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## A cladistic analysis of Rhodophyta: Florideophycidean orders

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# A Cladistic Analysis of Rhodophyta: Florideophycidean Orders

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A cladistic analysis of the orders of red algae is presented that concentrates on relationships among florideophyte taxa. The data matrix comprises 35 characters and 15 taxa. Biochemical and ultrastructural characters as well as features of vegetative and reproductive morphology and life history were used. Based on a previous cladistic analysis, Erythropeltidales are used as the outgroup to polarize characters among florideophyte orders. Batrachospermales, Ceramiales, Corallinales, Gelidiales, Hildenbrandiales and Nemaliales appear monophyletic, as do Palmariales with the possible exception of one or two genera. Recognition of Acrochaetiales and Bonnemaisoniales is supported, even though the former may be paraphyletic and the latter polyphyletic based on the inclusion of Naccariaceae. Gigartinales appear to be paraphyletic, and relationships among the constituent families need to be resolved to determine the status of the order. Rhodymeniales may not merit ordinal recognition, as all of the synapomorphies and autapomorphies that distinguish this taxon are also found in some taxa in Gigartinales, with the exception of tetrahedrally divided tetrasporangia. Relationships among syntagmatic taxa with two-layered plug caps are unresolved as is the relationship of these taxa with the zero-layered plug-cap lineage.

Phylogenetic analysis or cladistics has not been a widely used tool in red algal systematics. Garbary (1978) used this method (Hennigian argumentation) to support recognition of Acrochaetiales, although a formal cladistic analysis was not carried out. Gabrielson, Garbary & Scagel (1985) reported the results of the first cladistic analysis of red algae. They discussed mainly the relationships among bangiophyte taxa and demonstrated that the class/subclass Bangiophyceae/Bangiophycidae is paraphyletic, i.e. a group that includes a common ancestor and some but not all of its descendants (Farris, 1974). Because no synapomorphies (shared derived characters) were found that unite bangiophyte orders into a monophyletic group,

Gabrielson *et al.* (1985) recommended that a single class, Rhodophyceae, be recognized that includes all Rhodophyta. In this paper we continue to refer to bangiophyte orders and florideophyte orders, but only for convenience and do not mean to imply acceptance of these taxa as they have been defined in the past.

Gabrielson *et al.* (1985) demonstrated that florideophyte orders as a whole are monophyletic, with two features, presence of tetrasporangia and formation of a filamentous gonimoblast, serving as synapomorphies for the group. In this paper we discuss evolutionary relationships among florideophyte orders based on a cladistic analysis. In a few instances, bangiophyte orders will be referred to, but only as they relate to florideophyte orders or to previously published cladograms. We also

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discuss the reasons for revising some of the characters that we used in the previous analysis, and we will compare the topologies of the trees resulting from analyses of the two data matrices.

## MATERIALS AND METHODS

The 35 characters used in the analysis (Table I) were selected from the literature while preparing a comprehensive review of red algal systematics (Gabrielson & Garbary, 1986). Sources for the character states of each character are cited in the review. The data matrix comprises 15 taxa and 35 characters (Table II). Character states of each character were scored zero (0) for the primitive or plesiomorphic state, one (1) for the derived or apomorphic state or nine (9) if the character state was not applicable or unknown. Exceptions are characters 7 and 21–22 which are multistate rather than binary characters. Character 7 refers to the number of plug-cap layers (0, 1 or 2) that overlie pit plugs (Pueschel & Cole, 1982), and the character states were scored accordingly. Characters 21–22 express, using redundant linear coding (O'Grady & Deets, 1986), evolutionary relationships for the mode of cleavage of red algal tetrasporangia suggested by Guiry (1978). These relationships are represented by the tree in Fig. 1 and the corresponding character codes given in Fig. 2.

Numerous characters that were coded as occurring in one or another of two states in the previous analysis [e.g. isomorphic life history vs. heteromorphic life history (Gabrielson et al., 1985)] can occur in both states in a single order. In this analysis these characters were re-coded so that both states are represented in taxa where they co-occur. For each pair of characters, the states were coded to preserve the assigned polarity.

In cladistics the best method for determining character polarity is outgroup comparison (Wiley, 1981). One selects one or more sister taxa (i.e. those taxa most closely related to the group being studied) to serve as the outgroup and polarize the characters based on the polarity indicated by the outgroup. In this analysis Erythropeltidales were designated the outgroup, but using Compsopogonales, Rhodochaetales or Bangiales as the outgroup would not have affected the relationships among florideophyte orders as represented in the trees (see Gabrielson et al., 1985 for discussion). The data matrix was run using the PHYSYS program (copyright, J. S. Farris & M. F. Mickevich, 1983) and the tree building algorithm options designated "WAG" and "WAG.S".

TABLE I. List of characters used in the data matrix (Table II) with their designated character states. Character states listed first are usually considered to represent the plesiomorphic state

(1) Diffuse growth present (0), absent (1)
(2) Apical growth absent (0), present (1)
(3) B-phycoerythrin present (0), absent (1)
(4) R-phycoerythrin absent (0), present (1)
(5) Pit connections present (0), absent (1)
(6) Pit plugs present (0), absent (1)
(7) Plug caps 0, 1, or 2
(8) Outer cap layer not enlarged (0), enlarged (1)
(9) Dictyosomes unassociated with mitochondria (0), associated (1)
(10) Marine and freshwater (0), freshwater only (1)
(11) Sexual reproduction present (0), absent (1)
(12) Morphologically differentiated carpogonium absent (0), present (1)
(13) Morphologically differentiated carpogonial branch absent (0), present (1)
(14) Auxiliary cell absent (0), present (1)
(15) Auxiliary cell present before fertilization (0), after (1)
(16) Filamentous gonimoblast absent (0), present (1)
(17) Spermatangia and carposporangia syntagmatic (0), parenchymatous (1)
(18) Spermatangia obliquely divided (0), transversely divided (1)
(19) Monosporangia cleaved by a curved wall from an undifferentiated cell present (0), absent (1)
(20) Tetrasporangia absent (0), present (1)
(21–22) Mode of cleavage of tetrasporangia (see Figs 1, 2 and text for explanation)
(23) If zonate, successively divided (0), simultaneously divided (1)
(24) Spore germinates within its wall present (0), absent (1)
(25) Spore germinates out of its wall absent (0), present (1)
(26) Spore germinates into a filament present (0), absent (1)
(27) Spore germinates into a disc absent (0), present (1)
(28) Filamentous germling unipolar present (0), absent (1)
(29) Filamentous germling bipolar absent (0), present (1)
(30) Calcite absent (0), present (1)
(31) Female gametophyte macroscopic (0), microscopic (1)
(32) Thalli filamentous present (0), absent (1)
(33) Thalli syntagmatic absent (0), present (1)
(34) Heteromorphic life history present (0), absent (1)
(35) Isomorphic life history absent (0), present (1)

## RESULTS AND DISCUSSION

### Tree length and the consistency index

Six trees of equal length (= 62) resulted from the PHYSYS run using the WAG algorithm. The branch-swapping

TABLE II. Data matrix for red algal orders

| Taxa* | | | | | | | | | | | | | | | | | Characters | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 | 21 | 22 | 23 | 24 | 25 | 26 | 27 | 28 | 29 | 30 | 31 | 32 | 33 | 34 | 35 |
| ERY | 0 | 0 | 0 | 0 | 0 | 0 | 9 | 9 | 0 | 0 | 1 | 9 | 9 | 9 | 9 | 9 | 9 | 9 | 1 | 0 | 9 | 9 | 9 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 9 | 9 | 0 | 0 |
| COM | 0 | 0 | 9 | 9 | 1 | 0 | 9 | 9 | 0 | 1 | 0 | 9 | 9 | 9 | 9 | 9 | 9 | 9 | 1 | 0 | 9 | 9 | 9 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 9 | 9 | 9 | 9 | 9 |
| BAN | 0 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 9 | 9 | 9 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 |
| RHO | 1 | 1 | 9 | 9 | 1 | 0 | 9 | 9 | 0 | 0 | 1 | 0 | 0 | 0 | 9 | 0 | 0 | 0 | 1 | 0 | 9 | 9 | 9 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 1 |
| ACR | 1 | 1 | 0 | 1 | 1 | 1 | 2 | 0 | 1 | 0 | 1 | 1 | 0 | 0 | 9 | 1 | 0 | 1 | 0 | 1 | 0 | 0 | 9 | 1 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 |
| GEL | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 0 | 1 | 0 | 1 | 1 | 0 | 0 | 9 | 1 | 0 | 0 | 9 | 1 | 0 | 0 | 9 | 0 | 1 | 1 | 1 | 9 | 9 | 0 | 0 | 1 | 1 | 1 | 1 |
| NEM | 1 | 1 | 1 | 1 | 1 | 1 | 2 | 0 | 1 | 0 | 1 | 1 | 1 | 0 | 9 | 1 | 0 | 0 | 9 | 1 | 0 | 0 | 9 | 0 | 0 | 0 | 1 | 0 | 9 | 0 | 1 | 0 | 1 | 0 | 1 |
| PAL | 1 | 1 | 1 | 1 | 1 | 1 | 2 | 0 | 1 | 1 | 1 | 1 | 0 | 0 | 9 | 1 | 0 | 0 | 9 | 1 | 0 | 0 | 9 | 0 | 1 | 1 | 1 | 9 | 9 | 0 | 0 | 1 | 1 | 1 | 1 |
| BAT | 1 | 1 | 1 | 1 | 1 | 1 | 2 | 1 | 1 | 0 | 1 | 1 | 1 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 9 | 9 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 1 | 0 | 1 |
| GIG | 1 | 1 | 1 | 1 | 1 | 1 | 0 | 9 | 1 | 1 | 1 | 1 | 1 | 0 | 0 | 1 | 0 | 0 | 9 | 1 | 0 | 0 | 9 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 |
| RHY | 1 | 1 | 1 | 1 | 1 | 1 | 0 | 9 | 1 | 0 | 1 | 1 | 1 | 1 | 0 | 1 | 0 | 0 | 9 | 1 | 9 | 9 | 9 | 0 | 0 | 1 | 1 | 9 | 9 | 0 | 0 | 0 | 1 | 0 | 1 |
| BON | 1 | 1 | 1 | 1 | 1 | 1 | 0 | 9 | 1 | 0 | 1 | 1 | 1 | 0 | 9 | 1 | 0 | 0 | 9 | 1 | 0 | 1 | 9 | 0 | 0 | 0 | 1 | 1 | 1 | 0 | 0 | 1 | 1 | 0 | 1 |
| CER | 1 | 1 | 1 | 1 | 1 | 1 | 0 | 9 | 1 | 0 | 1 | 1 | 1 | 1 | 1 | 9 | 1 | 9 | 9 | 1 | 9 | 0 | 9 | 1 | 1 | 1 | 0 | 1 | 1 | 0 | 0 | 0 | 1 | 0 | 1 |
| HIL | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 0 | 0 | 9 | 9 | 9 | 9 | 9 | 9 | 9 | 9 | 1 | 0 | 1 | 0 | 1 | 1 | 1 | 1 | 9 | 9 | 0 | 9 | 1 | 1 | 9 | 1 |
| COR | 1 | 1 | 1 | 1 | 1 | 1 | 2 | 1 | 1 | 0 | 1 | 1 | 1 | 0 | 9 | 1 | 0 | 0 | 9 | 1 | 0 | 2 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 0 | 1 | 1 | 1 | 1 |

* ERY = Erythropeltidales; COM = Compsopogonales; BAN = Bangiales; RHO = Rhodochaetales; ACR = Acrochaetiales; GEL = Gelidiales; NEM = Nemaliales; PAL = Palmariales; BAT = Batrachospermales; GIG = Gigartinales; RHY = Rhodymeniales; BON = Bonnemaisoniales; CER = Ceramiales; HIL = Hildenbrandiales; COR = Corallinales.
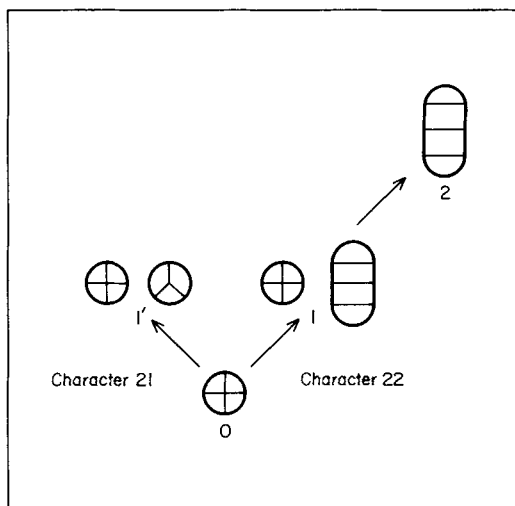
FIG. 1. Multistate character tree illustrating the postulated evolutionary relationships among cruciate, zonate and tetrahedral tetrasporangia.



FIG. 2. Matrix of character state codes for characters 21 and 22 showing the code for each character state.

algorithm (WAG.S) produced no trees of shorter length. Tree length refers to the number of steps (character changes) needed to produce the shortest tree using the character states provided in the data matrix. One of the six trees is illustrated in Fig. 3.

The other five cladograms differed from the one in Fig. 3 between nodes D and H, and three of these cladograms, representing the greatest variation, are illustrated in Figs 4, 5 and 6. They will be discussed below in the appropriate sections.

PHYSYS also provides a character consistency index (CI) for each tree and for each
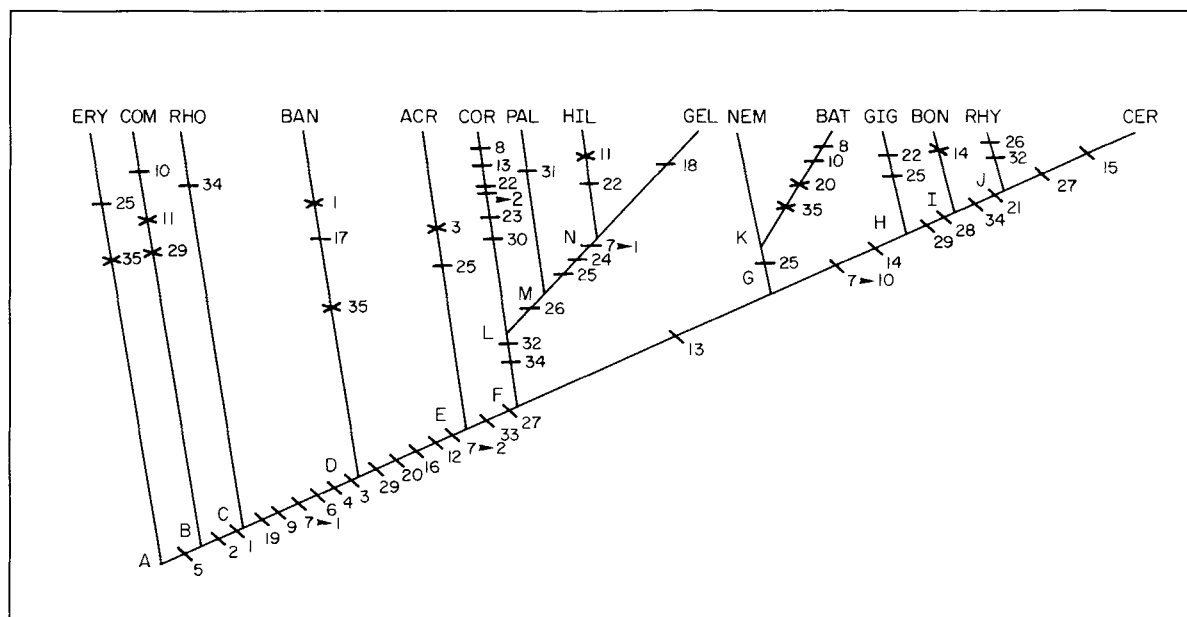


FIG. 3. One of six cladograms illustrating relationships among red algal orders that resulted from an analysis of the data matrix (Table II). Notes for Figs 3–7. Numbers on cladograms correspond to characters in Table I. Letters are to left of nodes they identify. Slashes represent character advances, crosses, character reversals or losses. Taxa abbreviations are same as in Table II. See text for discussion.
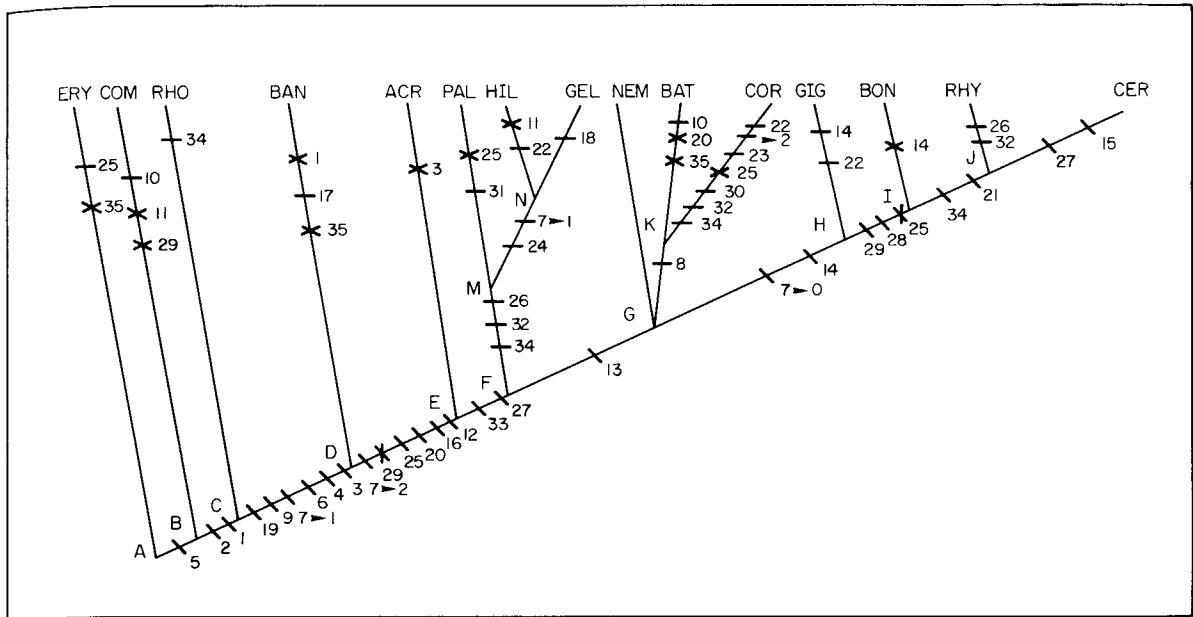
FIG. 4. One of six cladograms that resulted from an analysis of the data matrix.

character. This index is simply the number of times a character appears on the tree divided into the number of times the character appears in the data matrix, expressed as a percent. For example, character 15 (auxiliary cell present after fertilization) appears once in Fig. 3 and therefore has a CI of 100%, whereas character 8 (outer cap-layer enlarged) appears twice and has a CI of 50%. The overall CI of all six cladograms is c. 60%. Eighteen of the characters had a CI of 100%,



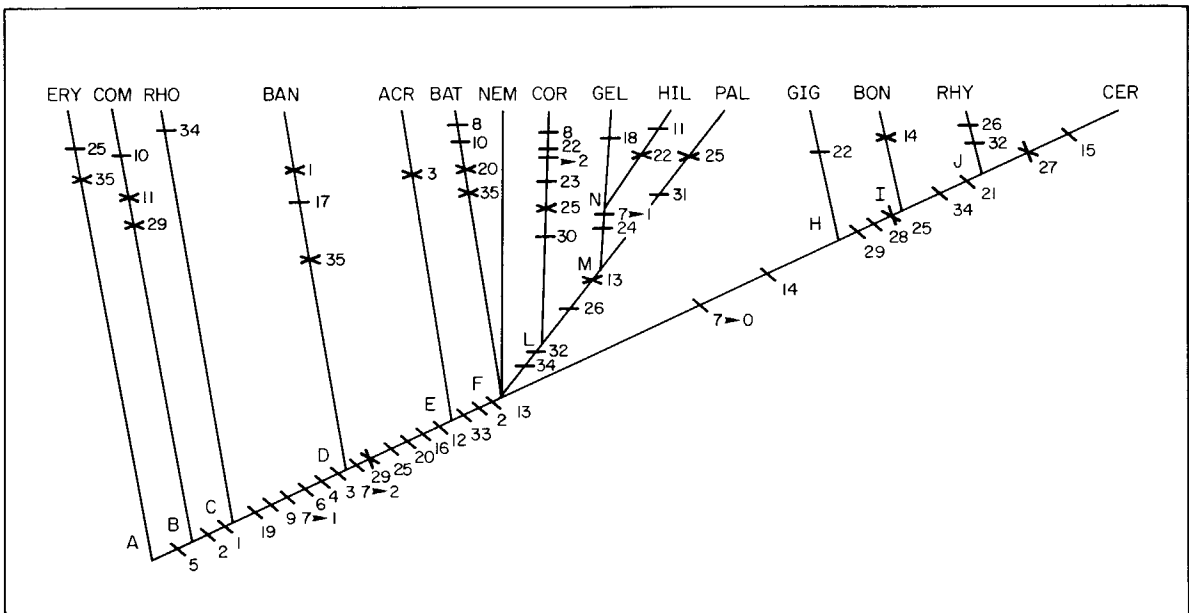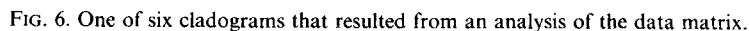FIG. 5. One of six cladograms that resulted from an analysis of the data matrix.

Fig. 6. One of six cladograms that resulted from an analysis of the data matrix.

two (characters 8 and 13) had a CI of 75%, nine had a CI of 50% and six had a CI of <50%. Characters with low CIs will be discussed where appropriate.

## Omission of the Porphyridiales and mitotic characters

The order Porphyridiales was omitted from the analysis because it is considered by us (Garbary, Hansen & Scagel, 1980; Gabrielson et al., 1985) and other workers (Fritsch, 1945, p. 438; Lee, 1974; Ott, 1976; Scott, 1986) to be a heterogeneous assemblage of taxa. In cladistics, it is a polyphyletic group, i.e. a group in which the most recent common ancestor is assigned to some other group and not to the group itself (Farris, 1974). Evidence supporting this view comes especially from recent ultrastructural studies on organelle associations (Scott, 1984 and pers. comm.) and mitosis (Schornstein & Scott, 1982; Scott, 1986) of porphyridialean taxa. Because so few taxa in the order have been examined for these features, and those that have are heterogeneous it is not currently possible to obtain a meaningful code of character states for an analysis.

Thus three features of mitosis [number of gaps in the nuclear envelope, morphology of the nucleus-associated organelle (NAO) and presence or absence of perinuclear endoplasmic reticulum (PER)] that were used in the previous study (Gabrielson et al., 1985), were removed from this analysis because of lack of information on mitosis in most florideophyte orders and the absence of reports on mitosis in any of the outgroups, making it impossible to polarize the characters. Previously we used the mitotic features of Porphyridium (Schornstein & Scott, 1982) to polarize these characters, but Porphyridiales has proven heterogeneous with regard to mitosis (Scott, 1986), and we consider the taxon to be polyphyletic.

Among florideophytes, only single species in Batrachospermales (Scott, 1983) and Rhodymeniales (Davis & Scott, 1986) and two species in Ceramiales (Dave & Godward, 1982; Scott et al., 1980) have been examined for ultrastructural features of mitosis. Of particular interest are the features of mitosis in Bangiales, the order that we currently consider to be the sister taxon to florideophytes based on the following synapomorphies: dictyosomes

associated with mitochondria and the presence of cellulose and R-phycoerythrin (Gabrielson et al., 1985). Knowledge of mitosis in Bangiales would support, or perhaps refute this hypothesis. We concur with Scott (1986), that mitosis in red algae is an evolutionary conservative process that will be useful in segregating taxa at higher ranks of classification.

## Plug caps

Pueschel & Cole (1982) showed that florideophyte red algae have pit plugs without a cap layer, with a single cap layer or with two cap layers. They used this feature to segregate families from orders that were heterogeneous regarding this character, e.g. Batrachospermaceae, Lemaneaceae and Thoreaceae from Nemaliales, and Hildenbrandiaceae from Cryptonemiales, and to support the recognition of orders that earlier had been established based on other features (e.g. Bonnemaisoniales, Corallinales, Gelidiales, and Palmariales). Character 7 (number of plug-cap layers) appears four times on all cladograms (Figs 3–6); once in the 0 state below node H; once in the 2 state below node E and twice in the 1 state, below nodes D and N. In the 1 state it is homoplasious, occurring first below node D and reversing to that state below node N. Caution is needed in interpreting this character, because while a diagnostic stain (phosphotungstic acid) was found for the outer cap layer of two-layered plug caps, and all of these stain consistently and are considered homologous, a diagnostic stain for the inner layer of two-layered plug caps and for one-layered plug caps has not been found. It is therefore not known whether the one-layered plug cap in Bangiales is homologous with the one-layered plug cap in Gelidiales or Hildenbrandiales, or with the inner cap layer of two-layered plug caps. In the data matrix (Table II) one-layered plug caps of Bangiales were not coded differently from one-layered plug caps found in Gelidiales or Hildenbrandiales. Therefore, the character state was considered homologous in the three taxa. Despite this assumption, in no cladogram did character 8 serve as a synapomorphy linking Bangiales with Gelidiales and Hildenbrandiales.

The importance of resolving homologies among taxa possessing at least one-layered plug caps is evident. Without such information, an understanding of the relationships among taxa possessing plug caps will not be forthcoming. Studies of the ontogeny of pit plugs and of cap layers may be the best approaches to answering these questions.

## Life histories

Feldmann (1952) was the first to hypothesize that a triphasic life history was primitive for red algae. He further considered an alternation of isomorphic generations, as found in some Acrochaetiales, to be primitive. Yet, despite the emphasis over the last 20 years on culturing the "missing" generations in red algal life histories, we are not closer to understanding whether an isomorphic or a heteromorphic life history is ancestral, or even a triphasic one. This was aptly stated by West & Hommersand (1981, p. 136) in their review of life histories, ". . . the possibility of a phylogenetic interpretation of red algal life histories seems almost to be receding from us".

Two characters (34 and 35) were used to code life histories, so that all orders are accurately represented, particularly those in which an alternation of both isomorphic and heteromorphic types are present. Compared with other characters in the analysis, these two have among the lowest CI values ($<35\%$). Some phylogenetically useful information, however, can be gained by examining the positions of these characters on the resulting cladograms. Among taxa above node D, character 34 (absence of a heteromorphic life history) appears either twice; below nodes J and L (Figs 3, 5, 6) or thrice; below nodes J and M and as an autapomorphy for Corallinales (Fig. 4). Thus, isomorphic life histories probably arose in at least two independent lineages of florideophytes. Note that only tetra-

sporangial thalli are known in Hilden-
brandiales, and therefore for the life history
characters (34 and 35), the character states
were scored "9" (unknown). Character 34 is
thus a synapomorphy below node L in Figs
3, 5 and 6, a lineage that includes
Hildenbrandiales. This does not mean that
Hildenbrandiales have an alternation of
isomorphic generations, but rather that their
ancestor likely exhibited such a life history.

If Bangiales is the sister group of
florideophyte orders as suggested by this
analysis (Figs 3–6) and the previous one
(Gabrielson et al., 1985), then an alternation
of heteromorphic generations would be the
ancestral condition among these orders.
With regard to all red algae, it is clear that
in the absence of an evident sister taxon, and
therefore with no means of polarizing the
isomorphic/heteromorphic     life     history
character, it may be impossible to determine
the life-history pattern of the progenitor of
Rhodophyta and to end this debate.

### Acrochaetiales

In all trees (Figs 3–6), Acrochaetiales are
supported as a distinct order and share a
common ancestry with all syntagmatic taxa
having filamentous gonimoblasts and tetra-
sporangia. A reversal in character 3
consistently appears as an autapomorphy of
the order (Figs 3–6) indicating the presence
of both R- and B-phycoerythrin in this
taxon. This is the only order that is
heterogeneous for the two types of phyco-
erythrin, perhaps suggesting that the order is
not monophyletic. Ultrastructural studies of
mitosis and pit plugs in the species of
Audouinella previously surveyed for phyco-
erythrin types (Glazer, West & Chan, 1982)
would be useful in resolving the question of
monophyly in the order. See Garbary (1978)
for further discussion and other proposals on
relationships of Acrochaetiales.

### Palmariales, Hildenbrandiales and Gelidiales

In all trees (Figs 3–6), a lineage leading to
Palmariales,       Hildenbrandiales       and

Gelidiales is consistently supported by
character 26 (absence of a filamentous
germling stage) below node M. In Fig. 4, the
lineage is additionally supported by
characters 32 (absence of filamentous thalli)
and 34 (absence of heteromorphic life
histories). Reasons for not supporting the
tree in Fig. 4 are discussed below. In Fig. 5
a reversal in character 13 (presence of a
morphologically differentiated carpogonial
branch) also supports the lineage.

By virtue of a unique life-history pattern
characterized by a microscopic female
gametophyte and apparent absence of a
carposporophyte generation, Palmariales are
clearly monophyletic. The palmarialean life
history is obviously derived from a
Polysiphonia-type life history, i.e. triphasic in
which gametophytes and tetrasporophytes
are isomorphic macroscopic plants, and this
is supported by all of the cladograms (Figs
3–6). There is some question, however,
regarding the assignment of Rhodophysema
(Silva, 1982) and Halosacciocolax (Hawkes
& Scagel, 1986) to the order.

The Hildenbrandiales–Gelidiales lineage is
supported in all trees (Figs 3–6) by the
presence of an empty spore pattern of
germination (character 25) and by the one-
layered plug-cap character state of character
7. The significance of the number of plug-cap
layers to red algal systematics was discussed
above, and Gabrielson & Garbary (1986)
have discussed spore germination patterns in
the same context. Empty spore germination
patterns are characteristic of a number of
orders and families of red algae and
Chemin's (1937) Gelidium-type pattern is
expecially distinctive. There is only a single
reliable study of spore germination patterns
in Hildenbrandia (Umezaki, 1969), and
additional species in this genus need to be
examined. New information may show that
each of these orders is a separate and distinct
lineage and that they did not share a
common ancestor. The Hildenbrandiales
and Gelidiales each appears to be mono-
phyletic with Hildenbrandiales unique
among red algae that possess pit plugs by
virtue of the absence of sexual reproduction

(character 11) in all taxa that have been studied. Gelidiales are unique in having transversely divided spermatangia (character 18, and see Gabrielson & Garbary, 1986).

## Corallinales

Corallinales are a well-defined and monophyletic order characterized by: (1) cell walls impregnated with calcite (character 30); (2) simultaneously, zonately divided tetrasporangia (characters 21–23); and (3) an enlarged outer plug cap layer (character 8) (also characteristic of Batrachospermales).

The position of Corallinales varied from node L (Figs 3, 5, 6), which it shared with the lineage leading to Gelidiales–Hildenbrandiales–Palmariales, to node O (Fig. 4), which it shared with Batrachospermales. Node L is supported in all trees (Figs 3–6) by the derived character states of character 32 (absence of filamentous thalli) and character 34 (absence of a heteromorphic life history). Node O (Fig. 4) is supported by character 8, but there is no evidence that this feature is homologous in the two orders. Other vegetative and reproductive characters also support Figs 3, 5, 6 and not Fig. 4.

The position of Corallinales in the cladograms is not affected regardless of whether the taxon is coded as having auxiliary cells (character 14) or lacking them. At present, there are two conflicting interpretations of post-fertilization development in Corallinales, by Lebednik (1977) and Woelkerling (1980). Lebednik observed an external connection (i.e. a connecting filament) between the carpogonium and supporting cell in *Mesophyllum*, whereas Woelkerling considered the transfer of the diploid nucleus between the carpogonium and supporting cell to occur internally in *Metamastophora*, as a result of cell fusions. Lebednik attempted to reconcile the placement of Corallinales (as Corallinaceae) in Cryptonemiales by emending the circumscription of the latter to include ". . . those species in which the auxiliary cell is located in a receptive carpogonial branch system or

in a reduced carpogonial branch system (= the accessory branch system of most authors)". Lebednik's concepts of evolutionary pathways within Cryptonemiales (including Corallinaceae) have not been followed, but neither have they been discussed in papers on other cryptonemialean or corallinalean taxa.

Based on Woelkerling's interpretation of post-fertilization events in Corallinales and the apparent stability of the plug cap character in the analysis, it is apparent that auxiliary cells of Corallinales are not homologous with those in Gigartinales (including Cryptonemiales), Rhodymeniales or Ceramiales. The position of Corallinales in Figs 3 and 6 calls into question the homology of carpogonial branches in this taxon as well as auxiliary cells. In both trees, character 13 (morphologically differentiated carpogonial branches) appears twice; as an autapomorphy for the Corallinales and as a synapomorphy below node G. In contrast, in Figs 4 and 5, character 13 occurs only once; below node G in the former and below node F in the latter, and thus morphologically differentiated carpogonial branches would appear to be homologous in all taxa above those nodes. Clearly there is a need for further studies of the ontogeny of carpogonial branches and "auxiliary cells" in Corallinales because of the conflicting interpretations by Lebednik (1977) and Woelkerling (1980) and the homoplasy of character 13 in our analysis. Cells from which gonimoblast and carposporangia are produced in Corallinales may need to be given a designation other than "auxiliary cells" to indicate that these structures are not homologous with those in Gigartinales, Rhodymeniales and Ceramiales. The precise relationship of Corallinales to other two-layered plug-cap taxa remains unresolved (Fig. 7).

## Nemaliales and Batrachospermales

The positions of these two orders varied the most in the trees resulting from the analysis (e.g. Figs 3, 5, 6). Arguments for
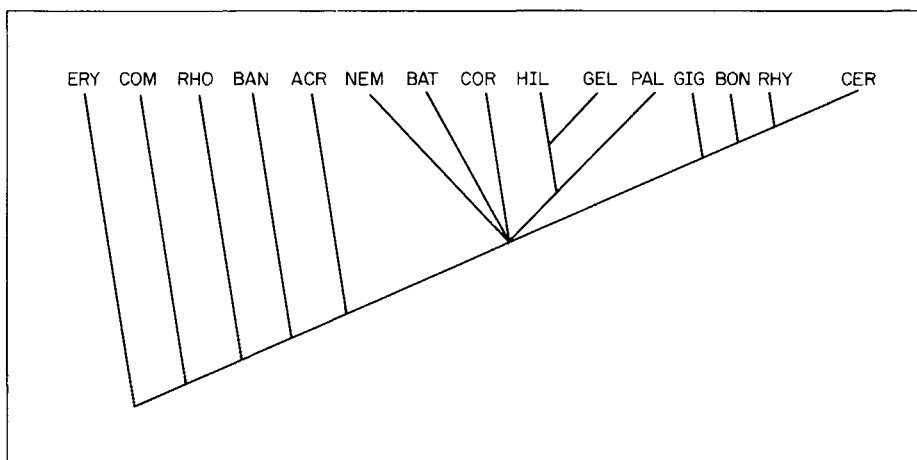
FIG. 7. Adams consensus tree based on congruence of cladograms in Figs 3-6.

rejecting the cladogram in Fig. 4 were discussed above. The tree in Fig. 5 has one less node compared with the cladograms in Figs 3 and 6, with a resulting quadrichotomy at node F. This results from the position of character 13 (morphologically differentiated carpogonial branches) which appears twice in Fig. 5, as a synapomorphy below node F and as a concomitant reversal below node M. The tree in Fig. 6 is better resolved with a trichotomy at node G, whereas the tree in Fig. 3 is fully resolved (dichotomous at all nodes). In the trees in Figs 3 and 6, character 13 also appears twice; as a synapomorphy below node G and as an autapomorphy of the Corallinales. Only in one tree (Fig. 3) was a synapomorphy present that supported a lineage leading to these two orders—character 25 (spores germinating outside of their walls) below node K. This character is very homoplasious and consequently had the lowest CI (<20%) of any character, appearing on all trees at least five times. Based on our analysis, there is no clear evidence that Batrachospermales and Nemaliales shared a common ancestry, particularly in light of the homoplasy of character 25.

Batrachospermales are clearly a monophyletic order with their taxa restricted to fresh water (character 10) and a life-history

pattern (character 20) that appears to be an adaptation to that habitat (Sheath, 1984). Nemaliales appear monophyletic, but there is no autapomorphy to distinguish this taxon. This may indicate the ancestral position of this order among those two-layered plug-cap taxa that have differentiated carpogonial branches. Even with the removal of Acrochaetiaceae, Gelidiaceae, Batrachospermaceae and Bonnemaisoniaceae and Naccariaceae to form the basis for other orders, Nemaliales still has considerable variation (e.g. Chiang, 1985) and may yet be paraphyletic.

## Gigartinales

Following Kraft & Robins (1985), we have included Cryptonemiales in Gigartinales, recognizing that the accessory nature of the auxiliary cell is not a feature that adequately distinguishes these orders. Gigartinales are supported by a single autapomorphy; the presence of both cruciately and zonately divided tetrasporangia (character 22). The bulk of the families, however, are characterized by one or the other type of tetrasporangial cleavage. We consider Gigartinales, both before and after inclusion of Cryptonemiales, to be paraphyletic (for further discussion see Gabrielson & Garbary, 1986). The relation-

ships among the families included in this taxon are clearly in need of further study.

## Bonnemaisoniales

Several authors, beginning with Feldmann & Feldmann (1942), have advocated removing the family Bonnemaisoniaceae from Nemaliales. Bonnemaisoniales was circumscribed with the families Bonnemaisoniaceae and Naccariaceae thought to be allied with Ceramiales. The proposal for ordinal status gained general acceptance with the demonstration by Pueschel & Cole (1982) that both families of Bonnemaisoniales lack plug-cap layers, in contrast to the two-layered plug caps present in all Nemaliales. Pueschel and Cole were careful to point out that the absence of plug-cap layers in itself does not necessarily support a close relationship between Bonnemaisoniales and Ceramiales, as Gigartinales and Rhodymeniales also lack plug-cap layers.

In all trees, Bonnemaisoniales share with Rhodymeniales and Ceramiales several spore-germination characters (25, 28 and 29), but they are segregated from the lineage leading to the other two orders by the presence, in some taxa, of heteromorphic life histories (character 34) and by the presence of only cruciately divided tetraporangia (the 0 state of character 21). Bonnemaisoniales are supported by a reversal in character 14, the absence of an auxiliary cell. Chihara & Yoshizaki (1972), however, interpret an auxiliary cell to be present in certain species of Bonnemaisoniaceae.

Bonnemaisoniales appear polyphyletic, there being what we consider only superficial vegetative and reproductive similarities that argue for the maintenance of the two families (Bonnemaisoniaceae and Naccariaceae) in the same taxon. Ultrastructural studies of vegetative development and of post-fertilization stages along the lines of Aghajanian & Hommersand (1980) and Ramm-Anderson & Wetherbee (1982) respectively, would enable us to compare these features in the two families. The Naccariaceae may prove to be more closely related to certain lower gigartinalean families than to the Bonnemaisoniaceae.

## Rhodymeniales

Rhodymeniales appear monophyletic following removal of two-layered plug-cap taxa to Palmariales, but may not merit recognition as a distinct order based on the sole criterion of the particular position of the auxiliary cell relative to the carpogonial branch (Kraft & Robins, 1985; Gabrielson & Garbary, 1986). There are other families in Gigartinales where auxiliary cells are found in characteristic positions (Searles, 1968; Gabrielson & Hommersand, 1982), and there is no suggestion that these taxa merit ordinal recognition.

In all trees (Figs 3–6), two characters, 26 (absence of a filamentous germling stage) and 32 (absence of filamentous thalli) serve as autapomorphies for Rhodymeniales, but these features are also found in numerous families in Gigartinales as are all synapomorphies below nodes I and J with the exception of the 1 state of character 21 (presence of cruciate and tetrahedral tetrasporangia). Tetrahedrally divided tetrasporangia are unknown in Gigartinales *sensu lato* (Guiry, 1978). Until certain orders, particularly Bonnemaisoniales, Gigartinales and Rhodymeniales are further studied and a phylogenetic analysis carried out at the family level, the relationships depicted in the cladograms in Figs 3–6 among zero-layered plug-cap taxa are our best approximation.

## Ceramiales

Ceramiales have been recognized as a monophyletic order since their removal from Rhodymeniales by Oltmanns (1904). They are uniquely characterized by having auxiliary cells cut off after fertilization (character 15) and are the only order in which all taxa exhibit bipolar spore germination. Our analysis agrees with the opinion expressed by nearly all red algal phycologists that Ceramiales represent a specialized and

highly derived group of Rhodophyceae with zero-layered plug caps.

## Consensus trees

A consensus tree is the result of taking all of the trees produced by an analysis, comparing them, and reducing to polytomies all areas of incongruence. Where branches of trees are congruent, the relationships among taxa remain resolved. The Adams consensus tree for the six trees resulting from the analysis of this data matrix is illustrated in Fig. 7 (see Miyamoto, 1985, for a discussion of Adams and Nelson consensus trees). A major polytomy occurs at node F, indicating the unresolved nature of relationships among the lineage leading to: (1) taxa with zero-layered plug caps (Bonnemaisoniales, Ceramiales, Gigartinales and Rhodymeniales); (2) the lineage leading to Palmariales–Gelidiales–Hildenbrandiales; and (3) the orders Batrachospermales, Corallinales and Nemaliales.

Except for Acrochaetiales, the consensus tree leaves unresolved the relationships among all taxa with two-layered plug caps. Orders with one-layered plug caps (i.e. Hildenbrandiales and Gelidiales), would appear to be derived from an ancestor that also gave rise to Palmariales. Using both light and electron microscopy, studies of early post-fertilization stages, of mitosis and of pit plugs and pit plug-cap layers will aid in supporting or refuting these proposed relationships. Relationships are resolved among zero-layered plug-cap orders as they are currently understood, but problems remain especially with the greatly enlarged order Gigartinales and among its constituent families. Much work is needed to clarify relationships among the families to determine whether the order is monophyletic, or paraphyletic as we have suggested. Studies on the two families of Bonnemaisoniales (Bonnemaisoniaceae and Naccariaceae) are important, since we suggest that the order is polyphyletic. Clarification of relationships of these families may have a strong bearing on

the situation in Gigartinales and in determining the outgroup for Ceramiales.

Above node F (Fig. 7), the question of whether zero-layered plug-cap taxa or two-layered plug-cap taxa represent the ancestral condition is unresolved. The most parsimonious solution is represented in Figs 3, 4, and 6, with two-layered plug-cap taxa giving rise to zero-layered plug-cap taxa by a loss of cap layers. For this hypothesis to be correct, the ancestral position of Acrochaetiales would have to be maintained with regard to all syntagmatic red algae. Studies of mitosis in both Bangiales and Acrochaetiales, as well as resolving the question of the homology of the one-layered plug cap, are critical to furthering our knowledge of relationships among these taxa.

A comparison of the consensus tree resulting from this analysis (Fig. 7) with the one previously published (Gabrielson et al., 1985, Fig. 4) shows many similarities and several significant differences. All changes resulted from altering the coding of some characters to better represent their occurrence in individual orders. Although characters that were changed are found in both bangiophyte and florideophyte orders, only relationships among the latter were affected. Therefore, the summary statements made in this paper about the former orders accurately reflect the current state of our knowledge. For a more complete discussion of those taxa, see Gabrielson et al. (1985).

Comparing the two consensus trees, the most significant differences are: (1) the resolved relationships among Gelidiales, Hildenbrandiales and Palmariales; (2) the unresolved relationship between Nemaliales and Batrachospermales; (3) the change in position of Bonnemaisoniales, Batrachospermales and Nemaliales; and (4) the absence of an outgroup for zero-layered plug-cap taxa. These resulted primarily from changes to the coding of characters dealing with spore germination patterns (characters 24–30) and tetrasporangia (characters 21–22). Thus, the consensus tree in the present analysis (Fig. 7) is preferred to the one from the previous analysis (Gabrielson

*et al.*, 1985, fig. 4), because it presents an evolutionary hypothesis based on improved coding for characters that are heterogeneous within individual orders.

## Concluding remarks

By presenting a cladistic analysis of red algal orders, we hope to stimulate further interest and research in red algal systematics. We consider the various phylogenetic trees that we have presented as evolutionary hypotheses and not as conclusions. Each tree suggests its own character homologies, convergences and parallelisms. The most useful application of these evolutionary models is to focus attention on characters and taxa that are of critical importance in resolving phylogenetic relationships of red algae.

## ACKNOWLEDGEMENTS

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