PHYLOGENETIC REASSESSMENT OF THE MASTOPHOROIDEAE (CORALLINACEAE, RHODOPHYTA) USING MOLECULAR AND MORPHOLOGICAL DATA

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ABSTRACT

Nuclear encoded small ribosomal subunit gene sequences were determined for six species of coralline algae (Corallinaceae, Rhodophyta) representing three genera classified in the Mastophoroideae and two species of *Amphiroa* (Lithophylloideae). These data were combined with previously published 18S rRNA gene sequences for 39 other coralline taxa and one unpublished sequence. Analyses were performed using parsimony and maximum likelihood methods to determine the phylogenetic position of the Mastophoroideae within the Corallinaceae as well as the relationships among *Hydrolithon, Metamastophora, Neogoniolithon,* and *Spongites*. In addition, partial sequences were determined for the nuclear-encoded large ribosomal subunit for five of the six species of mastophoroids and combined with unpublished 26S rRNA gene sequence data for 16 coralline taxa. Sequence data was analyzed using parsimony and maximum likelihood methods for an independent 26S rRNA data set and a taxonomically congruent combined 18S/26S rRNA data set. Results for the 26S and combined data sets were consistent with analysis of the 18S data. The Mastophoroideae is resolved as a polyphyletic taxon with four independent lineages. *Neogoniolithon* spp. were allied with geniculate members of the Corallinoideae and *Hydrolithon* spp. were resolved as sister to the geniculate Metagoniolithoideae. Based on these analyses, *Porolithon pachydermum* is transferred to *Hydrolithon* as *H. pachydermum* (Foslie) Bailey, Gabel *et* Freshwater. An independent morphological data set was also assembled to assess the phylogenetic significance of particular reproductive and vegetative characteristics within the Corallinales. This analysis implies that the mode of tetrasporangial conceptacle

development, orientation of cells lining the pore canal, location of formation of spermatangia within the male conceptacle, location of gonimoblast filament origin along the fusion cell, and appearance of the fusion cell provide additional features that are taxonomically and phylogenetically significant at the rank of subfamily.

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Adele Harvey at La Trobe University, Australia, provided technical assistance in tentatively identifying an unknown species I came across during the course of my research. Adele also kindly provided me with the 18S rRNA gene sequence for *Choreonema thuretii* prior to its public release.

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DEDICATION

This work is dedicated to the two people who have been instrumental in making it all possible: to my parents, Patricia and Paul Gabel. They imparted in me the type of work ethic and strong set of values that have enabled me to succeed at anything I put my mind to. They have been outstanding role models, always fostering in me the pursuit of knowledge and a greater understanding of life in general. I am indebted to them.

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Introduction

Morphology

Coralline red algae (Corallinales, Rhodophyta) are among the most diverse marine seaweeds and are found in all the world's oceans. Their thalli are pseudoparenchymous and composed of filaments that are differentiated into cortical, epithallial, and medullary cells. The vegetative cell walls of all coralline species are impregnated with calcium carbonate in the form of calcite. Two types of thalli are observed in coralline red algae. Geniculate (articulated) coralline algae have thalli comprised of larger calcified segments (intergenicula) separated by smaller uncalcified nodes (genicula). These branched fronds are flexible and are attached to the substrate by a holdfast. The second type includes nongeniculate (nonarticulated) coralline algae whose thalli are entirely calcified (except for reproductive structures) and most, but not all, take the form of crusts that adhere to the underlying substrate (Johansen 1981).

One concept regarding the classification of geniculate corallines includes the designation of three subfamilies based on three distinct types of genicula. The Corallinoideae includes taxa in which intergenicula are formed by similar length medullary cells that are arranged as tiers. In contrast, the genicula are comprised of a single tier of medullary cells that are many times longer in length than a single tier found in an intergeniculum. Genicula are produced by the apical meristem that regularly alternates between the production of calcified cells of the intergenicula and noncalcified cells that form the genicula. General characteristics of the geniculate Lithophylloideae

(Amphiroideae *sensu* Johansen 1969) include similar cell length observed between intergenicula and genicula, and genicula are formed via decalcification of specific portions of intergenicular cells that generally consist of more than one tier. The third geniculate subfamily, Metagoniolithoideae, is unique in that genicula are comprised of many unequal length cells that are not arranged into tiers, whereas the intergenicula are arranged into tiers. Another feature unique to this subfamily is that genicula are meristematic and may give rise to whorled branches. Thalli apices alternate between production of genicula and intergenicula, and genicula continue to elongate with age while intergenicula remain constant in length once established (Johansen 1981).

Nongeniculate coralline algae lack genicula. The most common types are epigenous, including crustose, protuberant, and taeniform thalli. Crustose forms (i.e., *Mesophyllum*, *Synarthrophyton*, *Lithothamnion*, *Hydrolithon,* and *Porolithon*) are most common and attach to the substrate via cell adhesion. Their encrusting thalli generally conform to the shape of the substrate. Nodules may be present on the thallus surface, however they are less distinct than those seen in protuberant types. The protuberant type possesses distinct branches that perpendicularly arise from the thallus and are cylindrical or compressed in shape. Protuberances in some species may be branched. Examples of genera including individuals of this type are *Titanoderma*, *Spongites*, *Lithophyllum*, and *Phymatolithon*. Taeniform thalli are characterized as having flattened (ribbon-like) branches (i.e., *Mastophora* and *Tenarea*), and in some cases attach to the substrate with a holdfast and possess a stipe (i.e., *Metamastophora* and *Mastophoropsis*) (Woelkerling 1988).

Ecology

Coralline red algae occur in a wide variety of marine ecosystems ranging from polar waters to warm tropical waters. In addition to the variety of latitudinal extremes, it has also been demonstrated that the coralline algae are tolerant of the lower boundaries of the photic zone. Nongeniculate forms occur deeper than geniculate forms, and the deepest known macrophyte is a nongeniculate coralline alga found at a record depth of 268 m (Bold & Wynne 1985; Littler et al. 1985). The morphology of species is highly variable and depends upon species-specific characteristics as well as local environmental conditions. Semi-endophytic species (i.e., those growing within the tissue of a host with reproductive structures exposed), epigenous species (i.e., those growing on top of a non-living substrate or as an epiphyte), or unattached species (known as rhodoliths or maerl) are common life forms found within the group (Woelkerling 1988).

One of the most fundamental roles of nongeniculate coralline algae is their function in reef structure. In high wave-energy environments crustose coralline algae proliferate into large "algal ridges" that are situated parallel to the axis of waves, serving as a barrier to lagoon erosion and providing protection to the flora and fauna present. Channels are cut into these algal ridges by waves, altering the hydrodynamics of the protected areas. Other species (i.e., *Hydrolithon*) form extensive pavements that may extend for kilometers in low energy environments. Crustose corallines act to cement the reef superstructure together and trap sediments resulting in further accretion (Johansen 1981). Furthermore, these algae provide food and shelter from predation to various reef denizens. In addition to structural roles, more recent studies have indicated that there may be a correlation between recruitment of invertebrate larvae (that are sessile as adults)

and crustose coralline algae (Morse 1990, 1991; Morse et al. 1994; Heyward & Negri 1999). Inducement of invertebrate larval/juvenile settlement, growth, and survival has also been observed in the green sea urchin, *Strongylocentrotus droebachiensis* (Hagen 1999). However, settlement of the ascidian *Herdmania curvata* is apparently inhibited by coralline substrates (Degnan & Johnson 1999). Although tropical coral reefs are extremely complex, species-rich systems, the members of crustose coralline algae that play the most important role in building the reef are relatively few including species of *Hydrolithon, Porolithon*, *Neogoniolithon*, *Sporolithon*, and *Lithophyllum* (Johansen 1981). The first four genera listed are members of the subfamily Mastophoroideae and *Lithophyllum* is a member of the subfamily Lithophylloideae.

Given the diversity and adaptability of this group it is not surprising that there is a long natural history associated with them. Because they are calcified, corallines are among the few macroalgal taxa for which an extensive fossil record exists. Extant corallines first appeared in the Jurassic period *ca*. 180 million years ago (mya) (Johnson 1956; Wray 1977; Johansen 1981). An apparent ancestral lineage, the Solenoporaceae, dates back to the Cambrian period, 543 mya, shortly after the first multicellular organisms evolved. Johansen (1981) and others have postulated that members of this extinct family gave rise to the extant members of the Corallinaceae. Fossil evidence is consistent with the hypothesis that melobesioid forms may be derived from *Solenopora* (an extinct genus within the Solenoporaceae) in the early Mesozoic (*ca*. 225 mya) and the lithophylloid and geniculate forms may be derived from *Parachaetetes* (late Paleozoic, *ca*. 353.7 mya) (Wray 1977). However, diagnoses for fossil taxa are based strictly on those anatomical features preserved in fossil specimens, including size, thickness, and

thallus shape as well as intercellular orientation and size. It is therefore difficult to integrate fossil taxa into modern classifications because reproductive (conceptacle) characteristics, one of the main diagnostic features used to classify extant coralline red algae, are poorly preserved or often absent in fossil specimens (Johansen 1981; Woelkerling 1988; Braga & Aguirre 1995).

Classification

The subfamilial classification of coralline algae is controversial. The presently accepted scheme is described in the following paragraphs. Conflicting viewpoints will be discussed later.

The predominant morphological difference that pervades the literature concerns the relationships among and between geniculate vs. nongeniculate morphotypes. Johansen (1981) and Woelkerling (1982, 1988) recognize seven subfamilies: Amphiroideae, Corallinoideae, Metagoniolithoideae, Choreonematoideae, Melobesiodeae, Mastophoroideae, and Lithophylloideae. The first three subfamilies include genera possessing genicula, whereas the remaining four include only nongeniculate genera. Other important taxonomic features include the presence or absence of secondary pit connections or cell fusions as well as uniporate vs. multiporate bi/tetrasporangial conceptacles and the production of conceptacle apical plugs (Woelkerling 1988). These characters and the subfamilies they pertain to are summarized in Table 1.

Throughout the history of classifying taxa within the Corallinaceae there has been much debate and many revisions of subfamilies that are too numerous and cumbersome

Table 1. Characteristics of subfamilies within the Corallinaceae (adapted from Johansen 1981, Woelkerling 1988, and Bailey 1999).

Subfamily	Secondary Pit Connections	Cell Fusions	Genicula	Pore Type	Apical Plug
Choreonematoideae	Absent	Absent	Absent	Uniporate	Present
Corallinoideae	Absent	Present	Present	Uniporate	Absent
Lithophylloideae	Present	Absent	Present/Absent	Uniporate	Absent
Mastophoroideae	Absent to rare	Present	Absent	Uniporate	Absent
Melobesiodeae	Absent to scattered	Present	Absent	Multiporate	Present
Metagoniolithoideae	Absent	Present	Present	Uniporate	Absent

to discuss in detail here. It suffices to mention that over 13 different higher-level arrangements have been proposed during the last 40 years, and generic and species concepts in most nongeniculate taxa are poorly understood. Because a taxonomic reassessment of the Mastophoroideae is the focus of this investigation, special attention is paid to this group.

Detailed investigations of vegetative and reproductive characteristics have been the basis for many reclassifications within this coralline subfamily (Chamberlain 1993, 1994; Chamberlain & Norris 1994; Penrose & Chamberlain 1993; Penrose & Woelkerling 1988, 1992; Woelkerling 1985; Woelkerling 1987). As noted earlier, Johansen and Woelkerling designated seven subfamilies within the Corallinaceae based solely on morphological characteristics, placing primary emphasis on the presence or absence of genicula. An alternative hypothesis to this classification scheme was proposed by Cabioch (1971, 1972, 1988) in which developmental criteria, in conjunction with morphological attributes, were used to delimit only four subfamilies: Corallinoideae, Lithophylloideae, Lithothamnioideae, and Sporolithoideae. Within this classification scheme, greater emphasis is placed on thallus ontogeny and the presence or absence of secondary pit connections and cell fusions rather than on the presence or absence of genicula. Furthermore, this scheme implies that certain geniculate and nongeniculate genera should be classified in the same subfamily and that genicula arose independently several times throughout the evolutionary history of the Corallinaceae. More recently, phylogenetic analyses have been used to reassess the systematics of the Corallinaceae using nuclear-encoded small subunit ribosomal rRNA (18S rRNA) gene sequences (Bailey & Chapman 1996; Bailey & Chapman 1998; Bailey 1999). Bailey (1999)

concluded that, based on molecular data, the Corallinaceae is composed of three major clades, one of which included geniculate and nongeniculate taxa. This monophyletic group includes taxa from Amphiroideae (*sensu* Johansen 1969) and Lithophylloideae. Species classified in each of these subfamilies possess secondary pit connections but lack cell fusions. Therefore, Bailey (1999) emended the diagnoses of these subfamilies, subsuming Amphiroideae into the Lithophylloideae (Lithophylloideae Setchell emend. J.C. Bailey). This proposal is consistent with Cabioch's (1972, 1988) conclusions. However, Bailey's data also indicates that the nongeniculate subfamily Melobesiodeae is monophyletic, as is the geniculate Metagoniolithoideae. These results are consistent with Johansen (1981) and Woelkerling's (1988) scheme, but are inconsistent with the classification proposed by Cabioch (1972, 1988).

Objectives

The classification of the Mastophoroideae has been debated since the 1970s. One scheme places the mastophoroids into their own subfamily, comprised entirely of nongeniculate coralline algae containing no cell fusions, no apical plug, and no haustoria (Johansen 1981; Woelkerling 1988). The second scheme suggests that the Mastophoroideae should not be considered its own subfamily and should be classified within the Corallinoideae, a subfamily including geniculate genera (Cabioch 1972, 1988). These two schemes emphasize different morphological characteristics and Cabioch includes ontogenetic characteristics in her scheme. Sequence data places *Spongites yendoi*, the only representative of the Mastophoroideae examined thus far, apart from all other coralline subfamilies (Bailey & Chapman 1996; Bailey & Chapman 1998; Bailey

1999). However, only one taxon from this subfamily was available to use in the phylogenetic analysis. Thus, the monophyly of the subfamily has not been tested, and the phylogenetic position of the subfamily remains uncertain.

A major issue regarding generic delineation within the Mastophoroideae revolves around the "*Spongites* complex." This complex is comprised of *Spongites, Porolithon, Hydrolithon,* and *Pseudolithophyllum*. These algae are important reef builders and for future scientific (i.e., ecological) investigations, it would be beneficial to know if we are dealing with one, two, three, or four different genera. Penrose and Woelkerling (1988) suggested that *Porolithon, Hydrolithon,* and *Pseudolithophyllum* be considered heterotypic synonyms of *Spongites*. Subsequent analysis suggested that *Porolithon* is synonymous with *Hydrolithon* and that, based upon tetraspore conceptacle features, *Hydrolithon* is distinct from *Spongites* (Penrose & Woelkerling 1992). In this study, additional members of the Mastophoroideae were analyzed using the nuclear genes coding for the small (18S rRNA) and large (26S rRNA) subunits of the ribosome. Parsimony and maximum likelihood methods were employed to reconstruct the relationships within this subfamily and between this subfamily and other Corallinaceae. In addition, a morphological data set was constructed for the Corallinales and analyzed using parsimony. The objectives of this investigation are to: 1) assess the monophyly of the Mastophoroideae, 2) determine the phylogenetic relationship of the Mastophoroideae to the other subfamilies within the Corallinales, 3) define the relationships among genera classified in the Mastophoroideae, especially those belonging to the "*Spongites* complex," and 4) examine character state evolution within the Corallinales.

This chapter deals with the phylogenetic relationships within the Corallinaceae as

inferred by analysis of the 18S rRNA gene. Particular relationships between the four mastophoroid lineages elucidated in this study with the remaining coralline taxa will be elaborated upon here, taking into special consideration particular trends observed with respect to sexual and asexual reproductive features. In Chapter 2, a smaller subset of the taxa investigated in the 18S rRNA analyses will be used to analyze the nuclear-encoded 26S large ribosomal subunit (26S rRNA) to draw further taxonomic inferences within the Corallinaceae. Chapter 3 focuses on trends in the character evolution within the Corallinaceae in an attempt to assess the homology of the traits discussed in this chapter.

Materials and Methods

A total of 10 taxa were added to Bailey's (1999) 18S rRNA dataset, two sequences of which were obtained from external sources. The small ribosomal subunit gene sequence for *Heydrichia homalopasta* was obtained from GenBank (Harvey et al. 2002) and the sequence for *Choreonema thuretii* was kindly provided by Adele Harvey, Department of Botany, La Trobe University, Australia (pers. comm.). For this investigation, two lithophylloid sequences and seven mastophoroid sequences were generated. See Table 2 for a complete list of taxa used in 18S rRNA analyses and their collection information, number of bases determined, and GenBank accession numbers.

DNA Extraction

Preservation of materials for DNA extraction is as follows: algae were completely dried in the sun immediately following collection then placed in separate collection bags

Table 2. Collection information, number of bases determined and GenBank accession numbers for the 49 taxa used in 18S rRNA analyses.

Taxon	Collection Location	Number of Bases Determined	GenBank Accession Number
CORALLINACEAE			
Corallinoideae			
Arthrocardia filicula (Lamarck) Johansen	South Africa	1649	U61258
Bossiella californica ssp. *schmittii* (Manza) Johansen	Stillwater Cove, Carmel, CA, USA	1652	U60945
Bossiella orbigniana ssp. *dichotoma* (Manza) Johansen	Laguna Beach, Orange Co., CA, USA	1688	U60746
Calliarthron cheilosporioides Manza	Laguna Beach, Orange Co., CA, USA	1620	U60943
Calliarthron tuberculosum (Postels et Ruprecht) Dawson	Vancouver Is., BC, Canada	1682	U60944
Cheilosporum sagittatum (Lamouroux) J. Areschoug	Pt. Lonsdale, Victoria, Australia	1655	U60745
Corallina elongata Ellis et Solander	Swakopmund, Namibia, South Africa	1704	U60946
Corallina officinalis Linnaeus	Peggy's Cove, Nova Scotia, Canada	1786	L26184
Haliptilon roseum (Lamarck) Garbary et Johansen	Pt. Lonsdale, Victoria, Australia	1599	U60947
Jania crassa Lamouroux	Palm Beach, Natal, South Africa	1717	U62113
Jania rubens (Linnaeus) Lamouroux	Galway, Ireland	1358	U61259
Serraticardia macmillanii (Yendo) Silva	MacKerracher St. Park, Mendocino Co., CA, USA	1725	U62114
Lithophylloideae			
Amphiroa sp. (AUS)	Pt. Lonsdale, Victoria, Australia	1717	U62115
Amphiroa sp. (SAF)	Pt. Alfred, South Africa	1581	U62116
Amphiroa fragilissima (Linnaeus) Lamouroux	Key Largo, FL, USA	1728	U60744
Amphiroa hancockii Taylor	Pillar Coral, Roatan, Honduras	1743	AY234233

Table 2 continued

Amphiroa tribulus Foslie et Howe	Turrumote, La Parguera, Puerto Rico, USA	1775	AY234234
Lithophyllum incrustans	Kimmeridge, Dorset, UK	1782	AF093410
Lithophyllum kotschyanum (Unger) Foslie	Fiji	1714	U62117
Lithothrix aspergillum J.E. Gray	Laguna Beach, Orange Co., CA, USA	1702	U61249
Titanoderma pustulatum	Kimmeridge, Dorset, UK	1775	AF093409
Mastophoroideae			
Hydrolithon onkodes (Heydrich) Penrose et Woelkerling	Summercloud Bay, South Australia, Australia	1768	AY234237
Hydrolithon samo_nse (Foslie) Keats et Chamberlain	Beachport, South Australia, Australia	1745	AY234236
Metamastophroa flabellata (Sonder) Setchell (clone 1)	Beachport, South Australia, Australia	1784	AY234239
Metamastophroa flabellata (Sonder) Setchell (clone 2)	Beachport, South Australia, Australia	1784	AY234240
Neogoniolithon brassica-florida (Harvey) Setchell et L.R. Mason	Nora Creina Bay, South Australia, Australia	1768	AY233346
Neogoniolithon spectabile (Foslie) Setchell et L.R. Mason	Little San Salvador Is., Half Moon Cay, Bahamas	1778	AY234238
Porolithon pachydermum Foslie	Media Luna, La Parguera, Puerto Rico, USA	1766	AY234235
Spongites yendoi (Foslie) Chamberlain	Holbaaipunt, South Africa	1712	U60948
Melobesiodeae			
Choreonema thuretii (Bornet) Schmitz	Smiths Beach, Phillip Island, Victoria, Australia	1765	XXXXX
Clathromorphum compactum (Kjellman) Foslie	Newfoundland, Canada	1636	U60742
Clathromorphum parcum (Setchell et Foslie) Adey	Horseshoe Cove, Sonoma Co., CA, USA	1666	U61252
Lithothamnion glaciale Kjellman	Labrador, Canada	1729	U60738
Lithothamnion tophiforme Unger	Labrador, Canada	1683	U60739
'Leptophytum acervatum' (Foslie) Chamberlain et Keats	South Africa	1723	U62119

Table 2 continued

'Leptophytum ferox' (Foslie) Chamberlain et Keats	South Africa	1676	U62120
Mastophoropsis canaliculata (Harvey et Hooker) Woelkerling	Beachport, South Australia, Australia	1423	U62118
Mesophyllum engelhartii (Foslie) Adey	South Africa	1647	U61256
Mesophyllum erubescens (Foslie) Lemoine	São Sebastio, Brazil	1624	U61257
Phymatolithon laevigatum (Foslie) Foslie	Kimmeridge, Dorset, UK	1685	U60740
Phymatolithon lenormandii (Areschoug) Adey	Kimmeridge, Dorset, UK	1678	U60741
Synarthrophyton patena (Hooker et Harvey in Harvey) Townsend	Beachport, South Australia, Australia	1623	U61255
Metagoniolithoideae			
Metagoniolithon chara (Lamarck) Ducker	Pt. Lonsdale, Victoria, Australia	1699	U60743
Metagoniolithon radiatum (Lamarck) Ducker	Beachport, South Australia, Australia	1518	U61250
Metagoniolithon stelliferum (Lamarck) Weber-van Bosse	Portsea, Victoria, Australia	1573	U61251
SPOROLITHACEAE			
Heydrichia homalopasta Townsend et Borowitzka	Jervis Bay, New South Wales	1224	AF411629
Heydrichia woelkerlingii Townsend, Chamberlain et Keats	Betty's Bay, South Africa	1758	U61253
Sporolithon durum (Foslie) Townsend et Woelkerling	Port MacDonnell, South Australia, Australia	1575	U61254
Incertae sedis			
Unidentified sp. (=*Sporolithon* sp.???)	Beachport, South Australia, Australia	1772	XXXXX

with silica gel to absorb moisture and maintain a dry environment to prevent DNA degradation. The protocol for DNA extraction was taken from Bailey and Chapman (1998) with the following modification: approximately 900 mL of coralline extraction buffer stock solution (12.5 mL 2M Tris-HCL, pH 7.5; 50 mL 0.5M EDTA, pH 8.0; 15 g SDS) and 8 μL 2-mercaptoethanol were added to 300 μL of ground tissue and incubated at 60°C for 1.5-2 h.

PCR Amplification and Gene Sequencing

Primers used for amplifying the 18S rRNA gene are described in Saunders and Kraft (1994). Primers were used at a 10 μm concentration in conjunction with the GeneAmp® PCR Core Reagent Kit (Perkin-Elmer, Branchburg, New Jersey). The thermocycling profile described in Bailey and Chapman (1998) has been modified as follows: initial DNA denaturation at 94ºC for 4 min and 35 cycles of 30 s at 94ºC, 30 s at 50ºC, 1.5 min at 72ºC. Amplified products were run out on a 0.8% agarose gel against a 1Kb DNA ladder (GIBCO BRL®, Life Technologies, Rockville, MD) to ensure amplification of the 18S rRNA gene. Products were purified with the GeneClean® II Kit (Bio 101, LaJolla, California) according to manufacturer's specifications and then resuspended in 25-30μL sterile water. Purified products were then sequenced using the ABI PRISM® BigDye^{TM} Terminator Cycle Sequencing Ready Reaction Kit (original version, Perkin-Elmer) according to manufactuer's instructions. Sequences were determined on an ABI PRISM® 377 Automated DNA Sequencer or an ABI PRISM® 3100 Genetic Analyzer.

PCR products for *Metamastophora flabellata* proved difficult to sequence. To

obtain complete sequences for this alga the pGEM® -T Vector System II (Promega, Madison, WI) was used to clone the 18S rRNA gene according to the manufacturer's specifications. In total, two complete sequences were generated for *M. flabellata*.

Sequence Alignment and Analysis

Sequences were manually edited and forward and reverse strands were independently assembled and then aligned together to form a consensus sequence. Consensus sequences were then aligned in the SeqApp program (Gilbert 1994) to 35 species of coralline algae available from GenBank (Bailey 1999).

Variable regions of the sequence matricies that could not be unambiguously aligned were excluded from subsequent analyses. Trees were rooted with *Heydrichia woelkerlingii* Townsend, Chamberlain et Keats (Corallinales, Rhodophyta) and the ingroup was constrained to monophyly. Parsimony analysis was performed using PAUP* (v**.** 4.0b10, Swofford 2002) with a heuristic search of 5,000 random sequence additions, MULTREES and tree-bisection-reconnection branch swapping algorithm. All characters were unordered and equally weighted and gaps were treated as missing data. Bootstrap proportion (BP) values for nodes of the trees were calculated based upon 10,000 pseudoreplicates using the "fast step-wise" addition option (Felsenstein 1985). Maximum likelihood (ML) analyses were performed in PAUP* (v**.** 4.0b10, Swofford 2002) using the general time-reversible (GTR) model with transition/transversion ratios, nucleotide frequencies, and gamma distributions inferred directly from the dataset using ModelTest (v. 3.06, Posada & Crandall 1998). The optimal ML tree was obtained from 10 separate searches of random sequence addition, MULTREES and tree-bissection-

reconnection branch swapping. Bootstrap proportion values were calculated using the heuristic "fast step-wise" addition option with 10 pseudoreplicates.

Results

Comparison of the 18S rRNA nucleotide sequences for the two clones of *Metamastophora flabellata* showed three single nucleotide polymorphisms between the two. This suggests that within the nuclear genome of *M. flabellata*, repeated units of the 18S rRNA gene are not identical to one another.

Of 1779 comparable sites, the 18S rRNA nucleotide sequences for *Neogoniolithon brassica-florida* and *N. spectabile* differed at 26 of those sites. This yielded a sequence divergence value of 1.5%. Comparison of *Hydrolithon onkodes* and *H. pachydermum* resulted in a sequence divergence of 0.34% (differing at six of the 1753 sites compared) and are considered to be very closely related. This contrasts with the relatively high sequence divergences of *H. samoense* with *H. pachydermum* (2.4%) and *H. onkodes* (3.9%). But taking the mean sequence divergence of all three *Hydrolithon* spp. (2.2%) and that of *Neogoniolithon* spp. (1.5%), these values are within the range (0.53-3.67%) reported for congeneric sequence divergences among coralline algae (Bailey 1999).

Parsimony and maximum likelihood analyses of the 18S rRNA data indicate that the Mastophoroideae is not monophyletic (Figs. 1, 2). Tree topologies for the two analyses differed with respect to the position of *Metamastophora flabellata* within the Corallinaceae as well as the relationship of *Neogoniolithon brassica-florida* and *N.*

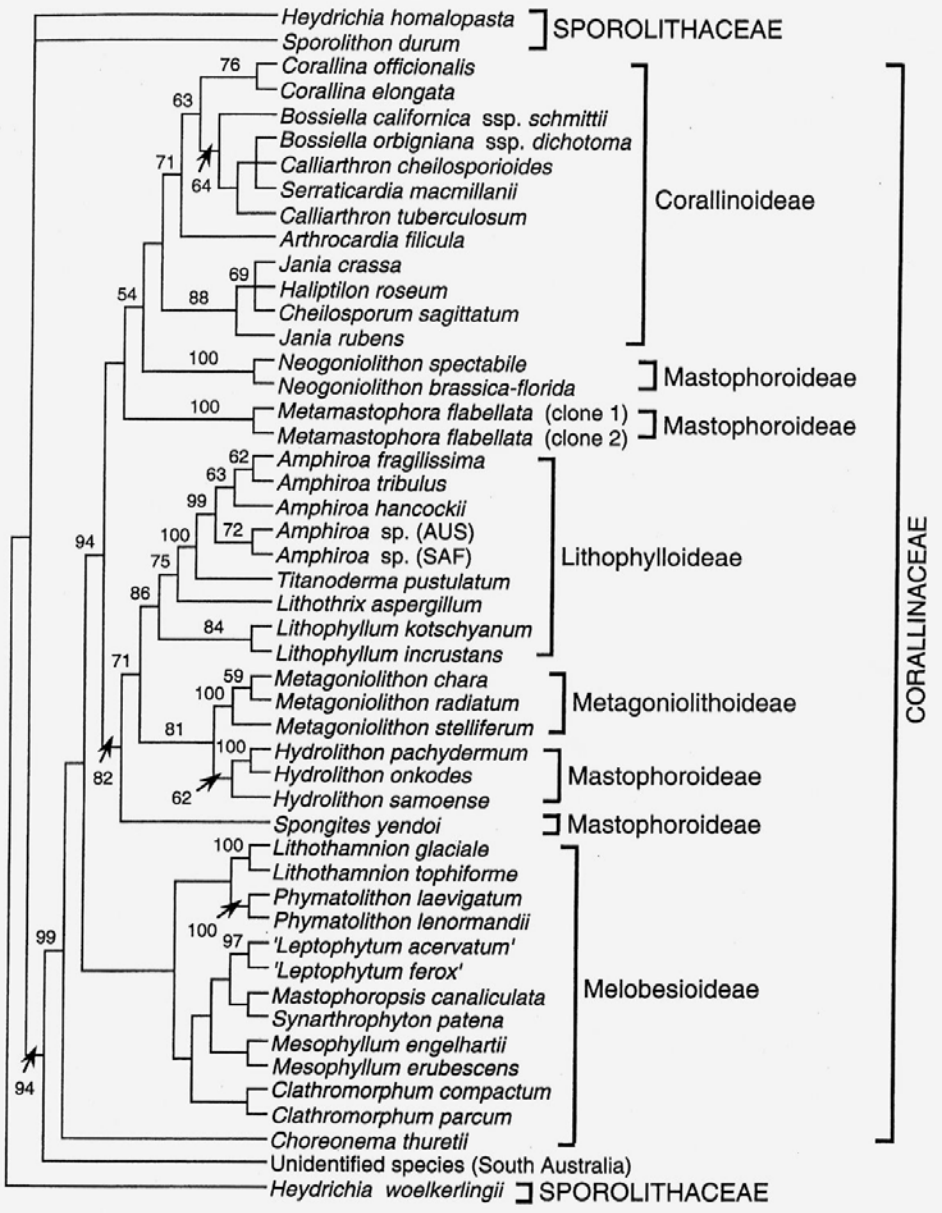

Figure 1. Majority rule of 12 equally parsimonious trees (CI= 0.515, RI= 0.754) obtained from cladistic analysis of the 18S rRNA gene for 48 species of coralline red algae. Bootstrap proportion values >50% for each node are given above the branches.

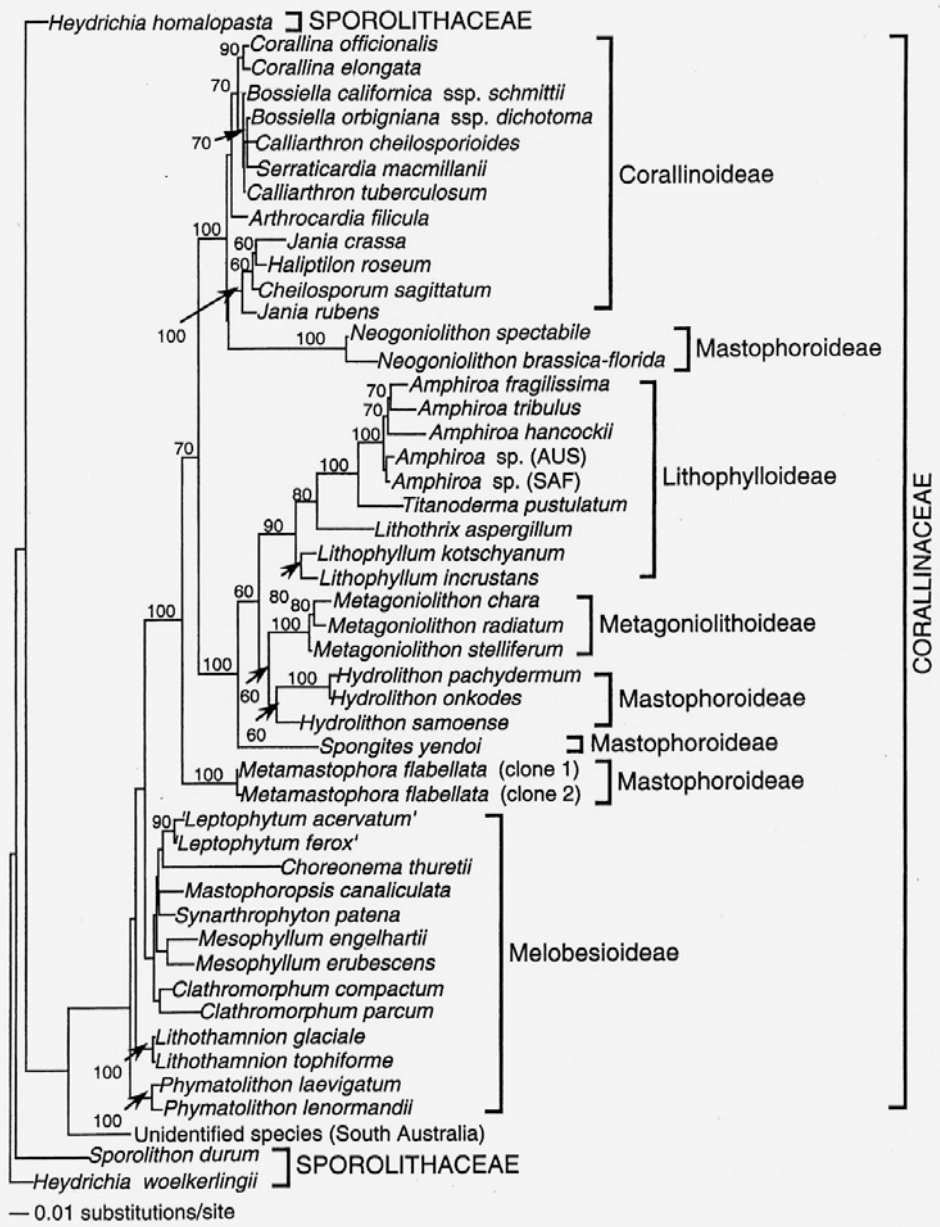

Figure 2. Maximum likelihood tree obtained from analysis of the 18S rRNA gene for 48 species of coralline red algae. Bootstrap proportion values >50% for each node are given above the branches.

spectabile to the Corallinoideae, however, the inferred positions of the remaining mastophoroids within the two trees were consistent.

Cladistic analysis yielded 12 equally parsimonious trees with a length of 1300 steps (CI= 0.515, RI= 0.754) and 335 phylogenetically informative positions. The 50% majority rule consensus tree is depicted in Figure 1. *Neogoniolithon spectabile* and *N. brassica-florida* are allied as sister taxa to members of the Corallinoideae with low support (BP= 54%). This arrangement differs slightly from the ML tree in which *Neogoniolithon spectabile* and *N. brassica-florida* are positioned within the Corallinoideae as sister taxa to members of the tribe Janieae (*Jania crassa, J. rubens, Haliptilon roseum,* and *Cheilosporum sagittatum*), however bootstrap support for this arrangement is less than 50% (Fig. 2).

In the parsimony analysis *Metamastophora flabellata* (clones 1 and 2) is placed as the sister taxon to a clade comprised of the Corallinoideae and *Neogoniolithon* spp., however there is less than 50% bootstrap support for the placement of this species at this position on the tree. The ML tree strongly supported the placement of *Metamastophora flabellata* as sister to a clade comprised of the Corallinoideae, Lithophylloideae, Metagoniolithoideae, and mastophoroid species (BP= 100%).

In both analyses *Hydrolithon pachydermum, H. onkodes,* and *H. samoense* are resolved as monophyletic (BP= parsimony 62%, ML 60%) and share a most recent common ancestor with the Metagoniolithoideae (BP= 81%, 60%). *Spongites yendoi* is positioned as sister taxon to a clade comprised of the Lithophylloideae, Metagoniolithoideae, and *Hydrolithon* spp. (BP= 82%, 100%).

Discussion

The molecular data presented here indicates that the Mastophoroideae is polyphyletic. Each of the four genera examined (*Hydrolithon, Metamastophora, Neogoniolithon,* and *Spongites*) do not share a most recent common ancestor and have diverged at different points along the evolutionary history of the Corallinaceae. The four lineages will be discussed separately with special attention paid to morphological features regarding tetrasporangial conceptacle development as well as reproductive features indicative of certain trends observed within the Corallinaceae.

It is important to note that because the relative position of *Metamastophora flabellata* changes depending upon the optimality criterion used, the maximum likelihood tree (Fig. 2) will be favored in discussing the taxonomic relationships of *M. flabellata* within the Corallinaceae. The rationale behind this decision is in part due to the strong bootstrap support (100%) for its position within the phylogram and partly due to the results of additional analyses that will be discussed in Chapter 2 that are congruent with this topology.

The Phylogenetic Position of *Neogoniolithon*

In both analyses, *Neogoniolithon brassica-florida* and *N. spectabile* were resolved as sister taxa (BP= 100%, 100%). Together, these two nongeniculate species are positioned as sister taxa to the geniculate Corallinoideae, although there is weak support for this arrangement (BP= 54%, <50%) (Figs. 1, 2).

According to Cabioch (1972, 1988), the presence or absence of genicula was not considered to be a phylogenetically informative character at the subfamilial level and she

classified *Neogoniolithon* as well as some other nongeniculate corallines within the Corallinoideae based on the presence of cell fusions, absence of secondary pit connections, and the occurrence of uniporate tetrasporangial conceptacles. The molecular data supports this proposal and is consistent with the placement of *Neogoniolithon* in the Corallinoideae but does not agree with the placement of other mastophoroid (*sensu* Woelkerling 1988) taxa within this subfamily. For example, Cabioch (1972, 1988) also placed *Hydrolithon* in the same lineage as *Neogoniolithon* but this is inconsistent with the molecular data that clearly indicate that *Hydrolithon* is derived from a separate lineage and is not closely related to the Corallinoideae (Figs. 1, 2).

There is a particular combination of gametangial and tetrasporangial conceptacle features that are found in *Neogoniolithon* but not in the other mastophoroid taxa. First, in *Neogoniolithon* tetrasporangial conceptacles arise from filaments that surround and grow over the fertile area, resulting in cells lining the pore canal that are oriented parallel to the thallus surface (Penrose 1992b). This mode of development has been described by Johansen (1981) as Type 1 and is found to occur in members of the Corallinoideae (*sensu* Woelkerling 1988) such as *Bosiella, Cheilosporum, Corallina,* and *Jania* (Ganesan 1967, 1968; Johansen 1977, 1981). In comparing the mode of tetrasporangial conceptacle development across different mastophoroid genera it becomes apparent that this feature is not consistent with the current circumscription of the Mastophoroideae. Although the majority of mastophoroids share in common this Type 1 mode of development (i.e., *Lesueuria*, *Mastophora, Metamastophora, Neogoniolithon,* and *Spongites*) (Woelkerling & Duker 1987; Woelkerling 1988; Penrose 1991; Penrose & Woelkerling 1991), a

different mode of tetrasporangial conceptacle development is found in others. This latter mode of development has been described by Johansen (1981) as Type 2, whereby the cells surrounding and interspersed throughout the fertile region form the conceptacle by a programmed cell elongation followed by the disintegration of cells forming the cavity. This mode of development results in cells lining the pore canal to be oriented perpendicular to the thallus surface. *Lithoporella* and *Hydrolithon* are two mastophoroid genera in which the Type 2 mode of tetrasporangial conceptacle development occurs (Woelkerling 1988; Penrose & Chamberlain 1993).

A second feature that *Neogoniolithon* shares with the Corallinoideae and no other mastophoroid taxa is the location of origin of spermatangial initials within the male conceptacle. There are two general trends observed within the Corallinaceae: spermatangial initials are confined to the floor of the conceptacle or they occur across the floor, walls, and roof of the conceptacle. In *Neogoniolithon* and members of the Corallinoideae, spermatangia are formed across the floor, walls, and roof of the conceptacle (Johansen 1981; Woelkerling 1988; Penrose 1992b). In all other mastophoroid taxa, spermatangia occur along the floor of the male conceptacle only (Woelkerling 1988; Penrose 1991; Penrose & Chamberlain 1993).

A third feature that sets *Neogoniolithon* apart from other mastophoroid taxa is the site of origin of diploid gonimoblast filaments. In *Neogoniolithon accretum* Adey et Vassar, *N. fosliei* (Heydrich) Setchell et Mason, and *N. pacificum* (Foslie) Setchell et Mason, gonimoblast filaments arise along the dorsal surface of an apparently discontinuous fusion cell (Masaki 1968; Penrose 1992b). This contrasts with features observed in other mastophoroid genera such as *Lesueuria* Woelkerling et Duker,

Metamastophora Setchell, *Pneophyllum* Kützing, and *Spongites* Kützing, in which gonimoblast filaments arise along the margin (or periphery) of a single, continuous fusion cell (Woelkerling 1980a; Woelkerling & Ducker 1987; Penrose 1991; Penrose & Woelkerling 1991). The origin of gonimoblast filaments may also provide a link between some taxa in the Corallinoideae with *Neogoniolithon*. In members of the tribe Corallineae (Corallinoideae), gonimoblast filaments are formed along the dorsal surface and margins of the fusion cell (Ganesan 1967, 1968; Johansen 1976, 1981; Murata & Masaki 1978). This feature is consistent with the observed origin of gonimoblast filaments in *Neogoniolithon,* but differs from what occurs in members of the tribe Janieae (Corallinoideae) where gonimoblast filaments arise along the margin of the fusion cell (Johansen 1977; Johansen & Silva 1978).

In summary, the reproductive features that ally *Neogoniolithon* with the Corallinoideae and provide morphological support to the inferred sister taxon relationship based on the molecular data (Figs. 1, 2) include: 1) Tetrasporangial cavity and roof formation follows the Type 1 mode of development as described by Johansen (1981). 2) Spermatangia occur not only across the floor of the male conceptacle but also along the walls and roof. 3) Gonimoblast filaments arise along the dorsal surface and margins of the fusion cell (with the exception of the Janieae where they are restricted to the margin of the fusion cell).

The Phylogenetic Position of *Hydrolithon*

The molecular data consistently resolves nongeniculate *Hydrolithon* spp. as sister to the geniculate Metagoniolithoideae with moderate to weak support (BP= 81%, 60%).

Taxonomic implications of the formation of tetrasporangial conceptacles with respect to numerous *Hydrolithon* spp. and other mastophoroid genera including *Fosliella* Howe, *Pneophyllum* Kützing, *Porolithon* Foslie, *Pseudolithophyllum* Lemoine emend. Adey, and *Spongites* Kützing have been discussed in previous studies (Penrose & Woelkerling 1988, 1991, 1992; Penrose 1992a; Penrose & Chamberlain 1993; Keats & Chamberlain 1994). In *Hydrolithon*, the conceptacle chamber is formed by filaments surrounding and interspersed throughout the fertile region (Type 2 mode of development). This results in cells lining the pore canal to be oriented more or less perpendicular to the thallus surface that do not protrude into the canal. This has been considered an important feature for not only delimiting *Hydrolithon* and *Spongites* (Penrose & Woelkerling 1992), where in *Spongites,* Type 1 mode of tetrasporangial conceptacle development occurs, but also in distinguishing *Pneophyllum* Kützing from *Spongites* (Penrose & Woelkerling 1991).

Penrose and Chamberlain (1993) constructed a revised taxonomic key to the genera of Mastophoroideae that not only took into account the mode of tetrasporangial conceptacle development, but also considered the location of spermatangia formation within the male conceptacle. In *Hydrolithon,* the formation of spermatangia is confined to the conceptacle floor. As previously mentioned, *Neogoniolithon* is the only mastophoroid genus where spermatangia are not confined to the floor of the male conceptacle but also occur along the walls and roof of the chamber. In the carposporangial conceptacles of *Hydrolithon*, gonimoblast filaments arise from the margins of the fusion cell, a feature held in common with most other mastophoroid taxa except *Neogoniolithon* (Woelkerling & Ducker 1987; Penrose & Woelkerling 1991;

Penrose 1992a, 1992b; Penrose & Chamberlain 1993).

In *Metagoniolithon* Weber van Bosse, similar morphological traits are observed in tetrasporangial and gametangial conceptacles. The tetrasporangial cavity is formed by filaments surrounding and interspersed throughout the fertile area (Type 2 mode of development) (Ganesan 1971; Ducker 1979). Spermatangia are restricted to the floor of the male conceptacle and following presumed karyogamy a continuous fusion cell forms, giving rise to gonimoblast filaments that occur along a peripheral ring of the fusion cell (Ganesan 1971; Ducker 1979).

It is interesting to note here that these three features also occur in members of the Lithophylloideae (Johansen 1976, 1981; Townsend 1981; Chamberlain 1991; Riosmena-Rodriguez & Siqueiros-Beltrones 1996). Townsend (1981) discussed the development of tetrasporangial conceptacles as an important character in delimiting subfamilial relationships between the Lithophylloideae (*sensu* Woelkerling 1988) and the Mastophoroideae. In this paper she referenced *Hydrolithon* spp. and *Porolithon* spp. as undergoing the Type 1 mode of development (calling it '*sur*' as referred to by Johansen 1976) along with *most* of the other mastophorid taxa, providing a useful subfamilial distinction from the Type 2 mode of tetrasporangial development (calling it '*col*' as referred to by Johansen 1976) common to all members of the Lithophylloideae. However, there are numerous studies that have shown that *Hydrolithon* spp. and *Porolithon* spp. in fact undergo the Type 2 mode of development (Penrose & Woelkerling 1988, 1992; Penrose 1992a; Penrose & Chamberlain 1993; Keats & Chamberlain 1994). Nonetheless, this character still seems to be useful in determining subfamilial relationships. According to the molecular data (Figs. 1, 2), in addition to the

sister taxa relationship of *Hydrolithon* with *Metagoniolithon,* a larger clade that includes *Hydrolithon, Metagoniolithon,* and members of the Lithophylloideae is resolved with moderate to weak support (BP= 71%, 60%).

In summary, reproductive features that may provide a link for the phylogenetic relationship of *Hydrolithon* to *Metagoniolithon,* as well as these two taxa to the Lithophylloideae include: 1) Type 2 mode of tetrasporangial conceptacle development whereby cavity cells disintegrate to form the chamber (Johansen 1981). 2) Spermatangial initials are confined to the floor of the male conceptacle. 3) Gonimoblast filaments are formed along the periphery of the fusion cell.

Hydrolithon pachydermum comb. nov.

The occurrence of *Porolithon pachydermum* (Foslie) Foslie is widespread and particularly common throughout the Carribbean where it tends to form vast pavements over reef substrates (Littler et. al. 1995; Littler & Littler 2000). For this investigation, *P. pachydermum* was collected in Puerto Rico (Table 1) and included in the analyses.

Despite the fact that some authors continue to recognize *Porolithon* as a distinct genus (i.e. Littler & Littler 2000), *Porolithon* is not recognized by others. Woelkerling (1985) and Penrose & Woelkerling (1988) reviewed the relationships between *Hydrolithon* Foslie, *Porolithon* Foslie, and *Spongites* Kützing by examining generitype specimens and found that vegetative thallus features (i.e., hypothallium arrangement, cell size, and occurrence/arrangement of trichocytes) were not reliable in distinguishing these three genera from one another. Therefore, Penrose & Woelkerling (1988) subsumed *Hydrolithon* and *Porolithon* in *Spongites*. It was not until later that Penrose &

Woelkerling (1992) compared the morphology of tetrasporangial conceptacles among these three genera and found that there were fundamental differences between the conceptacle chambers of *Hydrolithon* and *Spongites* that corresponded to the mode of development that occurs in each (discussed in other portions of this text). They considered *Porolithon* to be a heterotypic synonym of *Hydrolithon* and therefore subsumed *Porolithon* in *Hydrolithon*.

The molecular data in this study strongly supports the monophyly of '*Porolithon pachydermum'* and *Hydrolithon onkodes* (BP= 100%, 100%) and moderately supports the monophyly of the two aforementioned species and *H. samoense* (BP= 62%, 60%) (Figs. 1, 2). If it is assumed that an 18S rRNA molecular clock for coralline algae is a sequence divergence of 1% per million years (Bailey, unpubl. data), then *'P. pachydermum'* and *H. onkodes* may have diverged approximately 340,000 years ago. Comparison of the 18S rRNA nucleotide sequences between '*Porolithon pachydermum'* and *H. onkodes* shows that these two sequences differ at only six positions. Therefore, it is suggested that *Porolithon pachydermum* (Foslie) Foslie be transferred to *Hydrolithon* as *Hydrolithon pachydermum* (Foslie) Bailey, Gabel et Freshwater *comb. nov.*.

The Phylogenetic Positions of *Metamastophora* and *Spongites*

Sequence data for the 18S rRNA gene was generated for *Metamastophora flabellata* (Sonder) Setchell, the type species for the genus *Metamastophora*. Although the molecular data provide two different topologies for the phylogenetic position of *M. flabellata* within the Corallinaceae depending on the optimality criterion used (Figs. 1, 2), for reasons discussed in the following paragraphs as well as in Chapter 2, the maximum

likelihood tree will be favored for its placement in the Corallinaceae phylogeny. Thallus morphology of *Metamastophora* is unique in that it is the only mastophoroid that is branched and arborescent with a distinct holdfast and stipe (Woelkerling 1980a, 1988). Woelkerling (1980b) originally assigned *Metamastophora* to the subfamily Lithophylloideae based on the fact that both cell fusions and secondary pit connections were reported in *M. flabellata*, however, he did have some reservations as to the reliance of these types of cell connections in the delineation of subfamilies. Since the occurrence of secondary pit connections in *Metamastophora* was only occasional in older portions of the thallus, Woelkerling (1988) later moved this genus to the Mastophoroideae based on the predominant type of cell connection: cell fusions. As the Lithophylloideae is currently circumscribed as possessing secondary pit connections only (cell fusions are absent), with the presence or absence of genicula an unreliable feature at the subfamilial rank (Bailey 1999), *Metamastophora* clearly does not belong here. However, it is still uncertain whether or not *M. flabellata* should remain in the Mastophoroideae *sensu stricto*.

Reproductive features found in *Metamastophora* include the formation of uniporate tetrasporangial conceptacles initiated from a ring of filaments that surround and overgrow the fertile area (Woelkerling 1988), also referred to as "Type 1" mode of tetrasporangial conceptacle formation (Johansen 1981). A central columella is present within the conceptacle chamber and tetrasporangia are borne around the periphery of the chamber floor (Woelkerling 1980a, b, 1988). In the male conceptacle, perithallial cells that line the chamber floor give rise to spermatangial initials, resulting in the restriction of spermatangia formation to the chamber floor. Female carposporangial conceptacles

consist of a single, large, continuous fusion cell that gives rise to marginally located gonimoblast filaments bearing terminal carposporangia (Woelkerling 1980a, 1988).

With respect to the phylogenetic positioning of *Spongites* Kützing, the molecular data strongly resolves *S. yendoi* as sister taxon to a clade comprised of *Hydrolithon* spp., *Metagoniolithon* spp., and members of the Lithophylloideae (BP= 82%, 100%). Similar reproductive features described above for *Metamastophora* are also characteristic of *Spongites.* The formation of tetrasporangial conceptacles involves the upward growth of filaments situated along the periphery of tetrasporangial initials, resulting in the cells lining the pore canal to protrude into the canal and oriented parallel to the roof surface. Gametangial conceptacle features include the formation of spermatangial initials across the floor of the male coneptacle only and gonimoblast filaments arise along the periphery of a continuous fusion cell in the female conceptacle after presumed fertilization (Afonso-Carrillo 1988; Penrose & Woelkerling 1992; Chamberlain 1993).

With respect to the ontogeny of sexual and asexual conceptales, the trends observed in *Metamastophora* and *Spongites* are fundamentally similar. And although these two morphologically distinct taxa share in common these reproductive features, based on analysis of the 18S rRNA gene they do not belong to the same clade (Figs. 1, 2).

Investigations of coralline systematics using molecular analysis in this study and others have led to somewhat unconventional relationships being inferred among mastophoroids (*Hydrolithon* and *Spongites*), *Metagoniolithon,* and representatives of the Lithophylloideae (Bailey & Chapman 1996, 1998; Bailey 1999). As mentioned earlier, there is relatively strong support for this clade (BP= 82%, 100%), and there is in fact particular features that are common among these taxa. In the Lithophylloideae,

Metagoniolithon, and *Hydrolithon*, tetrasporangial conceptacle development proceeds by the Type 2 mode described by Johansen (1981). There are three distinct features resultant of this type of development: 1) The conceptacle chamber, pore, and pore canal are formed by cavity cells located among and surrounding the sporangial initials that elongate at first and then undergo a programmed cell death. 2) Apical pore plugs are absent. 3) Cells forming the conceptacle roof are derived from filaments located within the fertile area and are more or less oriented perpendicularly to the roof surface. The only taxon that does not fit this trend observed within this clade is *Spongites* in which Type 1 sporangial conceptacle development occurs. Here, the conceptacle chamber, pore, pore canal, and roof are formed from filaments that surround the fertile area that extend in an overarching fashion. This mode of development also occurs in members of the Corallinoideae, *Neogoniolithon*, and *Metamastophora*. The topologies inferred from the parsimony and maximum likelihood analyses (Figs. 1, 2) suggest that within the Corallinaceae, Types 1 and 2 mode of tetrasporangial conceptacle development are derived.

Furthermore, there are similar features concerning sexual conceptacle ontogeny whereby members within this clade (representatives of the Lithophylloideae, *Metagoniolithon, Hydrolithon,* and *Spongites*) all share in common. Spermatangial initials are confined to the floor in male conceptacles and in female conceptacles, gonimoblast filaments arise along the margin of the fusion cell. These characteristics are fundamentally different from what is observed in members of the Corallinoideae and *Neogoniolithon,* where spermatangial initials occur across the floor, walls, and roof of the male conceptacle. Within the female conceptacle, gonimoblast filaments arise primarily

along the dorsal surface of the fusion cell. Support for this clade is weak to strong, depending upon the optimality criterion used (BP= 54%, 100%).

Finally, in both parsimony and ML trees (Figs. 1, 2), the clade comprised of all members of the Corallinaceae excluding the Melobesiodeae is strongly supported in both analyses (BP= 94%, 100%). A unifying feature common to most taxa within this assemblage is the presence of a single, continuous fusion cell that forms following fertilization in the female conceptacle (Johansen 1981).

Reflections on the Melobesiodeae

As more and more taxa are added to the 18S rRNA dataset, character support for the monophyly of the Melobesiodeae declines. Bailey & Chapman (1996, 1998) and Bailey (1999) observed relatively weak bootstrap support that was marginally greater than 50% (their figures). In this study, the Melobesiodeae were resolved as a monophyletic taxon according to cladistic analysis (Fig. 1), however, bootstrap support for this clade is less than 50% while in the ML analysis the Melobesiodeae is not resolved as a monophyletic taxon (Fig. 2). And although the topology and resolution within this particular group may change again with the addition of more melobesiod taxa as well as more distantly related taxa in subsequent analyses, certain morphological features that are consistent within this subfamily and that make them distinct from all other members of the Corallinaceae are worth discussing here.

The mode of sporangial conceptacle development results in characteristic morphological features of the entire asexual reproductive organ. Cavity cells located within the fertile region elongate, and as the sporangia begin to enlarge the cavity cells

degenerate, forming the conceptacle chamber. The apex of each sporangium thickens to form a mucilagenous plug that blocks the pore until the bisporangia or tetrasporangia have matured (Johansen 1981; Woelkerling 1988). This process seems similar to the Type 2 mode of tetrasporangial conceptacle development (i.e., the fact that cavity cells that are interspersed throughout the fertile region elongate and then a portion degenerates to form the conceptacle chamber, resulting in the orientation of roof cells perpendicular to the thallus surface). However, the formation of a single ostiole with an apical pore plug over each tetrasporangium (resulting in a multiporate conceptacle) makes this subfamily unique enough for Johansen (1981) to call this mode of tetrasporangial conceptacle development "Type 3," characteristic of the Melobesiodeae.

The formation of gametangial conceptacles occurs from the overarching of filaments surrounding the fertile area. And although certain aspects of male conceptacle morphology among melobesiod taxa differ (i.e., differences in the appearance and formation of the spermatangial mother cell system), spermatangia occur across the floor and roof within the male conceptacle throughout the Melobesiodeae (Lebednik 1978). After presumed karyogamy, within the female conceptacle scattered or discontinuous fusion cells form from which gonimoblast filaments develop only along the periphery (Chamberlain & Keats 1994, 1995; Keats & Chamberlain 1997). Other accounts have provided different descriptions of a "fusion cell complex," an irregularly shaped fusion cell that appears discontinuous in section (Woelkerling & Harvey 1993), a single, centrally located fusion cell that gives rise to connecting cells (Townsend 1979; Chamberlain et al. 1995), the occurrence of several small fusion cells (Woelkerling & Foster 1989; Woelkerling & Harvey 1992), or no fusion cell is observed (Wilks &

Woelkerling 1994; Keats et al. 1996). Keats et al. (1996) noted that with the apparent absence of a fusion cell, gonimoblast filaments arise across the floor of the carposporangial conceptacle. However, within the literature the predominant location of gonimoblast filaments is along the periphery of the fusion cell or fusion complex (see references above). Lebednik (1977) provides a useful summation and description of the phenotypic plasticity of the appearance of fusion cells (or lack thereof) within melobesiod taxa.

This particular combination of features pertaining to sporangial and gametangial conceptacles is clearly unique to the Melobesiodeae, however, in this analysis this group is not resolved as monophyletic with very weak support for the positioning of taxa within the phylogeny (Figs. 1, 2). Greater resolution and support will perhaps follow with the addition of more melobesiod gene sequences.

Taxonomic and Evolutionary Implications

According to the 18S rRNA analyses, the Mastophoroideae is clearly a polyphyletic taxon with at least four independant lineages. Although the diagnosis for this subfamily inevitably needs to be changed, a new circumscription will not be included here. The reasoning for such a decision is in part due to the fact that if a taxonomic scheme is adopted now it will more than likely have to be revised in the near future when more representative mastophoroid genera are included in the analysis. There are also additional open-ended "problems" inherent with this analysis from a cladistic point of view. For example, according to the International Code of Botanical Nomenclature, rules of priority apply at the rank of family and below. The type genus for the

Mastophoroideae (erected by Setchell in 1943) is *Mastophora* Decaisne (1842), which was not sampled in this analysis and therefore it is uncertain as to which of the four lineages should be given priority in the recircumscription of this taxon, and which lineages should be included in the Mastophoroideae *sensu stricto*. Type species for *Neogoniolithon* (*N. fosliei* [Heydrich] Setchell *et* Mason) and *Spongites* (*S. fruticulosus* Kützing) have also yet to be examined. With the placement of *Neogoniolithon brassica-florida* and *N. spectabile* in this analysis (Figs. 1, 2) it could be argued that *Neogoniolithon* should be classified within the Corallinoideae, however, a recircumscription of this taxon will not be provided at this point because support at these nodes is particularly weak (BP= 54%, < 50%). Lastly, a number of propositions could be made for classifications within the clade comprised of *Spongites, Hydrolithon, Metagoniolithon,* and members of the Lithophylloideae (Figs. 1, 2). And although support for this clade is relatively strong (BP= 82%, 100%), the morphology and mode of development of tetrasporangial conceptacles differs between *Spongites* and the remaining taxa in that clade. Since the taxonomic implications of this particular feature are unclear at this point, revisions will be suspended for now.

CHAPTER 2. PHYLOGENETIC ANALYSIS OF THE CORALLINALES (RHODOPHYTA) BASED UPON INDEPENDENT ANALYSIS OF NUCLEAR-ENCODED 26S rRNA GENE SEQUENCES AND COMBINED 18S/26S rRNA SEQUENCE DATA

Introduction

Generic and subfamilial concepts for many members of the Corallinaceae (Corallinales, Rhodophyta) have undergone numerous taxonomic revisions over the past 30 years. The basis for most of these revisions has traditionally been comparative analysis of the anatomy and ontogeny of vegetative and reproductive features. With the advent of phylogenetic analysis using molecular data, additional taxonomic revisions were made, especially at the rank of subfamily (Bailey 1999). These molecular studies strongly imply that some of the vegetative characteristics relied so heavily upon in the past to delimit coralline taxa are phylogenetically unreliable and homoplasious.

Phylogenetic relationships inferred from molecular analyses have led to several important revisions concerning coralline algae including the broadening of the diagnosis of the Lithophylloideae to include geniculate and nongeniculate members (Bailey & Chapman 1998; Bailey 1999), establishment of the Mastophoroideae as a polyphyletic taxon (present study), and the erection of a new family within the Corallinales, the Hapalidiaceae (Broadwater et al., in press). However, these investigations have all involved the analysis of a single gene, the nuclear-encoded small ribosomal subunit.

More recent systematics studies are incorporating separate and combined analysis of two or more genes to determine relationships among taxa. With respect to algal systematics, Freshwater & Bailey (1998) were the first to use the combined analysis of several genes (18S rRNA, 26S rRNA, and *rbc*L) to determine relationships among red

algae. Only a handful of investigations have used this approach in phycological systematics (Harper & Saunders 2001, and references therein). Rousseau & De Reviers (1999) used partial 26S rRNA and 18S rRNA gene sequences to reevaluate relationships within the brown algal order Fucales and more recently, Harper & Saunders (2001, 2002) have also used this approach to investigate the systematics of red algae.

The purpose of this investigation is to assess the evolutionary relationships among representatives of the Mastophoroideae and other members of the Corallinaceae using additional gene sequence data obtained from the nuclear-encoded large ribosomal subunit. Separate parsimony and maximum likelihood analyses for the 26S rRNA dataset and a combined 18S/26S rRNA dataset were performed and compared to the results obtained from the 18S rRNA analyses in Chapter 1.

Materials and Methods

A total of six gene sequences for the large ribosomal subunit (26S rRNA) were determined and added to an existing dataset compiled by Drs. D. Wilson Freshwater and J. Craig Bailey, University of North Carolina at Wilmington. DNA extraction, PCR amplification profiles, and sequence alignments were performed as described in Chapter 1, and primers used for 26S rRNA amplification and gene sequencing were obtained from Freshwater and Bailey (1998). In total, 22 sequences were examined (Table 3).

The 26S rRNA dataset was analyzed independently and then a taxonomically congruent combined dataset was constructed by appending the 26S rRNA sequences to corresponding 18S rRNA gene sequences. Cladistic and maximum likelihood analyses of both the 26S-only and the combined 18S/26S rRNA datasets were performed in the same

Table 3. List of the taxa used in the LSU-only and combined SSU/LSU analyses with the number of bases determined for each. Collection information for each of the species is given in Table 2.

Taxon	Number of Bases Determined for the LSU	Number of Bases Determined for the SSU/LSU
CORALLINACEAE		
Corallinoideae		
Bossiella californica ssp. *schmittii* (Manza) Johansen	1207	3016
Calliarthron tuberculosum (Postels et Ruprecht) Dawson	1198	2963
Corallina elongata Ellis et Solander	1177	2982
Haliptilon roseum (Lamarck) Garbary et Johansen	1185	2979
Jania rubens (Linnaeus) Lamouroux	1166	2988
Lithophylloideae		
Amphiroa sp. (AUS)	1173	2970
**Lithophyllum incrustans*	1172	2976
Lithophyllum kotschyanum (Unger) Foslie	1169	2965
Lithothrix aspergillum J.E. Gray	1174	2976
**Titanoderma pustulatum*	1166	2967
Mastophoroideae		
Hydrolithon onkodes (Heydrich) Penrose et Woelkerling	1207	2981
Metamastophroa flabellata (Sonder) Setchell (clone 1)	1198	3002
Metamastophroa flabellata (Sonder) Setchell (clone 2)	1218	3002
Neogoniolithon brassica-florida (Harvey) Setchell et L.R. Mason	1211	2985
Neogoniolithon spectabile (Foslie) Setchell et L.R. Mason	1217	2993
Porolithon pachydermum Foslie	1212	2977
Spongites yendoi (Foslie) Chamberlain	1161	2956
Melobesiodeae		
Clathromorphum parcum (Setchell et Foslie) Adey	1179	2982

Table 3 continued

Lithothamnion tophiforme Unger	1182	2973
Phymatolithon lenormandii (Areschoug) Adey	1178	2976
Metagoniolithoideae		
Metagoniolithon chara (Lamarck) Ducker	1174	2965
SPOROLITHACEAE		
Heydrichia woelkerlingii Townsend, Chamberlain et Keats	1122	2934
Incertae sedis		
Unidentified sp. (=*Sporolithon* sp.???)	1273	3073

manner as the 18S rRNA analyses which are described in Chapter 1. The only difference pertaining to methodology with respect to ML analysis is as follows: optimal ML trees for the 26S rRNA and combined 18S/26S rRNA datasets were calculated using 50 random sequence addition replicates and bootstrap proportion values for these were calculated using the heuristic "fast step-wise" addition option with 100 pseudoreplicates.

Results

26S rRNA Analysis

Cladistic and ML analyses of the 26S data also imply that the Mastophoroideae do not share a most recent common ancestor with one another and is a polyphyletic taxon. The placement of *Neogoniolithon brassica-florida* and *N. spectabile* within the Corallinoideae, as well as the inferred evolutionary relationships of members of the Corallinoideae slightly differed between the two analyses. The remaining relationships were consistent in both analyses (Figs. 3, 4).

Cladistic analysis analysis yielded a total of three equally parsimonious trees with a length of 746 steps (CI= 0.613, RI= 0.703) and 240 phylogenetically informative positions. The 50% majority rule tree is depicted in Figure 3. The Corallinoideae is divided into two clades, one comprised of members from the tribe Corallineae and the other with *Neogoniolithon* spp. and representatives of the tribe Janieae, however, support for this arrangement is weak (BP= < 50%). Together, *N. brassica-florida* and *N. spectabile* are resolved as sister taxa to the Janieae with relatively weak bootstrap support (BP= 64%) whereas the Corallineae form a separate clade (BP= 68%). The topology of the Corallinoideae differs in the ML analysis where *Neogoniolithon* spp. are placed

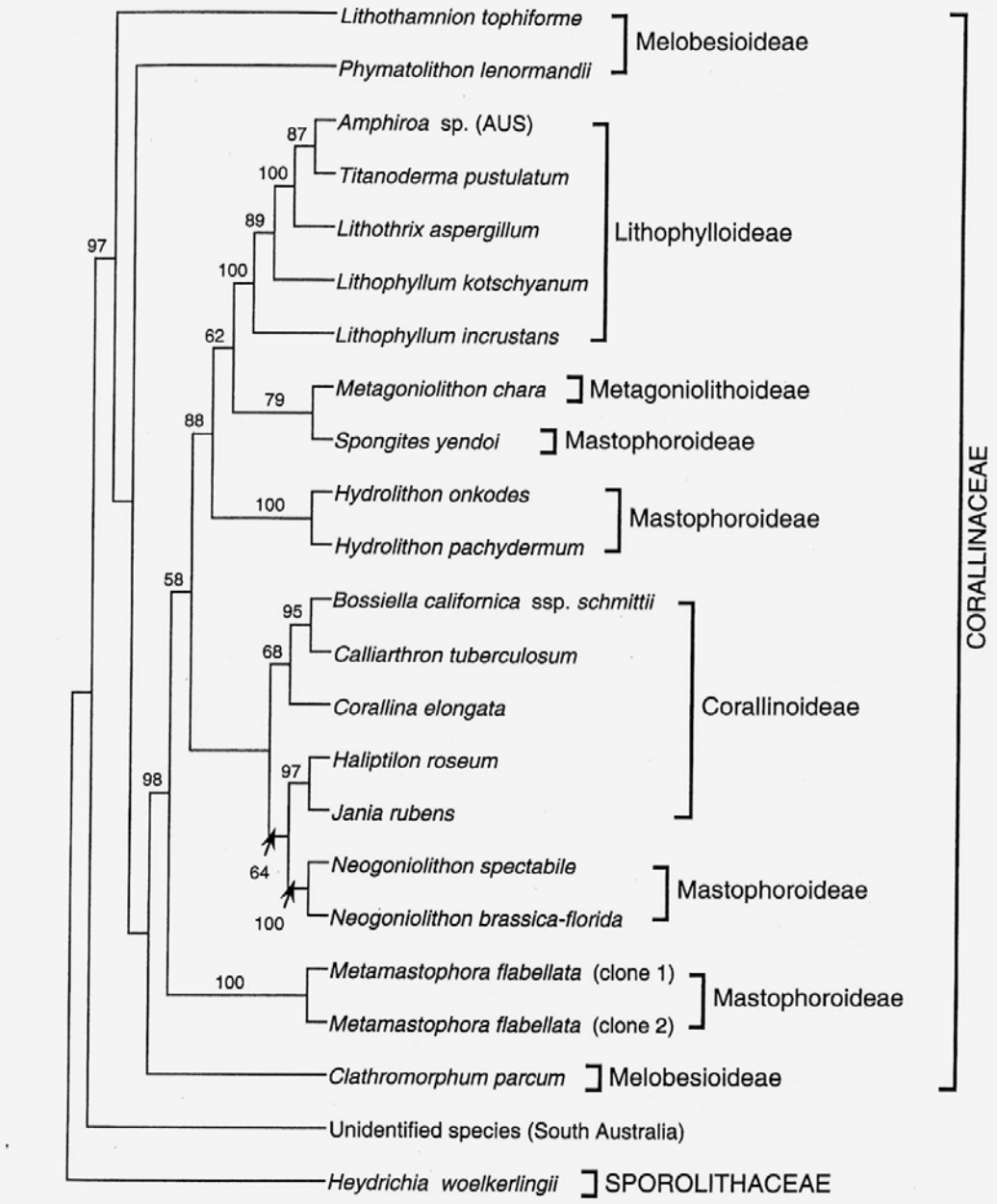

Figure 3. Majority rule of three equally parsimonious trees (CI= 0.613, RI= 0.703) obtained from cladistic analysis of the 26S rRNA gene for 22 species of coralline red algae. Bootstrap proportion values >50% for each node are given above the branches.

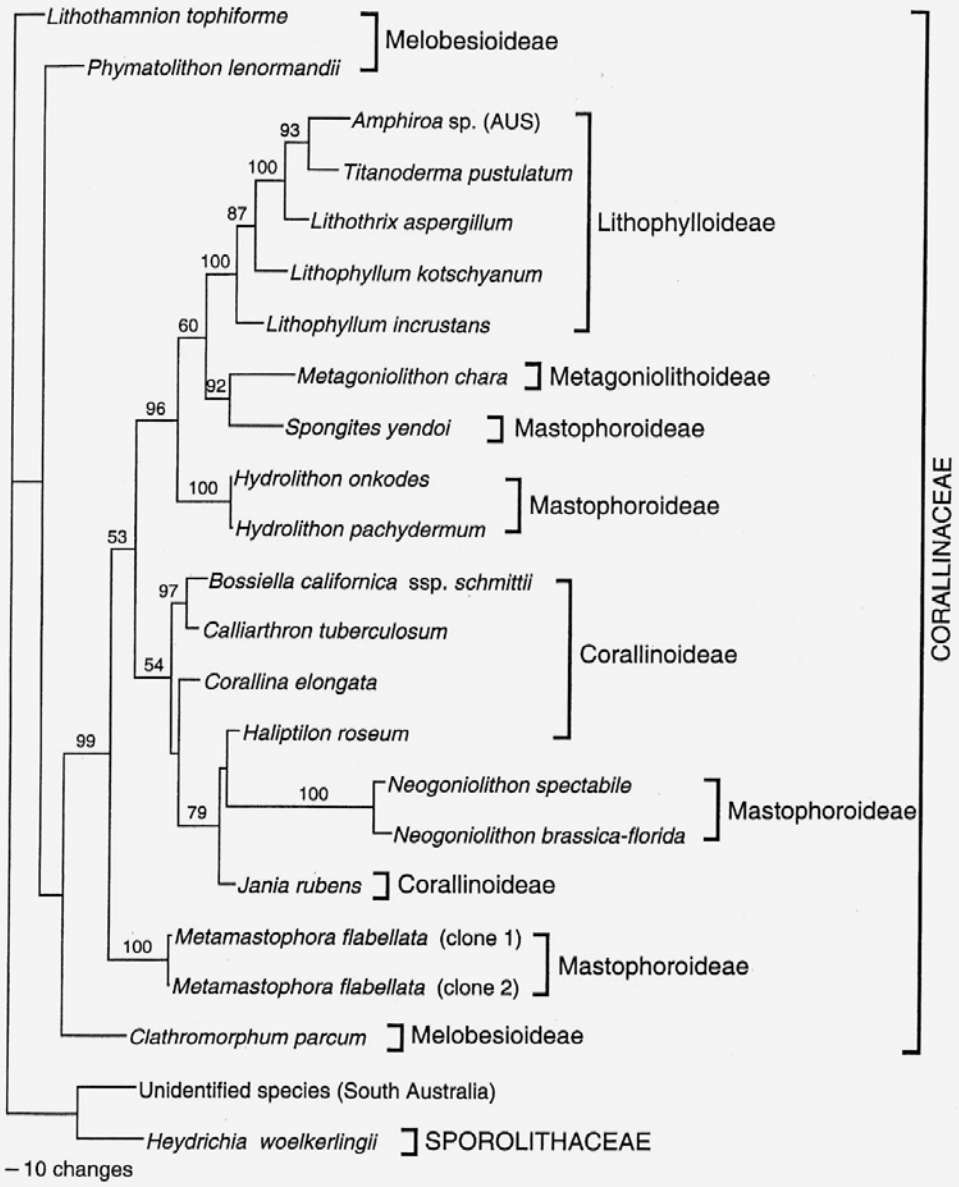

Figure 4. Maximum likelihood tree obtained from analysis of the 26S rRNA gene for 22 species of coralline red algae. Bootstrap proportion values >50% for each node are given above the branches.

within the Janieae as sister taxa to *Haliptilon roseum*, but support for this observation is weak (BP= < 50%). There is, however, relatively strong support for the clustering of the Janieae with *Neogoniolithon* spp. (BP= 79%) (Fig. 4). The ML tree also differs from the parsimony tree with respect to the placement of *Corallina elongata.* In this analysis, *C. elongata* is not monophyletic with *Bossiella californica* ssp. *schmittii* and *Calliarthron tuberculosum*, but there is very weak support for its position outside of the Corallineae (BP= < 50%).

In both parsimony and ML analyses, *Hydrolithon onkodes* and *H. pachydermum* are strongly supported as sister taxa (BP= 100%, 100%). Together, *H. onkodes* and *H. pachydermum* are positioned as sister to a clade comprised of the Lithophylloideae, Metagoniolithoideae, and *Spongites yendoi* with strong support for this arrangement (BP= 88%, 96%).

Metamastophora flabellata is resolved as an early diverging lineage within the Corallinaceae. In both analyses it is positioned at the base of a clade comprised of the Lithophylloideae, Metagoniolithoideae, Corallinoideae, and the additional representatives of the Mastophoroideae. Support for this arrangement is consistently strong between the two analyses (BP= 98%, 99%). The placement of *Spongites yendoi* is also consistent between the two trees, positioning it as sister taxon to *Metagoniolithon chara* with moderate to strong support (BP= 79%, 92%).

Representatives of the Melobesiodeae are resolved as the earliest diverging taxa within the Corallinaceae (Figs. 3, 4), however, they are not resolved as a monophyletic taxon in either cladistic or ML analyses of the 26S rRNA data and bootstrap support for their positions is less than 50% (except for the position of *Lithothamnion tophiforme* in

the parsimony tree where support for its early divergence from the rest of the Corallinaceae is strong [BP= 97%]).

Combined 18S/26S rRNA Analysis

Analysis of the nuclear encoded small ribosomal subunit gene in combination with the nuclear encoded large ribosomal subunit gene yielded consistent results in both cladistic and maximum likelihood trees (Figs. 5, 6). As with the exclusive 18S and 26S rRNA analyses, the Mastophoroideae are also resolved as a polyphyletic taxon in the combined 18S/26S analyses.

Cladistic analysis yielded two equally parsimonious trees with a length of 1624 steps (CI= 0.595, RI= 0.678) and 504 phylogenetically informative positions (Fig. 5). In both cladistic and maximum likelihood analyses, members of the Melobesiodeae are among the first taxa to diverge within the Corallinaceae but are not resolved as monophyletic (BP= < 50%, < 50%) (Figs. 5, 6).

Neogoniolithon brassica-florida and *N. spectabile* are resolved as sister taxa (BP= 100%, 100%), and both *Neogoniolithon* spp. are positioned as sister to the Janieae (BP= < 50%, 74%). Both analyses support the monophyly of *Neogoniolithon* and Corallinoideae species with weak to moderate support (BP= 60%, 81%).

The position of *Hydrolithon* in the combined 18S/26S analyses is consistent with its placement in the trees based on the 26S rRNA data alone. Here, *H. onkodes* and *H. pachydermum* are resolved as sister taxa (BP= 100%, 100%). Together, *Hydrolithon* spp. are positioned as sister to a clade comprised of the Lithophylloideae, Metagoniolithoideae and *Spongites yendoi* with strong support for this arrangement (BP= 96%, 99%).

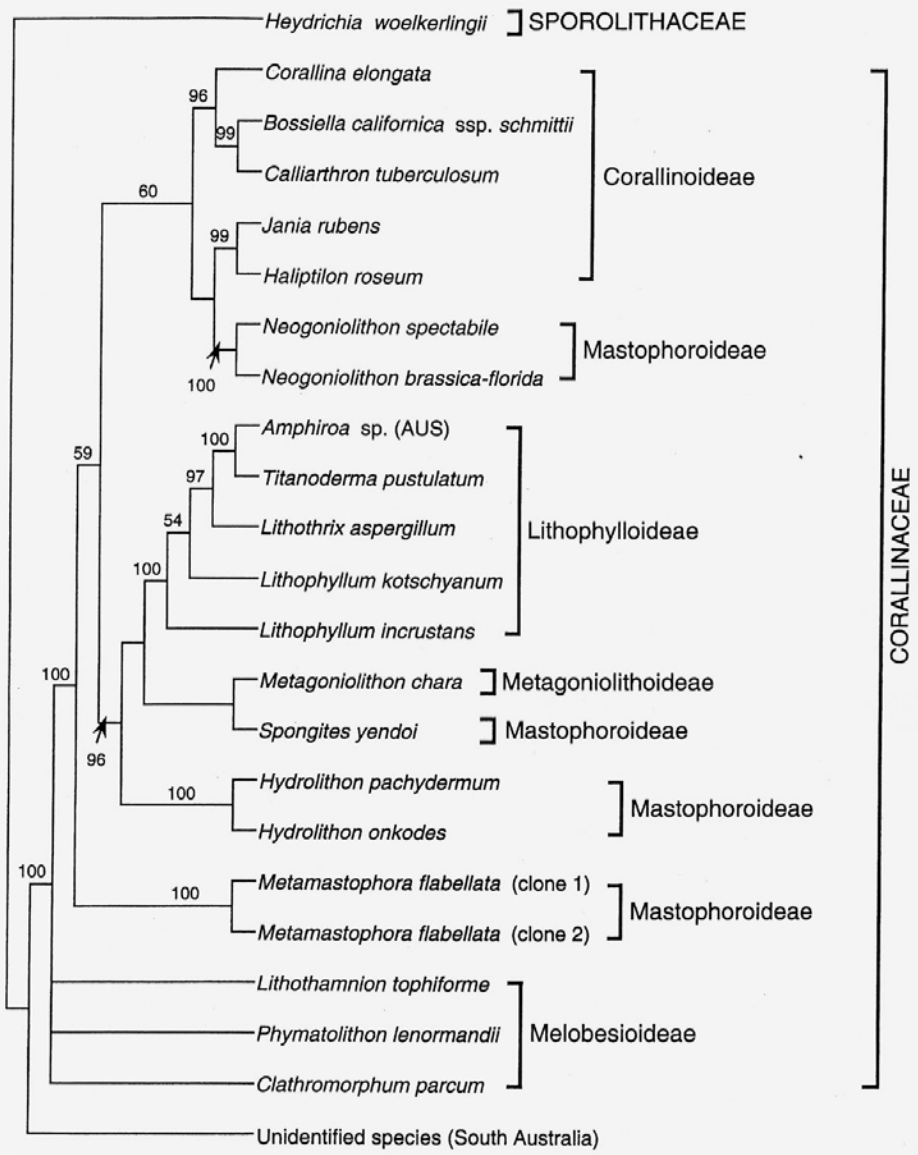

Figure 5. Majority rule of two equally parsimonious trees (CI= 0.595, RI= 0.678) obtained from cladistic analysis of the combined 18S/26S rRNA gene sequences for 22 species of coralline red algae. Bootstrap proportion values >50% for each node are given above the branches.

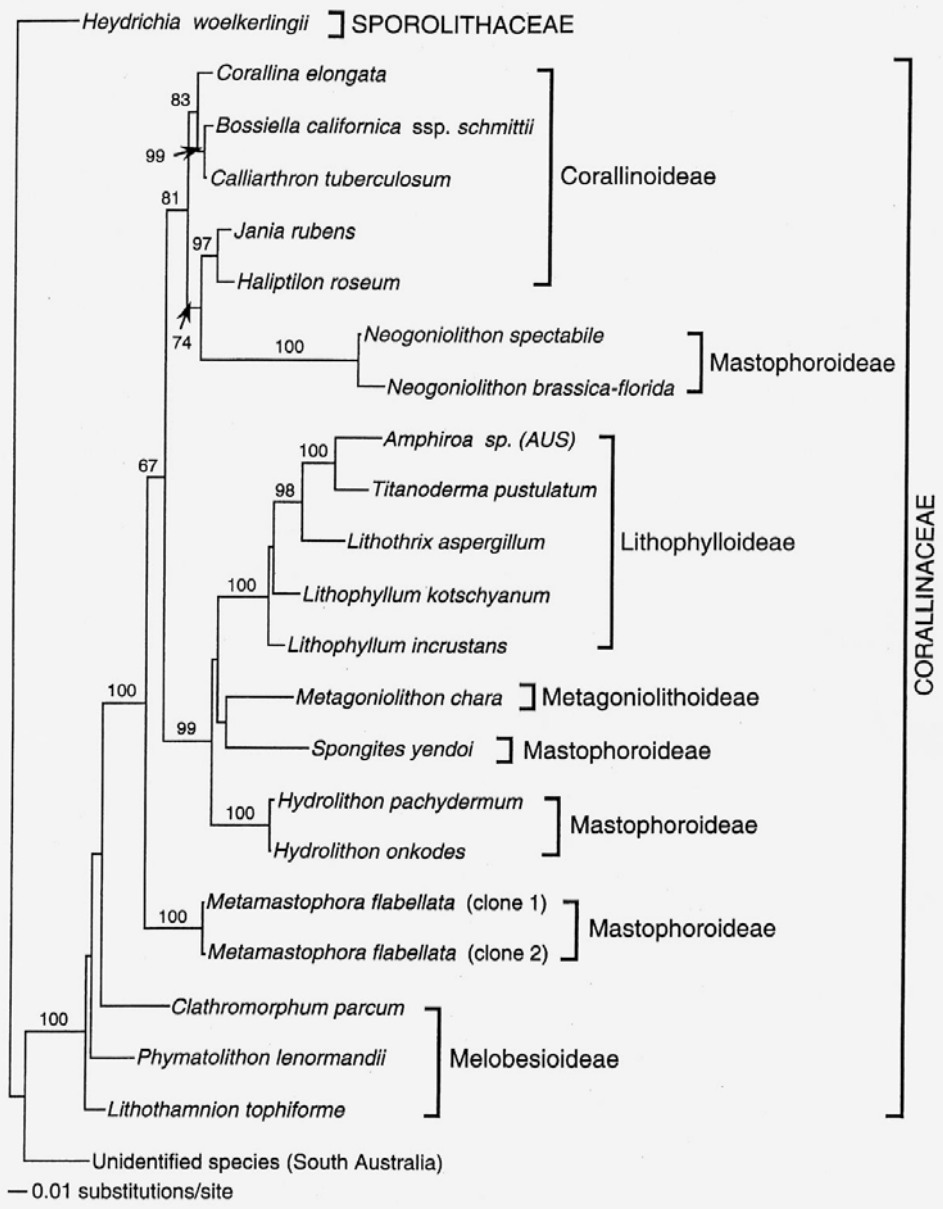

Figure 6. Maximum likelihood tree obtained from the combined analysis of 18S/26S rRNA gene sequences for 22 species of coralline red algae. Bootstrap proportion values >50% for each node are given above the branches.

Also in congruence with the 26S-only analyses (Figs. 3, 4) and 18S-only ML analysis (see Chapter 1, Fig. 2), *Metamastophora flabellata* is positioned as sister to a clade comprised of other members of the Corallinaceae excluding the Melobesiodeae. Support for this topology is strong in both parsimony and ML analyses (BP= 100%, 100%). *Spongites yendoi* is resolved as sister taxon to *Metagoniolithon chara,* and together these two taxa are placed as sister to the Lithophylloideae, but bootstrap support for these two arrangements is < 50% in both cladistic and ML analyses.

Discussion

The independant 26S rRNA and combined 18S/26S rRNA analyses (Figs. 3-6) are congruent with the results obtained in the 18S rRNA analyses (Figs. 1, 2) in the sense that the Mastophoroideae is resolved as a polyphyletic taxon with at least four independant lineages arising throughout the evolution of the Corallinaceae. A comparison of the trends observed in this study with those observed in Chapter 1 will follow, with mention of reproductive features that are characteristic of the taxa being discussed. However, specific details with respect to these features will not be repeated here as they have already been described in Chapter 1.

Phylogenetic Position of *Neogoniolithon*

According to the tree topologies that have been generated here (Figs. 3-6), it is inferred that *Neogoniolithon* shares its most recent common ancestor with members of the Janieae (Corallinoideae). This resolution is consistent with the topology generated by the 18S rRNA maximum likelihood analysis (Fig. 2), but differs from the 18S rRNA

parsimony analysis (Fig. 1). And although five out of the six topologies generated thus far place *Neogoniolithon* in a clade with the Janieae, bootstrap support values for that node are primarily weak (18S ML BP= < 50%, 26S parsimony BP= 64%, 26S ML BP= < 50%, 18S/26S parsimony BP= < 50%, 18S /26S ML BP= 74%).

With respect to reproductive features shared in common with *Neogoniolithon* and members of the Corallinoideae, all of these taxa undergo the Type 1 mode of tetrasporangial development and the development of spermatangia occurs along the floor, walls, and roof within the male conceptacle. And although detailed descriptions on gametangial and carposporangial conceptacle morphology in *Neogoniolithon* spp. is scarce, there is evidence that within the female conceptacle gonimoblast filaments arise from the dorsal surface of fusion cells (Penrose 1992b). The origin of gonimoblast filaments was a feature used by Johansen & Silva (1978) to delimit two tribes within the Corallinoideae, the Janieae whereby gonimoblast filaments arise along the periphery of the fusion cell and the Corallineae in which gonimoblast filaments can arise along the periphery of the fusion cell only or along the periphery and dorsal surface of the fusion cell. Because this unique set of tetrasporangial and male reproductive characters are consistent throughout all the Corallinoideae taxa and *Neogoniolithon*, it is probable that *Neogoniolithon* does in fact belong to the Corallinoideae and may be a direct descendant of the last common ancestor of this clade. However, the division of the Corallinoideae into two tribes on the basis of differences in gonimoblast filament origin, one might expect *Neogoniolithon* to be more closely allied with members of the Corallineae tribe rather than the Janieae. This is not the case in the majority of the analyses presented here, where *Neogoniolithon* is allied with members of the Janieae (Figs. 2-6).

With weak support across the board for the placement of *Neogoniolithon* as part of the Janieae clade, it is unwise to make any taxonomic revisions without the investigation of character state evolution within the Corallinaceae. At this point it is not known whether or not these morphological traits are indeed phylogeneically informative. This topic is addressed in Chapter 3.

Phylogenetic Positions of *Hydrolithon* and *Spongites*

The 26S and combined 18S/26S rRNA analyses are consistent with one another in their placement of *Hydrolithon* and *Spongites* within the trees generated in this study (Figs. 3-6). This particular topology differs with respect to the placement of these two taxa in the trees generated from analysis of the 18S data where the positions of *Hydrolithon* and *Spongites* are reversed (Figs. 1, 2).

In the 18S analyses (Figs. 1, 2), *Hydrolithon* spp. were resolved as sister taxa to the Metagoniolithoideae with moderate to weak support for this arrangement (BP= 81%, 60%), whereas 26S and 18S/26S rRNA analyses resolved *Spongites* as sister to *Metagoniolithon* with moderate to strong suppport for the 26S trees (BP= 79%, 92%) (Figs. 3, 4), and very weak support for the 18S/26S trees (BP= < 50%, < 50%) (Figs. 5, 6). Furthermore, the positioning of *Spongites* as sister to the clade comprised of the Lithophylloideae, *Metagoniolithon,* and *Hydrolithon* in the 18S trees (Figs. 1, 2) had relatively strong support (BP= 82%, 100%), but support for the placement of *Hydrolithon* as sister to the Lithophylloideae, *Metagoniolithon,* and *Spongites* clade in the 26S and 18S/26S analyses was also strong (26S BP= 88%, 96%; 18S/26S BP= 96%, 99%) (Figs. 3-6). With such relatively strong support for two different hypotheses, this discrepancy

might be difficult to reconcile. But the fact remains that only 23 taxa were included in the 26S and 18S/26S rRNA analyses whereas a total of 49 taxa were included in the 18S analyses, and a reduced taxon sampling versus more characters may be responsible for these incongruencies.

Taking into account reproductive features shared between *Hydrolithon* and *Metagoniolithon,* as well as members of the Lithophylloideae, the unique set of features that joins these taxa is as follows: Type 2 mode of tetrasporangial conceptacle development, spermatangia formation confined to the floor of the male conceptacle, and gonimoblast filaments arising along the periphery of the fusion cell in the female conceptacle (see references in Chapter 1). With respect to these features, *Spongites* differs from the members of this clade by demonstrating the Type 1 mode of tetrasporangial conceptacle development. Support for the clade comprised of the Lithophylloideae, *Metagoniolithon,* and *Hydrolithon* in the 18S-only analyses is moderate to weak (BP= 71%, 60%) (Figs. 1, 2), but in the substitution of *Spongites* for *Hydrolithon* in the 26S and 18S/26S analyses, support for the clade comprised of the Lithophylloideae, *Metagoniolithon,* and *Spongites* is weak (26S BP= 62%, 60%) (Figs. 3, 4) or not well supported at all (18S/26S BP= < 50%, < 50%) (Figs. 5, 6). The most parsimonious explanation of the observed reproductive features outlined here is found in the trees generated from the 18S analyses (Figs. 1, 2) where *Spongites* is placed as sister to the Lithophylloideae, *Metagoniolithon,* and *Hydrolithon* clade.

Phylogenetic Position of *Metamastophora*

Metamastophora was consistently placed as sister to a clade comprised of members of the Lithophylloideae and Corallinoideae, *Metagoniolithon, Hydrolithon,* and *Spongites* in all four 26S and 18S/26S rRNA analyses (Figs. 3-6) with strong support for this node (26S BP= 98%, 99%; 18S/26S BP= 100%, 100%). This topology is consistent with and strongly supported in the 18S ML tree (BP= 100%) (Fig. 2) but differs from the 18S parsimony tree. The 18S parsimony tree places *Metamastophora* as sister to a clade comprised of members of the Corallinoideae and *Neogoniolithon* spp. (Fig. 1). Since bootstrap support for this particular arrangement is less than 50% and all other analyses strongly support the former arrangement, it is inferred that *Metamastophora* is an early diverging lineage in the Corallinaceae and the topology obtained in Figs. 2-6 is therefore preferred.

Of the various combinations and trends seen in reproductive features within the Corallinaceae (discussed in Chapter 1), *Metamastophora* and *Spongites* share in common the following set of features: Type 1 mode of tetrasporangial conceptacle development, spermatangia formation restricted to the floor of the male conceptacle, and gonimoblast filaments arising along the periphery of the fusion cell. The one feature that links most members of the Corallinaceae, excluding the Melobesiodeae, in the clade subtended by *Metamastophora* is the presence of a single, continuous fusion cell in the carposporophytic conceptacle. The reliance of these features as to their utility in taxonomic delineation at the subfamilial rank will be addressed in the following chapter.

The primary purpose of this investigation was to evaluate the relationships of representative mastophoroid taxa within the Corallinaceae using additional gene sequene

data for the nuclear-encoded 26S rRNA. The results from the independent 26S and combined 18S/26S rRNA analyses differ slightly from those of the 18S analyses. Phylogenetic relationships with repect to the major clades formed within the Corallinaceae were consistent among all trees. This includes the alignment of *Neogoniolithon* with the Corallinoideae, *Hydrolithon* and *Spongites* with *Metagoniolithon* and the Lithophylloideae, and lastly, Metamastophora with all members of the Corallinaceae except for the Melobesiodeae. It is probable that *Neogoniolithon* does indeed share its most recent common ancestor with the Corallinoideae, even though support for its position remains weak with the analysis of an additional gene. Despite the fact of a smaller taxon sampling set was used here, the use of the 26S rRNA gene and the combination of the 18S rRNA and 26S rRNA genes provide similar topologies in the Corallinaceae as is resolved in the analysis of the nuclear-encoded 18S rRNA gene separately.

CHAPTER 3. THE SYSTEMATIC VALUE OF VEGETATIVE AND REPRODUCTIVE FEATURES FOR DIAGNOSING TAXA WITHIN THE CORALLINALES (RHODOPHYTA)

Introduction

Prior to the advent of modern molecular systematics techniques, particularly electron microscopy, high through-put DNA sequencing and computer-based analyses using objective optimality criteria, all classifications were based upon more-or-less subjective comparisons of morphological characters. Once coralline red algae were first recognized as plants (not coral animals) by Philippi, Kützing, and Decaisne from 1837-1842 (Woelkerling 1988), traditional comparative analyses of vegetative and reproductive features gave rise to original systems of classification for the group. Since the mid-1970's, numerous taxonomic revisions of the Corallinales have been published at the ranks of genus and above. During this period corallinologists gradually began to reject the premise that externally visible morphological features of vegetative thalli could be used to accurately identify and naturally classify coralline species. This conclusion was reached following the publication of taxonomic and ecological studies that clearly demonstrated that many features of mature vegetative thalli are phenotypically plastic. Although responses vary across species, it was shown that biotic and abiotic factors can influence gross morphology to the extent that distantly related species (particularly nongeniculate species) may resemble one another so closely that they cannot be identified with certainty. For this reason, and with a few notable exceptions, most recent classification systems for corallines have largely abandoned external morphology for the diagnosis of species. Instead, in dealing with different algal groups newer classifications have begun to incorporate, and clearly emphasize, features associated with reproduction

to infer relationships at the species level and higher taxonomic ranks (i.e. Saunders & Kraft 1997; Freshwater & Bailey 1998; Harper & Saunders 2002).

Recent molecular systematics studies have altered and improved our understanding of the evolution of coralline red algae (Bailey & Chapman 1996, 1998; Bailey 1999; Bailey et al. (in press); Chapters 1 & 2). These phylogenetic hypotheses, based upon 18S rRNA and 26S rRNA gene sequences, were, however, constructed largely without special consideration of morphological features. Thus, the question arises: If one objectively evaluates those vegetative and reproductive features presently used to circumscribe families, subfamilies and tribes etc. of coralline algae, do they support or contradict inferences based on molecular data alone?

In this study a data matrix including 18S rRNA sequences for 47 species of coralline algae was analyzed under the optimality criterion of maximum likelihood. Fourteen vegetative and reproductive features considered diagnostic of families and subfamilies were subsequently mapped onto the resulting ML tree to determine which morphological characters are phylogenetically informative and to identify those, if any, that are not.

Materials and Methods

A list of the morphological characters, their character states, and a binary data matrix for all species included in the 18S rRNA dataset is given in Table 4. Accession numbers for the 18S rRNA genes are provided in Table 2. A maximum likelihood tree was constructed using PAUP* (v. 4.0b10, Swofford 2002). The general time-reversible model was implemented with transition/transversion ratios, nucleotide frequencies, and

Table 4. Data matrix comprised of taxa used in this investigation and 14 character states coded for in binary format. The following states are as follows: 1. Tetrasporangial plugs: absent (0) or present (1). 2. Tetrasporangial development: does not occur within a calcified compartment (0) or occurs within a calcified compartment (1). 3. Tetrasporangial development: zonate (0) or cruciate (1). 4. Genicula: absent (0) or present (1). 5. Tetrasporangial conceptacles: absent (0), or uniporate (1), or multiporate (2). 6. Cell fusions: absent (0) or present (1). 7. Secondary pit connections: absent (0) or present (1). 8. Tetrasporangial conceptacle development: from filaments surrounding the fertile area and growing upwards to form a common chamber (0), or from filaments interspersed within the fertile area that disintegrate to form a common chamber (1), or from filaments interspersed within the fertile area that do not disintegrate to form a common chamber (2). 9. Cell orientation lining the tetrasporangial canal: perpendicular to roof surface and not protruding into pore canal (0), or parallel to roof surface, protruding into pore canal (1). 10. Location of spermatangia formation in male conceptacles: floor only (0), or floor, walls and roof (1). 11. Gonimoblast filaments: absent or apparently absent (0) or present (1). 12. Location of gonimoblast filament origin: periphery of fusion cell (0), or dorsal surface of fusion cell (1), or not applicable (2). 13. Fusion cell: absent or inconspicuous (0) or present (1). 14. Appearance of fusion cell: continuous (0), discontinuous (1), not applicable (2), or unknown (?).

	Character													
Taxon	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Sporolithaceae														
Heydrichia homalopasta Townsend et Borowitzka	1	1	1	0	0	0	1	2	0	1	0	2	0	2
Heydrichia woelkerlingii Townsend, Chamberlain et Keats	1	1	1	0	0	1	1	2	0	0	1	2	0	2
Sporolithon durum (Foslie) Townsend et Woelkerling	1	1	1	0	0	1	1	2	0	1	0	2	0	2
Corallinoideae														
Arthrocardia filicula (Lamarck) Johansen	0	0	0	1	1	1	0	0	1	1	1	1	1	0
Bossiella californica ssp. *schmittii* (Manza) Johansen	0	0	0	1	1	1	0	0	1	1	1	1	1	0
Bossiella orbigniana ssp. *dichotoma* (Manza) Johansen	0	0	0	1	1	1	0	0	1	1	1	1	1	0
Calliarthron cheilosporioides Manza	0	0	0	1	1	1	0	0	1	1	1	1	1	0
Calliarthron tuberculosum (Postels et Ruprecht) Dawson	0	0	0	1	1	1	0	0	1	1	1	1	1	0
Cheilosporum sagittatum (Lamouroux) J. Areschoug	0	0	0	1	1	1	0	0	1	1	1	0	1	0
Corallina elongata Ellis et Solander	0	0	0	1	1	1	0	0	1	1	1	1	1	0
Corallina officinalis Linnaeus	0	0	0	1	1	1	0	0	1	1	1	1	1	0
Haliptilon roseum (Lamarck) Garbary et Johansen	0	0	0	1	1	1	0	0	1	1	1	0	1	0
Jania crassa Lamouroux	0	0	0	1	1	1	0	0	1	1	1	0	1	0

Table 4 continued

Jania rubens (Linnaeus) Lamouroux	0	0	0	1	1	1	0	0	1	1	1	0	1	0
Serraticardia macmillanii (Yendo) Silva	0	0	0	1	1	1	0	0	1	1	1	1	1	0
Lithophylloideae														
Amphiroa sp. (AUS)	0	0	0	1	1	0	1	1	0	0	1	0	1	0
Amphiroa sp. (SAF)	0	0	0	1	1	0	1	1	0	0	1	0	1	0
Amphiroa fragilissima (Linnaeus) Lamouroux	0	0	0	1	1	0	1	1	0	0	1	0	1	0
Amphiroa hancockii Taylor	0	0	0	1	1	0	1	1	0	0	1	0	1	0
Amphiroa tribulus Foslie et Howe	0	0	0	1	1	0	1	1	0	0	1	0	1	0
Lithophyllum incrustans	0	0	0	0	1	0	1	1	0	0	1	0	1	0
Lithophyllum kotschyanum (Unger) Foslie	0	0	0	0	1	0	1	1	0	0	1	0	1	0
Lithothrix aspergillum J.E. Gray	0	0	0	1	1	0	1	1	0	0	1	0	1	0
Titanoderma pustulatum	0	0	0	0	1	0	1	1	0	0	1	0	1	0
Mastophoroideae														
Hydrolithon onkodes (Heydrich) Penrose et Woelkerling	0	0	0	0	1	1	0	1	0	0	1	0	1	0
Hydrolithon samoënse (Foslie) Keats et Chamberlain	0	0	0	0	1	1	0	1	0	0	1	0	1	0
Metamastophroa flabellata (Sonder) Setchell (clone 1)	0	0	0	0	1	1	1	0	1	0	1	0	1	0
Metamastophroa flabellata (Sonder) Setchell (clone 2)	0	0	0	0	1	1	1	0	1	0	1	0	1	0
Neogoniolithon brassica-florida (Harvey) Setchell et L.R. Mason	0	0	0	0	1	1	0	0	1	1	1	1	1	1
Neogoniolithon spectabile (Foslie) Setchell et L.R. Mason	0	0	0	0	1	1	0	0	1	1	1	1	1	1
Porolithon pachydermum Foslie	0	0	0	0	1	1	0	1	0	0	1	0	1	0
Spongites yendoi (Foslie) Chamberlain	0	0	0	0	1	1	0	0	1	0	1	0	1	0
Melobesioideae														
Choreonema thuretii (Bornet) Schmitz	1	0	0	0	2	0	0	1	0	1	1	0	1	?
Clathromorphum compactum (Kjellman) Foslie	1	0	0	0	2	1	0	1	0	1	1	0	1	1
Clathromorphum parcum (Setchell et Foslie) Adey	1	0	0	0	2	1	0	1	0	1	1	0	1	1
Lithothamnion glaciale Kjellman	1	0	0	0	2	1	0	1	0	1	1	0	1	1
Lithothamnion tophiforme Unger	1	0	0	0	2	1	0	1	0	1	1	0	1	1
'Leptophytum acervatum' (Foslie) Chamberlain et Keats	1	0	0	0	2	1	0	1	0	1	1	0	1	1
'Leptophytum ferox' (Foslie) Chamberlain et Keats	1	0	0	0	2	1	0	1	0	1	1	0	1	1
Mastophoropsis canaliculata (Harvey et Hooker) Woelkerling	1	0	0	0	2	1	0	1	0	1	1	0	1	1
Mesophyllum engelhartii (Foslie) Adey	1	0	0	0	2	1	0	1	0	1	1	0	1	1
Mesophyllum erubescens (Foslie) Lemoine	1	0	0	0	2	1	0	1	0	1	1	0	1	1
Phymatolithon laevigatum (Foslie) Foslie	1	0	0	0	2	1	0	1	0	1	1	0	1	1
Phymatolithon lenormandii (Areschoug) Adey	1	0	0	0	2	1	0	1	0	1	1	0	1	1

Table 4 continued

Synarthrophyton patena (Hooker et Harvey in Harvey) Townsend	1	0	0	0	2	1	0	1	0	1	1	0	1	1
Metagoniolithoideae														
Metagoniolithon chara (Lamarck) Ducker	0	0	0	1	1	1	0	1	0	0	1	0	1	0
Metagoniolithon radiatum (Lamarck) Ducker	0	0	0	1	1	1	0	1	0	0	1	0	1	0
Metagoniolithon stelliferum (Lamarck) Weber-van Bosse	0	0	0	1	1	1	0	1	0	0	1	0	1	0

gamma distributions inferred directly from the dataset in ModelTest (v. 3.06, Posada & Crandall 1998). All regions of the sequence matrix that could not be unambiguously aligned were excluded from the analysis. The binary data matrix was appended to the end of the 18S rRNA alignment (Table 2, excluding the unidentified species for which there presently are no morphological data) and then analyzed using MacClade (v. 3.0, Maddison & Maddison 1992). Fourteen morphological characters were independently mapped onto the ML tree.

Results

Individual vegetative and reproductive characters that have been optimized onto the maximum likelihood tree are depicted in Figs. 7-20. Of the 14 morphological features, the first seven (Figs. 7-13) are those features that have traditionally been used to delimit taxa at the ranks of family and subfamily.

The presence of tetrasporangial plugs occurs in members of the Sporolithaceae and the earliest divergent subfamily in the Corallinaceae, the Melobesiodeae. All other coralline taxa lack tetrasporangial plugs (Fig. 7). The development of tetrasporangia within calcified compartments (or sori, Fig. 8) and cruciate cleavage of the tetraspore mother cell (Fig. 9) are synapomorphic characters uniting all taxa classified in the Sporolithaceae. In the Corallinaceae multiple tetrasporangia develop in uncalcified chambers (conceptacles) and their tetraspores are produced by simultaneous zonate cleavage of the tetraspore mother cell (Figs 8, 9). Genicula are resolved as nonhomologous structures that have independently evolved at least three times (Fig. 10). The type of tetrasporangial conceptacle pore distinguishes the Melobesiodeae

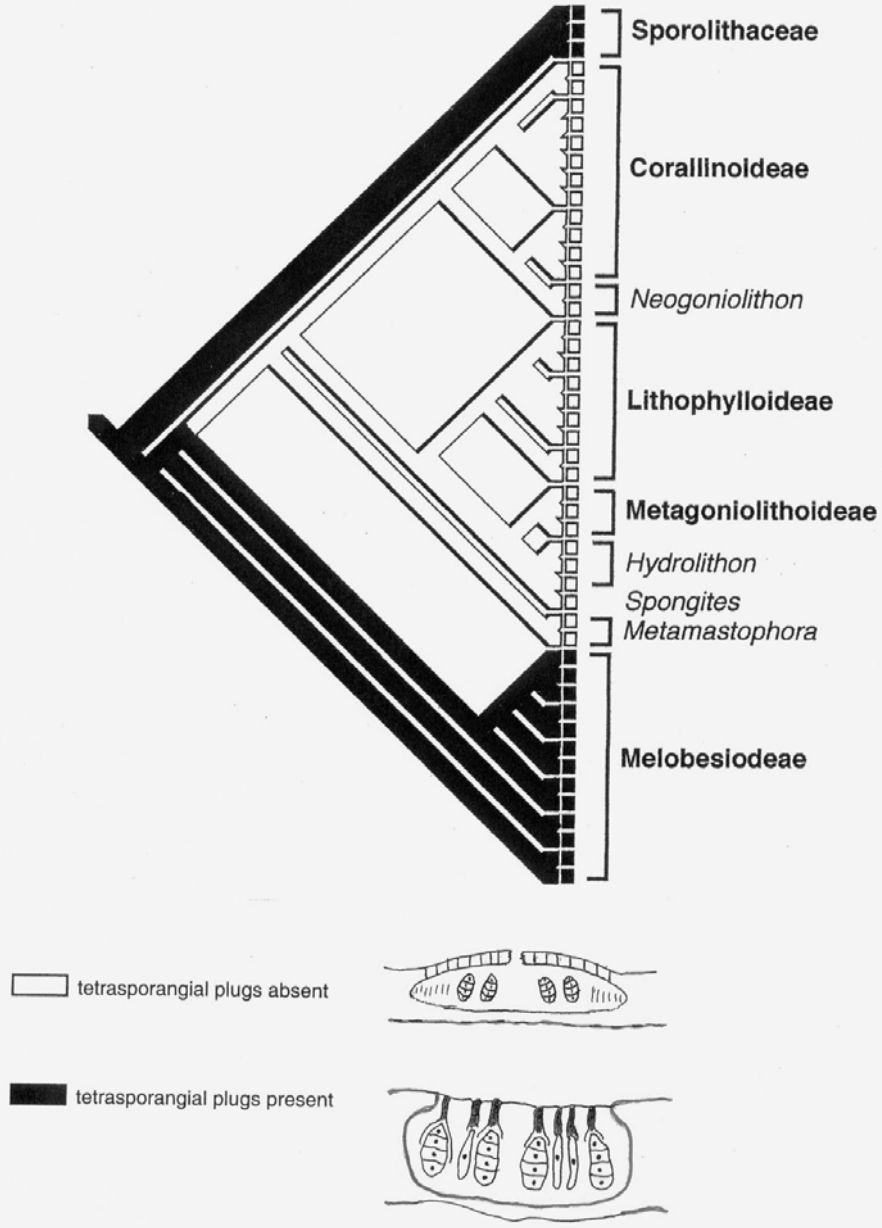

Figure 7. Depiction of morphological character 1 optimized onto the 18S rRNA maximum likelihood tree. Sketches accompanying the key are adapted from Johansen (1981).

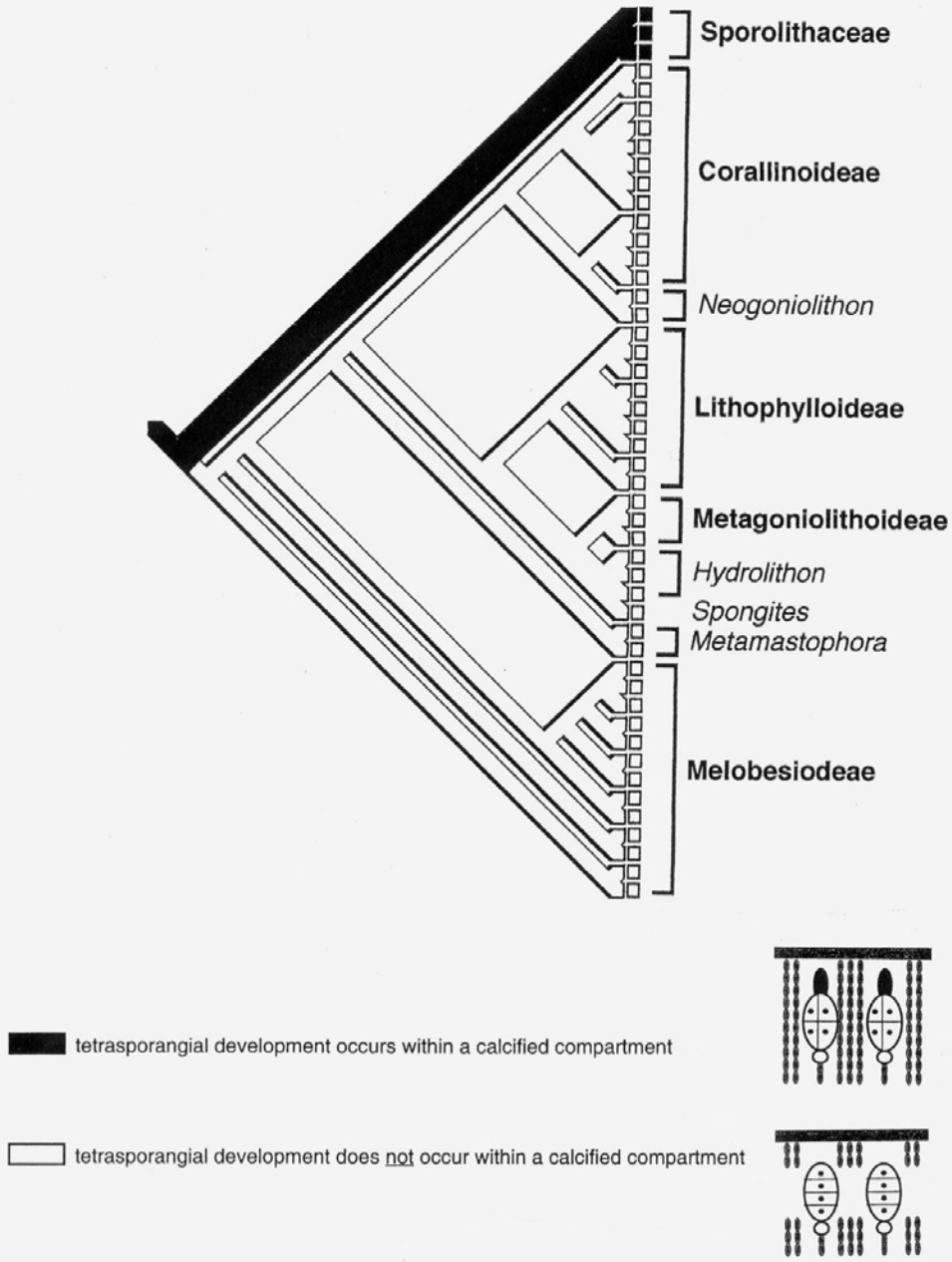

Figure 8. Depiction of morphological character 2 optimized onto the 18S rRNA maximum likelihood tree. Sketches accompanying the key are adapted from Johansen (1981).

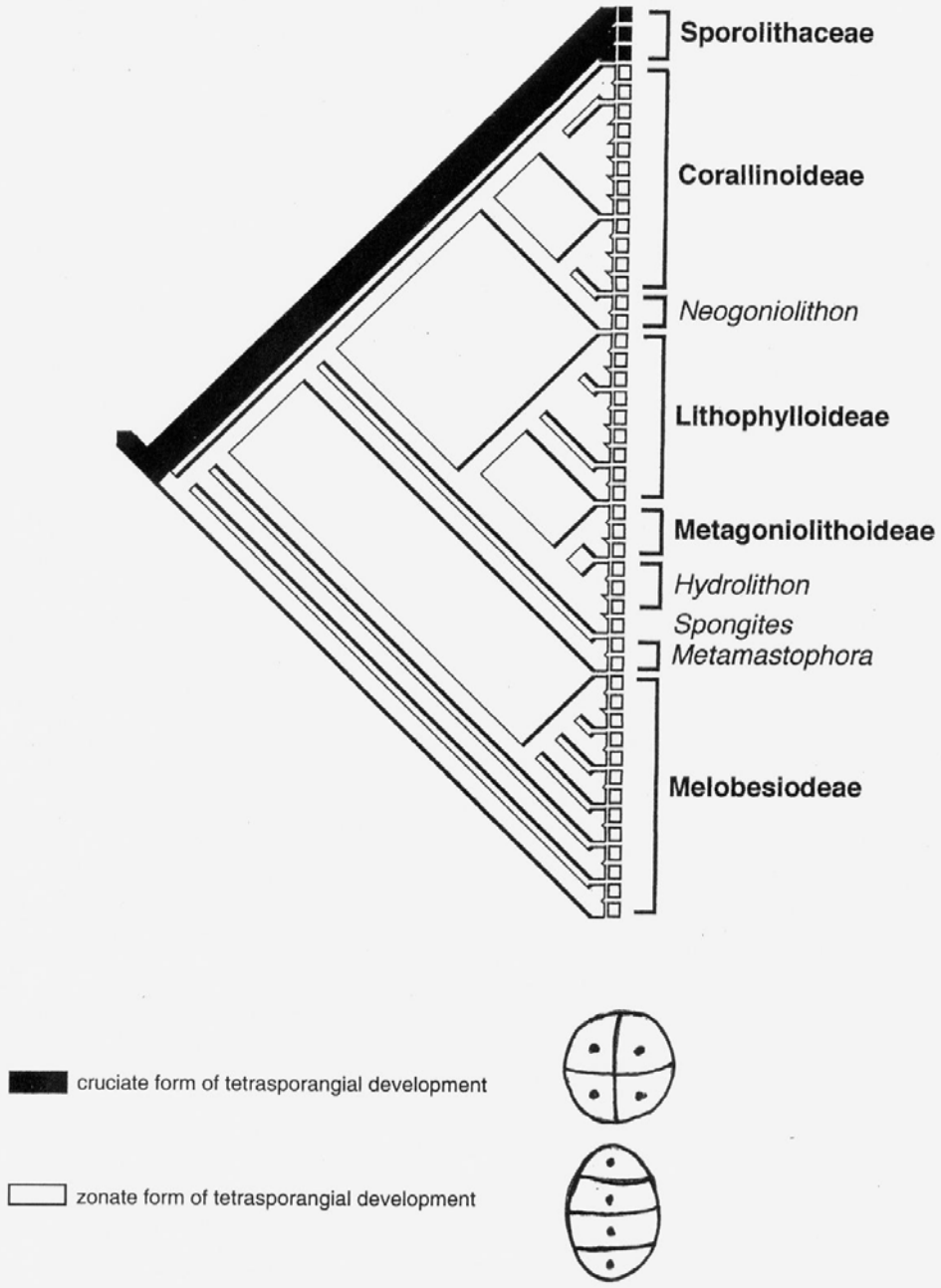

Figure 9. Depiction of morphological character 3 optimized onto the 18S rRNA maximum likelihood tree.

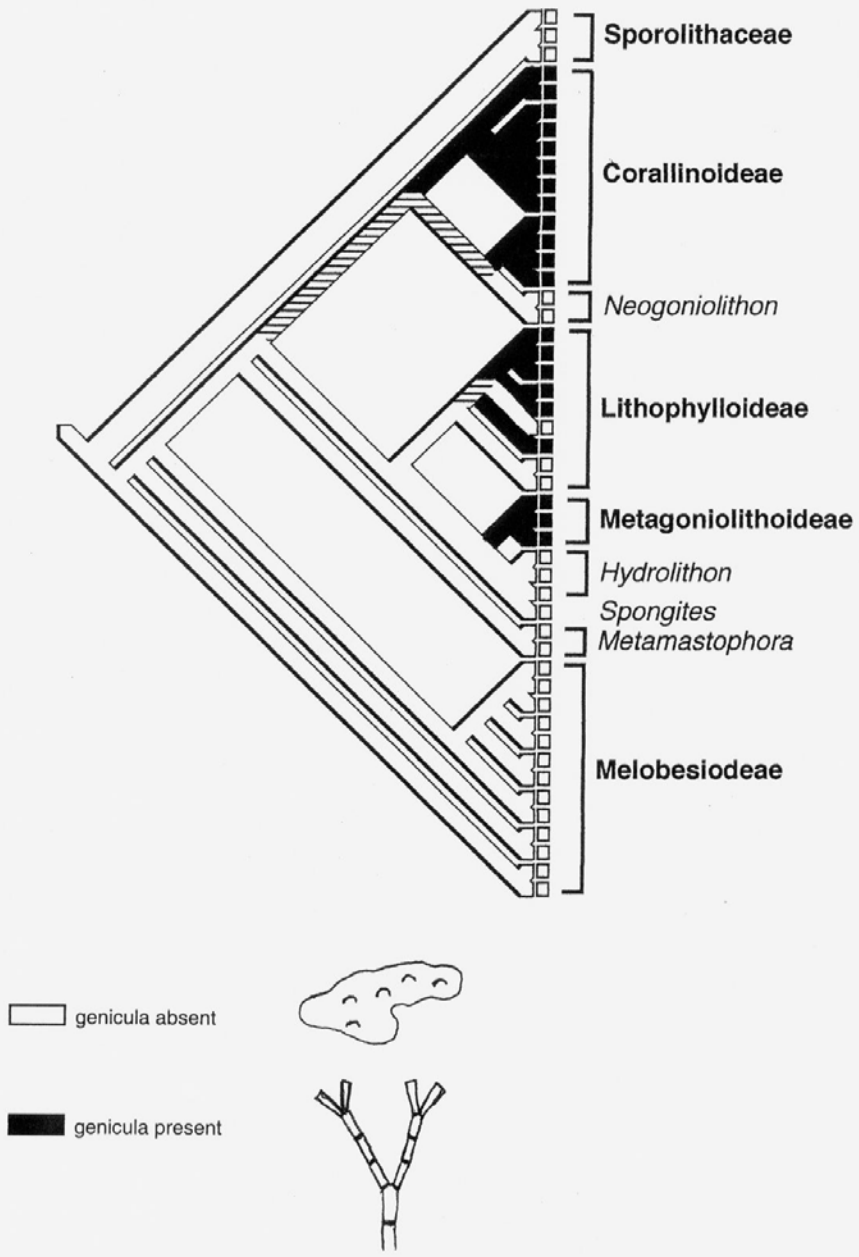

Figure 10. Depiction of morphological character 4 optimized onto the 18S rRNA maximum likelihood tree. Striping represents equivocal character state assignments for internal branches.

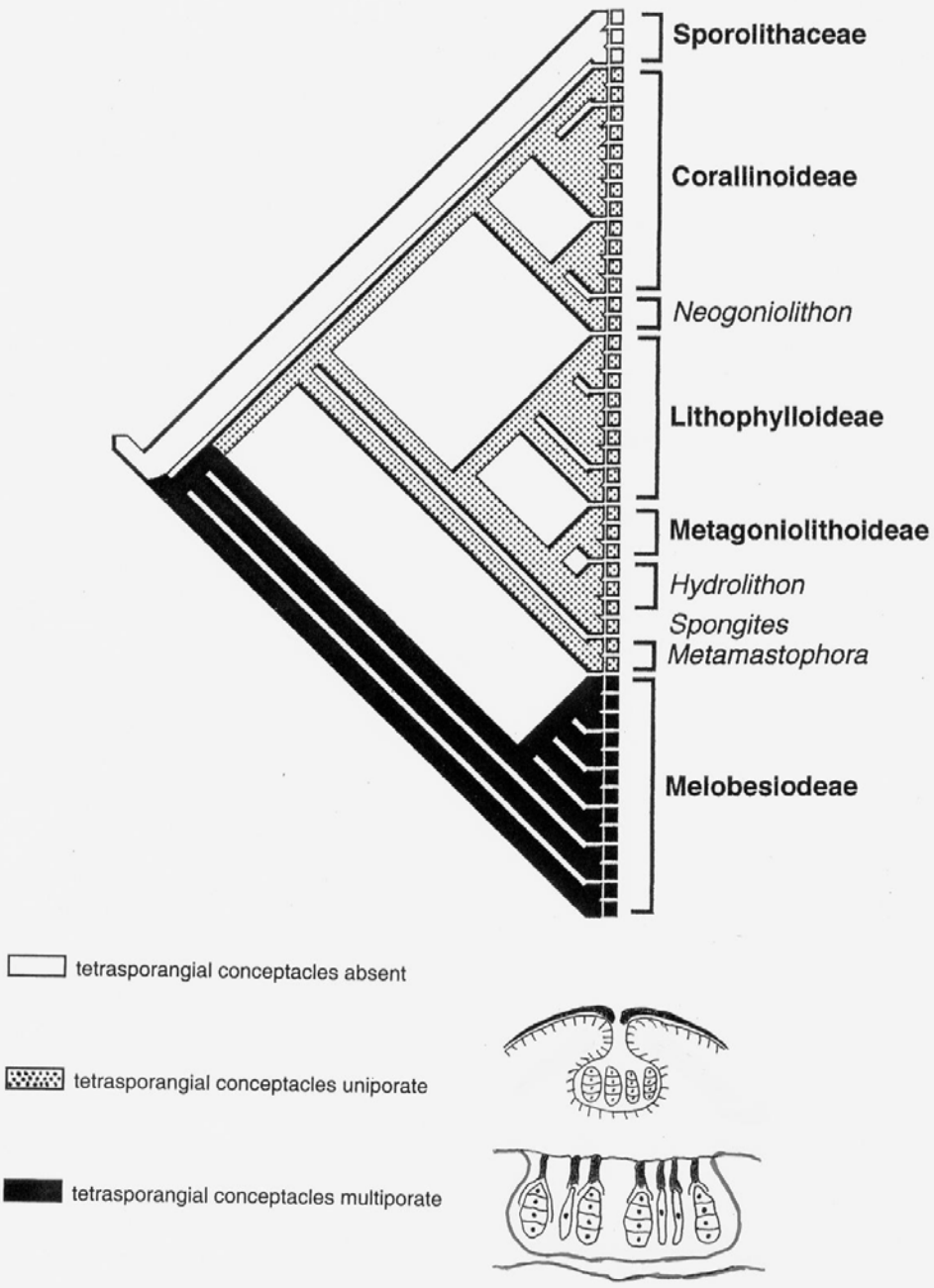

Figure 11. Depiction of morphological character 5 optimized onto the 18S rRNA maximum likelihood tree. Sketches accompanying the key are adapted from Johansen (1981).

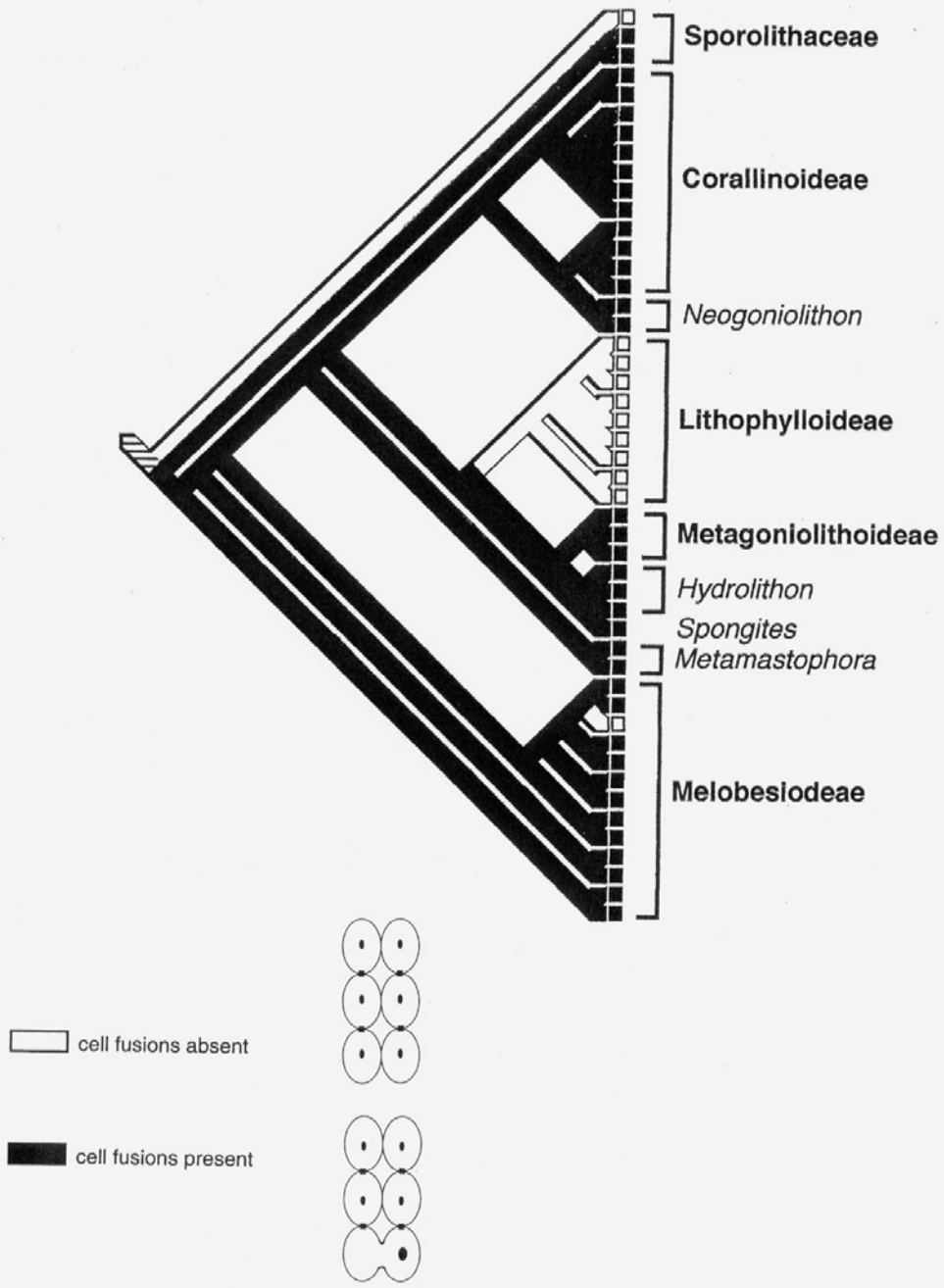

Figure 12. Depiction of morphological character 6 optimized onto the 18S rRNA maximum likelihood tree. Striping represents equivocal character state for internal branch.

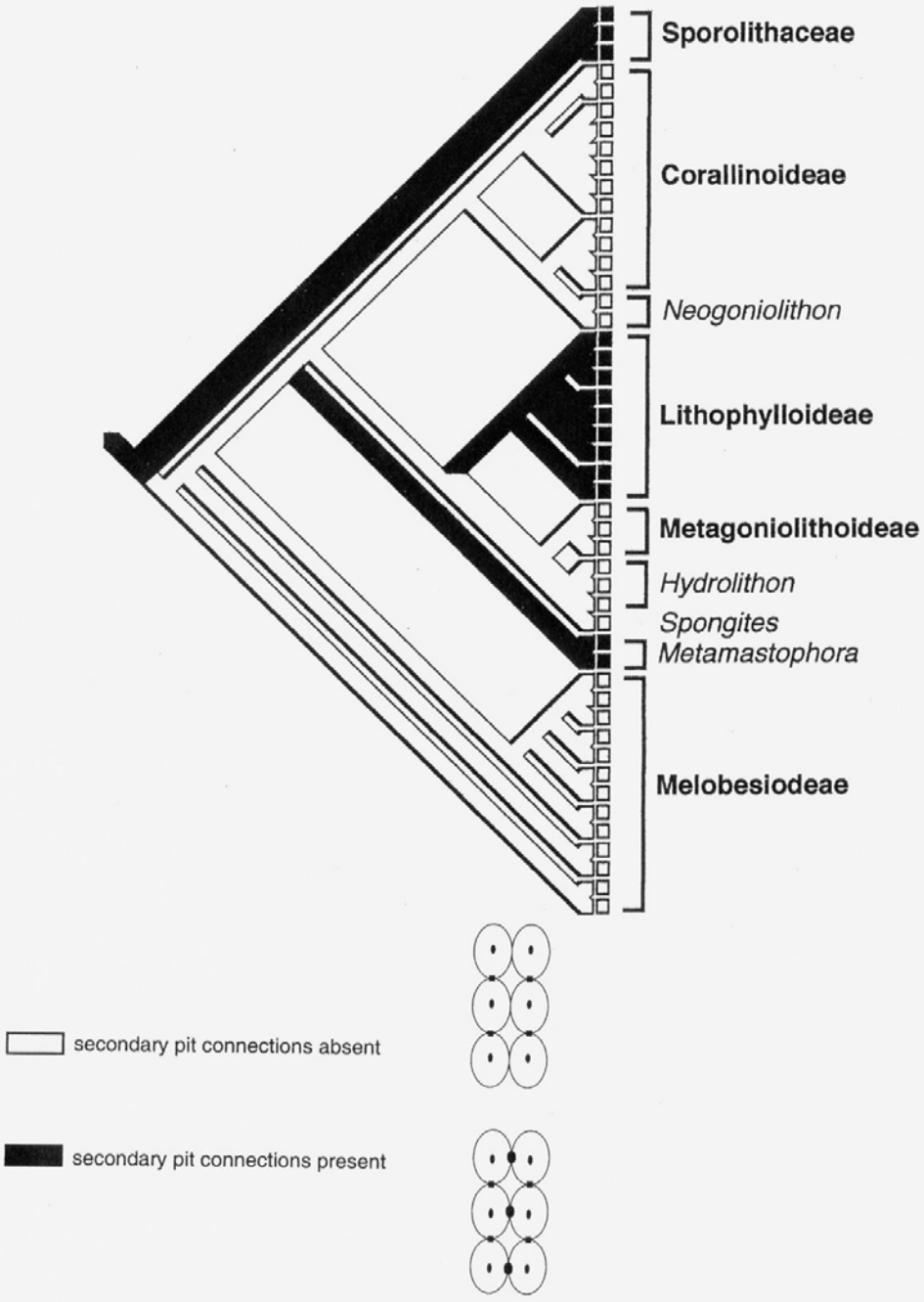

Figure 13. Depiction of morphological character 7 optimized onto the 18S rRNA maximum likelihood tree. Striping represents equivocal character state for internal branch.

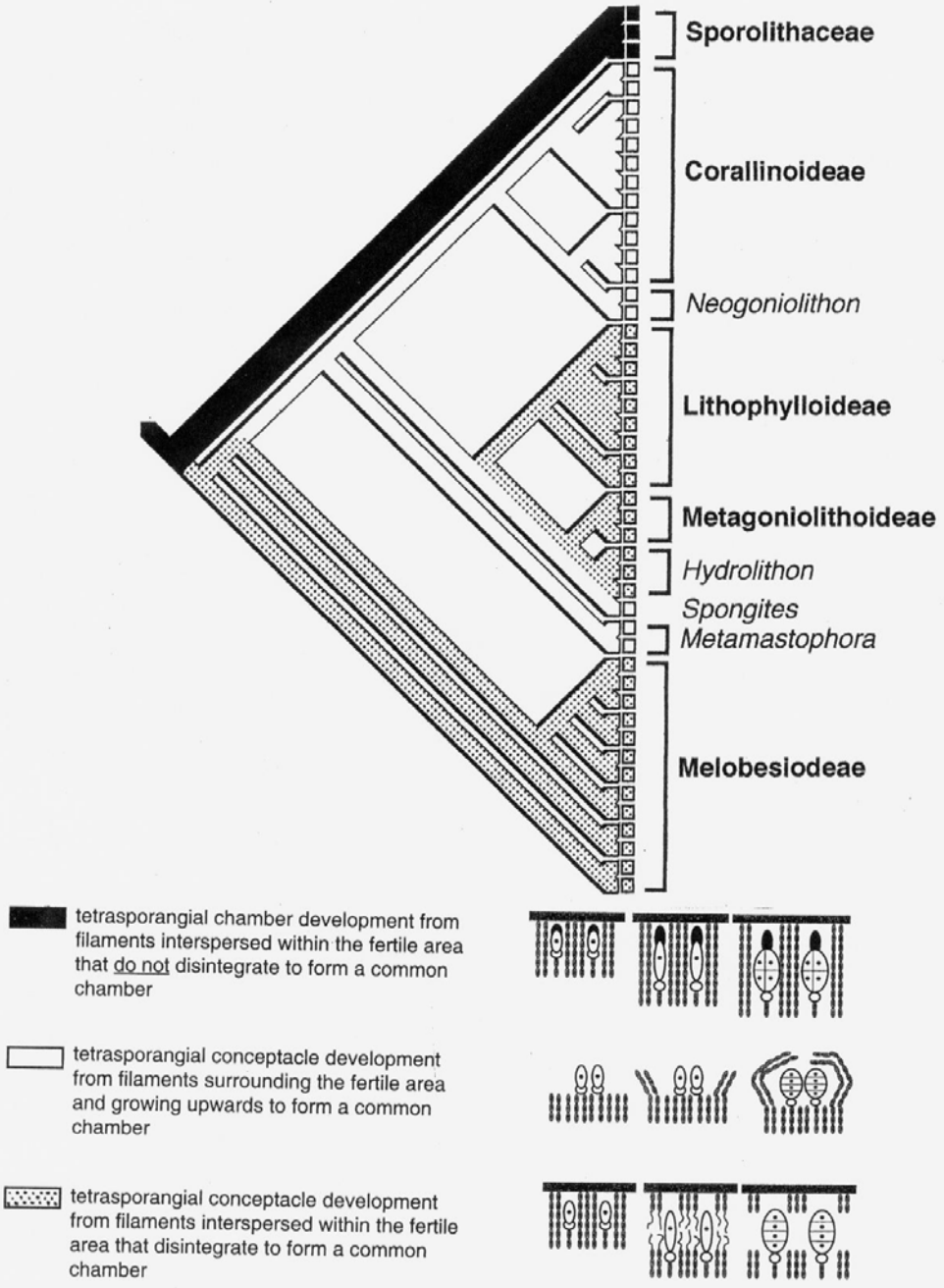

Figure 14. Depiction of morphological character 8 optimized onto the 18S rRNA maximum likelihood tree. Sketches accompanying the key are adapted from Johansen (1981).

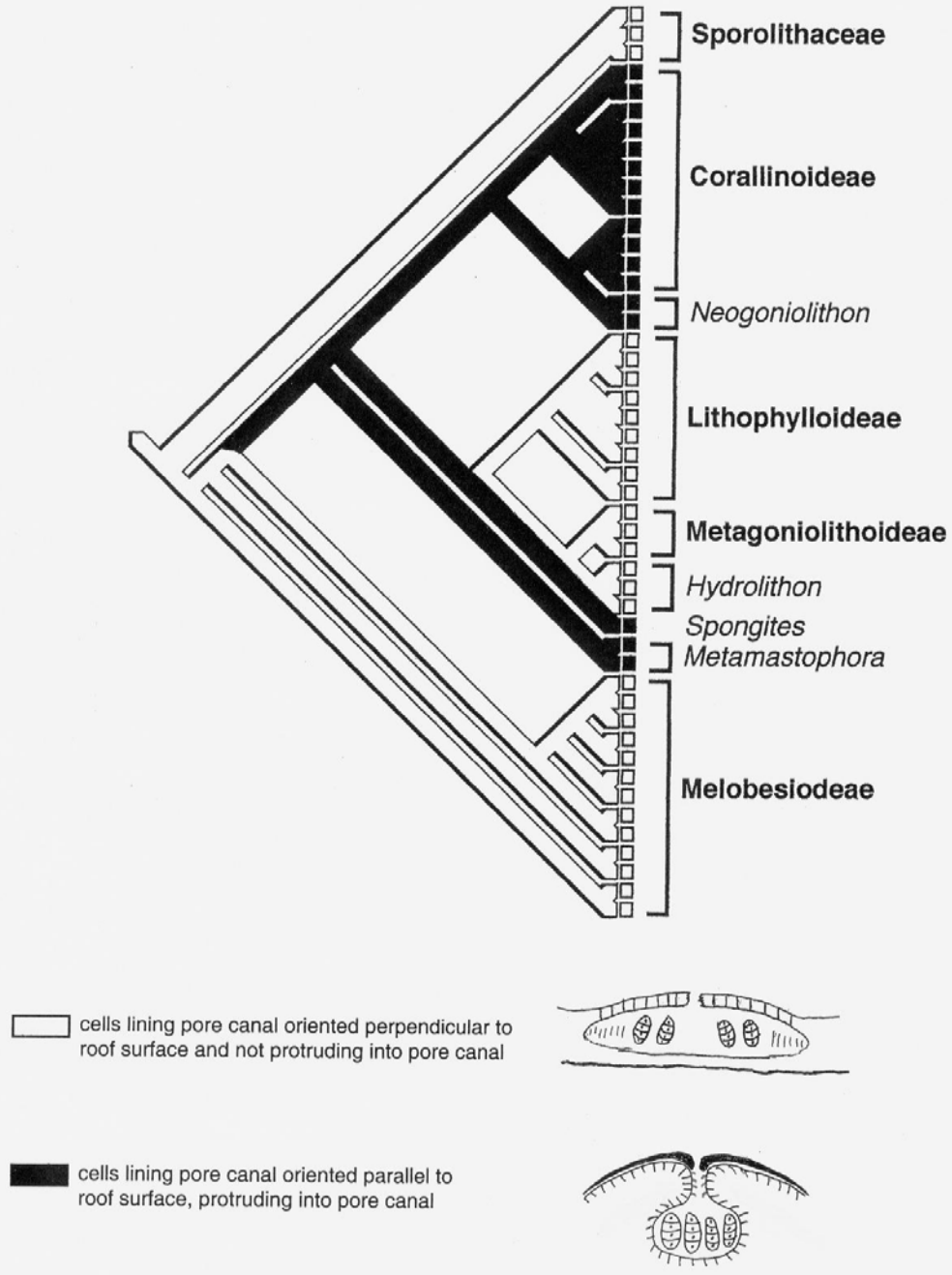

Figure 15. Depiction of morphological character 9 optimized onto the 18S rRNA maximum likelihood tree. Sketches accompanying the key are adapted from Johansen (1981).

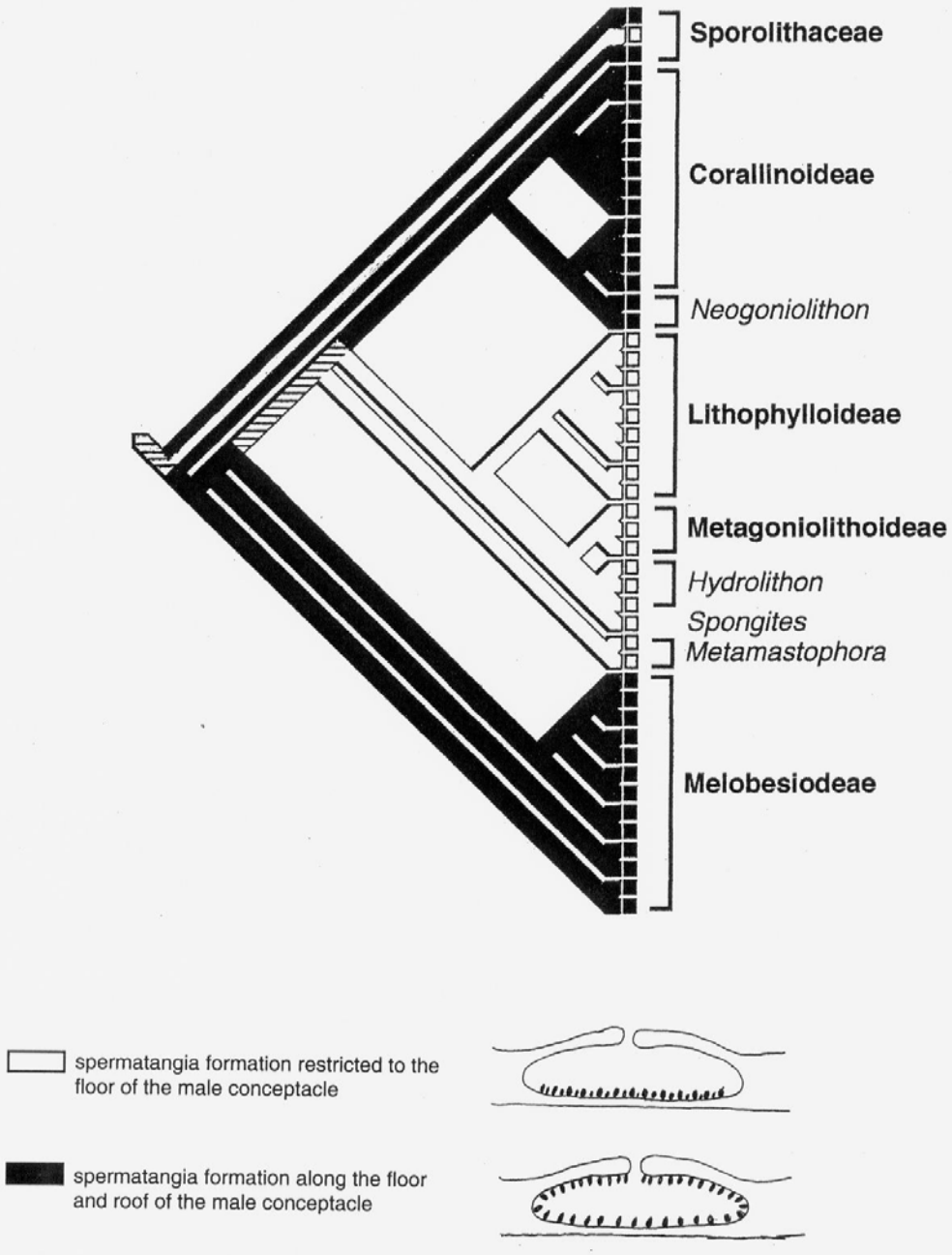

Figure 16. Depiction of morphological character 10 optimized onto the 18S rRNA maximum likelihood tree. Striping represents equivocal character states for internal branches.

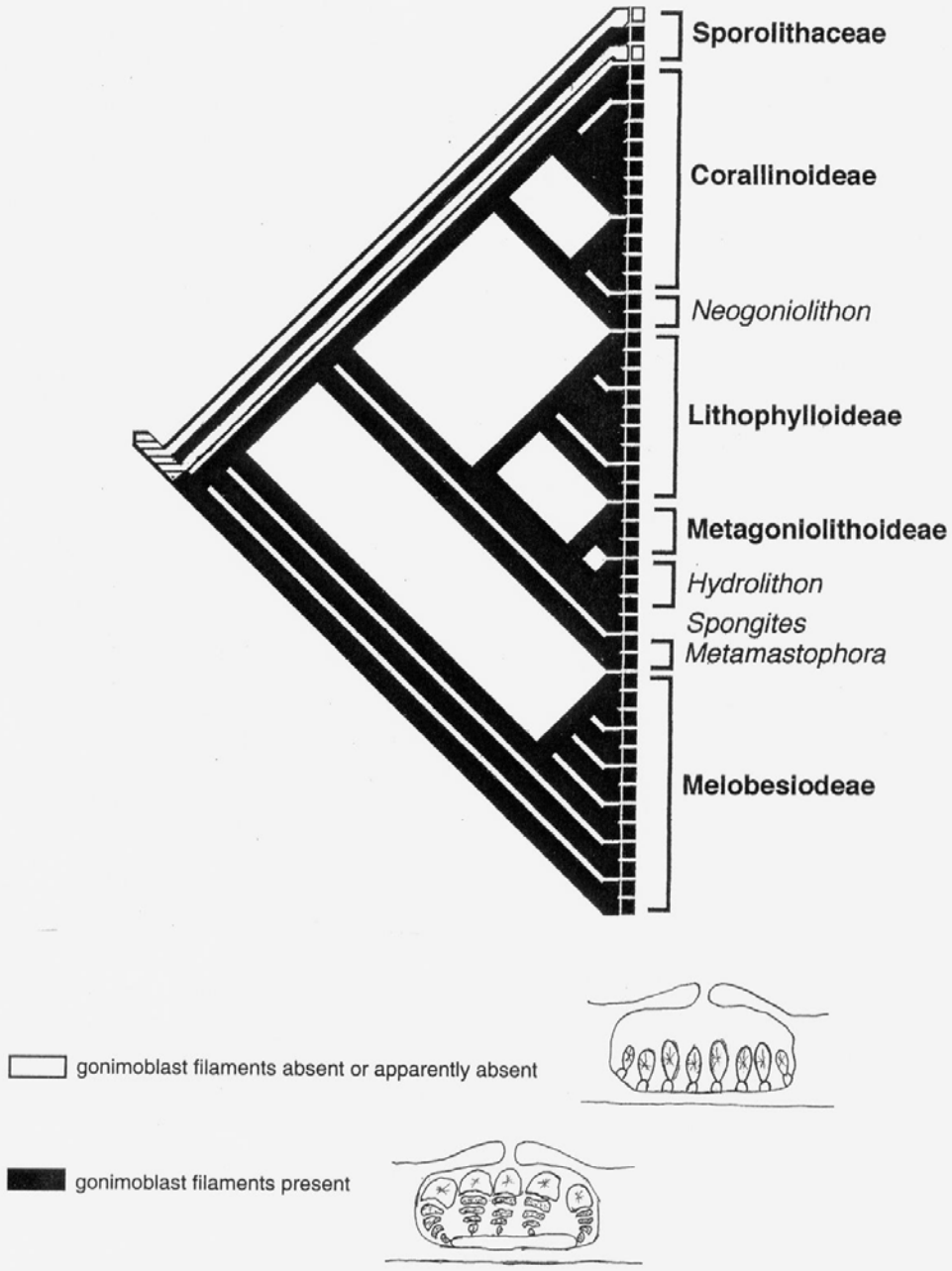

Figure 17. Depiction of morphological character 11 optimized onto the 18S rRNA maximum likelihood tree. Striping represents equivocal character states for internal branches.

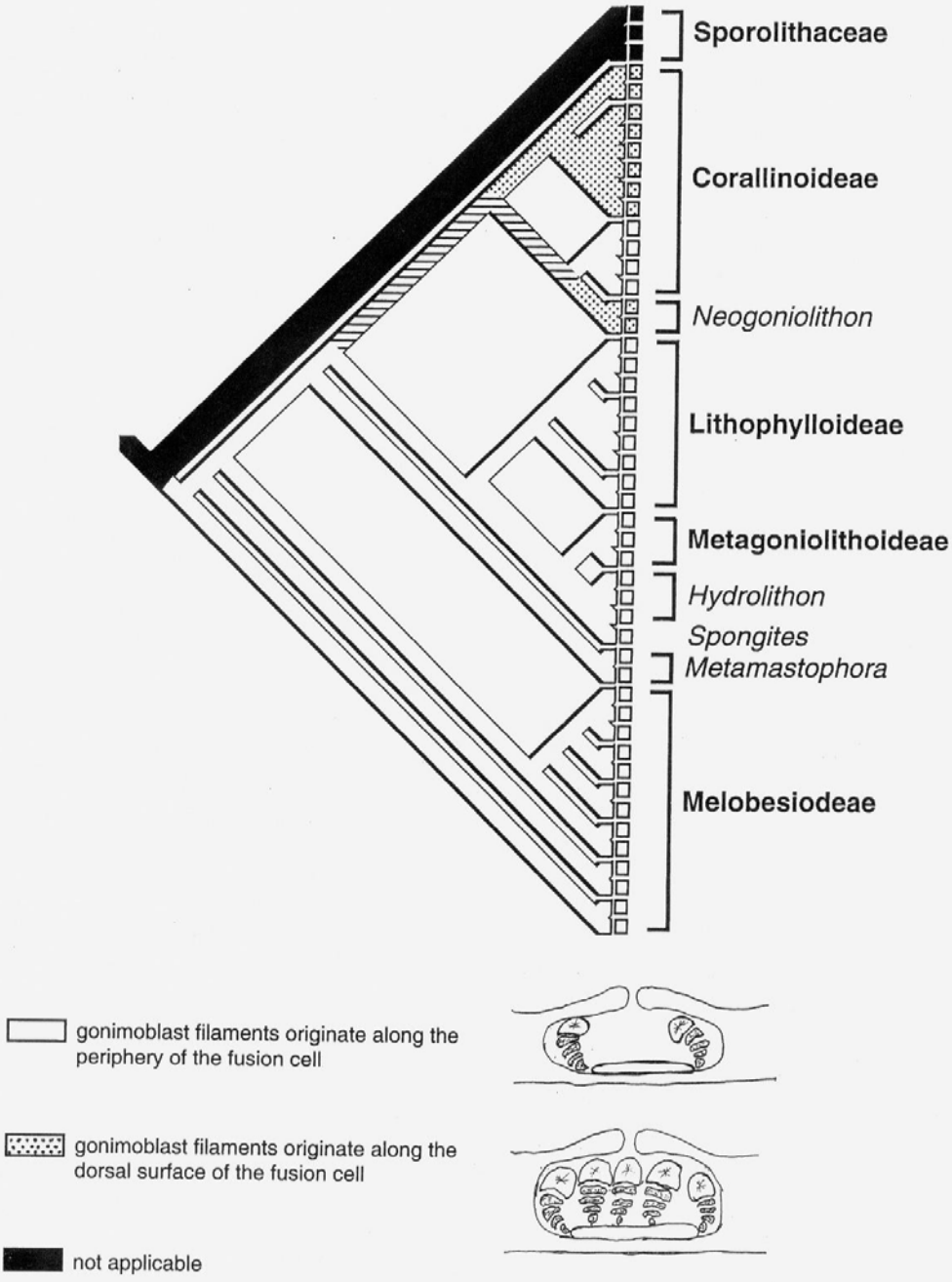

Figure 18. Depiction of morphological character 12 optimized onto the 18S rRNA maximum likelihood tree. Striping represents equivocal character states for internal branches.

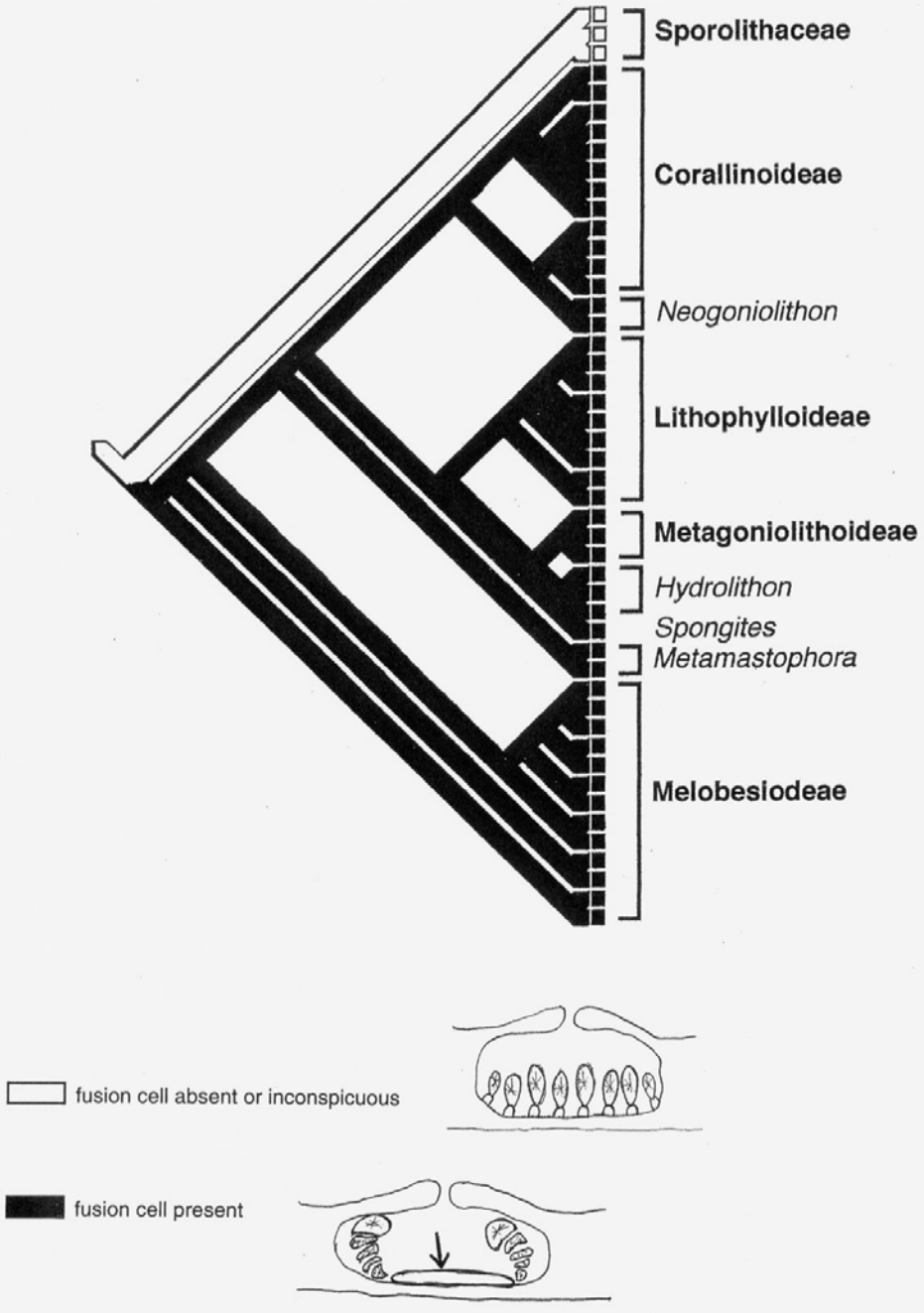

Figure 19. Depiction of morphological character 13 optimized onto the 18S rRNA maximum likelihood tree.

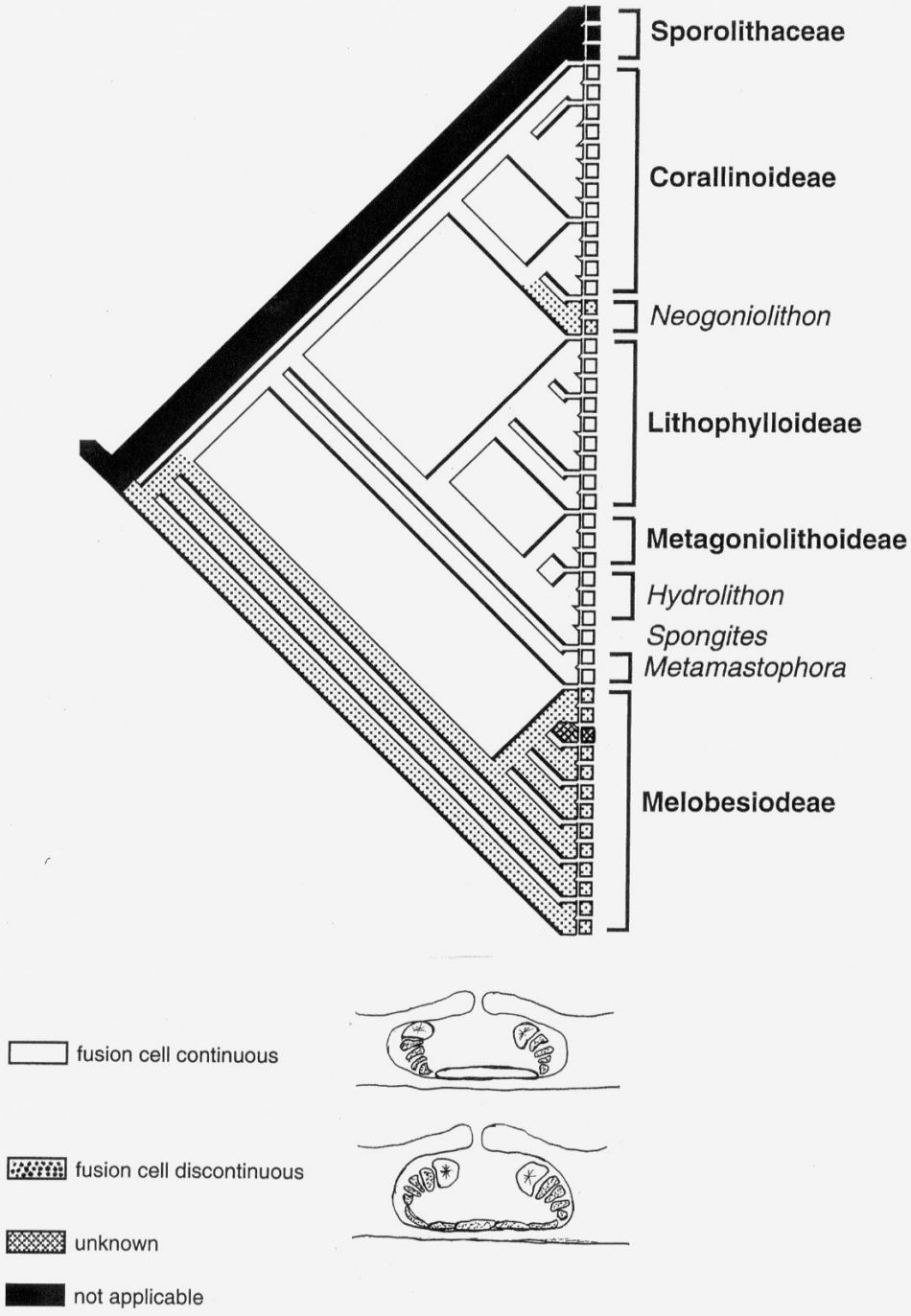

Figure 20. Depiction of morphological character 14 optimized onto the 18S rRNA maximum likelihood tree.

(multiporate conceptacle) from the remaining coralline taxa in which uniporate conceptacles are found (Fig. 11). Cell fusions are found in all subfamilies except for the Lithophylloideae *sensu stricto* in which only secondary pit connections occur (Figs. 12, 13). Two taxa, the Sporolithaceae and *Metamastophora*, possess both cell fusions and secondary pit connections (Figs. 12, 13). In fact, within the Sporolithaceae some taxa are unknown to undergo cell fusion whereas in other species both types of cell connection are apparent (see Fig. 12). The presence of both cell fusions and secondary pit connections in the Sporolithaceae suggests that this may have been the ancestral character state for the Corallinaceae (Figs. 12, 13). Interestingly all members of the Melobesioideae are characterized by cell fusions between cells of adjacent vegetative filaments, but cell fusions are unknown for the closely related *Choreonema thuretti* (Fig. 12). But in spite of this one feature that differs in *Choreonema,* there are 12 other characters this taxon shares with all other melodesiod taxa, one of which includes the single autapomorphic feature of the Melobesiodeae; the presence of a multiporate tetrasporangial conceptacle (Fig. 11). These results as well as other recent evidence suggest that *Choreonema,* the only representative of the Choreonematoideae, is closely related to the Melobesioideae (Chapter 1; Broadwater et al. 2002). *Choreonema* is an endophytic alloparasite that produces lenticular cells along a single vegetative filament. These lenticular cells apparently do not fuse with the host cell but instead produce fimbriate processes that penetrate host cells (Broadwater & LaPointe 1997; Broadwater et al. 2002). The absence of intrathallial cell fusions in *Choreonema* is apparently a derived condition and may be due to a change in this species' life history strategy from that of a free-living or epiphytic species to a parasitic one.

The mode of tetrasporangial conceptacle development is mapped onto the tree in Figure 14. Three modes of development are found within the Corallinales, one of which is autapomorphic for the Sporolithaceae (Fig. 14). In the Lithophylloideae, Metagoniolithoideae*, Hydrolithon,* and the Melobesiodeae, tetrasporangial conceptacles are formed from filaments that are interspersed throughout the fertile region that disintegrate to form a common chamber. In the Corallinoideae, *Neogoniolithon, Spongites,* and *Metamastophora*, the tetrasporangial conceptacle is formed by vegetative filaments that overgrow the fertile region.

The orientation of cells lining the tetrasporangial pore canal is mapped onto the tree in Figure 15. Two types of cellular orientations are observed and can be attributed to the mode of tetrasporangial conceptacle development. The Sporolithaceae, Lithophylloideae, Metagoniolithoideae, *Hydrolithon,* and the Melobesiodeae all have cells lining the pore canal that are oriented perpendicular to the roof surface and do not protrude into the canal (Fig. 15). This feature is the result of the developmental "program" by which the tetrasporangial chambers are constructed. In the Corallinoideae, *Neogoniolithon, Spongites,* and *Metamastophora*, cells lining the pore canal are oriented parallel to the roof surface and protrude into the pore canal (Fig. 15). Likewise, the latter orientation is a result of tetrasporangial conceptacle formation caused by the overgrowth of filaments surrounding the fertile region (Fig. 14).

Spermatangial inititial formation within the male conceptacles is restricted to the floor in some Corallinales, whereas in others spermatangia are formed across the floor and on the roofs and sidewalls of male conceptacles (Fig. 16). In the Sporolithaceae the formation of spermatangia may occur in either manner depending on the species. In two

of the taxa included in this analysis (*Sporolithon durum* and *Heydrichia homalopasta*) spermatangia are formed along the floor, walls, and roof of the male conceptacle (Fig. 16). In *Heydrichia woelkerlingii* spermatangia formation is restricted to the floor of the conceptacle. The formation of spermatangia also occurs along the floor, walls, and roof in the Corallinoideae, *Neogoniolithon*, and the Melobesiodeae. In the Lithophylloideae, Metagoniolithoideae, *Hydrolithon, Spongites,* and *Metamastophora*, spermatangia are restricted to the floor. With the exception of *H. woelkerlingii*, all taxa in the Sporolithaceae for which male conceptacles have been described possess spermatangia along the floor, walls, and roof in male conceptacles (Townsend et al. 1994; Harvey et al. 2002). Based on these observations and the assumption that the Melobesiodeae represent the earliest divergent taxon in the Corallinaceae (Wray 1977), these results suggest that the formation of spermatangia along the floor, walls, and roof of the male conceptacle may have been the ancestral character state in the Corallinaceae.

The presence or absence of gonimoblast filaments is mapped onto the tree in Figure 17. In the Sporolithaceae gonimoblast filaments are absent in two of the three taxa represented in this study (*H. homalopasta* and *S. durum*) and present in *H. woelkerlingii*. In all remaining taxa representative of the Corallinaceae gonimoblast filaments are present.

Two general trends with respect to the location of gonimoblast filament origination along the fusion cell which is mapped onto the tree in Figure 18. In the Sporolithaceae, a distinct fusion cell has not been reported and due to the fact that for many species female plants (and therefore their carposporangial features) have not been found, this character is not applicable. Only two taxa exhibit the formation of

gonimoblast filaments along the dorsal surface of the fusion cell and this is found in the tribe Corallineae (Corallinoideae) and in *Neogoniolithon*. In all other taxa, gonimoblast filaments arise along the periphery of the fusion cell.

The remaining two features pertain to the presence/absence of a fusion cell and the appearance of the fusion cell and are mapped onto the trees in Figures 19 and 20, respectively. The presence of a fusion cell in all members of the Corallinaceae and lack of a distinct fusion cell in the Sporolithaceae suggests that a fusion cell is a derived feature and appears to provide phylogenetic insight in delimiting between families within the Corallinales (Fig. 19). The fusion cell can appear as a single, continuous cell as observed in the Corallinoideae, Lithophylloideae, Metagoniolithoideae, *Hydrolithon, Spongites, Metamastophora*, and *Choreonema*. On the other hand, it can be discontinuous, in which case it is composed of supporting cells and basal cells that do not completely fuse to form a single multinucleate cell as observed in, for example, *Neogoniolithon* and the Melobesiodeae (Fig. 20). Although all other members of the Melobesiodeae possess a discontinuous fusion cell, Broadwater et al. (2002) reported a large fusion in *Choreonema thuretii*.

Discussion

The ML tree inferred based upon analysis of nuclear 18S rRNA gene sequences for 47 species of coralline red algae (Fig. 2) in combination with the morphological data (Figs. 7-20) suggest that these species are divided among four *major* lineages. These four primary lineages include: 1) the Sporolithaceae, 2) the Melobesioideae , 3) the Corallinoideae + *Neogoniolithon*, and 4) a clade including members of the

Lithophylloideae as well as *Metagoniolithon*, *Hydrolithon* and *Spongites*. *Metamastophora* is recognized as an independent fifth lineage.

In a previous study Bailey and Chapman (1998) examined the evolution of a subset of characters included in the analysis presented here. This investigation expands upon that work by including other morphological characters. In addition, 18S rRNA gene sequences were determined for 11 species not examined by Bailey (1999). In particular, six species belonging to the Mastophoroideae have been added to the data matrix as have species belonging to the Lithophylloideae, Melobesioideae and Sporolithaceae (see Table 2 for a complete list of taxa included in this study).

The results of this investigation of morphological character state evolution within the Corallinales are summarized below where each of the five lineages described above is treated separately.

The Families Sporolithaceae and Corallinaceae

The order Corallinales is divided into two families, the early-diverging Sporolithaceae and the Corallinaceae. This analysis indicates that the family Sporolithaceae can be diagnosed by a *combination* of nine features. Of these nine features two are autapomorphic for the Sporolithaceae. First, only in the Sporolithaceae do tetrasporangia develop in an individual calcified chamber (or sorus) containing one tetraspore mother cell (TMC) (Fig. 8). Although many sori may be grouped together in a fertile area of the thallus, the chambers are autonomous units and always contain but one TMC (Townsend et al. 1995). Second, only in members of the Sporolithaceae are the products of meiosis (tetraspores) cleaved from the TMC in a cruciate fashion (Fig. 9)

(Townsend et al. 1995). Seven other characters are found among species placed in Sporolithaceae; some (but not all) of these features are shared with at least one or more taxa belonging to the Corallinaceae. These features include (1) the presence of mucilaginous plugs occluding the sporangial pore canal, (2) the absence of genicula, (3) the presence of cell fusions and/or secondary pit connections between cells of adjacent filaments, (4) the perpendicular orientation of cells lining the sporangial pore canal, (5) the development of spermatangial initials on both the floor and roof of male conceptacles, (6) the presence or absence of gonimoblast filaments, and (7) the apparent absence of a well-defined fusion cell. This *combination* of nine features circumscribe the Sporolithaceae. In this study, and in previous studies, it has been well documented that *Sporolithon* and *Heydrichia* are very distantly related to other coralline algae and the fossil records indicate that this taxon evolved well before other coralline lineages began to diverge (Wray 1977). For these reasons, the characters listed above can be considered plesiomorphic for the Corallinales.

Our analysis indicates that there are three synapomorphic characters that unite all species placed in the family Corallinaceae. In all members of this group tetrasporangia are formed in "true" conceptacles that include many TMCs (Fig. 8), a fusion cell (continuous or discontinuous) is present (Fig. 19), and tetrasporangia are cleaved from the TMC by simultaneous zonate cell wall partitioning (Fig. 9).

Melobesioideae

The monophyly of the Melobesioideae is supported by a single autapomorphic character; in all members of this taxon tetrasporangia are formed in multiporate

tetrasporangial conceptacles (Fig. 11). The following combination of features defines the members of this subfamily (with the exception of *Choreonema,* which will be addressed below): 1) mucilaginous tetrasporangial plugs occluding the pore are present, 2) tetrasporangia develop within multiporate conceptacles, 3) the absence of genicula, 4) cell fusions occur between cells of adjacent filaments, 5) secondary pit connections between cells of adjacent filaments are absent, 6) the development of tetrasporangial conceptacles from filaments interspersed throughout the fertile area, 7) cells lining the pore canal oriented perpendicular to the roof surface, 8) the formation of spermatangia initials along the floor and roof of the male conceptacle, 9) the formation of gonimoblast filaments along the periphery of the fusion cell, and 10) the appearance of the fusion cell is discontinuous.

Based on the 18S rRNA analyses, *Choreonema* is most closely related to the Melobesiodeae, however, bootstrap support for its position within the Melobesiodeae is very weak (Figs. 1, 2). There are two primary differences between *Choreonema* and the remaining melobesiod taxa. First, *Choreonema* does not possess cell fusions; all other melobesiod taxa possess cell fusions (Fig. 12). Second, it has been reported that in *Choreonema* gonimoblast filaments arise along the periphery of a "large fusion cell" (Broadwater et al. 2002), which *implies* that the fusion cell is continuous. All other members of the Melobesiodeae possess a discontinuous fusion cell (Fig. 20). Aside from the dissimilarities mentioned above, all other vegetative and reproductive features found in *Choreonema* are consistent with those found in the Melobesiodeae (Figs. 7-11, Figs. 13-19), and in particular, the multiporate tetrasporangial conceptacle that is autapomorphic for the Melobesiodeae (Fig. 11). Since *Choreonema* is an endophytic

alloparasite with a highly reduced vegetative thallus, Broadwater et al. (2002) noted that the lack of cellular connections could be reconciled by its life history.

Corallinoideae and *Neogoniolithon*

The subfamily Corallinoideae is easily distinguished from other corallines, and particularly other geniculate corallines, by the structure of the geniculum. In this taxon the geniculum is composed of a single, very long tier of cells of roughly equal length. This character is synapomorphic for the subfamily *sensu* Johansen (1969).

In the ML anaylsis presented here *Neogoniolithon* is positioned as sister taxon to the tribe Janieae (including *Jania*, *Haliptilon* and *Cheilosporum*). In the 18S rRNA analysis, *Neogoniolithon* is placed at the base of the Corallinoideae (Fig. 1). The question of whether or not the Corallinoideae is monophyletic as presently circumscribed (i.e, Should *Neogoniolithon* be re-classified in the Corallinoideae?) has not been conclusively determined. Here the focus is on those characters that *Neogoniolithon* shares in common with some or all species placed in the Corallinoideae.

All taxa within this lineage possess the following *combination* of features: 1) the absence of mucilaginous tetrasporangial plugs, 2) tetrasporangial development within uniporate conceptacles, 3) the occurrence of cell fusions between cells of adjacent filaments, 4) the absence of secondary pit connections, 5) tetrasporangial conceptacles develop from filaments surrounding the fertile area, 6) cells lining the pore canal are oriented parallel to the roof surface, and 7) the development of spermatangia initials along the floor and roof of male conceptacles. Although these individual features are not exclusive (i.e., some are shared in common with other Corallinaceae), it is this particular

combination of features that sets this lineage apart from others. There is, however, one autapomorhic feature found only in this lineage and that is the occurrence of gonimoblast filaments along the dorsal surface of the fusion cell. The tribes Janieae and Corallineae were erected based on this feature with gonimoblast filaments arising along the dorsal surface of the fusion cell in the Corallineae and along the periphery of the fusion cell in the Janieae. *Neogoniolithon* is the only other taxon in the Corallinaceae in which gonimoblast filaments occur along the dorsal surface of the fusion cell. Aside from the features that unite this major lineage, there are two features that are autapomorphic for *Neogoniolithon* with respect to this clade. These features include the absence of genicula and the presence of a discontinuous fusion cell.

Lithophylloideae, *Metagoniolithon*, *Hydrolithon* and *Spongites*

The subfamily Lithophylloideae was recently emended by Bailey (1999) to include both nongeniculate (e.g., *Lithophyllum*, *Titanoderma*) and geniculate taxa (*Amphiroa*, *Lithothrix*). These analyses indicate that the Lithophylloideae is most closely related to an unorthodox assemblage of species placed in *Metagoniolithon*, *Hydrolithon* and *Spongites*. Furthermore, all analyses performed in this study imply that *Metagoniolithon* (geniculate) and *Hydrolithon* (nongeniculate) are sister taxa. The circumscription of the Lithophylloideae and the characters uniting taxa within that subfamily have been recently reviewed and will not be recounted here (Bailey 1999). However, it is within the scope of this study to determine which, if any, morphological characters ally the Lithophylloideae with *Metagoniolithon*, *Hydrolithon* and *Spongites*.

Results from this cladistic analysis indicate that *all* these taxa share five features in cmmon: 1) mucilaginous tetrasporangial plugs are absent, 2) tetrasporangia develop within uniporate conceptacles, 3) the formation of spermatangia initials are restricted to the floor of the male conceptacle, 4) gonimoblast filaments arise along the periphery of the fusion cell, and 5) the occurrence of a continuous fusion cell. This particular *combination* of features is found only in this lineage, however, there are a couple of vegetative/reproductive features that some of these taxa do not share in common with one another. For example, the Lithophylloideae possesses secondary pit connections but lacks cell fusions. The exact opposite is observed in *Metagoniolithon, Hydrolithon,* and *Spongites*; these taxa lack secondary pit connections but possess cell fusions. With respect to the mode of tetrasporangial conceptacle development, *Spongites* is the "odd-taxon-out" in this assemblage. In the Lithophylloideae, *Metagoniolithon,* and *Hydrolithon,* tetrasporangial conceptacles develop from filaments that are interspersed throughout the fertile region, resulting in cells lining the pore canal to be oriented perpendicular to the roof surface. In *Spongites*, tetrasporangial conceptacles are formed from filaments surrounding the fertile area, resulting in cells lining the pore canal to be oriented parallel to the roof surface. Although this feature is not consistent with the remaining taxa in this lineage, it is consistent with the mode of tetrasporangial conceptacle development found in *Metamastophora*, the Corallinoideae, and *Neogoniolithon*.

The Phylogenetic Position of *Metamastophora*

On the basis of morphlogical attributes alone, *Metamastophora* is unique in the sense that it is the only mastophoroid that exhibits a branched, arborescent thallus with a distinct holdfast and stipe (Woelkerling 1988). It is also the only taxon within the Corallinaceae that possesses both cell fusions and secondary pit connections. The following ten features are characteristic of the species *M. flabellata*: 1) mucilaginous tetrasporangial plugs are absent, 2) tetrasporangia develop within uniporate conceptacles, 3) genicula are absent, 4) cell fusions occur between cells of adjacent filaments, 5) secondary pit connections occur between cells of adjacent filaments, 6) tetrasporangial conceptacles are formed from filaments surrounding the fertile region, 7) cells lining the pore canal are oriented parrallel to the roof surface, 8) the formation of spermatangia initials are restricted to the floor of the male conceptacle, 9) gonimoblast filaments arise along the periphery of the fusion cell, and 10) the occurrence of a continuous fusion cell.

With the exception of the occurrence of secondary pit connections, all of the features listed above that circumscribe this lineage are also characteristic of *Spongites*.

Taxonomic Implications

With evidence from the combined molecular and morphological analyses that consistently unite *Neogoniolithon* and the Corallinoideae within a single lineage, it seems appropriate that *Neogoniolithon* be transferred to the Corallinoideae on the basis that the combination of reproductive features are more similiar to members of the Corallinoideae than they are to any other taxon within the Corallinaceae. The following features are shared between *Neogoniolithon* and the Corallinoideae: 1) mucilagenous tetrasporangial

plugs absent, 2) tetrasporangia development within uniporate conceptacles, 3) cell fusions occur between cells of adjacent filaments, 4) tetrasporangial conceptacles develop from filaments surrounding the fertile region, 5) cells lining the pore canal are oriented parallel to the roof surface, 6) spermatangia initial formation along the floor and roof of the male conceptacle, and 7) gonimoblast filaments arise along the dorsal surface of the fusion cell (Janieae excepted).

In treating the remaining mastophoroid taxa included in this analysis, with the exception of *Spongites,* it *could* be argued that based on the combination of morphological and molecular evidence the Lithophylloideae, Metagoniolithoideae, and *Hydrolithon* should be grouped within a single subfamily. This subfamily would be divided into two tribes based on the type of cellular connection that is found to occur: one tribe would include members of the Lithophylloideae for which only secondary pit connections occur, and the second tribe would include *Metagoniolithon* and *Hydrolithon* for which only cell fusions occur. On the other hand, it could also be suggested that within this lineage the Lithphylloideae should remain its own separate subfamily with *Hydrolithon* and *Metagoniolithon* classified within a single subfamily. This, however, does not reconcile the fact that based on the molecular evidence, *Spongites* is maintained within this major lineage and at this point it is noteworthy to indicate a few observations with respect to *Spongites* and the molecular/morphological investigations contained in this text. First, with respect to the molecular analyses, depending on the data used to analyze the phylogenetic relationships among coralline taxa, *Spongites* changed positions within the trees. For example, upon analysis of the 18S rRNA dataset, *Spongites* was positioned at the base of the lineage described in this chapter as well as in Chapter 1

(BP= 82%, 100%) (Figs. 1, 2). However, upon analysis of the 26S rRNA and combined 18S/26S rRNA data, *Spongites* was allied as sister taxon to *Metagoniolithon* (Figs. 3-6). And although bootstrap support was moderate to strong for this arrangement within the 26S rRNA analyses (parsimony = 79%, ML = 92%) (Figs. 3, 4), bootstrap support for the combined 18S/26S analyses were <50% (Figs. 5, 6). In addition to the change in the relative position of *Spongites* in the molecular analyses, *Metamastophora* also changed positions in the 18S rRNA analyses depending on the optimality criterion implemented. Using parsimony, *Metamastophora* was positioned as sister to a clade comprised of the Corallinoideae and *Neogoniolithon* (Fig. 1). However, since bootstrap support for this alignment was <50%, and all additional molecular analyses positioned *Metamastophora* as sister to a clade comprised of all taxa within the Corallinaceae excluding the Melobesiodeae with strong support (refer to Figs. 2-6 for BP support values), it was inferred that based on the overall molecular evidence, the topologies obtained in Figures 2-6 more accurately depicts the relationship between *Metamastophora* and the remaining members of the Corallinaceae. And so the question remains: What *could* be done with *Spongites, Metamastophora,* and *Hydrolithon*? The answer to that question is not clear-cut and only speculation can be offered at this time.

Four additional mastophoroid taxa for which the full complement of morphological features is documented (but not examined in this study) may provide some insight to this phylogenetic quandary with respect to *Spongites* and *Metamastophora*. *Fosliella*, which was considered a distinct genus within the Mastophoroideae (Woelkerling 1988) was subsumed in *Hydrolithon* based on observations with respect to the mode of tetrasporangial conceptacle development and orientation of cells lining the

pore canal (Penrose & Chamberlain 1993). *Lithoporella* and *Mastophora* were also distinguished as separate genera within the Mastophoroideae based on differences with repect to the mode of tetrasporangial conceptacle development (Turner & Woelkerling 1982). In *Lithoporella,* tetrasporangial conceptacles develop from filaments interspersed throughout the fertile region whereas in *Mastophora,* conceptacle development results from filaments surrounding the fertile area. *Lithoporella* shares in common all of the following features described for the Lithophylloideae, *Metagoniolithon,* and *Hydrolithon*: tetrasporangial conceptacle formation from filaments interspersed throughout the fertile region, orientation of cells lining the pore canal perpendicular to the roof surface, formation of spermatangia restricted to the floor of the male conceptacle, and gonimoblast filaments arising along the periphery of a continuous fusion cell. *Lesueria, Mastophora,* and *Pneophyllum* share in common the following combination of features found in *Metamastophora* and *Spongites*: tetrasporangial conceptacle formation from filaments that surround the fertile region, the orientation of cells lining the pore canal parrallel to the roof surface, spermatangia restricted to the floor of the male conceptacle, and gonimoblast filaments arising along the periphery of a continuous fusion cell (Turner & Woelkerling 1982; Woelkerling & Ducker 1987; Chamberlain 1994).

The premise for many of the reclassifications of genera within the Mastophoroideae has been based on the mode of tetasporangial conceptacle development and orientation of cells lining the pore canal (i.e., *Fosliella* and *Hydrolithon, Mastophora* and *Lithoporella,* and *Spongites, Hydrolithon,* and *Pneophyllum*). Since it is evident that with the addition of more taxa the phylogenetic position of *Spongites* changes (Chapters 1 & 2), it is conceivable that with the addition of gene sequence data for the

mastophoroid taxa described above, *Metamastophora* and *Spongites* could move their relative positions within the tree. Although speculative, it is evidence enough for this author to refrain from any formal taxonomic revisions with respect to the Mastophoroideae until the type specimen for this subfamily, *Mastophora*, and additional mastophoroid taxa are included in a more comprehensive molecular analysis. An informal classification of some taxa examined in this study is presented in Figure 21 and should serve as a working hypothesis for future systematics studies of the Corallinales.

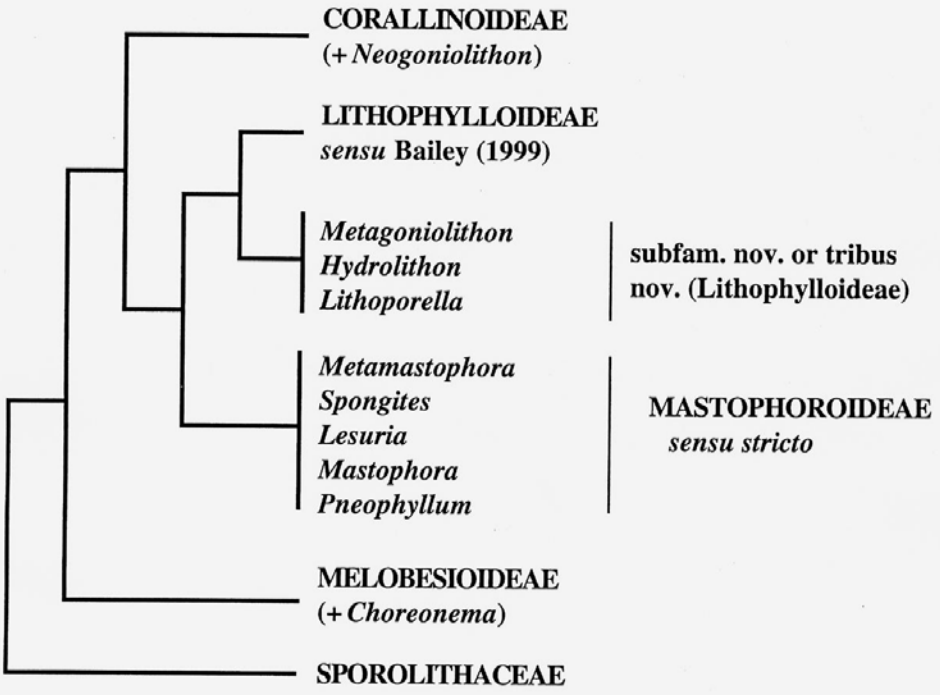

Figure 21. Possible phylogenetic relationships among mastophoroid genera and some other coralline algae based upon the author's interpretation of available molecular and morphological evidence.

REFERENCES

AFONSO-CARRILLO J. 1988. Structure and reproduction of *Spongites wildpretii* sp. nov. (Corallinaceae, Rhodophyta) from the Canary Islands, with observations and comments on *Spongites absimile* comb. nov.. *Br. Phycol. J.* **23**: 89-102.

BAILEY J.C. 1999. Phylogenetic positions of *Lithophyllum incrustans* and *Titanoderma pustulatum* (Corallinaceae, Rhodophyta) based on 18S rRNA gene sequence analysis, with a revised classification of the Lithophylloideae. *Phycologia* **38**: 208-216.

BAILEY J.C. & CHAPMAN R.L. 1996. Evolutionary relationships among coralline red algae (Corallinaceae, Rhodophyta) inferred from 18S rRNA gene sequence analysis. In Chaudhary, B.R. and S.B. Agrawal [eds] *Cytology, Genetics and Molecular Biology of Algae*, SPB Academic Publishing, Amsterdam, pp 363-376.

BAILEY J.C. & CHAPMAN R.L. 1998. A phylogenetic study of the Corallinales (Rhodophyta) based on nuclear small-subunit rRNA gene sequences. *J. Phycol*. **34**: 692-705.

BOLD H.C. & WYNNE M.J. 1985. *Introduction to the Algae*. Prentice-Hall, Englewood Cliffs, New Jersey.

BRAGA J.C. & AGUIRRE J. Taxonomy of fossil algal species: Neogene Lithophylloideae (Rhodophyta, Corllinaceae) from southern Spain. *Review of Paleobotany and Palynology* **86**: 265-285.

BROADWATER S.T. & LAPOINTE E.A. 1997. Parasitic interactions and vegetative ultrastructure of *Choreonema thuretii* (Corallinales, Rhodophyta). *J. Phycol*. **33**: 396-407.

BROADWATER S.T., HARVEY A.S., LAPOINTE E.A., & WOELKERLING WM.J. 2002. Conceptacle structure of the parasitic coralline red alga *Choreonema thuretii* (Corallinales) and its taxonomic implications. *J. Phycol.* **38**: 1157-1168.

CABIOCH J. 1971. Essai d'une nouvelle classification des Corallinacees actuelles. *Comptes Rendus Hebdomadaires des Seances de l'Academie des Sciences*, Paris 272D: 1616-1619.

CABIOCH J. 1972. *Etude sur les Corallinacees. Croissance et mode de realisation du thalle; consequences systematiques et phylogenetiques*. Imprimerie ROBIN & MAREUGE, Paris.

CABIOCH J. 1988. Morphogenesis and generic cocepts in coralline algae - a reappraisal. *Helgolander Meeresuntersuchungen* **42**: 493-509.

CHAMBERLAIN Y.M. 1991. Historical and taxonomic studies in the genus *Titanoderma*

(Rhodophyta, Corallinales) in the British Isles. *Bull. Br. Mus. Nat. Hist.* (Bot.) **21**: 1-80.

CHAMBERLAIN Y.M. 1993. Observations on the crustose coralline red alga *Spongites yendoi* (Foslie) comb. nov. in South Africa and its relationship to *S. decipiens* (Foslie) comb. nov. and *Lithophyllum natalense* Foslie. *Phycologia* **32**: 100-115.

CHAMBERLAIN Y.M. 1994. *Pneophyllum coronatum* (Rosanoff) D. Penrose comb. nov., *P. keatsii* sp. nov., *Spongites discoideus* (Foslie) D. Penrose et Woelkerling and *S. impar* (Foslie) Y. Chamberlain comb. nov. (Rhodophyta, Corallinaceae) from South Africa. *Phycologia* **33**: 141-157.

CHAMBERLAIN Y.M. & KEATS D.W. 1994. Three melobesiod crustose coralline red algae from South Africa: *Leptophytum acervatum* (Foslie) comb. nov., *L. foveatum* sp. nov. and *L. ferox* (Foslie) comb. nov.. *Phycologia* **33**: 111-133.

CHAMBERLAIN Y.M. & KEATS D.W. 1995. The melobesiod alga *Mesophyllum engelhartii* (Rhodophyta, Corallinaceae) in South Africa. *S. Afr. J. Bot.* **61**: 134-146.

CHAMBERLAIN Y.M. & NORRIS R.E. 1994. *Pneophyllum amplexifrons* (Harvey) comb. nov., a mastophoroid crustose coralline red algal epiphyte from Natal, South Africa. *Phycologia* **33**: 8-18.

CHAMBERLAIN Y.M., NORRIS R.E., KEATS D.W., & MANEVELDT G. 1995. *Clathromorphum tubiforme* sp. nov. (Rhodophyta, Corallinaceae) in South Africa with comments on generic characters. *Botanica Marina* **38**: 443-453.

DEGNAN B.M. & JOHNSON C.R. 1999. Inhibition of settlement and metamorphosis of the ascidian *Herdmania curvata* by non-geniculate coralline algae. *Biol. Bull*. **197**: 332-340.

DUCKER S.C. 1979. The genus *Metagoniolithon* Weber-van Bosse (Corallinaceae, Rhodophyta). *Aus. J. Bot.* **27**: 67-101.

FELSENSTEIN J. 1985. Confidence limits on phylogenies: an approach using the bootstrap. *Evolution* **39**: 783-791.

FRESHWATER D.W. & BAILEY J.C. 1998. A multigene phylogeny of the Gelidiales including large-subunit rRNA sequence data. *J. Applied Phycol.* **10**: 229-236.

GANESAN E.K. 1967. Studies on the morphology and reproduction of the articulated corallines - IV. *Serraticardia* (Yendo) Silva, *Calliarthron* Manza and *Bossiella* Silva. *Botanica Marina* **11**: 10-30.

GANESAN E.K. 1968. Studies on the morphology and reproduction of the articulated corallines - I I. *Corallina Linneaus emend. Lamouroux*. *Bol. Inst. Oceanog. Univ. Oriente* **7**: 65-97.

GILBERT D. 1994. SeqApp, v. 1.9a169. Program available via anonymous ftp at: ftp.bio.indiana.edu

HAGEN N.T. 1999. Survival and growth of juvenile green sea urchins on different macroalgal settlement substrates. *J. Shellfish Res*. **18**: 303.

HARPER J.T. & SAUNDERS G.W. 2001. Molecular systematics of the Florideophyceae (Rhodophyta) using nuclear large and small subunit rDNA sequence data. *J. Phycol.* **37**: 1073-1082.

HARPER J.T. & SAUNDERS G.W. 2002. A re-classification of the Acrochaetiales based on molecular and morphological data, and establishment of the Colaconematales *ord. nov.* (Florideophyceae, Rhodophyta). *Eur. J. Phycol.* **37**: 463-476.

HARVEY A.S., WOELKERLING WM.J., & MILLAR A.J.K. 2002. The Sporolithaceae (Corallinales, Rhodophyta) in south-eastern Australia: taxonomy and 18S rRNA phylogeny. *Phycologia* **41**: 207-227.

HEYWARD A.J. & NEGRI A.P. 1999. Natural inducers for coral larval metamorphosis. *Coral Reefs* **18**: 273-279.

JOHANSEN H.W. 1969. Morphology and systematics of coralline algae, with special reference to *Calliarthron. University of California Publications in Botany* **49**: 1-78.

JOHANSEN H.W. 1976. Current status of generic concepts in coralline algae (Rhodophyta). *Phycologia* **15**: 221-244.

JOHANSEN H.W. 1977. The articulated Corallinaceae (Rhodophyta) of South Africa: 1. *Cheilosporum* (Decaisne) Zanardini. *J. S. Afr. Bot.* **43**: 163-185.

JOHANSEN H.W. 1981. *The Coralline Algae, A First Synthesis*. CRC Press, Boca Raton, Florida.

JOHANSEN H.W. & SILVA P.C. 1978. Janieae and Lithotricheae: two new tribes of articulated Corallinaceae (Rhodophyta). *Phycologia* **17**: 413-417.

JOHNSON J.H. 1956. Ancestry of the coralline algae. *J. Paleontol.* **30**: 563-67.

KEATS D.W. & CHAMBERLAIN Y.M. 1994. Three species of *Hydrolithon* (Rhodophyta, Corallinaceae): *Hydrolithon onkodes* (Heydrich) Penrose and Woelkerling, *Hydrolithon superficiale* sp. nov., and *H. samoense* (Foslie) comb. nov. from South Africa. *S. Afr. J. Bot.* **60**: 8-21.

KEATS D.W. & CHAMBERLAIN Y.M. 1995. *Heydrichia groeneri* sp. nov.: a new species of crustose coralline alga (Rhodophyta, Sporolithaceae) from South Africa and Namibia.

Phycologia **34**: 51-57.

KEATS D.W. & CHAMBERLAIN Y.M. 1997. The non-geniculate coralline algae *Synarthrophyton eckloniae* (Foslie) comb. nov. and *S. magellanicum* (Foslie) comb. nov. (Rhodophyta) in South Africa including comparison with relevant types. *Eur. J. Phycol.* **32**: 55-79.

KEATS D.W., STENECK R.S., TOWNSEND R.A., & BOROWITZKA M.A. 1996. *Lithothamnion prolifer* Foslie: A common non-geniculate coralline alga (Rhodophyta: Corallinaceae) from the tropical and subtropical Indo-Pacific. *Botanica Marina* **39**: 187-200.

LEBEDNIK P.A. 1977. Postfertilization development in *Clathromorphum, Melobesia* and *Mesophyllum* with comments on the evolution of the Corallinaceae and the Cryptonemiales (Rhodophyta). *Phycologia* **16**: 379-406.

LEBEDNIK P.A. 1978. Development of male conceptacles in *Mesophyllum* Lemoine and other genera of the Corallinaceae (Rhodophyta). *Phycologia* **17**: 388-395.

LITTLER M.M., LITTLER D.S., BLAIR S.M., & NORRIS J.N. 1985. Deepest known plant life discovered on an uncharted seamount. *Science* **227**: 57-9.

LITTLER M.M., LITTLER D.S., & TAYLOR P.R. 1995. Selective herbivore increases biomass of its prey: a chiton-coralline reef-building association. *Ecology* **76**: 1666-1681.

LITTLER M.M. & LITTLER D.S. 2000. *Caribbean Reef Plants, An Identification Guide to the Reef Plants of the Caribbean, Bahamas, Florida and the Gulf of Mexico.* OffShore Graphics, Inc., Washington D.C. 542 pp.

MADDISON W.P. & MADDISON D.R. 1992. *MacClade,* version 3.0. Sinauer, Sunderland, Massachusetts.

MASAKI T. 1968. Studies on the Melobesioideae of Japan. *Mem. Fac. Fish. Hokkaido Univ.* **16**: 1-80.

MORSE A.N.C. 1991. How do planktonic larvae know where to settle? *Amer. Sci*. **79**: 154-157.

MORSE D.E. 1990. Recent progress in larval settlement and metamorphosis: closing the gaps between molecular biology and ecology. *Bull. Mar. Sci*. **46**: 465-483.

MORSE D.E., MORSE A.N.C., RAIMONDI P.T., & HOOKER N. 1994. Morphogen-based chemical flypaper for *Agaricia humilis* coral larvae. *Biol. Bull*. **186**: 172-181.

MURATA K. & MASAKI T. 1978. Studies of reproductive organs in articulated coralline algae of Japan. *Phycologia* **17**: 403-412.

PENROSE D. 1991. *Spongites fruticulosus* (Corallinaceae, Rhodophyta), the type species of *Spongites,* in southern Australia. *Phycologia* **30**: 438-448.

PENROSE D. 1992a. *Hydrolithon cymodoceae* (Foslie) comb. nov. (Corallinaceae, Rhodophyta) in southern Australia and its relationship to *Fosliella*. *Phycologia* **31**: 89-100.

PENROSE D. 1992b. *Neogoniolithon fosliei* (Corallinaceae, Rhodophyta), the type species of *Neogoniolithon*, in southern Australia. *Phycologia* **31**: 338-350.

PENROSE D. & CHAMBERLAIN Y.M. 1993. *Hydrolithon farinosum* (Lamouroux) comb. nov.: implications for generic concepts in the Mastophoroideae (Corallinaceae, Rhodophyta). *Phycologia* **32**: 295-303.

PENROSE D. & WOELKERLING WM.J. 1988. A taxonomic reassessment of *Hydrolithon* Foslie, *Poroloithon* Foslie and *Pseudolithophyllum* Lemoine emend. Adey (Corallinaceae, Rhodophyta) and their relationships to *Spongites* Kutzing. *Phycologia* **27**: 159-176.

PENROSE D. & WOELKERLING WM.J. 1991. *Pneophyllum fragile* in southern Australia: implications for generic concepts in the Mastophoroideae (Corallinaceae, Rhodophyta). *Phycologia* **30**: 495-506.

PENROSE D. & WOELKERLING WM.J. 1992. A reappraisal of *Hydrolithon* and its relationship to *Spongites* (Corallinaceae, Rhodophyta). *Phycologia* **31**: 81-88.

POSADA D. & CRANDALL K.A. 1998. Modeltest: testing the model of DNA substitution. *Bioinformatics* **14**: 817-818.

RIOSMENA-RODRIGUEZ R. & SIQUEIROS-BELTRONES D. 1996. Taxonomy of the genus *Amphiroa* (Corallinales, Rhodophyta) in the southern Baja California Peninsula, Mexico. *Phycologia* 35: 135-147.

ROUSSEAU F. & DE REVIERS B. 1999. Phylogenetic relationships within the Fucales (Phaeophyceae) based on combined partial SSU + LSU rDNA sequence data. *Eur. J. Phycol.* **34**: 53-64.

SAUNDERS G.W. & KRAFT G.T. 1994. Small subunit sequences from representatives of selected families of the Gigartinales and Rhodymeniales (Rhodophyta): I. Evidence for the Plocamiales ord. nov. *Can. J. Bot*. **75**: 1436-47.

SAUNDERS G.W. & KRAFT G.T. 1997. A molecular perspective on red algal evolution: focus on the Florideophycidae. In *Origins of Algae and their Plastids* (ed. Bhattacharya, D.), 115-138. Springer, New York.

SWOFFORD D.L. 2002. PAUP* 4.0b10, Computer program distributed by Sinauer Associates, Inc., Sunderland, Massachusetts.

TOWNSEND R.A. 1979. *Synarthrophyton*, a new genus of Corallinaceae (Cryptonemiales, Rhodophyta) from the southern hemisphere. *J. Phycol.* **15**: 251-259.

TOWNSEND R.A. 1981. Tetrasporangial conceptacle development as a taxonomic character in the Mastophoroideae and Lithophylloideae (Rhodophyta). *Phycologia* **20**: 407-414.

TOWNSEND R.A. & BOROWITZKA M.A. 2001. *Heydrichia homalopasta* sp. nov. (Sporolithaceae, Rhodophyta) from Australia. *Botanica Marina* **44**: 237-244.

TOWNSEND R.A., CHAMBERLAIN Y.M., & KEATS D.W. 1994. *Heydrichia woelkerlingii* gen. et sp. nov., a newly discovered nongeniculate red alga (Corallinales, Rhodophyta) from Cape Province, South Africa. *Phycologia* **33**: 177-186.

TOWNSEND R.A., WOELKERLING WM.J., HARVEY A.S., & BOROWITZKA M. 1995. An account of the red algal genus *Sporolithon* (Sporolithaceae, Corallinales) in southern Australia. *Aus. Sys. Bot.* **8**: 85-121.

TURNER J.A. & WOELKERLING WM.J. 1982. Studies on the *Mastophora* - *Lithoporella* complex (Corallinaceae, Rhodophyta). II. Reproduction and generic concepts. *Phycologia* **21**: 218-235.

VERHEIJ E. 1993. The genus *Sporolithon* (Sporolithaceae fam. nov., Corallinales, Rhodophyta) from the Spermonde Archipelago, Indonesia. *Phycologia* **32**: 184-196.

WILKS K.M. & WOELKERLING WM.J. 1994. An account of southern Australian species of *Phymatolithon* (Corallinaceae, Rhodophyta) with comments on *Leptophytum*. *Aus. Syst. Bot.* **7**: 183-223.

WOELKERLING WM.J. 1980a. Studies on *Metamastophora* (Corallinaceae, Rhodophyta). I. *M. flabellata* (Sonder) Setchell: Morphology and anatomy. *Br. Phycol. J.* **15**: 201-225.

WOELKERLING WM.J. 1980b. Studies on *Metamastophora* (Corallinaceae, Rhodophyta). II. Systematics and distribution. *Br. Phycol. J.* **15**: 227-245.

WOELKERLING WM.J. 1985. A taxonomic reassessment of *Spongites* (Corallinaceae, Rhodophyta) based on studies of Kützing's original collections. *Br. Phycol. J.* **20**: 123-53.

WOELKERLING WM.J. 1987. The status and disposition of *Paragoniolithon* (Corallinaceae, Rhodophyta). *Phycologia* **26**: 144-148.

WOELKERLING WM.J. 1988. The Coralline Red Algae, An Analysis of Genera and Subfamilies of Nongeniculate Corallinaceae. Oxford University Press, Oxford and British Museum (Natural History), London.

WOELKERLING WM.J. & DUCKER S.C. 1987. *Lesueuria minderiana* gen. et sp. nov. (Corallinaceae, Rhodophyta) from southern and western Australia. *Phycologia* **26**: 192-204.

WOELKERLING WM.J. & FOSTER M.S. 1989. A systematic and ecographic account of *Synarthrophyton schielianum* sp. nov. (Corallinaceae, Rhodophyta) from the Chatham Islands. *Phycologia* **28**: 39-60.

WOELKERLING WM.J. & HARVEY A. 1992. *Mesophyllum incisum* (Corallinaceae, Rhodophyta) in southern Australia: Implications for generic and specific delimitation in the Melobesiodeae. *Br. Phycol. J.* **27**: 381-399.

WOELKERLING WM.J. & HARVEY A. 1993. An account of southern Australian species of *Mesophyllum* (Corallinaceae, Rhodophyta). *Aust. Syst. Bot.* **6**: 571-637.

WRAY J.L. 1977. Calcareous Algae. Elsevier, Amsterdam.