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Nuclear small-subunit ribosomal RNA gene sequences from representative Ceramiaceae (Ceramiales, Rhodophyta)

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Recently published phylogenetic trees derived from nuclear small-subunit (SSU) ribosomal RNA gene sequences indicate particularly curious relationships for two species of the Ceramiaceae: *Ceramium nodulosum* and *Griffithsia globulifera*. To test these earlier results we have determined the SSU sequences for *Anotrichium furcellatum, Ceramium macilentum, Crouania attenuata* and *Griffithsia monilis*, all members of the Ceramiaceae, as well as for *Gelidium pusillum* of the Gelidiales. These are included in a multiple alignment containing representatives of all the 'higher' florideophyte orders. Our results indicate that an SSU sequence previously published for *Griffithsia globulifera* differs by only five nucleotide changes from a published gene for *Gelidium vagum*, as well as from the gene of *Gelidium pusillum* sequenced by ourselves. The two species of *Gelidium* differ from one another at four sites. In sharp contrast, our data for *Griffithsia monilis* strongly ally this species with other representative Ceramiales. The only other published ceramiacean SSU sequence, that of *Ceramium nodulosum*, allies only weakly, if at all, with the representative gene sequences available for members of the Dasyaceae, Delesseriaceae and Rhodomelaceae (Ceramiales) in phylogenetic analyses. Addition of the *Ceramium macilentum* gene to the multiple alignment strongly indicates monophyly for the Ceramiales (excluding *G. globulifera*) in all the phylogenies generated from the molecular data. We conclude that the lack of strength for a monophyletic Ceramiales in previous reports is largely attributable to the effects of the *Ceramium nodulosum* sequence on phylogenetic analyses.

Key words: Ceramiales, *Ceramium, Gelidium, Griffithsia*, phylogeny, Rhodophyta, small-subunit rRNA.

Introduction

There is a widely held belief among phycologists that monophyly for the red algal order Ceramiales is virtually beyond dispute (Kraft, 1981; Bold & Wynne, 1985; Garbary & Gabrielson, 1990). Since Kylin (1928) removed the family Bonnemaisoniaceae, and with the exception of a minor disagreement over whether the *Sarcomenia* Group should be placed in the Rhodomelaceae (Womersley & Shepley, 1959; Womersley, 1965) or the Delesseriaceae (Wynne, 1969), the order's component families—the Ceramiaceae, Delesseriaceae, Dasyaceae and Rhodomelaceae—have largely changed only by the addition of newly discovered genera and tribes. All four families themselves are generally regarded as monophyletic, the one possible exception being the proposal of Hommersand (1963) that the Delesseriaceae, Dasyaceae and Rhodomelaceae have evolved from independent lineages within the Ceramiaceae (this would render the Ceramiaceae paraphyletic: cf. Garbary & Gabrielson, 1990).

Contrary to views based on vegetative and reproductive morphology recent molecular data have not provided strong support for a monophyletic Ceramiales. Phylogenies derived from small-subunit (SSU) ribosomal RNA gene sequences, in particular, have varied in the degree to which they challenge this doctrine. These range from indications that representative Ceramiaceae, i.e. *Ceramium nodulosum* (Lightfoot) Ducluzeau [= *C. rubrum* (Hudson) C. Agardh *fide* Maggs & Hommersand (1993)], are only weakly, if at all, allied to the other families of the Ceramiales (Rice *et al.*, 1991; Ragan *et al.*, 1994; Saunders & Kraft, 1994, 1996; Millar *et al.*, 1996), to assertions that seem totally counter-intuitive. An example of the latter is the SSU gene sequence reported for *Griffithsia globulifera* which differs at only five sites from that of *Gelidium vagum* (Ragan *et al.*, 1994).

Although in previous papers we have promoted the virtues of molecular data for red algal systematics (Saunders *et al.*, 1992; Saunders & Kraft, 1994), we have also cautioned against uncritically accepting the molecular as the final or definitive word on algal phylogeny. We have opted rather for a more holistic approach in which all the vegetative, reproductive and ultrastructural information is considered in addition to molecular inputs (Saunders & Kraft, 1994, 1996). It would appear, for the case of SSU genes determined for species of the Ceramiaceae at least, that more attention needs to be paid to reconciling morphological and molecular data.

The existence of such disparity prompts an apparently naive question: which is more correct in these instances, the traditional or molecular perspectives? Towards addressing this conundrum we have generated SSU

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Table 1. Sample information for species investigated in this study

Species collected	Sample location	Sample	Genbank
CERAMIACEAE			
Anotrichium furcellatum (J. Agardh) Baldock	San Diego Bay, California, USA. 9 Mar. 1982. JAW culture 2600A3	2600A3	U32561
Ceramium macilentum J. Agardh	Subtidal, SCUBA (3 m), Williamstown, Victoria, Australia. 6 Feb. 1995	G0285	U32562
Crouania attenuata (C. Agardh). J. Agardh	Puerto Penasco, Sonora, Mexico. 20 Jun. 1966. JAW culture 540	G0284	U32563
Griffithsia monilis Harvey	Point Lonsdale, Victoria, Australia. Cultures from L. Goff	G0251	U32565
GELIDIACEAE			
Gelidium pusillum (Stackhouse) Le Jolis	Intertidal, Lone Cypress Point, Monterey County, California, USA. 25 Feb. 1994	G0159	U32564

sequence data for *Anotrichium furcellatum* and *Griffithsia monilis*, both of the ceramiaceous tribe Griffithsieae. Phylogenetic analyses of these sequences with a variety of others in our multiple alignment indicate that the SSU sequence previously reported for *G. globulifera* is closely allied to species of *Gelidium*. It is thus not remotely related to the two species of Griffithsieae that we have sequenced ourselves and which group strongly with the Ceramiales. We also generated SSU data from *Ceramium macilentum* and *Crouania attenuata* to use in addition to the published sequence for *Ceramium nodulosum* to determine whether expanded species sampling would buttress or further undermine monophyly of the Ceramiaceae and Ceramiales.

Materials and methods

Collection information is given in Table 1. Plants were processed and DNA extracted as previously described (Saunders, 1993). The SSU gene was polymerase chain reaction (PCR)-amplified as four overlapping fragments from total genomic DNA using primers G01–G10 (fragment L), G02–G14 (fragment M), G04–G13 (fragment N) and G06–G07 (fragment O). All primer sequence information has been provided previously (Saunders & Kraft, 1994). The Gene-Amp Kit (Perkin Elmer Cetus, Norwalk, CT) was employed following the manufacturer's recommendations, and the reaction profiles were as presented previously (Saunders & Kraft, 1996).

Aliquots of PCR product were agarose gel-purified according to the manufacturer's protocol using the Wizard PCR Preps DNA Purification System (Promega, Madison, WI). DNA cleaned by this method was sequenced with the Taq Dye Primer Cycle Sequencing Kit [Applied Biosystems (ABI), division of Perkin Elmer Cetus] following the manufacturer's recommendations. Sequence reactions were completed using both the PCR primers for each fragment, plus one additional primer for each fragment (L, G11; M, G03; N, G08; O, G12; Saunders & Kraft, 1994, 1996). Reactions were electrophoresed and the sequence data collected with the ABI Model 373A DNA sequencer (Saunders & Kraft, 1996).

Sequence data were compared and edited utilizing the SeqEd DNA Sequence Editor (ABI) software package, which allows sequences from both strands to be compared and overlapping fragments combined to derive SSU gene sequences for all five taxa. The new data were added to an alignment including 44 previously published red algal SSU sequences (Table 2).

The 1768 bp (base pairs) of aligned SSU sequences, excluding the 5′ and 3′ PCR primer regions (G01, G07; Saunders & Kraft, 1994, fig. 1), from the 49 species included in this study were converted to a distance matrix using DNADIST (PHYLIP computer package; Felsenstein, 1989). The KIMURA option was employed to compute evolutionary distances (Kimura, 1980) for pairwise comparisons of all taxa in the alignment, and this distance matrix was converted to a phylogenetic tree using the neighbor-joining algorithm (Saitou & Nei, 1987) of PHYLIP. Parsimony analysis was completed with the DNAPARS algorithm of PHYLIP using the global swapping option. Unrooted trees were calculated and the ingroup taxa subsequently rooted with reference to the outgroups. Distance and parsimony analyses were subjected to bootstrap resampling to determine the robustness of the inferred phylogenies (Felsenstein, 1985).

Results and discussion

The SSU genes determined in the current investigation varied in length from 1770 to 1775 bp. Complete sequence was obtained from both strands except for a region of 1–4 bp adjacent to the 5′ and 3′ PCR primers (cf. Saunders & Kraft, 1994). These same primer regions could not be determined by the method employed and were excluded from the alignment prior to phylogenetic analyses.

The newly determined sequences were added to a multiple alignment modified from Saunders & Kraft (1996). The alignment contained 44 additional sequences representative of the Ahnfeltiales (outgroup; Ragan *et al.*, 1994), Bonnemaisoniales, Ceramiales, Gelidiales, Gigartinales, Gracilariales, Halymeniales, Plocamiales and Rhodymeniales (Table 2). A sub-alignment including partial SSU gene sequences (complete alignment available on request) from all the included species of the Ceramiales

Table 2. Sources of additional SSU gene sequence data employed in the multiple alignment

Taxonomic affiliation	Species and authority	Reference
AHNFELTIALES		
Ahnfeltiaceae	*Ahnfeltia plicata* (Hudson) Fries	2
BONNEMAISONIALES		
Bonnemaisoniaceae	*Bonnemaisonia hamifera* Hariot	5
CERAMIALES		
Ceramiaceae	*Ceramium nodulosum* (Lightfoot) Ducluzeau	5
	Griffithsia globulifera Harvey	5
Dasyaceae	*Dasya baillouviana* (S. G. Gmelin) Montagne	5
Delesseriaceae	*Phycodrys rubens* (Linnaeus) Batters	5
Rhodomelaceae	*Rhodomela confervoides* (Hudson) P. Silva	5
GELIDIALES		
Gelidiaceae	*Gelidium vagum* Okamura	5
GIGARTINALES		
Dumontiaceae	*Dasyphloea insignis* Montagne	6
	Dilsea carnosa (Schmidel) O. Kuntze	7
	Farlowia mollis (Harvey *et* Bailey) Farlow *et* Setchell	7
Endocladiaceae	*Endocladia muricata* (Postels *et* Ruprecht) J. Agardh	7
	Gloiopeltis furcata (Postels *et* Ruprecht) J. Agardh	7
Furcellariaceae	*Furcellaria lumbricalis* (Hudson) Lamouroux	2
Gigartinaceae	*Chondrus crispus* Stackhouse	2
	Sarcothalia crassifolia (C. Agardh) Edyvane *et* Womersley	6
Kallymeniaceae	*Callophyllis rangiferina* (Turner) Womersley	7
Mychodeaceae	*Mychodea carnosa* Hooker *et* Harvey	7
Nizymeniaceae	*Nizymenia australis* Sonder	6
Petrocelidaceae	*Mastocarpus stellatus* (Stackhouse in Withering) Guiry	5
Phacelocarpaceae	*Phacelocarpus peperocarpos* (Poiret) Wynne, Ardré *et* Silva	6
Phyllophoraceae	*Schottera nicaeensis* (Lamouroux *ex* Duby) Guiry *et* Hollenberg	7
Schizymeniaceae	*Schizymenia dubyi* (Chauvin *ex* Duby) J. Agardh	7
Solieriaceae	*Areschougia congesta* (Turner) J. Agardh	6
Sphaerococcaceae	*Sphaerococcus coronopifolius* Stackhouse	6
GRACILARIALES		
Gracilariaceae	*Curdiea flabellata* Chapman	3
	Gracilaria tikvahiae McLachlan	1
	Gracilariopsis lemaneiformis (Bory) Dawson, Acleto *et* Foldvik	3
HALYMENIALES		
Halymeniaceae	*Carpopeltis phyllophora* (Hooker *et* Harvey) Schmitz	7
	Cryptonemia undulata Sonder	7
	Grateloupia filicina (Lamouroux) C. Agardh	7
	Halymenia plana Zanardini	7
Sebdeniaceae	*Sebdenia flabellata* (J. Agardh) Parkinson	7
PLOCAMIALES		
Plocamiaceae	*Plocamium angustum* (J. Agardh) J. D. Hooker *et* Harvey	6
	Plocamiocolax pulvinata Setchell	6
RHODYMENIALES		
Champiaceae	*Champia affinis* (Hooker *et* Harvey) J. Agardh	4
Lomentariaceae	*Lomentaria australis* (Kützing) Levring	7
	Lomentaria baileyana (Harvey) Farlow	5
Rhodymeniaceae	*Cephalocystis furcellata* (Harvey) Millar, Saunders, Strachan *et* Kraft	4
	Cordylecladia erecta (Greville) J. Agardh	4
	Epymenia wilsonis Sonder	7
	Erythrocolon podagricum (Harvey) J. Agardh	4
	Gloioderma fruticulosa (Harvey) De Toni	7
	Rhodymenia linearis J. Agardh	6

References:
1, Bird *et al.* (1990); 2, Bird *et al.* (1992*a*); 3, Bird *et al.* (1992*b*); 4, Millar *et al.* (1996); 5, Ragan *et al.* (1994); 6, Saunders & Kraft (1994); 7, Saunders & Kraft (1996).

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Gp  CGCTCGTAGT CGGAACTTGG CGGGCGACGC AGGGCGTCTT TGCGGACGGA TCTGT--ATC  652
Gv  .......... .......... .......... .......... .........T ..........  652
Gg  .......... .......... .......... .......... .......... ..........  652
Gm  .......... ..A.TT.... .....AC..T G.AT...TCG C.A.A.T.TT ...AC..G.T  655
An  .......... ....TT.... ......C..T GATTT...CG C.A...T.AT .T.AC..G..  658
Ca  .......... ..A.C..... ......GT.. GA...A..C. .A....T.AG CT.....GCT  662
Cn  .......... ....CG.... ......C.T. GA.T..G.C. CA...T..AT CT....TGG.  663
Cm  .......... ....CG.... ......T... .A.T..G.CG AAA..T..TT CT..A..GG.  657
Pr  .......... ..T.TT.... .A.CT.T..T ..AAT...CG CAA...TT.T .T..C..G..  657
Db  .......... ....TT.... ....A..... ...C....CG C.A.....A. CT..C..G..  655
Rc  .......... ....TT.... ..T.T.CTA. T.AAAC..C. AAAA..T.TT GA......G.  655

Gp  GGCCGCCTTT GTGGAGGGGG GCTTAG--CG GTGCTTTATT GCCT-TGCTA -AGTCGCTGC  708
Gv  .......... .......... .......... .......... .......... ..........  708
Gg  .......... .......... .......... .......G.. .......... ..........  708
Gm  .....T.... ...CGA..T. .C........ ..T..C.T.G .....A.C..  711
An  .....T.... ..A.GA..T. .......... .....C.TC. .T...A.C..  714
Ca  .....T.... ..C.G...T. ........C. .....C..CG .G...A.C..  718
Cn  .......... ......GT.. .......... .T.AC.C..T .T........  722
Cm  .......... ....T..... A.....C... .T.ATC.TGG ..........  714
Pr  ...T...... ........C. ..C.....T. .......... .....C.... .G..T...A.  713
Db  T.A....... .......... ....G...T. ........C. ..A......G ..........  711
Rc  T-........ .......... .TG.G...T. ..A..... .TA.....CG .CA.TT.C..  710

Gp  CACCGTTTAC TGTGAAAAAA TTGGAGTGTT CAAAGCAGGC G--TTTGCCT -TGTACACAT  765
Gv  .......... .......... .......... .......... .......... ..........  765
Gg  .......... .......... .......... .......... .......... ..........  765
Gm  .......... .......... ..A....... ......G... ...A.....A .C.A.T....  768
An  .......... .......... ..A....... ......G... ...A.....A T..A.T....  772
Ca  .......... .......... ..AAG..... ......G... T...G....A ...A.T....  775
Cn  .......... .......... ..A....... ......G... .........A ...A.T....  779
Cm  .......... .......... ..A....... ......G... .........A ...A.T....  771
Pr  .......... .......... ..A....... .......A.. .........C ...A.T....  770
Db  .......... ......T... ..A....... .......A.. A.A......C ...A.T....  769
Rc  ...T...... .......... ..A....... ......GA.. ...A.....A ...A.T....  767

Gp  TAGCATGGAA TAATAGAATA GGACCTGGTT -CTGTTT-TG TTGGTTT-GT GAGAATTAGG  822
Gv  .......... .......... .......... .......... .......... ..........  822
Gg  .......... .......... .......... .......... .......... ..........  822
Gm  .......... .......... .......TG. CT.A...... .......T.. T.AGT.C...  827
An  .......... .......... .......AG. CT.A..C... .......... T.AGT.C...  830
Ca  .......... .......... .......-G. C..T.....A .......... A..T..C...  832
Cn  .......... .......... .......-G. C.......... .......... ...T..C...  836
Cm  .......... .......... .......-G. C.......... .......... ...T..C...  828
Pr  .......... .......... .......-G. CT.A...... .......... ..AGT.C...  827
Db  .......... .......... .......-G. CT.....C.A .......... A.AGT.C...  827
Rc  .......... .......... .......-G. T........A .......... TG.T..C...  824
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Fig. 1. Sub-alignment for all species of the Ceramiales and Gelidiales included in the multiple alignment (positions 595 to 822 in Gp). A dash (–) indicates a gap in that sequence in the multiple alignment; a dot (·) that a species has the same character state for that site as in Gp. Numbers to the right indicate nucleotide position. See Table 3 for species abbreviations.

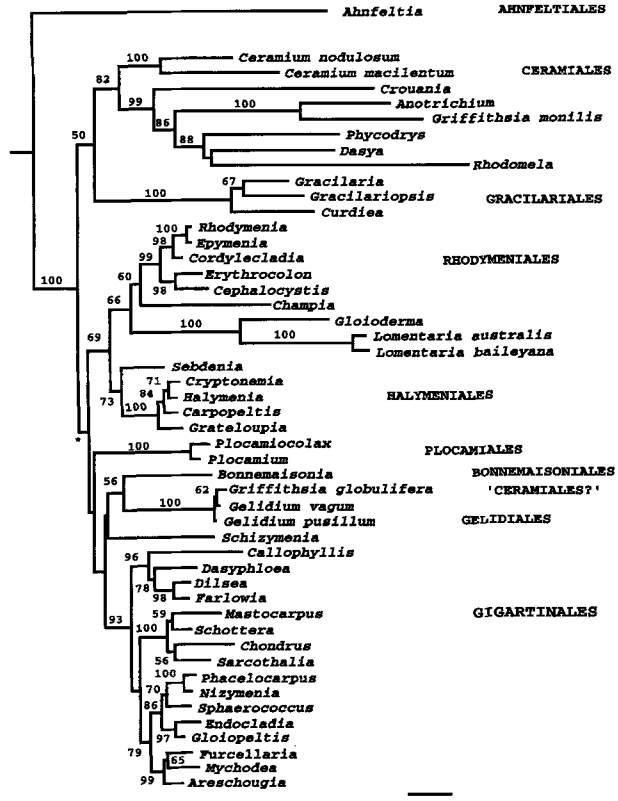

Fig. 2. Neighbor-joining tree. Numbers at branches are bootstrap values (100 replicates). Internal branches lacking numbers had bootstrap support in fewer than 50 replicates. An asterisk (*) indicates the position of pruning for the trees in Fig. 4. Scale bar represents 1% divergence.

and Gelidiales is provided (Fig. 1). Visual inspection of this sub-alignment reveals two groups of species. The first is a highly conserved assemblage that includes the two *Gelidium* species as well as *Griffithsia globulifera* (group 1); the second is much more diverse and contains the remainder of the Ceramiales (group 2). Actual pairwise distances computed between these species, including all substitutions, deletions and insertions, highlights further the discreteness of the two groups (Table 3). Group 2 is highly divergent, ranging from 90 to 233 nucleotide changes between species. The level of divergence within group 2 is almost of the same order as that observed between the two groups (133 to 232). Group 1, on the other hand, is highly conserved. The two species of *Gelidium*, which differ by four nucleotides, differ from *Griffithsia globulifera* at only five sites each (Table 3).

The complete multiple alignment (49 species with 1768 sites, excluding the 5′ and 3′ PCR primer regions) was converted to a distance tree (Fig. 2). In this tree *Griffithsia globulifera* was weakly (62 bootstrap replicates) allied to

Table 3. Actual pairwise distances (nucleotide changes) between the species included in the Ceramiales/Gelidiales sub-alignment (Fig. 1)

	Gp	Gv	Gg	Gm	An	Ca	Cn	Cm	Pr	Db	Rc
Gp	–										
Gv	4	–									
Gg	5	5	–								
Gm	210	209	212	–							
An	188	188	191	102	–						
Ca	204	205	207	222	199	–					
Cn	133	133	136	208	189	175	–				
Cm	144	144	147	206	189	186	90	–			
Pr	175	175	178	192	178	197	163	160	–		
Db	165	168	169	194	177	195	171	165	132	–	
Rc	228	229	232	231	208	233	211	217	175	177	–

Abbreviations for species: Gp, *Gelidium pusillum*; Gv, *Gelidium vagum*; Gg, *Griffithsia globulifera*; Gm, *Griffithsia monilis*; An, *Anotrichium furcellatum*; Ca, *Crouania attenuata*; Cn, *Ceramium nodulosum*; Cm, *Ceramium macilentum*; Pr, *Phycodrys rubens*; Db, *Dasya baillouviana*; Rc, *Rhodomela confervoides*.

Gelidium vagum, and these two species were strongly associated with *Gelidium pusillum* (100 replicates). These were only remotely related to a monophyletic (82 replicates) Ceramiales which included the two species of *Ceramium*, *Crouania*, *Anotrichium* and *Griffithsia monilis* of the Ceramiaceae, as well as single representatives of the Dasyaceae, Delesseriaceae and Rhodomelaceae. A similar result was obtained when the multiple alignment was subject to parsimony analysis (Fig. 3). On the basis of our data, we conclude that it is highly improbable that the SSU sequence presented for *Griffithsia globulifera* (Ragan *et al.*, 1994) is in fact correct. Considering the overwhelming vegetative, reproductive and ultrastructural differences between the Ceramiales and Gelidiales (Kraft, 1981; West & Hommersand, 1981; Bold & Wynne, 1985; Garbary & Gabrielson, 1990; Pueschel, 1990), our results are not at all surprising.

A further noteworthy result of both our distance (Fig. 2) and parsimony analyses (Fig. 3) is the absence of support for a monophyletic Ceramiaceae. Three distinct lineages are resolved for the included species which correspond to three recognized tribes of this family: Ceramieae, Crouanieae and Griffithsieae. Hommersand (1963) presented an evolutionary scenario for the Ceramiales which would in effect render the Ceramiaceae paraphyletic, proposing that the Dasyaceae, Delesseriaceae and Rhodomelaceae were each derived independently from within this lineage. Our data likewise do not support monophyly for the Ceramiaceae. However, they do not support, at least to date, the proposal of Hommersand, as they indicate that the Dasyaceae, Delesseriaceae and Rhodomelaceae are derived from a common ancestor within the Ceramiaceae.

We are also investigating the poor to absent support for monophyly of the order Ceramiales that has recently been reported by several authors (Rice *et al.*, 1991; Ragan *et al.*, 1994; Saunders & Kraft, 1994, 1996). Our studies show the Ceramiales to be monophyletic, with fair to strong support in both the distance (82 replicates) and parsimony (97 replicates) analyses. We completed a series of distance analyses on our multiple alignment in which we varied the component members of the Ceramiaceae to determine the effect that the various taxa might be having on the final phylogeny. Removal of *Ceramium macilentum* and *Crouania attenuata* from the alignment resulted in *Ceramium nodulosum* failing to group with the remaining Ceramiales, rendering the order polyphyletic (Fig. 4*a*) as has been noted in previous accounts (Rice *et al.*, 1991;

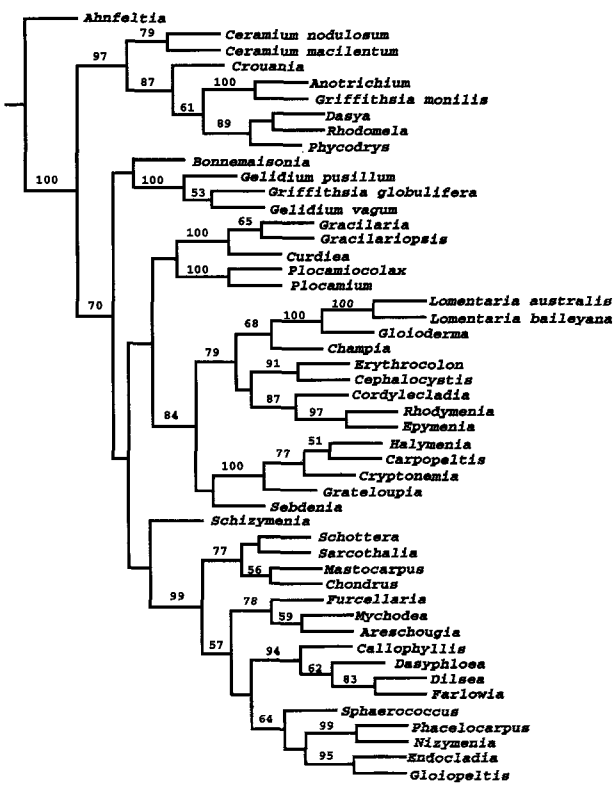

Fig. 3. Strict consensus tree of parsimony bootstrap analysis (consequently branches are not to scale). Numbers at branches are bootstrap values (100 replicates). Internal branches lacking numbers had bootstrap support in fewer than 50 replicates.

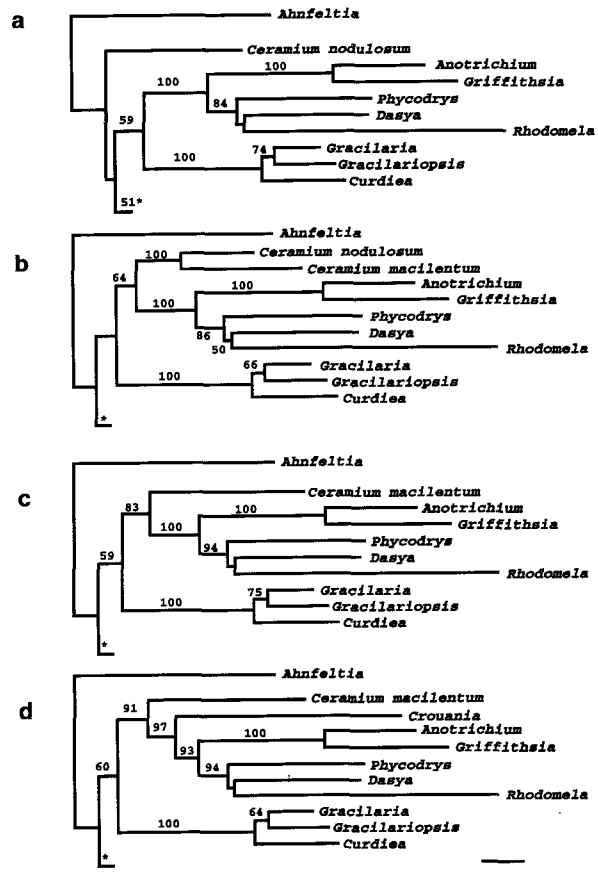

Fig. 4. Series of distance analyses in which representative Ceramiaceae were varied. All trees were pruned at the asterisk (*; compare Fig. 2). Relationships in the pruned portion of the tree changed little from that presented in Fig. 2. Numbers at branches are bootstrap values (100 replicates). Internal branches lacking numbers had bootstrap support in fewer than 50 replicates. Scale bar represents 1% divergence. (*a*) *Ceramium macilentum* and *Crouania attenuata* removed. (*b*) Only *Crouania attenuata* removed. (*c*) *Ceramium nodulosum* and *Crouania attenuata* removed. (*d*) Only *Ceramium nodulosum* removed.

Ragan *et al.*, 1994; Saunders & Kraft, 1994; 1996). Restoring the *Ceramium macilentum* sequence had the effect of drawing *Ceramium nodulosum* into the Ceramiales as its sister species, thus rendering the genus, as well as the order (support in only 64 bootstrap replicates), monophyletic (Fig. 4*b*). *Ceramium nodulosum* was then removed from this alignment, resulting in a substantial gain in support for a monophyletic Ceramiales (83 replicates; Fig. 4*c*). Finally, when *Crouania attenuata* was returned to the alignment, even greater support for ceramialean monophyly (91 replicates) resulted (Fig. 4*d*).

Acknowledging the dramatic effects that taxon sampling (Lecointre *et al.*, 1993) can have on phylogenetic determination, as well as recognizing the limitations of bootstrap resampling as an indicator of robustness (cf. Swofford & Olsen, 1990), it must be noted that there is an apparent destabilization of the Ceramiales whenever the *Ceramium nodulosum* sequence is included in the alignment. Given the consensus of contemporary phycologists with regard to accepting monophyly for the Ceramiales (Kraft, 1981; Bold & Wynne, 1985; Garbary & Gabrielson, 1990), it is necessary to consider carefully data which seem to run counter to this view. It is possible that the SSU sequence for this species is a poor representative of the genus. This would not be the first time such an anomaly was uncovered in determining relationships among red algae using molecular data (Saunders & Kraft, unpublished data), and is probable given the stochastic way which DNA evolves (Nei, 1987). Alternatively, lack of representation for the *Ceramium nodulosum* sequence could be the result of errors in the published, rather than the actual, SSU sequence of this species. Deciding between these two alternatives will require confirmation of the SSU gene sequence for *Ceramium nodulosum*.

In addition to the Ceramiales, the Bonnemaisoniales (unfortunately represented by only one species to date), Gelidiales, Gracilariales, Halymeniales, Plocamiales and Rhodymeniales all appear to be natural and justifiable orders. As for the Gigartinales, support for its monophyly varies with the analysis. Of the taxa included in the current phylogenetic investigation, only the Schizymeniaceae seems to present a problem. The remaining families group solidly together.

Conclusions

Until recently, speculations about the phylogenetic relationships of red algal families and orders have been based exclusively on anatomical, life-history, ultrastructural and biochemical features. Our molecular data have led us to challenge existing taxonomic systems at a number of points, although they confirm traditional thinking at others. Up to now, the indications of molecular data that have seemed to be at odds with established classification systems have directed our attention to new anatomical discoveries or emphases that have buttressed, rather than weakened, the cases for taxonomic revisions (Saunders & Kraft, 1994, 1996; Millar *et al.*, 1996; Saunders *et al.*, 1995).

In our investigation of the possible polyphyly of the order Ceramiales, our emphasis has shifted from an attempt to reconcile molecular and anatomical data to one of evaluating some seemingly improbable molecular findings of other authors in cases where the morphology of the organisms concerned is well documented. On reproductive anatomical grounds alone, the long history of meticulous, classical taxonomic work on the order throws up a very real challenge to the whole edifice of molecular phylogenetics: if so seemingly uniform a group is not demonstrably monophyletic, is there some basic flaw in the molecular approach and, if not, are there any grounds for hoping that careful anatomical study of red algae will ever have any relevance to the construction of phylogenetically based taxonomic systems?

Our data indicate that classical taxonomy is indeed a better guide to organism relatedness than has been the molecular data to date. But we have also shown that the question we originally posed was not the right one. Once our new sequences from a variety of ceramiaceous species are added to the analysis and molecular data of questionable validity is allowed for, either by elimination altogether or in conjunction with our new data, the molecular and traditional approaches no longer yield disparate results. The fundamental conclusion that we draw from this exercise applies equally to the traditional and molecular schools of systematics: the data must be collected with precision and accuracy, they must be repeated and reproducible, and they should in the end lend themselves to a holistic synthesis leading to a truer understanding of organismal phylogeny.

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