in Figure 1.

A phylogeny for red algae that contain sulfated galactans will require an understanding of the biosynthetic pathways that lead to formation of agarans, carrageenans, or mixtures of the two. The first step in the biosynthesis of these compounds is generally thought to be the formation of a regular polymer backbone having either alternating L and D residues (agarans) or containing all D residues (carrageenans). The second step is hypothesized to be the sulfation and methylation of several hydroxyl groups, with the final step being the enzymatic elimination of sulfate from position 6 on some 4-O-linked α-galactose residues to produce 3,6-anhydro-α-galactose units (McCandless & Craigie, 1979; Rees et al., 1982; Usov, 1992). According to this sequence of biosynthetic reactions, porphyrans are biogenetic precursors of agarose; a specific precursor produces either λ- or μ-carrageenan by a sulfotransferase reaction, and μ- and ν-carrageenans are precursors of κ- and ι-carrageenans (Craigie & Wong, 1979; Usov, 1992). In practice, the identification of red algal taxa as belonging either to the agaran or carrageenan group has rested primarily on the identification of 4-O-linked 3,6-anhydro-α-galactose residues as either L (agarans) or D (carrageenans). Methods have included partial hydrolysis to generate disaccharide repeats and characterization by retention time using gas-liquid chromatography and non-destructive procedures such as IR or ^{13}C-NMR spectroscopy (Whyte et al., 1985; Usov, 1992). ^{13}C-NMR spectra have been used not only to discriminate between agaran and carrageenan groups but to identify κ- and ι-carrageenans and their presumed biosynthetic precursors μ- and ν-fragments in hybrid carrageenans and to distinguish between κ- and λ-carrageenans and desulfated κ- and λ-carrageenans (Usov, 1984).

Gelling-strength agar is a major component in the orders Ahnfeltiales, Gelidiales and Gracilariales, and may be present in significant amounts in some Ceramiales (Craigie, 1990; Usov, 1992). Small amounts of agarobiose have been reported as agarobiitol following reductive hydrolysis in *Rhodymenia pertusa* (Postels et Ruprecht) J. Agardh, a typical member of the Rhodymeniales (Usov & Klochkova, 1992). This is the first evidence that the Rhodymeniales may belong in the agaran family. Agarobiitol has also been found in the Dumontiaceae and some members of the Kallymeniaceae, two families previously placed in the Cryptonemiales (Usov & Klochkova, 1992). These are in addition to earlier reports of the presence of mixed or hybrid agarans and carrageenans in *Grateloupia divaricata* Okamura (Halymeniaceae, Cryptonemiales) and *Endocladia muricata* (Postels et Ruprecht) J. Agardh (Endocladiaceae, Cryptonemiales). Mixed or hybrid agarans/carrageenans are probably even more common than reports in the literature would indicate (Craigie, pers. comm.). In as much as agarans are found in the primitive order Bangiales and occur in the outgroup order Ahnfeltiales, it is possible that they represent the primitive condition among the higher Florideophycideae, and agarophyte taxa may be ancestral to taxa that contain carrageenan in their cell walls. The presence of small amounts of agarobiose in groups that are known primarily as carrageenophyte taxa affords further evidence supporting this opinion.

Phycocolloids belonging to the carrageenan group are often heterogeneous and complex, with several carrageenan types present in the same or in different life history stages of a species (McCandless, 1978; Craigie, 1990). Different carrageenans have traditionally been designated by letters from the Greek alphabet. Recently Knutsen et al. (1994) proposed a modified system in which the different disaccharide repeating units are given names and letter codes. The older system using Greek letters is followed here. Carrageenans have, for the most part, been designated as members of either the kappa or lambda group. Kappa-type carrageenans may have galactose units esterified with sulfate at the 4-position, whereas lambda-type carrageenans do not (McCandless, 1978; Usov, 1992). Kappa and iota carrageenans contain significant amounts of 3,6-anhydro-D-galactose residues and can undergo thermally reversible gelation in the presence of K^+ and certain other cations, whereas lambda-type carrageenans contain smaller amounts of 3,6-anhydro-D-Galactose residues, or none, and will not gel (Rees et al., 1982; Craigie, 1990).

Lambda carrageenan was originally characterized from cell wall polyaccharides obtained from tetrasporophytes of members of the Gigartinaceae. Lambda-like carrageenans is a term used by some authors to refer to any highly viscous, non-gelling polysaccharide that may contain carrageenan, xyloglycan or, in some cases, agaran residues. As such, it is a structurally heterogeneous assemblage that exhibits a variety of sulfation patterns, sugar types, and linkage patterns. An unusual λ-like carrageenan composed predominantly of $(1 \rightarrow 3)$-linked-D-galactopyranose units with sulfation at carbon 4 was reported by Whyte et al. (1984) from *Schizymenia* (Schizymeniaceae) and *Plocamium* (Plocamiaceae) based on infrared, methylation and gas chromatographic analyses. More recently, λ-like carrageenans have been reported to be present in the Sarcodiaceae (Liao et al., 1993) and also in the Halymeniaceae and the Kallymeniaceae (Usov, 1992; Chopin et al., 1994).

We have included additional taxa for the Sarcodiaceae and Plocamiaceae in Figure 2. The Sarcodiaceae is largely a Southern Hemisphere family containing two genera, *Sarcodia* and *Trematocarpus*. *Kallymenia antarctica* is misplaced in the Kallymeniaceae and clearly belongs in *Sarcodia* based on morphological (Lamb & Zimmerna, 1977; Fredericq, pers. obs.) and molecular evidence. Accepting *Kallymenia antarctica* as a *Sarcodia*, the generic distinction between broad, largely subtidal species *(Sarcodia)* and narrow, dichotomously branched, largely intertidal species *(Trematocarpus)* is not supported. *Sarcodia dentata* (Suhr) R.E. Norris from the east coast of South Africa is resolved as sister to the tropical species *S. ceylanica* Harvey from the Western Pacific and Indian Oceans, and both are distinct from the type species, *S. montagneana* (Hooker et Harvey) J. Agardh, from New Zealand. The Plocamiaceae with two genera, *Plocamium* and *Plocamiocolax*, has recently been elevated to ordinal status (Saunders & Kraft, 1994). Relationships among the small number of *Plocamium* species sampled are fully resolved in the minimal *rbc*L tree but are not well supported (Figure 2). Although no particular species distribution pattern is evident from this limited data set, the use of *Plocamium cartilagineum* (L.) Dixon for taxa from different parts of the world is not supported.

Figure 3 expands the number of taxa in the Schizymeniaceae and Halymeniaceae, families that are resolved together in the overall tree (Figure 1).

The Schizymeniaceae was recently elevated to family rank by Masuda & Guiry (1995) to include *Schizymenia, Platoma* and, perhaps, *Titanophora.* The inclusion of *Titanophora* in the Schizymeniaceae is supported by decay and bootstrap analysis of the *rbc*L data (Figure 3). Like *Plocamium, Schizymenia* appears to contain λ-like carrageenans based on infrared spectra and methylation analysis (Whyte et al., 1984). The Halymeniaceae is resolved as a monophyletic group with two lineages, both of which are reported to contain λ-like carrageenans. The one that includes *Aeodes* possesses special carrageenans referred to as aeodans, which differ from typical λ-carrageenans in that all of the sulfated groups are located on the 3-linked residues and are, therefore, stable to alkaline treatment (Chopin et al., 1994). While the first group has a world-wide distribution, the *Aeodes*-group is found primarily in the Southern Hemisphere in Australia, New Zealand, southern Africa, southern South America and islands of the West Wind Drift. An *Aeodes* group was first identified by Chiang (1970), who distinguished this assemblage on the basis of the type of auxiliary cell ampulla. Whereas most Halymeniaceae possess auxiliary cells with few ampullary filaments composed of relatively long cells, in the *Aeodes* group the auxiliary cells bear up to five orders of ampullary filaments composed of short, ovoid cells (Chiang, 1970; Womersley, 1994). The *Aeodes* group of Chiang includes *Aeodes, Pachymenia, Polyopes*, and *Dermocorynus occidentalis* Hollenberg from Southern California.

The large clade situated in the upper half of Figure 1 appears to contain only carrageenophyte taxa. Included are moderately well to well-supported clades that contain the families: (1) Phacelocarpaceae, Gloiosiphoniaceae, and Endocladiaceae; (2) the Sphaerococcaceae; (3) the Polyideaceae, Kallymeniaceae, Dumontiaceae and Rhizophyllidaceae; (4) the Gigartinaceae and Phyllophoraceae, and (5) the Furcellariaceae, Tichocarpaceae, Caulacanthaceae, Hypneaceae, Cystocloniaceae and Solieriaceae. Not all of the taxa in this part of the tree contain κ-type carrageenans; however, it appears as though all taxa known to contain carrageenans belonging to the kappa group, defined as containing 3-linked galactans esterified with sulfate at the 4-position, reside in this clade. The cell wall composition of the Gloiosiphoniaceae, Phacelocarpaceae, and Sphaerococcaceae is unknown; however, the Nizymeniaceae, a family thought to be related to the Phacelocarpaceae, has been reported to contain branched, highly sulfated xylogalactans having properties similar to λ-like carrageenans as major cell wall components (Chiovitti et al., 1995). *Endocladia* is peculiar in that it is reported to contain desulfated κ-carrageenan (β-carrageenan) together with 6% agarobiose (Stancioff & Stanley, 1969; Whyte et al., 1985).

With the recent report of low yields of λ-carrageenan having high sulfate content in *Kallymenia westii* Ganesan (Chopin et al., 1994) and *K. reniformis* (Turner) J. Agardh (Deslandes et al., 1990) and the presence of λ-like carrageenans in *Dumontia* and λ-like plus κ-type (μ) carrageenans in *Dilsea carnosa* (Schmidel) Kuntze (Zinoun et al., 1990), the clade containing the Kallymeniaceae and Dumontiaceae appears to have a special relationship to the traditional carrageenophytes. The presence of μ-carrageenan in *Dilsea* is interesting in that this compound is said to be a precursor in the biosynthetic pathway leading to κ-carrageenan (Usov, 1992). Although still undefined, the cell wall polysaccharides of *Polyides* and *Portieria* are thought to be λ-like carrageenans (Craigie, 1990).

The synthesis of κ-type carrageenans presumably requires additional biosynthetic steps not found in taxa that produce only λ-like carrageenans. It is possible that the biosynthetic pathway leading to κ-carrageenan evolved only once and that κ-type carrageenophytes are monophyletic. On the other hand, the *rbc*L tree in Figure 1 suggests that the Endocladiaceae forms a distinct line, and that the family complexes that comprise clades centered around the Gigartinaceae and Solieriaceae may represent independent evolutionary lines. The cell walls of members of the Gigartinaceae and Phyllophoraceae contain κ-type carrageenans in the gametophytic phase and λ-carrageenans in the tetrasporophytic phase of the life cycle (McCandless, 1978), whereas families belonging to the Solieriaceae complex often contain a heterogeneous assemblage of carrageenans, with the same types occurring in both gametophytic and tetrasporophytic phases (Craigie, 1990).

We have previously shown that the Gigartinaceae forms a monophyletic clade containing seven described genera found primarily in temperate regions of the world (Hommersand et al., 1994). The related family Phyllophoraceae contains the genus *Mastocarpus* in addition to genera traditionally placed in this family. Accordingly, the Petrocelidaceae with one genus, *Mastocarpus*, resides within the Phyllophoraceae (Fredericq & Ramíez, 1996).

The cluster in Figure 1 that contains the Solieriaceae is subdivided into two clades, one centered around *Furcellaria* and the other around *Caulacanthus.*

One of these includes the families Furcellariaceae, Tichocarpaceae and two closely related genera, *Opuntiella* and *Turnerella*, which are currently misplaced in the Solieriaceae. The cell walls of *Furcellaria* and *Tichocarpus* both contain κ/β-hybrid carrageenans (Craigie, 1990; Usov, 1992). Kylin (1956) placed the Tichocarpaceae in the Cryptonemiales because the carpogonial branch and auxiliary cell were said to be borne together (procarpic condition) on an 'accessory' branch; that is, a branch having an adventitious origin. In *Furcellaria, Turnerella* and *Opuntiella*, in contrast, the auxiliary cell is an ordinary vegetative cell that is remote from the carpogonial branch (non-procarpic condition). These genera were placed by Kylin (1956) in the Gigartinales. Linkage between the carpogonium and the auxiliary cell takes place by means of connecting filaments in these genera. The evolution of *Tichocarpus* from a *Furcellaria*-like ancestor could have involved the loss of connecting filaments and the transformation of a cell born in a special, adventitious filament adjacent to the carpogonium into an auxiliary cell. Similar-appearing adventitious filaments occur in *Opuntiella* and *Rissoella*, but do not, however, contain auxiliary cells.

The assemblage that includes the Caulacanthaceae, Cystocloniaceae, Hypneaceae and Solieriaceae share a suite of characters in common. All possess auxiliary cells in which the gonimoblasts develop inwardly from a single gonimoblast initial, and all possess zonately divided tetrasporangia (Kylin, 1956). This group, along with the Furcellariaceae, was previously identified in an *rbc*L tree as a sister taxon to the Gigartinaceae complex (Freshwater et al., 1994); however, their sister relationship is only weakly supported in the present analysis (Figure 1). Cell wall phycocolloids are heterogeneous and complex in this assemblage and include a range of κ- and κ/β-types (Craigie, 1990; Liao et al., 1993).

The results reported here are preliminary and require confirmation. Even so, they lend support to the suggestion originally expressed by Stoloff and Silva (1957) that the water soluble polysaccharides may be significant for the classification of the red algae at higher taxonomic levels. We foresee a time when a phylogeny based on the chemistry and biosynthetic pathways of red algal phycocolloids and that based on molecular and morphological characters agree.

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Appendix

Species, authorities, collection information, percent of *rbc*L sequence produced and GenBank accession numbers (see Benson et al., 1994) for taxa analyzed in this study.

Aeodes orbitosa (Suhr) Schmitz, Blaubergstrand, Table Bay, Cape Province, South Africa, coll. M. H. Hommersand, 16.vii.93, 59% (U21599)

Agardhiella subulata (C. Agardh) Kraft et Wynne, Federal Basin, New Hanover Co., NC, USA, coll. D. W. Freshwater, iii.91, 96% (U04176)

Ahnfeltia fastigiata (Postels et Ruprecht) Makiendo, Bodega Head, Sonoma Co., CA, USA, coll. M. H. Hommersand, 23.v.92, 88% (U04167)

Ahnfeltia plicata (Hudson) Fries, Broadhaven, Pembrokeshire, Wales, coll. C. A. Maggs, 9.ii.93, 91% (U04168)

Ahnfeltiopsis devoniensis (Greville) Silva et DeCew, Ile Vêrte, Roscoff, Brittany, France, coll. J. Cabioch, 22.vi.93, 86% (U21697)

Antithamnion sp. published sequence (Kostrzewa et al., 1990)

Bonnemaisonia hamifera Hariot, Penmarch, Brittany, France, coll. M. H. Hommersand, 21.vi.93, 74% (U04044)

Calliblepharis jubata (Goodenough et Woodward) Kuetzing, Penmarch, Brittany, France, coll. M. H. Hommersand, 21.vi.93, 80% (U04189)

Callophycus africanus (Schmitz) Hewitt, Palm Beach, Natal, South Africa, coll. M. H. Hommersand, 23.vii.93, 91% (U21591)

Callophyllis violacea (J. Agardh) Kylin, Pigeon Point, San Mateo Co., CA, USA, coll. M. H. Hommersand, 21.xii.92, 90% (U04191)

Carpopeltis maillardii (Montagne et Millardet) Chiang, Magang, Taiwan, coll. S. Fredericq, 11.viii.93, 51% (U22097)

Caulacanthus ustulatus (Turner) Kuetzing, Carantec, Brittany, France, coll. J. Cabioch, 22.vi.93, 68% (U04188)

Chondria dasyphylla (Woodward) C. Agardh, Bogue Sound, Carteret Co., NC, USA, coll. D. W. Freshwater, iv.90, 95% (U04021)

Chondrus crispus Stackhouse, Bally Castle, Co. Antrim, N. Ireland, coll. C. A. Maggs, 20.i.92, 92% (U02984)

Cordylecladia erecta (Greville). J. Agardh, Black Head, Co., Clare, Ireland, coll. M. D. Guiry, 64% (U21638)

Delisea pulchra (Greville) Montagne, Magang, Taiwain, coll. S. Fredericq, 2.viii.93, 83% (U21706)

Dudresnaya japonica Okamura, Ye Liu, Taiwan, depth 3–6 m, coll. S. Fredericq & S.-M. Lin, 7.vii.94, #SF-7-7-94-1-1, 80% (U21691)

Dumontia contorta (S. G. Gmelin) Ruprecht, Manomet Bluffs, Plymouth Co., MA, USA, coll. M. H. Hommersand, 23.iv.93, 85% (U26823)

Endocladia muricata (Postels et Ruprecht) J. Agardh, Bodega Head, Sonoma Co., CA, USA, coll. M. H. Hommersand, 23.v.92, 81% (U04193)

Eucheuma denticulatum (Burman) Collins et Hervey, Danajan Reef, northern Bohol, Philippine Islands, coll. D. F. Kapraun, i.92, 91% (U04177)

Farlowia conferta (Setchell) Abbott, Pigeon Pt, San Mateo Co., CA, USA, coll. M. H. Hommersand, 21.xii.92, 91% (U04213)

Furcellaria lumbricalis (Hudson) Lamouroux, Penmarch, Brittany, France, coll. M. H. Hommersand 21.vi.92, 81% (U04194)

Gastroclonium subarticulatum (Turner) Kuetzing, Pigeon Pt, San Mateo Co., CA, USA, coll. M. H. Hommersand, 21.xii.92, 88% (U04178)

Gelidium sesquipedale (Clemente) Thuret, Aramar, Asturias, Spain, coll. J. Rico, 98% (L22071)

Gigartina pistillata (S. G. Gmelin) Stackhouse, Santec, Brittany, France, coll. J. Cabioch, 6.iv.92, 90% (U03429)

Gloiosiphonia verticillaris Farlow, Seal Rock, Lincoln Co., OR, USA, coll. E. C. Henry, 24.v.93, 84% (U04196)

Gracilaria tikvahiae McLachlan, Fort Fisher, New Hanover Co., NC, USA, coll. D. W. Freshwater, iii.90, 90% (U04172)

Gracilariopsis tenuifrons (Bird et Oliveira) Fredericq et Hommersand, Araya Peninsula, Sucre, Venezuela, coll. D. W. Freshwater, v.90, 82% (U04171)

Grateloupia filicina (Lamouroux) C. Agardh, Sebastian Inlet, Indian River Co., FL, USA, coll. D. F. Kapraun, 27.iii.92, 86% (U04212)

Grateloupia longifolia Kylin, Swakopmund, Namibia, coll. M. H. Hommersand, 7.vii.93, 77% (U21597)

Hymenocladiopsis crustigena Moe, Pta Peñon, Bahía Fildes, King George I., South Shetland Is., Antarctic Peninsula, coll. S. Fredericq & J. Rodríguez, 9.ii.94, #SF-2-9-94-2-6 (U21640)

Hypnea musciformis (Wulfen) Lamouroux, Fort Fisher, New Hanover Co., NC, USA, coll. D. W. Freshwater, iii.90. 97% (U04179)

Kallymenia reniformis (Turner) J. Agardh, dredged, Pighet, Brittany, France, coll. J. Cabioch, 22.vi.93, 67% (U26827)

'Kallymenia' antarctica Hariot, Pta Collins, Bahía Fildes, King George I., South Shetland Is., Antarctic Peninsula, coll. S. Fredericq & J. Rodríguez, 15.ii.94, #SF-2-15-94-1-1, 97% (U21698)

Lomentaria hakodatensis Yendo, Berkeley Pier, Contra Costa Co., CA, USA, coll. M. H. Hommersand, 23.xii.92, 91% (U04180)

Opuntiella californica (Farlow) Kylin, Friday Harbor, WA, USA, coll. S. C. Lindstrom, x.93, 87% (U21589)

Pachymenia cornea (Kuetzing) Chiang, Buffles Bay, Cape Peninsula, South Africa, coll. M. H. Hommersand, 14.vii.93, 88% (U21588)

Pachymenia crassa Lindauer, Northwest Bay, Great I., Three Kings Is., New Zealand, coll. V. Staines, 18.i.94, 56% (U21598)

Pachymenia sp., Pta Peñon, Bahía Fildes, King Georges I., South Shetland Is., Antarctic Peninsula, coll. S. Fredericq & J. Rodríguez, 14.ii.94, #SF-2-14-94-1-7, 80% (U22096)

Phacelocarpus tortuosus Endlicher et Diesing, The Kowie, Port Alfred, Cape Provence, S. Africa, coll. M. H. Hommersand, 19.vi.93, 84% (U26826)

Phyllophora crispa (Hudson) Dixon, Spiddall, Co. Galway, Ireland, coll. M. D. Guiry, 7.iii.93, 91% (U02990)

Plocamium angustum (J. Agardh) Hooker f. et Harvey, Aramoara, Otago, New Zealand, coll. W. Nelson, 29.iii.94, 54% (U26821)

Plocamium cartilagineum (Linnaeus) Dixon, Spiddall, Co. Galway, Ireland, coll. M. D. Guiry, 28.ii.93, 90% (U21701); Pigeon Pt, San Mateo Co., CA, USA, coll. M. H. Hommersand, 21.xii.92, 90% (U04211); Bahía Elefante, King George I., South Shetland Is., Antarctic Peninsula, coll. S. Fredericq & J. Rodríguez, 5.ii.94, #SF-2-5-94-1-11, 90% (U26818); Isla Negra, Prov. San Antonio, Chile, coll. S. Fredericq & M. E. Ramírez, 26.i.94, #SF-1-26-94-2-4, 90% (U26817)

Plocamium corallorhiza (Turner) Harvey in Hooker et Harvey, The Kowie, Port Alfred, South Africa, coll. M. H. Hommersand, 19.vii.93, 89% (U21700)

Plocamium cornutum (Turner) Harvey, Buffles Bay, Cape Peninsula, South Africa, coll. M. H. Hommersand, 74% (U21704)

Plocamium telfairiae (Harvey) Harvey in Kuetzing, Tokawa, Choshi, Japan, coll. S. Fredericq & M. Yoshizaki, 2.ix.93, 68% (U21702)

Polyides rotundus (Hudson) Greville, Penmarch, Brittany, France, coll. M. H. Hommersand, 20.vi.93, 66% (U04214)

Polyneura latissima (Harvey) Kylin, Crissie Field, Fort Point, San Francisco Co., CA, USA, coll. M. H. Hommersand, 23.xii.92, 84% (U04022)

Portieria japonica (Harvey) Silva, Tokawa, Choshi, Chiba Pref., Japan, coll. M. Yoshizaki, 22.v.93, 85% (U26825)

Prionitis lanceolata (Harvey) Harvey, Pigeon Pt, San Mateo Co., CA, USA, coll. M. H. Hommersand, 21.xii.92, 85% (U04216)

Prionitis lyallii Harvey, Horseshoe Cove, Bodega Head, Sonoma Co., CA, USA, coll. M. H. Hommersand, 22.xii.92, 60% (U04217)

Pterocladia capillacea (S.G. Gmelin) Bornet et Thuret, La Jolla, San Diego Co., CA, USA, coll. R. A. Lewin, ii.92, 98% (U01896)

Ptilonia magellanica (Montagne) J. Agardh, Pta. Dungeness, Magellanes, Chile, coll. S. Fredericq & M. E. Ramírez, 18.ii.94, 88% (U21699)

Rhodopeltis borealis Yamada, Hwa Pin Yen, Hsiao Liuchiu I., Taiwan, coll. S. Fredericq & S.-M. Lin, 23.viii.93, 74% (U26824)

Rhodymenia pseudopalmata (Lamouroux) Silva, Wrightsville Beach, New Hanover Co., NC, USA, coll. D. W. Freshwater, vi.91, 68% (U04184)

Sarcodia dentata (Suhr) R. E. Norris, The Kowie, Port Alfred, South Africa, coll. M. H. Hommersand, 10.vii.93, 98% (U26816)

Sarcodia ceylanica Harvey ex Kuetzing, Long-Shia Dong, Hsiao Liuchiu I., Taiwan, coll. S. Fredericq, 23.viii.93, #SF-8-23-93-3-2, 97% (U26819)

Sarcodia montagneana (Hooker f. et Harvey) J. Agardh, Taipa, New Zealand, coll. W. Nelson, 2.xii.93, 88% (U21705)

Sarcodiotheca gaudichaudii (Montagne) Gabrielson, Pigeon Pt, San Mateo Co., CA, USA, coll. M. H. Hommersand, 21.xii.92, 80% (U04184)

Schizymenia dubyi (Chauvin ex Duby) J. Agardh, dredged, Pighet, Brittany, France, coll. J. Cabioch, 22.vi.93, 64% (U21592); Jodogahama, Miyako, Iwate-ken, Japan, coll. M. H. Hommersand & M. Yoshizaki, 10.vii.94, 60% (U21594)

Schizymenia obovata (J. Agardh) J. Agardh, Swakopmund, Namibia, coll. M. H. Hommersand, 6.vii.93, 64% (U21593)

Schizymenia pacifica (Kylin) Kylin, drfit, Whiffin Spit, Vancouver I., British Columbia, Canada, coll. S. C. Lindstrom, 15.iv.94, 73% (U21596)

Sebdenia monardiana (Montagne) Berthold, Lachea I., Catania, Italy, coll. G. Furnari & M. Cormaci, x.94, 79% (U21600)

Solieria filiformis (Kuetzing) Gabrielson, Harbor Branch, Indian River Co., FL, USA, coll. R. Lewis, x.92, 77% (U04185)

Sphaerococcus coronopifolius Stackhouse, Finavarra, Co. Clare, Ireland, coll. M. Guiry, 7.ii.93, 87% (U04218)

Tichocarpus crinitus (S. G. Gmelin) Ruprecht in Middendorff, Muroran, Hokkaido, Japan, coll. S. Fredericq, 6.ix.93, #SF-9-6-93-1-2, 87% (U21590)

Titanophora weberae Boergesen, Wai Hai Xiang, Taiwan, depth 12 m, coll. S. Fredericq & S.-M. Lin, 8.vii.94, #SF-7-8-94-1-1, 64% (U21708)

Trematocarpus dichotomus Kuetzing, Horcón, Prov. Valparaíso, Chile, coll. S. Fredericq & M. E. Ramírez, 27.i.94, #SF-1-27-94-1-3, 98% (U26814)

Trematocarpus fragilis (C. A. Agardh) DeToni, Buffles Bay, Cape Peninsula, South Africa, coll. M. H. Hommersand, 14.vii.93, 86% (U26815)

Turnerella mertensiana (Postels et Ruprecht) Schmitz, Juneau Alaska, USA, coll. S. C. Lindstrom, iv.94, 77% (U21707)