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A molecular phylogeny of the marine red algae (Rhodophyta) based on the nuclear small-subunit rRNA gene

(Bangiophycidae/Florideophycidae/taxonomy/Kishino–Hasegawa test/Templeton–Felsenstein test)

MARK A. RAGAN*†, CAROLYN J. BIRD*†, ELLEN L. RICE‡, ROBIN R. GUTELL§, COLLEEN A. MURPHY*, AND RAMA K. SINGH*

*Institute for Marine Biosciences, National Research Council of Canada, 1411 Oxford Street, Halifax, NS Canada B3H 3Z1; ‡Biological Sciences Branch, Scotia-Fundy Region, Fisheries and Oceans Canada, P. O. Box 550, Halifax, NS Canada B3J 2S7; and §Department of Molecular, Cell and Developmental Biology, Campus Box 347, University of Colorado, Boulder, CO 80309

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ABSTRACT **A phylogeny of marine Rhodophyta has been inferred by a number of methods from nucleotide sequences of nuclear genes encoding small subunit rRNA from 39 species in 15 orders. Sequence divergences are relatively large, especially among bangiophytes and even among congeners in this group. Subclass Bangiophycidae appears polyphyletic, encompassing at least three lineages, with Porphyridiales distributed between two of these. Subclass Florideophycidae is monophyletic, with Hildenbrandiales, Corallinales, Ahnfeltiales, and a close association of Nemaliales, Acrochaetiales, and Palmariales forming the four deepest branches. Ceramiales may represent a convergence of vegetative and reproductive morphologies, as family Ceramiaceae is at best weakly related to the rest of the order, and one of its members appears to be allied to Gelidiales. Except for Gigartinales, for which more data are required, the other florideophyte orders appear distinct and taxonomically justified. A good correlation was observed with taxonomy based on pit-plug ultrastructure. Tests under maximum-likelihood and parsimony of alternative phylogenies based on structure and chemistry refuted suggestions that Acrochaetiales is the most primitive florideophyte order and that Gelidiales and Hildenbrandiales are sister groups.**

The Rhodophyta is a large, morphologically diverse assemblage of eukaryotes, with 2500–6000 species in about 680 genera (1). Although the division as a whole is well delimited (1, 2), its taxonomy at the levels of subclass and order has been unstable. Traditionally, two subclasses have been recognized, Bangiophycidae and Florideophycidae, with four and 14 orders, respectively. Recently, the former has been adjudged untenable (3–5) because it is not distinguished by synapomorphic characters. Alternatively, three new subclasses have been proposed to replace the Bangiophycidae and Florideophycidae on the basis of the degree of cellular transformation into spores (6). At the ordinal level (7), six new orders have been described since 1978 (8–12), and the large classical order Cryptonemiales has been subsumed into the similarly large Gigartinales (13), creating a heterogeneous assemblage of families that requires further resolution. Ordinal changes have arisen mainly from increasing appreciation of the significance of life-history variations and ultrastructure (5, 7, 9). However, taxonomic instability in Rhodophyta has also been ascribed to a lack of association with phylogenetic hypotheses, and attempts have been made (4, 6, 7) to infer phylogenetic relationships from morphological, anatomical, ultrastructural, life history, and chemical characters. Molecular sequences, particularly of nuclear genes encoding small subunit rRNA (SSU rDNAs) have proven useful in resolving phylogenetic relationships within other problematic groups (14–16). In a general survey of molecular relationships among marine Rhodophyta, we have determined the nucleotide sequence of SSU rDNAs¶ from 52 representatives in 15 orders and now present inferences on phylogenetic relationships within the Rhodophyta.

MATERIALS AND METHODS

Plant Materials. At least one species (see *Appendix*) was selected from each order except Rhodochaetales (monotypic and obscure), Batrachospermales (exclusively freshwater), and Rhodogorgonales (based on newly discovered genera and too recently described for inclusion). When possible, orders were represented by a member of their type genus. To estimate intraordinal variability, several genera or species were examined in Palmariales, Ceramiales, Gigartinales, Gracilariales, and all three bangiophyte orders. Fresh, frozen (−80°C) and freeze-dried material was used (17). Voucher specimens of macroalgae were deposited in the herbarium of the Institute for Marine Biosciences [NRCC (National Research Council of Canada)].

DNA Methods. DNA was extracted (18), and SSU rDNAs were amplified by using eukaryote-specific primers (19) as described (20). Amplification products were cloned into pUC and sequenced fully on both strands (17). Problematic regions were resolved by use of custom primers, nucleotide analogues, and/or direct sequencing.

Sequence Analysis. Sequences were initially aligned for maximum primary- and secondary-structural similarity by manual iteration against an extensive data base of aligned eukaryote rDNAs (21, 22). Trees were inferred based on this initial alignment; the red algal rDNA sequences were reordered in accordance with the most stable features of these trees, and the alignment was further adjusted to maximize similarities. The alignment was progressively optimized as new sequences were added to the data base and thereafter was largely stabilized by two iterations of tree inference and realignment.

From 52 red algal rDNAs in our initial data base, we selected 39 (*Appendix*) that provide a broad, balanced sample. Representatives of all 15 available orders and 21 available families were included; the majority of sequences excluded were of the order Gracilariales, analyzed elsewhere (17). Sequences of cryptomonad nucleomorph rDNAs from *Cryptomonas* ϕ (23) and *Pyrenomonas salina* (24) grouped with bangiophycidean rDNAs and were retained; the corre-

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Abbreviations: rDNA, DNA encoding ribosomal RNA; SSU, small subunit; NAP complex, Nemaliales–Acrochaetiales–Palmariales complex.

†To whom reprint requests should be addressed.

¶The sequences reported in this paper have been deposited in the GenBank data base (accession nos. given in *Appendix*).

Table 1. Ranges of G+C% values for individual sequences in the full, more conservative, and most conservative matrices.

	Matrices		
	Full	More conservative	Most conservative
All sequences	43.1–52.4	43.3–51.8	44.4–50.4
All rhodophytes	44.8–52.4	45.0–51.8	45.5–50.4
Bangiophytes + nucleomorphs	43.1–48.4	43.3–47.7	44.4–47.0
Florideophytes	47.3–52.4	47.8–51.8	47.8–50.4

sponding nuclear rDNAs were included as outgroups. Empty columns and positions corresponding to amplification primers were removed, yielding a "full" matrix of 43 sequences × 1787 positions. From this we deleted 18 regions where columns were mostly empty, gaps were ambiguous (largely in variable loop regions), or the alignment was especially problematic to obtain a 43 × 1566 "more conservative" matrix. Finally, we removed all remaining positions where secondary or higher order structure was not well defined across most eukaryote SSU rDNAs (including stably aligned regions among some red algae, especially Florideophycidae), yielding a 43 × 1200 "most conservative" matrix. Thereby, the G+C% range among all sequences in our matrix was reduced, and the overlap between bangiophycidean and florideophycidean rDNAs was eliminated (Table 1).

Trees were inferred with PHYLIP version 3.5 (25) unless otherwise indicated, with randomized orders of sequence input and global rearrangements. Distances were corrected for multiple substitution at individual sites according to Felsenstein's "maximum likelihood" model. Topologies of consensus parsimony trees inferred with PHYLIP were put into PAUP (26) for calculation of branch lengths and fit indices. All distance, neighbor-joining, and parsimony analyses were statistically verified by 100 bootstrap iterations (27), and majority-rule consensus trees were calculated. Maximum-likelihood inference was bootstrapped (n = 10 iterations) from only the most conservative data set by using fastDNAml (28). For all methods, consensus topologies were used as user trees for calculation of distances, tree lengths, or likelihoods. Alternative hypotheses of relationships were tested by input of user-specified trees with statistical analysis as described by Kishino and Hasegawa (29) for maximum likelihood and by Felsenstein (30) following Templeton (31) for parsimony. These methods compute the value and standard deviation (SD) of a measure of tree goodness for the best tree; alternative hypotheses are rejected if they require a log likelihood, or minimum number of steps, of more than 1.96 SD worse.

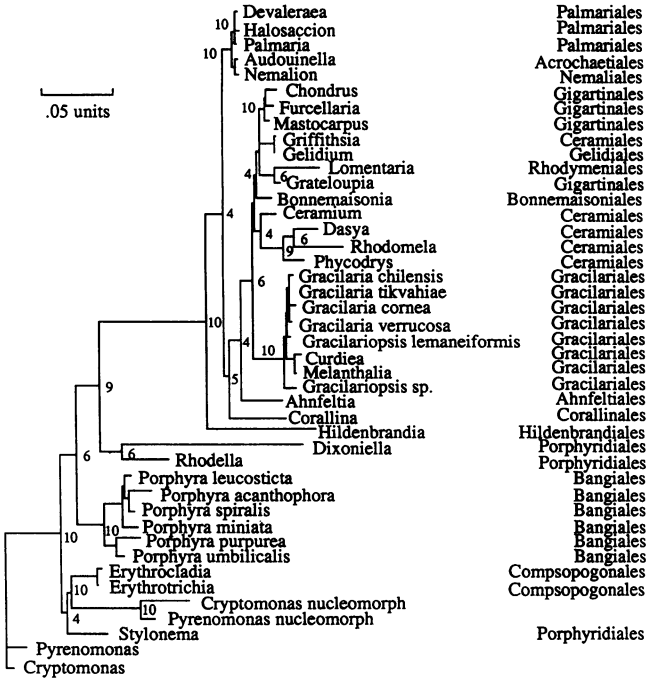

FIG. 1. Bootstrap analysis of maximum-likelihood tree (fastDNAml, CONSENSE) inferred from the most conservative matrix. Bootstrap values <4 (40%) are not shown.

RESULTS

Complete SSU rDNA exon sequences varied from 1760 (*Lomentaria baileyana*) to 1844 (*Porphyra acanthophora*) nucleotides, including 46 nucleotides in amplification primers. In all trees, the most basal rhodophyte branches represent rDNAs of bangiophytes. In maximum-likelihood (Fig. 1) and some distance trees, bangiophycidean rDNAs diverge as three branches, with Compsopogonales, some Porphyridiales, and the cryptomonad nucleomorphs forming the most basal branch, Bangiales diverging thereafter, and the porphyridialean unicells *Dixoniella* and *Rhodella* diverging third. Parsimony trees (e.g., Fig. 2) differ only in showing further fragmentation of the most basal branch into two or three independent branches. Branching order among bangiophyte lineages differs slightly in some Fitch–Margoliash distance and neighbor-joining analyses, probably owing to attraction effects of the long *Dixoniella* and *Rhodella* branches, which sometimes diverge separately (although with low bootstrap confidence levels). In all distance trees, bangiophyte taxa are widely divergent compared with florideophytes.

Subclass Florideophycidae is monophyletic with 100% bootstrap support in all trees and is distant from Bangiophy-

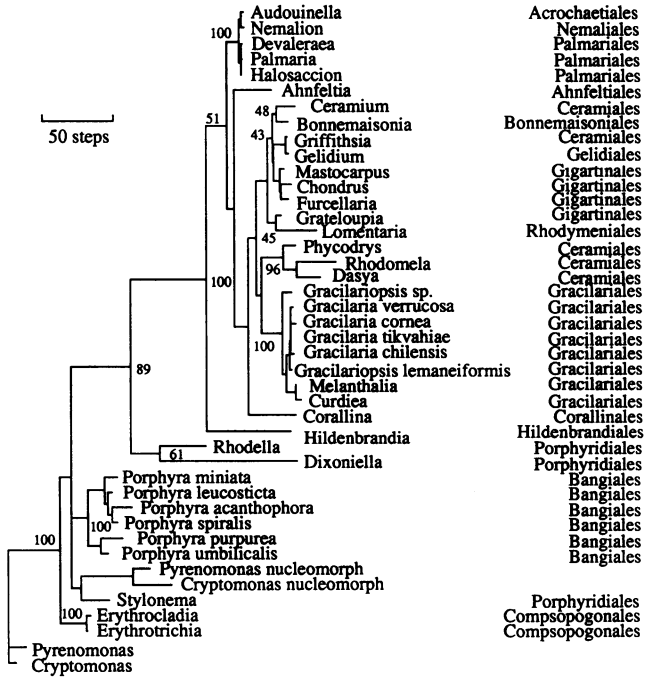

FIG. 2. Bootstrap analysis of parsimony tree (DNAPARS, CONSENSE) inferred from the most conservative matrix (474 varying characters, of which 302 are potentially informative). Length, 1198 steps; consistency index (CI), 0.481 based on informative characters; retention index (RI), 0.800. Scale shows branch lengths (horizontal axis) in steps, with optimization based on accelerated transformation (ACCTRAN). Bootstrap values < 40% are not shown.

cidae. Support is excellent for some orders (e.g., Gracilariales) and groups of orders (Nemaliales–Acrochaetiales–Palmariales = "NAP complex"), but bootstrap confidence values are low for most nodes along the backbone of the tree with even the full matrix. The rDNAs of Hildenbrandiales, Corallinales, the NAP complex, and Ahnfeltiales constitute the four most basal florideophycidean branches except in parsimony analysis of the most conservative matrix, where *Corallina* moves into the crown of the tree owing to loss of characteristic nucleotides.

Based on these results and against the background of red algal systematics and interordinal relationships inferred from phenotypic characters (7), we selected five key hypotheses to be tested against our data. To control for alignment effects, all three versions of our data matrix were used in each test.

Hypothesis 1. *Hildenbrandiales, Corallinales, the NAP complex, and Ahnfeltiales, respectively, form the four most basal branches within Florideophycidae.* Initial results showed that except for the artifact with Corallinales (above), moving any of these branches to the base or crown of the tree or inserting other branches among them, greatly increased overall tree length. Thus, we restricted hypothesis-testing to all 24 possible branching orders among these four lineages and did not change other topological features from the corresponding most parsimonious or maximally likely trees. Most trees passing the tests showed *Hildenbrandia* and *Corallina* forming the two most-basal branches, although a few cases with *Ahnfeltia* or the NAP complex as the deepest florideophyte lineage could not be rejected.

Because of the difficulty in establishing the branching order among these four branches, all 24 possibilities were retained in the analysis when testing *Hypotheses 2–5*.

Hypothesis 2. *Bangiophycidae are monophyletic.* We considered three subcases embodying the three possible ways in which the three bangiophycidean lineages shown in Fig. 1 could be related within a monophyletic Bangiophycidae; in combination with the 24 possibilities from *Hypothesis 1*, this yielded 72 test trees. For computational reasons, alternative possibilities in which the bangiophycidean groups shown do not remain intact were not considered. With all three variants of the data matrix, a monophyletic Bangiophycidae could be rejected by the Kishino–Hasegawa test under maximum likelihood; most alternative topologies considered were 4–5 SD worse than the corresponding maximally likely tree. Using the Templeton–Felsenstein test under parsimony, 29 of the 72 alternative topologies were acceptable based on the full data matrix. With the more conservative (less noisy) matrix, however, only one topology incorporating a monophyletic Bangiophycidae passed the test (22 steps longer than the corresponding most parsimonious tree, with rejection at 23.5 steps). With the most conservative matrix, three topologies were just acceptable (14, 14, and 15 steps longer; rejection at 15.6, 16.3, and 16.5 steps respectively). Thus, our data imply that it is unlikely, but not impossible, that Bangiophycideae is monophyletic.

Hypothesis 3. *Acrochaetiales represents the earliest florideophycidean lineage.* This relationship has been proposed from cladistic analysis of red algal structure, biochemistry (4, 7), and reproductive features (6). Only one acrochaetialean SSU rDNA, from *Audouinella dasyae*, was available in this matrix; it differs by only 31–39 nucleotides (1.8–2.2%) from two other acrochaetialean rDNAs (G. W. Saunders, C.J.B., E.L.R., and M.A.R., unpublished data), and thus adequately represents the order. With all three forms of the data matrix, *Hypothesis 3* can be rejected under both parsimony and maximum likelihood; trees incorporating this topology were 3.5–8 SD worse than the corresponding most parsimonious or maximally likely trees.

Hypothesis 4. *Gelidiales and Hildenbrandiales are sister groups.* Red algal systematics has traditionally been based on female reproductive morphology and postfertilization development (32). This has not been helpful with order Hildenbrandiales, wherein sexual reproduction is unknown. Placement of Hildenbrandiales as specific relatives of Gelidiales (4, 7) was based on structure and biochemistry and, in the absence of sexual reproductive characters, was influenced strongly by the single pit-plug cap layer and details of spore germination. As tests of *Hypothesis 1* (above) indicated that *Hildenbrandia* rDNA represents one of the more basal florideophycidean lineages, *Gelidium* rDNA was forced to group specifically with *Hildenbrandia* and not vice versa. Trees based on all 24 such permutations ranged from about 5–6 SD worse than the corresponding best trees with the full data set to 7–12 SD worse with the most conservative data set. We conclude that the rDNA sequences strongly rule out any specific relationship between Gelidiales and Hildenbrandiales.

Hypothesis 5. Griffithsia *and* Ceramium *are members of the same monophyletic family or order.* Ceramiales is often considered to be the most clearly defined rhodophyte order, founded on a four-celled carpogonial branch with formation of the auxiliary cell from a supporting cell after fertilization. The latter feature is unique to Ceramiales, the former feature being not unique but unusual among other red algae. Based on the further lack of cellular encasement around the carposporophyte, *Ceramium* and *Griffithsia* are classified in family Ceramiaceae.

In none of our trees did rDNAs of *Griffithsia* and *Ceramium* group together. *Ceramium* associates weakly (e.g., Fig. 1) or not at all (e.g., Fig. 2) with a monophyletic grouping of its conordinals *Phycodrys* (Delesseriaceae), *Rhodomela* (Rhodomelaceae), and *Dasya* (Dasyaceae), and 100% bootstrap support is evident for association of *Griffithsia* with *Gelidium* (Gelidiales). When *Griffithsia* was forced to group specifically with *Ceramium*, tests under maximum likelihood and parsimony revealed this topology to be 4–5 SD worse than the corresponding best trees with the most conservative data set, and up to 6–8 SD worse with the full matrix. We also tested a weaker variant of this hypothesis that would compromise family Ceramiaceae as presently constituted, but preserve order Ceramiales; thus, *Griffithsia* was moved to be the deepest branch in Ceramiales. These topologies ranged from about 2.5–4 SD worse than the corresponding best trees with the most conservative data matrix, or up to 5–7 SD worse with the full matrix. Thus, if our *Griffithsia* sequence is correct and representative, a monophyletic Ceramiales can be rejected with all variants of the data matrix under either test.

DISCUSSION

Red algae clearly constitute one of the major radiations of eukaryotes. Measured by divergence of SSU rDNA sequences within the most conservative regions, Rhodophyta are more divergent among themselves than are (*i*) fungi or (*ii*) green algae and green plants together. Of the 1200 most conservative positions, 1050 (87.5%) are identical between the chytrid *Blastocladiella emersonii* and the imperfect fungus *Aspergillus fumigatus*, 1110 (92.5%) between the volvocalean green alga *Characium perforatum* and the cycad *Zamia pumila*, and 1107 (92.25%) between the slime mold *Acanthamoeba castellani* and the monocot *Glycine max*. By contrast, rDNAs of *Hildenbrandia rubra* and *Dixoniella grisea* are identical in only 937 (78.1%) of these positions, and *H. rubra* and *Porphyra purpurea* are identical in 997 (83.1%).

The primary dichotomy between subclasses Bangiophycidae and Florideophycidae has been based on nuclear condition (uninucleate in Bangiophycidae vs. usually multinucleate in Florideophycidae), plastid number (usually single vs. usually multiple, respectively), plastid shape (typically

stellate vs. typically discoidal), plastid location (typically axile vs. usually peripheral), pattern of cell division (intercalary vs. mostly apical), pit connections (usually absent vs. present), thallus complexity (uni- or multicellular vs. multicellular), sexual reproduction (absent, rare, or controversial except in Bangiales vs. widespread except in Hildenbrandiales), a filamentous gonimoblast (absent vs. usually present), and tetrasporangia (absent vs. usually present) (3, 33). Remarkably, none is a taxonomically absolute, stable, and positive synapomorphy.

Our results suggest that Bangiophycidae is probably polyphyletic, although not in the way suggested by Magne (6), and encompasses three or more individual lineages, one of which includes cryptomonad nucleomorphs. Topological details in the basal region of the red algal tree depend strongly on selection of outgroups (unpublished), and correct inference is complicated by long branches leading to *Dixoniella* and *Rhodella*. Other key bangiophycidean rDNAs have not yet been examined—e.g., Rhodochaetales and additional Porphyridiales.

Our results strongly suggest that order Porphyridiales as currently constituted is at least biphyletic, as suggested earlier (7). In our trees, the filamentous *Stylonema* (Porphyridiales) tends to associate with filamentous *Erythrotrichia* and *Erythrocladia* (Compsopogonales) and with the cryptomonad nucleomorphs, while the unicells *Dixoniella* and *Rhodella* (Porphyridiales) diverge from the main red algal lineage later than *Porphyra* (Bangiales). rDNA sequences promise to provide much-needed resolution among lineages of these structurally simple organisms.

Order Bangiales, represented here by six *Porphyra* rDNAs, is solidly monophyletic but shows a remarkable degree of internal divergence. Additional *Porphyra* rDNA sequences (unpublished data) further support this group. These six *Porphyra* rDNAs are identical in as few as 1140 of the 1200 most conservative positions. Including less conservative sites and length variation, identities as low as 84.5% are found.

Florideophycidean rDNAs form a monophyletic group with 100% bootstrap support in our trees. Thus, despite its paucity of morphological or biochemical synapomorphies, this subclass appears well-founded. The distinctiveness of Florideophycidae is especially clear in distance-based and maximum-likelihood analyses; even in trees inferred from the most conservative matrix, the internodal distance between the least basal bangiophycidean and the most basal florideophycidean branches is >4 times longer than any other internodal distance along the backbone of the tree. This could indicate that florideophytes appeared much later in evolution, that the rate of acceptance of mutations increased dramatically early in the florideophycidean lineage and slowed again thereafter, or that a minor or phase-specific rDNA species became fixed within this lineage.

Within Florideophycidae, rDNAs from Hildenbrandiales, Corallinales, the NAP complex, and Ahnfeltiales form the four most basal lineages in most of our analyses. Additional sequences, particularly from Hildenbrandiales and Corallinales, should help to stabilize topology in this region. Group IC1 introns (34) occur at identical sites in SSU rDNA genes of *Hildenbrandia rubra* (35) and the bangiophytes *Porphyra* species (36, 37) and *Bangia atropurpurea* (unpublished data), but they probably arose by lateral transfer (37), and their occurrence need not imply that Hildenbrandiales is the deepest radiation within Florideophycidae.

The very close relationship among morphologically distinct Nemaliales, Acrochaetiales, and Palmariales was not generally expected from classical phenetic characters. However, together with Corallinales (likewise one of the most basal florideophycidean branches) and Batrachospermales, these organisms uniquely exhibit a two-layered pit-plug cap (38). The outer cap layer is enlarged into a dome-like structure in Corallinales, Batrachospermales, and some Acrochaetiales but is thin in Nemaliales, Palmariales, and other Acrochaetiales. This correlation raises the possibility that these five orders may have diverged monophyletically from the main red algal lineage, a hypothesis consistent with our data and requiring only one additional step under parsimony.

Among the remaining orders in the crown of the tree, Ceramiales and Gigartinales appear polyphyletic in our analyses. Representatives of three of the four ceramialean families (Dasyaceae, Delesseriaceae, and Rhodomelaceae) group together with 96–100% bootstrap support, but the rDNA of *Ceramium* (Ceramiaceae) is at best weakly associated (Fig. 1), while that of *Griffithsia* (Ceramiaceae) lies outside the group (*Hypothesis 5*). The absence of carpospore encasement in the type family Ceramiaceae has been suggested (39, 40) as indicating a dichotomy between it and the "other" ceramialean families. If our result is confirmed, the distinctive pattern of auxiliary cell formation and the four-celled carpogonial branch must be convergent.

In Gigartinales, three carrageenophytes (*Chondrus*, *Furcellaria*, and *Mastocarpus*) representing three families form a distinct group, but another family, Halymeniaceae (represented by *Grateloupia*), is associated with Rhodymeniales. As Halymeniaceae is the type family of the former order Cryptonemiales, this result appears to support the classical separation of Cryptonemiales and Gigartinales. However, since Gigartinales *sensu lato* comprises >40 families, our sample is clearly unrepresentative, and additional sequences are required to resolve ordinal limits and phylogeny.

The usefulness of SSU rDNA sequences in defining and relating red algal orders appears mixed. Among those represented by two or more sequences, Bangiales, Compsopogonales, Gracilariales, and the NAP complex are well supported, as is Ceramiales minus Ceramiaceae. Hildenbrandiales, Corallinales, Ahnfeltiales, and (in some trees) Bonnemaisoniales, so far represented by single sequences, likewise appear distinctive. Recent phylogenetic concepts supported by our analyses are the primitive nature of Ahnfeltiales, postulated from pit-plug structure (10, 38), and the elevation of Gelidiales, along with Bonnemaisoniales, to "higher" Rhodophyta (41).

The actual degree of divergence correlates poorly with present taxonomic rank (42); species within genus *Porphyra* vary by up to 5.0% over the most conservative 1200 positions, while representatives of the NAP complex vary by no more than 0.7%. We have examined intrafamilial variation in detail only in Gracilariaceae (17) and emphasize that more sequences are required to assess the validity of taxa by phylogenetic criteria.

APPENDIX

Taxa (subclass, order, family, and genus and species) from which SSU rDNA sequences were obtained, with GenBank accession numbers for rDNAs. Collection sites and culture sources are available from C.J.B.

BANGIOPHYCIDAE BANGIALES (Bangiaceae): *Porphyra acanthophora* Oliv. & Coll, L26197; *P. leucosticta* Thur. in Le Jol., L26199; *P. miniata* (C. Ag.) C. Ag., L26200; *P. purpurea* (Roth) C. Ag., L26201; *P. spiralis* Oliv. & Coll var. *amplifolia* Oliv. & Coll, L26177; *P. umbilicalis* (L.) J. Ag., L26202. COMPSOPOGONALES (Erythropeltidaceae): *Erythrocladia* sp., L26188; *Erythrotrichia carnea*, L26189. PORPHYRIDIALES (Porphyridiaceae): *Dixoniella grisea* (Geitler) Scott *et al.*, L26187; *Rhodella maculata* Evans; *Stylonema alsidii* (Zanard.) Drew, L26204.

FLORIDEOPHYCIDAE. ACROCHAETIALES (Acrochaetiaceae): *Audouinella dasyae* (Dillw.) Woelk., L26181. AHNFELTIALES (Ahnfeltiaceae): *Ahnfeltia plicata* (Huds.) Fr.,

Z14139. BONNEMAISONIALES (Bonnemaisoniaceae): *Bonnemaisonia hamifera* Hariot, L26182. CERAMIALES (Ceramiaceae): *Ceramium nodulosum* (Lightf.) Ducluz., L26183; *Griffithsia globulifera* Harv., L26192; (Dasyaceae): *Dasya baillouviana* (Gmel.) Mont., L26185; (Delesseriaceae): *Phycodrys rubens* (L.) Batt., L26198; (Rhodomelaceae): *Rhodomela confervoides* (Huds.) Silva, L26203. CORALLINALES (Corallinaceae): *Corallina officinalis* L., L26184. GELIDIALES (Gelidiaceae): *Gelidium vagum* Okam., L26190. GIGARTINALES (Furcellariaceae): *Furcellaria lumbricalis* (Huds.) Lamour., Z14141; (Gigartinaceae): *Chondrus crispus* Stackh., Z14140; (Halymeniaceae): *Grateloupia filicina* (Lamour.) C. Ag. var. *luxurians* A. & E. S. Gepp, L26191; (Petrocelidaceae): *Mastocarpus stellatus* (Stackh. in With.) Guiry, L26195. GRACILARIALES (Gracilariaceae): *Curdiea flabellata* Chapm., L26207; *Gracilaria chilensis* Bird *et al.*, L26217; *G. cornea* J. Ag., L26212; *G. tikvahiae* McLachlan, M33640; *G. verrucosa* (Huds.) Papenf., M33638; *Gracilariopsis lemaneiformis* (Bory) Dawson *et al.*, L26214; *Gracilariopsis* sp., M33639; *Melanthalia obtusata* (Labillard.) J. Ag., L26215. HILDENBRANDIALES (Hildenbrandiaceae): *Hildenbrandia rubra* (Sommerf.) Menegh., L19345. NEMALIALES (Liagoraceae): *Nemalion helminthoides* (Vell. in With.) Batt., L26196. PALMARIALES (Palmariaceae): *Devaleraea ramentacea* (L.) Guiry, L26186; *Halosaccion glandiforme* (Gmel.) Rupr., L26193; *Palmaria palmata* (L.) Kuntze, Z14142. RHODYMENIALES (Lomentariaceae): *Lomentaria baileyana* (Harv.) Farl., L26194.

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1. Woelkerling, W. J. (1990) in *Biology of the Red Algae*, eds. Cole, K. M. & Sheath, R. G. (Cambridge Univ. Press, New York), pp. 1–6.
2. Gabrielson, P. W., Garbary, D. J., Sommerfeld, M. R., Townsend, R. A. & Tyler, P. L. (1990) in *Handbook of the Protoctista*, eds. Margulis, L., Corliss, J. O., Melkonian, M. & Chapman, D. J. (Jones & Bartlett, Boston), pp. 102–118.
3. Gabrielson, P. W., Garbary, D. J. & Scagel, R. F. (1985) *BioSystems* **18**, 335–346.
4. Gabrielson, P. W. & Garbary, D. J. (1987) *Br. Phycol. J.* **22**, 125–138.
5. Scott, J. & Broadwater, S. (1989) *Protoplasma* **152**, 112–122.
6. Magne, F. (1989) *Cryptogam. Algol.* **10**, 101–115.
7. Garbary, D. J. & Gabrielson, P. W. (1990) in *Biology of the Red Algae*, eds. Cole, K. M. & Sheath, R. G. (Cambridge Univ. Press, New York), pp. 477–498.
8. Guiry, M. D. (1978) in *Modern Approaches to the Taxonomy of Red and Brown Algae*, eds. Irvine, D. E. G. & Price, J. H. (Systematics Assoc., London), Spec. Vol. 10, pp. 111–144.
9. Pueschel, C. M. & Cole, K. M. (1982) *Am. J. Bot.* **69**, 703–720.
10. Maggs, C. A. & Pueschel, C. M. (1989) *J. Phycol.* **25**, 333–351.
11. Fredericq, S. & Hommersand, M. H. (1989) *J. Phycol.* **25**, 213–227.
12. Fredericq, S. & Norris, J. N. (1994) *Cryptogem. Bot.* **81**, in press.
13. Kraft, G. T. & Robins, J. (1985) *Phycologia* **24**, 67–77.
14. Woese, C. R. (1987) *Microbiol. Rev.* **51**, 221–271.
15. Cedergren, R., Gray, M. W., Abel, Y. & Sankoff, D. (1988) *J. Mol. Evol.* **28**, 98–112.
16. Wainright, P. O., Hinkle, G., Sogin, M. L. & Stickel, S. A. (1993) *Science* **260**, 340–342.
17. Bird, C. J., Rice, E. L., Murphy, C. A. & Ragan, M. A. (1992) *Phycologia* **31**, 510–522.
18. Rice, E. L. & Bird, C. J. (1990) *Phycologia* **29**, 501–510.
19. Sogin, M. L. (1990) in *PCR Protocols. A Guide to Methods and Applications*, eds. Innes, M. A., Gelfand, D. H., Sninsky, J. J. & White, T. J. (Academic, San Diego), pp. 307–314.
20. Bird, C. J., Rice, E. L., Murphy, C. A., Liu Q.-Y. & Ragan, M. A. (1990) *Nucleic Acids Res.* **18**, 4023–4024.
21. Gutell, R. R. (1993) *Nucleic Acids Res.* **21**, 3051–3054.
22. Larsen, N., Olsen, G. J., Maidak, B. L., McCaughey, M. J., Overbeek, R., Macke, T. J., Marsh, T. L. & Woese, C. R. (1993) *Nucleic Acids Res.* **21**, 3021–3023.
23. Douglas, S. E., Murphy, C. A., Spencer, D. F. & Gray, M. W. (1991) *Nature (London)* **350**, 148–151.
24. Maier, U. G., Hofmann, C. J. B., Eschbach, S., Wolters, J. & Igloi, G. L. (1991) *Mol. Gen. Genet.* **230**, 155–160.
25. Felsenstein, J. (1989) *Cladistics* **5**, 164–166.
26. Swofford, D. L. (1991) PAUP: *Phylogenetic Analysis Using Parsimony* (Illinois Nat. Hist. Surv., Champaign), Version 3.1.
27. Felsenstein, J. (1985) *Evolution* **39**, 783–791.
28. Olsen, G. J., Matsuda, H., Hagstrom, R. & Overbeek, R. (1994) *Comput. Appl. Biosci.* **10**, 41–48.
29. Kishino, H. & Hasegawa, M. (1989) *J. Mol. Evol.* **29**, 170–179.
30. Felsenstein, J. (1985) *Syst. Zool.* **34**, 152–161.
31. Templeton, A. R. (1983) *Evolution* **37**, 221–244.
32. Schmitz, F. (1892) in *Syllabus der Pflanzenfamilien*, ed. Engler, A. (Bornträger, Berlin), pp. 16–23.
33. Bold, H. C. & Wynne, M. J. (1985) *Introduction to the Algae* (Prentice-Hall, Englewood Cliffs, NJ), 2nd Ed., p. 720.
34. Michel, F. & Westhof, E. (1990) *J. Mol. Biol.* **216**, 585–610.
35. Ragan, M. A., Bird, C. J., Rice, E. L. & Singh, R. K. (1993) *Nucleic Acids Res.* **18**, 3898.
36. Stiller, J. W. & Waaland, J. R. (1993) *J. Phycol.* **29**, 506–517.
37. Oliveira, M. C. & Ragan, M. A. (1994) *Mol. Evol. Biol.* **11**, 195–207.
38. Pueschel, C. M. (1989) *J. Phycol.* **25**, 625–636.
39. Papenfuss, G. F. (1944) *Madroño* **7**, 193–214.
40. Hommersand, M. H. (1963) *Univ. Calif. Publ. Bot.* **35**, 165–366.
41. Coomans, R. J. & Hommersand, M. H. (1990) in *Biology of the Red Algae*, eds. Cole, K. M. & Sheath, R. G. (Cambridge Univ. Press, New York), pp. 275–304.
42. Saunders, G. W., Kraft, G., Tan, I. H. & Druehl, L. D. (1992) *BioSystems* **28**, 109–116.