FRONTISPIECE: *Laminaria setchellii* Silva

RIBOSOMAL DNA PHYLOGENY OF THE PLANT DIVISION PHAEOPHYTA

by

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Ribosomal DNA Phylogeny of the Plant Division Phaeophyta

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Abstract

The phylogenetic relationships among the brown algae (Phaeophyta) at various taxonomic levels were explored using DNA sequence data from the cytoplasmic small-subunit ribosomal DNA (18S rDNA). Existing controversial phylogenetic hypotheses among the brown algae were based on phenotypic characters of extant taxa. DNA sequence data were used as an independent data set to address various phylogenetic issues from the generic to the ordinal level. Twenty-two taxa representing 14 of the 16 recognized orders of the division Phaeophyta were represented in the study.

The current placement of *Ralfsia fungiformis* (Gunnerus) Setchell et Gardner and *Analipus japonicus* (Harvey) Wynne in the Ectocarpales is questionable. The inferred ribosomal DNA phylogeny supported the contention that *Ralfsia* and *Analipus* should not be placed in the Ectocarpales.

The complete 18S rDNA gene sequences from *Chorda tomentosa* Lyngbye (Chordaceae) and *Saccorhiza polyschides* (Lightfoot) Batters (Phyllariaceae) were determined and compared with published sequences representing two other kelp families (Alariaceae and Lessoniaceae) and 12

other brown algal orders to better understand kelp evolution at the familial level. Results suggested that the Laminariales is paraphyletic.

At the ordinal level, the inferred ribosomal DNA phylogeny showed the 14 studied brown algal orders segregated into two main lineages. Representatives from the Ectocarpales, Chordariales, Dictyosiphonales and Scytosiphonales (ECDS) made up one lineage, while the remaining taxa formed the other. The separation of these orders into two lineages was well supported (100%) by the bootstrap analyses. The ECDS lineage was characterized by the presence of normal pyrenoids, and the other lineage was characterized by the absence of pyrenoids or the presence of rudimentary pyrenoids. This study provided evidence to support the proposal that the Laminariales, Desmarestiales and Sporochnales are close relatives and disputed their placement in different phylogenetic lines.

Results suggested that the traditional criterion of thallus organization is not as important in the delimitation of brown algal orders as was originally supposed. Instead, ultrastructural characters such as the possession of pyrenoids and eyespots in certain life history stages were proposed as important criteria to circumscribe the orders.

Dedication

This thesis is dedicated to my parents.

"It seems probable that ultimately there will be a significant rearrangement of phylogenetic affinities within the Phaeophyta."

Müller, Clayton & Germann 1985

"A revision of our concept of the fundamental characteristics and phylogeny of the Phaeophyceae is needed"

Kawai 1991

Acknowledgments

I am grateful to Dr. Gayle Hansen for introducing me to the world of phycology. I was most fortunate to experience my very first phycological moment by Execution Rock, Barkley Sound; I was in awe witnessing Gayle leaping and dashing across slippery seaweed- covered boulders with such graceful ballerina jetés while yelling "oooh, there's my darling". That was my very first phycological moment.

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INTRODUCTION

The Phaeophyta circumscribes a division of algae commonly known as brown algae. Brown algae are found along the shallow coasts or continental shelves of all oceans, and they are major producers of organic material in coastal environments (Sze 1993). For example, subtidal kelp systems produce 1000 grams of carbon per square metres per year (Mann 1982). Further, species of the giant kelp *Macrocystis* form extensive submarine forests along the Pacific coast of North and South America. Kelp forests are important habitats for a diversity of marine organisms including invertebrates, fish, other algae and mammal species. Darwin (1860) compared these submarine forests with terrestrial ones: " I can only compare these great aquatic forests of the southern hemisphere, with the terrestrial ones in the inter-tropical regions. Yet if in any country a forest was destroyed, I do not believe nearly so many species of animals would perish as would here, from the destruction of the kelp".

Brown algae are so named because they have an abundance of the brown-coloured photosynthetic accessory pigment, fucoxanthin. Additional distinguishing brown algal characteristics include the occurrence of alginates

and phlorotannins in cell walls and physodes (spherical bodies within the cells) respectively (Clayton 1989). Overall body size ranges from uniseriate branched filaments, as found in the genus *Ectocarpus*, to large prominent thalli (up to sixty metres in length) found in the giant kelp, *Macrocystis*. Commercially valuable species of brown algae, notably kelp species (Laminariales), are currently harvested for alginates (used in food processing), fertilizers and food supplements.

Brown algae possess numerous morphological, reproductive and developmental characteristics. For example, *Ectocarpus* (Ectocarpales) is filamentous, grows by diffuse cell division, and reproduces by bearing identical biflagellated gametes (isogamous). Conversely, the kelp (Laminariales) are parenchymatous, grow by cell division restricted to the intercalary meristem, and reproduce by sperm and eggs (oogamy). Traditionally this heterogeneous collection of characters was used for phylogeny inference; however, the inferred phylogenies were subjected to scrutiny and controversy because they were inconsistent and questionable. For example, Kylin (1933 as cited by Clayton 1984) considered thallus organization and life history pattern as important criteria for delimiting and reflecting phylogenetic relationships among the orders. Conversely, Manton

(1965) and Kawai (1992) emphasized the importance of using ultrastructural and cytological characters (e.g., pyrenoid distribution) to infer brown algal phylogeny. Reports contradicting the classical and traditional phylogenetic hypotheses (Kylin 1933, Papenfuss 1953, Wynne and Loiseaux 1976) led several investigators to conclude that, "Relationships among the brown algae are clearly in need of reassessment involving careful evaluation of the relative weights attributed to various taxonomic characters" (Müller et al. 1985a), and, "A revision of our concept of the fundamental characteristics and phylogeny of the Phaeophyceae is needed" (Kawai 1991).

Recent phylogenetic investigations (Lim et al. 1986, Stam et al. 1988, Saunders and Druehl 1992) using molecular characters were reported for the Phaeophyta. These studies used DNA sequence data which were independent of the traditional morphology-based characters for phylogenetic inference. DNA sequences, such as the cytoplasmic small subunit ribosomal DNA (18S rDNA) sequences, were used extensively for phylogenetic studies among a diversity of prokaryotes and eukaryotes including the algae (Bhattacharya et al. 1990, Zechman et al. 1990, Saunders and Druehl 1992). In addition, a rapidly growing database of 18S rDNA sequences was available for comparative analysis. In spite of the recent molecular reports (Lim et al.

1986, Stam et al. 1988, Saunders and Druehl 1992), many questions remain unanswered regarding brown algal evolutionary relationships. For instance, how are the currently recognized orders of brown algae related to each other based on molecular characters? Do these molecular phylogenies contradict traditional brown algal phylogenies? In order to address such phylogenetic issues, representatives from most of the recognized brown algal orders were selected for this study.

The purpose of this study was to address phylogenetic relationships at three taxonomic levels within the division Phaeophyta, specifically at the ordinal, familial and finally generic levels. Twenty-two representative taxa from fourteen orders were selected to address the interordinal relationships (Table 1). These taxa represent most of the universally recognized brown algal orders with the exception of the Durvillaeales and Ascoseirales. At the familial level, sequence data from two kelp families (Chordaceae and Phyllariaceae) were determined and compared with other published kelp sequences [Alariaceae and Lessoniaceae (Saunders and Druehl 1992)] in order to explore the relationships among the kelp families. Lastly, at the generic level, the controversial taxonomic placements of the two brown algal

Table 1. Brown algal (Phaeophyta) taxa included in the phylogenetic study (Bold and Wynne 1985).

Representatives	**Order**
Ectocarpus siliculosus (Dillwyn) Lyngbye	Ectocarpales
Scytosiphon lomentaria (Lyngbye) Areschoug	Scytosiphonales
Colpomenia peregrina (Sauvageau) Hamel	Scytosiphonales
Asperococcus bullosus Lamouroux	Dictyosiphonales
Punctaria expansa Setchell *et* Gardner	Dictyosiphonales
Leathesia difformis (Linnaeus) Areschoug	Chordariales
Haplogloia andersonii (Farlow) Levring	Chordariales
Elachista fucicola (Velley) Areschoug	Chordariales
Sporochnus comosus C. A. Agardh	Sporochnales
Desmarestia ligulata (Lightfoot) Lamouroux	Desmarestiales
Taonia atomaria (Woodward) J. Agardh	Dictyotales
Sphacelaria furcigera Kützing	Sphacelariales
Syringoderma phinneyi Henry *et* Müller	Syringodermatales
Analipus japonicus (Harvey) Wynne	Ralfsiales
Ralfsia fungiformis (Gunnerus) Setchell *et* Gardner	Ralfsiales
Tilopteris mertensii (Turner in Smith) Kützing	Tilopteridales
Cutleria multifida (Smith) Greville	Cutleriales

Table 1. (Continued)

Representatives	**Orders**
Chorda tomentosa Lyngbye	Laminariales
Saccorhiza polyschides (Lightfoot) Batters	Laminariales
Alaria marginata Postels *et* Ruprecht[1]	Laminariales
Macrocystis integrifolia Bory[2]	Laminariales
Sargassum vestitum (R. Brown ex Turner) C. Agardh[3]	Fucales
Fucus gardneri Silva[4]	Fucales

[1,2] Saunders and Druehl 1992; [3] Saunders and Kraft 1995; [4]Bhattacharya et al.1992.

genera, *Analipus japonicus* (Harvey) Wynne and *Ralfsia fungiformis* (Grunnerus) Setchell et Gardner were addressed.

Two different sets of 18S rDNA sequence data were subjected to a variety of phylogenetic inference methods. The first data set consisted of partial 18S rDNA sequences from 21 taxa representing 14 orders. This data set originated from the pilot study for the current research; the latter half of the 18S rDNA, including a variable region (at the 3' end) which might be valuable for phylogeny inference among the studied taxa, was used in this study. The inferred ribosomal DNA phylogeny showed the orders segregating into two disparate lineages; however, certain branching orders within each lineage were not fully resolved. Therefore, the entire 18S rDNA was included in the analysis with anticipation of including more variable and informative sites found in the 5' end of the 18S rDNA to the analysis; the second data set consisted of complete 18S rDNA sequences representing 16 taxa from 14 orders.

Taxonomically important characters

Brown algal orders were traditionally distinguished by four morphological and developmental characters: life history pattern, thallus (plant body) organization, sexuality and growth types (Table 2). There are three different life history patterns: an isomorphic alternation of generations, a heteromorphic alternation of generations and a gametic life cycle. Brown algae with morphologically similar gametophyte (haploid phase which produces gametes via mitosis) and sporophyte (diploid phase which produces meiospores via meiosis) phases have an isomorphic alternation of generations (e.g., Ectocarpales, Dictyotales, Sphacelariales). Algae with morphologically distinct gametophyte and sporophyte phases have a heteromorphic alternation of generations (e.g., Desmarestiales, Laminariales, Chordariales). Phaeophytes which exhibit a single dominant phase in their life history (having no free-living haploid phase), such as members of the Fucales, Durvillaeales and Ascoseirales, have a gametic life cycle.

Brown algae display three types of growth: diffuse, apical and intercalary. Those with diffuse growth have new cell production occurring throughout the thallus (e.g., *Ectocarpus*). Apical and intercalary growth types

Table 2. Traditional characters (with various modes) used by different authors to delimit brown algal (Phaeophyta) orders as well as to infer phylogenetic relationships among the orders (Kylin 1933, Papenfuss 1954, Wynne and Loiseaux 1976). See text for description of terms.

CHARACTERS	MODES		
a) Life history:	isomorphic alternation of generations	heteromorphic alternation of generations	gametic life cycle
b) Thallus organization:	filamentous	pseudoparenchymatous	parenchymatous
c) Sexuality:	isogamous	anisogamous	oogamous
d) Growth:	diffuse	apical	intercalary

are characterized by actively dividing cells restricted to specific regions of the thallus called meristems. Apical growth is initiated from meristematic cells found at the terminal ends of the thallus (e.g., *Dictyota*). These occur as either a single prominent apical cell or as a row of cells at the edge of the thallus. Meristems within the thallus contribute to intercalary growth. Intercalary growth is characteristic of the Laminariales. Most frequently the intercalary meristem of the kelp is located between the stipe and the blade. A unique type of intercalary growth is seen in the Desmarestiales. Here, the intercalary meristem is found at the base of a terminal hair; it is also known as trichothallic meristem.

Phaeophytes are categorized into filamentous, pseudoparenchymatous or parenchymatous algae. These categories are basically different modes of thallus organization as a result of cell division. Filamentous forms are typically composed of linear rows of cells which divide in one plane. Superficially, filamentous brown algae appear thread or filament-like; *Ectocarpus* has the classical filamentous form. Parenchymatous phaeophytes have cells which are capable of dividing in three planes. A cross section through parenchymatous tissue shows that cells are arranged randomly and compactly. Pseudoparenchymatous phaeophytes have tissues which appear

parenchymatous (e.g., compact) but are composed of intertwined branched filaments which grew by cell division in one plane. All three modes of thallus organization are achieved by various types of early development of the spores or zygotes. The heterotrichous type of early development involves the spores or zygotes germinating into horizontal filaments which later give rise to upright filaments. In contrast, upright filaments growing directly from spores or zygotes is termed, the erect type of early development. Discal-type describes upright filaments arising from a disc.

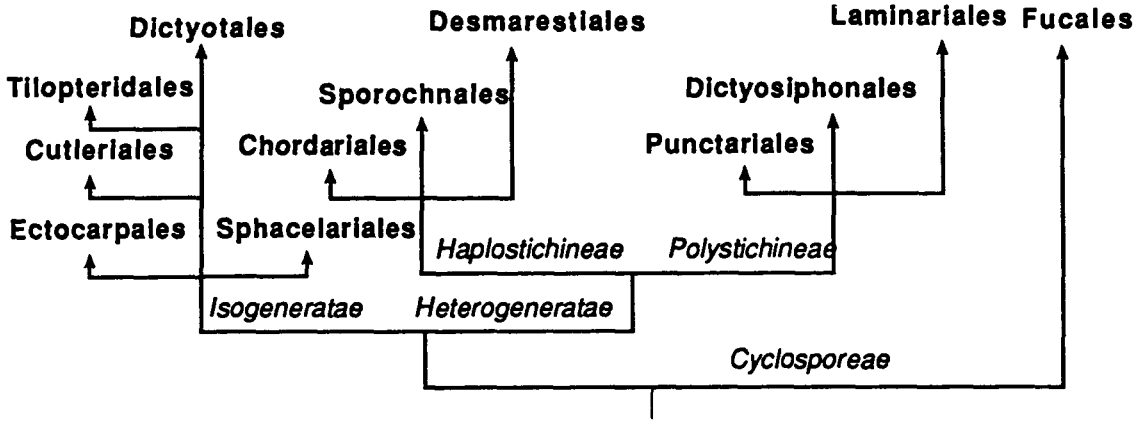
Three types of sexuality or gametic conditions are found in a brown algal life history: isogamy, anisogamy and oogamy. Brown algal gametes have either two or no flagella (projections from the cell which serve locomotive purposes); those with two flagella have one flagellum with mastigonemes (hair-like projections) and one without. Both flagella are laterally inserted. Only biflagellated gametes are motile. Biflagellation is the universal rule among the male gametes, whereas the female gametes can be either biflagellated or non-flagellated. Isogamy exists when both male and female gametes are identical in morphology: both gametes have two flagella and both are identical in size. Anisogamy describes slight morphological differences between the male and female gametes: both are biflagellated but

differ slightly in size. Oogamy characterizes a biflagellated male gamete and a non-flagellated female gamete (egg). The eggs are generally much larger than the male gametes.

Classical brown algal phylogeny

Kylin (1933 as cited by Clayton 1984) proposed a brown algal phylogeny based on life history patterns and thallus organization types (Fig. 1) which is still the most widely accepted hypothesis of the evolutionary relationships among the brown algae. Kylin classified the brown algal orders into 3 classes (Isogeneratae, Heterogeneratae and Cyclosporeae) on the basis of life history patterns. The Isogeneratae included orders with an isomorphic alternation of generations (Ectocarpales, Sphacelariales, Cutleriales, Tilopteridales and Dictyotales) while the Heterogeneratae delimited orders with a heteromorphic alternation of generations (Desmarestiales, Sporochnales, Chordariales, Laminariales, Dictyosiphonales and Punctariales). The Cyclosporeae contained a single order, the Fucales, because of its gametic life cycle. Orders within the

Fig. 1. Phylogenetic hypothesis among the brown algal (Phaeophyta) orders according to Kylin (1933). Kylin emphasized life history pattern and thallus organization to reflect the ordinal relationships.

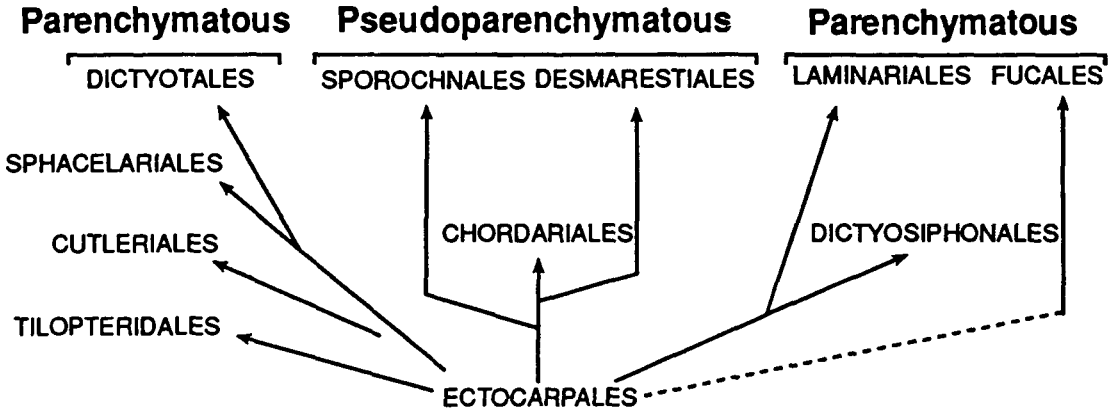

Dictyotales
Desmarestiales
Laminariales
Fucales
Tilopteridales
Sporochnales
Dictyosiphonales
Cutleriales
Chordariales
Punctariales
Ectocarpales
Sphacelariales
Haplostichineae
Polystichineae
Isogeneratae
Heterogeneratae
Cyclosporeae

Kylin (1933)

Heterogeneratae were further separated into different subclasses based on modes of thallus organization: Haplostichineae (pseudoparenchymatous) and Polystichineae (parenchymatous).

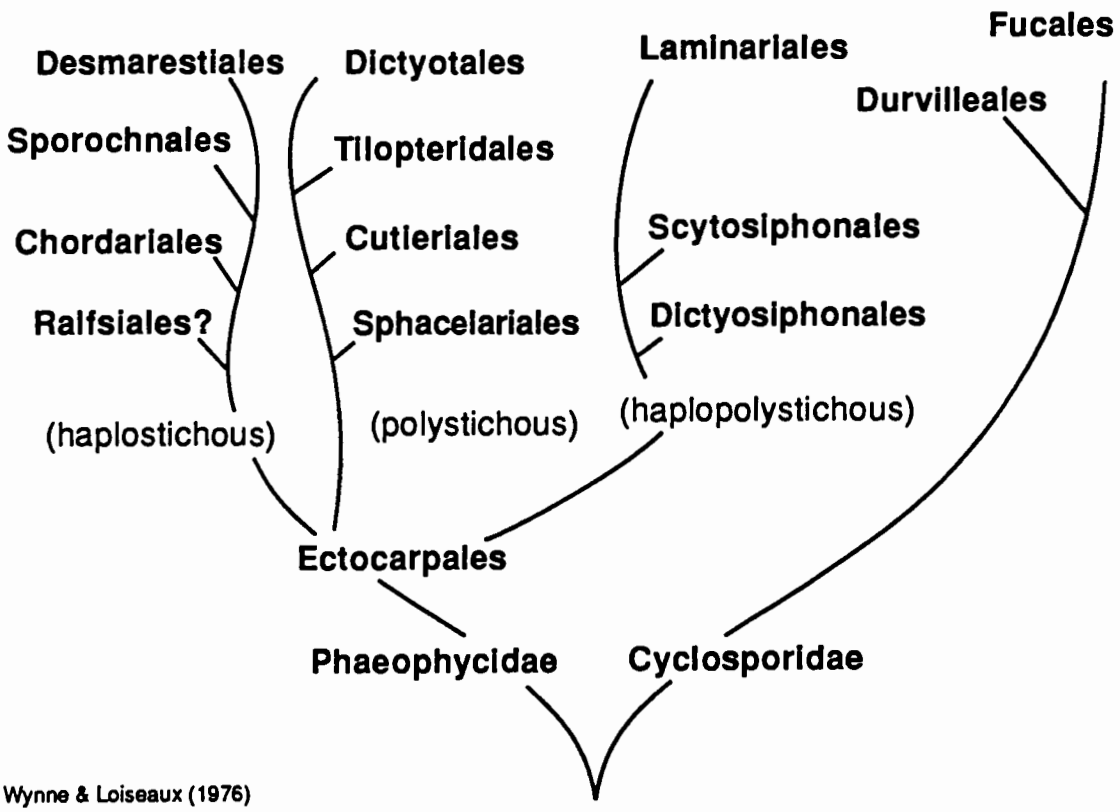
Subsequent phylogenetic hypotheses (Papenfuss 1953, Wynne and Loiseaux 1976) followed Kylin's (1933) scheme with some modifications (Fig. 2,3). These newer proposals also used the modes of thallus organization to separate the brown algal orders into different lineages. Papenfuss (1953) and Wynne and Loiseaux (1976) proposed that members of the Ectocarpales retained various ancestral brown algal characteristics, and thus, designated the Ectocarpales as the most 'primitive' brown algal order. Papenfuss (1953) disregarded life history pattern as an important phylogenetic characteristic, and rather chose modes of thallus organization to reflect brown algal relationships. Conversely, Wynne and Loiseaux (1976) separated the orders into two groups (subclasses) based on life history patterns: Phaeophycidae and Cyclosporidae (Fig. 3). The former subclass included those orders with an alternation of generations, while the latter subclass have orders with a gametic life cycle. The Cyclosporidae contained only 2 orders, the Fucales and Durvillaeales. The Phaeosporidae was further subdivided into three groups based on modes of thallus

Fig. 2. Phylogenetic hypothesis among the brown algal (Phaeophyta) orders according to Papenfuss (1953). Papenfuss chose only thallus organization to reflect the ordinal phylogeny.



Papenfuss (1953)

Fig. 3. Phylogenetic hypothesis among the brown algal (Phaeophyta) orders according to Wynne and Loiseaux (1976). Wynne and Loiseaux separated the orders into two subclasses, Phaeophycidae and Cyclosporidae, based on life history pattern; the Phaeophycidae was further divided into 3 groups based on thallus organization.



Wynne & Loiseaux (1976)

organization. The Laminariales and Scytosiphonales were grouped together because they have pseudoparenchymatous and parenchymatous phases (haplopolystichous). For example, the Laminariales have pseudoparenchymatous gametophytes and parenchymatous sporophytes. In contrast, the Desmarestiales and Sporochnales were grouped together because they have only pseudoparenchymatous phases (haplostichous), and the Dictyotales and Cutleriales were grouped together because they have only parenchymatous phases (polystichous).

Inferred phylogenies based on phenotypic and developmental characters remain inconsistent and questionable because of the differing character-selection process employed by each author (Kylin 1933, Papenfuss 1953, Wynne and Loiseaux 1976). In addition to the morphological and developmental characters, Manton (1965) and Evans (1966, 1968) emphasized the importance of using ultrastructural characters for phylogeny inference. Evans (1966, 1968) proposed pyrenoid distribution as a diagnostic character to classify the brown algae. Kawai (1991) also stressed the importance of including cytological characteristics in the discussion of brown algal phylogeny, and he reported that pyrenoid distribution is generally constant among the brown algae irrespective of life history stage.

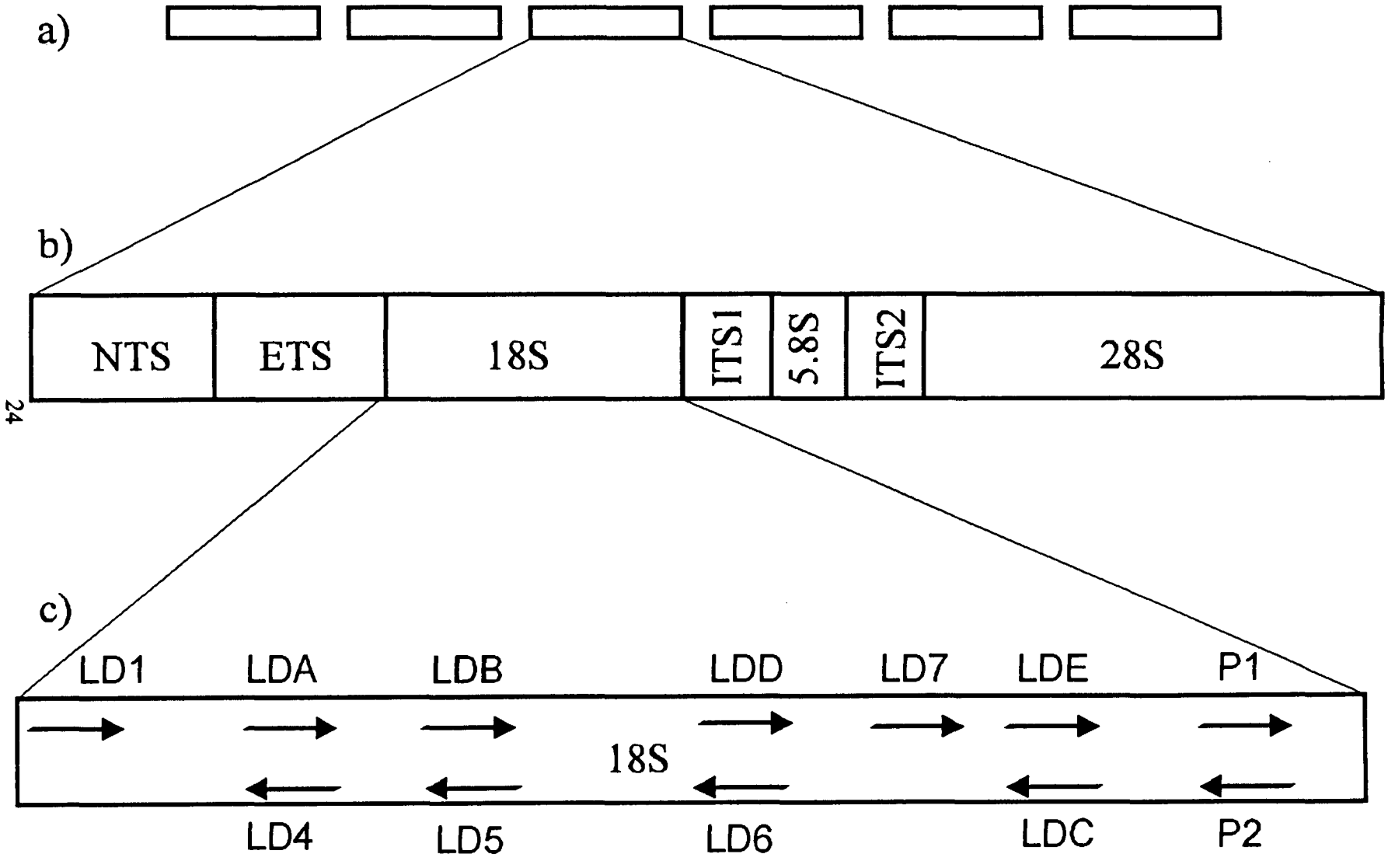
Unfortunately the poor fossil record of the Phaeophyta cannot provide corroboration for the suitability of either morphological or ultrastructural characters chosen by the authors to indicate evolutionary relationships (Clayton 1984).

Small-Subunit Ribosomal DNA

Ribosomes are involved in protein synthesis within the cell. Protein synthesis is an essential sustaining life process, and therefore, ribosomes (and rRNAs) are universally found in all living organisms (Hillis and Dixon 1991). Ribosomes are composed of structural RNA [ribosomal RNA (rRNA)] coupled with proteins. Free ribosomes exist as two separate subunits: the small-subunit (SSU) and the large-subunit (LSU). The small-subunit and large-subunit are also known as the 18S rRNA and the 25-28S rRNA respectively. These two subunits appear to exist as separate entities within the cytoplasm when they are not complexed with mRNA. The small-subunit is responsible for binding the mRNA, and the large-subunit contains the enzyme which catalyzes the formation of peptide bonds between amino acids.

The small-subunit ribosomal DNA (18S rDNA) is one of three ribosomal DNAs within the ribosomal cistron (Fig. 4). The other two rDNAs include the large-subunit (28S) and the 5.8S. Nuclear ribosomal cistrons are arranged head to tail in tandemly repeating units. The number of tandem repeats varies widely across taxa and among individuals. For example, within an individual (e.g., angiosperm) the number can range from 200 to 22,000 copies per cell (Rogers and Bendich 1987). The evolution of these tandemly arranged ribosomal cistrons occurs in a concerted manner; as a result, each copy of the ribosomal cistron is very similar to the other copies (Arnheim 1983). Concerted evolution results in the homogenization of such gene sequences, initially within a genome and then among individuals within populations (Dowling et al. 1990), and it is believed to be a result of unequal crossing over and gene conversion (Dover 1982a,b). Each region of the ribosomal cistron is under a different level of functional constraint; the 18S rDNA has the greatest constraint among the rDNAs. The 18S rDNA has been used extensively to infer phylogenetic relationships among different groups of organisms (Hasegawa et al. 1985, Pace et al. 1986, Woese 1987, Pashley et al. 1993) including the algae (Bhattacharya et al. 1990, Zechman et al. 1990, Saunders and Druehl 1992, Saunders and Kraft 1995). Hasegawa et

Fig. 4. Schematic representation of the nuclear ribosomal cistron. a) tandemly repeating units of ribosomal cistrons; b) arrangement of various encoding and spacer regions within a cistron: 18S (small-subunit) rDNA, 5.8S rDNA, 28S (large-subunit) rDNA, NTS (non-transcribed spacer), ETS (external transcribed spacer), ITS1 (Internal transcribed spacer 1) and ITS2 (Internal trancribed spacer 2); c) location and orientation of the oligonucleotide primers used to amplify (PCR) and sequence the 18S rDNA. Oligonucleotide primer sequences are presented in Table 5.


a)
b)
NTS
ETS
18S
ITS1
5.8S
ITS2
28S
24
c)
LD1
LDA
LDB
LDD
LD7
LDE
P1
18S
LD4
LD5
LD6
LDC
P2

al. (1985) reconstructed the phylogenetic relationships among the eukaryotic kingdoms based on the comparison of 18S rDNA sequences. At the ordinal level Pashley et al. (1993) inferred the phylogenetic relationships among nine holometabolous (with a pupal stage marking transformation from the feeding larval stage to the reproductive adult stage) insect orders. Similarly, Zechman et al. (1990) inferred the phylogeny among the green algal orders based on analysis of 18S rDNA sequences. Both ordinal studies showed the 18S rDNA provided adequate phylogenetic signal to reflect phylogeny among the orders (Zechman et al. 1990, Pashley et al. 1993). In addition, Saunders and Kraft's (1995) study indicated that the 18S rDNA provided suitable divergence for assessing interfamilial relationships within the brown algal order Fucales. Furthermore, Sogin (1989) proposed the 18S rDNA as a suitable molecular marker for phylogenetic studies because the gene satisfied several criteria. For instance, the 18S rDNAs are evolutionary homologues which mutate slow enough to impart genetic divergence between the compared sequences, they do not undergo transfer between species and they have a significant number of variable sites (Sogin 1989).

Molecular Phylogeny

Molecular characters are used extensively to reconstruct phylogenetic trees to reflect evolutionary relationships among organisms (Hillis 1987, Hillis & Moritz 1990). Determination of molecular sequence data has become a popular means of assessing evolutionary relatedness among taxa, surpassing other molecular techniques such as nucleic acid hybridization and immunological tests in sensitivity (Pace et al. 1986). Straightforward and quantitative assessments can be made of the discrete changes between aligned homologous sequences (sequences which share a common ancestry). Nei (1987) suggested that nucleotide changes occur randomly and are not subjected to the same selection processes as phenotypic characters. Thus, similarity between homologous sequences can be interpreted as a function of relatedness and not the result of selection driven convergent evolution. Zuckerkandl and Pauling (1965) stated that phylogenies inferred from the comparison of macromolecular sequences are the most reliable and accurate. In addition, numerous phylogenetic issues previously considered intractable by morphological characters can now be addressed by molecular characters (Hillis 1987, Patterson 1987).

Lim et al. (1986) were the first to address the controversial brown algal ordinal phylogeny utilizing molecular data. They inferred a phylogeny based on comparisons of cytoplasmic 5S ribosomal RNA gene sequences from five brown algae representing the Laminariales, Fucales, Ectocarpales, Chordariales and Dictyosiphonales. The branching order among the five orders was unresolved in that the percent similarity among the compared sequences ranged from 96% to 99%. In addition to concluding that the brown algae diverged very recently from one another, they also suggested that the 5S rRNA gene was too conserved for estimating the precise phylogenetic relationships among the studied taxa. Similarly, the 5S rRNA gene was suggested as too short in sequence to provide adequate signal for phylogenetic inference (Halanych 1991, Steele et al. 1991).

The brown algal 18S rDNA is about 15 times longer than its 5S counterpart thus, the longer 18S rDNA sequence has potentially more phylogenetic information for phylogeny inference among the brown algal orders. Furthermore, a growing 18S rDNA database (Maidak et al. 1994, Van de Peer et al. 1994) provides additional sequences for phylogenetic studies among a diversity of organisms.

Various methods are available for reconstructing evolutionary relationships based on the comparison of such sequences (Felsenstein 1988). Two such methods include the distance matrix methods and maximum parsimony methods. Distance matrix methods involve the pairwise comparison of aligned sequences; distance values (established from sequence dissimilarity) were calculated with corrections (e.g., Kimura 1980) to reflect the estimate of substitutions between the compared sequences. The resulting matrix of distance values is used by a clustering algorithm, such as the neighbor-joining method (Saitou and Nei 1987), to reconstruct phylogenetic trees. The clustering algorithm groups taxa with the highest similarity. Saitou and Nei's (1987) neighbor-joining method does not assume uniform rates of nucleotide change among sequences and was found to be the most efficient at inferring the true phylogenetic tree as compared to other methods (Saitou and Imanishi 1989, Kim et al. 1993).

Maximum parsimony methods use character states instead of distance values for reconstruction of phylogenetic trees (Nei 1987). Only the character states of nucleotides at informative sites are used. Informative sites are sites where two or more orthologous (share a common ancestry with divergence based on speciation) sequences share a character state different from that of

the other compared sequences (Nei 1987). Taxa with the same character states are grouped together. The inferred tree with the fewest changes is preferred (Felsenstein 1988).

Phylogenetic issues

i) Relationships among the brown algal orders

Delimitation of the brown algal orders remains inconsistent and controversial; even the number of orders within the division is debatable (Fritsch 1945, Wynne 1969, Russell and Fletcher 1975, Parke and Dixon 1976, Gabrielson et al. 1989). Membership within each order is based on life history pattern, thallus organization, sexuality and growth types (Table 3). Van den Hoek and Jahn (1978) demonstrated graphically the indistinctive boundary delimiting the orders, especially among the Ectocarpales, Chordariales, Dictyosiphonales and Scytosiphonales (ECDS) (Fig. 5). The relationships among the ECDS were subjected to controversy (Fritsch 1945, Scagel 1966) and, to date, membership within these orders remains uncertain

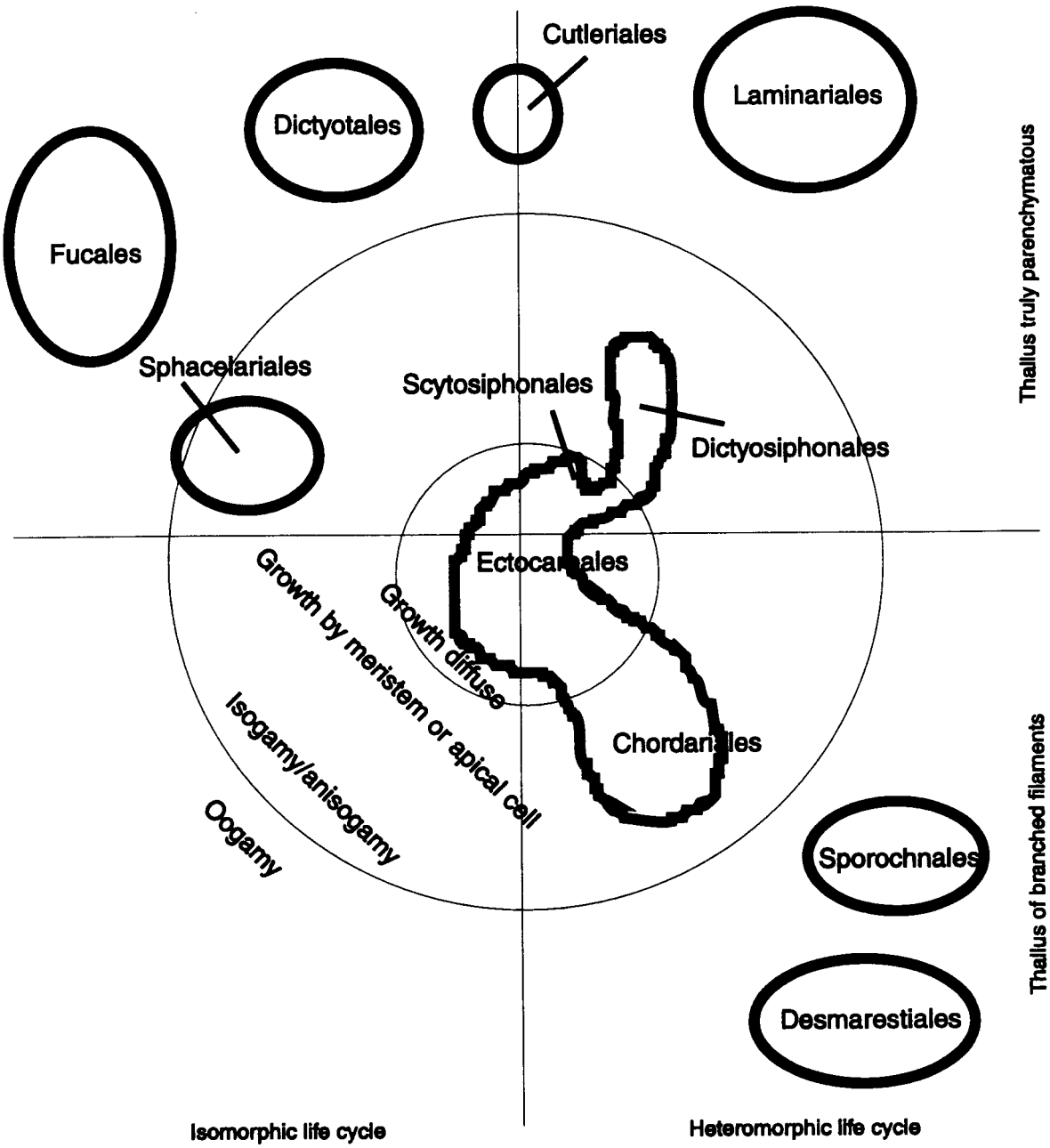
Table 3. Distribution of traditional characteristics among the studied taxa.

	life history	**thallus organization**	**sexuality**	**growth**
Ectocarpales	isomorphic	filamentous	iso/anisogamous	diffuse
Chordariales	isomorphic	pseudoparenchymatous	isogamous	variable
Dictyosiphonales	heteromorphic	parenchymatous	iso/anisogamous	variable
Scytosiphonales	heteromorphic	parenchymatous	iso/anisogamous	diffuse
Laminariales	heteromorphic	parenchymatous	oogamous	intercalary
Desmarestiales	heteromorphic	pseudoparenchymatous	oogamous	trichothallic
Sporochnales	heteromorphic	pseudoparenchymatous	oogamous	trichothallic
Tilopteridales	isomorphic	filamentous	oogamous	trichothallic
Cutleriales	heteromorphic	parenchymatous	anisogamous	trichothallic
Sphacelariales	isomorphic	parenchymatous	isogamous	apical
Syringodermatales	heteromorphic	pseudoparenchymatous	isogamous	apical

Table 3. (Continued)

	life history	**thallus organization**	**sexuality**	**growth**
Dictyotales	isomorphic	parenchymatous	oogamous	apical
Fucales	gametic	parenchymatous	oogamous	apical

Fig. 5. Distribution of traditional characteristics (life history pattern, thallus organization, growth and sexuality) and interrelationships among the brown algal (Phaeophyta) orders. Figure modified from van den Hoek and Jahn (1978).


Cutleriales
Laminariales
Dictyotales
Fucales
Sphacelariales
Scytosiphonales
Dictyosiphonales
Ectocarpales
Chordariales
Growth by meristem or apical cell
Growth diffuse
Isogamy/anisogamy
Oogamy
Sporochnales
Desmarestiales
Thallus truly parenchymatous
Thallus of branched filaments
Isomorphic life cycle
Heteromorphic life cycle

and confusing (Scagel 1966, Wynne 1969, Russell and Fletcher 1975, Parke and Dixon 1976, Boney 1978, Gabrielson et al. 1989). Taxa described as ectocarpoids on the basis of their morphological features by some workers (Fritsch 1945, Russell and Fletcher 1975, Parke and Dixon 1976, Gabrielson et al. 1989) were elevated to ordinal status by other authors (Scagel 1966, Wynne 1982). For example some of these ectocarpoids (*sensu* Fritsch 1945) were placed in the Chordariales, Ectocarpales, Dictyosiphonales, Scytosiphonales (Scagel 1966), Punctariales (Kylin 1933 as cited by Fritsch 1945) and Ralfsiales (Nakamura 1972). Fritsch (1945) proposed to merge the ECDS into one order: Ectocarpales. He was of the opinion that heterotrichy and the absence of true oogamy (eggs and sperm) in these four orders justified their ordinal treatment as one order. Scagel (1966) challenged Fritsch's proposal in that he did not consider type of early development (heterotrichy) and sexuality as important taxonomic characters to delimit brown algae at the ordinal level. Rather, he stressed the importance of overall thallus organization (filamentous, pseudoparenchymatous or parenchymatous) and life history pattern as distinguishing criteria to delimit brown algae at the ordinal level. Since the ectocarpoids (*sensu* Fritsch 1945) vary in type of

thallus organization and sexuality, Scagel (1966) recommended they remain in separate orders.

The phylogenetic relationships among the Sporochnales, Desmarestiales and Laminariales (SDL) are also disputed (Clayton 1984, Motomura et al. 1985, Müller et al. 1985a, Kawai 1992). These three orders were traditionally classified in different phylogenetic lineages because they have different types of thallus organization (Scagel 1966, Wynne and Loiseaux 1976). The Sporochnales and Desmarestiales, being pseudoparenchymatous, were associated with other pseudoparenchymatous orders such as the Chordariales, whereas the parenchymatous Laminariales was associated with other parenchymatous orders such as the Scytosiphonales. However, the SDL were proposed as close relatives because they share other characters which were typically used to delimit brown algal orders: sexual reproduction features and life history pattern (Clayton 1984, Motomura et al. 1985, Kawai 1992). All three orders are oogamous and have an alternation of heteromorphic generations between a microscopic gametophyte and a macroscopic sporophyte.

The phylogenetic affinity of the Dictyotales is enigmatic (Clayton 1984). Members of this order have uniflagellated sperm (plus the presence of

a vestigial second flagellum) in contrast to the typical biflagellated brown algal sperm (Manton 1965). On the basis of overall thallus morphology and spermatozoid and spore ultrastructural features, the Dictyotales have been closely associated with the Cutleriales (Phillips et al. 1990, Phillips and Clayton 1991). However, the life history pattern within the Cutleriales differs from the Dictyotales in that the Cutleriales have heteromorphic alternation of generations while the Dictyotales have isomorphic alternation of generations. In addition, the Dictyotales produce small numbers of non-motile meiospores [with the exception of *Homoeostrichus olsenii* (Phillips and Clayton 1994)] in contrast to typical brown algal meiospores which are produced in great numbers and are motile (Clayton 1984). These differences led Clayton (1984) to suggest that the Dictyotales represent a distinct line of evolution which is not closely associated with the other brown algal orders. Members of the monogeneric Syringodermatales were previously classified within the Dictyotales and proposed as close relatives to the Sphacelariales (Walker and Henry 1978). Members of all three orders have apical growth; however, they differ in other traditional ordinal characteristics. The Syringodermatales has a heteromorphic alternation of generations while the Dictyotales and Sphacelariales have isomorphic alternation of generations (Henry 1980).

The life history within the Tilopteridales deviates from the typical brown algal life history (Kuhlenkamp et al. 1993) in that members (three monospecific genera) demonstrate a sequence of gradually reduced life histories. *Phaeosiphoniella cryophila* Hooper, Henry et Kuhlenkamp reproduces primarily through fragmentation with only vestigial sexual reproductive structures observed in this alga (Hooper et al. 1988). *Tilopteris mertensii* (Turner in Smith) Kützing exists only in the gametophytic phase, and the gametophytes develop from unfertilized eggs, and *Haplospora globosa* Kjellman has an isomorphic alternation of generations (Kuhlenkamp et al. 1993). Superficially, the Tilopteridales resemble the Sphacelariales; however, the former order has an intercalary meristem while the latter one has prominent apical cells. Furthermore, its marked anisogamous or oogamous gametes resemble those of the Laminariales, Cutleriales, Desmarestiales and Sporochnales, and thus, the phylogenetic affinities of the Tilopteridales remain questionable.

The Fucales is universally viewed as representing a distinct evolutionary line within the Phaeophyta by phycologists (Kylin 1933, Papenfuss 1953, Wynne and Loiseaux 1976) (Fig. 1,2,3). This view is based on the unique gametic life cycle among members of the Fucales (no free-living

haploid phase). However, the Fucales do share other similar morphological characteristics with orders such as the Chordariales and Dictyosiphonales (Clayton 1984). For example, the presence of conceptacles (cavities containing gamete producing structures) and cryptostomata (sterile conceptacles) are regarded as unique characteristics among members of the Fucales; however, similar structures are also found in certain members of the Chordariales and Dictyosiphonales (Clayton 1984). These phylogenetic issues have not been resolved by analysis of traditional characteristics; thus, an independent approach using molecular characters (DNA sequence data) is used to explore these questions.

ii) Evolutionary relationships within the Laminariales

Due to an impoverished fossil record, the taxonomic and evolutionary relationships among the kelp (Laminariales) are based primarily on morphological similarity among extant species (Clayton 1984). However, the morphology based taxonomy of the order has been acknowledged to be

inconsistent (Saunders and Druehl 1992 from Setchell and Gardner 1925). As a result, laminarialean evolution and phylogeny are poorly understood. A variety of phylogenetic issues has stimulated numerous evolutionary studies within the Laminariales (Druehl 1970, Estes and Steinberg 1988, Lüning and tom Dieck 1990, Druehl and Saunders 1992, Saunders and Druehl 1992, Mayes 1993).

Molecular phylogeny within the Laminariales has been actively pursued by several research groups (Lim et al. 1986, Fain et al. 1988, Stam et al. 1988, Bhattacharya et al. 1991, Saunders and Druehl 1992). To date, the branching orders within the inferred molecular phylogeny of the Laminariales remain unresolved (Saunders and Druehl 1992). Saunders and Druehl (1992) examined the so-called 'advanced' families: Alariaceae, Laminariaceae and Lessoniaceae (ALL), whose members are considered to possess derived kelp features. Further investigation is needed to elucidate the evolutionary relationships among the kelp. Resolution of divergence and evolutionary relationships among the kelp can be pursued further by incorporating sequence data from other kelp families not included in the Saunders and Druehl (1992) study. These families include the Chordaceae and Phyllariaceae whose members are considered to have retained ancestral kelp

features; they are commonly known as 'primitive' kelp families. Ancestral kelp features include the presence of eyespots in meiospores. Meiospores with eyespots are found in brown algal orders such as the Ectocarpales (ectocarpoids are universally perceived as 'primitive' brown algae because of their simple filamentous thalli). On the other hand, the ALL are considered derived taxa because of their more complex parenchymatous thalli which feature trumpet hyphae (which function as photosynthate translocating vessels), the Chordaceae and Phyllariaceae lack trumpet hyphae. In addition to having meiospores with eyespots, the Chordaceae and Phyllariaceae lack mucilage gland cells and ducts whereas the ALL possess mucilage gland cells and ducts.

iii) Phylogenetic affinities of *Analipus* and *Ralfsia*

The taxonomic placement of *Analipus japonicus* and *Ralfsia fungiformis* is frequently disputed (Nakamura 1972, Nelson 1982, Kawai 1989). Nakamura (1972) separated these two genera from the order

Ectocarpales and placed them in his newly established order Ralfsiales. He argued that *Analipus* and *Ralfsia* are highly distinct from the other members of the Ectocarpales. Both genera differ from the ectocarpoids in several aspects: type of early development, chloroplast shape, pyrenoid distribution and reproductive organs (Nakamura 1972). For example, *Analipus* and *Ralfsia* have discal-type early development, while the ectocarpoids have heterotrichous type early development; both *Analipus* and *Ralfsia* lack pyrenoids, while the ectocarpoids have pyrenoids . However, Nelson (1982) challenged the validity of the Ralfsiales as she noted "inconsistencies in the delimitaion of the Ralfsiales", and therefore, proposed the reinstatement of *Analipus* and *Ralfsia* to the Ectocarpales. Adding to the controversy, Kawai (1991) stated that "the order Ralfsiales itself remains invalid because of the lack of a Latin diagnosis (ICBN, Art. 36.2; see Greuter 1988)". Prior to Nelson's (1982) challenge, the acceptance of the Ralfsiales as an order, was equivocal, with recognition by some authors (Bold and Wynne 1978, Tanaka and Chihara 1982) but not by others (John and Lawson 1974, Russell and Fletcher 1975, Abbott and Hollenberg 1976). In spite of Nelson's proposal, the recognition of this order remains equivocal (Pritchard and Bradt 1984, Gabrielson et al. 1989).

The disagreement among phycologists regarding the validity of the Ralfsiales as well as the unresolved relationships of this putative order to other brown algal orders, was the impetus for investigating these questions with molecular tools. Specifically, partial 18S rDNA sequence data from *Analipus japonicus* and *Ralfsia fungiformis* were compared with putative close relatives: members of the Ectocarpales, Chordariales, Dictyosiphonales and Scytosiphonales (ECDS). The taxonomic treatment of these taxa is confusing because it varies among different authors. For example, Gabrielson et al. (1989) placed members of these 5 orders (*sensu* Wynne 1982), including the Ralfsiales, into 9 different families within the order Ectocarpales (Table 4). The relationships among the Ralfsiales, the ECDS and 8 other orders (Dictyotales, Sphacelariales, Syringodermatales, Desmarestiales, Sporochnales, Tilopteridales, Cutleriales and Fucales) were examined by comparison of partial 18S rDNA sequences.

Table 4. Different taxonomic treatments of selected brown algal (Phaeophyta) taxa (Wynne 1982, Gabrielson et al 1989), their voucher specimen accession numbers and their GenBank accession numbers for partial 18S rDNA sequences (Bilofsky and Burks 1988). Members of the Ectocarpales, Chordariales, Dictyosiphonales and Scytosiphonales (*sensu* Wynne) were placed in different families within the order Ectocarpales by Gabrielson et al. (1989).

	Wynne (1982)	**Gabrielson et al. (1989)**	**UBC Herbarium accession numbers**	**GenBank accession numbers**
Ectocarpus siliculosus	Ectocarpales	Ectocarpales Ectocarpaceae	A81059	L17015
Scytosiphon lomentaria	Scytosiphonales	Ectocarpales Scytosiphonaceae	A80359	L17016
Colpomenia peregrina	Scytosiphonales	Ectocarpales Scytosiphonaceae	A80361	L17014
Asperococcus bullosus	Dictyosiphonales	Ectocarpales Dictyosiphonaceae	A80357	L17013
Punctaria expansa	Dictyosiphonales	Ectocarpales Punctariaceae	A80363	L17010
Leathesia difformis	Chordariales	Ectocarpales Leathesiaceae	A80362	L16915
Haplogloia andersonii	Chordariales	Ectocarpales Chordariaceae	A80365	L17012

Table 4. (Continued)

	Wynne (1982)	Gabrielson et al. (1989)	UBC Herbarium accession numbers	GenBank accession numbers
Elachista fucicola	Chordariales	Ectocarpales Elachistaceae	A80360	L17011
Analipus japonicus	Ralfsiales	Ectocarpales Heterochordariaceae	A81060	L17009
Ralfsia fungiformis	Ralfsiales	Ectocarpales Ralfsiaceae	A81056	L17019
Taonia atomaria	Dictyotales	Dictyotales	A81058	L17011
Sphacelaria furcigera	Sphacelariales	Sphacelariales	A81055	L17020
Syringoderma phinneyi		Syringodermatales	A81057	L17017

METHODS AND MATERIALS

Specimen preparation

Brown algal taxa included in the study included both field and culture samples (Table 1). Culture samples were provided by Drs. Eric Henry (Oregon State University) and Dieter Müller (University of Konstanz). The field collected samples were cleaned by removing obvious epiphytes and rinsing several times with distilled water. Samples which were not processed (DNA extraction) immediately were air dried at room temperature. All the received cultures were maintained with f/2 enrichment medium (Fritz Chemical Company, Dallas, Texas, USA). Voucher specimens for most of the studied taxa were deposited in the University of British Columbia Herbarium (UBC Herbarium), Vancouver, British Columbia, Canada.

Genomic DNA extraction

Prior to DNA extraction, the cultures were compacted and collected by centrifugation at 3,000 x g for 2 minutes. Both fresh and dried plant materials were ground in liquid nitrogen to fine powder with a mortar and pestle. About 0.02 g of ground material was deposited and further ground in a 1.5 ml microcentrifuge tube together with 5 µl of Proteinase K (Sigma P0390; 20mg.ml^{-1} stock) and 100 µl of protease buffer [50 mM EDTA, 100 mM tris hydroxymethyl aminomethane (tris buffer) pH 8.5 (calibrated with HCl), 200 mM NaCl and 1% lauryl sulphate (SDS)] (Emmons et al. 1979) with a disposable pellet pestle mixer (VWR Scientific KT95050-99). The ground mixture was topped up with an additional 400 µl of protease buffer before incubating at 65° C water bath for 1 hour with frequent inversions to facilitate organelle lysis. Proteins were removed from the lysate with a series of phenol, followed by chloroform-isoamyl alcohol extractions (Maniatis et al. 1982). A minimum of 3 phenol extractions were conducted or until no whitish flocculent material was detected at the aqueous/phenol interphase. Centrifugation between each extraction was for 2 minutes at 12,000 x g in a microcentrifuge. The final phenol extraction was followed by a chloroform-

isoamyl alcohol (24:1 v/v) extraction. The aqueous phase was drawn into a clean 1.5 ml microcentrifuge tube; 0.4 vol 5M ammonium acetate and 2 vol 95% ethanol (stored at -20° C) were added. Precipitation of DNA was carried out by storing at -20° C for 2-3 hours. DNA was pelleted by centrifugation at 12,000 x g for 30 minutes in a microcentrifuge. The slightly coloured (brownish) pellet was washed with 1.5 ml of 70% ethanol (stored at -20° C) and recentrifuged as above. The pellets were air dried at room temperature. Fifty µl of autoclaved double distilled water was added to resuspend the dried pellet.

The crude genomic DNA extract was gel-purified to remove contaminating polysaccharides prior to amplification via the polymerase chain reaction (PCR) methods (Saiki et al. 1988). The DNA sample was electrophoresed on a 0.8% agarose gel (0.2 µg/ml ethidium bromide) with 1 x TBE (100 mM Tris base, 100 mM boric acid, 2 mM disodium EDTA) electrophoresis buffer (Saunders 1993). The high molecular weight DNA was excised from the gel under ultraviolet illumination (>300nm). The DNA was then recovered from the gel slice using Sephaglas BandPrep™ (Pharmacia) following the manufacturer's protocol. This gel-purified DNA was ready for PCR. Gel-purification of the crude DNA extract can be circumvented by

simply diluting the crude DNA extract 500 - 1000 fold in distilled water prior to PCR.

Polymerase Chain Reaction

The 18S rDNA was amplified by PCR in 2 sections by using specific oligonucleotide primers: LD1 + LDC and LDD + LDF (Table 5). The PCR reactions were performed using the Gene-Amp® Kit (Perkin-Elmer Cetus) following the manufacturer's recommendations. Amplification was conducted on an automated thermocycler following these settings: initial cycle (denature at 95° C for 5 min; anneal at 55° C for 30 sec; extension at 72° C for 2 min), 28 cycles (denature at 95° C for 30 sec; anneal at 55° C for 30 sec; extension at 72° C for 2 min) and final cycle (denature at 95° C for 30 sec; anneal at 55° C for 30 sec; extension at 72° C for 10 min). A negative control, using all the primers and PCR reagents minus template DNA, was included in every PCR reaction. The double stranded PCR products were gel-purified using either Sephaglas BandPrep™ (Pharmacia) or Prep-A-Gene® (Biorad) DNA purification matrix protocols and kits.

Table 5. Sequences of oligonucleotide primers (Saunders and Druehl 1992) used in polymerase chain reaction (PCR) methods (Saiki et al. 1985) and direct sequencing via the dideoxynucleotide chain terminating protocol (Sanger et al. 1977).

Coding strand complement

Primer	Sequence
LD1	$^{5'}$AATCTGGTTGATCCTGCCAG$^{3'}$
LDA	$^{5'}$CGATTCCGGAGAGGGAGCCTG$^{3'}$
LDB	$^{5'}$GTCTGGTGCCAGCAGCCGCGG$^{3'}$
LDD	$^{5'}$CAGAGGTGAAATTCTTGGAT$^{3'}$
LD7	$^{5'}$CTGAAACTTAAAGAAATTGACCG$^{3'}$
LDE	$^{5'}$GGTGGTGGTGCATGGCCGTTC$^{3'}$
P1	$^{5'}$TAATCTGTTGAACGTGCATCG$^{3'}$

Noncoding strand complement

Primer	Sequence
LDF	$^{5'}$GATCCTTCTGCAGGTTCACCTAC$^{3'}$
P2	$^{5'}$CTATCACGATGCACGTTCAACAG$^{3'}$
LDC	$^{5'}$GAACGGCCATGCACCACCACC$^{3'}$
LD6	$^{5'}$ATCCAAGAATTTCACCTCTG$^{3'}$
LD5	$^{5'}$CCGCGGCAGCTGGCACCAGAC$^{3'}$
LD4	$^{5'}$TCAGGCTCCCTCTCCGG$^{3'}$

Direct sequencing of PCR products

The purified PCR products were sequenced directly using the Sequenase® kit (United States Biochemicals) by following a modified version (T. Snutch, University of British Columbia, Vancouver, B.C., Canada) of the dideoxynucleotide chain terminating protocol (Sanger et al. 1977) using ^{35}S dATP. The modified sequencing protocol incorporated dimethyl sulphoxide [DMSO (BDH Inc.)] in the initial denaturing step of the double stranded template. Thirteen oligonucleotide primers (Table 5) were used in sequencing the two PCR fragments. Labeled fragments were subsequently separated in 6% acrylamide gels with 1 X TBE buffer at 60 W.

Sequence Analysis

All the sequences were manually read from the autoradiographs and aligned manually using the multisequence editing program [Eyeball Sequence Editor (ESEE)] (Cabot and Beckenbach 1989). The aligned sequences were subjected to both distance matrix analysis and maximum parsimony analysis

for phylogeny inference. Even though the neighbor-joining method (Saitou and Nei 1987) was shown to be more efficient at inferring the correct phylogeny compared to other methods (Saitou and Imanishi 1989, Kim et al. 1993), the parsimony method was included in the analysis for comparison purposes. However, parsimony analysis was shown to be effective at using derived characters to infer phylogenetic trees (see review by Stewart 1993).

The distance matrix analysis was done with various computer programs in the MEGA [Molecular Evolutionary Genetics Analysis version 1.02 (Kumar et al. 1993)] package. SAV2MEGA (Andrew Beckenbach, Institute of Molecular Biology and Biochemistry, Simon Fraser University, Burnaby, B.C., Canada) was used to convert the saved ESEE files to MEGA formatted input files. A Kimura (1980) 2-parameter distance matrix was generated from the aligned sequences. The resulting distance matrix was subjected to the neighbor-joining algorithm to reconstruct a phylogenetic tree. Bootstrap analysis (500) replicates was used to generate estimates of confidence intervals on the distance matrix trees (Felsenstein 1985, Sanderson 1989). Unrooted neighbor-joining trees were drawn by the DRAWTREE program of PHYLIP 3.5c [Phylogeny Inference Package (Felsenstein 1993)]. The input files for PHYLIP were formatted by the export option in MEGA. For the

purposes of this study, the partial 18S rDNA sequence from the xanthophyte *Tribonema aequale* Pascher (Aritztia et al. 1991) was used as an outgroup.

Parsimony analysis of the 18S rDNA sequences was done with the PAUP 3.1.1 computer package (Swofford 1993). The input files for PAUP were formatted by the export option in MEGA. The branch-and-bound and heuristic search options were used. The RANDOM TREES feature of PAUP was used to generate 100,000 random trees and to calculate g1 skewness statistic from the set of all possible trees.

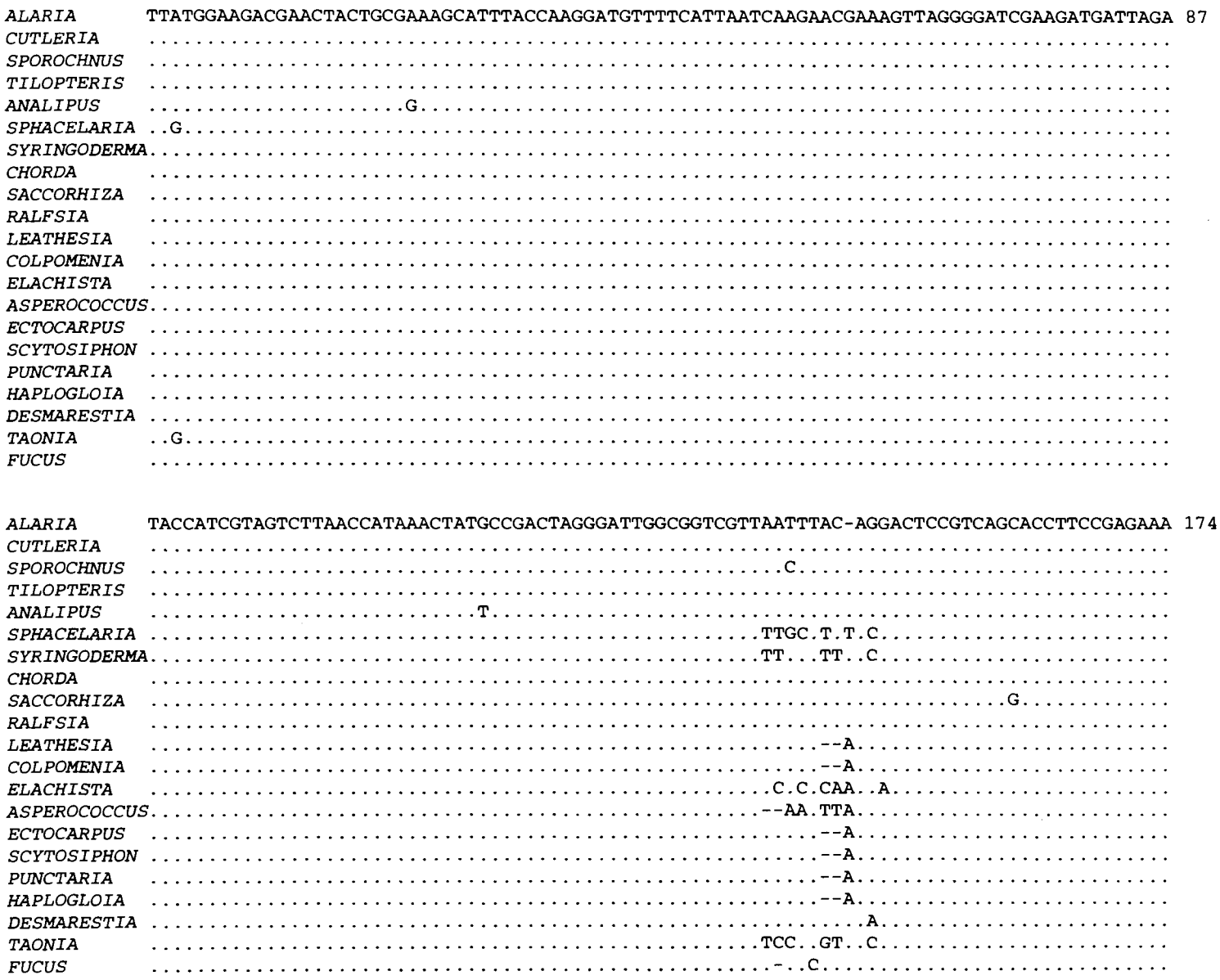
RESULTS

Analysis of partial 18S rDNA sequences

The partial 18S rDNA sequences (870 nucleotides) of 19 brown algae representing 13 orders were determined (Table 4) and compared with published brown algal sequences (Bhattacharya et al. 1992, Saunders and Druehl 1992) (Fig. 6). Pairwise distance values (numbers of substitution per site) between the aligned sequences were generated by MEGA (Table 6); the greatest divergence between any two brown algae was 6.69% (*Fucus gardneri* vs *Ectocarpus siliculosus*), and the least divergence between any two brown algae was 0.12% (*Punctaria expansa* vs *Leathesia difformis*).

A neighbor-joining tree (unrooted) was generated by PHYLIP ver. 3.4 (Fig. 7). The studied taxa were separated into two distinct groups. Members of the Ectocarplaes, Chordariales, Dictyosiphonales and Scytosiphonale (ECDS) were grouped into one lineage while the other taxa were grouped into the other lineage. The branching orders within each group were examined by the bootstrap analysis (500 replicates) done by MEGA. *Fucus gardneri* was used as an outgroup in the neighbor-joining analysis; the bootstrapped

Fig. 6. Sequence alignment of partial 18S rDNA sequences from 21 brown algal taxa representing 14 orders. *Alaria* sequence was determined by Saunders and Druehl (1992) and the *Fucus* sequence was determined by Bhattacharya et al. (1992).


ALARIA TTATGGAAGACGAACTACTGCGAAAGCATTTACCAAGGATGTTTTCATTAATCAAGAACGAAAGTTAGGGGATCGAAGATGATTAGA 87
CUTLERIA
SPOROCHNUS
TILOPTERIS
ANALIPUS
SPHACELARIA
SYRINGODERMA
CHORDA
SACCORHIZA
RALFSIA
LEATHESIA
COLPOMENIA
ELACHISTA
ASPEROCOCCUS
ECTOCARPUS
SCYTOSIPHON
PUNCTARIA
HAPLOGLOIA
DESMARESTIA
TAONIA
FUCUS
ALARIA TACCATCGTAGTCTTAACCATAAACTATGCCGACTAGGGATTGGCGGTCGTTAATTTAC-AGGACTCCGTCAGCACCTTCCGAGAAA 174
CUTLERIA
SPOROCHNUS
TILOPTERIS
ANALIPUS
SPHACELARIA
SYRINGODERMA
CHORDA
SACCORHIZA
RALFSIA
LEATHESIA
COLPOMENIA
ELACHISTA
ASPEROCOCCUS
ECTOCARPUS
SCYTOSIPHON
PUNCTARIA
HAPLOGLOIA
DESMARESTIA
TAONIA
FUCUS

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ALARIA        TCAAAGTCTTTGGGTTCCGGGGGGAGTATGGTCGCAAGGCTGAAACTTAAAGAAATTGACGGAAGGGCACCACCAGGAGTGGAGCCT 261
CUTLERIA      ...............................................................................................
SPOROCHNUS    ...............................................................................................
TILOPTERIS    ...............................................................................................
ANALIPUS      ...............................................................................................
SPHACELARIA   ...............................................................................................
SYRINGODERMA...............................................................................................
CHORDA        ...............................................................................................
SACCORHIZA    ...............................................................................................
RALFSIA       ..............................................................................................A.
LEATHESIA     ...............................................................................................
COLPOMENIA    ...............................................................................................
ELACHISTA     ...............................................................................................
ASPEROCOCCUS..............................................................................................
ECTOCARPUS   ...............................................................................................
SCYTOSIPHON  ...............................................................................................
PUNCTARIA    ...............................................................................................
HAPLOGLOIA   ...............................................................................................
DESMARESTIA  ...............................................................................................
TAONIA       ...............................................................................................
FUCUS        ...............................................................................................

ALARIA        GCGGCTTAATTTGACTCAACACGGGGAAACTTACCAGGTCCGGACATAGTGAGGATTGACAGATTGAGAGCTCTTTCTTGATTCTAT 348
CUTLERIA      ...............................................................................................
SPOROCHNUS    ...............................................................................................
TILOPTERIS    ...............................................................................................
ANALIPUS      ...............................................................................................
SPHACELARIA   ...-...........................................................................................
SYRINGODERMA.....................A.....................................................................
CHORDA        ...............................................................................................
SACCORHIZA    ...............................................................................................
RALFSIA       ...............................................................................................
LEATHESIA     ...-...........................................................................................
COLPOMENIA    ...-...........................................................................................
ELACHISTA     ...............................................................................................
ASPEROCOCCUS..............................................................................................
ECTOCARPUS   ...-...........................................................................................
SCYTOSIPHON  ...............................................................................................
PUNCTARIA    ...............................................................................................
HAPLOGLOIA   ...............................................................................................
DESMARESTIA  ...............................................................................................
TAONIA       ...............................................................................................
FUCUS        ...............................................................................................
```

```
ALARIA        GGGTGGTGGTGCATGGCCGTTCTTAGTTGGTGGAGTGATTTGTCTGGTTAATTCCGTTAACGAACGAGACCCCCGCCTGCTAAATAG 435
CUTLERIA      ...............................................................................
SPOROCHNUS    ...............................................................................
TILOPTERIS    ...............................................................................
ANALIPUS      ...............................................................................
SPHACELARIA   ...............................................................................
SYRINGODERMA...............................................................................
CHORDA        ...............................................................................
SACCORHIZA    ...............................................................................
RALFSIA       ...............................................................................
LEATHESIA     ...............................................................................
COLPOMENIA    ...............................................................................
ELACHISTA     ...............................................................................
ASPEROCOCCUS...............................................................................
ECTOCARPUS    ...............................................................................
SCYTOSIPHON   ...............................................................................
PUNCTARIA     ...............................................................................
HAPLOGLOIA    ...............................................................................
DESMARESTIA   ...............................................................................
TAONIA        ...............................................................................
FUCUS         ...............................................................................

ALARIA        TGTGGCTTACGCTTCTGTGTAGGT-GCTCGCTTCTTAGAGGGACTTTCGGTGACTAACCGAAGGAAGTTGGGGGCAATAACAGGTCT 522
CUTLERIA      .....TC.......T..C......A-.....................................................
SPOROCHNUS    ..............T..C.....................................................
TILOPTERIS    ..............T..C......A-.....................................................
ANALIPUS      ..............T..C......A-.....................................G.A.....
SPHACELARIA   ..............T..-......A-.....................................-.....
SYRINGODERMA...............T..C......A-.....................................
CHORDA        ..............T..C.....................................-.....
SACCORHIZA    ..............TA.C.....-.....................................-.....
RALFSIA       ..............T..C......A-.....................C..T.....
LEATHESIA     ..............T..-.....................CT.....AG.....
COLPOMENIA    ..............T..C.....................CT.....AG.....
ELACHISTA     ..............T..C.....................CT.....AG.....
ASPEROCOCCUS...............T..C.....................CA.....TG.....
ECTOCARPUS    ..............T..C......A-.....................CT.....AG.....
SCYTOSIPHON   ..............T..-.....................CT.....AG.....
PUNCTARIA     ..............T..C.....................CT.....AG.....
HAPLOGLOIA    ..............T..-......A-.....................CT.....AG.....
DESMARESTIA   ..............T..C......A-.....................-.....
TAONIA        ..G...........T..-......A-.....
FUCUS         ..G........T....AC.....CA.....
```

```
ALARIA          GTGATGCCCTTAGATG-TCCTGGGCCGCACGCGCGCTA-CACTGATGCATGCAACGAGTTCTTTTTTTTCCTGGGTCGAGAGGCCCG 609
CUTLERIA        ..............................................................---...C.................
SPOROCHNUS      ..............................................................C.....................
TILOPTERIS      ..............................................................C.....................
ANALIPUS        ..............................................................T.--C.................
SPHACELARIA     ..............................................................TCA..G..-.................
SYRINGODERMA    ..............................................................TA---.G.......C........G...
CHORDA          ..............................................................--.....................-.
SACCORHIZA      ..............................................................-.....................
RALFSIA         ..............................................................AC--..................
LEATHESIA       ..............................................................T.-...................
COLPOMENIA      ..............................................................T.-A..................
ELACHISTA       ..............................................................TC--..................
ASPEROCOCCUS    ..............................................................---...................
ECTOCARPUS      ..............................................................T.-AC.................
SCYTOSIPHON     ...............A..............................................T.-A........A...........T.
PUNCTARIA       ..............................................................T.---.................
HAPLOGLOIA      ...........................................T..................T.---.................
DESMARESTIA     ..............................................................AC--..................
TAONIA          ..............................................................TC-GACGC..............-.
FUCUS           ..............................................................CAA.C.C......C..GA...T...

ALARIA          GGTAATCTGTTGAACGTGCATCGTGATAGGGATAGATCATTGCAATTATTGATCTTGAACGAGGAATTCCTAGTAAACGCGAGTCAT 696
CUTLERIA        ................................................................................
SPOROCHNUS      ..........G.....................................................................
TILOPTERIS      ................................................................................
ANALIPUS        ................................................................................
SPHACELARIA     ..............................................................C.................
SYRINGODERMA    ................................................................................
CHORDA          ..............................................................................AT
SACCORHIZA      ................................................................................
RALFSIA         ................................................................................
LEATHESIA       ................................................................................
COLPOMENIA      ........T.......................................................................
ELACHISTA       ................................................................................
ASPEROCOCCUS    ................................................................................
ECTOCARPUS      ................................................................................
SCYTOSIPHON     ..-.....T.......................................................................
PUNCTARIA       ........T.......................................................................
HAPLOGLOIA      ................................................................................
DESMARESTIA     ................................................................................
TAONIA          .....C..T.............................T...T......C.............................
FUCUS           .....C..T.............................TT...T..A....A.............................
```

```
ALARIA        CAGCTCGCATTGATTACGTCCCTGCCCTTTGTACACACCGCCCGTCGCACCTACCGATTGAATGTTTCGGTGAAGATTCCGGACTGT 783
CUTLERIA      ..............................................................................C..
SPOROCHNUS    ..............................................................................
TILOPTERIS    ..............................................................................C..
ANALIPUS      ..............................................................................C..
SPHACELARIA   ..............................................................................C..
SYRINGODERMA..............................................................................C..
CHORDA        ..............................................................................C..
SACCORHIZA    ..............................................................................C..
RALFSIA       ..............................................................................C..
LEATHESIA     ..........................................................CA........G.........T.CC
COLPOMENIA    ..........................................................CA........G...CT....T.T.
ELACHISTA     ..........................................................CA........G.........T.T.
ASPEROCOCCUS..............................................................CA........G.........T.CC
ECTOCARPUS    ..........................................................CA........G...CT....T.T.
SCYTOSIPHON   ..........................................................CA........G...CT....T.T.
PUNCTARIA     ..........................................................CA........G.........T.CC
HAPLOGLOIA    ..........................................................CA........G.........T.CC
DESMARESTIA   ..............................................................................C..
TAONIA        ..............................................................................C..
FUCUS         ..............................................................................C.G

ALARIA        GGCTCGCGTGCTTCACGGCGC-TCTTGCCGTGGGAAGTTATCTAAACCTCAACATTTAGAGGAAGGTGAAGTCGTAACAAGGTTTCC 870
CUTLERIA      ........C.....................................................................
SPOROCHNUS    ........C.........CG...C....A.....................................................
TILOPTERIS    ........C.....................................................................
ANALIPUS      ........C.....................................................................
SPHACELARIA   ......T.C............G.C.....G.....................................................
SYRINGODERMA......T.C..........-...C.....................................................
CHORDA        ........C.........CG..........................................................
SACCORHIZA    ........C.........CG..........................................................
RALFSIA       ........C...........C.........................................................
LEATHESIA     .TAG.TTAC..........T..T..TA..AAAA........C........TG.........................
COLPOMENIA    .TT.TTTA...........T..TCA.A..A.A........C........TG.........................
ELACHISTA     .TTG.TTAC....T.....T..T...A..AAA........C........TG.........................
ASPEROCOCCUS.TAG.TTAC..........T..T..TA..AAAA........C........TG.........................
ECTOCARPUS    .TTG.TTAC.-........T..T.C.A..A.A.....C...C........TG.........................
SCYTOSIPHON   .TT.TTTAC..........T..TCA.A..A.A.....C...C........TG.........................
PUNCTARIA     .TAG.TTAC..........T..T..TA..AAAA........C........TG.........................
HAPLOGLOIA    .T.ATTTAC..........T..TC..AT.AAA........C........TG.........................
DESMARESTIA   ....T...C...........C.............................T.........................
TAONIA        ....A...C..............G.........................................................
FUCUS         AA..T.A.C.....G.......A..A..GAA.................................................
```

Table 6. Pairwise distance values between partial small-subunit ribosomal DNA sequences of selected brown algae*. Pairwise distance values (numbers of substitution per site), generated by MEGA (Kumar et al. 1993) using the Kimura (1980) two-parameter model, are expressed as percentages.

	AL	CM	SC	TM	AJ	SF	SPh	CT
AL		0.59	0.83	0.36	0.95	1.79	1.43	0.71
CM			0.95	0.24	0.83	1.67	1.31	0.59
SC				0.71	1.31	1.79	1.55	0.83
TM					0.59	1.43	1.07	0.35
AJ						1.79	1.43	0.95
SF							1.43	1.79
SPh								1.43
CT								

Table 6. (Continued).

	SPo	RF	LD	CP	EF	AB	ES	SL
AL	0.71	0.95	3.51	3.51	3.64	3.63	3.51	3.88
CM	0.59	0.83	3.63	3.88	3.76	3.75	3.64	4.01
SC	0.83	1.31	3.88	3.88	4.01	3.87	3.88	4.01
TM	0.35	0.59	3.38	3.63	3.51	3.5	3.39	3.76
AJ	0.95	1.07	3.5	3.76	3.63	3.87	3.51	3.88
SF	1.79	1.67	4.25	4.25	3.88	4.37	4.26	4.38
SPh	1.43	1.43	4.0	4.0	4.0	4.37	4.0	4.13
CT	0.47	0.95	3.75	4.0	3.88	3.87	3.75	4.13
SPo		0.95	3.75	4.0	3.88	3.87	3.75	4.13
RF			3.88	4.13	3.76	4.12	3.88	4.26
LD				1.67	1.07	0.59	1.19	1.79
CP					1.55	2.28	0.83	0.36
EF						1.55	1.07	1.67
AB							1.79	2.4
ES								0.71
SL								

Table 6. (Continued).

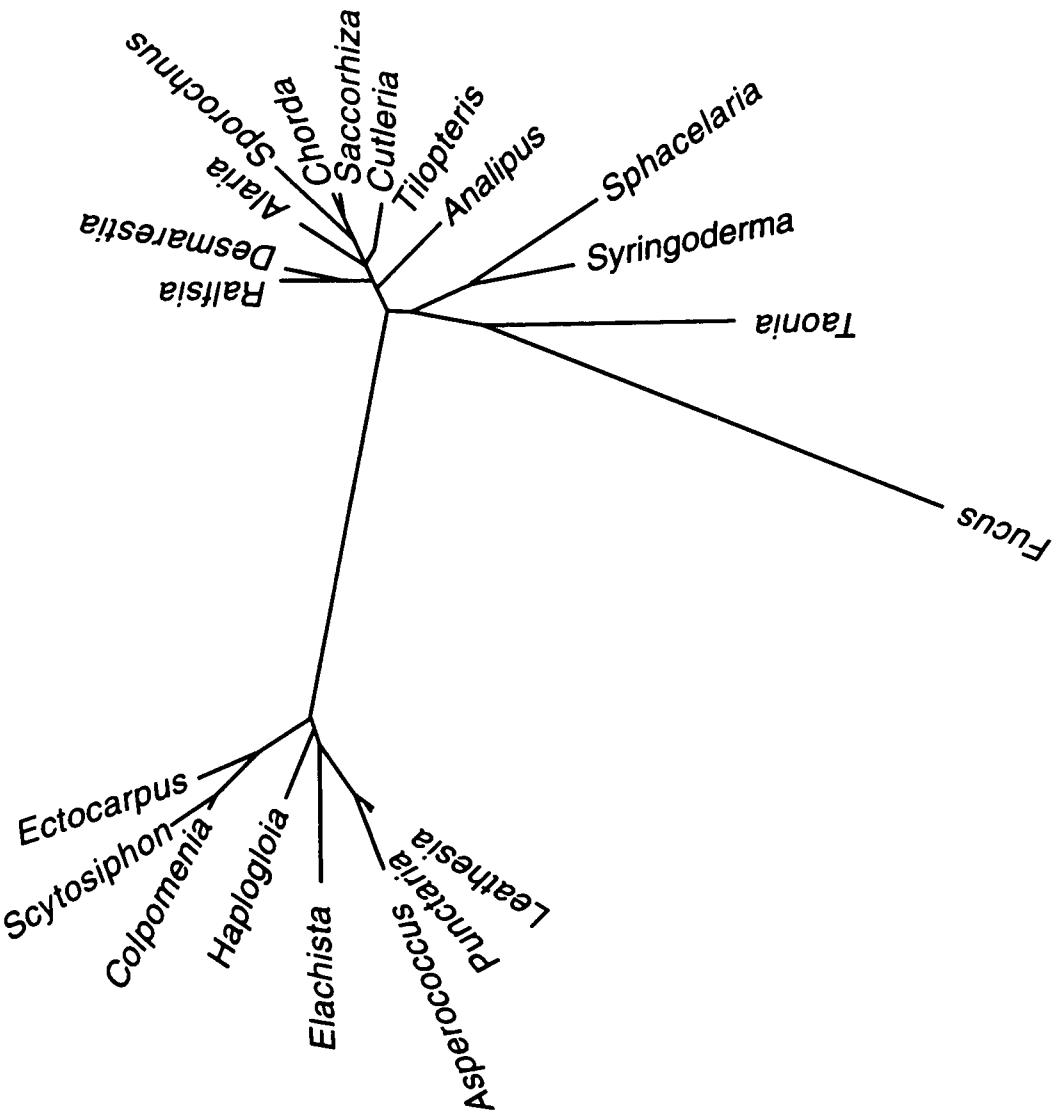
	PE	HA	DL	TA	FG
AL	3.63	3.51	0.95	2.28	3.64
CM	3.75	3.64	0.83	2.16	3.76
SC	4.0	3.63	1.31	2.4	4.01
TM	3.5	3.39	0.59	1.91	3.51
AJ	3.63	3.51	1.07	2.28	4.14
SF	4.38	4.0	1.55	2.15	4.64
SPh	4.12	3.75	1.31	2.15	4.25
CT	3.88	3.75	0.95	2.28	3.88
SPo	3.88	3.75	0.95	2.28	3.88
RF	4.0	3.88	0.71	2.15	4.13
LD	0.12	0.83	3.75	5.13	6.42
CP	1.55	1.31	3.76	4.89	6.05
EF	1.19	1.31	3.63	5.01	6.69
AB	0.71	1.43	4.0	5.38	6.54
ES	1.31	1.44	3.76	5.01	6.43
SL	1.67	1.43	3.88	5.02	6.18
PE		0.95	3.88	5.01	6.3

Table 6. (Continued).

	DL	TA	FG
HA	3.51	5.01	6.04
DL		1.91	3.88
TA			3.89
FG			

*Taxa are abbreviated as follows: AM=*Alaria marginata*; CM=*Cutleria multifida*; SC=*Sporochnus comosus*; TM=*Tilopteris mertensii*; AJ=*Analipus* japonicus; SF=*Sphacelaria furcigera*; SPh=*Syringoderma phinneyi*; CT=*Chorda tomentosa*; SPo=*Saccorhiza polyschides*; RF=*Ralfsia fungiformis*; LD=*Leathesia difformis*; CP=*Colpomenia peregrina*; EF=*Elachista fucicola*; AB=*Asperococcus bullosus*; ES=*Ectocarpus siliculosus*; SL=*Scytosiphon lomentaria*; PE=*Punctaria expansa*; HA=*Haplogloia andersonii*; DL=*Desmarestia ligulata*; TA=*Taonia atomaria*; FG=*Fucus gardneri*.

Fig. 7. Unrooted neighbor-joining tree based on partial 18S rDNA sequences. The studied taxa were separated into 2 clusters. Cluster on the left included representatives of the Ectocarpales,Chordariales, Dictyosiphonales and Scytosiphonales. The other studied taxa were grouped the other cluster (right).


Sporochnus
Alaria
Chorda
Saccorhiza
Cutleria
Tilopteris
Analipus
Sphacelaria
Syringoderma
Taonia
Fucus
Ralfsia
Desmarestia
Ectocarpus
Scytosiphon
Colpomenia
Haplogloia
Elachista
Asperococcus
Punctaria
Leathesia

consensus tree is shown in Figure 8. The 50% majority-consensus tree from the neighbor-joining analysis showed *Punctaria expansa* (Dictyosiphonales), *Asperococcus bullosus* (Dictyosiphonales) and *Leathesia difformis* (Chordariales) grouped together in 85% of the 500 bootstrap replicates (Fig. 9). Within this assemblage, *L. difformis* and *P. expansa* formed a clade 97% of the time. Similarly, the two members of the Scytosiphonales, *Scytosiphon lomentaria* and *Colpomenia peregrina*, grouped together 87% of the time. Both *Analipus japonicus* and *Ralfsia fungiformis* were not included within the ECDS clade. *Analipus* and *Ralfsia* were shown to be associated with members of the Desmarestiales, Laminariales, Sporochnales, Tilopteridales and Cutleriales. The bootstrap values among these taxa were mostly less than 50% except for the association between *Chorda tomentosa* (Chordaceae, Laminariales) and *Saccorhiza polyschides* (Phyllariaceae, Laminariales) and between *Ralfsia fungiformis* (Ralfsiales) and *Desmarestia ligulata* (Desmarestiales). *Sphacelaria furcigera* (Sphacelariales) formed a clade with *Syringoderma phinneyi* (Syringodermatales) 56% of the time. *Taonia atomaria* (Dictyotales) was excluded from the studied taxa (except for *Fucus gardneri*) 73% of the time. *F. gardneri* was shown to be most diverged of all

Fig. 8. Neighbor-joining tree with bootstrap values (500 replicates) based on partial 18S rDNA sequences. *Fucus* was used as an outgroup taxon in the analysis.

Scale bar = 1% divergence.

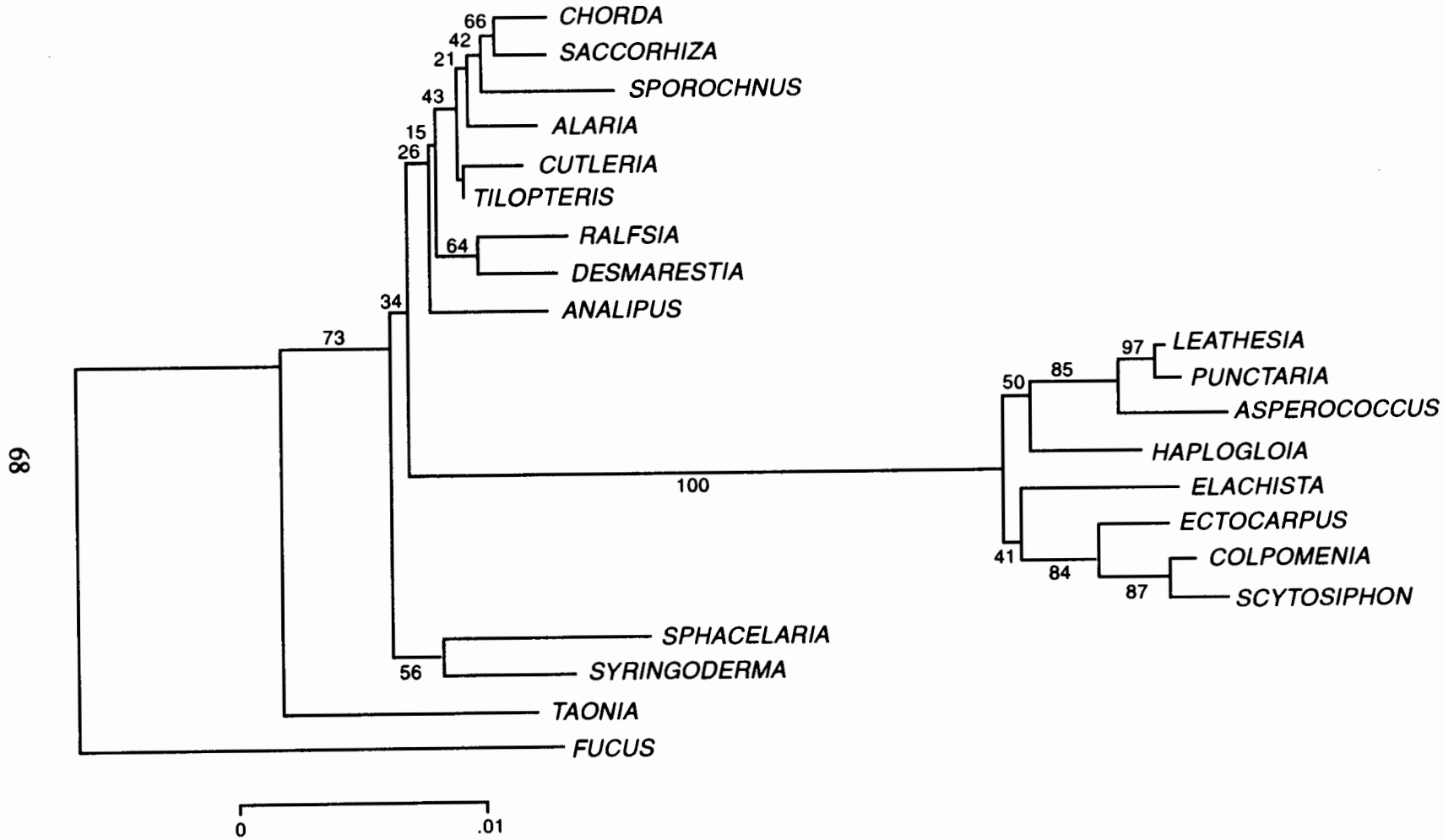
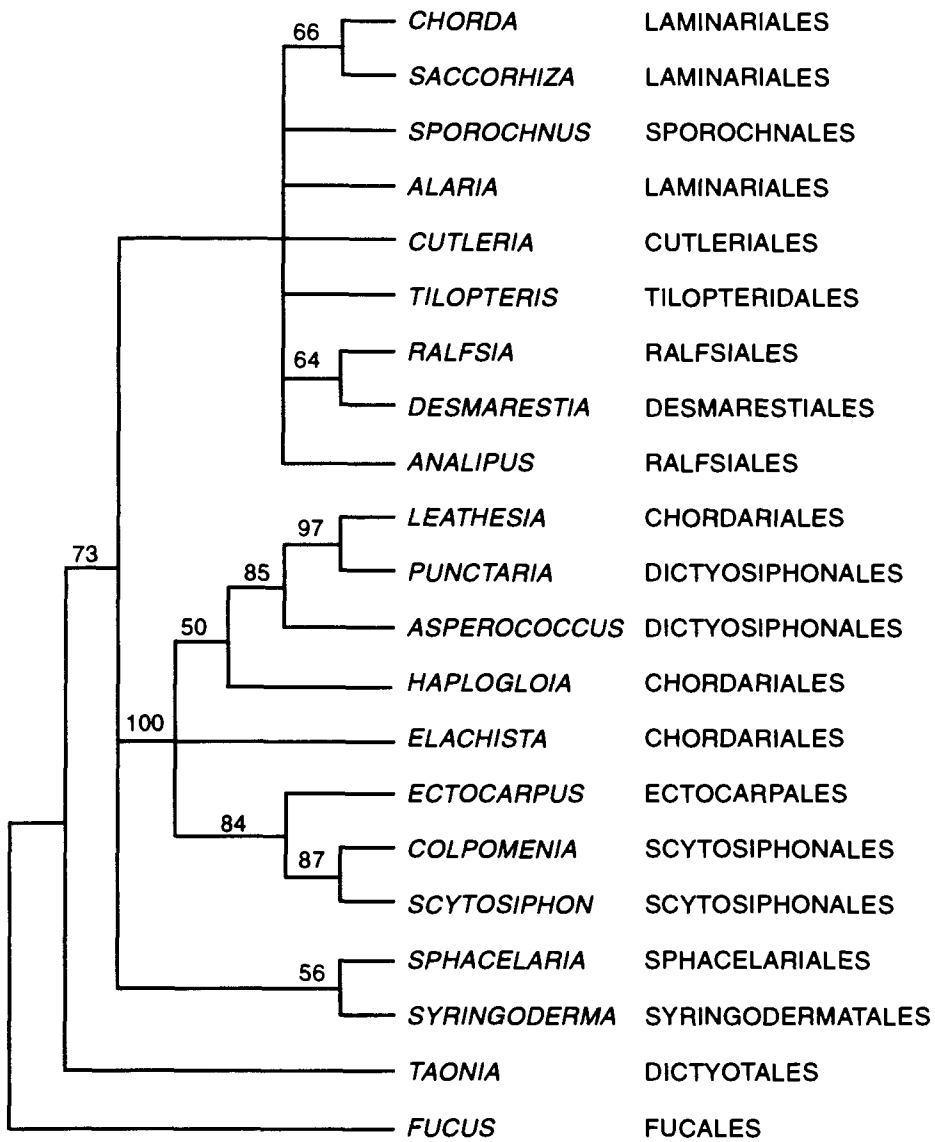

CHORDA
SACCORHIZA
SPOROCHNUS
ALARIA
CUTLERIA
TILOPTERIS
RALFSIA
DESMARESTIA
ANALIPUS
LEATHESIA
PUNCTARIA
ASPEROCOCCUS
HAPLOGLOIA
ELACHISTA
ECTOCARPUS
COLPOMENIA
SCYTOSIPHON
SPHACELARIA
SYRINGODERMA
TAONIA
FUCUS
66
42
21
43
15
26
64
34
73
97
85
50
100
41
84
87
56
89
0
.01

Fig. 9. 50% majority-rule consensus tree (neighbor-joining). Nodes with bootstrap values of less than 50% were collapsed to form a polytomy.


66
CHORDA
LAMINARIALES
SACCORHIZA
LAMINARIALES
SPOROCHNUS
SPOROCHNALES
ALARIA
LAMINARIALES
CUTLERIA
CUTLERIALES
TILOPTERIS
TILOPTERIDALES
64
RALFSIA
RALFSIALES
DESMARESTIA
DESMARESTIALES
ANALIPUS
RALFSIALES
97
LEATHESIA
CHORDARIALES
73
85
PUNCTARIA
DICTYOSIPHONALES
50
ASPEROCOCCUS
DICTYOSIPHONALES
HAPLOGLOIA
CHORDARIALES
100
ELACHISTA
CHORDARIALES
ECTOCARPUS
ECTOCARPALES
84
87
COLPOMENIA
SCYTOSIPHONALES
SCYTOSIPHON
SCYTOSIPHONALES
56
SPHACELARIA
SPHACELARIALES
SYRINGODERMA
SYRINGODERMATALES
TAONIA
DICTYOTALES
FUCUS
FUCALES

the studied taxa (Table 6). An outgroup taxon (*Tribonema aequale*) and an additional fucalean sequence [*Sargassum vestitum* (R. Brown ex Turner) C. Agardh: Saunders and Kraft (1995)] were added to the analysis, while *Colpomenia* and *Leathesia* were removed from the data set. The resulting unrooted neighbor-joining tree (Fig. 10) was congruent with the previous unrooted tree (Fig. 7); in addition, the former tree showed *Sargassum* and *Tribonema* forming an unresolved trifurcation with the *Fucus/Taonia* clade. Bootstrap analysis (500 replicates) provided 52% support for the *Fucus/Taonia* clade and 62% of the time, *Sargassum* was excluded from all the studied brown algal taxa (Fig. 11).

Among the 870 sites determined for the parsimony analysis, 57 sites were phylogenetically informative; 772 sites were invariant and 41 sites were variable but noninformative. 741 equally parsimonious trees were obtained using PAUP's heuristic search option (tree length 167 steps; CI, excluding informative characters = 0.778; RI = 0.847; trees not shown). CI equals 1 when a particular tree explains the data as well as any tree possibly could, and RI equals 0 when a character fits the tree as poorly as possible (Swofford 1993). The frequency distribution of tree lengths (g1 = -0.575) indicates that the data had a great amount of phylogenetic signal; more negative g1 values

Fig. 10. Unrooted neighbor-joining tree based on partial 18S rDNA sequences. An xanthophyte sequence was added to the analysis: *Tribonema aequale* (Aritztia et al. 1990).

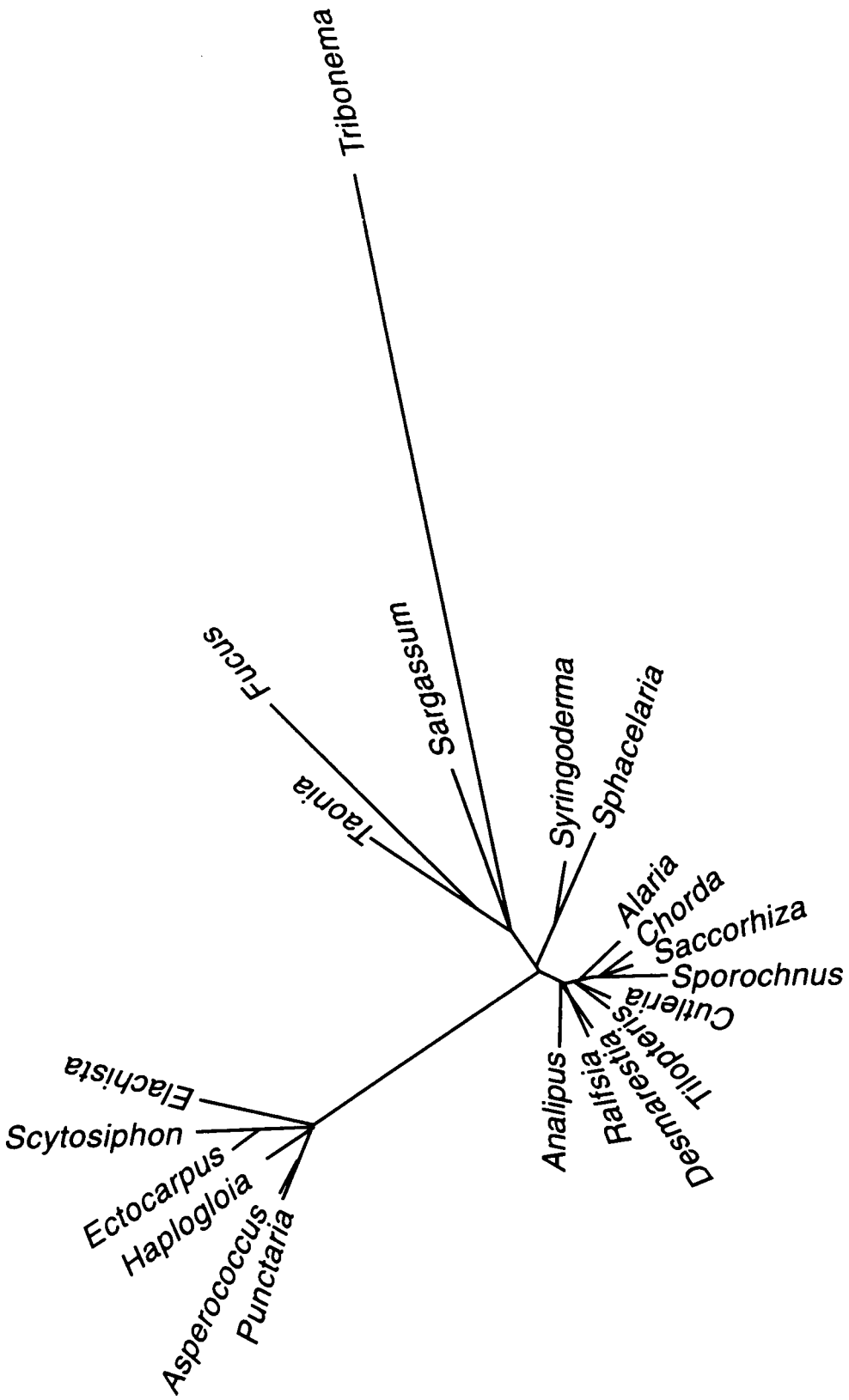
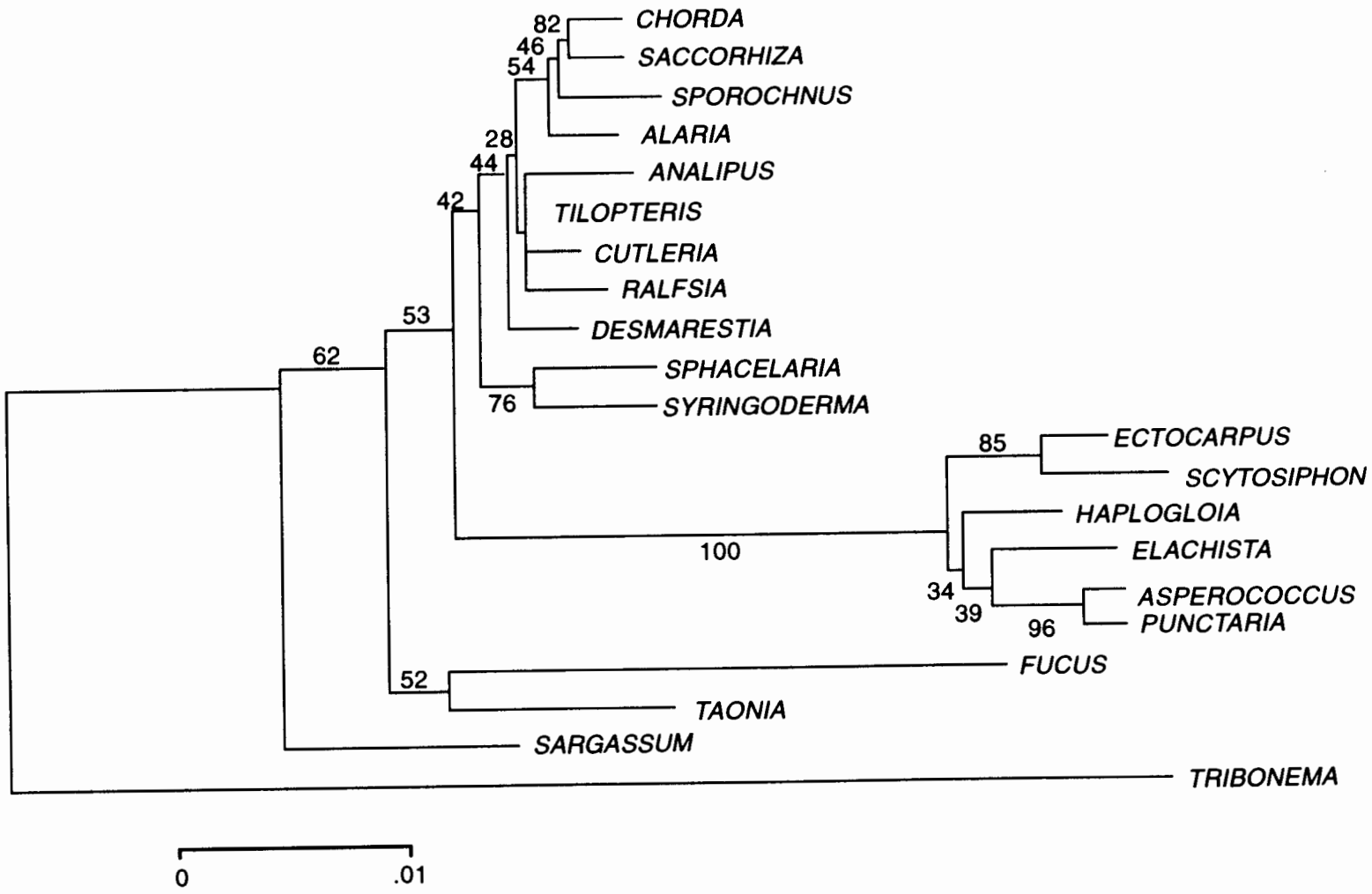

Tribonema
Sargassum
Fucus
Taonia
Syringoderma
Sphacelaria
Alaria
Chorda
Saccorhiza
Sporochnus
Cutleria
Desmarestia
Tilopteridales
Ralfsia
Analipus
Elachista
Scytosiphon
Ectocarpus
Haplogloia
Asperococcus
Punctaria

Fig. 11. Neighbor-joining tree with bootstrap values (500 replicates). *Tribonema aequale* (a xanthophyte; Aritztia et al. 1990) was used as an outgroup taxon in the analysis.

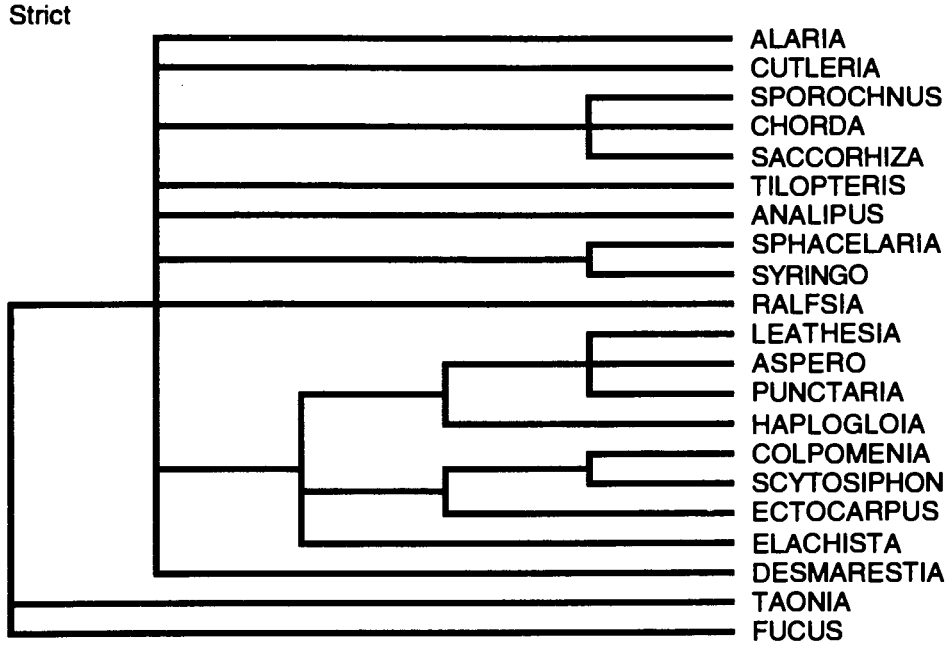
Scale bar = 1 % divergence.


82
46
54
28
44
42
53
62
76
85
100
34
39
96
52
CHORDA
SACCORHIZA
SPOROCHNUS
ALARIA
ANALIPUS
TILOPTERIS
CUTLERIA
RALFSIA
DESMARESTIA
SPHACELARIA
SYRINGODERMA
ECTOCARPUS
SCYTOSIPHON
HAPLOGLOIA
ELACHISTA
ASPEROCOCCUS
PUNCTARIA
FUCUS
TAONIA
SARGASSUM
TRIBONEMA
0
.01

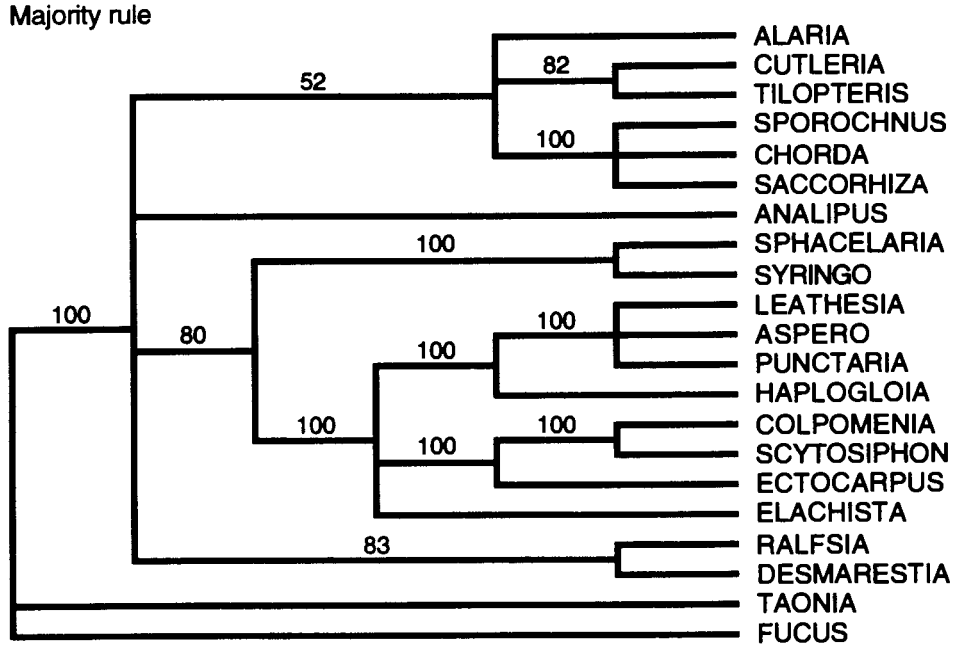
suggest greater phylogenetic signal than random noise (Hillis and Huelsenbeck 1992, Swofford 1993). The strict consensus tree and 50% majority-rule consensus tree grouped members of the ECDS together 100% of the time (Fig. 12). Within this assemblage, *Leathesia difformis* (Chordariales) formed a trifurcation with members of the Dictyosiphonales (*Punctaria expansa* and *Asperocossus bullosus*). *Haplogloia andersonii* (Chordariales) was a sister taxon to this trifurcation. 100% of the parsimonious trees grouped members of the Ectocarpales and Scytosiphonales together; the Scytosiphonales was shown to be monophyletic. The 50% majority-rule consensus tree grouped all the orders together with the exception of *Taonia atomaria* (Dictyotales) and *Fucus gardneri* (Fucales). Representatives from the Laminariales, Cutleriales, Tilopteridales and Sporochnales formed an unresolved trifurcation (52%). Within this assemblage, *Cutleria multifida* associated with *Tilopteris mertensii* (82%) while *Sporochus comosus* formed a trifurcation with *Chorda tomentosa* and *Saccorhiza polyschides* (100%). *Sphacelaria furcigera* (Sphacelariales) and *Syringoderma phinneyi* (Syringodermatales) formed a clade (100%), and *Ralfsia fungiformis* (Ralfsiales) and *Desmarestia ligulata* (Desmarestiales) formed a clade (83%). Both *Taonia atomaria* (Dictyotales) and *Fucus*

Fig. 12. Consensus trees from maximum parsimony analysis (heuristic, PAUP 3.1.1; Swofford 1993); 741 equally parsimonious trees of 167 steps, CI = 0.778 and RI = 0.847). Strict = strict consensus tree; majority rule = 50 % majority consensus tree.

Strict



Majority rule



gardneri (Fucales) were consistently (100%) excluded from the assemblage containing the studied taxa. Several branching orders among the studied taxa were unresolved. Therefore, entire 18S rDNA sequences (1792 nucleotides) were obtained for 14 taxa in order to determine if complete gene sequences provided a sufficient level of variation for resolution at the ordinal level.

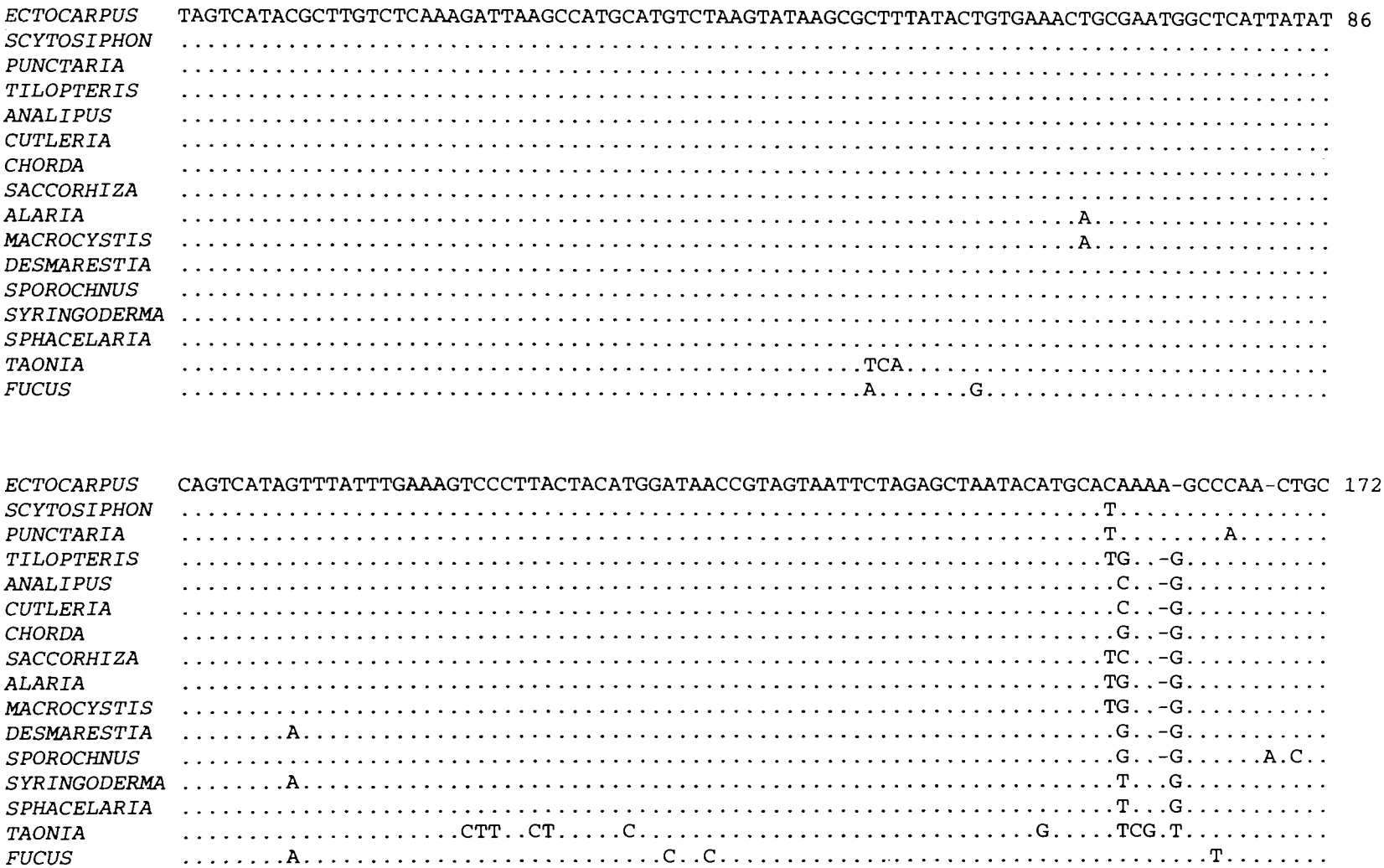
Analysis of complete 18S rDNA sequences

Complete 18S rDNA sequences (1792 nucleotides) from 13 brown algae representing 13 orders were determined (Table 7) and compared with 3 published brown algal sequences [*Fucus gardneri* (Fucales), Bhattacharya et al. 1992; *Alaria marginata* and *Macrocystis integrifolia* (Laminariales), Saunders and Druehl 1992) (Fig. 13). Pairwise distance values between the aligned sequences were generated by MEGA (Table 8); the greatest divergence between any two brown algae was 5.58% (*Fucus gardneri* vs *Taonia atomaria*) while the least divergence (0.4%) occurred between *Alaria marginata* and *Macrocystis integrifolia*.

Table 7. Selected brown algal (Phaeophyta) taxa with complete 18S rDNA sequences determined and their GenBank accession numbers (Bilofsky and Burks 1988).

	Order	**GenBank accession numbers**
Ectocarpus siliculosus	Ectocarpales	L43062
Scytosiphon lomentaria	Scytosiphonales	L43066
Punctaria expansa	Dictyosiphonales	
Leathesia difformis	Chordariales	
Sporochnus comosus	Sporochnales	L43061
Desmarestia ligulata	Desmarestiales	L43060
Taonia atomaria	Dictyotales	
Sphacelaria furcigera	Sphacelariales	
Syringoderma phinneyi	Syringodermatales	
Analipus japonicus	Ralfsiales	
Tilopteris mertensii	Tilopteridales	
Cutleria multifida	Cutleriales	
Chorda tomentosa	Laminariales	L43056
Saccorhiza polyschides	Laminariales	L43059

Fig. 13. Sequence alignment of complete 18S rDNA of 16 brown algal taxa representing 13 orders. *Fucus* sequence determined by Bhattacharya et al. (1992); *Alaria* and *Macrocystis* sequences determined by Saunders and Druehl (1992).


ECTOCARPUS TAGTCATACGCTTGTCTCAAAGATTAAGCCATGCATGTCTAAGTATAAGCGCTTTATACTGTGAAACTGCGAATGGCTCATTATAT 86
SCYTOSIPHON
PUNCTARIA
TILOPTERIS
ANALIPUS
CUTLERIA
CHORDA
SACCORHIZA
ALARIA
MACROCYSTIS
DESMARESTIA
SPOROCHNUS
SYRINGODERMA
SPHACELARIA
TAONIA
FUCUS
ECTOCARPUS CAGTCATAGTTTATTTGAAAGTCCCTTACTACATGGATAACCGTAGTAATTCTAGAGCTAATACATGCACAAAA-GCCCAA-CTGC 172
SCYTOSIPHON
PUNCTARIA
TILOPTERIS
ANALIPUS
CUTLERIA
CHORDA
SACCORHIZA
ALARIA
MACROCYSTIS
DESMARESTIA
SPOROCHNUS
SYRINGODERMA
SPHACELARIA
TAONIA
FUCUS

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ECTOCARPUS    C-TC-GGCGGACGGGTTGCATTGATTAGACCGAAACCAATGCGTCTTCGGAC-GGTTTTGTGGTGAATCATAATCACTTGCGGATC 258
SCYTOSIPHON   ...............C.....................................................................
PUNCTARIA     ............T.................T.....................................................
TILOPTERIS    T..............C.....................................................................
ANALIPUS      T...TC.........C.....................................................................
CUTLERIA      T..............C.....................................................................
CHORDA        T..............C.....................................................................
SACCORHIZA    ...............C.....................................................................
ALARIA        T..............C.....................................................................
MACROCYSTIS   T..A.C.........C.....................................................................
DESMARESTIA   T..............C.....................................................................
SPOROCHNUS    T...G.........C.....................................C...............................
SYRINGODERMA  .C..G.........C.............TT.............C......G.................................
SPHACELARIA   TC..G.........C.............TT........C...C......G.................................
TAONIA        ...............C.............T.............C......G.T....C.....................T....
FUCUS         .T..T.........C.............T.............C.....................................T....
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ECTOCARPUS    GCACGCTTCGGCGGCGACGTTTCATTCAAGTTTCTGCCCTATCAGCTTTGGATGGTAGGGTATTGGCCTACCATGGCTTTAACGGG 344
SCYTOSIPHON   ...................................................................................
PUNCTARIA     ...................................................................................
TILOPTERIS    ...................................................................................
ANALIPUS      ...................................................................................
CUTLERIA      ...GC...TT.........................................................................
CHORDA        ...................................................................................
SACCORHIZA    ...................................................................................
ALARIA        ...................................................................................
MACROCYSTIS   ...................................................................................
DESMARESTIA   ...................................................................................
SPOROCHNUS    ...................................................................................
SYRINGODERMA  ...T...............................................................................
SPHACELARIA   ...T...............................................................................
TAONIA        ...T...............................................................................
FUCUS         ...................................................................................
```

```
ECTOCARPUS    TAACGGGGAATTGGGGTTCGATTCCGGAGAGGGAGCCTGAGAGACGGCTACCACATCCAAGGAAGGCAGCAGGCGCGTAAATTACC 430
SCYTOSIPHON   ................................................................................
PUNCTARIA     ................................................................................
TILOPTERIS    ................................................................................
ANALIPUS      ................................................................................
CUTLERIA      ................................................................................
CHORDA        ................................................................................
SACCORHIZA    ................................................................................
ALARIA        ..............................................A.................................
MACROCYSTIS   ..............................................A.................................
DESMARESTIA   ................................................................................
SPOROCHNUS    ................................................................................
SYRINGODERMA  ................................................................................
SPHACELARIA   ................................................................................
TAONIA        ................................................................................
FUCUS         ................................................................................

ECTOCARPUS    CAATCCTGACACAGGGAGGTAGTGACAATAAATAACAATGCCGGGCTTTTACAAGTCTGGCAATTGGAATGAGAGCAATTTAAATC 516
SCYTOSIPHON   ................................................................................
PUNCTARIA     ................................................................................
TILOPTERIS    ................................................................................
ANALIPUS      ................................................................................
CUTLERIA      ................................................................................
CHORDA        ................................................................................
SACCORHIZA    ................................................................................
ALARIA        ..............................................A.................................
MACROCYSTIS   ..............................................A.................................
DESMARESTIA   ..............................................T.........G.....................
SPOROCHNUS    ..............................................................................C.
SYRINGODERMA  ..............................................T.................................
SPHACELARIA   ..............................................T.................................
TAONIA        ..............................................T.................................C.
FUCUS         ..............................................C.................................
```

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ECTOCARPUS    CATCATCGAGGATCAATTGGAGGGCAAGTCTGGTGCCAGCAGCCGCGGTAATTCCAGCTCCAATAGCGTATATTAAAGTTGCTGCA 602
SCYTOSIPHON   ....................................................................................
PUNCTARIA     ....................................................................................
TILOPTERIS    ....................................................................................
ANALIPUS      ....................................................................................
CUTLERIA      ....................................................................................
CHORDA        ....................................................................................
SACCORHIZA    ....................................................................................
ALARIA        ....................................................................................
MACROCYSTIS   ....................................................................................
DESMARESTIA   ....................................................................................
SPOROCHNUS    ....................................................................................
SYRINGODERMA  ....................................................................................
SPHACELARIA   ....................................T...............................................
TAONIA        ....................................................................................
FUCUS         ....................................................................................

ECTOCARPUS    GTTAAAAAGCTCGTAGTTGGATTTGTGGCGCGGCCGTCCGGCGGGGCTCCTTCATTGGGGCCGTTTGTC-TGGTTTTTCGGCCGCA 688
SCYTOSIPHON   ..................................T.............A.T.C.............................T
PUNCTARIA     ..................................T.....................A.........................A.....
TILOPTERIS    ..................................T.....................C.....................-..A....C
ANALIPUS      ..............................................T.C.....................-.........
CUTLERIA      ..................................T.....................T.C.....................-..A....T
CHORDA        ..............................................T.C.....................................T
SACCORHIZA    ..................................T.............A.T.C.....................-..A....T
ALARIA        ..............................................T......A...........C-......-.......T
MACROCYSTIS   ..............................................T......A.........C.C-.............C
DESMARESTIA   ..................................T.............T..T.C.A.....................-..A.....
SPORODHNUS    ..............................................C.....................................T
SYRINGODERMA  ..........................................G....G.....T.C...TC.......C.C-.....A-.......T
SPHACELARIA   ..............................................T.-..C..A.C.........C.......-.......T
TAONIA        ........................AA..................G.......C...TC.........C.......-.......T
FUCUS         ..............................................TC.TC.G..A.....................-.......G
```

```
ECTOCARPUS    CCATTCTCGGGTA--GTGTGTCG-CTGGCATTAGGTTGTCGGCTTCTTCA-CGCCCGTCGTTTACTGTGAAAAAATTAGAGTGTTC 774
SCYTOSIPHON   ................................................................................
PUNCTARIA     ................................................................................
TILOPTERIS    ................C...............................G.....................................
ANALIPUS      ................C...............................G.....................................
CUTLERIA      ................C...............C...............G.....................................
CHORDA        ................C...............................G.....................................
SACCORHIZA    ................C........G........A.....T........G.....................................C....
ALARIA        ................C...............................G.....................................
MACROCYSTIS   ................C.....T.....................G.....G.....................................
DESMARESTIA   ................C....G.....................C.....G.....................................
SPOROCHNUS    ................C....G.....................G.....................................
SYRINGODERMA  ..T............C....G.....................CG....G.GC......T.....................
SPHACELARIA   ..T............C....G.....................T....G....G.........T.....................
TAONIA        T.T.........TA.C..A.T.G.....................CG....GG.C.....................
FUCUS         .......TC.AG...AA.CA...G.....................T.CGA.TG..T..GA.....................

ECTOCARPUS    AAAGCAGGCTTAGGCCATTGGATACATTAGCATGGAATAATGAGATAGGGCCACGACGGTCTATTTTGTTGGTTTGCACGTTGTGG 860
SCYTOSIPHON   ................................................................................
PUNCTARIA     ................G...............................................................
TILOPTERIS    ................G...............................................................
ANALIPUS      ................G...............................................................
CUTLERIA      ................G...............................................................
CHORDA        ................G...............................................................
SACCORHIZA    ................G...............................................................C.A..
ALARIA        ................G...............................................................
MACROCYSTIS   ................G...............................................................
DESMARESTIA   ................G...............................................................
SPOROCHNUS    ................G.....................................G.............T.............
SYRINGODERMA  ................G...............................................................
SPHACELARIA   ................G.........................A.....................................
TAONIA        ................G.....................................G.....................
FUCUS         ................G.....................................A.....................
```

```
ECTOCARPUS    TAATGATTAACAGGAACGGTTGGGGGTATTCGTATTCAATTGTCAGAGGTGAAATTCTTGGATTTATGGAAGACGAACTACTGCGA 946
SCYTOSIPHON   ...............................................................................................
PUNCTARIA     ..........................C....................................................................
TILOPTERIS    ...............................................................................................
ANALIPUS      ..............................................................................................G
CUTLERIA      ...............................................................................................
CHORDA        ..........................-....................................................................
SACCORHIZA    ..........................-....................................................................
ALARIA        ..........................-....................................................................
MACROCYSTIS   ..........................-....................................................................
DESMARESTIA   ...............................................................................................
SPOROCHNUS    ...............................................................................................
SYRINGODERMA  ...............................................................................................
SPHACELARIA   .....G.........TA.....................................................G.....................
TAONIA        ..........................?.....................................................G.....................
FUCUS         ...............................................................................................
```



```
ECTOCARPUS    AAGCATTTACCAAGGATGTTTTCATTAATCAAGAACGAAAGTTAGGGGATCGAAGATGATTAGATACCATCGTAGTCTTAACCATA 1032
SCYTOSIPHON   ...............................................................................................
PUNCTARIA     ...............................................................................................
TILOPTERIS    ...............................................................................................
ANALIPUS      ...............................................................................................
CUTLERIA      ...............................................................................................
CHORDA        ...............................................................................................
SACCORHIZA    ...............................................................................................
ALARIA        ...............................................................................................
MACROCYSTIS   ...............................................................................................
DESMARESTIA   ...............................................................................................
SPOROCHNUS    ...............................................................................................
SYRINGODERMA  ...............................................................................................
SPHACELARIA   ...............................................................................................
TAONIA        ...............................................................................................
FUCUS         ...............................................................................................
```

```
ECTOCARPUS    AACTATGCCGACTAGGGATTGGCGGTCGTTAATTTA--AGGACTCCGTCAGCACCTTCCGAGAAATCAAAGTCTTTGGGTTCCGGG 1118
SCYTOSIPHON   ...............................................................................
PUNCTARIA     ...............................................................................
TILOPTERIS    ..................................C............................................
ANALIPUS      ......T..........................C............................................
CUTLERIA      ..................................C............................................
CHORDA        ..................................C............................................
SACCORHIZA    ..................................C............G...............................
ALARIA        ..................................C............................................
MACROCYSTIS   ..................................C............................................
DESMARESTIA   ..................................C..A.........................................
SPOROCHNUS    ................................C...C.........................................
SYRINGODERMA  ..............................TT...TT..C.....................................
SPHACELARIA   ..............................TTGC.TCT.C.....................................
TAONIA        ..............................TCC..G.T.C.....................................
FUCUS         ................................-..C.C.....................................
```



```
ECTOCARPUS    GGGAGTATGGTCGCAAGGCTGAAACTTAAAGAAATTGACGGAAGGGCACCACCAGGAGTGGAGCCTGCG-CTTAATTTGACTCAAC 1204
SCYTOSIPHON   ...............................................................................G.............
PUNCTARIA     ...............................................................................G.............
TILOPTERIS    ...............................................................................G.............
ANALIPUS      ...............................................................................G.............
CUTLERIA      ...............................................................................G.............
CHORDA        ...............................................................................G.............
SACCORHIZA    ...............................................................................G.............
ALARIA        ...............................................................................G.............
MACROCYSTIS   ...............................................................................G.............
DESMARESTIA   ...............................................................................G.............
SPOROCHNUS    ...............................................................................G.............
SYRINGODERMA  ...............................................................................G.............
SPHACELARIA   ...............................................................................G.............
TAONIA        ...............................................................................G.............
FUCUS         ...............................................................................G.............
```

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ECTOCARPUS    ACGGGGAAACTTACCAGGTCCGGACATAGTGAGGATTGACAGATTGAGAGCTCTTTCTTGATTCTATGGGTGGTGGTGCATGGCCG 1290
SCYTOSIPHON   ................................................................................
PUNCTARIA     ................................................................................
TILOPTERIS    ................................................................................
ANALIPUS      ................................................................................
CUTLERIA      ................................................................................
CHORDA        ................................................................................
SACCORHIZA    ................................................................................
ALARIA        ................................................................................
MACROCYSTIS   ................................................................................
DESMARESTIA   ................................................................................
SPOROCHNUS    ................................................................................
SYRINGODERMA .....A..........................................................................
SPHACELARIA   ................................................................................
TAONIA        ................................................................................
FUCUS        ................................................................................

ECTOCARPUS    TTCTTAGTTGGTGGAGTGATTTGTCTGGTTAATTCCGTTAACGAACGAGACCCCCGCCTGCTAAATAGTGTGGCTTACGCTTTTGC 1376
SCYTOSIPHON   ................................................................................-
PUNCTARIA     ................................................................................
TILOPTERIS    ................................................................................
ANALIPUS      ................................................................................
CUTLERIA      ..............................................................................TC..........
CHORDA        ................................................................................
SACCORHIZA    ..............................................................................A..
ALARIA        ..............................................................................C..T
MACROCYSTIS   ..............................................................................C..T
DESMARESTIA   ................................................................................
SPOROCHNUS    ................................................................................
SYRINGODERMA ................................................................................
SPHACELARIA   ..............................................................................-
TAONIA        ............................................................................G.............-
FUCUS        ............................................................................G.....T..C.A.
```

```
ECTOCARPUS    GTAGGTA-CTCGCTTCTTAGAGGGACTTCTGGTGACTAACCAGAGGAAGTTGGGGGCAATAACAGGTCTGTGATGCCCTTAGATG- 1462
SCYTOSIPHON   .......-G.....................................................................A
PUNCTARIA     .......-G.....................................................................
TILOPTERIS    ............................TC...........GA.....................................
ANALIPUS      ............................TC...........G..A..................................
CUTLERIA      ............................TC...........GA.....................................
CHORDA        .......-G....................TC...........GA..-.................................
SACCORHIZA    .......-G....-...............TC...........GA..-.................................
ALARIA        .......-G....................TC...........GA.....................................
MACROCYSTIS   .......-G....................TC...........GA.....................................
DESMARESTIA   ............................TC...........GA..-.................................
SPOROCHNUS    .......-G....................TC...........GA.....................................
SYRINGODERMA  ............................TC...........GA.....................................
SPHACELARIA   ............................TC...........GA..-.................................
TAONIA        ............................TC...........GA.....................................
FUCUS         .....C.G....................TC...........GA.....................................

ECTOCARPUS    TCCTGGGCCGCACGCGCGCTACACTGATGCATGCAACGAGTTTT-ACTTTTCCTGGGTCGAGAGGCCCGGGTAATCTGTTGAACGT 1548
SCYTOSIPHON   ..............................................T.......A............T...-.....T........
PUNCTARIA     ..............................................--..............................T........
TILOPTERIS    ..........................................C.TTT...C.....................................
ANALIPUS      ..............................................-.....................................
CUTLERIA      ..........................................C..--...C.....................................
CHORDA        ..........................................C..-T.....................-.................
SACCORHIZA    ..........................................C..TT......................................
ALARIA        ..........................................C.TTT......................................
MACROCYSTIS   ..........................................C.TTT......................................
DESMARESTIA   ..........................................AC.-T......................................
SPOROCHNUS    ..........................................C.TT.....................................G......
SYRINGODERMA  ..............................................-..T.G.......C........G.................
SPHACELARIA   ..........................................C..T.G.....................................
TAONIA        ..........................................C.GACGC.................-......C..T........
FUCUS         ..........................................C.C.A.C.C......C..GA...T........C..T........
```

```
ECTOCARPUS    GCATCGTGATAGGGATAGATCATTGCAATTATTGATCTTGAACGAGGAATTCCTAGTAAACGCGAGTCATCAGCTCGCATTGATTA 1634
SCYTOSIPHON   ................................................................................
PUNCTARIA     ................................................................................
TILOPTERIS    ................................................................................
ANALIPUS      ................................................................................
CUTLERIA      ................................................................................
CHORDA        ..........................................................AT....................
SACCORHIZA    ................................................................................
ALARIA        ................................................................................
MACROCYSTIS   ................................................................................
DESMARESTIA   ................................................................................
SPOROCHNUS    ................................................................................
SYRINGODERMA  ................................................................................
SPHACELARIA   ..........................................................C.....................
TAONIA        ......................T...T......C..............................................
FUCUS         ......................TT...T..A....A..............................................

ECTOCARPUS    CGTCCCTGCCCTTTGTACACACCGCCCGTCGCACCTACCGATTGAATCATTCGGTGAGGATCTCGGATTTTGTTGCTTACG-TTCA 1780
SCYTOSIPHON   ..............................................................................TT.....C....
PUNCTARIA     ..........................................................TC......CC..A.......C....
TILOPTERIS    ..................................................GT........A...TC....CCG..GCT.GCG..C....
ANALIPUS      ..................................................GT........A...TC....CCG..GCT.GCG..C....
CUTLERIA      ..................................................GT........A...TC....CCG..GCT.GCG..C....
CHORDA        ..................................................GT........A...TC....CCG..GCT.GCG..C....
SACCORHIZA    ..................................................GT........A...TC....CCG..GCT.GCG..C....
ALARIA        ..................................................GT........A...TC....C.G..GCT.GCGT.C....
MACROCYSTIS   ..................................................GT........A...TC....C.G..GCT.GCGT.C....
DESMARESTIA   ..................................................GT........A...TC....CCG..GCTTGCG..C....
SPOROCHNUS    ..................................................GT........A...TC....C.G..GCT.GCG..C....
SYRINGODERMA  ..................................................GT........A...TC....CCG..GCT.G.G..C....
SPHACELARIA   ..................................................GT........A...TC....CCG..GCT.G.G..C....
TAONIA        ..................................................GT........A...TC....CCG..GCTAGCG..C....
FUCUS         ..................................................GT........A...TC....CCGGAACTTGAG..C...G
```

```
ECTOCARPUS     CGGCGT-TTTCGACGAGAGAAGTCATCCAAACCTCATGATTTAGAGGAAGGTGAAGTCGTAACAAGGTTTCC 1792
SCYTOSIPHON    .........CA.....................................................................
PUNCTARIA      ..........TT....A.A....T........................................................
TILOPTERIS     .....C..C.T.C..T.G.....T...T........AC..........................................
ANALIPUS       .....C..C.T.C..T.G.....T...T........AC..........................................
CUTLERIA       .....C..C.T.C..T.G.....T...T........AC..........................................
CHORDA         ....CG..C.T.C..T.G.....T...T........AC..........................................
SACCORHIZA     ....CG..C.T.C..T.G.....T...T........AC..........................................
ALARIA         .....C..C.T.C..T.G.....T...T........AC..........................................
MACROCYSTIS    .....C..CCT.C..T.G.....T...T........AC..........................................
DESMARESTIA    .....CC.C.T.C..T.G.....T...T........AT..........................................
SPOROCHNUS     ....CG..CCT.C.AT.G.....T...T........AC..........................................
SYRINGODERMA   .....-..CCT.C..T.G.....T...T........AC..........................................
SPHACELARIA    .....CG-CCT.C..G.G.....T...T........AC..........................................
TAONIA         .....C..C.G.C..T.G.....T...T........AC..........................................
FUCUS          .....C.AC.A.CGA..G.....T...T........AC..........................................
```

Table 8. Pairwise distance values between complete small-subunit ribosomal DNA sequences of selected brown algae. Pairwise distance values (numbers of substitution per site), generated by MEGA (Kumar et al. 1993) using the Kimura (1980) two-parameter model, are expressed as percentages.

	ES	SL	PE	TM	AJ	CM	CT
ES		0.74	1.09	2.32	2.2	2.73	2.32
SL			1.5	2.5	2.43	2.85	2.44
PE				2.32	2.55	2.85	2.67
TM					0.74	0.63	0.51
AJ						0.97	0.63
CM							0.8
CT							

Table 8. (Continued)

	SP	AM	MI	DL	SC	SP	SF
ES	2.85	2.44	2.79	2.74	2.67	3.62	3.69
SL	2.61	2.67	2.97	2.85	2.79	3.62	3.81
PE	2.96	2.73	3.08	2.85	3.03	3.86	3.92
TM	0.97	0.75	1.03	0.86	0.98	2.2	2.2
AJ	1.21	1.09	1.32	1.09	1.21	2.14	2.26
CM	1.21	1.26	1.67	1.21	1.38	2.37	2.49
CT	0.92	0.8	1.21	0.98	0.8	2.02	2.14
SP		1.38	1.79	1.55	1.5	2.55	2.79
AM			0.4	1.21	1.15	2.49	2.37
MI				1.5	1.44	2.61	2.67
DL					1.44	2.2	2.43
SC						2.37	2.32
SP							1.61
SF							
TA							
FG							

Table 8. (Continued)

	TA	FG
ES	4.84	5.27
SL	4.9	5.21
PE	5.14	5.45
TM	3.33	3.99
AJ	3.38	4.17
CM	3.62	4.29
CT	3.26	3.98
SP	3.8	4.47
AM	3.63	4.05
MI	3.98	4.41
DL	3.56	4.23
SC	3.32	4.47
SP	2.73	4.95
SF	3.15	5.32
TA		5.58
FG		

* Taxa are abbreviated as follows: ES=*Ectocarpus siliculosus*; SL=*Scytosiphon lomentaria*; PE=*Punctaria expansa*; TM=*Tilopteris mertensii*; AJ=*Analipus japonicus*; CM=*Cutleria multifida*; CT=*Chorda tomentosa*; SP=*Sacchoriza polyschides*; AM=*Alaria marginata*; MI=*Macrocystic integrifolia*; DL=*Desmarestia ligulata*; SC=*Sporochnus comosus*; SP=*Syringoderma phinneyi*; SF=*Sphacelaria furcigera*; TA=*Taonia atomaria*; FG=*Fucus gardneri*.

The unrooted tree (neighbor-joining; PHYLIP 3.4) separated the studied taxa into two groups (Fig. 14), congruent with the partial sequence data set. The resulting bootstrapped (500 replicates) consensus tree is shown in Figure 15. The 50% majority-consensus tree showed members of the Ectocarpales, Dictyosiphonales and Scytosiphonales grouped in an assemblage 100 % of the time (Fig. 16). Representatives from the Tilopteridales, Cutleriales, Laminariales, Ralfsiales, Desmarestiales and Sporochnales also formed an assemblage; the branching orders among these taxa were unresolved except for the association between *Alaria marginata* and *Macrocystis integrifolia* (99%). *Taonia atomaria* (Dictyotales) formed an assemblage with *Syringoderma phinneyi* (Syringodermatales) and *Sphacelaria furcigera* (Sphacelariales) 95% of the time, with *S. phinneyi* forming a clade with *S. furcigera* (59%). *F. gardneri* was shown to be the most diverged from all the studied taxa (Table 8).

Among the 1792 sites determined for the parsimony analysis, 94 sites were phylogenetically informative; 1589 sites were invariant and 109 sites were variable but noninformative. 153 equally parsimonious trees were obtained using PAUP (tree length 312 steps; CI, excluding informative characters = 0.766; RI = 0.631; trees not shown). The frequency distribution

Fig. 14. Unrooted neighbor-joining tree based on comparison of complete 18S rDNA sequences.

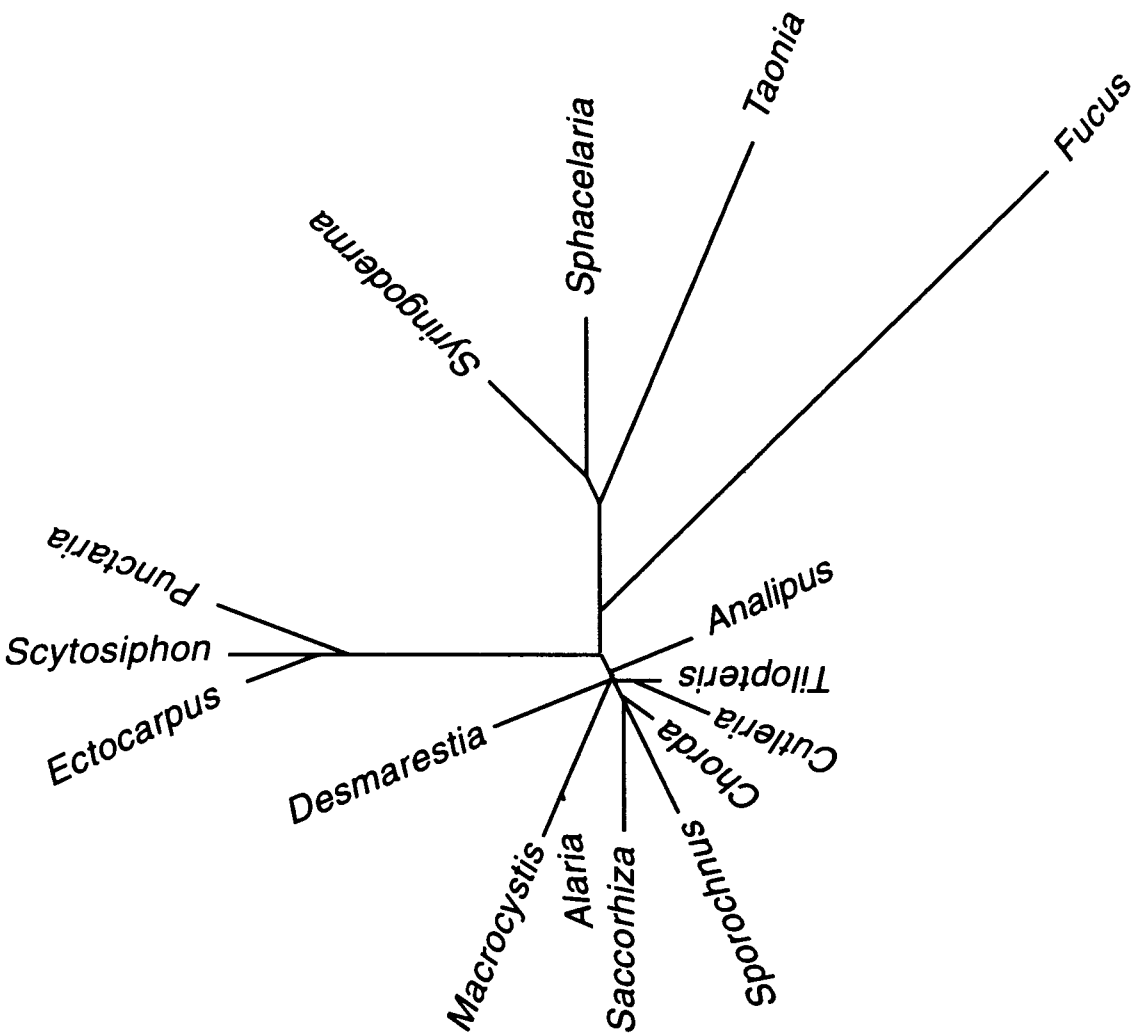

Fucus
Taonia
Sphacelaria
Syringoderma
Punctaria
Scytosiphon
Ectocarpus
Desmarestia
Macrocystis
Alaria
Saccorhiza
Sporochnus
Chorda
Cutleria
Tilopteris
Analipus

Fig. 15. Neighbor-joining tree with bootstrap values (500 replicates) based on comparison of complete 18S rDNA sequences. Fucus (Bhattacharya et al. 1992) was used as an outgroup taxon in the analysis.

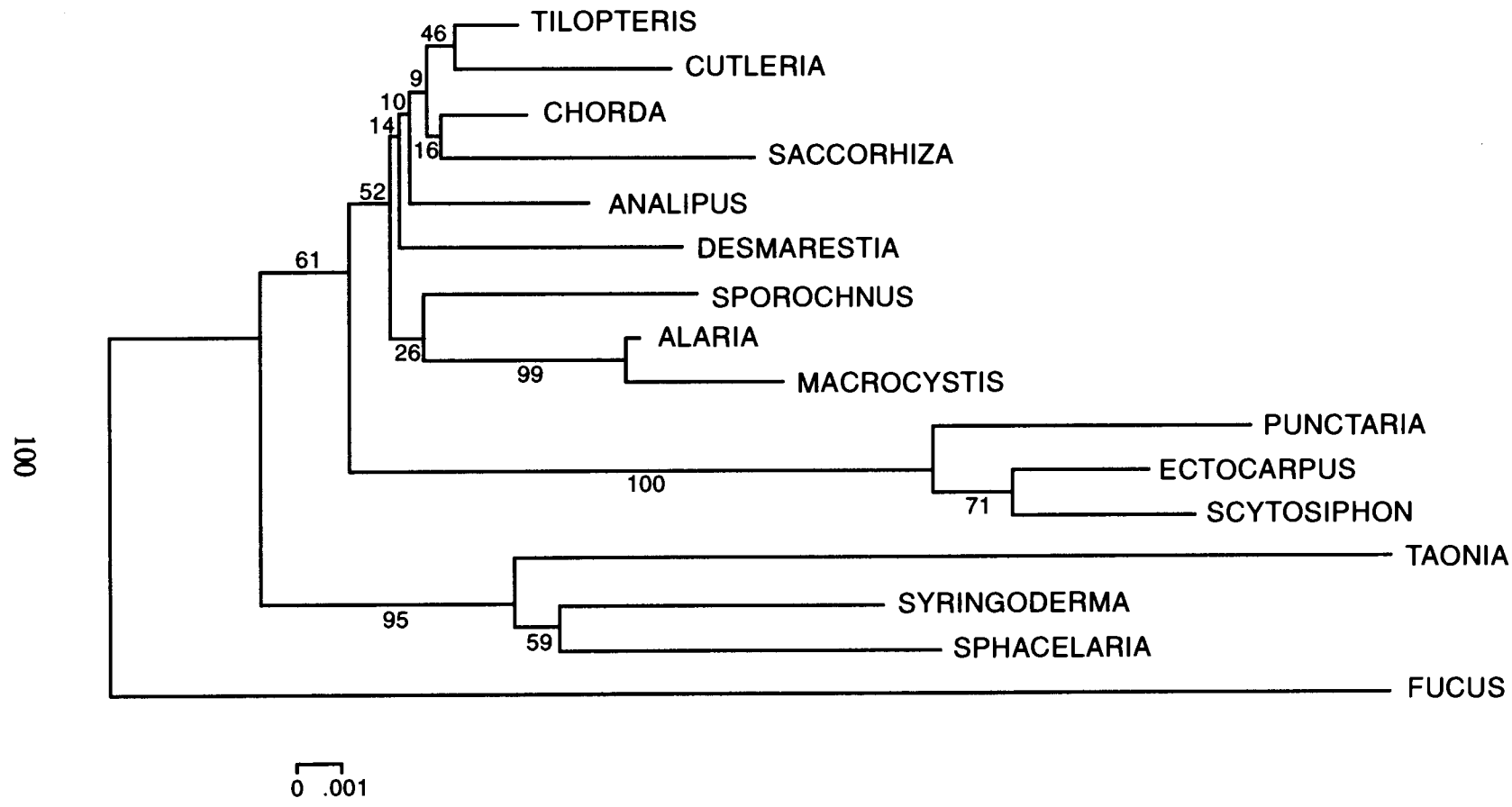
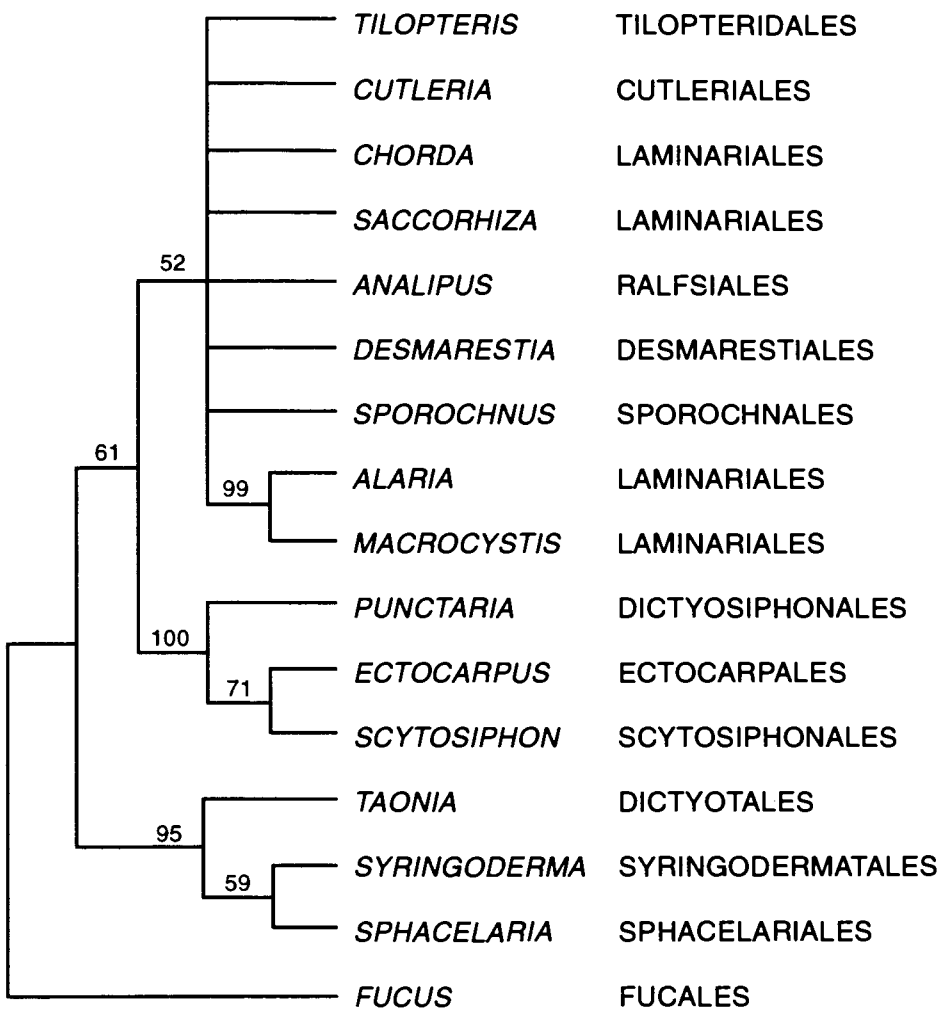

TILOPTERIS
CUTLERIA
CHORDA
SACCORHIZA
ANALIPUS
DESMARESTIA
SPOROCHNUS
ALARIA
MACROCYSTIS
PUNCTARIA
ECTOCARPUS
SCYTOSIPHON
TAONIA
SYRINGODERMA
SPHACELARIA
FUCUS
46
9
10
14
16
52
61
26
99
100
71
95
59
100
0 .001

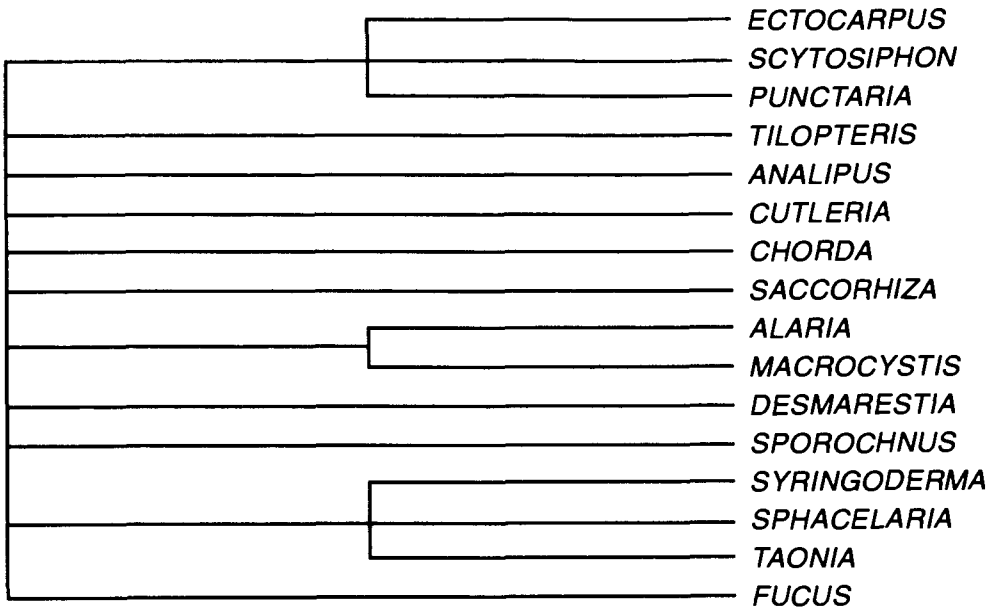
Fig. 16. 50 % majority-rule consensus tree (Neighbor-joining). Nodes with bootstrap values of less than 50% were collapsed to indicate a polytomy.


TILOPTERIS
TILOPTERIDALES
CUTLERIA
CUTLERIALES
CHORDA
LAMINARIALES
SACCORHIZA
LAMINARIALES
52
ANALIPUS
RALFSIALES
DESMARESTIA
DESMARESTIALES
SPOROCHNUS
SPOROCHNALES
61
99
ALARIA
LAMINARIALES
MACROCYSTIS
LAMINARIALES
PUNCTARIA
DICTYOSIPHONALES
100
ECTOCARPUS
ECTOCARPALES
71
SCYTOSIPHON
SCYTOSIPHONALES
TAONIA
DICTYOTALES
95
SYRINGODERMA
SYRINGODERMATALES
59
SPHACELARIA
SPHACELARIALES
FUCUS
FUCALES

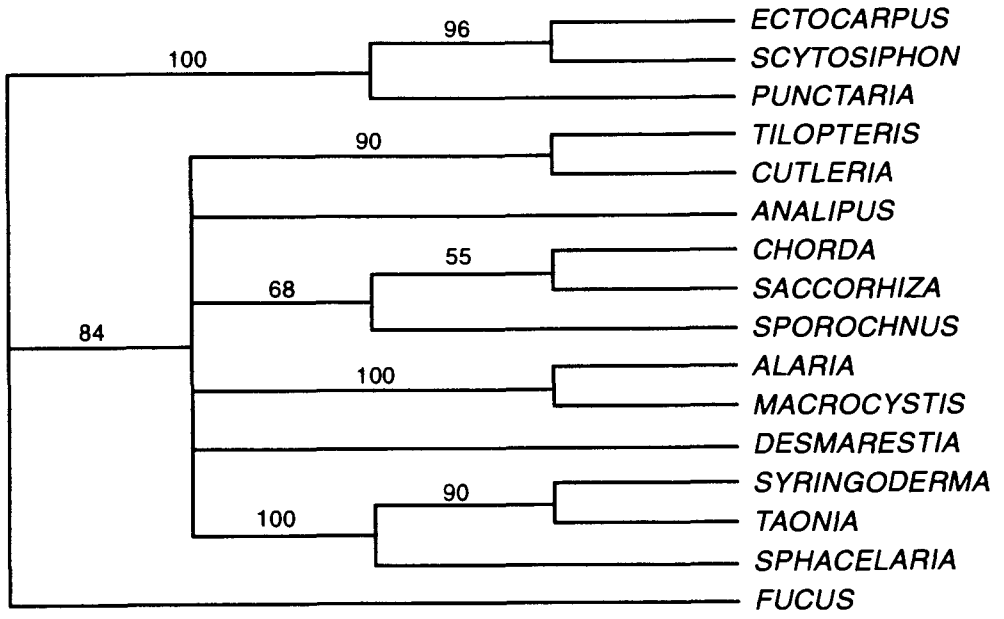
of tree lengths showed g1 = -1.588; based on the g1 value, this data set of complete 18S rDNA has greater phylogenetic signal that the data set with partial 18S rDNA sequences. The strict consensus tree showed the associations between *Ectocarpus/Scytosiphon/Punctaria*, *Alaria/Macrocystis* and *Syringoderma/Sphacelaria/Taonia* (Fig. 17A). The 50% majority-rule consensus tree showed members of the Tilopteridales, Cutleriales, Laminariales, Sporochnales, Ralfsiales and Desmarestiales grouped in a cluster (Fig. 17B). Included in this cluster was the *Syringoderma/Sphacelaria/Taonia* assemblage. Within the cluster, *Tilopteris mertensii* grouped together with *Cutleria multifida* (90%) and *Chorda tomentosa*, *Saccorhiza polyschides* and *Sporochnus comosus* grouped together (68%). The branching orders among *Analipus japonicus* and *Desmarestia ligulata* and the other taxa were unresolved.

Fig. 17. Consensus trees from maximum parsimony analysis (branch-and-bound, PAUP 3.1.1; Swofford 1993); 153 equally parsimonious trees of 312 steps, CI = 0.766 and RI = 0.631. (A) tree is the strict consensus tree; (B) is the 50 % majority-rule consensus tree.

A


ECTOCARPUS
SCYTOSIPHON
PUNCTARIA
TILOPTERIS
ANALIPUS
CUTLERIA
CHORDA
SACCORHIZA
ALARIA
MACROCYSTIS
DESMARESTIA
SPOROCHNUS
SYRINGODERMA
SPHACELARIA
TAONIA
FUCUS

B


96
100
90
55
68
84
100
90
100
ECTOCARPUS
SCYTOSIPHON
PUNCTARIA
TILOPTERIS
CUTLERIA
ANALIPUS
CHORDA
SACCORHIZA
SPOROCHNUS
ALARIA
MACROCYSTIS
DESMARESTIA
SYRINGODERMA
TAONIA
SPHACELARIA
FUCUS

DISCUSSION

Ordinal relationships

The present study is the first comprehensive examination to address the evolutionary relationships among the orders of the Phaeophyta using 18S ribosomal DNA sequences. This study exceeds previous work (Lim et al. 1986) both in scope (14 orders compared to 5 orders) and in the sensitivity of method (DNA sequences versus RNA sequences) allowing an expanded phylogenetic analysis of this division and, consequently, more definitive hypotheses of evolutionary relationships within the Phaeophyta. Lim et al. (1986) were the first to address the controversial brown algal relationships using molecular data (5S ribosomal RNA sequence data) from 5 taxa representing 5 of the 16 brown algal orders (Laminariales, Fucales, Chordariales, Ectocarpales and Dictyosiphonales). Due to the limited phylogenetic signal of the 5S rRNA sequence data, percent similarity among the orders was estimated between 96 - 99 %, leading the authors to conclude that these brown algae diverged very recently from one another. However, both distance matrix and maximum parsimony analyses of the current 18S rDNA sequences resulted in phylogenetic trees which consistently separated

the 14 included orders into two disparate clusters. One of the clusters included representatives of the Ectocarpales, Chordariales, Dictyosiphonales and Scytosiphonales. The other cluster included representatives from the Cutleriales, Tilopteridales, Ralfsiales, Sporochnales, Desmarestiales, Laminariales, Sphacelariales, Syringodermatales, Dictyotales and Fucales.

i) Ectocarpales, Chordariales, Dictyosiphonales and Scytosiphonales

Results offer valuable insights into the controversial phylogenetic relationships among the Ectocarpales, Chordariales, Dictyosiphonales and Scytosiphonales (ECDS). Bootstrap analysis strongly supported the association of members in the ECDS cluster (100% in both distance and parsimony analyses) suggesting that they are more closely related to one another than to members of the other orders. Within the ECDS complex the neighbor-joining trees suggested that the Chordariales and Dictyosiphonales are not natural taxa. The Dictyosiphonales and Chordariales were shown to be paraphyletic (a paraphyletic taxon does not contain all the descendants of one ancestral species). *Asperoccoccus bullosus* and *Punctaria expansa*,

which belong to the Dictyosiphonales, formed a clade with *Leathesia difformis*, a member of the Chordariales. Similarly, *Haplogloia andersonii* (Chordariales) was shown to be a sister taxon to the *Aperococcus/Punctaria/Leathesia* clade. Current results suggest that the Scytosiphonales may be a monophyletic taxon (it contains one ancestral species and all its descendants) based only on the two scytosiphonalean species included in the study: *Scytosiphon lomentaria* and *Colpomenia peregrina*.

The ECDS complex is characterized by having heterotrichous development and the possession of normal pyrenoids. In heterotrichous brown algae, spores and zygotes germinate into horizontal prostrate filaments which later give rise to erect filaments. Members of the ECDS exhibit heterotrichy in both gametophytic and sporophytic stages. Fritsch (1945) suggested the close relationships among members of the ECDS based on the presence of heterotrichy and the lack of oogamy among members of the ECDS. He proposed merging these four orders into one single order; the Ectocarpales. Scagel (1966) challenged the merging of the ECDS, and he argued that members of other orders, such as the Laminariales, also have heterotrichous development as well while members of the ECDS have very

different types of thallus organization. However, heterotrichy is present only in the gametophytic stages within the Laminariales, whereas, heterotrichy is present in both the gametophytic and sporophytic stages among members of the ECDS. Members within the ECDS complex do indeed have very different types of thallus organization. For example, *Ectocarpus siliculosus* (Ectocarpales) is filamentous, *Leathesia difformis* (Chordariales) is pseudoparenchymatous and *Punctaria expansa* (Dictyosiphonales) is parenchymatous. The inferred ribosomal DNA phylogenies suggested that thallus organization is not an important character for the delimitation of brown algae at the ordinal level. The importance of thallus organization as a taxonomic character is also questioned in other orders, such as the Desmarestiales where most members are pseudoparenchymatous with the exception of a parenchymatous Antarctic genus, *Himantothallus* (Müller et al. 1985a).

Members of the ECDS complex have normal conspicuous pyrenoids while the other studied taxa not included in this cluster have either rudimentary or no pyrenoids at all (Table 9). Brown algal pyrenoids are thought to be associated with polysaccharide synthesis and accumulation. Evans (1966, 1968) hypothesized that the absence/presence of pyrenoids was

Table 9. Distribution of pyrenoids and stigmata (eyespots) in meiospores among the studied taxa (Hori 1971, Kawai 1992). Those with pyrenoids were categorized as having either normal or rudimentary pyrenoids (see text for discussion between the two types of pyrenoid).

	pyrenoid distribution	**stigmata in meiospores**
Ectocarpales	normal	+
Chordariales	normal	+
Dictyosiphonales	normal	+
Scytosiphonales	normal	+
Laminariales	rudimentary*	-/+[1]
Desmarestiales	absent	+
Sporochnales	absent	+
Tilopteridales	absent	+
Cutleriales	rudimentary**	+
Sphacelariales	rudimentary	+
Syringodermatales	absent	+
Dictyotales	absent	+
Fucales	rudimentary***	+

Table 9. (Continued).

* absent in most members except *Undaria pinnatifida*, ** absent in most members except *Cutleria cylindrica*, *** absent in most members except *Cystoseira tamariscifolia;* [1]meiospores without stigmata in the Alariaceae, Laminariaceae and Lessoniaceae; meiospores with stigmata in the Chordaceae and Phyllariaceae.

an important feature to reflect brown algal phylogenies. According to Evans, presence of pyrenoids is an ancestral brown algal feature, and therefore, the ECDS are more 'primitive' than other orders without pyrenoids. Furthermore, there are two types of brown algal pyrenoids: normal ones and rudimentary ones. Normal pyrenoids are larger in size as compared to rudimentary ones ranging from 1.5 to 3.0 μ in width and 1.5 to 2.5 μ in length while rudimentary pyrenoids range from 0.5 to 1.0 μ in diameter (Hori 1971). In addition, normal pyrenoids are present only in isogamous or anisogamous brown algae, while rudimentary pyrenoids are found only in markedly anisogamous and oogamous brown algae. For example, the cortical cells of *Cutleria cylindrica* (an anisogamous brown alga) have rudimentary pyrenoids (Hori 1971), and the eggs of *Cystoseira tamariscifolia* (an oogamous brown alga) have rudimentary pyrenoids as well (Evans 1968). Kawai (1991) also regarded the absence/presence of pyrenoids as a "good taxonomic character". He reported that this character is constant among members of an order. Current results support absence/presence and type of pyrenoids as a useful character to distinguish major lineages of brown algae (the ECDS lineage or the 'other' lineage).

ii) Sporochnales, Desmarestiales and Laminariales

Members of the Sporochnales, Desmarestiales and Laminariales (SDL) were consistently grouped in the same cluster together with members of the Cutleriales, Tilopteridales and Ralfsiales. The inferred ribosomal DNA phylogenies supported the contention (Clayton 1984, Müller et al. 1984a) that the SDL have close phylogenetic ties; however, the branching orders among the SDL and associated taxa were not fully resolved. Only the branching order of the *Alaria/Macrocystis* clade within the assemblage was strongly supported by bootstrap resampling.

Clayton (1984) compiled evidence pointing to "a common ancestor linking the Laminariales with the Desmarestiales and the Sporochnales". This evidence included types of sexual reproduction and sporeling development. Members of these three orders have an alternation of generations between a microscopic gametophyte and a macroscopic sporophyte. The oogamous gametophytes, among the three orders, are morphologically and physiologically indistinguishable (Müller et al. 1985a). However, at the ultrastructural level, the gametophytes of the Sporochnales differ from the Laminariales and Desmarestiales in that they have lobed parietal chloroplasts

(Kawai pers. comm.). In all three orders, antheridium dehiscence is stimulated by the sexual pheromone secreted by the oogonia: lamoxirene in the Alariaceae, Laminariaceae and Lessoniaceae, desmarestene in the genus *Desmarestia* (Müller et al. 1982) and caudoxirene in the Sporochnales (Müller et al. 1988). Despite the fact that the SDL do not have the same pheromone, their events of sexual reproduction are similar (Müller et al. 1985b). In addition to stimulating the release of sperm, these different pheromones also attract the sperm to the eggs. These sperm lack eyespots while sperm with eyespots are found among the other studied phaeophytes (Table 9). Sperm of the Sporochnales, Desmarestiales and Laminariales have a shorter anterior and longer posterior flagellum. Other phaeophyte sperm have a longer anterior and shorter posterior flagellum (Henry and Cole 1982, Kawai 1992). Fertilization follows the attraction of sperm to the eggs, and the resulting zygotes develop *in situ* on the female gametophyte. The sporophytes of the Sporochnales, Desmarestiales, Alariaceae and Lessoniaceae grow by means of a localized meristem (Clayton 1984); however, *Chorda tomentosa* (Chordaceae) does not have an obvious localized meristem (Maier 1984b) but *C. filum* does (South & Burrows 1967).

Specifically, the Sporochnales and Desmarestiales have trichothallic growth while the Laminariales has an intercalary meristem.

The similarity of sexuality and sporeling development among these three groups seems to be reflected by their close evolutionary relationships (Clayton 1984, Motomura et al. 1985, Müller et al. 1985a). Russell and Fletcher (1975) and Parke and Dixon (1976) treated the Sporochnales as a family within the Desmarestiales based on such similarities. The inferred ribosomal DNA phylogenies do not support the continued treatment of the Sporochnales, Desmarestiales and Laminariales in different phylogenetic lines (Scagel 1966, Wynne and Loiseaux 1976). Instead, results suggest the view that these three orders are closely related taxa (Clayton 1984, Motomura et al. 1985, Müller et al. 1985a, Kawai 1992). However, the evolutionary relationships between the SDL and other associated taxa such as the Cutleriales, Tiloteridales and Ralfsiales were not resolved by comparison of 18S rDNA sequences.

iii) Sphacelariales, Syringodermatales, Dictyotales and Fucales

The inferred ribosomal DNA phylogenies grouped the four apically growing brown algal orders into an assemblage; however, the branching order among the four taxa remained unresolved. Bootstrap analyses (neighbor-joining) based on partial and complete 18S rDNA sequences supported the association between *Sphacelaria furcigera* (Sphacelariales) and *Syringoderma phinneyi* (Syringodermatales). However, the parsimony analysis on complete 18S rDNA sequences supported the association between *Syringoderma phinneyi* and *Taonia atomaria* (Dictyotales). The taxonomic placement of *Syringoderma* is in a state of flux (Henry and Müller 1983, Henry 1984). For example, Delépine (1968 as cited by Walker and Henry 1978) and Wynne (1972) proposed to place the genus *Syringoderma* in the order Sphacelariales on the basis of its apical growth and isogamy. The Sphacelariales is parenchymatous, while the Syringodermatales is pseudoparenchymatous; however, thallus organization used as an ordinal character is being questioned based on the results of this study. Furthermore, Levring (1940 as cited by Henry 1984) assigned *Syringoderma* to the Dictyotales, also, based on the presence of an apical meristem. Nonetheless,

Henry (1984) established the order Syringodermatales to delimit the genus *Syringoderma* on the basis of its life history pattern, sexuality and thallus organization. For example, both the Dictyotales and Sphacelariales are parenchymatous, while *Syringoderma* is pseudoparenchymatous. In addition, the oogamous Dictyotales and isogamous Sphacelariales have an isomorphic alternation of generations. The recently established Syringodermatales has a heteromorphic alternation of generations with isogamous species (Henry 1984). Additional representatives from each order to the current molecular analysis is required in order to elucidate the phylogenetic and taxonomic affinities among the three orders.

The Dictyotales were perceived as unique within the Phaeophyta because they possessed uniflagellated sperm whereas typical brown algal sperm were biflagellated (Fritsch 1945). However, recent ultrastructural study by Phillips and Clayton (1991) showed biflagellated sperm in the dictyotalean species, *Zonaria angustata* (Kützing) Papenfuss. Phillips and Clayton (1991) proposed that the biflagellated sperm of *Zonaria* linked the Dictyotales to other brown algal orders. The inferred ribosomal DNA phylogenies support Phillips and Clayton's (1991) suggestion that the Dictyotales are not as far removed from the other brown algal orders as

previous thought. However, the branching order among *Taonia atomaria* (Dictyotales) and the Sphacelariales and Syringodermatales is not conclusive based on the present work. On this basis it is favorable to include *Zonaria angustata* in the molecular analysis to elucidate the phylogenetic affinities of the Dictyotales.

The Fucales are generally perceived as having diverged from the rest of the Phaeophyta early in the evolution of the division. Members of the Fucales demonstrate only one dominant diploid phase (gametic life cycle) while most other brown algae have an alternation of diploid and haploid phases. Comparison of the 18S rDNA sequence of *Fucus gardneri* (Fucales) (Bhattacharya et al., 1992) with the sequences determined in this study showed the fucalean sequence being the most divergent. However, Clayton (1984) suggested that certain members of the Chordariales and Dictyosiphonales might have links with the Fucales based on the various morphologically similarities such as conceptacles and cryptostomata. The inferred ribosomal DNA phylogenies show *Fucus* (Fucales) to be only distantly related to the studied members of the Chordariales and Dictyosiphonales. However, Clayton's comments referred to *Splachnidium* (Chordariales) and *Scytothamnus* and *Adenocystis* (Dictyosiphonales), three

taxa which were not included in the present study. In this context it would be of interest to include these three taxa in the subsequent molecular analyses.

Laminarialean family relationships

Saunders and Druehl (1992) demonstrated that the 18S rDNA was too conserved to resolve the phylogenetic relationships among members of the Alariaceae, Laminariaceae and Lessoniaceae (ALL) of the Laminariales. However, the same gene system, used in the present study, provided adequate sequence divergence to distinguish two other laminarialean families, the Chordaceae and Phyllariaceae, from the ALL. The inferred ribosomal DNA phylogenies consistently grouped *Alaria marginata* and *Macrocystis integrifolia* into an assemblage. Inclusion of the other kelp sequences (Alariaceae and Lessoniaceae) from Saunders and Druehl's (1992) study resulted in an unresolved assemblage with *A. marginata* and *M. integrifolia* (results not shown). *Chorda tomentosa* (Chordaceae) and *Saccorhiza polyschides* (Phyllariaceae) formed an unresolved assemblage with *Sporochnus* (Sporochnales). The strong support for the *Alaria/Macrocystis*

cluster substantiates the notion that the Chordaceae and Phyllariaceae are "phylogenetically isolated" from the ALL (Müller et al. 1985b).

It is widely accepted that the Chordaceae and Phyllariaceae are quite unique within the Laminariales and the phylogenetic relationships between them and the ALL are enigmatic (Druehl and Saunders 1992). On the basis of sexual reproduction studies, Maier (1984a as cited by Müller et al. 1985a) reported that, "there are several distinct species groupings within the order Laminariales". Moreover, molecular evidence indicated that *Chorda filum* had a greater degree of chloroplast DNA divergence from the ALL than from *Fucus gardneri* of the Fucales (Fain 1986). The inferred 18S rDNA trees showed the *Alaria marginata/ Macrocystis integrifolia* clade forming an unresolved association with the other two 'primitive' kelp *Chorda tomentosa* and *Saccorhiza polychides* and members of the Cutleriales, Tilopteridales, Ralfsiales, Sporochnales and Desmarestiales. This suggested that the Laminariales might be paraphyletic.

Chorda tomentosa and *Saccorhiza polyschides* possess several atypical kelp features. For example, *C. tomentosa* and *S. polyschides* have eyespots in their meiospores, however eyespots are absent in the meiospores of the ALL. Meiospores with eyespots are considered an ancestral kelp feature (Kawai

1992). In addition, eyespots are present in meiospores of the Desmarestiales and Sporochnales. Physiologically the Chordaceae and Phyllariaceae have different means for translocation purposes than the ALL. In place of the trumpet hyphae which are found in the ALL, *C. tomentosa* simply has "elongated" hyphae and *S. polyschides* has solenocysts and allelocysts (Emerson et al. 1982). Likewise, instead of secreting lamoxirene, the ALL sexual pheromone, *C. tomentosa* secretes multifidene and *S. polyschides* secretes ectocarpene (Maier et al. 1984a). Lamoxirene promotes egg secretion within the Laminariales, but it has no effect on the monoecious gametophytes of *C. tomentosa* (Müller et al. 1985b). Furthermore, lamoxirene is not a structurally related compound to either ectocarpene or multifidene (Müller et al. 1985b). Current molecular data together with these other findings question the taxonomic affinities of *C. tomentosa* and *S. polyschides* within the Laminariales and, also between other brown algal orders.

Specific genera relationships: *Analipus* and *Ralfsia*

The order Ralfsiales was established based on early development of the thallus, ultrastructural features and reproductive organs (Nakamura 1972). Both *Analipus* and *Ralfsia* share these common characters, and therefore, they were classified in the Ralfsiales by Nakamura (1972). However, Nelson (1982) concluded that the characters used to establish the order Ralfsiales were not consistent. Instead, she recommended classification of both *Analipus* and *Ralfsia* in the order Ectocarpales. My study, based on analysis of 18S rDNA sequences, provided an independent method to address this controversy. *Analipus japonicus* (Heterochordariaceae) and *Ralfsia fungiformis* (Ralfsiaceae) were not incorporated in the clade which included all other representatives of the order Ectocarpales (*sensu* Gabrielson et al. 1989) (Table 4) in the consensus trees (Fig. 8,10). Instead, *A. japonicus* and *R. fungiformis* were associated with members of the Desmarestiales, Dictyotales, Fucales, Laminariales, Sphacelariales and Syringodermatales. However, the proposed close phylogenetic relationship between *Analipus* and *Ralfsia* (Nakamura 1972) remains to be elucidated. The current phylogenetic

analysis failed to provide evidence that *A. japonicus* is closely related to *R. fungiformis* in that the branching order between these two algae is unresolved.

An assessment of the sexual pheromones of *Analipus* suggested a departure from the pheromone bouquet associated with other representatives of the Ectocarpales (Müller et al. 1990). When Kawai (1989) described the species *Heteroralfsia saxicola* (Okamura et Yamada) Kawai, he was hesitant to place it in the order Ectocarpales because it would only make the order "more heterogeneous". In addition, he also stated that, "the order Ralfsiales itself remains invalid because of the lack of a Latin diagnosis (ICBN, Art. 36.2; see Greuter 1988)". Current results suggest that *A. japonicus* and *R. fungiformis* not be placed in the order Ectocarpales (*sensu* Gabrielson et al. 1989). In addition, we agree with Kawai that the ordinal systematic affinities of *Ralfsia* and its relatives should be reconsidered.

The inferred tree topologies might have resulted from one of several different evolutionary processes. The presence/absence of pyrenoids seems to be one of these processes. *Ectocarpus*, *Asperococcus*, *Punctaria*, *Leathesia*, *Elachista*, *Colpomenia*, *Scytosiphon* and *Haplogloia* were all grouped in the same clade 100% of the time. Members of this clade have normal pyrenoids (Evans 1966). The other brown algae which were not

grouped into this clade lack normal pyrenoids or have rudimentary pyrenoids (discrepancies of pyrenoid presence/absence in *Sphacelaria bipinnata* reported by Simon 1954 as cited by Evans 1966). Both *Analipus* and *Ralfsia* lack pyrenoids as well (Kawai 1989). Thus, it appears that presence of pyrenoids is an important character among the ectocarpoids. Similarly, Evans (1966, 1968) proposed that pyrenoids were a significant taxonomic feature for certain ordinal and familial treatments. He regarded the presence of pyrenoids as an ancestral character. For example, he considered the order Ectocarpales to be more 'primitive' than either the orders Laminariales or Fucales. Members of the Ectocarpales possess pyrenoids whereas members of the Laminariales and Fucales lack pyrenoids or have rudimentary ones.

Developmental pattern seems to be another evolutionary process which has contributed to the observed tree topologies. Nakamura (1972) suggested developmental pattern to be an ordinal criterion among certain phaeophytes. *Analipus* and *Ralfsia* both possess discal-type development; for that reason Nakamura (1972) separated both algae from the other heterotrichous ectocarpoids (*sensu* Gabrielson et al. 1989) and placed them in a separate order, the Ralfsiales. However, Fritsch (1945) included *Analipus* and *Ralfsia* within the order Ectocarpales together with the other heterotrichous

ectocarpoids in his taxonomic treatment of the Ectocarpales. Current analysis separated *Analipus* and *Ralfsia* from the heterotrichous ectocarpoids and grouped them with non-heterotrichous phaeophytes (e.g., *Desmarestia*, *Alaria*, *Sporochnus*).

Based on the current analysis of available 18S rDNA sequences for certain ectocarpoids, the order Ectocarpales (*sensu* Gabrielson et al. 1989) is not a monophyletic taxon. *Analipus. japonicus* (Heterochordariaceae) and *R. fungiformis* (Ralfsiaceae) should not be classified within the Ectocarpales. Assuming that taxonomy reflects phylogenetic relationship, I recommend the removal of these two taxa from the order Ectocarpales. Further studies are required for the re-evaluation of the ordinal systematic affinities of *A. japonicus* and *R. fungiformis*.

CONCLUSIONS

This present work used molecular characters (DNA sequence data), as an independent approach, to address various controversial phylogenetic issues within the plant division Phaeophyta (brown algae). Existing phylogenetic hypotheses among the brown algae were based on morphological and physiological characters (Kylin 1933 as cited by Clayton 1984, Papenfuss 1954, Nakamura 1972, Wynne and Loiseaux 1976, Clayton 1984); however, these hypotheses remain inconsistent and controversial (Fritsch 1945, Clayton 1984, Kawai 1992). Results from the current analysis of 18S rDNA sequence data provide valuable insight to controversial phylogenetic issues among the brown algae from the ordinal to the generic levels.

At the ordinal level, the evolutionary relationships among 14 of the 16 universally recognized orders were inferred from the comparison of 18S rDNA sequences. The 18S rDNA was shown to be too conserved to resolve phylogenetic relationships among various brown algal taxa, but it is divergent enough to distinguish the twenty-two studied brown algal taxa into two disparate clusters. Members of the Ectocarpales, Chordariales, Dictyosiphonales and Scytosiphonales (ECDS) formed a cluster. The other

studied taxa were grouped in another major cluster which included representatives from the Tilopteridales, Cutleriales, Ralfsiales, Laminariales, Sporochnales, Desmarestiales, Syringodermatales, Sphacelariales, Dictyotales and Fucales.

The inferred 18S ribosomal DNA phylogenies supported Fritsch's (1945) controversial proposal that members of the ECDS are closely related taxa in relation to other brown algae. The association of these taxa is further supported by ultrastructural and developmental characteristics such as pyrenoid distribution and type of early development. Members of the ECDS have large conspicuous pyrenoids, and their spores and zygotes germinate by growing horizontal filaments which later give rise to erect filaments (heterotrichy). The inferred 18S rDNA phylogenies also showed that the Chordariales and Dictyosiphonales are not monophyletic taxa.

Current results provided another piece of evidence, in addition to data from sexual reproduction studies, to support the contention (Clayton 1984, Müller et al. 1985a) that the Sporochnales, Laminariales and Desmarestiales are more closely related to one another than previously thought. These three orders were separated into different lineages on the basis of different modes of thallus organization (Kylin 1933, Papenfuss 1954, Wynne and Loiseaux

1976). Present work suggests that mode of thallus organization is not an important criterion for delimiting brown algae at the ordinal level.

At the familial level, the evolutionary relationships among the kelp (Laminariales) families were explored by comparison of 18S rDNA sequences. The 18S rDNA was divergent enough to distinguish the 'primitive' kelp families, Chordariaceae and Phyllariaceae, from the 'advanced' kelp families, Alariaceae and Lessoniaceae. Furthermore, the inferred rDNA phylogenies showed that the order Laminariales is paraphyletic. Current results are further supported by ultrastructural and physiological characters such as the presence/absence of eyespots in meiospores and the different pheromones involved in sexual reproduction.

The controversial taxonomic and phylogenetic affinities of the two brown algal genera, *Analipus japonicus* and *Ralfsia fungiformis*, were addressed by analysis of 18S ribosomal DNA sequences. The inferred phylogenies supported Nakamura's (1972) proposal that both *A. japonicus* and *R. fungiformis* are not close relatives of the ECDS. However, current results suggest that the order Ralfsiales established by Nakamura to delimit *A. japonicus* and *R. fungiformis* is not monoplyletic. Branching orders among these two taxa and members of the Cutleriales, Tilopteridales, Sporochnales,

Desmarestiales and Laminariales are not conclusive based on the present work.

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