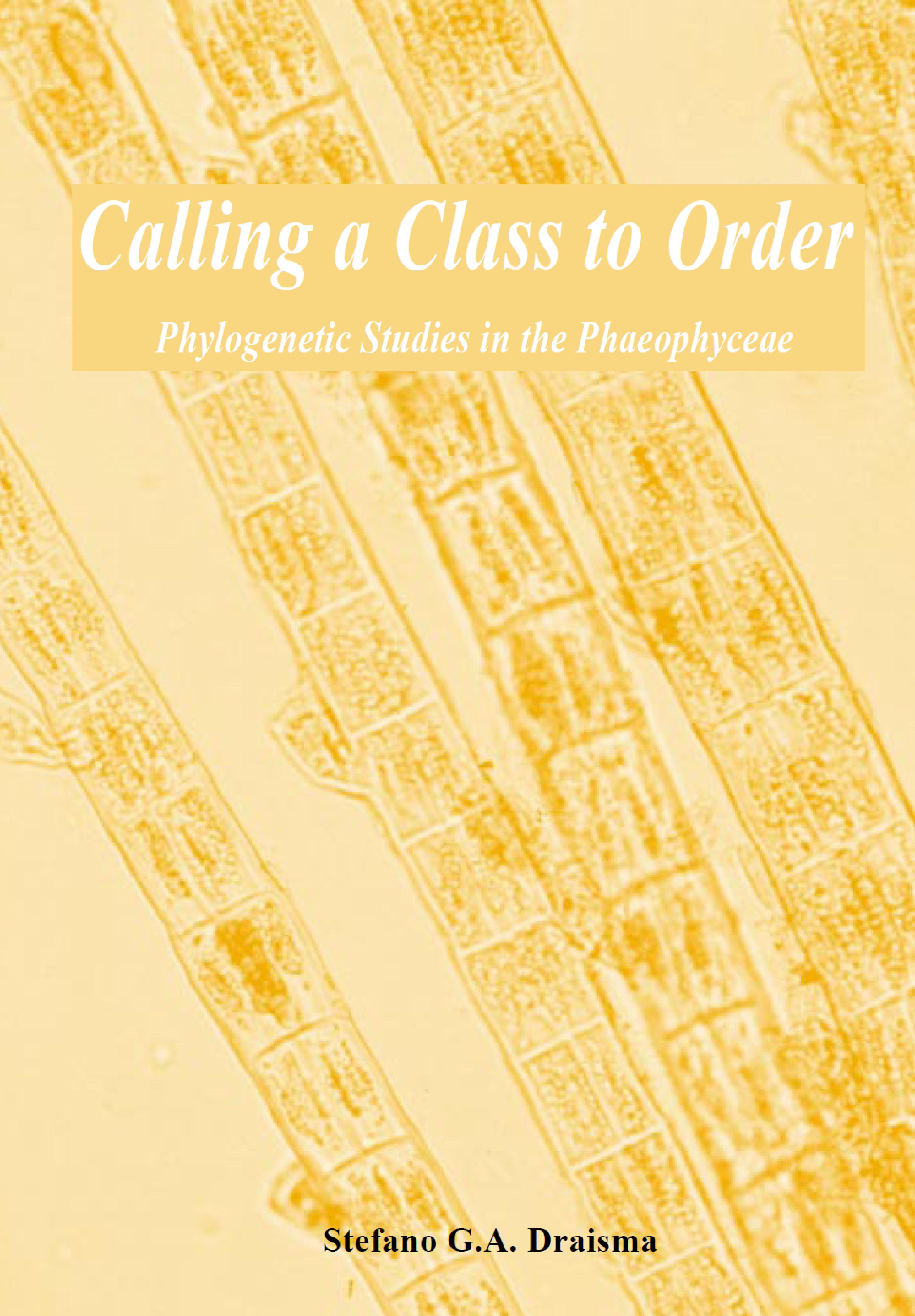


Calling a Class to Order

Phylogenetic Studies in the Phaeophyceae

Stefano G.A. Draisma

STELLINGEN

behorend bij het proefschrift *Calling a Class to Order – Phylogenetic Studies in the Phaeophyceae*
van Stefano Draisma

I

De Sphacelariales *sensu lato* zijn niet monofyletisch.
Dit proefschrift.

II

De morfologische kenmerken waarop geslachten en secties van de Sphacelariales gebaseerd zijn, zijn niet allemaal geschikt om hun fylogenie te reconstrueren.
Dit proefschrift.

III

Moleculaire fylogenieën tonen niet consequent aan dat de Fucales de meest basale bruinwierorde is.
Dit proefschrift; Contra: Peters en Clayton (1998)

IV

Homoplasie (saturatie) in de derde codon positie is nodig om een fylogenie te reconstrueren met diepe en ondiepe *clades*.
Dit proefschrift; Källersjö et al*. (1999).*

V

Bij fylogenetische studies moet het testen van de congruentie van verschillende datasets (d.w.z. of ze wel of niet een tegenstrijdig fylogenetisch signaal bevatten) een standaardprocedure worden, alvorens de datasets samen te voegen in één analyse. Indien dit niet wordt gedaan dient elke dataset apart te worden geanalyseerd.

VI

De rang van divisie is, vanuit het perspectief van een algoloog, veel te veel eer voor de hogere planten (Bryophyta en Tracheophyta). De rang van klasse binnen de Chlorophyta is voldoende erkenning.

VII

DNA extraheren uit bruinwieren is net zo makkelijk als koffie zetten. Goed DNA uit bruinwieren extraheren is net zo moeilijk als goede koffie zetten.

VIII

De groenwiergeslachten *Enteromorpha* (darmwier) en *Ulva* (zeesla) dienen samen in één geslacht (*Ulva*) ondergebracht te worden.
Malta et al. *(1999).*

IX

Het promotiereglement van de Universiteit Leiden bestaat uit vele ongeschreven regels.

X

In verband met de dreigende stijging van de zeespiegel verdient het aanbeveling nader onderzoek te doen naar het absorptievermogen van de in zee levende sponzen.

XI

De psychologische afstand tussen Leiden en Groningen is in beide richtingen even groot.
Contra: mijn Groningse vriendenkring.

XII

Een rondje aan een draaiende bar hoeft niets te kosten.

Calling a Class to Order

Phylogenetic Studies in the Phaeophyceae

PROEFSCHRIFT

ter verkrijging van
de graad van Doctor aan de Universiteit Leiden,
op gezag van de Rector Magnificus Dr. D.D. Breimer,
hoogleraar in de faculteit der Wiskunde en
Natuurwetenschappen en die der Geneeskunde,
volgens besluit van het College voor Promoties
te verdedigen op dinsdag 25 juni 2002
te klokke 16.15 uur

door

STEFANO GIOVANNI ANGELO DRAISMA
geboren te Sneek/Snits in 1970

PROMOTIECOMMISSIE:

Promotores: Prof. dr P. Baas
Prof. dr J.L. Olsen (Rijksuniversiteit Groningen)

Co-promotores: Dr W.F. Prud'homme van Reine
Dr W.T. Stam (Rijksuniversiteit Groningen)

Referent: Prof. dr E. Coppejans (Universiteit Gent, België)

Overige leden: Prof. dr E. Gittenberger
Prof. dr D.J. Kornet

Cover: *Sphacelaria tsengii* (Photo: S.G.A. Draisma) and on the back Fig. 8.1 (see Chapter 8).
Printed by: Optima Grafische Communicatie, Rotterdam, The Netherlands.
ISBN: 90-77017-66-6.

The research reported in this thesis was carried out at:
Nationaal Herbarium Nederland – Universiteit Leiden branch.
Department of Marine Biology – University of Groningen.

This study was supported by grant No. 805-42.025 from the Foundation of Earth- and Life-Sciences (ALW), which is subsidised by the Netherlands Organization for the Advancement of Research (NWO).

CONTENTS

Oh! call us not weeds but flowers of the sea,
for lovely, and gay, and bright tinted we are:
Our blush is as deep as the rose of thy bowers,
then call us not weeds, we are ocean's gay flowers.
Not nursed like the plants of the summer parterre,
whose gales are but sighs of an evening air:
Our exquisite, fragile and delicate forms
are nurs'd by the ocean, and rock'd by the storms.

Anonymous, XIX Century.

Chapter 1

GENERAL INTRODUCTION

INTRODUCTION

'Algae' is the common name for a diverse polyphyletic assemblage of photosynthetic prokaryotic and eukaryotic lineages that occur in virtually all imaginable habitats. Among the eukaryotic, multicellular macrophytes or seaweeds, three main groups are recognised on the basis of their photosynthetic pigmentation (Ben Ali *et al.* 2001), *viz.* the Chlorophyta (green algae), Rhodophyta (red algae), and Chromophyta (brown and golden-brown algae). The latter group, which is paraphyletic, includes many unicellular phytoplankton which are further subdivided into many smaller lineages (compare circumscriptions and terminology between van den Hoek *et al.* [1995] and Graham and Wilcox [2000]) on the basis of cell coverings, specialised pigments, plastid and flagellar ultrastructure, and phylogenies based on comparative DNA sequences (Bhattacharya *et al.* 1992, Leipe *et al.* 1994, Bhattacharya and Medlin 1995, Medlin *et al.* 1997, Cavalier-Smith 1998, Ben Ali *et al.* 2001). The Phaeophyceae or brown algae belong to this last group.

One of the brown algal orders for which the evolutionary history has remained unexplored is the Sphacelariales. This order has been variously considered as relatively primitive to relatively advanced based on the significance attached to morphological complexity and the mode of tissue formation. Moreover, the monophyly of this order has not been established nor its position within the Phaeophyceae. Therefore, the first task of the thesis was to 'call the class to order'.

The extensive culture collection of Sphacelariales specimens available at the *Universiteit Leiden* branch of the *Nationaal Herbarium Nederland* provided an excellent opportunity to undertake such a study. The subsequent analyses presented in this thesis were undertaken in a descending hierarchical order from class to species. One of the major hindrances in algal taxonomy is the presence of relatively few diagnostic, morphological characters combined with high levels of convergence (= homoplasy). This makes identification difficult and has resulted in the proliferation of different circumscriptions based on taxonomic opinion rather than formal analyses. The only way around the so-called 'low morphology' problem is via the addition of comparative DNA sequence analysis. Although sequence data are also subject to homoplasy in varying degrees, their independence from morphology provides a basis from which to evaluate both the diagnostic and phylogenetic value of particular morphological characters. Taken together, morphological and molecular data sets provide the standard for modern taxonomic and phylogenetic studies.

The aims of this thesis were to: (1) explore evolutionary relationships within the Phaeophyceae; (2) determine the position of the Sphacelariales within the brown algae;

(3) establish the (non)monophyly of the Sphacelariales; (4) examine species radiations within the Sphacelariales; and (5) reassess the role of morphological characters in the taxonomy and classification of this ecologically broad group.

THE BROWN ALGAE

Brown algae form the class Phaeophyceae or Fucophyceae. One or many (most often discoid) chloroplasts occur per cell and are golden-brown in colour, because the chlorophyll (a, c_1 and c_2) is masked by the accessory pigment fucoxanthin. Some species have chloroplasts associated with pyrenoids. Motile cells are produced as gametes or zoospores, bearing two dissimilar flagella. There are more than 1,500 species of brown algae distributed over more than 250 genera (Wynne 1982). They vary from tiny filamentous forms to gigantic thalli >60 meters in length. Brown algae are almost entirely restricted to the intertidal and sublittoral zones, particularly of rocky shores. Only six genera are known from freshwaters. The Phaeophyceae exhibit the highest diversity in regard to number of species in the cold-temperate oceans of both hemispheres. Although not represented by many species in the tropics, brown algae are often among the most dominant seaweeds.

The characteristics that are most useful in distinguishing the orders of brown algae include thallus construction, mode of growth and the type of life history. Three types of thallus construction are recognised: filamentous, parenchymatous and pseudoparenchymatous. Filamentous thalli are uniseriate, i.e., they consist of a single row of cells initiated from a basal, intercalary or apical meristematic region. In contrast, parenchymatous thalli are composed of true tissue in which cell divisions occur in more than one plane. Pseudoparenchymatous tissues consist of closely compacted uniseriate filaments, and thus superficially resemble a true parenchyma. Parenchymatous tissues are also referred to as polystichous, pseudoparenchymatous and filamentous as haplostichous, and predominantly uniseriate filaments as oligostichous. The Sphacelariales are characterised by parenchymatous thalli and apical growth which will be discussed later.

The life cycle of most brown algae involves an alternation of free-living generations (= diplohaplontic life history). In species with an isomorphic diplohaplontic life history the sporophytic stage and the gametophytic stage are indistinguishable (apart from the reproductive structures). In species with a heteromorphic diplohaplontic life history the two alternating generations are different. A diplontic life history is a third kind of sexual life history occurring in brown algae in which only a single diploid phase is formed. Sexual phases appear to be absent in some groups. The Sphacelariales have a mainly isomorphic alternation of free-living generations.

The currently recognised brown algal orders are: the Ascoseirales (1 family), Cutleriales (1 family), Desmarestiales (2 families), Dictyotales (3 families), Ectocarpales (23 families), Fucales (7–8 families), Laminariales (8 families), Ralfsiales (2 families), Scytothamnales (2 families), Sphacelariales (3–4 families), Sporochnales (1 family), Syringodermatales (1 family), and Tilopteridales (1 family). The Chordariales (8 families), Dictyosiphonales (including the Punctariales) (11 families), and

Scytosiphonales (2 families) are presently included in the Ectocarpales (de Reviers and Rousseau 1999). Peters and Ramírez (2001) have recently proposed a reduction in the number of families within the Ectocarpales to five. The orders Durvillaeales (1 family) and Notheiales (1 family) are currently included in the Fucales (de Reviers and Rousseau 1999). Characteristics of brown algal orders are summarised in Figure 1.1.

Evolutionary relationships among brown algal orders are still a subject of discussion. Over the years, a number of phylogenetic hypotheses have been put forth but in the absence of any formal analysis of homology. For example, Wynne and Loiseaux (1976) envisioned two deep sister lineages, one including the Fucales and Durvillaeales (together called Cyclosporidae), and one including all the remaining orders (together called Phaeophycidae) with the Ectocarpales *sensu stricto* (*s.s.*) most primitive. They based these two groups on the life history: the Cyclosporidae having a diplontic life history and the Phaeophycidae having an alternation of generations. Clayton (1984) suggested that the Dictyotales represented an independent evolutionary lineage, not closely related to any of the other orders. She based her hypothesis on the presence of unique uniflagellate spermatozoids and meiosporangia that differ from the more typical unilocular sporangia found in brown algae. Van den Hoek *et al.* (1995) considered the Ectocarpales *s.s.* to be the most primitive brown algae based on their simple filamentous construction. Likewise they considered the Fucales and Durvillaeales as the most advanced based on the lack of a free-living gametophytic phase. The remaining orders have been variously intercalated based on the relative importance attached to pyrenoids, growth forms, and oogamy.

With the advent of DNA sequencing a number of phylogenetic studies have emerged over the past decade. Most of the studies, particularly those from the early 1990s, opted for a survey approach in which only one or two species were used to represent entire orders (references in Table 2.1, Chapter 2). More recent studies, however, have focused on particular orders with more complete taxon sampling. These include the Ectocarpales (Stache-Crain *et al.* 1997, Siemer *et al.* 1998), the Desmarestiales (Peters *et al.* 1997), the Fucales (Rousseau and de Reviers 1999a), the Laminariales (Boo *et al.* 1999a, b, Lane *et al.* 2001), and the Scytothamnales (Müller *et al.* 1998). The unevenness of the exemplar approach to taxon sampling combined with (often) incomplete sequences and/or different sequences and different taxon sampling have resulted in a mosaic view of the Phaeophyceae in which sister-group relationships have remained uncertain. Resolving this problem was one of the main challenges of this thesis.

THE SPHACELARIALES

The order Sphacelariales owes its name to the genus *Sphacelaria* (Gk. σφακελος = gangrene). Thalli are heterotrichous varying from small tufts (<2 cm) to relatively large plants (30–40 cm). Epiphytic, endophytic, and epilithic forms occur, and occasionally all three habits occur within a single species. Species occur from polar to tropical regions, but mainly in temperate regions. The main centres of distribution

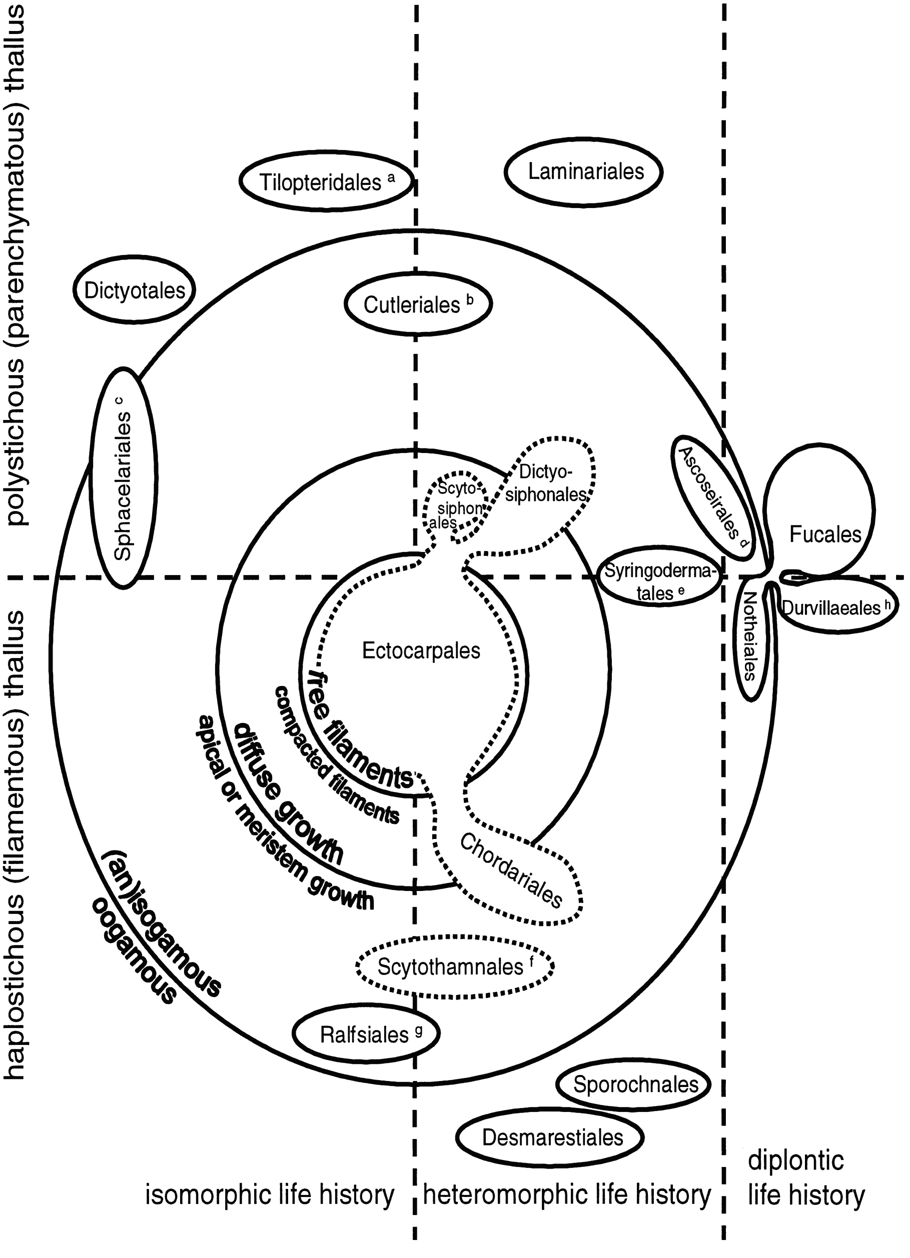


Fig. 1.1. Conceptual overview of relationships among classical orders of Phaeophyceae with respect to morphological grades of organisation and life history features. The figure is a modification of van den Hoek and Jahns (1978, p. 146, fig. 37), Womersley (1987, p. 21, fig. 1) and de Reviers and Rousseau (1999, pp. 132–134, table 5). Connected orders have recently been merged on the basis of phylogenetic analyses. Orders containing pyrenoids are indicated with a dotted outline. Superscripts refer to the additional comments listed on the opposite page.

are along European coasts and in southern Australia and New Zealand (Fig. 1.2). Two freshwater species have also been described (Jao 1943, Schloesser and Blum 1980). There appears to be a disjunct distribution between Europe and Australia (Prud'homme van Reine 1993). Whether this represents a sampling artefact or a true vicariance remains unclear. All tropical species belong to the *Sphacelaria* subgenus *Propagulifera* and are circumtropical with dominance in the Indo-West Pacific.

Growth, branching, and cell division

The Sphacelariales are characterised by temporarily blackening of the cell walls when treated with bleaching liquid (Eau de Javelle, aqueous sodium hypochlorite) and growth by conspicuous apical cells. Transverse divisions of the subapical cells (or primary segments) produce secondary segments that may undergo longitudinal and secondary transverse divisions (Fig. 3.1, Ch. 3). In this manner a distinctive parenchymatous construction is produced. The cells contain a large number of disc-shaped chloroplasts with no obvious or only rudimentary pyrenoids. Branches in the Sphacelariales originate either from the apical cell (acroblastic) or from one of its derivatives (hypacroblastic) (Fig. 3.1). Two types of hypacroblastic branching are recognised: hemiblastic and meriblastic. In the hemiblastic method lateral branches (whether determinate or indeterminate) arise from a cell occupying the entire height of a superior secondary segment, thus half the length of a primary segment. The meriblastic method involves the development of a lateral from only a part of a secondary segment rather than the entire segment. Two types of acroblastic branching are recognised: acroheteroblastic and acrohomoblastic. Acroheteroblastic branching involves an apical cell that cuts off a small apical lenticular branch-initial. In an early stage this branch-initial divides horizontally, cutting off a small cell on its upper side. The lower and larger cell of the branch-initial form the lateral, while the upper and smaller cell may give rise to a bundle of hairs, to one or more zoidangia, to one small lateral, or to a group of resting cells. The larger part of the original apical cell, located below and to one side of the small lenticular cell, elongates and functions again as the apical cell of the main axis

---

Explanation of the superscripts in Figure 1.1.

[a] *Haplospora* is isomorphic; only the gametophytic phase exists in *Tilopteris* (Kuhlenkamp and Müller 1985); only vegetative propagation in *Phaeosiphoniella* (Hooper *et al.* 1988).

[b] *Zanardinia* is isomorphic (Fritsch 1945); *Cutleria* is heteromorphic; only the sporophyte of *Microzonia* is known (O'Donnell 1954), its inclusion in the Cutleriales is a matter of debate (Eichenberger *et al.* 1993).

[c] *Sphacella* is haplostichous (Prud'homme van Reine 1982); *Stypocaulon* is oogamous (Prud'homme van Reine 1993).

[d] No free gametophytic phase.

[e] *Syringoderma floridana* E. C. Henry and *S. abyssicola* (Setchell *et* Gardner) Levring have a very reduced gametophytic phase which is retained on the sporophyte (Henry 1984); oligostichous.

[f] *Splachnidium* is heteromorphic (Price and Ducker 1966); the gametophyte and sporophyte of *Scytothamnus* are morphologically similar (Clayton 1986).

[g] *Ralfsia* has a direct life cycle (sexuality unknown); *Nemoderma* is isomorphic (Kuckuck 1912); *Analipus* is also isomorphic (Abe 1935, 1936), but its inclusion in the Ralfsiales is a matter of debate (Nelson 1982, Tan and Druehl 1994, de Reviers and Rousseau 1999).

[h] Intercalary, diffuse growth, but mainly in the apical zone.

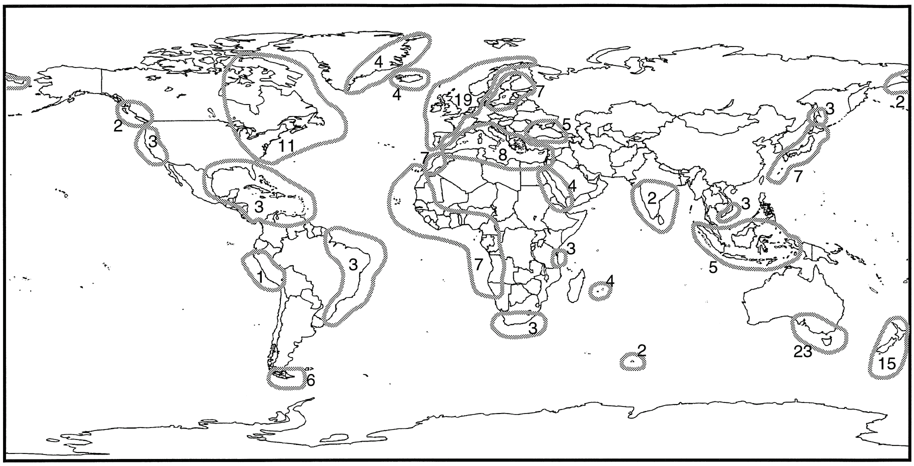


Fig. 1.2. Worldwide distribution of Sphacelariales. Numbers associated with outlined geographical areas indicate the number of species found in each area. Redrawn from Prud'homme van Reine (1982, p. 4, fig. 1).

so that the small lenticular branch-initial is pushed into a lateral position. Acrohomoblastic branching involves the formation of only one kind of structure (a hair or a lateral) from the lenticular initial cell. A special case of acrohomoblastic branching is dichotomy occurring in the apical cell (dichoblastic branching). Phaeophycean hairs are present in several sphacelarialean species and are usually formed by acroblastic branching.

Two different patterns of division by the longitudinal cell walls can be observed in transverse section of the multiseriate (polysiphonal) secondary segments: a radial pattern and a periclinal one (Fig. 3.1, Ch. 3). In both patterns the first longitudinal wall is in principle radial. The two next walls are usually perpendicular to the primary longitudinal wall. In the radial type later walls form acute angles with the ones formed before, but in the periclinal type all walls are perpendicular to each other.

In many Sphacelariales the segments exhibit almost no further enlargement after their formation. In these so-called leptocaulous Sphacelariales the diameter of the mature segments of the filaments equals or slightly exceeds the diameter of the apical cell at the time of formation of these segments. The length of the secondary segments does not alter in the leptocaulous Sphacelariales. In another group of Sphacelariales, called auxocaulous, the secondary segments can enlarge conspicuously both in length and in diameter. The only means of conspicuous secondary enlargement for the leptocaulous Sphacelariales is by downward growing corticating filaments (a pseudocortex) that originate from the peripheral cells of the axial filaments.

Life history

The complete life history of several species has been followed in culture: Sphacelaria *rigidula* Kützing (van den Hoek and Flinterman 1968), *Cladostephus spongiosus* (Hudson) C. Agardh (Gibson 1994), *Halopteris filicina* (Grateloup) Kützing (Keum *et al.* 1995) and *Onslowia endophytica* Searles (Henry 1987a). A slightly heteromorphic, diplohaplontic life history with vegetative propagules has been demonstrated for *Sphacelaria rigidula* (as *S. furcigera*) from The Netherlands, involving anisogamy and with a dependence on temperature (Fig. 1.3) (van den Hoek and Flinterman 1968). The dioecious gametophytes were found to be smaller than the sporophytes. The female macrogametangia and the male microgametangia developed in cultures at 4 and 12 °C (under a 12 h daylength), whereas propagules were produced by the gametophytes grown at 20 °C. Although unfused microgametes did not survive, macrogametes were able to germinate (parthenogenesis), giving rise to presumptive female gametophytes or haploid sporophytes bearing unilocular sporangia. These latter plants represent an evolutionary dead end in the life cycle though they may persist in nature for long periods. Nothing is known about the evolutionary fate of these plants. Sporophytes formed unilocular sporangia at 4 °C (under a 12 h daylength) but only propagules at 12 and 20 °C. In a subsequent publication by Colijn and van den Hoek (1971) it was observed that propagules were formed only at higher temperatures (12 and 17 ℃) and under intermediate (12 h daylength) or long day (16 h daylength) conditions. Propagules were not formed at lower temperatures (4 °C) or under short day conditions (8 h daylength) at higher temperatures (both 12 and 17 °C). On the basis of these studies along with often incomplete evidence from other publications an isomorphic or slightly heteromorphic diplohaplontic life history as in *S. rigidula* has been hypothesised for

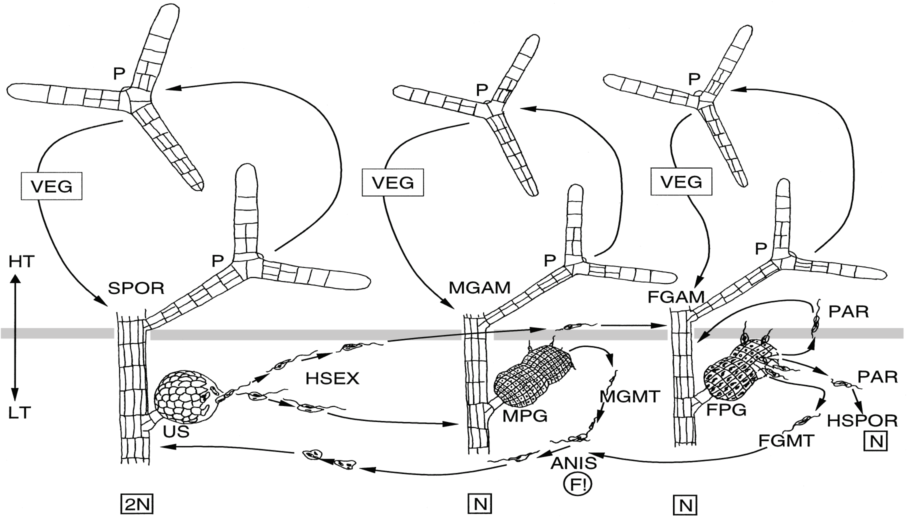


Fig. 1.3. Life history of *Sphacelaria rigidula*. 2N = diploid; ANIS = anisogamy; F! = fertilisation; FGAM = female gametophyte; FGMT = female (macro-)gamete; FPG = female plurilocular gametangium (macrogametangium); HSEX = haplogenotypic sex determination; HSPOR = haploid sporophyte; HT = high temperature, e.g., 12–20 °C; LT = low temperature, e.g., 4–12 °C; MGAM = male gametophyte; MGMT = male (micro-)gamete; MPG = male plurilocular gametangium (microgametangium); N = haploid; P = propagule; PAR = parthenogenesis; SPOR = sporophyte; US = unilocular sporangium; VEG = vegetative reproduction. (After van den Hoek *et al.* 1995, p. 183, fig. 12.11).

all Sphacelariales (Prud'homme van Reine 1982). Vegetative propagation in the Sphacelariales occurs in the subgenus *Propagulifera* of the genus *Sphacelaria* and in the Choristocarpaceae. This occurs by the production of multicellular branchlets (vegetative 'mini-thalli') termed propagules (Fig. 1.3).

Traditional circumscriptions of the families

The Sphacelariales is subdivided into four families: the Cladostephaceae, Sphacelariaceae, Stypocaulaceae, and Choristocarpaceae. The inclusion of the latter family in the Sphacelariales has been questioned by Prud'homme van Reine (1993) with the result that he referred to the Sphacelariales *sensu stricto* (excluding the Choristocarpaceae) and *sensu lato* (including the Choristocarpaceae). Examples of some taxa belonging to the Sphacelariales are shown in Figure 1.4.

Fig. 1.4. **A**. 1–13 on p. 13, **B**. 14–24 on p. 14, and **C**. 25–33 on p. 15**.** Range of morphological types in the Sphacelariales.

1–24. Sphacelariaceae; 1–23. *Sphacelaria*; 1–6. Subgenus *Sphacelaria*; 1, 2. *Sphacelaria reticulata* Lyngbye (= type species); 1. Habit; 2. Dichoblastic branching; 3, 4. *Sphacelaria radicans* (Dillwyn) C. Agardh; 3. Unilocular zoidangia; 4. Basal disk; 5. *Sphacelaria caespitula* Lyngbye, unilocular zoidangia; 6. *Sphacelaria nana* Naegeli *ex* Kützing, young plant; 7–13. Subgenus *Pseudochaetopteris*; 7–10. Section *Pseudochaetopteris*; 7–9. *Sphacelaria plumosa* Lyngbye; 7. Longitudinal section of corticated main filament; 8. Part of erect filament; 9. Habit; 10. *Sphacelaria plumigera* Holmes, habit; 11–13. Section *Racemosae*; 11, 12. *Sphacelaria racemosa* Greville; 11. Plurilocular zoidangia; 12. Part of erect filament; 13. *Sphacelaria arctica* Harvey, habit; 14–15. Subgenus *Battersia*; 14, 15. *Sphacelaria mirabilis* (Reinke *ex* Batters) Prud'homme van Reine; 14. Longitudinal section of polystromatic disk with unilocular zoidangia; 15. Habit on stone; 16. *Incertae sedis* (freshwater); 16. *Sphacelaria fluviatilis* Jao, part of a filament with propagation cells; 17–23. subgenus *Propagulifera*; 17–18. section *Propagulifera*; 17, 18. *Sphacelaria cirrosa* (Roth) C. Agardh; 17. Germinating propagule and a detached mature propagule; 18. Habit on *Cystoseira baccata* (S.G. Gmelin) P.C. Silva; 19–20. Section *Furcigerae*; 19, 20. *Sphacelaria rigidula* Kützing; 19. Habit on *Cystoseira* spec.; 21–23. Section *Tribuloides*; 21–23. *Sphacelaria tribuloides* Meneghini; 21. Propagule; 22. Habit; 23. Germinating propagule; 24. *Sphacella subtilissima* Reinke, erect filament with unilocular zoidangia; 25–27. Cladostephaceae; 25–27. *Cladostephus spongiosus* (Hudson) C. Agardh; 25. Longitudinal section; 26. Transverse section; 27. Habit; 28–32. Stypocaulaceae; 28. *Alethocladus corymbosus* (Dickie) Sauvageau, acrohomoblastic branching; 29, 30. *Halopteris filicina* (Grateloup) Kützing; 29. Acroheteroblastic branching; 30. Habit; 31, 32. *Stypocaulon scoparium* (Linnaeus) Kützing; 31. Apex of a vegetative branch; 32. Habit; 33. Choristocarpaceae; 33. *Choristocarpus tenellus* (Kützing) Zanardini, filament with unilocular zoidangia.

Vertical scale bars represent 100 µm; thin horizontal scale bars represent 1 mm; and thick horizontal scale bars represent 10 mm.

Figures 8, 9 and 27 scanned from Lyngbye (1819); Figure 32 from Hauck (1885); Figure 14 from Reinke (1891); Figures 1, 12, 25, 26, 28 and 29 from Sauvageau (1900–1914); Figure 33 from Kuckuck (1929); Figure 16 from Jao (1943); Figures 17, 30 and 31 from Fritsch (1945); Figures 2–7, 10, 11, 13, 15 and 18–24 from Prud'homme van Reine (1982).

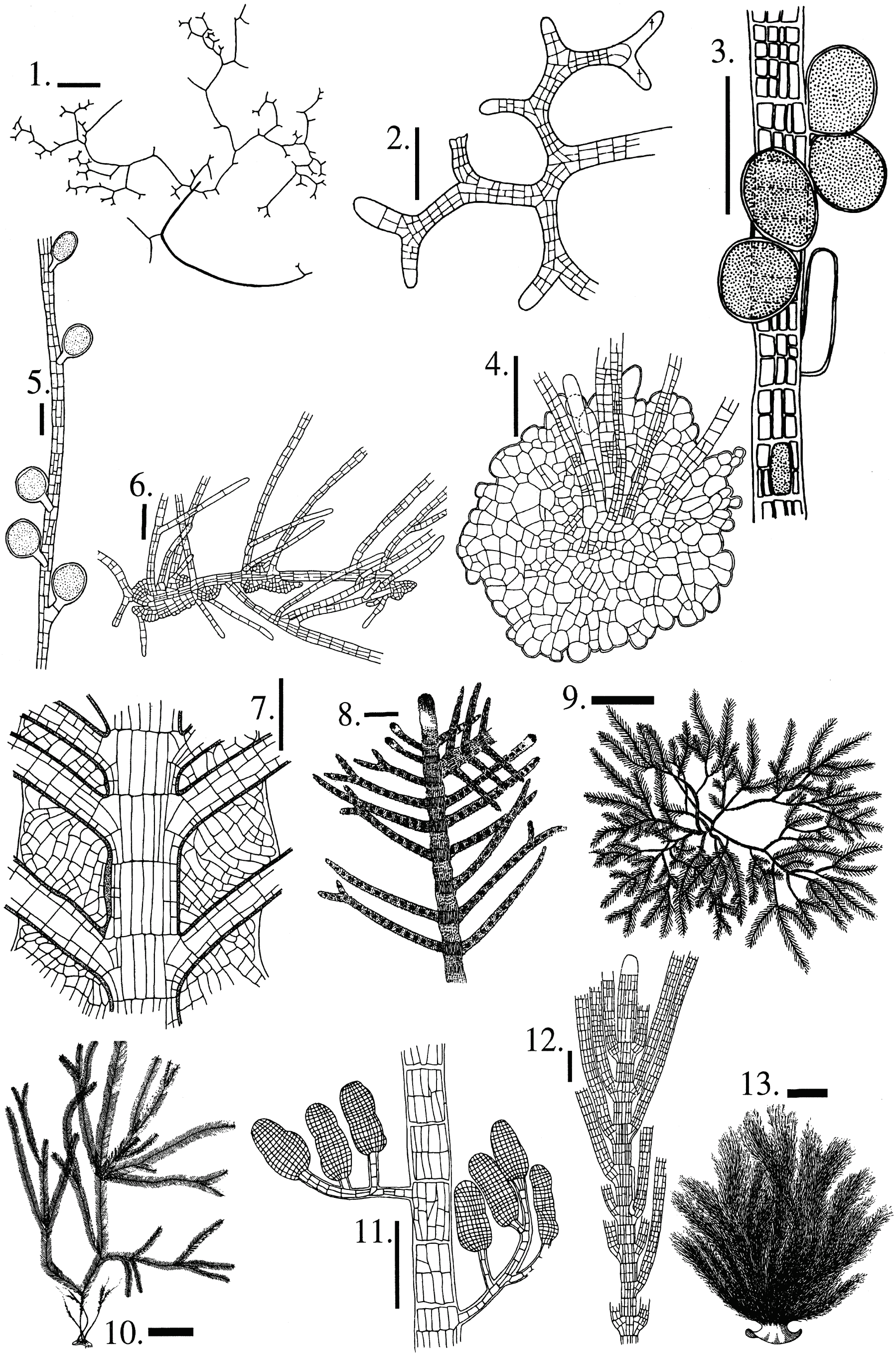


Fig. 1.4. A (legend on p. 12).

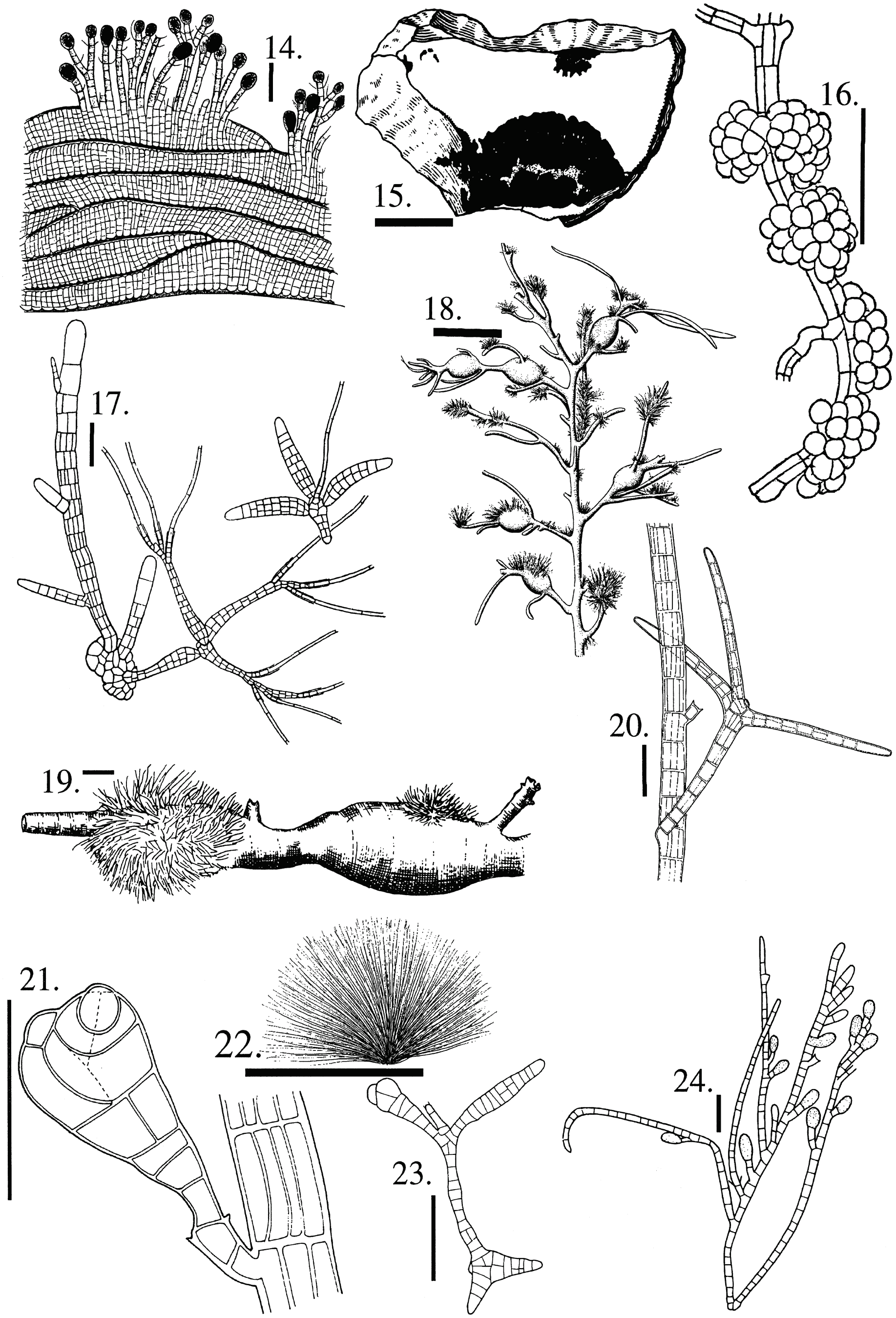


Fig. 1.4. B (legend on p. 12).

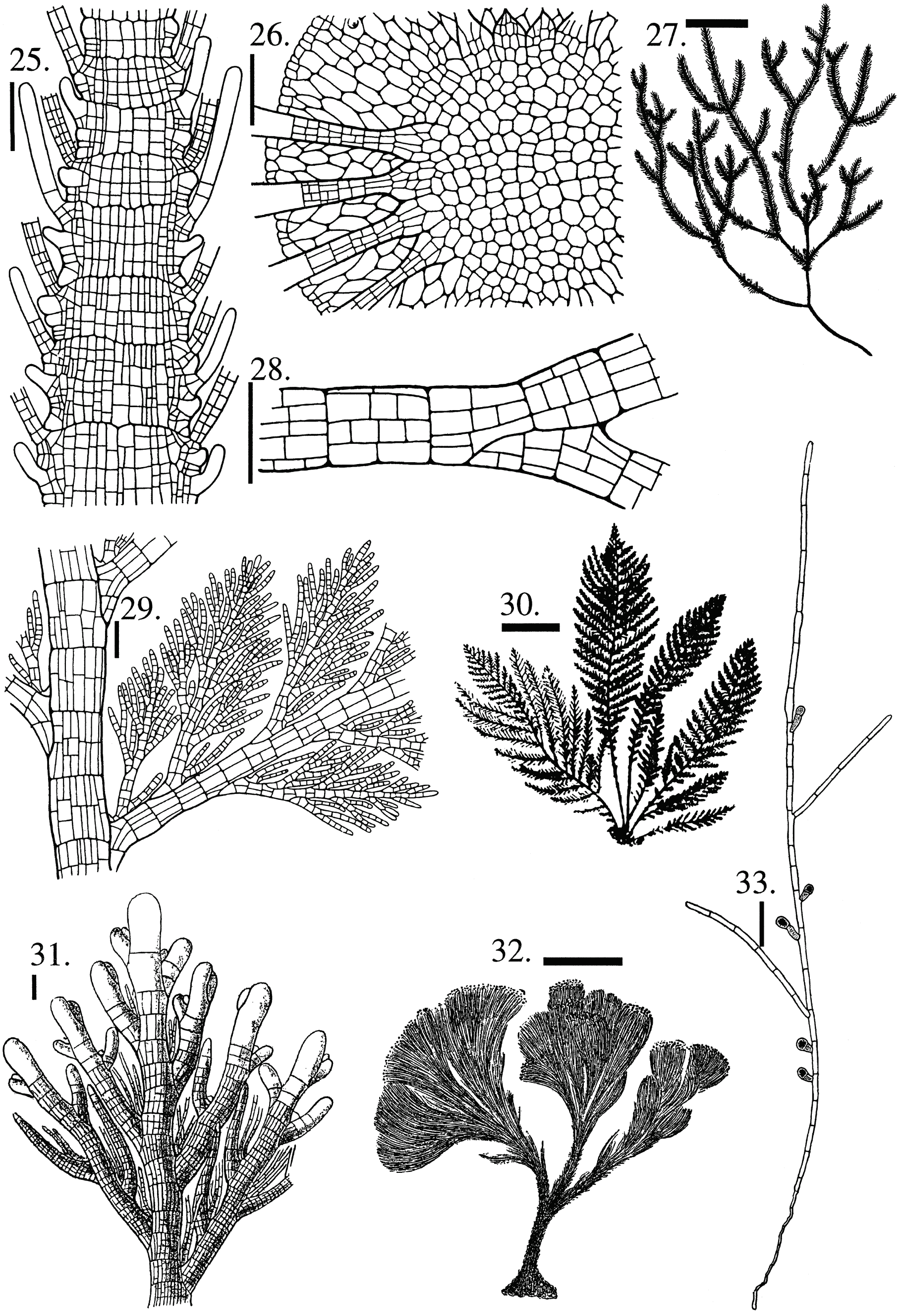


Fig. 1.4. C (legend on p. 12).

The following generalisation can be made based on classical data (Prud'homme van Reine 1982, 1993).

Cladostephaceae: Monotypic, characterised by auxocaulous growth and simultaneous occurrence of several different branching modes, an isomorphic diplohaplontic life history with isogametes (isomorphic, but physiologically differentiated) and perhaps also neutral plurilocular zoidangia. Parthenogenesis and ephebogenesis (the latter implying germination of unfused male gametes) have been observed.

Sphacelariaceae: Characterised by strict leptocaulous growth and hemiblastic formation of laterals, contains only two genera, the large genus *Sphacelaria* (over 40 species) and the monotypic genus *Sphacella*. Isomorphic or slightly heteromorphic diplohaplontic life history with isogamy or anisogamy. Occurrence of neutral plurilocular zoidangia is probable in several species. Parthenogenesis has been observed and vegetative propagation by propagules occurs in several species. *Sphacella* is characterised by the absence of longitudinal cell walls. *Sphacelaria* is subdivided into six subgenera.

Stypocaulaceae: Containing 4–5 different genera, characterised by acroblastic branching modes, either leptocaulous or auxocaulous. Life history with anisogamy or oogamy. Parthenogenesis and ephebogenesis have been observed. The genera are the monotypic *Alethocladus* and *Ptilopogon*, *Phloiocaulon* (three species), *Halopteris* (four species), and *Stypocaulon* (five species). *Stypocaulon*, the only oogamous genus, is merged with *Halopteris* by some authors.

Choristocarpaceae: Containing the genus *Onslowia* (two species) and three monotypic genera, *viz. Verosphacela*, *Choristocarpus*, and *Discosporangium*. The latter two genera are uniseriate, the former two oligostichous, all are leptocaulous. Characterised by apical growth without transverse division of subapical cells. The cell walls do not blacken when treated with bleaching liquid. Life history supposedly isomorphic diplohaplontic with unilocular and plurilocular zoidangia. *Choristocarpus*, *Onslowia*, and *Verosphacela* form propagules. Unilocular zoidangia have not been observed in *Discosporangium*.

IDENTIFICATION, CLASSIFICATION AND NOMENCLATURE

Taxonomy encompasses identification, classification and nomenclature. Identification of taxa, especially of species, is traditionally based on comparative morphology. The taxonomist decides which characters are diagnostic and how much morphological variation is allowed within a taxon. The degree to which this is qualitative or quantitative varies greatly. Reliable diagnostic characters are, of course, essential. If they cannot be found, then the quality of the taxonomy and subsequent classification suffers. Taxonomic classifications are hypotheses of relationship with or without an evolutionary basis. In the traditional context of the type-method, new specimens were compared against the standard, i.e., the holotype, and assigned accordingly. If sufficient discontinuities were found, then a new species and/or new circumscription was made based on taxonomic expertise and judgement. With the advent of numerical taxonomy (Sokal and Sneath 1963), phenetic classifications based on overall similarity operationalised the analysis of larger data sets and generally improved and organised various circumscriptions. However, in the 1970s, the writings of Hennig (1950, 1966) set the stage for a fundamental revolution in classification with the advent of cladistics. The core aspect of this school, which is now recognised more generally as phylogenetic systematics (Wiley *et al.* 1991, Hillis *et al.* 1996a), is the requirement that classifications reflect identity by descent or shared common ancestry through the formal recognition of shared homologies, i.e., patristic similarity and not just overall similarity. Moreover, explicit rules for character analysis and subsequent phylogenetic reconstruction were outlined, thus lending objectivity and independent reproducibility. These theoretical and methodological developments further coincided with the new field of molecular systematics in the 1980s, an approach to taxonomy independent from morphology (Moritz and Hillis 1996, Avise 1994). As will be shown, the approach to data analysis as well as the availability of different types of data have revolutionised taxonomy/ systematics but have created havoc (in many cases) by the creation of unstable classifications and nomenclatural nightmares that are difficult to reconcile under the Linnaean system. Rules governing the application of scientific names to plants, algae and fungi are embodied in the *International Code of Botanical Nomenclature* (ICBN) (Greuter 2000). The basic aim of the ICBN is to achieve stability of names and to provide formal means for changing names without losing the trail of earlier applied names.

Recently, a phylogenetic code of biological nomenclature has been proposed; the PhyloCode (Forey 2001). At the moment it is only in draft form and can be downloaded from the web at www.ohio.edu/PhyloCode. This code only recognises monophyletic groups. The PhyloCode will not be applied in this thesis, because it is not yet operational and rules governing species names have not yet been formulated.

PHYLOGENETICS

Phylogenetics aims to unravel the shared evolutionary history among taxa. Unravelling shared ancestry is done through the assessment of homology and ultimately, the recognition of nested monophyletic groups. Determination of homology is achieved either by structure or position. In the case of morphological characters, structural comparisons are the most common, especially if the ontogeny is known. Where this is not possible, characters and character-states can be coded in an unordered manner. In the case of DNA sequence data, homology is assessed by position, i.e., the position of a nucleotide in an alignment where characters are the position in the sequence of interest and character-states are the nucleotides A, C, T and G. Different evolutionary models of sequence evolution can be utilised.

The quality of the phylogenetic analysis depends on both data quality and quantity. Careful analysis and coding of morphological characters and their states and/or careful alignment of DNA sequences are paramount in combination with adequate taxon sampling.

In any data set, there will be at least some conflicts in character-state distributions across the taxa being compared. This is convergence or homoplasy, which leads to multiple possible tree topologies. Even in small data sets involving as few as ten taxa the number of potential ‘trees’ is huge: 2,027,025 unrooted trees or 34,459,425 rooted trees (Page and Holmes 1998). In larger data sets, exhaustive searches are not feasible;

heuristic approaches must be used. Most tree-drawing algorithms attempt to minimise conflicts among character-state distributions between the data matrix and the topology of the tree.

Standard approaches to tree construction include both distance and character-based approaches. The most common are neighbour joining (NJ) (Saitou and Nei 1987), maximum parsimony (MP) (Farris 1970, Swofford and Olsen 1990) and maximum likelihood (ML) (Cavalli-Sforza and Edwards 1967, Felsenstein 1981a, b, Kishino and Hasegawa 1989). Advantages and disadvantages of each approach depend on the type, quality and quantity of the data as well as the size of the group under study. Although ML methods are generally favoured because they are related to a specific underlying, evolutionary model, there are still computational limitations on the size of the data sets that can be analysed and the method is not applicable to morphological data. Maximum parsimony methods look for the shortest tree, i.e., the minimum number of character-state changes that have to occur to support a particular topology. The advantage of the approach is that it can handle large data sets, especially using heuristic search approaches and using optimality criteria (in this case minimising the number of character-state transformations) which produce an estimate of relative support. The major disadvantage is that it does not show how much better a particular tree is than another tree of the same length or slightly longer. Neighbor-joining is a distance method (the other two are character-based) that calculates trees based on estimates of the total number of evolutionary events that have occurred following divergence. The advantage of NJ is that it is quick and generally yields only one tree, the disadvantage is that it is a pure algorithmic method that does not invoke optimality criteria, i.e., does not show how much better the reconstructed tree performs than other possible trees.

The bootstrap and the jackknife are tools to estimate how well the data fit the trees. The bootstrap approach (Felsenstein 1985) involves a random resampling of characters from the data set with replacement. Each new data set is reanalysed resulting in a new phylogeny. This process of resampling and analysis is repeated ≥1000 times and a 50% majority-rule consensus tree is made from all resulting trees. Bootstrap support is expressed as a percentage. There is no general agreement among researchers about the cut off value of bootstrap support values. Felsenstein (1985) originally suggested that a bootstrap value of 95% should be taken as robust support. However, Hillis and Bull (1993) showed, using empirical data and computer simulations, that almost every branch with bootstrap support >80% defined a true clade, and that >95% of the clades with bootstrap support >70% were correct.

The character jackknife (Farris *et al.* 1996) is another resampling technique. Jackknifing involves a random resampling of the data without replacement. However, only a fraction of the original data is used in each reanalysis. Again a consensus tree is made from all resulting trees. The number of retained clades is dependent upon the proportion of characters that was deleted in each replicate data set.

Decay analysis or Bremer support (Bremer 1988) is used to test the reliability of MP trees. Support for a clade is expressed as the number of extra steps that are needed in order to collapse that clade. A difficulty with this index is that it is not clear how large a value must be for the group to be considered well supported.

DATA

The concern in phylogenetics is to find homologous characters or synapomorphies that define monophyletic groups and thereby reflect shared common ancestry. Data sets that have too much or too little variation, and therefore an insufficient number of phylogenetically informative characters, will reduce the resolution of the tree. Characters that are unique to a single species (= autapomorphies), for example, define those species and are excellent identification markers while being useless as phylogenetic markers because each taxon becomes its own group but there is no way of knowing what the relationships among the groups are. At the other extreme, a character that is present in all species has little phylogenetic value within that group because there is no discriminatory power. Between these extremes are synapomorphies, i.e., shared homologous characters that sequentially and hierarchically define monophyletic groups. Unfortunately, characters can be gained and lost as well as arising more than one time. This is convergence or homoplasy. The amount of homoplasy in a data set further affects the ability to accurately reconstruct phylogeny. The major shortcoming of phenetic approaches, based on overall similarity of shared characters associated with clustering and the old numerical taxonomy, is that homologous and convergent characters remain mixed.

In the Sphacelariales, as in many other algae, the number of morphological characters is limited and the potential for convergent morphological traits is high. This constraint cannot be altered. While the search for new characters is sometimes fruitful, the net gain is generally small. At the DNA level, however, the number of characters is limited only by the availability of suitable sequences, i.e., those having enough variation for the level of the question being addressed. Highly conserved sequences, for example, will not be appropriate for constructing phylogenies at the species level, whereas rapidly evolving sequences may present too much variation at the family or ordinal levels. Since some sequences will have more or less resolution, it is also desirable to use multiple sequences whenever possible and preferably from both nuclear and organellar compartments. This has two functions. First, it provides more data and thus, potentially more resolving power; and second, it acts as a check on whether the gene tree and species tree can be regarded as equivalent (Hillis *et al.* 1996b, Slowinski and Page 1999). Differences between trees may also signal lateral gene transfer, hybridisation events and so forth, which are confounding for the phylogeny but of interest biologically. For these reasons DNA sequences from both the nuclear encoded ribosomal cistron and the chloroplast encoded RUBISCO-gene were used.

The nuclear ribosomal cistron (Fig. 1.5)**.**
Within eukaryotic genomes, ribosomal DNA (rDNA) is organised in long arrays of hundreds or even thousands of tandem repeats (Soltis and Soltis 1998). Intergenic spacers (IGS) separate these rDNA repeats. A single eukaryotic rDNA repeat consists of three genes: the small subunit (SSU or 18S), 5.8S, and large subunit (LSU or 26S). The distal ends of the IGS, which are called the external transcribed spacers, flank the 5’-end of the SSU and the 3’-end of the LSU. Between the three rRNA genes lie the two internal transcribed spacers (ITS1 and ITS2). The entire rDNA repeat is transcribed into a precursor rRNA (pre-rRNA). The pre-rRNA is subsequently processed into the

different mature rRNA types by removal of all transcribed spacer regions. Mature eukaryotic SSU rRNA binds with about thirty different proteins to yield the small subunit of the ribosome. For the assembly of the eukaryotic large ribosomal subunit, 26S and 5.8S rRNA, and 5S rRNA bind together with about fifty different ribosomal proteins.

In most eukaryotes, the 5S rRNA gene is found at a different locus than that of larger rDNAs on the nuclear genome. However, physical linkage of the 5S rRNA and the other rRNA genes has been reported in several eukaryotes among which the brown alga *Scytosiphon lomentaria* (Lyngbye) Link (Kawai *et al.* 1995). A linked 5S rRNA can be hypothesised for all Phaeophyceae or even all heterokonts for it has also been reported in the pseudo-fungi Oomycetes, although also unlinked 5S rRNA genes have been reported in the Oomycetes (Belkhiri *et al.* 1992).

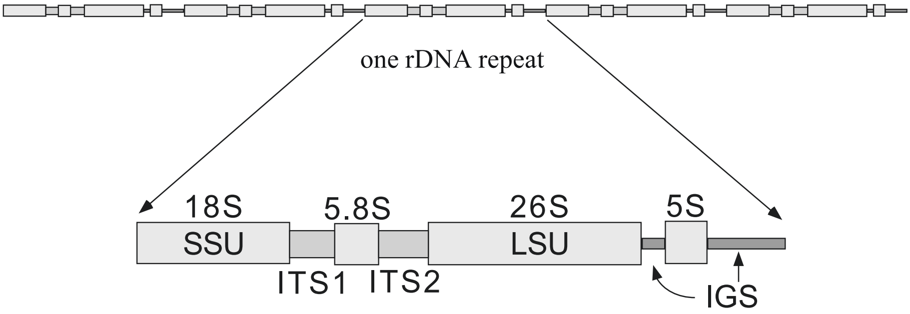


Fig. 1.5. Schematic representation of the nuclear ribosomal DNA cistron in the brown alga *Scytosiphon lomentaria*. See text for abbreviations. (After Kawai *et al.* 1995).

The regions of the ribosomal cistron provide a wide range of phylogenetic resolution and have been used from the level of populations to kingdoms. The SSU, 5.8S, and LSU genes are the most conserved regions of the cistron and have been used to unravel deep relationships. Highly conserved and more variable areas can be discerned within the rRNA genes (van de Peer *et al.* 1997, Ben Ali *et al.* 1999). The ITS regions are relatively free of evolutionary constraints and can show considerable length variation. The ITS1 and ITS2 are among the most widely used sequences for phylogenetic reconstruction at the species level in algae (van Oppen *et al.* 1995, Peters *et al.* 1997, Olsen *et al.* 1998, Malta *et al.* 1999, Meusnier *et al.* 2001).

Although the rDNA repeats are homogenised via processes of concerted evolution (Zimmer *et al.* 1980, Li 1997, Page and Holmes 1998), intra-individual variation due to non-homogenised repeats is potentially problematic and must always be checked for by sequencing several clones per individual. Intra-individual polymorphism is most commonly due to incomplete lineage sorting through hybridisation, prolonged asexuality or the possibility of cistrons being located at multiple loci on different chromosomes (Sang *et al.* 1995, Wendel *et al.* 1995, Quijada *et al.* 1997, Hugall *et al.* 1999, Diekmann *et al.* 2001). The empirical experience with ITS sequences in algae is species specific. In long separated species within *Desmarestia* (Phaeophyceae), virtually no intra-individual variation was detected (Peters *et al.* 1997), whereas in a recent study within the *Caulerpa racemosa* (Forsskal) J. Agardh complex (Chlorophyta) (Famà *et al.* 2000), intra-specific and intra-individual variation were extremely high.

The RUBISCO-gene (Fig. 1.6).
D-ribulose 1,5-bisphosphate carboxylase-oxygenase (abbreviated as RuBisCo, Rubisco, or RUBISCO) is a bifunctional enzyme (EC 4.1.1.39). It catalyses the carboxylation of ribulose 1,5-bisphosphate (RuBP) in the Calvin cycle and, in the presence of high levels of O_2 and low levels of CO_2, the oxygenation and cleavage of RuBP to form phosphoglycolate and 3-phosphoglycerate (Kellogg and Juliano 1997). RUBISCO from most sources consists of large and small subunits (LSU and SSU). In green algae and land plants the gene coding for the SSU (*rbc*S) is located in the nucleus and the gene coding for the LSU (*rbc*L) in the chloroplast. However, in chromophytes and rhodophytes *rbc*L and *rbc*S are both encoded by the chloroplast genome and a transcribed spacer separates them: the RUBISCO-spacer. The two genes are co-transcribed (Valentin and Zetsche 1989, Hwang and Tabita 1991, Kostrzewa *et al.* 1990).

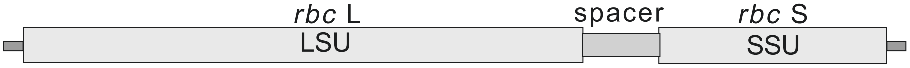


Fig. 1.6. Schematic representation of the RUBISCO-operon in chromophytes. See text for abbreviations.

The RUBISCO-genes and -spacer provide an opportunity to assess the phylogeny of photosynthetic organisms at different hierarchical levels, because the genes are more conserved than the spacer and within protein-coding genes first and second codon positions are more conserved than third codon positions. A benefit of protein-coding genes over rDNA is the relative ease of alignment, because required gaps are always a multiple of three nucleotide positions long (i.e., the length of a codon). Evolution of *rbc*L is strongly constrained by function. The maximum number of informative sites may be about one quarter less than the total number of bases (Kellogg and Juliano 1997). *Rbc*L has been widely used in algae (Hommersand *et al.* 1994, McCourt *et al.* 1995, Bailey and Freshwater 1997, Daugbjerg and Andersen 1997, Nozaki *et al.* 1997, Bailey *et al.* 1998, Siemer *et al.* 1998, Kogame *et al.* 1999, Hanyuda *et al.* 2000, Daugbjerg and Guillou 2001) and the RUBISCO-spacer has proven moderately useful in the brown algal orders Ectocarpales (Stache-Crain *et al.* 1997, Siemer *et al.* 1998), Fucales (Phillips 1998, Lee *et al.* 1999), and Laminariales (Yoon and Boo 1999, Yoon *et al.* 2001).

TOTAL EVIDENCE APPROACHES

Different data sets can be analysed separately or together. Ideally, one expects to obtain identical topologies from each separate analysis as well as the combined analysis. In addition, the combined analysis might actually provide more resolution.

This is the 'total evidence' (also called 'simultaneous analysis') argument (Donoghue and Sanderson 1992, Eernisse and Kluge 1993, Johnson and Soltis 1998, Page and Holmes 1998). If, for example, one character set resolves nodes closer to the tips of the tree and another data set is more useful for basal branching, the combination of the two may substantially improve the resolution of the complete tree. Another argument for a combined analysis is that weak, but true, signals may be present in different data sets, but the signal within any single data set may be masked. The main argument against a combined analysis is that random or systematic errors may increase. In the worst case, a combined analysis could actually yield a worse estimate if particular characters cancel one another out by chance. Prior to a combined analysis one can assess the degree to which data sets are in agreement (i.e., congruent) or disagreement using the partition homogeneity test (PHT) (Farris *et al.* 1995). The PHT is a bootstrap approach which randomly partitions characters. It tests the null hypothesis that a given partition of a data set (for example, rDNA and *rbc*L data sets) represents a random partition of the data. If two data sets are highly incongruent, then the sum of their minimal trees should be significantly shorter than that of the sum of tree lengths from random partitions of the combined data and the null hypothesis will be rejected (Hoot *et al.* 1997).

Total evidence analyses that combine molecular data with morphological data tend to follow the stronger data set, usually the molecular data set which has more characters (Swofford 1991). Where morphological convergence is high, the morphological data do not add to the overall resolution of the tree. In general, it is more useful to analyse the data sets separately and then map the morphological characters on to the molecular tree. This approach has been successfully used in the algae (Peters *et al.* 1997, Hillis *et al.* 1998) and will be explored in the Sphacelariales using the program MacClade (Maddison and Maddison 1992). MacClade allows to reconstruct the evolution of a character in the data matrix and display the reconstruction graphically by tracing onto a tree the inferred character states of ancestors. The tracing displays the ancestral character states that imply the smallest number of character state changes. These reconstructions are estimates based on parsimony considerations, they are not observed facts.

OUTLINE OF THIS THESIS

In Chapter 2 the phylogeny of the Phaeophyceae is assessed using nuclear rRNA genes and the chloroplast encoded *rbc*L. The specimens analysed ($n = 58$ for rDNA and $n = 48$ for *rbc*L, excluding outgroup) represent a taxon coverage of approximately 75% and 60%, respectively, at the ordinal level and 75% and 40% at the familial level (following the familial subdivision of the Ectocarpales *s.l.* by Peters and Ramírez [2001]). A critical comparison is made of the different genes in terms of their suitability and information content for phylogenetic inference in the brown algae. Sister-group relationships were assessed and a suitable outgroup established for the Sphacelariales. Chapter 3 focuses on relationships within the Sphacelariales using morphological characters, *rbc*L sequences and partial RUBISCO-spacer sequences. The phylogenetic informativeness of the different morphological characters is assessed in relation to the molecular phylogeny and a series of possible circumscriptions are discussed including the nomenclatural consequences in each case. Chapter 4 explores the suitability of the ribosomal internal transcribed spacers for phylogenetic studies within the Sphacelariales. In Chapter 5 the *Sphacelaria* subgenus *Propagulifera* is explored using *rbc*L and RUBISCO-spacer sequences. The status and utility of a subdivision into sections are discussed. Chapter 6 presents an inventory of the species of *Sphacelaria* from China, a biogeographic area that has only begun to be explored in the genus. This study includes the description of a new species and a discussion on the morphology of propagules. In Chapter 7 a new family is described, Onslowiaceae, based on the results of Chapter 2. Chapter 8 provides an update of the *rbc*L-based phylogeny and includes a number of phaeophycean *rbc*L sequences from a number of colleagues that were released after the publication of Chapter 2. This new phylogeny sets the stage for a new classification of the brown algae. This is discussed in Chapter 9 in which ‘the class is called to order’.

Chapter 2

A REASSESSMENT OF PHYLOGENETIC RELATIONSHIPS WITHIN THE PHAEOPHYCEAE BASED ON RUBISCO LARGE SUBUNIT AND RIBOSOMAL DNA SEQUENCES [1]

In order to better assess the current state of phaeophycean phylogeny, we compiled all currently available *rbc*L, 18S and 26S rDNA sequences from the EMBL/GenBank database; and added 21 new *rbc*L sequences of our own. We then developed three new alignments that were designed to maximise taxon sampling while minimising information loss due to partial sequences. Phylogenetic analyses were performed on separate and combined data sets (with and without taxa from the sister classes Tribophyceae and Phaeothamniophyceae as outgroups) using a variety of assumption sets, tree-drawing algorithms (parsimony, neighbor joining and likelihood) and resampling methods (bootstrap, decay, jackknife). Partition homogeneity testing (PHT) by codon position within *rbc*L showed that all positions could be used despite mild third position saturation. PHT by gene and domain within rDNA showed that the 26S D1 and D2 regions do not enhance phylogenetic signal even when combined with the 18S. The *rbc*L and rDNA (excluding the 26S D1 and D2) could be combined under PHT. The topology of the combined tree was the same as that of the *rbc*L tree alone but bootstrap support was consistently higher in the combined analysis, applied to more branches, and enabled the establishment of sister-group relationships among six orders. Although the taxon sampling for the combination tree was lower ($n = 22$) than for individual gene analyses ($n = 58$ for *rbc*L and $n = 59$ for rDNA), results show that the Laminariales (previously reported) and Sphacelariales (new) are both paraphyletic. *Choristocarpus tenellus* is the most basal phaeophyte and the Dictyotales the most basal order. In contrast, the Laminariales *sensu stricto* and Ectocarpales *sensu lato* are the most derived. For phylogenetic studies in the Phaeophyceae, *rbc*L has more resolving power than rDNA though the reason for this is unclear based on the fact that both genes are highly conserved.

INTRODUCTION

The heterokont class Phaeophyceae, better known as the brown algae, have continued to fascinate systematists throughout the last century for the simple reason that they present an extensive range of morphological form—from microscopic filaments to the giant, structurally complex thalli of kelps. The comparative morphological approach

---

[1] *Also published as:*
S.G.A. Draisma, W.F. Prud'homme van Reine, W.T. Stam & J.L. Olsen 2001. A reassessment of phylogenetic relationships within the Phaeophyceae based on RUBISCO large subunit and ribosomal DNA sequences. *Journal of Phycology* 37: 586–603.

has, therefore, been supplied with a rich source of characters including thallus construction, modes of growth, type of sexual reproduction and characteristics of the chloroplast (Bold and Wynne 1985). Silva and de Reviers (2000) reviewed the classical taxonomic literature and catalogued some 39 ordinal names, including 17 descriptive names and 22 names based solely on specific genera. Numerous classifications have been proposed (Bold and Wynne 1985, van den Hoek *et al.* 1995, de Reviers and Rousseau 1999, Graham and Wilcox 2000). At present, 13–17 orders of Phaeophyceae are recognised: Ascoseirales, Chordariales, Cutleriales, Desmarestiales, Dictyosiphonales, Dictyotales, Durvillaeales, Ectocarpales, Fucales, Laminariales, Ralfsiales (*nomen nudum*), Scytosiphonales, Scytothamnales, Sphacelariales, Sporochnales, Syringodermatales and Tilopteridales. De Reviers and Rousseau (1999) downgraded the Durvillaeales (and the Notheiales) to family status within the Fucales. They merged the Chordariales, Dictyosiphonales (including the Punctariales), Ectocarpales and Scytosiphonales into the Ectocarpales *sensu lato* (*s.l.*) although they were not the first authors to propose this. Fritsch (1945) already merged these taxa into Ectocarpales and also included the members of the Ralfsiales, which did not have ordinal status at that time. Newton (1931) even included the Desmarestiales and Sporochnales in the Ectocarpales. Russell and Fletcher (1975) also merged the Chordariales, Dictyosiphonales, Ectocarpales *sensu stricto* (*s.s.*) and Scytosiphonales into the Ectocarpales *s.l.*, but also included the Ralfsiales and the Tilopteridales. Members of the recent erected Scytothamnales (Peters and Clayton 1998) were previously classified in the Chordariales. Russell and Fletcher (1975) compared 132 species using a clustering analysis of traditional morphological characters. Results of their phenetic approach established seven orders but left many taxa in strange alliances or as outliers. These perennial reshuffling of taxa within and among brown algal orders signal the difficulties of circumscription due to morphological convergence, let alone the difficulties in the establishment of an evolutionary tree.

Evolutionary relationships within the Phaeophyceae have long been a source of discussion and speculation. Various phylogenetic hypotheses have been put forth, based on a limited number of characters and in the absence of any formal analyses. For example, Wynne and Loiseaux (1976) envisioned two deep sister lineages, one including the Fucales and Durvillaeales and the other including all the remaining orders with the Ectocarpales *s.s.* most primitive. Clayton (1984) suggested that the Dictyotales represented an independent evolutionary lineage, not closely related to any of the other orders. This was based on the presence of unique uniflagellate spermatozoids and meiosporangia that differed from the more typical unilocular sporangia found in brown algae. She also hypothesised that the Fucales were derived from the Chordariales or Dictyosiphonales. Based on their simple filamentous construction, van den Hoek *et al.* (1995) considered the Ectocarpales *s.s.* to be the most primitive brown algae; likewise they considered the Fucales and Durvillaeales as the most advanced based on the lack of a free-living gametophytic phase.

With the advent of DNA sequence data and formal analysis methods in the early 1990s, the picture of the evolutionary history of the brown algae has been steadily emerging (Table 2.1). The first studies were coarse and involved extremely limited taxon sampling. Gradually, however, representatives from all of the principal orders have been surveyed. The nuclear rDNA small subunit gene (18S) has provided the bulk of the data. Unfortunately, the resolving power of the 18S has proven disappointing because of to the apparent relative recency of the various phaeophycean lineages. Partial sequences of the rDNA large subunit gene (26S) have also been used with similar results (Rousseau and de Reviers 1999a, b, Rousseau *et al.* 2000). The rDNA phylogeny of de Reviers and Rousseau (1999) provides the best taxon sampling so far, but the tree remains poorly resolved, there are no support values and there are no nonphaeophycean outgroups. The authors suggested that rapid divergence has resulted in a "brown algal crown radiation". The chloroplast encoded, large subunit RUBISCO gene (*rbc*L) has recently been added to the arsenal (e.g. Siemer *et al.* 1998) of useful sequences for brown algal phylogeny, along with faster evolving rDNA internal transcribed spacer (ITS) (e.g., Peters *et al.* 1997) and RUBISCO spacer sequences (e.g., Stache-Crain *et al.* 1997, Lee *et al.* 1999, Yoon and Boo 1999, Kraan and Guiry 2000) for generic and species-level comparisons. To date, the available *rbc*L data have been restricted to a few groups so that full-scale comparisons across the phaeophytes have necessarily remained limited. The observation that *rbc*L has a slightly faster mutation rate than ribosomal genes within the phaeophytes may yet allow us to recover some of the branching order within the 'brown algal crown radiation'.

The main aim of this article is (re)assess the current state of brown algal phylogeny. Comparisons among previously published studies have remained difficult because of differences in taxon sampling, use of different genes, use of partial sequences (but then from different regions of the same gene in different studies), different outgroup taxa, and different analysis methods. In order to reconcile these problems we use all currently available 18S and 26S rDNA, and *rbc*L sequences from the EMBL/GenBank database, plus 21 new *rbc*L sequences generated by us. Criteria are established for use of partial sequences and new alignments are constructed. Phylogenetic analyses are performed under different assumption sets and evolutionary models for the separate data sets, as well as for the combined data sets. In particular, we statistically assess the degree to which rDNA and *rbc*L are congruent under partition homogeneity testing (PHT) and, therefore, should or should not be combined (Wendel and Doyle 1998, Johnson and Soltis 1998). Because we want to know which lineages are relatively older and younger, we include non-phaeophycean outgroups. We address three questions: Which lineages are monophyletic? Which orders are sister taxa? Which lineages are ancestral or derived?

MATERIALS AND METHODS

Taxon sampling

New sequences (*n* = 36) were collected from 21 species (Table 2.2). The remaining sequences were retrieved from the EMBL/GenBank Nucleotide Sequence database (*n* = 134) or from printed publications (*n* = 4) (Table 2.3).

DNA extraction

DNA was newly extracted from 10 species (Table 2.2). Tissue (0.2–2 g) from a unialgal culture was ground in liquid nitrogen and incubated in 4 mL CTAB-PVPP extraction buffer (2% hexadecyltrimethylammonium bromide [CTAB; w/vol], 0.1%

Table 2.1. Summary of molecular phylogenetic studies among phaeophycean orders since 1993. Only those publications that include representatives of at least three brown algal orders are cited. The number of representative taxa per order are indicated as 1 •, 2 •, etc. Lineages not belonging to the Phaeophyceae are named by their class. The Chordariales, Dictyosiphonales, Ectocarpales *s.s.*, Punctariales, and Scytosiphonales are included here as Ectocarpales *s.l.*

Author(s)/year	DNA fragment	Outgroup taxa	Ingroup taxa
Tan and Druehl (1993)	18S (partial)	1 • Bacillariophyceae, 1 • Tribophyceae	1 • Dictyotales, 8 • Ectocarpales *s.l.*, 1 • Fucales, 1 • Laminariales, 1 • Sphacelariales, 1 • Syringodermatales [a]
Tan and Druehl (1994)	18S (partial)	1 • Bacillariophyceae, 1 • Tribophyceae	1 • Fucales, 8 • Ectocarpales *s.l.*, 1 • Laminariales, 2 • Ralfsiales [b], 1 • Sphacelariales, 1 • Syringodermatales
Saunders and Kraft (1995)	18S (partial)	—	1 • Dictyotales, 3 • Ectocarpales *s.l.*, 5 • Fucales, 1 • Laminariales, 1 • Sphacelariales, 1 • Syringodermatales
Tan and Druehl (1996)	18S	1 • Tribophyceae	1 • Desmarestiales, 2 • Ectocarpales *s.l.*, 1 • Fucales, 4 • Laminariales, 1 • Sporochnales
Druehl *et al.* (1997)	18S (partial)	—	1 • Cutleriales, 1 • Desmarestiales, 1 • Dictyotales, 8 • Ectocarpales *s.l.*, 1 • Fucales, 3 • Laminariales, 2 • Ralfsiales, 1 • Sphacelariales, 1 • Sporochnales, 1 • Syringodermatales, 1 • Tilopteridales
Medlin *et al.* (1997)	18S	4 • Oomycetes	1 • Desmarestiales, 1 • Ectocarpales, 1 • Fucales, 1 • Laminariales, and 46 other heterokont algae
van de Peer and de Wachter (1997)	18S	—	2 • Ectocarpales *s.l.*, 1 • Desmarestiales, 1 • Fucales, 3 • Laminariales *s.l.*, 1 • Sporochnales, 1 • Tribophyceae, 23 other heterokont algae and many other eukaryotes
Rousseau *et al.* (1997)	26S (partial)	1 • Cutleriales, 3 • Laminariales, 1 • Sphacelariales	10 • Fucales
Andersen *et al.* (1998)	18S (partial)	2 • Oomycetes	1 • Ectocarpales *s.l.*, 1 • Fucales, 2 • Laminariales *s.l.*, 1 • Phaeothamniophyceae, 5 • Tribophyceae, and many other heterokonts
Burkhardt and Peters (1998)	18S (partial), ITS	1 • Tribophyceae	1 • Fucales, 15 • Ectocarpales *s.l.* [c], 1 • Laminariales
Horiguchi and Yoshida (1998)	18S	1 • Ectocarpales *s.l.*, 1 • Laminariales	11 • Fucales
Müller *et al.* (1998)	18S + 26S (partial)	—	5 • Ectocarpales *s.l.*, 2 • *Incertae sedis*, 6 • Scytothamnales

(continued)

(Table 2.1 continued)

Peters (1998)	18S, ITS (partial)	1 • Tribophyceae [d]	3 • Desmarestiales, 4 • Ectocarpales *s.l.*, 1 • Fucales, 6 • Laminariales, 2 • Sporochnales
Peters and Burkhardt (1998)	18S (partial), ITS	1 • Tribophyceae	1 • Fucales, 11 • Ectocarpales *s.l.* [c], 1 • Laminariales
Peters and Clayton (1998)	18S, ITS	1 • Tribophyceae	5 • Ectocarpales *s.l.*, 1 • Fucales, 1 • Laminariales, 4 • Scytothamnales
Siemer *et al.* (1998)	*rbc*L, RUBISCO-spacer (partial)	1 • Raphidophyceae, 2 • Tribophyceae	22 • Ectocarpales *s.l.*, 1 • Tilopteridales
Boo *et al.* (1999)	18S	1 • Ectocarpales *s.l.*	1 • Desmarestiales, 13 • Laminariales, 1 • Sporochnales
Ehara *et al.* (1999)	18S, *cox*1 [e] amino acid sequence	3 • Prymnesiophyta	2 • Bacillariophyceae, 3 • Eustigmatophyceae, 1 • Raphidophyceae, 6 • Tribophyceae, 1 • Desmarestiales, 4 • Ectocarpales *s.l.*, 1 • *Incertae sedis*, 6 • Laminariales, 1 • Sporochnales [f]
De Reviers and Rousseau (1999) [g]	18S + 26S (partial)	—	1 • Ascoseirales, 1 • Cutleriales, 5 • Desmarestiales, 3 • Dictyotales, 13 • Ectocarpales *s.l.*, 21 • Fucales, 4 • *Incertae sedis*, 7 • Laminariales, 1 • Ralfsiales, 3 • Scytothamnales, 3 • Sphacelariales, 1 • Sporochnales, 1 • Syringodermatales, 3 • Tilopteridales
Rousseau and de Reviers (1999a)	18S + 26S (partial)	8 • Ectocarpales *s.l.*	1 • Cutleriales, 1 • Durvillaeales, 24 • Fucales, 9 • Laminariales, 2 • Scytothamnales, 3 • Sphacelariales
Rousseau and de Reviers (1999b)	18S + 26S (partial)	1 • Cutleriales, 2 • *Incertae sedis*, 6 • Laminariales, 3 • Scytothamnales, 2 • Sphacelariales	13 • Ectocarpales *s.l.*
Rousseau *et al.* (2000)	18S + 26S (partial)	1 • Cutleriales, 4 • Laminariales, 2 • Scytothamnales	13 • Ectocarpales *s.l.*

---

[a] Desmarestiales was initially thought to be included, but later this turned out to be based on a misidentification (Peters *et al.* 1997).

[b] *Ralfsia* and *Analipus* as Ectocarpales.

[c] Including kelp endophytes of unknown affinity.

[d] As Chrysophyceae.

[e] Mitochondrial cytochrome oxydase subunit I.

[f] Different taxa were included in the 18S analysis and the *cox*1 amino acid sequence analysis.

[g] A hypothetical tree is presented based on unpublished sequences; Durvillaeales (2x) and Notheiales (1x) were included in the Fucales.

Table 2.2. Species for which new sequences were obtained in the present study. Taxonomic authorities are provided in Table 2.3.

Species	Collection site	Collector/identifier, collection year	DNA extraction method
Alethocladus corymbosus	Astrolabe I., Antarctica	D. Moe, 1985	This study
Ascophyllum nodosum	Tjärnö, western Sweden	J.L. Olsen 1999	Olsen (unpublished)
Choristocarpus tenellus	La Nave, Ischia, Italy	E.C. Henry 1987	This study
Cladostephus spongiosus	Oosterschelde, Zeeland, The Netherlands	W.F. Prud'homme van Reine, 1966	This study
Desmarestia aculeata	Helgoland, Germany	D.G. Müller, 1978	Peters *et al.* (1997)
Desmarestia ligulata	Brittany, France	A.F. Peters, 1990	Peters *et al.* (1997)
Desmarestia viridis	Helgoland, Germany	K. Lüning, 1978	van Oppen *et al.* (1993)
Dictyota cervicornis	Zanzibar, Tanzania	O. de Clerck, 1997	de Clerck (1998)
Dictyota dichotoma	Oosterschelde, Zeeland, The Netherlands	O. de Clerck, 1996	de Clerck (1998)
Eisenia arborea	Santa Catalina I., CA, USA	J.A. Coyer, 1997	Coyer *et al.* (1994)
Himantothallus grandifolius	Isla Rey Jorge, Antarctica	C. Wiencke, 1986	Peters *et al.* (1997)
Macrocystis pyrifera	Santa Catalina I., CA, USA	J.A. Coyer, 1997	Coyer *et al.* (1994)
Nereocystis luetkeana	Friday Harbor, San Juan I., WA, USA	M. Dethier, 1998	Coyer *et al.* (1994)
Onslowia endophytica	Fort Pierce, FL, USA	E.C. Henry, 1981	This study
Pelagophycus porra	Santa Catalina I., CA, USA	J.A. Coyer, 1993	Coyer *et al.* (1994)
Sargassum muticum	Oosterschelde, Zeeland, The Netherlands	S.G.A. Draisma, 1999	This study
Sphacelaria cirrosa	Fife Ness, Fife, Scotland	S.G.A. Draisma, 1997	This study
Sphacella subtilissima	Banco Amanay, Fuerteventura, Canary Islands	D.G. Müller, 1991	This study
Stypocaulon scoparium	Porto Santo, Madeira Archipelago	W.F. Prud'homme van Reine, 1994	This study
Syringoderma phinneyi	Henderson Point, BC, Canada	A. Whittick, 1982	This study
Verosphacela ebrachia	Vero Beach, FL, USA	E.C. Henry, 1987	This study

polyvinylpolypyrrolidone [PVPP; w/vol], 100mM Tris-HCl, 20 mM EDTA, 1.4 M NaCl, pH 8.0), 8 • L • -mercapto-ethanol and 40 • L 10% SDS (w/vol) while slowly rotating at room temperature. After 1 h an equal volume of chloroform:isoamyl alcohol (24:1 vol/vol) was added, mixed, and centrifuged for 15 min at 1800*g*. The upper aqueous phase was transferred to a clean tube, and the chloroform:isoamyl alcohol extraction step was repeated two more times.

CsCl purification

Further purification of the DNA with CsCl was essential, and successful purification was obtained only when the crude DNA extract was not precipitated. In this step 2.5 mL of the aqueous phase was mixed with 2.5 g CsCl, 0.1 mL Triton X-100, and 50 • L ethidium bromide (10 $g \cdot L^{-1}$); transferred to a Beckman Quick-Seal™ polyallomer centrifuge tube (Beckman Instruments, Palo Alto, CA, USA); and centrifuged at 20 °C for 4 h at 100,000 rpm (TLN 100 rotor) in a Beckman Optima™ TL Ultracentrifuge

(Beckman Instruments) with no braking. The DNA band was visualised under UV light, aspirated with a syringe, and transferred to a 2-mL Eppendorf tube. Ethidium bromide was removed by isopropanol extraction, and the DNA solution was dialysed for 36 h at 4 °C against 1.5 L 1 • TE (Tris-EDTA) with two changes of TE. DNA was precipitated over night at 4 °C with 1/10 volume of 4 M NaAc (pH 5.2) and 2/3 volume of isopropanol (-20 °C) and pelleted by centrifugation (30 min, 12,000*g*, 4 °C). The pellet was washed twice with 70% cold ethanol, air dried, and redissolved in 200 • L 0.1 • TE buffer. DNA yield varied from 400 to 2000 ng. A 1/10 or 1/100 dilution was used for PCR amplification.

DNA amplification

Double-stranded DNA amplifications were performed in a Hybaid OmniGene thermocycler (Hybaid, Franklin, MA, USA). A 100-• L reaction volume contained 1–4 • L diluted (see above) genomic DNA, 200 • M of each of the four dNTPs, 0.5 • M of each primer, 10 • L 10• reaction buffer (Promega Corp., Madison, WI, USA), 1.5 mM $MgCl_2$, and 0.48 • L of Taq DNA polymerase (5u·• L^{-1}, Promega). Reactions were overlaid with mineral oil to minimise evaporation. An initial denaturation step of 96 °C for 3 min was followed by 27 cycles: 1 min at 96 °C, 2 min at 42 °C, and 2 min at 72 °C. The amplification was ended with a final step of 72 °C for 10 min.

Amplification primers are listed in Table 2.4. For the amplification of the *rbc*L, one of the forward primers, RbcL68F or L188F, was combined with the reverse primer S3R. The partial rDNA 18S 3'-end fragments were obtained by using the forward primer B2 and the reverse primer RITS1. The partial rDNA 26S 5'-end fragments were amplified with the forward primer AB28r and the reverse primer TW13.

PCR products were checked for length and yield by electrophoresis on 1.5% agarose gels. Reamplifications were performed in the case of low yields. In that case, 1–2 • L of the first PCR amplification was used as seed DNA for a second PCR amplification with the same reagents, the same primers and amplification conditions. Fragments were cut from the gel and the DNA eluted from the gel slice by centrifugation over siliconised glass wool. Eluted PCR products were purified using the Quiaquick PCR purification Kit (Qiagen, GmbH, Düsseldorf, Germany) following the manufacturer's instructions and resuspended in 5 • L MilliQ water (Millipore, Bedford, MA, USA).

Cloning and plasmid isolation

Purified PCR products were ligated into a pGEM-T Vector (Promega) and transformed into JM109 competent cells (Promega) following the manufacturer's instructions, except that 40 • L of competent cells was used instead of 50 • L.

Colonies were tested for the presence of inserts using colony PCR. A 20-• L PCR reaction was set up as previously described but with the forward and reverse vector primers (T7 and SP6). Colony PCR's were carried out in a Perkin-Elmer Cetus thermocycler (PE Applied Biosystems, Foster City, CA, USA) with an initial denaturation step of 95 °C for 3 min followed by 27 cycles: 1 min at 95 °C, 2 min at 50 °C, and 2 min at 74 °C. Plasmid DNA from positively transformed clones was isolated with a Flexiprep kit (Amersham, Pharmacia Biotech, Uppsala, Sweden) following the manufacturer's protocol. Isolated plasmids were resuspended in 50 • L 0.1 • TE buffer.

Table 2.3. (p. 32–35). Species used in the present study. EMBL accession numbers for new sequences (this study) are shown in bold. Length of partial sequence given in parentheses.

Class and order	Species and taxonomic authorship	EMBL accession number		
		18S sequences **(DS43994)**[a]	26S sequences **(DS43994)**[a]	*rbc*L sequences **(DS43993)**[a]
Phaeophyceae				
Ascoseirales	*Ascoseira mirabilis* Skottsberg	AJ229126 (414 nt)	AJ229141 (556 nt)	—
Cutleriales	*Cutleria multifida* (J.E. Smith) Greville	AF073326 (433 nt)	AF053119 (559 nt)	—
Desmarestiales	*Arthrocladia villosa* (Hudson) Duby	Z99448 (394 nt)	AJ229142 (475 nt)	—
	Desmarestia aculeata (L.) J.V. Lamouroux	Z99449 (260 nt)	AJ229143 (550 nt)	**AJ287847** (1158 nt)
	Desmarestia ligulata (Lightfoot) J.V. Lamouroux	L43060 (438 nt)	**AJ287434** (559 nt)	**AJ287848** (1250 nt)
	Desmarestia viridis (O.F. Müller) J.V. Lamouroux	—	—	**AJ287849** (1246 nt)
	Himantothallus grandifolius (A. Gepp *et* E. S. Gepp) Zinova	**AJ287432** (439 nt)	**AJ287433** (559 nt)	**AJ287850** (1255 nt)
	Phaeurus antarcticus Skottsberg	AJ229111 (438 nt)	AJ229146 (420 nt)	—
Dictyotales	*Dictyota cervicornis* Kützing	**AJ287435** (270 nt)[b]	**AJ287436** (600 nt)	**AJ287851** (1371 nt)
	Dictyota dichotoma (Hudson) J.V. Lamouroux	**AJ287437** (439 nt)	**AJ287438** (580 nt)	**AJ287852** (1368 nt)
Ectocarpales *s.l.*	*Adenocystis utricularis* (Bory) Skottsberg	AF073322 (439 nt)	AF071778 (529 nt)	—
	Chordaria flagelliformis (O.F. Müller) C. Agardh	AJ229129 (332 nt)	AJ229129 (555 nt)	—
	Coelacladia arctica Rosenvinge	—	—	AF055395 (1399 nt)
	Delamarea attenuata (Kjellman) Rosenvinge	—	—	AF055396 (1399 nt)
	Dictyosiphon foeniculaceus (Hudson) Greville	DFZ99463 (311 nt)	AJ229137 (553 nt)	AF055397 (1399 nt)
	Ectocarpus siliculosus (Dillwyn) Lyngbye	—	—	X52503 (1399 nt)
	Elachista fucicola (Velley) Areschoug	—	—	AF055398 (1399 nt)
	Giraudia sphacelarioides Derbès *et* Solier	—	—	AF055399 (1399 nt)
	Hecatonema sp.	—	—	AF055400 (1399 nt)
	Hincksia granulosa (J.E. Smith) P.C. Silva *in* P.C. Silva *et al.*	AF115433 (415 nt)	AF071782 (546 nt)	—
	Hummia onusta (Kützing) Fiore	—	—	AF055402 (1399 nt)
	Isthmoplea sphaerophora (Harvey) Kjellman	—	—	AF055403 (1399 nt)
	Laminariocolax tomentosoides (Farlow) Kylin	—	—	AF055404 (1399 nt)

(continued)

(Table 2.3 continued)

	Leptonematella fasciculata (Reinke) P.C. Silva	—	—	AF055405 (1376 nt)
	Litosiphon pusillus (Lyngbye) Harvey	—	—	AF055406 (1398 nt)
	Myrionema strangulans Greville	—	—	AF055407 (1399 nt)
	Myriotrichia clavaeformis Harvey	Z99475 (265 nt)	AJ229138 (555 nt)	AF055408 (1399 nt)
	Petalonia fascia (O.F. Müller) O. Kuntze	AF115431 (437 nt)	AB022271 (551 nt)	—
	Pylaiella littoralis (L.) Kjellman	AF115434 (414 nt)	AF071782 (558 nt)	X55372 (1399 nt)
	Pogotrichum filiforme Reinke	—	—	AF055409 (1399 nt)
	Punctaria latifolia Greville	AF115432 (437 nt)	AF071158 (550 nt)	—
	Punctaria plantaginea (Roth) Greville	—	—	AF055410 (1399 nt)
	Scytosiphon lomentaria (Lyngbye) Link	L43066 (438 nt)	AF071159 (550 nt)	—
	Sorocarpus micromorus (Bory) Silva	—	—	AF055411 (1399 nt)
	Sphaerotrichia divaricata (C. Agardh) Kylin	—	—	AF055412 (1283 nt)
	Stictyosiphon soriferus (Reinke) Rosenvinge	—	—	AF055413 (1399 nt)
	Streblonema maculans (Hamel) South *et* Tittley	AF073323 (436 nt)	AF071784 (557 nt)	—
	Streblonema tenuissimum Hauck	—	—	AF055414 (1399 nt)
	Striaria attenuata (Greville) Greville	AJ229139 (265 nt)	Z99478 (555 nt)	AF055415 (1399 nt)
	Utriculidium durvillei (Bory) Skottsberg	AF073321 (439 nt)	AF071157 (529 nt)	—
Fucales *s.l.*	*Anthophycus longifolius* (Turner) Kützing	AF091294 (442 nt)	AF091273 (559 nt)	—
	Ascophyllum nodosum (L.) Le Jolis	AF091297 (441 nt)	AF053106 (558 nt)	**AJ287853** (1374 nt)
	Axillariella constricta (J. Agardh) Silva	AF091293 (442 nt)	AF091281 (561 nt)	—
	Bifurcaria bifurcata Ross	AF091288(442 nt)	AF053113 (556 nt)	—
	Bifurcaria brassicaeformis (Kützing) Barton	AF091292 (442 nt)	AF091280 (563 nt)	—
	Caulocystis cephalornithos (Labillardière) J. Agardh	(439 nt)[c]	AF091279 (558 nt)	—
	Durvillaea potatorum (Labillardière) Areschoug	AF091290 (440 nt)	AF091283 (553 nt)	—
	Fucus vesiculosus (L.)	AF091296 (440 nt)	AF053105 (557 nt)	—
	Himanthalia elongata (L.) S. F. Gray	AF091287 (443 nt)	AF053108 (556 nt)	—
	Hormosira banksii (Turner) Decaisne	(441 nt)[c]	AF091270 (579 nt)	—
	Notheia anomala Harvey *et* Bailey	(440 nt)[c]	AF091282 (555 nt)	—
	Sargassum echinocarpum J. Agardh	—	—	AF076689 (1140 nt)
	Sargassum muticum (Yendo) Fensholt	AF091295 (442 nt)	AF053109 (558 nt)	**AJ287854** (1355 nt)
	Sargassum polyphyllum J. Agardh	—	—	AF076690 (1139 nt)

(continued)

(Table 2.3 continued)

Class and order	Species and taxonomic authorship	EMBL accession number		
		18S sequences **(DS43994)**[a]	26S sequences **(DS43994)**[a]	*rbc*L sequences **(DS43993)**[a]
	Seirococcus axillaris (R. Brown *ex* Turner) Montagne *ex* Harvey	AF091291 (440 nt)	AF091284 (572 nt)	—
	Turbinaria ornata (Turner) J. Agardh	—	—	AF076688 (1399 nt)
	Turbinaria turbinata (L.) O. Kuntze	AF091300 (442 nt)	AF091272 (545 nt)	—
	Xiphophora chondrophylla (R. Brown *ex* Turner) Montagne *ex* Harvey	AF091289 (442 nt)	AF091271 (558 nt)	—
Laminariales	*Alaria esculenta* (L.) Greville	AF115427 (428 nt)	AF071151 (551 nt)	AF064745 (1161 nt)
	Chorda filum (L.) Stackhouse	AF073324 (436 nt)	AF053117 (554 nt)	—
	Eisenia arborea Areschoug	—	—	**AJ287855** (1253 nt)
	Halosiphon tomentosus (Lyngbye) Jaasund	L43056 (436 nt)	AF071156 (559 nt)	—
	Laminaria digitata (L.) Lamouroux	AF091286 (425 nt)	AF071153 (554 nt)	—
	Laminaria ochroleuca Bachelot de La Pylaie	AF091301 (435 nt)	AF071154 (554 nt)	—
	Macrocystis pyrifera (L.) C. Agardh	AF115430 (432 nt)	AF053116 (555 nt)	**AJ287856** (1255 nt)
	Nereocystis luetkeana (Mertens) Postels *et* Ruprecht	—	—	**AJ287857** (1370 nt)
	Pelagophycus porra (Leman) Setchell	—	—	**AJ287858** (1274 nt)
	Saccorhiza polyschides (Lightfoot) Batters	L43059 (437 nt)	AF053118 (557 nt)	—
Scytothamnales	*Scytothamnus australis* (J. Agardh) J.D. Hooker *et* Harvey	AF073325 (439 nt)	AF071780 (553 nt)	—
	Splachnidium rugosum (L.) Greville	AF073327 (432 nt)	AF071781 (555 nt)	—
	Stereocladon rugulosus (Bory de Saint-Vincent) Hariot	Z99454 (300 nt)	AJ229132 (554 nt)	—
Sphacelariales *s.l.*	*Alethocladus corymbosus* (Dickie) Sauvageau	**AJ287439** (437 nt)	**AJ287440** (566 nt)	**AJ287860** (1256 nt)
	Choristocarpus tenellus (Kützing) Zanardini	**AJ287441** (439 nt)	**AJ287442** (569 nt)	**AJ287861** (1374 nt)
	Cladostephus spongiosus (Hudson) C. Agardh	AF091298 (439 nt)	AF053115 (546 nt)	**AJ287863** (1375 nt)
	Onslowia endophytica Searles	**AJ287443** (439 nt)	**AJ287444** (557 nt)	**AJ287864** (1373 nt)
	Sphacelaria cirrosa (Roth) C. Agardh	AF115426 (413 nt)	AF071150 (560 nt)	**AJ287865** (1372 nt)
	Sphacella subtilissima Reinke	—	**AJ287447** (558 nt)	**AJ287931** (1371 nt)
	Stypocaulon scoparium (L.) Kützing	AF091299 (439 nt)[d]	AF091285 (552 nt)[d]	**AJ287866** (1374 nt)
	Verosphacela ebrachia E.C. Henry	—	**AJ287445** (558 nt)	**AJ287867** (1255 nt)

(continued)

(Table 2.3 continued)

Syringodermatales	*Syringoderma phinneyi* E.C. Henry *et* D.G. Müller	L17017 (437 nt)	**AJ287446** (559 nt)	**AJ287868** (1256 nt)
Tilopteridales	*Haplospora globosa* Kjellman	—	—	AF055400 (1132 nt)
Incertae sedis	*Asteronema ferruginea* (Harvey) Delépine *et* Asensi	AFA229134 (414 nt)	AFA229114 (556 nt)	—
	Asteronema rhodochortonoides (Borgesen) Müller *et* Parodi	ARA229117 (405 nt)	ARA229135 (553 nt)	—
	Asterocladon lobatum D.G. Müller, E.R. Parodi *et* A.F. Peters	AJ229120 (315 nt)	AJ229136 (386 nt)	—
Tribophyceae				
Order unknown	*Botrydium stoloniferum* Mitra	—	—	AF064743 (1348 nt)
Mischococcales	*Botrydiopsis intercedens* Vischer *et* Pascher	—	—	AF015587 (1350 nt)
	Bumilleriopsis filiformis Vischer	—	—	U89900 (1374 nt)
	Mischococcus sphaerocephalus Vischer	—	—	AF064744 (1399 nt)
Tribonematales	*Tribonema aequale* Pascher	M55286 (436 nt)	Y07979 (597 nt)	AF084611 (1383 nt)
	Xanthonema debile (Vischer) Silva	—	—	AF084612 (1399 nt)
Vaucheriales	*Vaucheria bursata* (O.F. Müller) C.A. Agardh	—	—	AF015589 (1387 nt)
Phaeothamniophyceae				
Phaeothamniales	*Phaeoschizochlamys mucosa* Lemmermann	—	—	AF064747 (1313 nt)
	Phaeothamnion confervicola Lagerheim	—	—	AF069446 (1398 nt)
	Stichogloea globosa Starmach	—	—	AF155584 (1399 nt)

---

[a] EMBL accession number for the alignment.

[b] Sequence taken from de Clerck (1998), but not previously submitted to EMBL.

[c] No EMBL accession numbers, taken from Saunders and Kraft (1995).

[d] As *Halopteris scoparia* in Rousseau and de Reviers (1999a).

Table 2.4. Amplification and sequencing primers. F, forward; R, reverse.

Primer	Direction	Sequence (5'–3')	Approximate annealing position	Reference
RbcL68F	F	TGC CWA AAT GGG RWA YTG GGA TGC	*rbc*L 68	This study
RbcL188F	F	TCT ACT GCA ACA TGG AC	*rbc*L 188	Siemer *et al.* (1998)[a]
RbcL454F	F	ACT TTC CAA GGA CCT GCR ACA	*rbc*L454	This study
RbcL496F	F	AGA TTA GAY AMA TTT GGW CGT	*rbc*L 496	This study
RbcL1359R	R	GYT TTT AAW GGW CCA CAA KTY	*rbc*L 1359	This study
RbcL1380R	R	TAT CTT WCC ATA AAT CTA AMG C	*rbc*L 1380	This study
S3R	R	AAA CAT CCT TGT GTA ACT CTC	*rbc*S 23	Siemer *et al.* (1998)[b]
B2	F	GCG AAA CAC GAA CTA CTG CG	18S 916	White *et al.* (1990)
RITS1	R	TTC CGC AGG TTC ACC TAC GGA	18S 1762	White *et al.* (1990)
AB28r	F	ACC CGC TGA ACT TAA GCA TAT	26S 48	This study
TW13	R	GGT CCG TGT TTC AAG ACG	26S 639	White *et al.* (1990)

[a] As BLSrbcL212F.
[b] As BLSrbcLS3R.

DNA sequencing

The purified plasmids were sequenced over both strands using the forward and reverse vector primers and the ABI Prism BigDye Terminator Cycle Sequencing Ready Reaction Kit (PE Applied Biosystems). For *rbc*L, additional internal primers were used (Table 2.4): RbcL454F and RbcL1359R for the Sphacelariales and the Syringodermatales, and RbcL496F and RbcL1380R for the other orders. Sequence reactions were run on either an ABI 310 or ABI 377 automated sequencer (PE Applied Biosystems). Sequences were assembled using Sequence Navigator version 1.0 (PE Applied Biosystems).

Alignment

Sequences of *rbc*L (Table 2.3) were aligned manually using Sequence Navigator version 1.0. The complete alignment is submitted under EMBL accession number DS43993. Ribosomal sequences of the partial 18S and 26S genes (Table 2.3) were aligned using the BioEdit Sequence Alignment Editor (Hall 1999). Because many partial sequences of these genes can be found in the EMBL data base, we selected only those sequences for which both the 3'-end of the 18S and the 5'-end of the 26S were available. Alignments were based on Rousseau and de Reviers (1999a) and the adopted secondary structure models of van de Peer *et al.* (1997) and de Rijk *et al.* (1999). The complete alignment is submitted under EMBL accession number DS43994. A combined *rbc*L–18S–26S alignment was made with a subset of species for which all three sequences were available. This alignment differed from the single alignments by only five gaps (Table 2.5), which did not affect subsequent analyses. Therefore, the alignment was not deposited in EMBL.

Tests for possible mutational saturation were done by plotting the pair-wise uncorrected distances (p-distances) versus inferred Tajima-Nei distances (Tajima and

Nei 1984). For *rbc*L, this test was performed initially with the first and second codon positions alone and thereafter with the third codon position alone. The distance values were obtained in PAUP* version 4.0b4a (Swofford 1998).

Phylogenetic analyses

Phylogenetic analyses were done with PAUP* version 4.0b4a. Maximum parsimony (MP) analyses were done as heuristic searches, using the tree bisection-reconnection branch-swapping algorithm, with random sequence addition (100 replicates) and treating gaps as missing data. For the neighbor-joining (NJ) analyses, the Tajima-Nei model was used. For the maximum likelihood (ML) analyses (used for the combined data set only), the nucleotide substitution model used was first determined using MODELTEST version 2.1 (Posada and Crandall, 1998).

Sampling error and precision

Decay analysis of MP trees was performed with AutoDecay version 4.0 (Erikson 1998). Bootstrapping (Felsenstein 1985) was performed in PAUP* using random sequence addition (10 times) and 1000 replicates. For ML, only 100 replicates were possible. Parsimony jackknifing, a complementary method to bootstrapping, which samples without replacement, is particularly suitable for large data sets. This method was also used to assess saturation effects in third codon positions, which is relevant to the *rbc*L dataset (Källersjö *et al.* 1999). Parsimony jackknifing was performed according to the method of Farris *et al.* (1996) in the program Xac (Farris 1997). Ten random sequence additions and 1000 replicates were performed. Character deletions per jackknife replicate were set at e^{-1} (= ±37%) according to Farris *et al.* (1996). Congruence of data sets was evaluated using the PHT (Farris *et al.* 1995, Cunningham 1997). This approach uses a resampling method to estimate the degree to which two data sets (or subsets of data sets) are in agreement (i.e. congruent or homogeneous) or in disagreement (i.e. incongruent or heterogeneous). The difference between the numbers of steps required by individual and combined analyses is the incongruence length difference (ILD_{MF}) (Mickevitch and Farris 1981). The distribution of the ILD statistic can be estimated by permutation. Values of $P > 0.05$ indicate congruence thus allowing combination of the data sets in question. Conversely, values of $P < 0.05$ indicate a significant difference between the data sets and suggest that they should not be analysed together. We apply this test in three ways: (1) partitioning of the *rbc*L gene by codon position (2000 replicates, simple taxon addition, maxtrees set at 250); (2) partitioning between the variable D1 and D2 domains of the 26S and the remainder of the rDNA (2000 replicates, simple taxon addition, maxtrees set at 250); and (3) between *rbc*L and rDNA in the combined data set (10,000 replicates, random taxon addition [10 replicates], no maxtrees). In all analyses, invariant characters were first deleted.

Outgroups

Representatives of the Tribophyceae (= Xanthophyceae) and the Phaeothamniophyceae (Table 2.3) were selected as outgroup taxa, being the sister classes of the Phaeophyceae (Daugbjerg and Andersen 1997, Potter *et al.* 1997, Saunders *et al.* 1997a, b, van de Peer and de Wachter 1997, Andersen *et al.* 1998, Bailey *et al.* 1998, Ehara *et al.*

Table 2.5. Summary of data properties and parsimony analyses for the three alignments and their subsets. Numbers in parentheses are for the Phaeophyceae only. CI/RI/RC, consistency index/retention index/rescaled consistency index of most parsimonious trees (MPTs); n.d. = not determined.

	*rbc*L alignment			rDNA alignment		*rbc*L + rDNA
	All positions	First + second positions	Third positions	All positions (18S and 26S)	Excluding D1 and D2 region of 26S[a]	All positions (*rbc*L + 18S and 26S)
Number of taxa	58 (48)	58 (48)	58 (48)	59 (58)	57 (56)	22 (21)
Length of alignment	1399	932	467	1139	659	2533
Variable positions	656 (585) = 47% (42%)	239 (187) = 26% (20%)	417 (398) = 89% (85%)	496 (441) =41% (39%)	182 (156) = 28% (24%)	979 (884) = 39% (35%)
Informative sites	536 (449) = 38% (32%)	157 (107) = 17% (11%)	379 (342) = 81% (73%)	326 (319) = 29% (28%)	105 (102) = 16% (15%)	669 (626) (25%) = 26% (25%)
Number of MPTs	227	19,700	12	16	61,600	1
MPTs within 1 step	4458	n.d.	274	1084	n.d.	2
Length of MPTs	3109	680	2461	1969	480	2759
CI/RI/RC	0.36/0.64/0.21	0.38/0.74/0.34	0.30/0.61/0.18	0.43/0.64/0.28	0.45/0.75/0.41	0.52/0.51/0.26
Tree topology	Figs 2.1 and 2.2	Not shown	Not shown	Figs 2.3 and 2.4	Not shown	Fig. 2.5

[a] Analysis without *Verosphacela ebrachia* and *Sphacella subtilissima*.

1999). For the combined 18S–26S analysis, sequences were available from only one tribophyte, *Tribonema aequale*, and no phaeothamniophyte. 26S-sequences from other heterokont classes are unalignable (see also de Reviers and Rousseau 1999).

RESULTS

Three alignments were made, one for *rbc*L, one for the combined partial 18S and 26S, and one that combined all three genes. The partial 18S and 26S sequences cover the same regions as in de Reviers and Rousseau (1999), Rousseau and de Reviers (1999a, b) and Rousseau *et al.* (2000). Properties of the alignments are summarised in Table 2.5. Because all three sequences were not available for all taxa, the combined analysis included only 22 species.

Alignment properties: *rbc*L

In heterokont algae, *rbc*L is 1467 base pair (bp) in length, except in diatoms and their sister class the Bolidophyceae where it is 1473 bp (Daugbjerg and Guillou 2001). No stop codons or gaps were found in the *rbc*L sequences in the present study; therefore, the alignment was unambiguous. In 15 of the 58 sequences, circa 150 nucleotides were missing from either the 5’- or the 3’-ends (but not both ends simultaneously in one sample). However, inspection of the alignment confirmed no significant loss of phylogenetic information because the variation is evenly distributed over the whole gene and our alignment covered >95% of the gene. The *rbc*L alignment of the present study covers 467 amino acids of which 198 are variable or, in terms of nucleotides, 656 variable positions out of 1399. Base composition deviated somewhat from 25% (average: A, 0.29; T, 0.32; C, 0.16; G, 0.22), which justified the use of the Tajima-Nei distances in the saturation tests and later NJ analyses. The average transition/transversion (ti/tv) ratio for the *rbc*L alignment was 1.21 (excluding the outgroup) and 1.05 (including the outgroup). The ti/tv ratio is expected to decrease with increasing sequence distance because transversions erase the record of the more frequent transitions. Values between 1 and 2 are typical and do not indicate saturation. Values < 1 do indicate some saturation (Holmquist 1983, Bakker *et al.* 1995). Because *rbc*L is a protein-coding gene, saturation was further investigated in relation to codon position. Plots of uncorrected versus corrected pair-wise distance estimates for first and second codon positions showed no mutational saturation, whereas for the third position, a clear leveling off of the curve was found (not shown). However, Källersjö *et al.* (1998, 1999) showed that rapidly evolving third codon positions in *rbc*L of higher plants often contain most of the phylogenetic signal in the data and that this can be used even in the presence of some saturation. Therefore, the third position was included and excluded in some analyses to evaluate its contribution.

Alignment properties: rDNA

Complete sequences for the 18S gene are available for only about 30 species of brown algae, whereas more than 120 additional, partial sequences are known. A similar situation exists for the 26S gene. There is only one complete sequence known, that is, *Scytosiphon lomentaria* (Kawai *et al.* 1995), and about 60 partial sequences. These traverse the 5’-end and the most variable structural domains (i.e., D1 and D2). Partial sequences present a problem for the construction of a more comprehensive alignment because the missing areas between sequences are not necessarily the same ones. This limits the number of taxa and the length of the alignment. The number of species and the length of homologous sequences available for the present study represent only about 25% of the 18S (436–444 bp across the V9 region) and 25% of the 26S (552–600 bp across the D1 and D2 regions). Fortunately, these domains are among the most variable portions of both molecules.

As shown in Table 2.5, the number of informative sites in the combined 18S–26S alignment among Phaeophyceae was about 70% of that found in the *rbc*L alignment. In the 18S–26S alignment, 57 sites were gapped as a result of the incorporation of the outgroup. In particular the sequences of *Choristocarpus tenellus*, the two *Dictyota* species and the outgroup taxon *Tribonema aequale* were difficult to align due to length differences in the D1 and D2 domains of the 26S. The alignment is, therefore, not completely unambiguous. No 18S sequences were obtained for *Sphacella subtilissima* and *Verosphacela ebrachia*, but because the 26S contained the major part of the information, these two taxa were included in the analysis of the combined 18S–26S alignment anyway. The average base composition was as follows: A, 0.23; T, 0.23; C, 0.24; G, 0.30. The average ti/tv ratio for the rDNA alignment was 1.20, including the outgroup *Tribonema aequale* and 1.21, excluding the outgroup.

Combined *rbc*L–18S–26S alignment

The combined three-gene alignment was predictably much smaller in number of taxa (Table 2.5) but almost doubled the number of characters. Combining the genes across the reduced set of taxa did not significantly change the alignment (five gaps less in the rDNA).

Phylogenetic analysis: *rbc*L

Three different MP analyses were performed: (1) first + second codon positions only, (2) third codon position only, and (3) all three codon positions together (Table 2.5). The sub-analyses gave slightly different topologies in the 50% majority-rule consensus trees (not shown).

Analysis of the first and second positions only, yielded poorly resolved trees, whereas most of the structure recovered using all three codon positions could also be recovered by analysing third positions alone. An analysis at the amino acid level gave no more resolution than the analysis based on the first and second codon positions only. Bootstrap analysis and parsimony jackknife analysis gave the same results, that is, third positions complemented and strengthened the information from the first two positions. A PHT in which trees based on first and second codon positions were compared against those based on the third positions alone yielded a *P*-value of 0.062. This indicates that the two subsets are not in conflict and can be combined. The result of the MP analysis using all three codon positions is shown in Figures 2.1 and 2.2. One of the 227 most parsimonious trees (MPTs) is shown in phylogram style in Figure 2.1 to show internodal branch lengths in relation to the number of character-state changes along branches. The 50% majority-rule consensus tree (Fig. 2.2) provides alternative measures of branch support. Pruning of the (internally) highly unstable

Ectocarpales *s.l.* clade to, for example, five species reduced the number of MPTs to one, thus giving the same tree topology as the tree in Figure 2.1. An NJ tree of the complete *rbc*L alignment (not shown) yielded the same topology as the tree in Figure 2.2 with the exception of the position of the *Onslowia endophytica* + *Verosphacela ebrachia* clade, which formed a sister group to the Syringodermatales. However, that relationship had no bootstrap support. An analysis using ML was not possible because of the large size of the data set.

Eight phaeophycean orders are represented in Figure 2.2. The Laminariales are artificially monophyletic in this analysis as a result of the taxon sampling. The Sphacelariales *s.l.* are not monophyletic, being split into three different clades: (1) *Choristocarpus tenellus*, (2) *Onslowia endophytica* + *Verosphacela ebrachia*, and (3) all other representatives (i.e., the Sphacelariales *s.s.*). *Syringoderma phinneyi* consistently groups with the Sphacelariales *s.s.*, though there is no bootstrap support. The grey lines in Figure 2.2 show the minimal topological structure of the consensus tree for which there is bootstrap support.

Phylogenetic analysis: rDNA

MP analyses were performed with and without gaps. The best results in all analyses were obtained when gaps were treated as missing data. A PHT was then performed between the variable 26S D1 and D2 domains and the remainder of the rDNA. Surprisingly, $P = 0.002$, indicating that the subsets are incongruent. Although the resolution of the tree in Figure 2.3 appears to be quite good, the 50% majority-rule consensus tree shown in Figure 2.4 reveals how little bootstrap support there actually is for ordinal relationships. Exclusion of the difficult to align D1 and D2 regions based on the result of the PHT gave an even worse result of 61,600 MPTs (tree not shown), but this is probably a consequence of the further reduction of the data set. However, *Choristocarpus tenellus* was then the most basal taxon (as in the *rbc*L based trees).

The NJ tree (not shown), based on all positions, differed significantly from the MP trees. Its topology is as follows: outgroup (Dictyotales [Fucales {*Choristocarpus* + Sphacelariales *s.s.* (*Onslowia* + *Verosphacela* + *Asterocladon* [Syringodermatales {Laminariales *s.s.* (*Chorda* [*Asteronema rhodochortonoides* + Ectocarpales *s.l.* {Ascoseirales (*Asteronema ferruginea* + Scytothamnales [Desmarestiales {*Halosiphon* (*Sacchoriza* + Cutleriales)}])}])}])}]). For *Verosphacela ebrachia* there was no 18S sequence and for *Asterocladon lobatum* the number of sequenced nucleotides was also limited (Table 2.3). This may explain the attraction of these two taxa in the NJ tree, for which no close relationship was expected. The short internodes (0.00–0.02) that separated the orders, suggest low phylogenetic signal (Bakker *et al.* 1995) that can be further exacerbated by the application of distance methods. We did not do a ML analysis because of the large size of the data set.

Ten phaeophycean orders and three species of unknown affinity are represented in the rDNA tree (Fig. 2.4). It is important to note that the taxon sampling is not identical with that used in the *rbc*L analysis (Fig. 2.2). The Laminariales *s.l.* are paraphyletic as has been shown by other authors and the Sphacelariales *s.l.* was split into two clades: (1) *Choristocarpus tenellus* + Sphacelariales *s.s.* and (2) *Onslowia endophytica* +

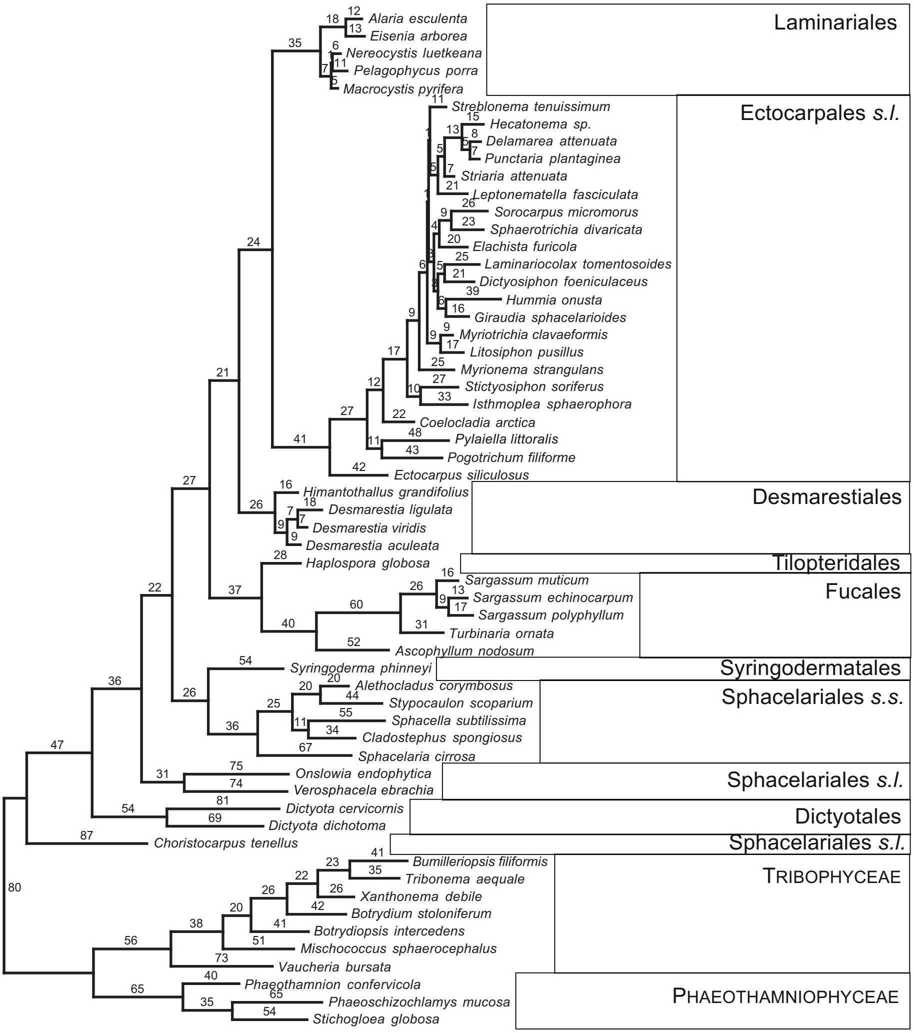


Fig. 2.1. *Rbc*L tree for the Phaeophyceae. One of 227 most parsimonious trees shown in phylogram style with branch lengths given above branches.

Verosphacela ebrachia. Again the grey lines in Figure 2.4 represent the minimum topology for which there is bootstrap support. A comparison with the *rbc*L tree in Figure 2.2 makes it clear that there is less resolution in the rDNA tree.

Phylogenetic analysis: combined *rbc*L–18S–26S

The PHT for the partition *rbc*L and 18S-26S (including the 26S D1 and D2 regions), gave a *P* value of 0.0006, which means that the genes are incongruent. When the test was repeated on the partition of *rbc*L and 18S-26S (excluding the 26S D1 and D2 regions), *P* = 0.89, indicating congruence. However, subsequent MP analyses of both

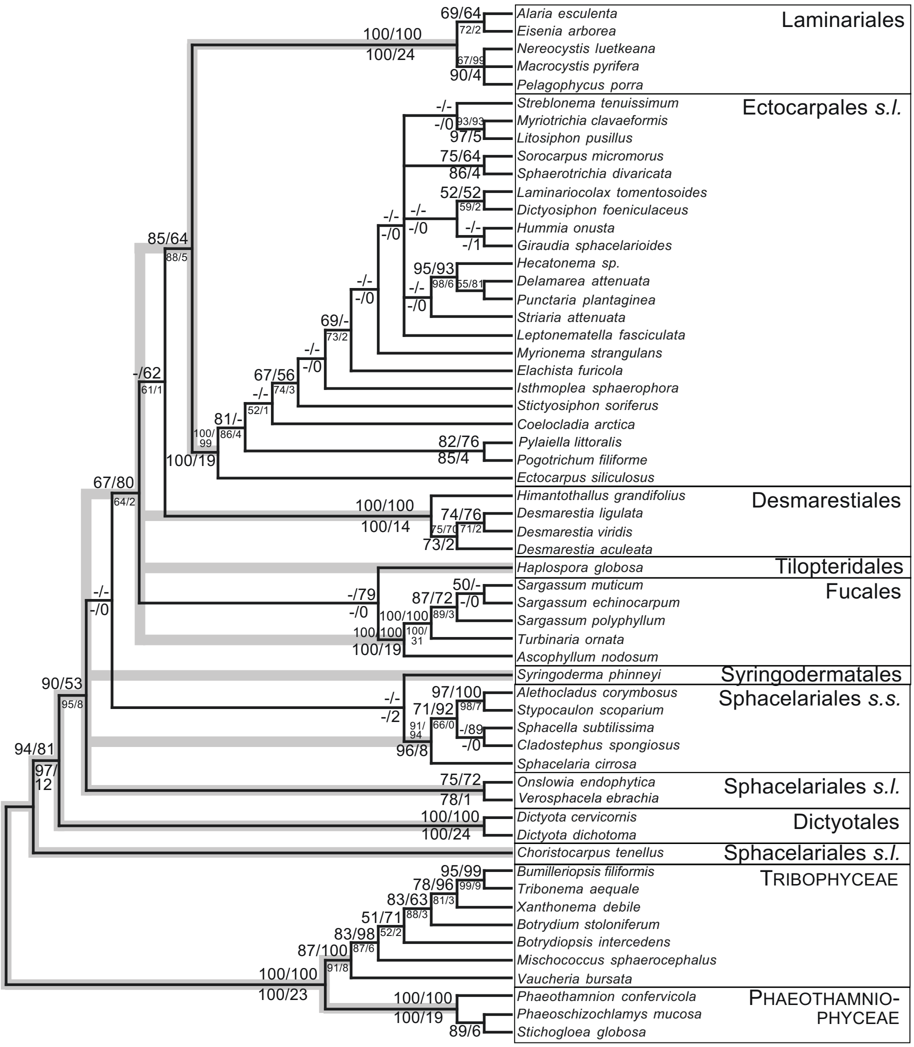


Fig. 2.2. *Rbc*L consensus tree for the Phaeophyceae. A 50% majority-rule consensus tree of the 227 most parsimonious trees is shown. Tree statistics are given in Table 2.5. Outgroup = Tribophyceae and Phaeothamniophyceae; bootstrap percentages are given above branches, MP (left) and NJ (right); below branches parsimony jackknife percentages (left) and decay index values (right) are given. Dashes (-) indicate percentages of <50% or that the internode did not occur in the NJ or jackknife tree. The underlying backbone tree (grey) indicates the minimal topology for which there is support.

versions of the *rbc*L–18S–26S alignment, resulted in the same single MPT (Fig. 2.5). The backbone of the tree is the same as for the *rbc*L analysis (Fig. 2.2), except that the combined *rbc*L–18S–26S analysis did not include Tilopteridales. The NJ tree (not shown) also showed the same tree topology as the tree in Figure 2.5 with the exception of *Onslowia endophytica* + *Verosphacela ebrachia*, which formed a sister group of

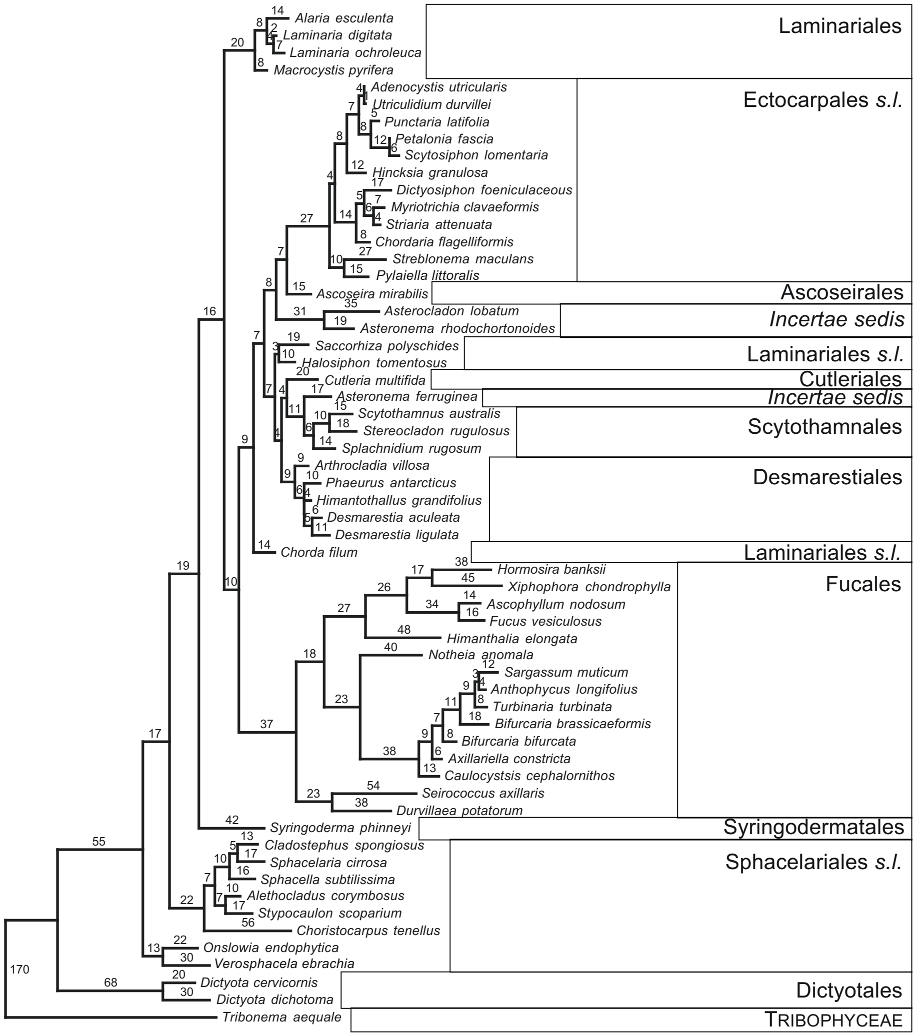


Fig. 2.3. rDNA tree for the Phaeophyceae. One of 16 most parsimonious trees based on partial 18S and 26S sequences shown in phylogram style with branch lengths given above branches.

Syringodermatales (same as the NJ analysis of the *rbc*L alignment). Again, that relationship had no bootstrap support. In a ML analysis, the general-time-reversible model of DNA substitution (Rodríguez *et al.* 1990) was used, following a gamma distribution and invariable sites. The ML tree (not shown) differed from the MP tree only in the position of the Dictyotales clade, which formed a weakly supported sister group with the *Onslowia endophytica* + *Verosphacela ebrachia* clade. The seven orders represented in the combined tree (Fig. 2.5), mirror the analysis of *rbc*L alone, but bootstrap/jackknife support is better for the node that separates the basal clades

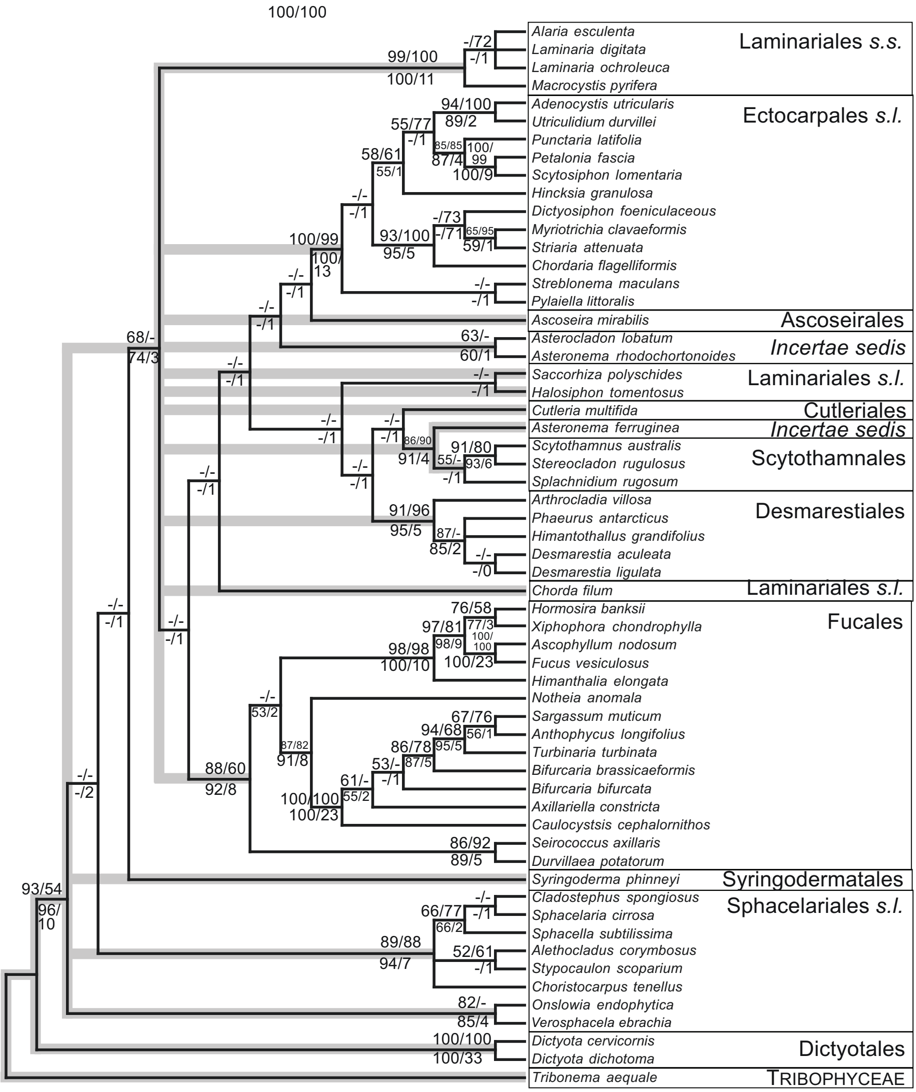


Fig. 2.4. rDNA consensus tree for the Phaeophyceae. A 50% majority-rule consensus tree of 16 most parsimonious trees based on partial 18S and 26S sequences is shown. Tree statistics are given in Table 2.5. Outgroup = *Tribonema aequale*; bootstrap percentages are given above branches, MP (left) and NJ (right); below branches parsimony jackknife percentages (left) and decay index values (right) are given. Dashes (-) indicate percentages of <50% or that the internode did not occur in the NJ or jackknife tree. The underlying backbone tree (grey) indicates the minimal topology for which there is bootstrap support.

(Dictyotales, Sphacelariales *s.l.*, Syringodermatales) from the upper clades (Fucales, Desmarestiales, Ectocarpales *s.l.*, Laminariales). Again, the grey lines in Figure 2.5 represent the minimum topology for which there is good bootstrap support.

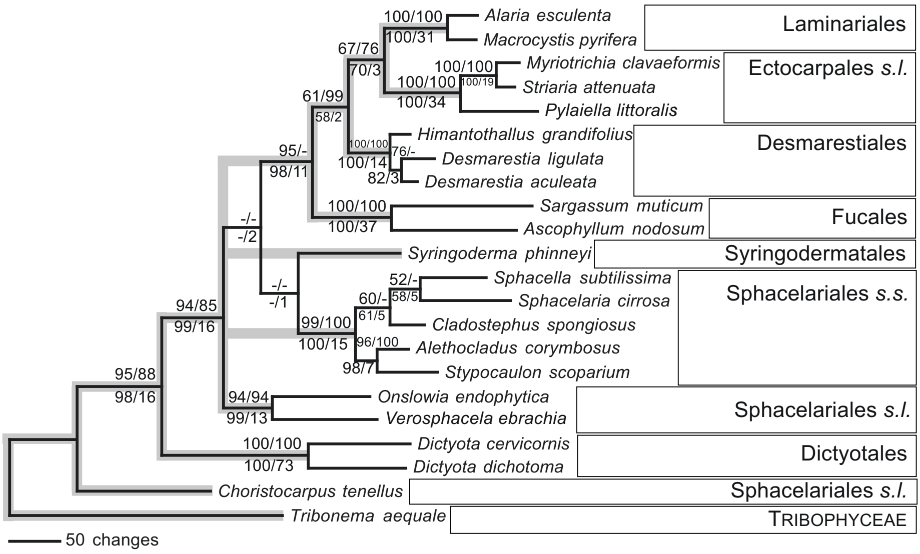


Fig. 2.5. Single most parsimonious tree of the Phaeophyceae based on combined *rbc*L and partial 18S and 26S rDNA sequences. Tree statistics are presented in Table 2.5. Outgroup = *Tribonema aequale*; bootstrap percentages are given above branches, MP (left) and NJ (right); below branches parsimony jackknife percentages (left) and decay index values (right) are given. Dashes (-) indicate percentages of <50% or that the internode did not occur in the NJ or jackknife tree. The underlying backbone tree (grey) indicates the minimal topology for which there is bootstrap support.

DISCUSSION

Tree comparisons

The *rbc*L tree (Figs 2.1 and 2.2) and the rDNA tree (Figs 2.3 and 2.4) are congruent with one another, but the *rbc*L tree has a higher resolution. Although taxon sampling is different and less comprehensive in the *rbc*L tree, both trees place the Fucales, Desmarestiales, Ectocarpales *s.l.,* and Laminariales in the upper part of the tree and the Dictyotales, Sphacelariales, and Syringodermatales in the lower portion of the trees. When both data sets were combined on a smaller set of taxa, the same relationships were maintained but with higher bootstrap support (Fig. 2.5). This increase in robustness of this tree is an effect of combining the two data sets and not the result of the reduction of the number of taxa. This has been verified by running the separate *rbc*L and rDNA analysis with exactly the same number of taxa as in the combined analysis. An examination of the relative number of polytomies in the backbone portions of the bootstrap-supported trees (Figs 2.2, 2.4, and 2.5, grey lines), reveals the most conservative version of the respective analyses. Here again, it is clear that *rbc*L has more resolving power with fewer polytomies, particularly in the basal part of the tree. The overall agreement of the *rbc*L and rDNA trees lends support to the idea that the gene trees are representative of the species tree.

The incorporation of multiple outgroups outside of the Phaeophyceae was an important consideration. Many of the studies listed in Table 2.1 focused on particular orders within the brown algae rather than on the phylogeny of the Phaeophyceae as a whole and so used sequences of other brown algae as ‘internal’ outgroups. As noted in Materials and Methods, members of the Tribophyceae and Phaeothamniophyceae are the closest known sister taxa to the Phaeophyceae. Use of multiple outgroups in the *rbc*L tree consistently placed *Choristocarpus tenellus* as the most basal phaeophyte. Unfortunately, this was not possible in the rDNA tree where only one outgroup sequence was available. Moreover, the inclusion of *Tribonema aequale* was problematic because the alignment of its rDNA sequence in the D1 and D2 regions was difficult. An attempt to check the position of *Choristocarpus* based only on partial 18S and 26S (minus the D1 and D2) resulted in a polytomy (61,000 MPTs), as a result of a >50% reduction in the number of characters. However, *Choristocarpus* was then most basal. The combination analysis of *rbc*L and rDNA, with or without the D1 and D2 regions, always placed *Choristocarpus* as the most basal. To test this further, we used the program MacClade version 3.08a (Maddison and Maddison 1992) to force *Choristocarpus tenellus* to the base of the rDNA tree. This was done at the cost of 15 extra steps, which is <1% of the total number of 1969 steps, and therefore acceptable.

The incongruence found within rDNA when the D1 and D2 regions were included is puzzling. These data sets are expected to be compatible because they are part of the same cistron and thus not independent. Cunningham (1997) suggests that incongruence can arise from insufficient taxon sampling, differences in nucleotide substitution rates or inaccurate alignment and thus questionable homology. Taxon sampling was uniform and there was no evidence for differences in substitution based on nucleotide frequencies. This leaves the alignment. As noted previously, there was some difficulty with the inclusion of *Choristocarpus tenellus* and *Dictyota* in the D1 and D2 domains due to length variation. Secondary structure was taken into account and some gaps were required. However, inclusion or exclusion in the analyses did not alter the topology, and attempts to improve the alignment had no effect. Still, the PHT revealed incongruence. This result suggests that adding more sequence is not necessarily better and means that congruence tests should become routine anytime that multiple sequences are used. It can also serve as a check on hidden alignment problems that may be correctable.

Internal relationships

Establishment of monophyly, paraphyly, and sister group relationships is wholly dependent on the taxon sampling, which in practice, is never complete. It has been estimated that there are 1500–2000 species of brown algae in 265 genera and circa 60 families (Bold and Wynne 1985, van den Hoek *et al.* 1995, de Reviers and Rousseau 1999). This means that overall taxon sampling of the phaeophycean lineage is well below 10%. Nevertheless, presently available taxon sampling is generally good at the ordinal level (>90%) and probably does provide a good cross-section of phaeophycean diversity. Unfortunately, the same cannot be said for the family level. With some exceptions, for example, the Alariaceae (Yoon and Boo 1999), Desmarestiaceae (Peters *et al.* 1997), Fucaceae (Serrão *et al.* 1999), and Sphacelariaceae (Draisma *et al.* 2002/Ch. 3), family-level studies are limited. A further caveat is that we do not actually know how accurate or inaccurate classical taxonomic categories are at the ordinal-family level, and therefore we use the terminology mainly for convenience. Results of the present study and previous studies (Table 2.1) show, for example, that well-known groups, such as the Laminariales, are paraphyletic assemblages of homogeneous taxa, whereas the Ectocarpales *s.l.* has turned out to be a monophyletic, albeit heterogeneous assemblage of previously separate species. Another interesting observation is the growing number of species that have no obvious affinities (i.e. *incertae sedis*). These species are often members of obscure genera with only one or a few described species (e.g. *Asteronema, Choristocarpus*), but there are also cases that involve well-known genera (e.g. *Ascoseira, Cutleria, Chorda*). Considering the combined results of all of our analyses, 11 'orders' could be distinguished: Ascoseirales, Cutleriales, Desmarestiales, Dictyotales, Ectocarpales *s.l.*, Fucales *s.l.*, Laminariales *s.s.*, Scytothamnales (including *Asteronema ferruginea*), Sphacelariales *s.s.*, Syringodermatales, and Tilopteridales. 'Orders' missing from our analyses include Ralfsiales and Sporochnales. Clades (often consisting of single branches) for which the species are of uncertain affinity (*incertae sedis*) with respect to an order and to their relative position in the trees include *Asterocladon lobatum* + *Asteronema rhodochortonoides*, *Chorda filum*, *Choristocarpus tenellus*, *Halosiphon tomentosus*, *Saccorhiza polyschides*, and *Onslowia endophytica* + *Verosphacela ebrachia*.

The paraphyletic status of the Laminariales was first detected by Tan and Druehl (1996). This is confirmed in Figure 2.4 where *Chorda filum*, *Halosiphon tomentosus*, and *Saccorhiza polyschides* do not group with the Laminariales *s.s.* De Reviers and Rousseau (1999) considered these taxa as *incertae sedis*. However, a closer examination of the supported topology in the rDNA tree (Fig. 2.4, grey lines) shows that all the clades between the Fucales and Laminariales *s.s.* are equidistant and unresolved. A similar backbone topology holds for the *rbc*L tree (Fig. 2.2, grey lines) but because the taxon sampling does not include the three aforementioned species, it remains unclear as to whether the Laminariales will remain paraphyletic. It is possible that increased taxon sampling in the Laminariales *s.l.* and the use of *rbc*L would restore monophyly to some of the Laminariales. Relationships within the Laminariales *s.s.* have also been evaluated using RUBISCO-spacer sequences (Yoon and Boo 1999, Boo and Yoon 2000, Kraan and Guiry 2000). The study by Yoon and Boo (1999) used *Chorda filum* as the outgroup. Their results did not support the current familial system of the Laminariales *s.s.* Boo and Yoon (2000) suggested that the Laminariales *s.s.* should be downgraded to family status or possibly that eight independent families should be erected.

The Sphacelariales *s.l.* are also paraphyletic. In the rDNA tree, two clades were recovered (Fig. 2.4), whereas in the *rbc*L tree three well-supported clades were recovered (Fig. 2.2). The three groups are further supported in the combined gene analysis (Fig. 2.5). *Onslowia* and *Verosphacela* form a well-supported monophyletic group separate from the other Sphacelariales and *Choristocarpus* splits off basally. The position of *Choristocarpus tenellus* is of particular interest because it appears to be the most basal taxon in the Phaeophyceae. De Reviers and Rousseau (1999) also suggested the possible separation of the family Choristocarpaceae (including *Onslowia* [two species] and the monotypic genera *Choristocarpus*, *Discosporangium*, and *Verosphacela*) from the Sphacelariales *s.s.*, but they had no sequence data available.

Onslowia and *Verosphacela* cannot be incorporated in a single monophyletic group that also includes *Choristocarpus* and the position of *Discosporangium* in the Choristocarpaceae remains unknown. However, the separation of the Choristocarpaceae from the order Sphacelariales is clearly justified from the present analyses and also on morphological grounds. Two key ordinal characters that define the Sphacelariales, transverse division of subapical cells into secondary segments and darkening of cell walls when placed in bleach (Reinke 1890), are absent in the Choristocarpaceae. For the time being, the *Choristocarpus* and *Onslowia* + *Verosphacela* clades must be regarded as *incertae sedis*.

Relationships among 'orders' is of interest in helping to understand the evolutionary trends of the Phaeophyceae in general and in establishing sister groups and outgroups for familial-level studies. Again, taxon sampling directly affects the result, but some trends and relationships can be discussed. The *rbc*L and combined analysis (Figs 2.2 and 2.5) unambiguously place the morphologically complex Laminariales *s.s.* as sister group to the morphologically simple, but diverse, Ectocarpales *s.l.* This may not be such a radical concept as it first seems when carefully compared with traditional morphological classifications. For example, Scagel (1966) and Wynne and Loiseaux (1976) considered the Dictyosiphonales and Scytosiphonales to be sister orders to the Laminariales. Because these two groups are now considered as part of the Ectocarpales *s.l.* (Rousseau and de Reviers 1999b), the relationship is consistent. On the other hand, van den Hoek *et al.* (1995) hypothesised that the Laminariales should be more related to Desmarestiales and Sporochnales. The *rbc*L (Fig. 2.2), and especially the combined analysis (Fig. 2.5), show that the Desmarestiales are basal (with high support) to the Laminariales *s.s.* and Ectocarpales *s.l.* clade. Because Sporochnales are missing, we cannot determine what their effect might be. A general comparison of ordinal resolution between the *rbc*L (Fig. 2.2) and rDNA (Fig. 2.4) analyses shows better resolution in the *rbc*L tree. Relationships in the combined analysis (Fig. 2.5) are even stronger although the reduced taxon sampling may also be playing a role. The inter-ordinal relationships shown in Figure 2.5 are strong, particularly among the upper four orders Laminariales *s.s.,* Ectocarpales *s.l.*, Desmarestiales and Fucales. The Desmarestiales have been extensively studied (Peters *et al.* 1997; rDNA-ITS sequences). The Fucales, a very large group, have been most extensively studied by Rousseau and de Reviers (1999a; rDNA-18S and 26S sequences) and to a lesser extent by Rousseau *et al.* (1997; rDNA-26S) and Horiguchi and Yoshida (1998; rDNA-18S). Relationships among the Sphacelariales *s.s.* and *s.l.* along with Syringodermatales are well resolved but bootstrap support for two of the clades (Fig. 2.5) is weak. In all analyses, the Dictyotales come out basal in the tree, just above *Choristocarpus*.

Sequence divergence, branch length and the ancestral Phaeophyceae

Figures 2.1, 2.3 and 2.5 are presented in phylogram style to show actual branch lengths. In most ordinal groups terminal branches are short and taxa are separated by short internodes, for example, the Laminariales *s.s.*, Ectocarpales *s.l.*, and Desmarestiales. In contrast, the Fucales are characterised by long terminal branches and long internodes. In general, shallow topologies are indicative of recent speciation, whereas deep internodal topologies are indicative of old events. Long terminal branches can also be indicative of extinctions (i.e., a surviving lineage that was once more speciose). Within a monophyletic group such as an order or family, rates of evolutionary change are usually assumed to be uniform. However, as one moves to more distant groupings, the assumption of rate uniformity becomes increasingly questionable and is why molecular clocks (Zuckerkandl and Pauling 1965, Sarich and Wilson 1967), even local clocks, are so difficult to apply.

From rDNA-5S sequence divergence Lim *et al.* (1986) estimated that the Phaeophyceae diverged within the last 200 million years. On the basis of rDNA-18S sequence divergences, Medlin *et al.* (1997) estimated the earliest probable origin of the brown algae at 155 million years ago. They calibrated their molecular clock by regressing branch lengths from taxa with a fossil record (diatoms) from a linearised NJ tree against first appearance dates. Unfortunately, their taxon sampling focused on the Bacillariophyceae and included only one representative from the phaeophycean orders Desmarestiales, Ectocarpales, Fucales, and Laminariales (Table 2.1). The Fucales were the most basal phaeophycean clade in their study. Inclusion of representatives of the Dictyotales, Sphacelariales and/or Syringodermatales may have resulted in a slightly older estimation. Saunders and Druehl (1992) applied a molecular clock to 18S data from the Laminariales and estimated a recent divergence at about 16–20 million years ago. This is in accord with Miocene deposits (7–10 million years old) of Laminariales and Fucales (van den Hoek *et al.* 1995). Taken together, these data show that the Phaeophyceae are an ancient lineage, but that most extant lineages are of fairly contemporary origin. The exception is the Fucales, with their deep branching patterns. Since 1995, molecular studies that have included a nonphaeophycean outgroup (Table 2.1) have placed the Fucales at the base of the Phaeophyceae. As it turns out this was an artifact of taxon sampling because the Dictyotales, Sphacelariales, and Syringodermatales were not included. However, the general relationship among the three aforementioned groups has been shown previously by Tan and Druehl (1994) and de Reviers and Rousseau (1999).

In our analyses, *Choristocarpus tenellus* is the most basal brown alga. In terms of its morphology it generally fits the classic view of creeping filaments, apical growth, and an isomorphic life history. This fits with the notion of van den Hoek *et al.* (1995), who speculated that the Ectocarpales *s.s.* was ancestral to all other brown algae ("… meaning that the Ectocarpales are thought to have changed least since the origin of the Phaeophyceae"). According to van den Hoek *et al.*, "The ancestral ectocarpalean brown alga consisted of creeping filaments with apical and intercalary growth, and an isomorphic, diplohaplontic life cycle". Given that thallus complexity, intercalary versus apical growth mode and life history are among the classic characters used in the discussion of brown algal classification and evolution, we consider them briefly with respect to the molecular tree.

Working up from the base of the tree (Figs 2.2, 2.4, or 2.5), the Dictyotales, Sphacelariales *s.l.*, and Syringodermatales all show apical growth. The Dictyotales and Sphacelariales *s.l.* have isomorphic diplohaplontic life histories. However, some authors have considered the life history of the Sphacelariales *s.s.* to be heteromorphic (De Reviers and Rousseau 1999, Rousseau and de Reviers 1999b), an opinion most probably inspired by the study of van den Hoek and Flinterman (1968) in which they described the life-history of *Sphacelaria rigidula* Kützing (as *S. furcigera*) as slightly heteromorphic, because the haploid gametophytic phase was relatively slender as compared with a more robust diploid sporophytic phase. We consider diameter an unreliable character and therefore consider all Sphacelariales to be isomorphic. All four species of Syringodermatales appear to have a heteromorphic life history, of which two species lack a free gametophytic phase (Henry 1984). The Desmarestiales, Ectocarpales *s.l.*, and Laminariales have various combinations of diffuse, intercalary and apical growth, and all have heteromorphic life cycles, except for the Ectocarpales *s.s. Haplospora globosa* (Tilopteridales) is isomorphic. In *Tilopteris mertensi* (Turner) Kützing only the gametophyte is known, and a third tilopteridalean species, *Phaeosiphoniella cryophila* Hooper, Henry *et* Kuhlenkamp, is only known to reproduce asexually. It is clear, therefore, that considerable diversity exists even within groups. In sharp contrast, the Fucales have strongly apical growth and a monophasic life history, and they sit in the middle of the tree. However, the Durvillaeaceae (Fucales *s.l.*) show intercalary diffuse growth but mainly in the apical zone. These many observations make it obvious that morphological grades of organisation (simplicity or complexity); diffuse, intercalary, or apical modes of growth; and type of life history have evolved and/or have been lost independently and repeatedly in the Phaeophyceae. While these characteristics are crucial for identification and for understanding the ecology of the brown algae, they are not useful in the phylogenetic sense, that is, they are not identical by descent. What this kind of deep homoplasy signals, however, is the great diversity of life history strategies that have evolved and continue to be maintained under similar selective regimes at different times; or perhaps as evolutionary accidents that have become fixed.

Diplohaplontic life histories have generally been classified as more primitive and less successful from an evolutionary point of view (Valero *et al.* 1992, Klinger 1993) and that diploidy (as in diatoms, fucoids, and multicellular animals) will eventually prevail. Modelling (Valero *et al.* 1992) has shown that diplohaplontic life histories in algae (Klinger 1993) are not *per se* transitional but an evolutionary stable strategy. The fact that the Fucales are not the most recent/derived group, despite their putatively advanced, oogamous, monophasic, diploid life history, and the fact that isomorphic and heteromorphic life histories are characteristic of many species independent of their phylogenetic relationships opens a new window on the study of life history evolution in the algae.

Where next?

Our results show that even though the crown radiation of brown algae occurred quite rapidly, as assessed by short internodes in both gene trees, the search for phylogenetic resolution in the Phaeophyceae is not a lost cause. The information content of *rbc*L is greater than that of rDNA even though both genes are considered to be quite conserved. Used together, the overall resolution of the tree was not higher than the *rbc*L tree alone, but bootstrap support was generally improved and previously unsupported branches gained support. Although much has been learned about the Phaeophyceae from ribosomal DNA sequences, we suggest that future efforts concentrate on *rbc*L sequences and perhaps *rbc*S. Both of these genes are coded for in the chloroplast of brown algae, and along with the separating RUBISCO spacer, are likely to provide strong phylogenetic signal both between and within orders, families, and genera.

With regard to taxon sampling, it will be essential to concentrate on small and/or exotic groups that are currently *incertae sedis* (i.e., Ascoseirales, *Asterocladon*, *Asteronema*, *Bachelotia*, Cutleriales, Ralfsiales, Scytothamnales, Sporochnales, Syringodermatales, and Tilopteridales), on groups that show paraphyly (Laminariales *s.l.*), and on large groups for which there is still poor representation (Dictyotales, the Durvillaeaceae and Seirococcaceae within the Fucales). In our opinion, the Desmarestiales, Ectocarpales *s.l.,* Laminariales *s.s.*, and the Sphacelariales *s.s.* have been adequately sampled. It is also important to avoid single-taxon sampling. Even if the group in question is monotypic, an effort should still be made to sequence different individuals (preferably from different geographic areas) to establish a strong clade that will remain stable in the tree. It is also desirable to try to break the long branch of *Choristocarpus tenellus* and find more support for its ancestral status. *Discosporangium mesarthrocarpum* (Meneghini) Hauck may be a sister taxon or it may represent yet another independent lineage. The inclusion of a few freshwater brown algae (*Heribaudiella fluviatilis* [Areschoug] Svedelius [Pueschel and Stein 1983, Wehr and Stein 1985], two species of *Sphacelaria*, i.e., *S. fluviatilis* Jao [1943] and *S. lacustris* Schloesser *et* Blum [1980], *Pseudobodanella peterfii* Gerloff [1967], and *Porterinema fluviatile* [Porter] Waern [Dop 1979]) and the obligate parasitic species, *Herpodiscus durvillaeae* (Lindauer) South, is also of interest. In conclusion, our understanding of phylogenetic relationships within the Phaeophyceae is steadily improving. Careful taxon selection along with emphasis on *rbc*L sequences should produce a comprehensive view in the near future.

Finally, the addition of new faster evolving genes, possibly from mitochondrial DNA, is desirable. Ehara *et al.* (1999) surveyed the *cox*I gene in 18 species of the Heterokontophyta, including six species in the Phaeophyceae. Resolution within the Phaeophyceae was low. Although *cox*I is conserved, the potential of algal mtDNA remains promising with comparative genomics being the most exciting new development. Complete (or nearly complete) mitochrondrial genome sequences are now available for *Pylaiella littoralis* (58,507 bp) (Oudot-Le Secq *et al.* 2001) and *Laminaria digitata* (38,007 bp) (M.-P. Oudot-Le Secq, University of Groningen, pers. comm.). Mitochondrial genomes in *Ectocarpus, Sphacelaria, Fucus* and *Dictyota* are also being sequenced (W.T. Stam and J.L. Olsen). Although the genomics approach severely restricts taxon sampling, it will provide a unique opportunity to explore mitochondrial evolution and deep-phylogeny in the heterokont algae more generally.

Note added in proof

The *rbc*L sequences of 14 species of Scytosiphonales (representing two families and six genera) published by Kogame *et al.* (1999; *Phycologia* 38: 496–502) were not yet released by the EMBL/GenBank/DDBJ databases when we completed the phylogenetic analyses for the present study. A preliminary analysis of our data set together with these new Scytosiphonales sequences showed that this order forms a monophyletic sister group to the rest of the Ectocarpales *s.l.*

After acceptance of this article three more papers dealing with phaeophycean phylogenetics were published. One by Kawai and Sasaki (2000; *Phycologia* 39: 416–28), who provide *rbc*L sequences of *Akkesiphycus*, *Halosiphon*, *Sporochnus*, and three species of *Desmarestia*. This study showed that the Laminariales *s.l.* are nonmonophyletic. Another study by Rousseau *et al.* (2001; *C. R. Acad. Sci. Paris, Sciences de la vie* 324: 1–15) presents more 18S and 26S sequences of various Phaeophyceae. Using *Tri-*

bonema aequale as outgroup they confirmed our finding that the Dictyotales is the most basal phaeophycean order followed by Sphacelariales *s.s.*, Syringodermatales, and then a crown radiation of all other orders. A third article dealing with phylogenetics of *Chorda* by Kawai *et al.* (2001; *J. Phycol.* 37: 130–42) provides *rbc*L sequences of eight species of Laminariales *s.l.*

Acknowledgements
Supported by grant no. 805-42.025 from the Foundation of Earth- and Life-Sciences (ALW), which is subsidised by the Netherlands Organisation for the Advancement of Research (NWO). We thank the following persons for supplying algal cultures and/or DNA extracts: Olivier de Clerck, Jim Coyer, Eric Henry, Dieter Müller, and Akira Peters. We are grateful to Mari Källersjö and Steve Farris for their help with the parsimony jackknifing.

Chapter 3

PHYLOGENETIC RELATIONSHIPS WITHIN THE SPHACELARIALES (PHAEOPHYCEAE): *rbc*L, RUBISCO-SPACER AND MORPHOLOGY [1]

Phylogenetic relationships within the Sphacelariales *sensu stricto* were investigated using both a molecular and a morphological approach. Twenty species were included, representing all three families (i.e., Cladostephaceae, Sphacelariaceae and Stypocaulaceae) and six of the eight genera. The outgroup consisted of six species representing the Syringodermatales, Dictyotales, Choristocarpaceae and Onslowiaceae. DNA sequences of partial *rbc*L (1255–1375 nt) and the adjacent RUBISCO-spacer (40-842 nt) were determined in order to assess the molecular phylogeny. Only the 3'-end of the spacer (112 nt positions) was alignable for the ingroup taxa. Partition homogeneity testing showed that *rbc*L and RUBISCO-spacer sequences could be combined. However, analysis of *rbc*L alone or in combination with the RUBISCO-spacer gave the same results with only slight differences in support (bootstrap, jackknife, decay). Support was low at the base of the ingroup. Four basal clades could be discerned: (1) Stypocaulaceae, (2) *Sphacelaria radicans*, (3) *Sphacelaria caespitula*, and (4) all other included taxa; (*Sphacella subtilissima* + the *Sphacelaria* subgenus *Propagulifera*) and (*Cladostephus spongiosus* + *Sphacelaria nana* + the *Sphacelaria* subgenera *Battersia* + *Pseudochaetopteris*). The independent morphological analysis (using 23 unordered morphological characters) revealed high homoplasy and an almost completely unresolved tree in which only the subgenus *Propagulifera* was supported. The morphological characters were subsequently mapped onto the *rbc*L tree in order to identify diagnostic or phylogenetically informative characters. Bleaching response and the presence of secondary segments were found to be basal synapomorphies for the Sphacelariales; presence of propagules with a lenticular central apical cell defines the *Propagulifera*; and strict acroblastic branching mode and axillary zoidangia define the Stypocaulaceae. The remaining characters have been gained or lost multiple times. This study highlights the problem of extreme morphological convergence and/or plasticity. Four options for a new circumscription including nomenclatural changes are discussed. It is concluded that none of the options will lead to greater clarity either with respect to identification or classification.

INTRODUCTION

The order Sphacelariales (Phaeophyceae) was erected by Migula (1909) and is characterised by blackening of the cell walls when treated with bleaching liquid and growth by conspicuous apical cells (Prud'homme van Reine 1993). Transverse divisions of

---

[1] *Also published as:*
S.G.A. Draisma, J.L. Olsen, W.T. Stam & W.F. Prud'homme van Reine 2002. Phylogenetic relationships within the Sphacelariales (Phaeophyceae): *rbc*L, RUBISCO-spacer and morphology. *European Journal of Phycology* 37: in press.

the subapical cells (or primary segments) produce secondary segments which may undergo longitudinal and secondary transverse divisions. In this manner the distinctive parenchymatous construction is produced (Fig. 3.1). Other general features of the order include an isomorphic diplohaplontic life history and asexual reproduction from distinctively shaped vegetative propagules in some genera. Oltmanns (1922) divided the order into three families based on modes of growth and distinctive branching patterns (Fig. 3.1). These were the Cladostephaceae, the Sphacelariaceae and the Stypocaulaceae. The family Choristocarpaceae Kjellman 1891 was also placed in the Sphacelariales by Fritsch (1945) based on growth from prominent apical cells. However, members of the Choristocarpaceae do not have the characteristic ordinal character of transverse division of subapical cells, nor blackening of their cell walls with bleach treatment.

In a recent molecular phylogenetic study of the Phaeophyceae, Draisma *et al.* (2001/Chapter 2) have shown that the Sphacelariales is paraphyletic. In a reanalysis of the entire Phaeophyceae (including nine species and genera and all families of the Sphacelariales), using both chloroplast and nuclear encoded DNA sequences, they were able to identify three clades. These included the Sphacelariales *s.s.* (i.e., including the families Cladostephaceae, Sphacelariaceae and Stypocaulaceae), a clade containing the genera *Onslowia* and *Verosphacela* and a clade containing *Choristocarpus* by itself at the base of the rooted phaeophycean tree. Therefore, it is clear that the Choristocarpaceae do not belong to the Sphacelariales. Draisma and Prud'homme van Reine (2001/Chapter 7) have separated *Onslowia* and *Verosphacela* from the Choristocarpaceae and placed them in the newly created family Onslowiaceae. Both the Choristocarpaceae and the Onslowiaceae are considered *incertae sedis*.

The Sphacelariales *s.l.* (Table 3.1) have been comprehensively reviewed by Prud'homme van Reine (1993). Newly described species since Prud'homme van Reine's review (1993) include *Sphacelaria nipponica* Kitayama (1994), *Sphacelaria tsengii* Draisma *et al.* (1998/Chapter 6) and *Sphacelaria recurva* Keum *et al.* (2001); all belonging to the subgenus *Propagulifera*. Prud'homme van Reine (1993) did not include *Halopteris congesta* (Reinke) Sauvageau, but this species would have been included in the genus *Stypocaulon*. The type species of the genus *Phloiocaulon* (i.e., *Phloiocaulon squamulosum* [Suhr] Geyler), is now called *Phloiocaulon suhrii* (J. Agardh) P. Silva (Silva *et al.* 1996). Prud'homme van Reine was not aware of the description of *Sphacelaria kovalamensis* V. Krishnamurthy *et* Baluswami in Krishnamurthy (1992), which had been previous described in Krishnamurthy and Baluswami (1988) without a Latin diagnosis and therefore initially invalid. Its membership in the Sphacelariales is not clear.

The aim of the present paper is to expand the phylogenetic analysis within the Sphacelariales *s.s.* by including representatives of all families and as many species as possible. We take both a molecular and a morphological approach. First we assess phylogenetic relationships based on sequences of the chloroplast-encoded large subunit of the RUBISCO-gene (*rbc*L) and its adjacent RUBISCO-spacer; next we assess phylogenetic relationships based on unordered morphological characters; and finally we compare the two analyses. Our objectives were to determine the best estimate of phylogeny, to establish which morphological features are useful for identification *vs.*

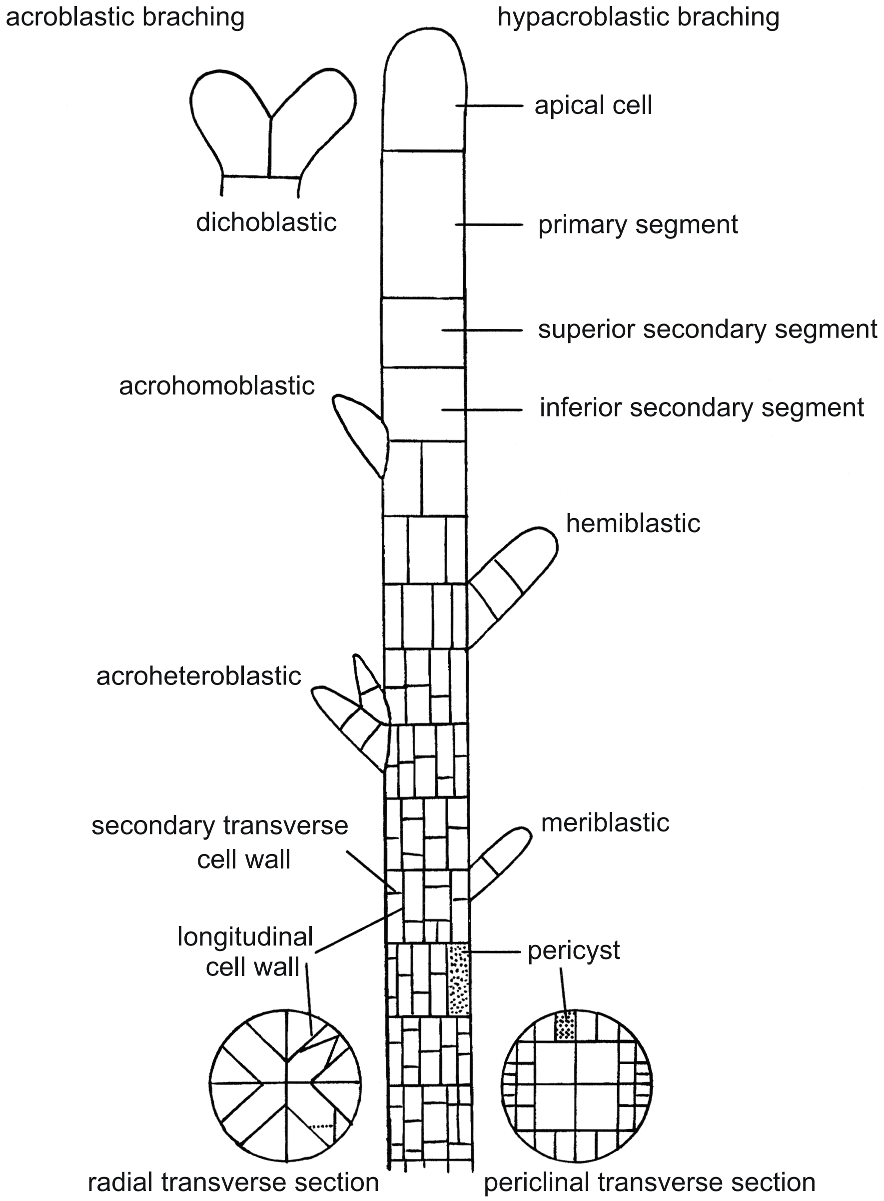


Fig. 3.1. Diagrammatic representation of anatomical characters used in taxonomic descriptions of the Sphacelariales.

those that are useful for phylogenetic reconstruction, and to assess nomenclatural consequences under different circumscriptions.

Table 3.1. Overview of the Sphacelariales *sensu lato* and notes on their distribution (Prud'homme van Reine 1993; Kitayama 1994; Draisma *et al.* 1998/Ch. 6; Keum *et al.*, 2001; Draisma and Prud'homme van Reine 2001/Ch. 7). Genera and subgenera labelled with an asterisk have one or more representatives in the present study (see Table 3.2). N., S., W. and E. indicate north(ern), south(ern), west(ern) and east(ern) respectively.

Family, Genus, Subgenus	Number of species	Distribution
Cladostephaceae		
*Cladostephus**	1	Bipolar in temperate waters
Sphacelariaceae		
*Sphacelaria**	42–47	Cosmopolitan
*Sphacelaria**	4	Temperate to cold N. Atlantic
*Pseudochaetopteris** (2 sections*)*	4	Temperate to cold N. Atlantic and circumpolar
*Battersia**	1	Temperate N. Atlantic
*Propagulifera** (3 sections*)*	17–22	Worldwide temperate to tropical. Some species are endemic in small areas. *S. cirrosa* shows a disjunct distribution in temperate N. Atlantic and S. Australia
Bracteata	3	S.E. Australia and New Zealand, except *S. sympodiocarpa* which is endemic in Gulf of Biscay.
Reinkea	6	Australia and New Zealand. *S. bornetii* also in S. America.
Incertae sedis	7	2 freshwater species (China and U.S.A.), other species endemics of Kerguelen, New Zealand, Pacific Mexico, and Indonesia
*Sphacella**	1	W. Mediterranean, Canary Islands and S. Australia
Stypocaulaceae		
*Alethocladus**	1	Antarctic and sub-Antarctic regions
*Halopteris**	4	S. Australia, New Zealand, and S. America. Exception is *H. filicina* which occurs in warm temperate N.E. Atlantic, Korea, and Japan.
Phloiocaulon	3	S. Australia and S. Africa.
Ptilopogon	1	S.E. Australia and New Zealand
*Stypocaulon**	5	3 in S. hemisphere and 2 in N. hemisphere temperate waters.
Choristocarpaceae		
*Choristocarpus**	1	Mediterranean and Canary Islands
Discosporangium	1	Warm temperate N. Atlantic and S. Australia
Onslowiaceae		
*Onslowia**	2	Warm temperate Atlantic U.S.A. and Bahamas
*Verosphacela**	1	Endemic in Florida

MATERIALS AND METHODS

Taxon sampling

Taxa used in the phylogenetic analyses are listed in Table 3.2, including taxa with previously published sequences. Table 3.3 lists additional specimens for which (partial) *rbc*L and RUBISCO-spacer sequences were obtained that were (nearly) (see Results) identical to sequences of specimens listed in Table 3.2 and were therefore not included in the analyses.

DNA extraction, DNA amplification, cloning and plasmid isolation, DNA sequencing

All steps from DNA extraction to DNA sequencing are described in Draisma *et al.* (2001/Ch. 2). Field material was used for *Sphacelaria plumigera* and *Sphacelaria mirabilis*. Furthermore, DNA from the encrusting species *S. mirabilis* was extracted in only 1 ml CTAB-PVPP extraction buffer, because only a tiny piece of crust (± 6 mm^2) was available. The same protocol was followed as described by Draisma *et al.* (2001/Ch. 2) up to and including the three CIA cleaning steps. From this step onwards, DNA was directly precipitated. DNA extracts of *S. mirabilis*, *Sphacelaria caespitula* 4, *Sphacelaria nana* 2 and 3, *Halopteris filicina* 3, and *Stypocaulon scoparium* 3, were PCR amplified with the BirF forward primer (= BLS*rbc*L1277F in Siemer *et al.* 1998) (5'–CAG CTA ACC GTG TTG C–3') instead of the RbcL68F or RbcL188F forward primers (see Draisma *et al.*, 2001/Ch. 2) which were used for the other taxa.

Alignment and phylogenetic analysis

Sequences were aligned manually and edited using Sequence Navigator version 1.0 (PE Applied Biosystems, Foster City, CA, USA).

Phylogenetic analyses were done with PAUP* version 4.0b4a (Swofford 1998). Maximum parsimony (MP) analysis was done as heuristic searches, using the tree bisection-reconnection (TBR) branch-swapping algorithm, with random sequence addition (100 replicates) and treating gaps as missing data. For the Maximum Likelihood (ML) analyses the nucleotide substitution model was first determined using MODELTEST version 2.1 (Posada and Crandall 1998). The general-time-reversible model of DNA substitution (Rodríguez *et al.* 1990) was used for ML, including a gamma distribution (G) and a proportion of invariable sites (I).

Draisma *et al.* (2001 / Ch. 2) concluded that mutational saturation at *rbc*L third codon sites did not negatively affect the phylogenetic signal when comparing between phaeophycean orders. Therefore, mutational saturation at third codon sites is not considered a problem at a lower taxonomic level as is the matter in this study.

Decay analysis of MP trees was performed with AutoDecay version 4.0 (Erikson 1998). Bootstrapping (Felsenstein 1985) and parsimony jackknifing (Farris *et al.* 1996) was performed in PAUP* using respectively five and ten random sequence additions and 10,000 replicates. Character deletions per jackknife replicate were set at 37%. For ML bootstrapping only 100 replicates were possible.

Congruence of *rbc*L and RUBISCO-spacer sequences was evaluated using the partition homogeneity test (Farris *et al.* 1995, Cunningham 1997). This approach utilises a resampling method in order to estimate the degree to which two data sets (or subsets of data sets) are in agreement (i.e., congruent or homogeneous) or disagreement (i.e., incongruent or heterogeneous). The difference between the numbers of steps required by individual and combined analyses is the incongruence length difference (ILD_{MF}) (Mickevitch and Farris 1981). The distribution of the ILD statistic can be estimated by permutation. P-values >0.05 indicate congruence thus allowing combination of the data sets in question. Conversely, P-values <0.05 indicate a significant difference between the data sets and suggest that they should not be analysed together. Constant characters were deleted prior to the analysis, a random sequence addition (10 replicates) was applied and 10,000 replicates were performed.

Table 3.2. Species used in the molecular and morphological phylogenetic analyses.

Species	Collection site	Collector/identifier, collection year	EMBL accession number and length	
			*rbc*L	RUBISCO-spacer
Ingroup taxa (Sphacelariales *s.s.*)				
Cladostephus spongiosus (Hudson) C. Agardh	Oosterschelde, Zeeland, The Netherlands	W.F. Prud'homme van Reine, 1966	AJ287863[a] (1375 nt)	AJ287932 (315 nt)
Sphacelaria radicans (Dillwyn) C. Agardh	Øystese, Norway	W.F. Prud'homme van Reine, 1967	AJ287874 (782 nt)	AJ287922 (201 nt)
Sphacelaria caespitula Lyngbye	Espegrend, Norway	W.F. Prud'homme van Reine, 1967	AJ287870 (1255 nt)	AJ287919 (173 nt)
Sphacelaria nana Naegeli *ex* Kützing	Øystese, Norway	W.F. Prud'homme van Reine, 1967	AJ287875 (1255 nt)	AJ287923 (367 nt)
Sphacelaria plumigera Holmes	St. Andrews, Scotland	S.G.A. Draisma, 1997	AJ287878 (1375 nt)	AJ287926 (842 nt)
Sphacelaria plumosa Lyngbye	Espegrend, Norway	W.F. Prud'homme van Reine, 1967	AJ287879 (1255 nt)	AJ287927 (345 nt)
Sphacelaria racemosa Greville	St. Andrews, Scotland	W.F. Prud'homme van Reine, 1971	AJ287880 (1374 nt)	AJ287928 (423 nt)
Sphacelaria arctica Harvey	Askö, Sweden	W.F. Prud'homme van Reine, 1967	AJ287881 (1375 nt)	AJ287929 (331 nt)
Sphacelaria mirabilis (Reinke *ex* Batters) Prud'homme van Reine	St. Andrews, Scotland	S.G.A. Draisma, 1997	AJ287882 (190 nt)	AJ287930 (272 nt)
Sphacelaria cirrosa (Roth) C. Agardh	Fife Ness, Scotland	S.G.A. Draisma, 1997	AJ287865[a] (1372 nt)	AJ287946 (255 nt)
Sphacelaria rigidula Kützing	Galway, Ireland	W.F. Prud'homme van Reine, 1966	AJ287883 (1366 nt)	AJ287941 (252 nt)
Sphacelaria divaricata Montagne	Tsuyazaki, Kyushu, Japan	S. Kawaguchi, 1989	AJ287889 (1374 nt)	AJ287949 (238 nt)
Sphacelaria yamadae Segawa	Ogi, Sado, Japan	T. Kitayama, 1990	AJ287890 (1375 nt)	AJ287950 (255 nt)
Sphacelaria tribuloides Meneghini	Sesoko, Okinawa, Japan	W.F. Prud'homme van Reine, 1993	AJ287891 (1375 nt)	AJ287947 (257 nt)
Sphacelaria californica Sauvageau *ex* Setchell *et* Gardner	Oiso, Awajishima, Japan	W.F. Prud'homme van Reine, 1993	AJ287893 (1255 nt)	AJ287951 (276 nt)
Sphacella subtilissima Reinke	Fuerteventura, Canary I.	D.G. Müller, 1991	AJ287869 (1250 nt)	AJ287931 (210 nt)
Alethocladus corymbosus (Dickie) Sauvageau	Astrolabe I., Antarctica	D. Moe, 1985	AJ287860[a] (1255 nt)	AJ287933 (135 nt)
Halopteris filicina (Grateloup) Kütz. (1)	Dock-do I., South Korea	Y.-S. Keum, 1993	AJ287894 (1375 nt)	AJ287934 (196 nt)
(2)	St. Lunaire, BRZ, France	W.F. Prud'homme van Reine, 1968	AJ287895 (1233 nt)	AJ287936 (211 nt)
Stypocaulon scoparium (L.) Kützing	Porto Santo, Madeira Arch.	W.F. Prud'homme van Reine, 1994	AJ287866[a] (1374 nt)	AJ287939 (195 nt)
Stypocaulon durum (Rüprecht) Okamura[b]	Bonne Bay, Newfoundland, Canada	E.C. Henry, 1987	AJ287897 (1255 nt)	AJ289737 (140 nt)

(continued)

(Table 3.2 continued)

Outgroup taxa				
Choristocarpus tenellus (Kützing) Zanardini	La Nave, Ischia, W. Italy	E.C. Henry, 1987	AJ287861 [a] (1374 nt)	AJ287915 (40 nt)
Dictyota cervicornis Kützing	Zanzibar, Tanzania	O. de Clerck, 1997	AJ287851 [a] (1371 nt)	AJ287905 (161 nt)
Dictyota dichotoma (Hudson) J.V. Lamouroux	Oosterschelde, Zeeland, The Netherlands	O. de Clerck, 1996	AJ287852 [a] (1368 nt)	AJ287906 (174 nt)
Onslowia endophytica Searles *in* Searles *et* Leister	Fort Pierce, FL, USA	E.C. Henry, 1981	AJ287864 [a] (1373 nt)	AJ287916 (159 nt)
Syringoderma phinneyi Henry	Henderson Pt, BC, Canada	A. Whittick, 1982	AJ287868 [a] (1255 nt)	AJ287914 (133 nt)
Verosphacela ebrachia Henry	Vero Beach, FL, USA	E.C. Henry, 1987	AJ287867 [a] (1255 nt)	AJ287917 (144 nt)

[a] Previously published in Draisma *et al.* (2001/Ch. 2).

[b] The same individual as isolate CAN1 (referred to as *Stypocaulon scoparium*) in Novaczek *et al.* (1987).

Table 3.3. Specimens with sequences that were (nearly) identical to those in Table 3.2 and were therefore not included in the phylogenetic analyses.

			EMBL accession number for	
Species	Collection site	Collector/identifier, collection year	*rbc*L	RUBISCO-spacer
Sphacelaria caespitula 2	St. Andrews, Scotland	S.G.A. Draisma, 1997	AJ287871 (1374 nt)	AJ287919 (173 nt)
Sphacelaria caespitula 3	Cherbourg, Normandy, France	W.F. Prud'homme van Reine, 1968	AJ287872 (1255 nt)	AJ287920 (173 nt)
Sphacelaria caespitula 4	Espegrend, Norway	W.F. Prud'homme van Reine, 1967	AJ287873 (190 nt)	AJ287921 (173 nt)
Sphacelaria nana 2	Espegrend, Norway	W.F. Prud'homme van Reine, 1967	AJ287876 (190 nt)	AJ287924 (371 nt)
Sphacelaria nana 3	Tjomme, Norway	W.F. Prud'homme van Reine, 1967	AJ287877 (173 nt)	AJ287925 (365 nt)
Halopteris filicina 3	Roscoff, Brittany, France	W.F. Prud'homme van Reine, 1968	AJ287896 (190 nt)	AJ287935 (211 nt)
Stypocaulon scoparium 2	Canary Islands	I. Germann/E.C. Henry, 1987	AJ287898 (1254 nt)	AJ287938 (190 nt)
Stypocaulon scoparium 3	Rade de Brest, Brittany, France	W. F. Prud'homme van Reine, 1967	AJ287899 (190 nt)	AJ287940 (195 nt)

Representatives of the Syringodermatales, Dictyotales, Choristocarpaceae and Onslowiaceae were selected as outgroup taxa (Table 3.2). Draisma *et al.* (2001/Ch. 2) have shown that these taxa branch-off prior to the Sphacelariales *s.s.* in a rooted phylogeny of the Phaeophyceae.

Morphological characters

Morphological characters and their character states are shown in Table 3.4 and the data matrix in Table 3.5. Morphological character states were obtained from the literature. MP analysis based on the matrix of Table 3.5 was done using PAUP* under the polymorphism option and simple taxon addition (reference taxon *Choristocarpus tenellus*). Bootstrap and parsimony jackknife (JK) analyses were performed with the following settings: 10,000 replicates, 5 random sequence additions per replicate, maxtrees =100, and for JK nine characters [= 39%] were deleted in each replicate.

Each morphological character was mapped onto the *rbc*L tree using the MacClade version 3.08a computer program (Maddison and Maddison 1992).

RESULTS

Alignment properties

In brown algae the *rbc*L is 1467 base pairs in length. The length of the *rbc*L alignment in the present study is 1375 nucleotides (nt), excluding the RbcL68F primer. No gaps were found, therefore, the alignment was unambiguous. The first 120 nt were excluded from the present phylogenetic analyses because these nt were undetermined for ten taxa (those that were amplified with the RbcL188F primer). EMBL accession numbers of *rbc*L sequences are listed in Table 3.2 and 3.3. Only 782 nt of the *rbc*L sequence were determined for *Sphacelaria radicans* and 190 nt for *Sphacelaria mirabilis*. The final alignment used 1255 nt (1375 minus 120). At the nucleotide level 484 out of 1255 nt were variable and 313 phylogenetically informative among 26 taxa (*S. mirabilis* not included). At the amino acid level 84 sites were variable and 44 phylogenetic informative. Seventy-one sites were informative at the first or second codon positions and 242 at third codon positions. Within the Sphacelariales *s.s.* (20 taxa, *S. mirabilis* not included) there are 331 variable sites of which 187 are informative, 41 at first and second codon positions and 146 at third codon positions.

Average base composition of *rbc*L was: A 0.30; T 0.33; C 0.15; G 0.22. The average transition/transversion (ti/tv) ratio for the 1255 nt *rbc*L alignment was 1.19, for the ingroup taxa alone 1.48, and for the outgroup taxa alone 0.80. The ti/tv ratio is expected to decrease with increasing sequence distance because transversions erase the record of the more frequent transitions. Values between 1 and 2 are typical and do not indicate saturation. Values <1 do indicate some saturation (Holmquist 1983, Bakker *et al.* 1995).

The RUBISCO-spacer sequences varied in length from 40 nt in the outgroup taxon *Choristocarpus tenellus* to 842 nt in *Sphacelaria plumigera*. Average base composition of the spacers was: A 0.50; T 0.33; C 0.08; G 0.10. The spacer sequences of the outgroup taxa were unalignable with those of the Sphacelariales *s.s.* Complete RUBISCO-spacer sequences were alignable for the species of the *Sphacelaria* subgenus *Propagulifera* only (not shown). Spacer sequences of other Sphacelariales species differed considerably from each other at the 5'-end. The 3'-end of the spacer (94–105 nt) is best conserved and could be aligned for the 21 taxa of the Sphacelariales *s.s.* resulting in an alignment of 112 nt positions of which 36 were gapped, 78 variable and 51 phylogenetic informative. The alignment is submitted to EMBL under accession number ALIGN 000127. RUBISCO-spacer sequence lengths and EMBL accession numbers are listed for each specimen in Table 3.2 and 3.3. All four *Sphacelaria caespitula* specimens showed identical RUBISCO-spacer sequences, as well as the two French *Halopteris filicina* specimens (2 and 3). Alignment of the spacers of the French and the Korean (1) *H. filicina* specimens (not shown) required 12 gaps (7 • one, 4 • two, and 1 • eight position(s) in length) and showed 33 substitutions. The RUBISCO-spacer of the *Sphacelaria nana* specimens only differed by indels (of one and of five nt positions). Spacer sequences of the *Stypocaulon scoparium* specimens differed by one substitution and one indel of six nt positions.

Phylogenetic analysis – *rbc*L and RUBISCO-spacer

Maximum parsimony (MP) analysis of *rbc*L was performed for all three codon positions together and resulted in 21 most parsimonious trees (MPTs). One of these trees is shown in Figure 3.2. A 50% majority-rule consensus tree of the 21 MPTs has the same topology as the tree shown in Figure 3.2, except that the branch joining *Sphacelaria arctica* and *Sphacelaria racemosa* collapses. The Stypocaulaceae is monophyletic and the monotypic Cladostephaceae is nested within the Sphacelariaceae. The genus *Sphacella* is positioned within the genus *Sphacelaria* and is sister to the subgenus *Propagulifera*. The *Sphacelaria* subgenus *Propagulifera* is monophyletic. The subgenus *Pseudochaetopteris* is monophyletic here because *Sphacelaria mirabilis* (subgenus *Battersia*) was not included in this analysis. An analysis with the 190 nt of *Sphacelaria mirabilis* grouped it with the species of the subgenus *Pseudochaetopteris*. The subgenus *Sphacelaria* is paraphyletic. An analysis excluding the nucleotide positions that are undetermined for *Sphacelaria radicans* gave the same tree topology. The maximum likelihood (ML) tree (not shown) differed from the MP tree only in the position of *Sphacelaria caespitula*, which formed a single taxon clade basal to all the Sphacelariales *s.s*. However, this difference did not occur in the MP and ML bootstrap trees. Additional differences between the ML and MP tree were found within the Stypocaulaceae and *Propagulifera*, but again these differences were not bootstrap supported.

An analysis of the partial RUBISCO-spacer sequences (including *S. mirabilis* and using the Stypocaulaceae + *S. radicans* as outgroups) resulted in 834 MPTs (173 steps, CI = 0.69, RI = 0.71, RC = 0.50) (trees not shown). The 50% consensus tree was unresolved except for the *Propagulifera* clade which maintained 100% bootstrap support. The short length of the alignment provided very little signal.

The partition homogeneity test (PHT) comparing *rbc*L and RUBISCO-spacer (excluding the outgroup taxa and *Sphacelaria mirabilis*) yielded a P-value of 0.196, indicating that the two subsets are not in conflict and can be combined.

MP analysis of *rbc*L and RUBISCO-spacer together including the outgroup taxa (having 112 uncertain nt in the RUBISCO-spacer part of the alignment) resulted in 1 MPT (1497 steps, CI = 0.52, RI = 0.55, RC = 0.29) (tree not shown) with the same

Table 3.4. Morphological characters used in the morphological phylogenetic analysis. Indented characters depend upon character states of the above characters that are outlined to the left.

Nr	Character	Character states
1	Bleaching response	0: no, 1: yes
2	Transverse division of primary segments into secondary segments	0: no, 1: yes
3	Secondary length growth of secondary segments	0: no, 1: yes
4	Secondary transverse cell walls in secondary segments	0: frequent, 1: absent or scarce
5	Longitudinal cell walls	0: no, 1: yes
6	Longitudinal cell walls in transverse section	0: radial pattern, 1: periclinal pattern
7	Growth	0: leptocaulous, 1: auxocaulous
8	Branching mode	0: hypacroblastic, 1: acroblastic, 2: dichoblastic
9	Branching pattern	0: irregular, 1: regular
10	Acroblastic branching	0: acrohomoblastic, 1: acroheteroblastic
11	Hypacroblastic branching	0: hemiblastic, 1: meriblastic
12	Pericysts	0: absent (rare), 1: present
13	Differentiation in medulla and cortex	0: no, 1: yes
14	Holdfast	0: disc, 1: filamentous, rhizoidal
15	Rhizoids in upper part of plant	0: no, 1: divaricate, 2: appressed to the filament
16	Propagules	0: no, 1: with large apical cell, 2: without large or central apical cell, 3: with lenticular central apical cell
17	Propagules with central apical cell	0: with apical hair, 1: with long arms, 2: with short arms/horns
18	Hairs	0: absent, 1: solitary, 2: in bundles
19	Zoidangia	0: sessile or in thallus, 1: on one-celled stalk, 2: on multicellular stalk
20	Stalked zoidangia	0: on unbranched stalks, 1: on branched stalks or in cymose stands
21	Zoidangia in axils	0: no, 1: yes
22	Plurilocular zoidangia	0: with equal sized loculi, 1: with different sized loculi, 2: presence of oogonia
23	Life cycle	0: isomorphic, 1: heteromorphic

topology as the tree in Figure 3.2. Bootstrap support values were not higher. When the analysis was repeated, excluding the original outgroup and substituting the Stypocaulaceae and *Sphacelaria radicans* as new outgroups, three MPTs (859 steps, CI = 0.62, RI = 0.64, RC = 0.40) were recovered (trees not shown). These trees differed slightly from the tree in Figure 3.2 with respect to the position of *Cladostephus spongiosus*. In the clade with *S. nana* and the subgenus *Pseudochaetopteris*, *Cladostephus spongiosus* became the sister taxon to the subgenus *Pseudochaetopteris* species, but this position is not well supported. The affiliation of *Sphacella* with the subgenus *Propagulifera* as shown in Figure 3.2 was confirmed by high support values on the clade leading to these taxa (bootstrap = 93%, jackknife = 99%, decay value = 8 steps). Also the support for the clade leading to (*Sphacella*, *Propagulifera*) + (*Pseudochaetopteris*, *S. nana*, *Cladostephus*) receives significantly more support (respectively 86%,

Table 3.5. Data matrix for the morphological analysis. Character numbers refer to the characters listed in Table 3.4. Genera with only a single representative (Table 3.2) are referred to by the genus name only. A dash (-) indicates that the character is not relevant to the taxon under consideration. A question mark (?) indicates that the character state is unknown or doubtful. More than one number for a character means that more than one character state has been reported in the species. Sources of character states are Sauvageau (1900–1914), van den Hoek and Flinterman (1968), Searles and Leister (1980), Prud'homme van Reine (1982, 1993, personal observation), Henry and Müller (1983), Henry (1987a, b), Womersley (1987), Kitayama (1994), Gibson (1994), Keum *et al.* (1995, 1999), de Clerck (1998), Kawai and Prud'homme van Reine (1998).

	Character number																						
Taxon	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23
Sphacelaria caespitula	1	1	0	0	1	0	0	0	0	–	1	1	0	0	0	0	–	0	1,2	1	0	0	0
S. radicans	1	1	0	0	1	0	0	0,1,2	0	1	1	1	0	0	1	0	–	2	0,1,2	1	0	0	0
S. nana	1	1	0	0,1	1	?	0	0	0	–	?	?	0	0	1	0	–	1	1,2	1	0	0	0
S. mirabilis	1	1	–	–	1	–	0	0	–	–	–	–	–	0	–	0	–	–	1,2	1	–	0	0
S. plumigera	1	1	0	0	1	1	0	0	1	–	0	0	1	0	2	0	–	1,2	2	1	0	0	0
S. plumosa	1	1	0	0	1	1	0	0	1	–	0	0	1	0	2	0	–	1,2	1,2	1	0	0	0
S. arctica	1	1	0	0	1	1	0	0	0,1	–	0	0	1	0	2	0	–	1,2	2	1	0	0	0
S. racemosa	1	1	0	0	1	1	0	0	0	–	0	0	1	0	1,2	0	–	1,2	2	1	0	0	0
S. cirrosa	1	1	0	1	1	0	0	0	0	–	0	0	0	0,1	1,2	3	0,1	1	1,2	0,1	0	1	0
S. rigidula	1	1	0	1	1	0	0	0	0	–	0	0	0	0,1	1	3	1	1	1,2	0,1	0	0,1	0
S. divaricata	1	1	0	1	1	0	0	0	0	–	0	0	0	1	1	3	1	1	1,2	0	0	1	0
S. yamadae	1	1	0	1	1	0	0	0	0	–	0	0	0	0,1	1	3	1	1	2	0,1	0	1	0
S. tribuloides	1	1	0	1	1	0	0	0	0	–	0	0	0	1	0	3	2	1	1,2	0,1	0	1	0
S. californica	1	1	0	1	1	0	0	0	0,1	–	0	0	0	0,1	0	3	2	1	2	1	0	?	0
Sphacella	1	1	0	–	0	–	0	0	0	–	–	–	–	1	0	0	–	0	1	–	0	–	0
Cladostephus	1	1	1	0	1	1	1	0,1	0	1	0,1	1	1	0,1	2	0	–	1,2	1,2	1	0	1	0
Alethocladus	1	1	?	0	1	1	0	1	0	0	–	0	1	1	2	0	–	0	1	0	1	?	0
Halopteris	1	1	1	0	1	1	0	1	1	1	–	0	1	0,1	2	0	–	1,2	1,2	0	1	1	0
Stypocaulon scoparium	1	1	1	0	1	1	0	1	1	1	–	1	1	1	2	0	–	2	2	0	1	2	0
S. durum	1	1	1	0	1	1	0	1	0	1	–	1	1	0	2	0	–	2	2	0	1	?	0
Syringoderma	0	0	–	–	0	–	0	1	0	0	–	–	–	1	0	0	–	0	0	–	0	0	1
Onslowia	0	0	–	–	1	–	0	0	0	–	–	–	–	1	0	2	–	1	0,1,2	0	0	0	0
Verosphacela	0	0	–	–	1	–	0	0	0	–	–	–	–	1	0	2	–	1	0	–	0	0	0
Dictyota cervicornis	0	0	–	–	1	–	1	2	–	–	–	1	1	1	0	0	–	2	1	0	0	?	0
D. dichotoma	0	0	–	–	1	–	1	2	–	–	–	1	1	1	0	0	–	2	1	0	0	2	0
Choristocarpus	0	0	–	–	0	–	0	1	0	–	–	–	–	0	0	1	–	0	0	–	0	0	0

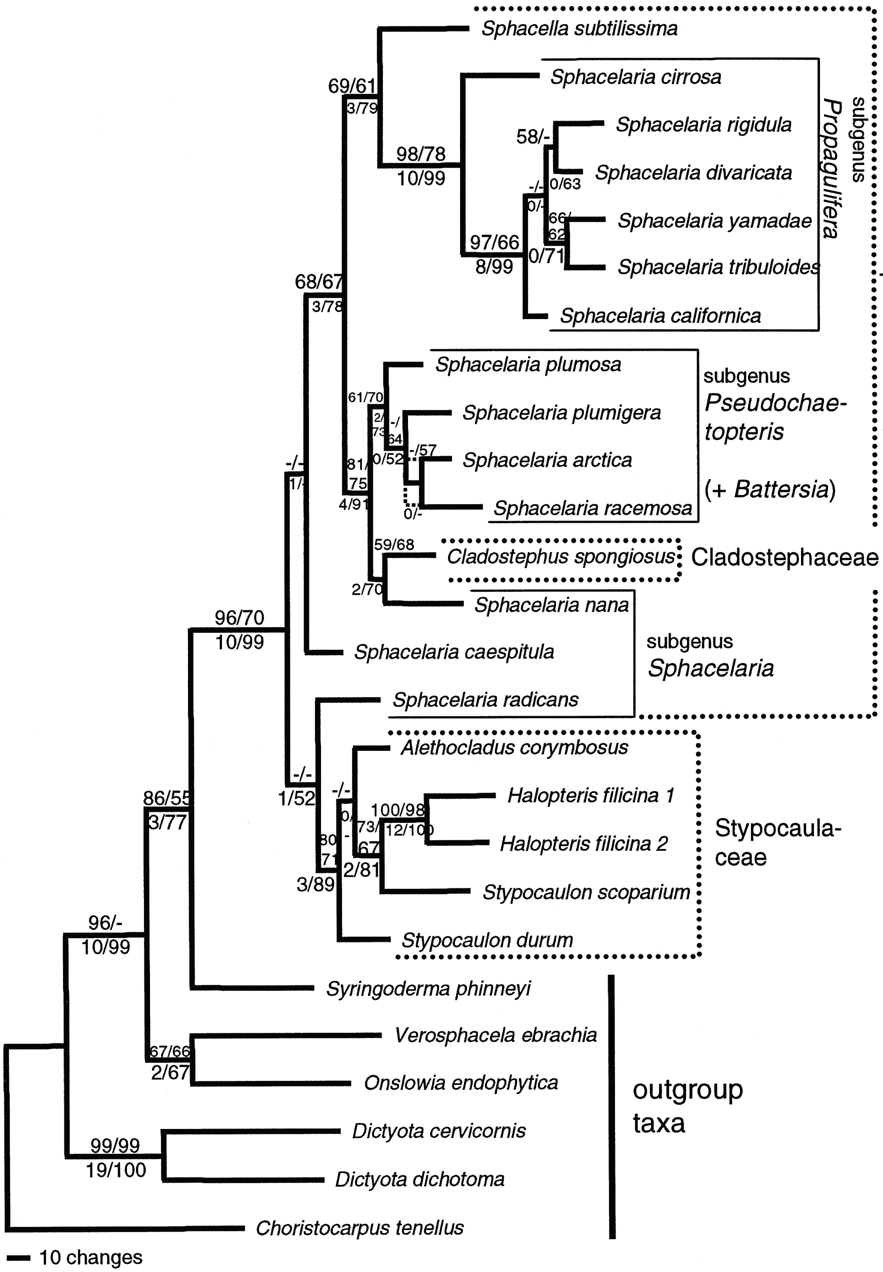


Fig. 3.2. *rbc*L tree for the Sphacelariales. One of 21 most parsimonious trees (length = 1312 steps; consistency index = 0.50; retention index = 0.53; rescaled consistency index = 0.27). The 50% majority-rule consensus tree is identical except where shown with dotted lines. Branch lengths are proportional to the number of nucleotide changes. Bootstrap percentages are given above branches (MP left and ML right); decay index values are given below branches (left) and parsimony jackknife percentages (right); dashes (-) indicate percentages <50% or that the internode did not occur in the ML tree. Decay values of 0 indicate where the strict consensus tree collapses.

93% and 4 steps). A similar analysis with the reduced taxon set (i.e., without the original outgroup) with only the *rbc*L resulted in 9 MPTs (679 steps, CI = 0.61, RI = 0.64, RC = 0.39) (trees not shown) of which the consensus tree had the same topology as in the combined analysis, except that now *Cladostephus* stayed with *S. nana*. Again here very good support values for the (*Sphacella*) + (*Propagulifera*) and the (*Sphacella*, *Propagulifera*) + (*Pseudochaetopteris*, *S. nana*, *Cladostephus*) clades were found. Apparently, adding the RUBISCO-spacer to the data set did not significantly influence the outcome of the phylogenetic analyses.

Phylogenetic analysis – morphology

Maximum parsimony analysis of the ingroup and outgroup taxa using 23 morphological characters (Tables 3.4 and 3.5) resulted in 119,400 MPTs of 92 steps. The 50% majority-rule consensus tree is shown in Figure 3.3A and the bootstrap and parsimony jackknife (JK) tree in Figure 3.3B. Nineteen character state changes (involving thirteen characters) could be traced unambiguously on the tree in Figure 3.3A of which eleven (involving nine characters) within the ingroup. When the outgroup was excluded from the MP analysis 19,493 MPTs of 73 steps were recovered. The 50% majority-rule consensus tree (Figure 3.4A) and the bootstrap and parsimony JK tree (Figure 3.4B) reveal an unsupported tree except for the *Propagulifera*. Ten characters state changes (involving eight characters: 5, 7, 10, 12, 14, 15, 18, 22) could be traced unambiguously on the consensus tree in Figure 3.4A. However, this was not the same subset of characters that changed unambiguously within the ingroup in Figure 3.3A (characters: 7, 9, 11, 12, 15, 18, 21, 22).

In order to further identify and analyse homoplasy, each character was mapped onto the topology of the *rbc*L-based tree (Fig. 3.5). The tracing displays the ancestral character states that imply the smallest number of character state changes. Characters 1, 2 and 23 are constant within the ingroup (synapomorphic for the order Sphacelariales *s.s.*) and thus not phylogenetically informative. The characters 5, 7 and 10 are not informative within the ingroup either (autapomorphic in one species). As can be seen in Figure 3.5, a few characters are useful within sub-clades. In general, however, all of the remaining characters show homoplasy, i.e., character states that have been gained or lost repeatedly.

DISCUSSION

RUBISCO-spacer

RUBISCO-spacer sequences are, by themselves, not informative enough for a phylogenetic analysis of the Sphacelariales *s.s.* This is mainly related to extreme length variation which affected the alignment and the subsequent number of useable positions. Lengths ranged from 135–842 nt (Table 3.2). The general cause of length variation in spacer sequences is related to duplications, indels or slipped-strand mispairing which have little effect on these presumptively nonfunctional regions. In the present case, we were not able to detect major duplications, even in the very long spacers.

The RUBISCO-spacer length of *Choristocarpus tenellus* (40 nt) is comparable to that of nonphaeophycean heterokont algae studied to date. The RUBISCO-spacer is

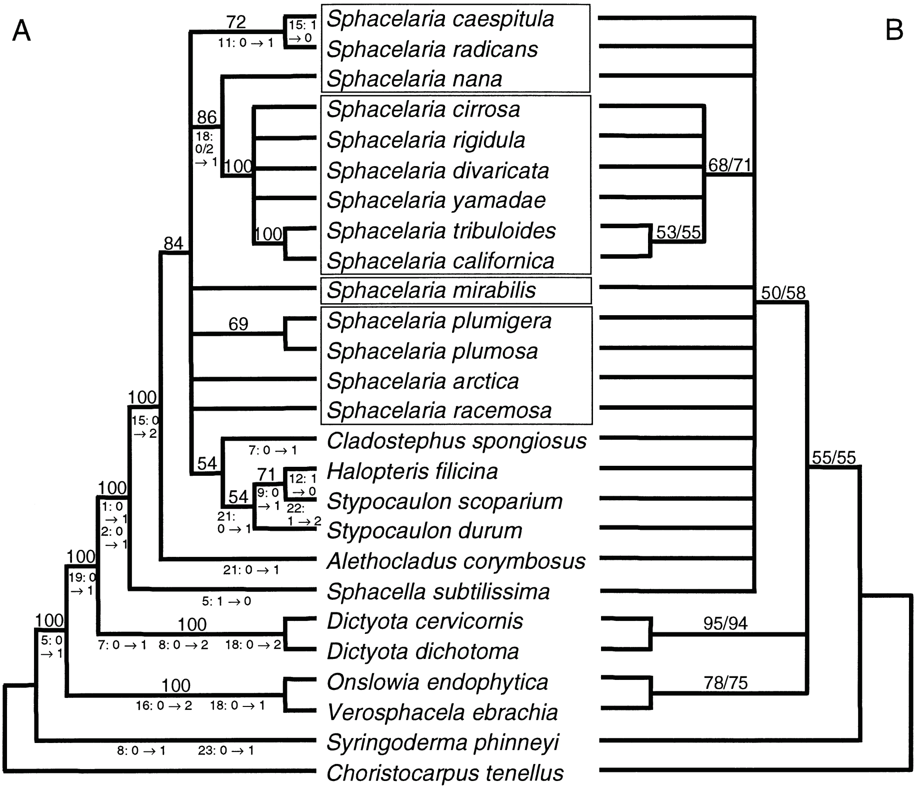


Fig. 3.3. Morphological tree for the Sphacelariales *s.l.* Parsimony analysis based on 23 unordered morphological characters (Table 3.4 and 3.5) with the six most basal taxa used as paraphyletic outgroup. Boxes indicate conventionally recognised *Sphacelaria* subgenera. A. 50% majority-rule consensus tree of 119,400 most parsimonious trees (length = 92; CI = 0.77; RI = 0.81; RC = 0.63) with group frequencies above branches and characters that change unambiguously below branches (character numbering as in Table 3.4). B. 50% bootstrap and parsimony jackknife tree with support values; bootstrap left, jackknife right.

54 nt long in *Heterosigma akashiwo* (Hada) Hada *ex* Sournia (as *H. carterae*) (Raphidophyceae) (Chesnick *et al.* 1996), 38–43 nt in 5 species of Bacillariophyceae (Daugbjerg and Guillou 2001), 38–40 nt in 2 species (3 strains) of *Bolidomonas* (Bolidophyceae) (Daugbjerg and Guillou 2001), and 38–43 nt in 2 species (14 and 3 strains) of Pelagophyceae (Bailey and Andersen 1999). This observation is in accordance with the basal position of *C. tenellus* in the *rbc*L-based Phaeophyceae tree presented by Draisma *et al.* (2001/Ch. 2). In other brown algae the RUBISCO-spacer length ranges from 138–204 nt in the Ectocarpales *s.l.* (Stache-Crain *et al.* 1997, Siemer *et al.* 1998, Kogame and Masuda 2001, Peters and Ramírez 2001), 122–200 nt in the Fucales (Phillips 1998, Lee *et al.* 1999), 144–231 nt in the Desmarestiales (Sasaki *et al.* 2001, Draisma unpublished/Appendix I), 319–438 nt in the Scytothamnales (Peters

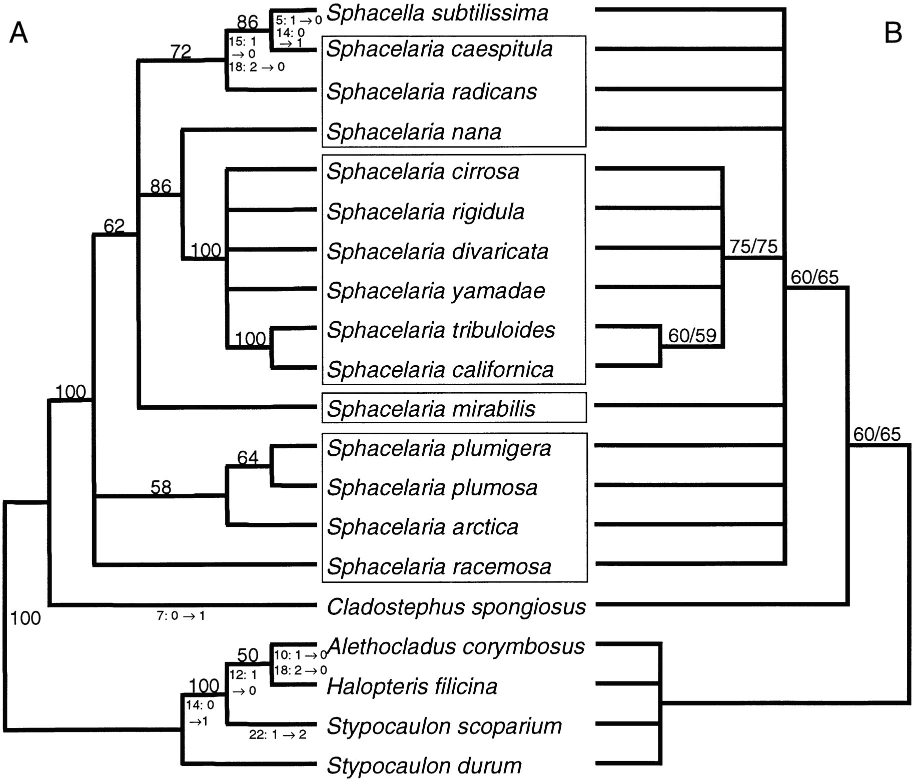
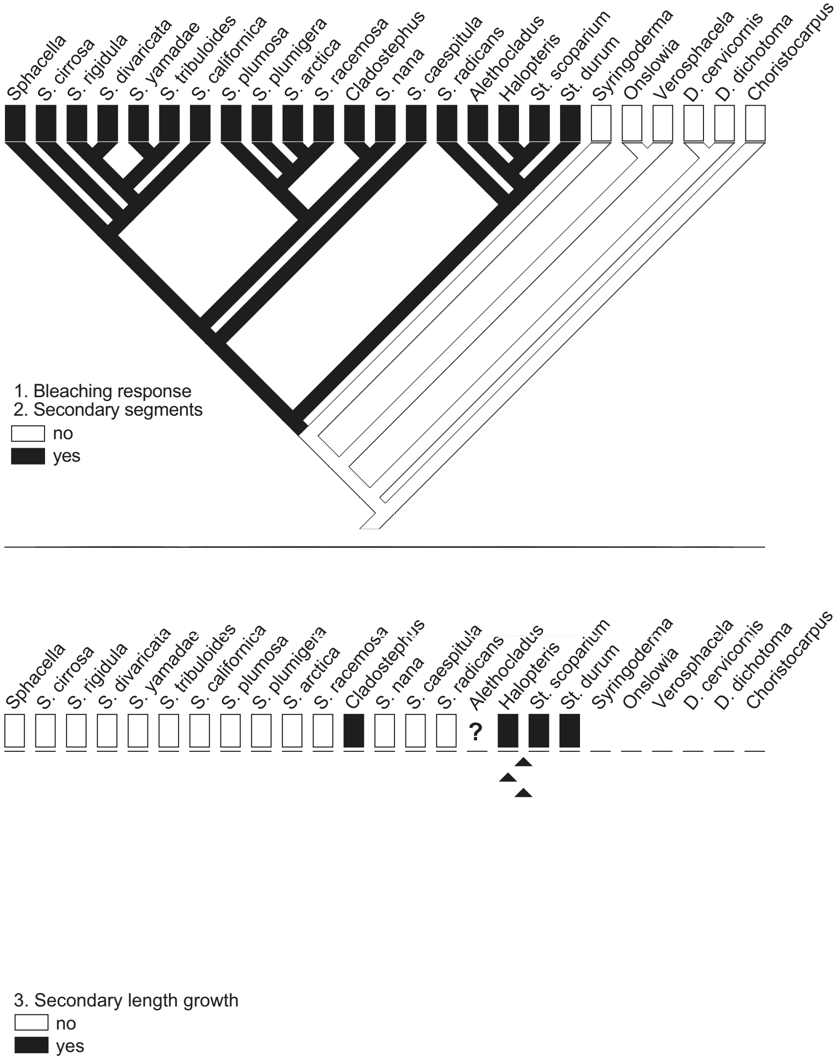


Fig. 3.4. Morphological trees for the Sphacelariales *s.s.* Parsimony analysis based on 23 unordered morphological characters (Table 3.4 and 3.5) with the four basal taxa used as monophyletic outgroup. Boxes indicate conventionally recognised *Sphacelaria* subgenera. A. 50% majority-rule consensus tree of 19,493 most parsimonious trees (length = 73; CI = 0.85; RI = 0.84; RC = 0.71) with group frequencies above branches and characters that change unambiguously below branches (character numbering as in Table 3.4). B. 50% bootstrap and parsimony jackknife tree with support values; bootstrap left, jackknife right.

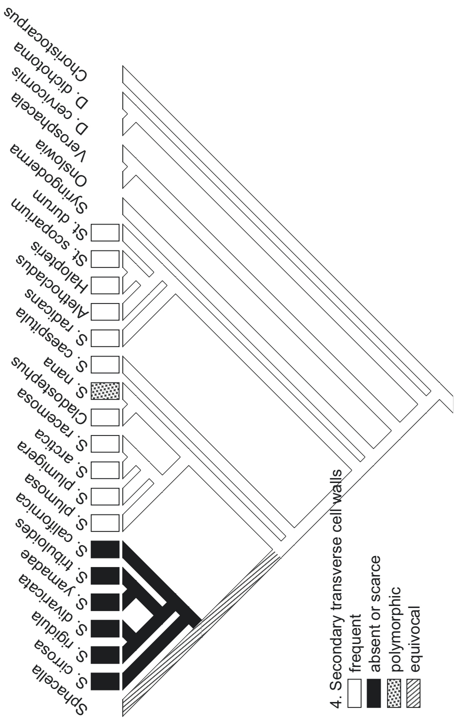
and Ramírez 2001), 190–234 nt in the Sporochnales (Sasaki *et al.* 2001), 220–225 nt in the Tilopteridales (Sasaki *et al.* 2001), 160 nt in *Asteronema rhodochortonoides* (Boergesen) Müller *et* Parodi (*incertae sedis*) (Peters and Ramírez 2001), 265–289 nt in the advanced Laminariales (i.e., Alariaceae, Laminariaceae and Lessoniaceae) (Yoon and Boo 1999; Kraan and Guiry, 2000; Kawai *et al.*, 2001; Draisma unpublished/ Chapter 8, Box 8.1), and in the other Laminariales 148–150 nt, 192 nt, 131 nt, 177–180 nt, and 202 nt in respectively the Chordaceae, the Pseudochordaceae, the Halosiphonaceae, the Phyllariaceae, and the Akkesiphycaceae (Yoon and Boo 1999; Kawai and Sasaki, 2000; Kawai *et al.*, 2001; Sasaki *et al.* 2001). We have no explanation for these extreme length differences. Members of the Sphacelariales *s.s.* are definitely on the high end with averages above 250 nt.

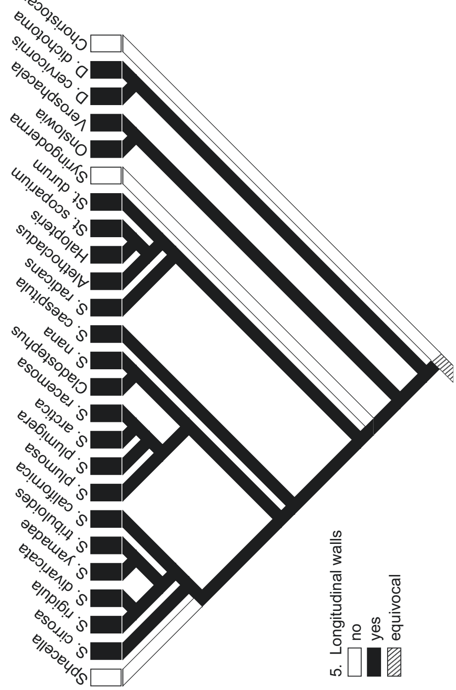


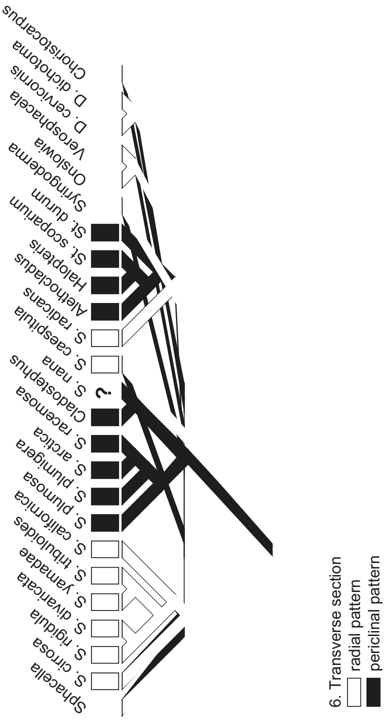
(continued)

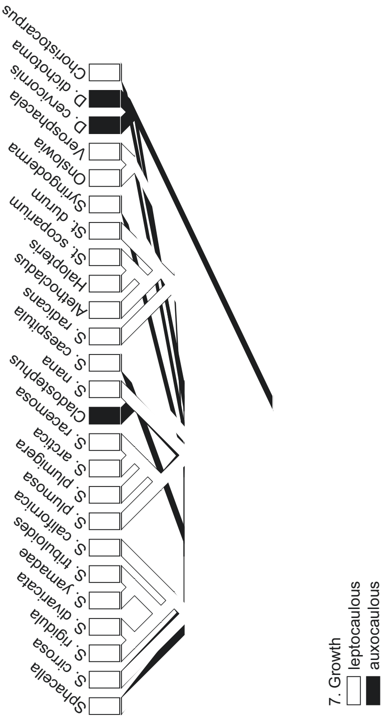
Fig. 3.5 (divided over p. 70–75)**.** Character mapping on the *rbc*L-based tree. Each of the 23 morphological characters from Table 3.4 and 3.5 were mapped onto the *rbc*L tree from Figure 3.2. *Halopteris filicina* 1 and 2 are presented as a single taxon. Each branch is shaded (or patterned) to indicate what states are most parsimoniously placed at the node terminating the branch. Equivocal means that more than one option is equally parsimonious. The boxes just under each terminal taxon name show the state(s) observed in that taxon. If a character is absent in a taxon (indicated with '-' in Table 3.5) no box is shown and if the state is unknown ('?' in Table 3.5) a question mark is shown.

(Fig. 3.5 continued)



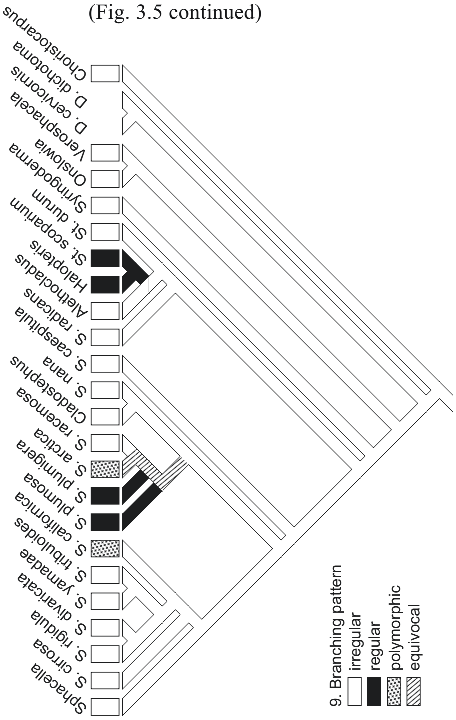


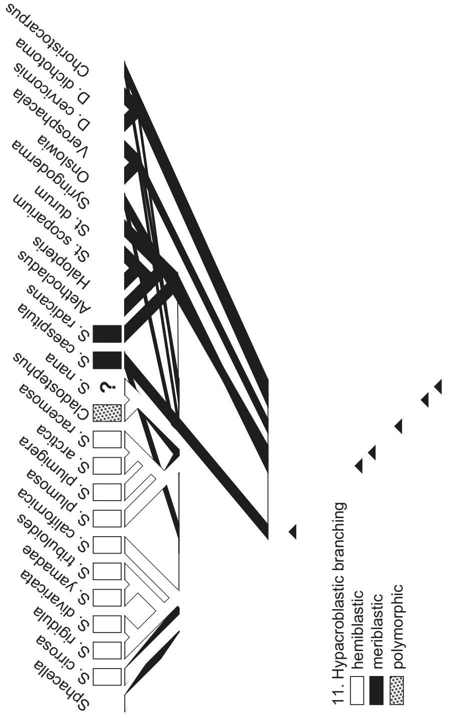


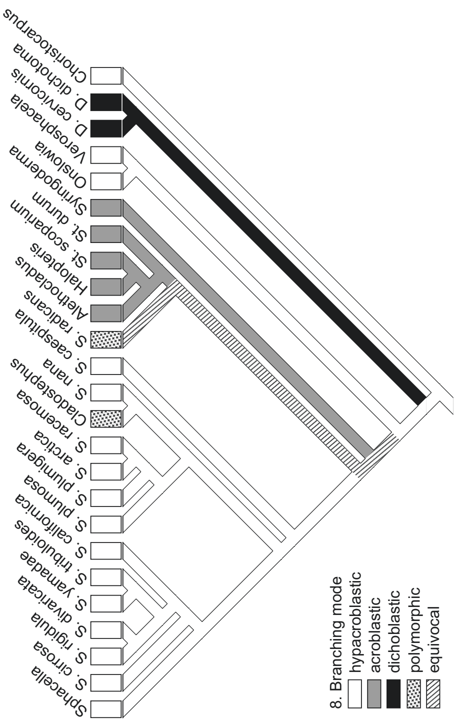


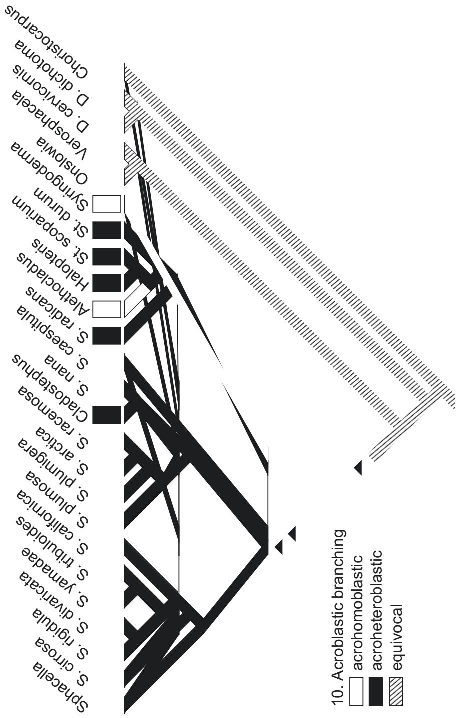
(continued)

(Fig. 3.5 continued)



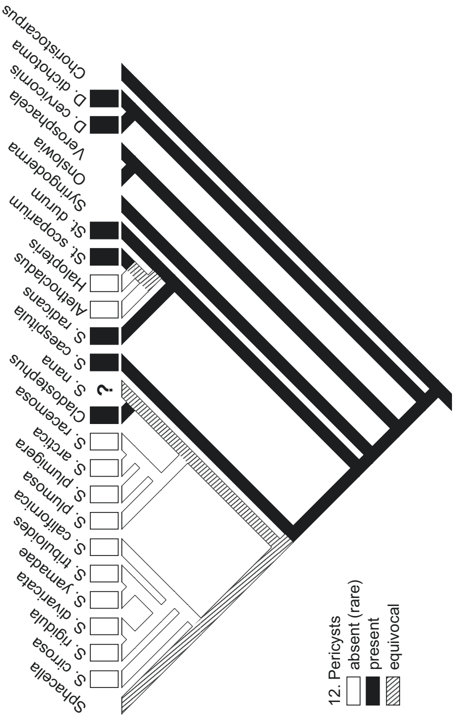


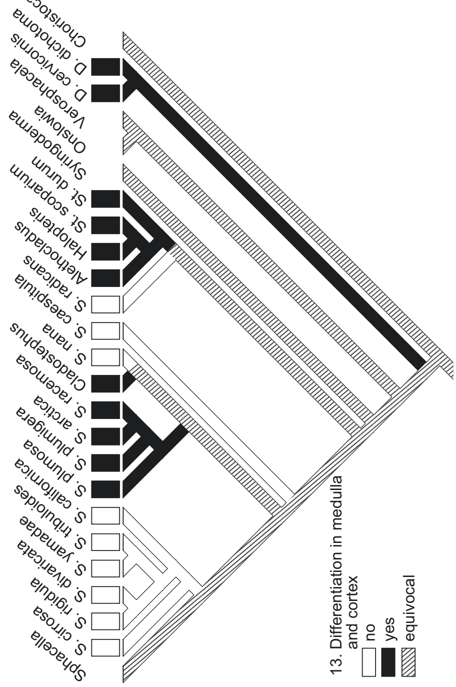


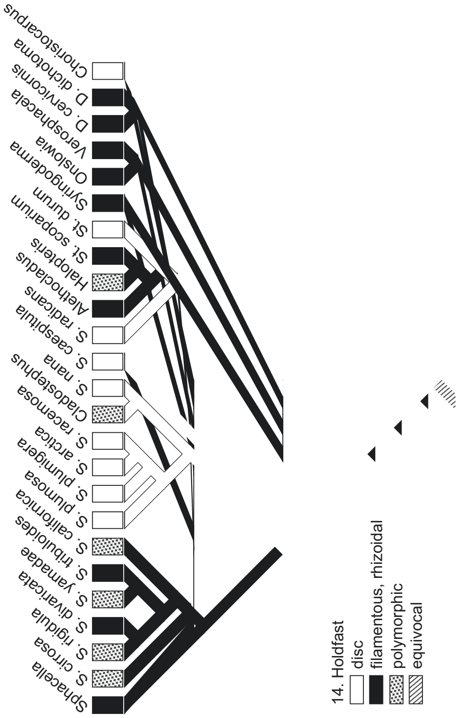


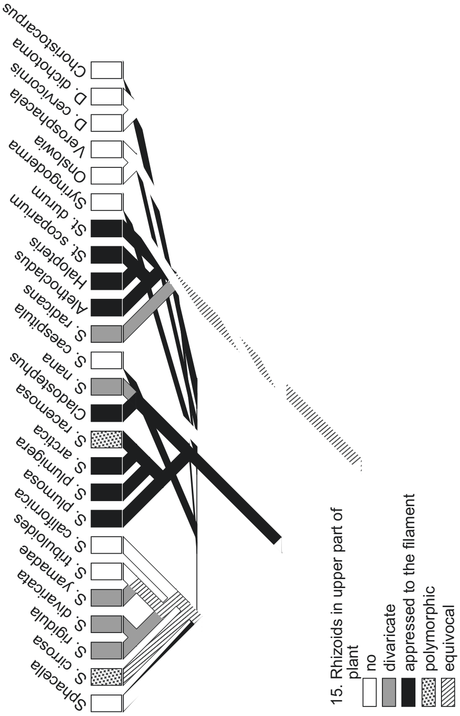
(continued)

(Fig. 3.5 continued)



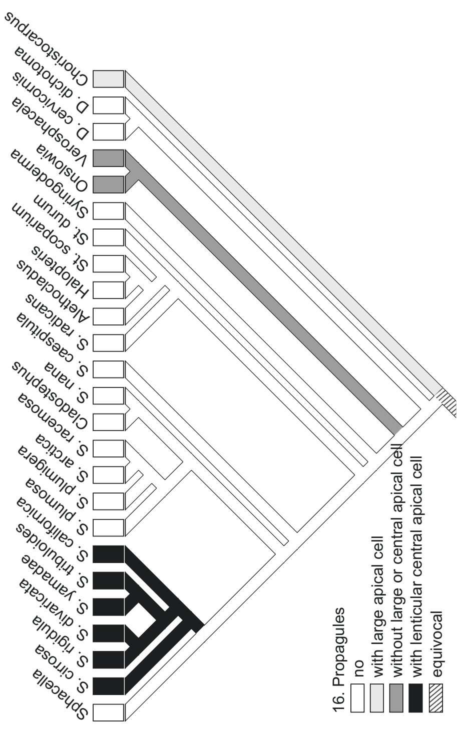


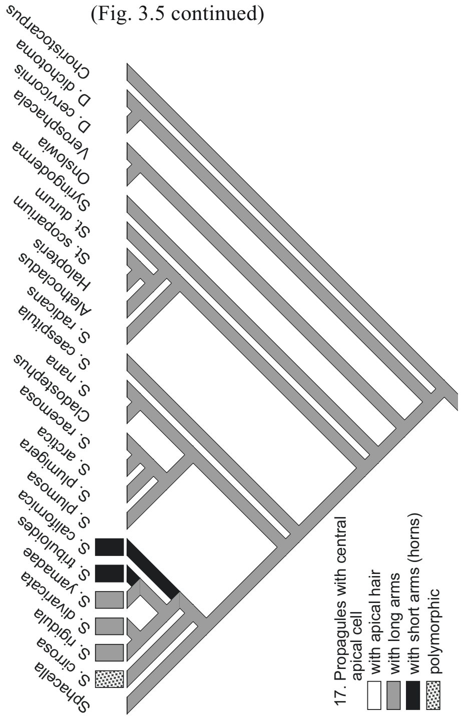


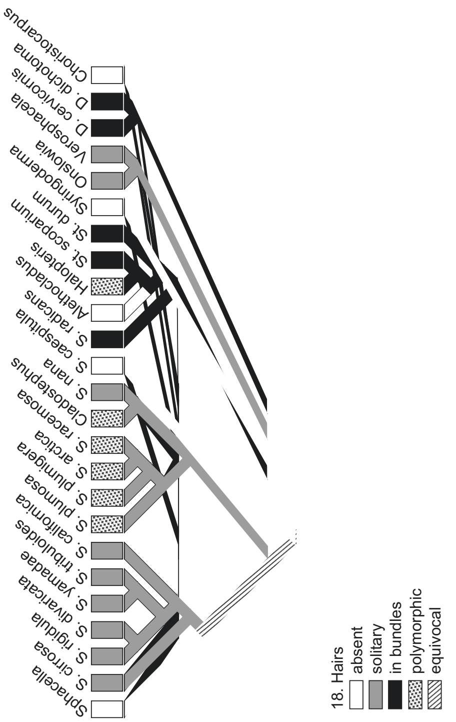


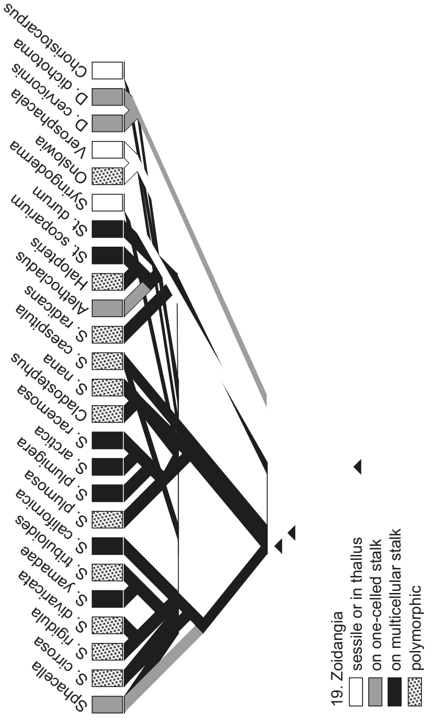
(continued)

(Fig. 3.5 continued)



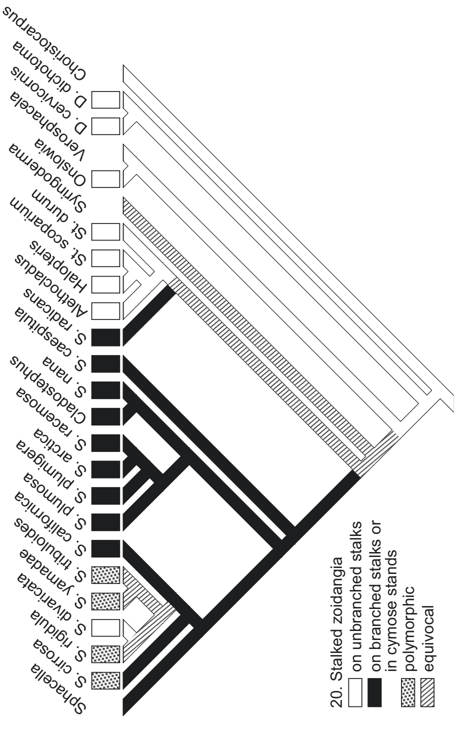


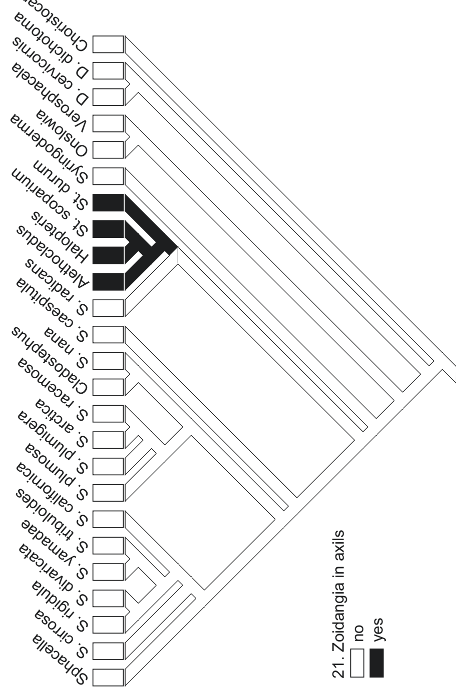


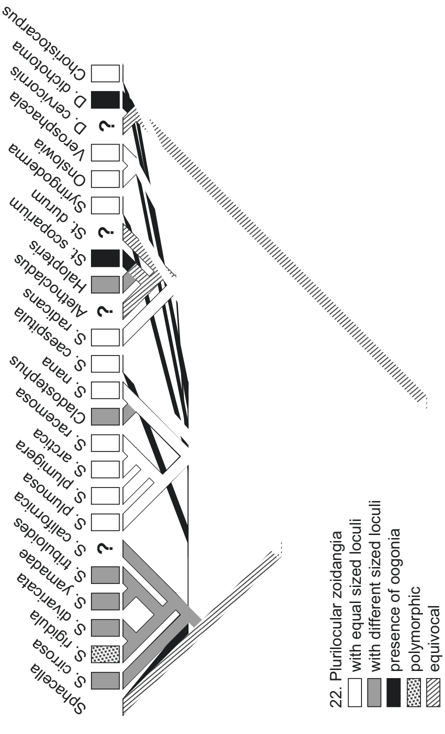


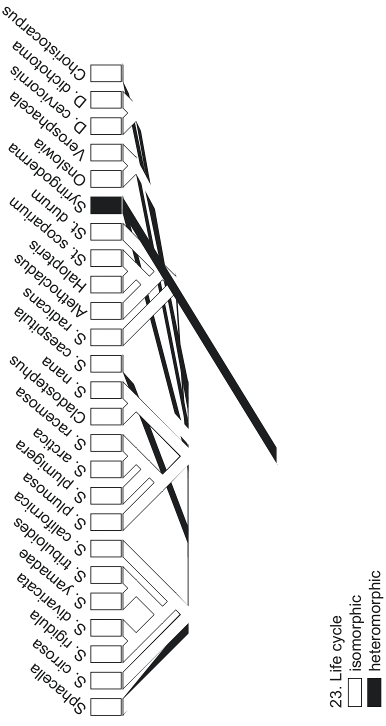
(continued)

(Fig. 3.5 continued)


20. Stalked zoidangia


21. Zoidangia in axils


22. Plurilocular zoidangia


23. Life cycle

*Rbc*L phylogeny

The *rbc*L tree in Figure 3.2 represents the best estimate of the Sphacelariales *s.s.* currently available and will serve as the basis in the later discussions. An analysis that included both *rbc*L + partial RUBISCO-spacer sequences resulted in the same topology. The bootstrap and parsimony jackknife support percentages signal that the resolution in the tree is not as good as it appears. This is particularly true in the basal portion of the ingroup. We will return to these issues later on.

Morphological phylogeny

Analysis of the morphological data yielded much less resolution than the molecular data. The only ingroup clade with moderate bootstrap support was the *Sphacelaria* subgenus *Propagulifera* (Fig. 3.3B). The analysis without outgroup (Fig. 3.4B) of the morphological data additionally showed weak support for the Stypocaulaceae. The difficulty in establishing monophyletic relationships was influenced by three factors. First, there were relatively few unambiguous characters and character states which led to many MPTs and low bootstrap support. Of 23 characters, more than 50% showed homoplasy, i.e., gains and losses of character states two or more times. Second, defining taxa by the absence of characters (e.g., *Sphacella*) and/or autapomorphies (e.g., *Cladostephus*) are weak criteria. Whether or not either of these genera should continue to be recognised is questionable. Third, natural phenotypic plasticity and/or polymorphism in characters among different individuals of a species reduces utility (e.g., occurrence of more than one branching mode, solitary *vs.* bundled hairs, rhizoidal vs. discoid holdfast). Polymorphism occurred in 11 of the 23 characters. It must be concluded, therefore, that high levels of morphological convergence combined with few synapomorphies greatly restrict the usefulness of morphological characters for phylogenetic classification. Nevertheless, it remains a fact that morphology is the basis for identification.

In order to further explore the reliability of traditional characters, we mapped each morphological character onto the molecular tree (Fig. 3.5). This was justified on the grounds that the molecular tree is the best estimate of the phylogeny currently available and on the expectation that at least a few morphological characters do carry a phylogenetic signal. Bleaching response and formation of secondary segments (characters 1 and 2) are good synapomorphies defining the Sphacelariales *s.s.* Presence of propagules with a lenticular, central apical cell (character 16) defines the subgenus *Propagulifera*. Strict acroblastic branching of the main axes (character 8) and axillary zoidangia (character 21) define the Stypocaulaceae. The remaining characters are either autapomorphies or have been gained or lost multiple times.

There is no good basis for the recognition of *Sphacella*. It is nested within *Sphacelaria*. Apart from character 5 there are no other characters that contradict the grouping of *Sphacella* with *Sphacelaria*. Character 5 is autapomorphous in *Sphacella*. Most characters that occur in *Sphacelaria* but are missing in *Sphacella* (*viz.* characters 4, 6, 11, 12, and 13) are due to the absence of longitudinal cell walls (character 5) in *Sphacella*. *Cladostephus* is also nested within *Sphacelaria*. Unlike *Sphacella*, however, it has well-defined morphological features that separate it from *Sphacelaria*. These specifically include the co-occurrence of different branching modes and the arrangement in whorls of determinant laterals. The traditional recognised subgenus

Sphacelaria (which includes the type) is also paraphyletic. Considering *S. nana* as part of *Cladostephus* or considering *S. radicans* as belonging to the Stypocaulaceae are untenable on morphological grounds but not on molecular grounds.

The Stypocaulaceae

Pacific and Atlantic Halopteris. *Halopteris filicina* is the single species in the present study for which isolates from different oceanic basins have been used. Although the Pacific and the Atlantic specimens form a monophyletic clade (Fig. 3.2), they could represent two separate cryptic species, because their genetic distance of the *rbc*L is higher than in 22 other pair-wise species comparisons. It is, however, precarious to base the distribution of two haplotypes solely on one or two isolates per region (Kooistra *et al.* 1992, van Oppen *et al.* 1995). Whether or not the two haplotypes of *H. filicina* indeed are indistinguishable on the basis of their morphology needs further investigation. Mathias (1935) and Ernst-Schwarzenbach (1957) reported that gametophytes of Mediterranean *H. filicina* plants were more slender than sporophytes, whereas Keum *et al.* (1995) observed that the gametophytes and sporophytes were almost identical in Korean field and culture specimens. Keum *et al.* (1995) also reported the occurrence of propagule-like structures in Korean plants, resembling those of *Choristocarpus tenellus*.

Pacific and Atlantic Stypocaulon. Novaczek *et al.* (1989) found that the temperature ranges for the northern and southern distribution limits of *S. scoparium* were lower in the west Atlantic than in the east Atlantic. On the basis of geographic distributions and differences in temperature tolerance, they suggested that eastern and western Atlantic populations are two different entities, with a possible Tethyan- and north Pacific origin, respectively. Moreover, a conspecificity of the northwest Atlantic *Stypocaulon* and the Pacific *S. durum* seems apparent because (1) Kawai and Prud'homme van Reine (1998) determined that the range in temperature tolerance of Pacific *S. durum* is similar to that of the northwest Atlantic *Stypocaulon* sp. and (2) Prud'homme van Reine (unpublished) observed discoid holdfasts (characteristic for *S. durum*) on all northwest Atlantic (Canadian) herbarium specimens of *Stypocaulon*. The observed difference between the *rbc*L sequence of the Canadian *S. durum* and the European *Stypocaulon* isolates (Tables 3.2 and 3.3, Fig. 3.2) is in favour of the hypothesised conspecificity, but the actual proof can only be given by including the *rbc*L sequence of a Pacific *S. durum* isolate.

Halopteris vs. Stypocaulon. The genera *Halopteris* and *Stypocaulon* were first described by Kützing (1843), but Sauvageau (1903) merged the species of both genera in the single genus *Halopteris*. Prud'homme van Reine (1991, 1993) proposed to reinstate the genus *Stypocaulon*, mainly based on the absence (*Halopteris*) or presence (*Stypocaulon*) of oogonia. The *rbc*L data do not lead us out of this dilemma. The mostly poorly supported topology within the Stypocaulaceae makes it impossible to assess the validity of these two genera. This also counts for the Antarctic genus *Alethocladus*.

Options for a new circumscription: lumping or splitting?

This part of the Discussion is based on the *rbc*L phylogeny in Figure 3.2 and redrawn in Figure 3.6.

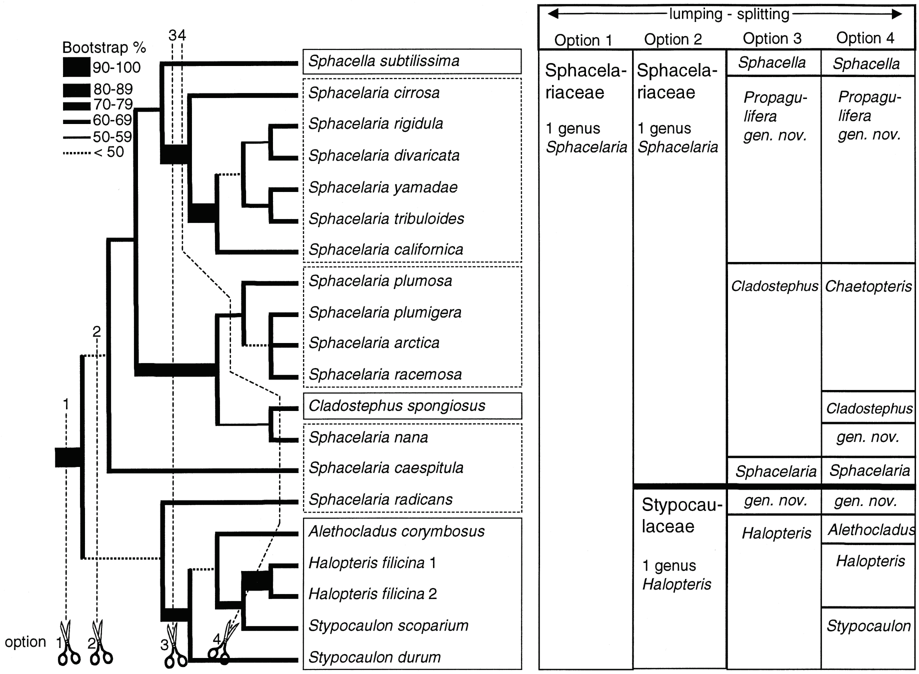


Fig. 3.6. Four options for a revised classification of the Sphacelariales *s.s.* based on the topology of the *rbc*L 50% majority-rule consensus tree (Fig. 3.2). The thickness of horizontal branches indicates the level of bootstrap support. The dashed boxes indicate the traditional classification in *Sphacelaria* subgenera. The scissors indicate where the generic limits are drawn for each option. See text for a more extensive explanation.

Option 1. A radical option would be to transfer all taxa to the genus *Sphacelaria*. However, under the International Code of Botanical Nomenclature (Greuter 2000), the name *Cladostephus* Agardh 1817 would take nomenclatural priority over *Sphacelaria* H.C. Lyngbye 1818. Since *Sphacelaria* is the best-known name, a proposal for conservation could be submitted. Option 1 would maintain the greatest stability with respect to nomenclatural changes and preserves monophyly. From the morphological perspective, however, the lumping of all the genera would take some adjustment.

Option 2. A second solution would be to recognise two groups (Fig. 3.6), the Sphacelariaceae with one genus *Sphacelaria* and the Stypocaulaceae with one genus *Halopteris*. The main difficulty here is the paraphyletic position of *S. radicans*. There is also another potential problem. *Sphacelaria radicans* is the putative sister-species to *S. reticulata* Lyngbye in Hornemann 1818, the type species for *Sphacelaria*. Live material of S. *reticulata* is unlikely to be forthcoming as the last reported finding occurred near Hofmansgave (Fyn, Denmark) in 1867. Silva (1980) proposed to conserve *S. caespitula* Lyngbye 1819 as the type species. That proposal was formally rejected in 1986 on the grounds of priority. In a proposal to choose another type species for *Sphacelaria* it can be stated that the combination *S. reticulata* does not have to be considered as a *nomen specifico-genericorum* (a species description that is at the same time also a genus description), because it was not the intention of Lyngbye himself. Hence, *S. caespitula* would be the best choice for a new type. It is also the most basal species of *Sphacelaria*.

Option 3. In this solution *Sphacella* would continue to be recognised, the subgenus *Propagulifera* would be upgraded to the genus level, the next clade (Fig. 3.6) would become *Cladostephus* with concomitant new combinations and only *S. caespitula* would be recognised as the true *Sphacelaria* (including issues around the type of the genus *Sphacelaria* as discussed under Option 2). *Sphacelaria radicans* would have to become a new genus and the remaining clade would be merged under *Halopteris*.

Option 4. This solution is the most divisive. As shown in Figure 3.6, the *Cladostephus* clade in Option 3 would be further subdivided into three genera. The name *Chaetopteris* F.T. Kützing 1843 would take priority for the four species of the subgenus *Pseudochaetopteris* (and *S. mirabilis*) and a new genus would have to be proposed for *S. nana*. The genera of the Stypocaulaceae would not be changed.

Conclusion

Ideally a classification should reflect shared evolutionary history of the group and simultaneously provide a basis for clear taxonomic identification. This combination is not always achievable within a single classification. To continue to recognise *Cladostephus* and *Sphacella* will result in irreconcilable paraphyly within *Sphacelaria*. Circumscriptional Options 1, 2, 3 and 4 solve this problem by only recognising monophyletic groups. The preference of any option is largely a matter of preference rather than of necessity. Therefore, we do not propose the adoption of any formal change in nomenclature at this time.

From an evolutionary standpoint, the phylogenetic relationships within the Sphacelariales *s.s.* reveal a complex paraphyletic assemblage based on *rbc*L and RUBISCO-spacer sequences, and an almost completely unresolved tree based on morphology. Highly convergent morphology and often weak diagnostic characters are a common problem in almost all algal groups. For this reason, comparative molecular data are indispensable. At the outset of the study, rDNA ITS sequences were planned to be used in addition to *rbc*L and RUBISCO-spacer sequences. Complete and partial ITS sequences of six species were obtained (Chapter 4). However, length variation was extreme. ITS1 ranged from 235 to >340 and ITS2 from 265 to >627 nts. In addition there were many mono-, di-, tri-, and tetra-nucleotide repeats which hampered sequence reactions and made unambiguous alignment impossible. These results bode that ITS sequences of any nonsphacelarialean taxon would be unalignable with sphacelarialean ITSs and, therefore, an outgroup would not be available. B.L. Siemer (personal communication) obtained a similar result in her studies of the Ectocarpales *s.l.* and Tilopteridales (Siemer *et al.* 1998) which caused her to abandon ITS altogether. Reasons for the extreme length variation in these groups is not known but is possibly related to the life cycle in combination with organisation of the ribosomal cistrons on different chromosomes, polyploidy, and extended periods of asexual reproduction. Although an additional gene or spacer sequence is highly desirable, we have no recommendation for what that might be.

The taxon sampling in the present study covers only Northern Hemisphere species, with the exception of *A. corymbosus*. A comparison study focusing on the Southern Hemisphere representatives (South Africa, Australia, and New Zealand) is conspicuously missing. Although it is tempting to speculate where these might fit, results from the molecular phylogeny make it clear that predictions are unlikely to be correct. The evolutionary trajectories of these lineages are undoubtedly different. At present it is unclear whether or not *Sphacelaria* is of Northern or Southern Hemisphere origin.

Acknowledgements

This work was supported by grant No. 805-42.025 from the Foundation of Earth- and Life-Sciences (ALW), which is subsidised by the Netherlands Organisation for the Advancement of Research (NWO). We thank the following persons for supplying algae or DNA: A.M. Breeman, O. de Clerck, E.C. Henry, S. Kawaguchi, Y.-S. Keum, T. Kitayama, and D.G. Müller.

Chapter 4

THE INTERNAL TRANSCRIBED SPACERS OF THE RIBOSOMAL CISTRON ARE UNSUITABLE FOR PHYLOGENETIC STUDIES IN THE SPHACELARIALES (PHAEOPHYCEAE) [1]

The suitability of the internal transcribed spacers (ITS1 and ITS2) of the ribosomal cistron for phylogenetic studies in the Sphacelariales was investigated. ITS sequences were determined for two species of Stypocaulaceae and four species of *Sphacelaria* (Sphacelariaceae). ITSs were found to be extremely variable and very difficult to align due to length differences, large indels and long stretches of repeat motifs. Intra-specific and intra-individual ITS variation was observed in the stypocaulacean *Halopteris filicina.* Phylogenetic analyses with selected parts of the alignment confirmed earlier determined phylogenetic relationships based on RUBISCO-gene sequences. Still it is concluded that, due to the inferior quality of the alignment, ITS sequences are not suitable the determination of the phylogenetic relationships at the genus level within the Sphacelariales. ITS sequences may be suitable for phylogenetic studies in the Sphacelariales from the sub-generic to the intra-specific level.

INTRODUCTION

Species of the brown algal order Sphacelariales are characterised by blackening of the cell walls when treated with bleaching liquid and growth by conspicuous apical cells (Prud'homme van Reine 1982). Transverse divisions of the subapical cells (or primary segments) produce secondary segments, which may undergo longitudinal and secondary transverse divisions. The Sphacelariales are subdivided into three families based on morphological characteristics, *viz*. the Sphacelariaceae (2 genera), the Stypocaulaceae (5 genera) and the monotypic Cladostephaceae. Draisma *et al.* (2002/Chapter 3) reconstructed the phylogeny of the order using partial sequences of the chloroplast encoded large subunit of the RUBISCO-gene (*rbc*L) and its adjacent RUBISCO spacer, including 19 species of Sphacelariales representing all three families and six out of eight genera. Maximum parsimony analysis of *rbc*L revealed four basal clades with moderate to high support: (1) Stypocaulaceae, (2) *Sphacelaria radicans*,

---

[1] *Submitted to* Cryptogamie Algologie *as:*
S.G.A. Draisma, W.F. Prud'homme van Reine, J.L. Olsen & W.T. Stam. The internal transcribed spacers of the ribosomal cistron are unsuitable for phylogenetic studies in the Sphacelariales (Phaeophyceae).

(3) *Sphacelaria caespitula*, and (4) all other included taxa; (*Sphacella subtilissima* Reinke + the *Sphacelaria* subgenus *Propagulifera*) and (*Cladostephus spongiosus* [Hudson] C. Agardh + *Sphacelaria nana* + *Sphacelaria* subgenera *Battersia* and *Pseudochaetopteris*). The phylogenetic relationships between these groups could not be established unambiguously. Although the RUBISCO-spacer is more variable than the *rbc*L gene, there was no enhancement in the resolution of the trees when the spacer sequences were analysed on their own or in combination with the *rbc*L sequences. This illustrates the need for an additional phylogenetic marker and the Internal Transcribed Spacers (ITS1 and ITS2) of the ribosomal cistron are the primary candidates. ITS1 and ITS2 are under fewer functional constraint then the rRNA genes and therefore evolve at a faster rate. ITS sequences can be amplified relatively easily using primers that anneal on the conservative flanking genes. DNA sequences of the ITS1 and ITS2 have been successfully applied in phylogenetic studies of brown algae at various taxonomic levels (Peters *et al.* 1997, 2000; Stache-Crain *et al.* 1997; Serrão *et al.* 1999; Kawai and Sasaki 2000; Kawai *et al.* 2001; Sasaki *et al.* 2001; Uwai *et al.* 2001). However, not all attempts were successful. In the Ectocarpales, ITS sequences were often unalignable because of extreme differences in length variation due to inserts, repeated motifs and deletions that may be encountered between species, within species and within individuals (B.L. Siemer pers. comm.). The utility of ITS sequences for phylogenetic studies must, therefore, be evaluated on a case by case basis.

The aim of the present study was to determine the suitability of ITS sequences for phylogenetic studies in the Sphacelariales. ITS sequences were determined for four species of Sphacelariaceae and for two species of Stypocaulaceae, representing all four clades mentioned above. For *Halopteris filicina*, ITS sequences were determined for two different isolates and for one isolate two cloned fragments were sequenced. These sequences were tested for their phylogenetic information content and compared with chloroplast encoded *rbc*L sequences.

MATERIALS AND METHODS

From taxon sampling to alignment of DNA sequences

Taxa are listed in Table 4.1. DNA extraction and all following steps, up till aligning of the obtained sequences, followed the protocol as described in Draisma *et al.* (2001/ Ch. 2) with the following exceptions: (1) The DNA was extracted in only 1 ml CTAB-PVPP and (2) the DNA was precipitated directly after the third CIA extraction and used for PCR amplifications. The ITS1 and ITS2 region was amplified by using the C1 forward primer (5'- GTA CAC ACC GCC CGT CGC TCC - 3') and the ITS4 reverse primer (5'- TCC TCC GCT TAT TGA TAT GC -3') (White *et al.* 1990). The primers C1 and ITS4 anneal at position 1624 (18S) and 52 (26S) in the ribosomal DNA of *Chlamydomonas reinhardtii* (van Oppen 1995), respectively. From *Halopteris filicina* two isolates (isolates 2 and 3) were sequenced and from the amplification product of isolate 2, two cloned fragments were sequenced (2a and 2b). The sequences were aligned by eye using the BioEdit Sequence Alignment Editor (Hall 1999). The ends of the coding and non-coding regions were determined based on Saunders and Druehl (1993).

Table 4.1. Species used. All species were collected by W. F. Prud'homme van Reine. Length numbers in bold indicate the length of the complete ITS. Lengths printed in non-bold and followed by '+' indicates that the ITS is only partially sequenced.

Family Species	Origin	Collection year	Length ITS1	Length ITS2	EMBL accession number
Sphacelariaceae					
Sphacelaria caespitula Lyngbye	Espegrend, Norway	1967	**235**	**292**	AJ311819
S. nana Naegeli *ex* Kützing	Espegrend, Norway	1967	**301**	**265**	AJ311816
S. racemosa Greville	St. Andrews, Scotland	1971	**285**	**275**	AJ311817
S. radicans (Dillwyn) C. Agardh	Øystese, Norway	1967	**259**	**427**	AJ311818
Stypocaulaceae					
Halopteris filicina (Grateloup) Kützing (isolate 2)	St. Lunaire, Brittany, France	1967			
(cloned fragment 2a)			198+	546+	AJ311821[a], AJ311822 [b]
(cloned fragment 2b)			84+	193+	AJ311823 [a], AJ311824 [b]
H. filicina (isolate 3)	Roscoff, Brittany, France	1968	221+	627+	AJ311825 [a], AJ311826 [b]
Stypocaulon scoparium (Linnaeus) Kützing	Rade de Brest, Brittany, France	1967	**340**	**415**	AJ311820

[a] for ITS1.
[b] for ITS2.

*Rbc*L sequences were taken from Draisma *et al.* (2002, table 2/Ch. 3, Table 3.2). These include *Halopteris filicina* (Brittany isolate, EMBL accession number: AJ287895), *Sphacelaria caespitula* (AJ287870), *Sphacelaria nana* (AJ287875), *Sphacelaria racemosa* (AJ28780), *Sphacelaria radicans* (AJ287874), and *Stypocaulon scoparium* (AJ287866). The nuclear and chloroplast encoded sequences of each species are in most cases not represented by the same isolates. From the *rbc*L sequences 1255 nt of the 3'-end were used in the phylogenetic analyses.

Phylogenetic analysis

Phylogenetic analyses of the complete or partial (see Results) sequences of the combined ITS1 and ITS2 were done with PAUP* version 4.0b6 (PPC) (Swofford 1998). The nuclear encoded ITS sequences were also analysed in combination with the plastid encoded *rbc*L sequences and the latter were also analysed separately with the same taxon set. Maximum Parsimony (MP) analyses were done as exhaustive searches, including or excluding gapped positions and treating gaps as missing or new state. For the Maximum Likelihood (ML) analyses the nucleotide substitution model to be used was first determined using MODELTEST version 3.0 (Posada and Crandall 1998). The two stypocaulacean species *H. filicina* and *S. scoparium* were appointed as outgroup taxa.

Sampling error and precision

Decay analysis of MP trees was performed with AutoDecay version 4.0 (Erikson 1998). MP and ML bootstrapping (Felsenstein 1985) was performed in PAUP* using 10 (MP) or 5 (ML) random sequence additions and 10,000 (MP) or 1000 (ML) replicates.

Congruence of data sets was evaluated using the partition homogeneity test (PHT) (Farris *et al.* 1995, Cunningham 1997) which is implemented in PAUP*. Constant characters were deleted prior to the analysis, the random sequence addition option (10 replicates) was used and 10,000 replicates were performed.

RESULTS

The sequenced region of the ribosomal DNA covered 149–151 nucleotides (nt) of the 18S, the complete ITS1, 5.8S (160 nt) and ITS2, and 38 nt of the 26S, excluding the annealing positions of the amplification primers. Incomplete ITS1 and ITS2 sequences (and subsequently no 5.8S sequences) were obtained for the *Halopteris filicina* specimens, because the determined sequences from the forward and the reverse sequence reactions did not overlap. No effort was made to bridge the gap by doing sequence reactions using internal sequence primers. The number of ITS nucleotides (nt) determined per taxon is shown in Table 4.1 along with EMBL accession numbers of the sequences. The partial ITS1 (5'-end) and partial ITS2 (3'-end) of the *H. filicina* sequences were submitted separately. The sequences obtained from the cloned fragments 2a and 2b from *H. filicina* were very short and therefore excluded from the alignment and subsequent phylogenetic analyses.

Alignment properties

Available coding regions of the ribosomal cistron were conservative and alignable for all taxa, but did not contain any phylogenetic information. The ITSs were extremely variable and very difficult to align due to length differences, large indels and long stretches of repeat motifs. Aligning of ITS sequences was most feasible for the species combinations *S. caespitula* + *S. radicans* and *S. nana* + *S. racemosa*. The alignment of ITS1 is shown in Figure 4.1 and that of ITS2 in Figure 4.2. Properties of different parts of the (combined) alignments are summarised in Table 4.2. Reliably alignable parts of the ITS regions, indicated with asterisks (*) in Figures 4.1 and 4.2 and referred to with 'selection', were used for some analyses (see next section). Uncorrected 'p' distances between species ranged from 0.15 to 0.35 for the complete ITS1 (0.07–0.21 for ITS1 selection) and from 0.09 to 0.38 for ITS2 (0.05–0.28 for ITS2 selection). Intra-individual variation was found between the ITSs of the cloned fragments of isolate 2 of *H. filicina.* This variation was most conspicuous in the difference in number of repeat motifs.

Phylogenetic analysis

A preliminary PHT analysis between the ITS1 + ITS2 selection and the *rbc*L indicated that both data sets were congruent (*P*-value = 0.60) and could be combined for phylogenetic analyses. The results of the various phylogenetic analyses are summarised in Table 4.2, including the selected models of DNA substitution for the Maximum Likelihood (ML) analysis. The most conservative analysis (i.e., ITS1 + ITS2 selection and gapped positions excluded) resulted in two most parsimonious trees (MPTs), which are shown in Figure 4.3. All additional analyses with more extended data sets gave one or two MPTs, each with the topology identical to Figure 4.3A and/or B, except for the analysis based on *rbc*L alone (see footnote ^{f} in Table 4.2). The two trees in Figure 4.3 differ from each other in the relative and unsupported positions of *S. radicans* and *S. caespitula*. Only in the analysis in which gaps were considered a new state, the position of *S. radicans* was well supported as a sister species to all other *Sphacelaria* species (Fig. 4.3A), with the clade leading to these three species obtaining a bootstrap value of >90%.

DISCUSSION

Data properties

The incomplete ITS2 sequence of *Halopteris filicina* 3 (> 627 nt) exceeds the longest brown algal ITS2 sequence reported thus far. In other brown algae, ITS sequences vary in length from 186–283 nt (ITS1) and 291–358 (ITS2) in the Desmarestiales (Peters *et al.* 1997, 2001), 206–789 and 252–485 in the Ectocarpales *sensu lato* (Stache-Crain *et al.* 1997, Burkhardt and Peters 1998, Peters and Burkhardt 1998, Kogame and Masuda 2001, Peters and Ramírez 2001, Uwai *et al.* 2001), 401–515 and 322–409 in the Fucales (Leclerc *et al.* 1998, Serrão *et al.* 1999), 238–360 and 255–486 in the Laminariales *sensu lato* (Saunders and Druehl 1993, Peters 1998, Yotsukura *et al.* 1999, Kawai and Sasaki 2000, Coyer *et al.* 2001, Kawai *et al.* 2001,

Fig. 4.1. Alignment of ITS1 sequences of Sphacelariales. The 3'-end of the *H. filicina* (isolate 3) sequence is incomplete. Dashes (-) indicate gaps to restore alignment. Question marks (?) indicate undetermined regions of unknown length. *Sp.* = *Sphacelaria*, *St.* = *Stypocaulon*, *H.* = *Halopteris*. Asterisks (*) indicate reliably alignable parts and are referred to with 'selection' in the text and in Table 4.2.

```
                ********** *******  20         30         40         50         60         70         80
Sp. radicans    CCGTTTTT-G CGCGC-CCTT GC-TTT---- ---------- -----CAC-- TAGCA----- --GA------ ----------
Sp. caespitula  CCGTTTTT-G CTTGCTCGCG GC-------- ---------- ----GGACGT TG-------T TCGACGTCC- GCCGC-----
Sp. nana        CCGTTTTT-G CTAGCTGCCC TCGTTTGCGT CTCGCAGTAG GTGAGTGCCT TTAGGGCCGC -CGAGGGCCT GACCGACGCA
Sp. racemosa    CCGTTTTT-G CTCGC-CCAC ACCTTTGCGC CTCGTTTTCG TCGCGGACAA TAGCATCGTG TCGAGA-CCT GGCGTGGTGT
St. scoparium   CCGTTTTT-G GAT-CTCGGG CTTTGTTGTC CGAGGTCCTC CCCGTCGCCG CTGTTGTTGT TGTTTGTCTT CCATTCTTCG
H. filicina     CCGTTTTTTG C-CGCTC--- ----GTTGTT ---------- ---GTTGTTG TTGTTGTTGT TGTT-GTAAC AAGCGATGAT

                         90        100        110        120        130         * ********** **********
Sp. radicans    ---------- ---------- ---------- ---GGC---G A--------- ---CA-AA-C CAACCCCGCG AA--TTCTTC
Sp. caespitula  ---------- ---------- ---------- ---GGC---A A--------- ---CG-AA-C CAACCCCGCG AACGCTCGAT
Sp. nana        GC-------- ---------- ---------- ---GGCAGCA G--------- ---CACAA-C CAACCCCGCG AAACTGTTTT
Sp. racemosa    G--------- ---------- ---------- ---GGTTG-A A--------- ---C--AT-C CAACCCCGCG AAACTGTTTT
St. scoparium   GTCGCTACAT GTGTGGTGGC CGGCGTTTGT GTGGGCAACG ACGACGACGC GCG-ACAACC CAACCCCGCG AACGTTTCTT
H. filicina     GATGATGATG ATGATGATGA TGC------- ---GGCAA-A G--------- ---C-C--CC GAAACCCGCG AAA-TGTTTT

                ********** ********** ********** ********** ********** *       220        230     *****
Sp. radicans    TTTTT----- ----TTATCC GATGTCGGGC GA-GTCTGCG CC-TCGCTGA GCCGTTTGCG TTGATTCGTT CGGGCTCGAA
Sp. caespitula  TATTTTT-CT -CAATTATCC GAAGCCGGGC GA-GTCTGCG TC-TCGCCGA GCC---TGC- ---AA----- --GGCTCGAA
Sp. nana        TTAATTTTCA ACMATTATCC -AAGCCGGGC SA-RTCTGCS TC-TCGCTGA GAAAC---C- STAAAAGGGG TCT--TCGAA
Sp. racemosa    TATTACT-CA -CAATTATCC GAAGCCGGGC GA-GTCTGCG TC-TCGCCGA GGGCA--GCT GTTGCTC--- -CT-CTCGAA
St. scoparium   TGTTTA--CA -CAATTATCC GAAGTCGGGC GA-GTCTGCG TCCTCGCCGA GTC-TCTTCT C--GA----- --GGCTCTAA
H. filicina     TTG------A ACAATTATCA CAAGTCGGGC GAAGTGTGCC TS-TCGCTGA GCCTGC---- ---AA----- --GGCTCGAA

                ********** ********** ********** ********** ********** ********300        310        320
Sp. radicans    CCAAAGCGCG CCCCCCACTT T-CAACCCCA AAACCAAACT CTGAATCTGA ACTCTAAGGG GCGTTTTTTT TCTCTCTGTC
Sp. caespitula  CCAAAGCGCA CCCC--ACTT TTCAACCCCA AAAGCAAACT CTGAATCTGA ACCTTAAAGG GGA-CCG--- GCC-------
Sp. nana        TCAAAGCGCA CCCC--ACTT TTNAACCCCA AAAGCAAACT CTGAATCTGA ACCTTAAGAG GTGGCTGCCT G--CC----G
Sp. racemosa    CCAAAGCGCA CCCC--ACTT TTCAACCCCA AAAGCAAACT CTGARTCTGA ACCTTAAAGG -TGGCCGCCC GCCG------
St. scoparium   CCAAAGCGCA CCCTCTTTTT CCAAACCCNN -AACCAAACT CTGAGTCTGA ACCTTGAATG GNGGGCGGNT TAACCTGTTG
H. filicina     ACAAAGTGCA CG----ACTT TT-AACCCCA AATGCAKA-- CTGAATCTGA ACTTTTAA?? ?????????? ??????????

                        330        340        350        360        370
Sp. radicans    TCTCTCTTGA TTGATTGATT GATTGACGC- CCTCCAACCT TTT--AA-CG TTGT
Sp. caespitula  --------AA -AA---G--- -CTGGGC-C- CCTCCAACC- TTA--AA-CG TTGT
Sp. nana        TC------AA TAATACGACG GC-GGGCCCT CCTCCAACCA TTA--AA-CG TTGT
Sp. racemosa    TC------AA ----ACG--- -C-GGGCCCT CCTCCAACCA TAA--AA-CG TTGT
St. scoparium   TCAAGACNAA TAA------- ------CCC- CCTCC-ACC- TTANNAANCG NNGT
H. filicina     ?????????? ?????????? ?????????? ?????????? ?????????? ????
```

Table 4.2. Summary of data properties and maximum parsimony and maximum likelihood analyses for different alignments. For all alignments six taxa were used (*H. filicina* represented by isolate 3). sel. = 'Selection' and refers to the parts of the alignment indicated with * in Figures 4.1 and 4.2.; comp. = complete alignment analysed; gapped positions are included or excluded (= excl.); when gapped positions were included, the gaps were treated as missing data (miss.) or as new state (= new.); ITSs = ITS1 + ITS2; n.d. = not determined.

Alignment			Maximum parsimony					Maximum likelihood			
Region	Length	Number of variable (%)/ informative (%) positions	Number of MPTs [a]	Length of MPTs [a]	CI/RI/RC [b]	Topology	g_1 [c]	Selected model [d]	-ln L [e]	Topology	g_1 [c]
ITSs sel.; excl.	251	80 (32%)/24 (10%)	2	117	0.89/0.55/0.49	Fig. 4.3A&B	-0.60	K2P + G	877.7	as Fig. 4.3B	-1.41
ITSs sel.; miss.	325	115 (35%)/30 (9%)	1	150	0.91/0.60/0.54	as Fig. 4.3B	-0.70	K2P + G	1116.3	as Fig. 4.3B	-1.18
ITSs sel.; new.	325	158 (49%)/61 (19%)	1	234	0.89/0.64/0.57	as Fig. 4.3A	-0.63	n.d.	n.d.	n.d.	n.d.
ITSs comp.; excl.	371	156 (42%)/50 (13%)	1	234	0.87/0.47/0.41	as Fig. 4.3A	-0.47	n.d.	n.d.	n.d.	n.d.
ITSs comp.; miss.	1236	388 (31%)/83 (7%)	2	539	0.92/0.54/0.50	as Fig. 4.3A&B	-0.58	n.d.	n.d.	n.d.	n.d.
*rbc*L	1255	190 (15%)/60 (5%)	2	247	0.89/0.58/0.51	as Fig. 4.3A & not shown [f]	-1.10	GTR + G	2981.5	as Fig. 4.3A	-1.42
ITSs sel. + *rbc*L; miss.	1580	305 (19%)/90 (6%)	1	398	0.89/0.57/0.51	as Fig. 4.3A	-1.02	TIM + G	4166.4	as Fig. 4.3B	-1.44

[a] MPT = most parsimonious tree.

[b] CI/RI/RC = consistency index / retention index / rescaled consistency index of MPTs.

[c] g_1 = skewness statistic (Hillis and Huelsenbeck 1992).

[d] GTR = general-time-reversible model (Rodríguez *et al.* 1990); K2P = Kimura 2-parameter model (Kimura 1980); TIM = transitional model (Rodríguez *et al.* 1990); G = shape parameter of the gamma distribution.

[e] - ln L = - log likelihood.

[f] the topology 'not shown' is the same as the one in Figure 4.3A, except that *S. caespitula* and *S. radicans* changed position in the tree.

Fig. 4.2. Alignment of ITS2 sequences of Sphacelariales. The 5’-end of the *H. filicina* (isolate 3) sequence is incomplete. Symbols and abbreviations as in Figure 4.1.

```
                        10         20         30         40         50         60         70         80
Sp. radicans    AACTCCACTC GCCAGGCCCC CCTTTTTT-- CTT------- ---------- ---------- ---------- ----------
Sp. caespitula  AACTCCACTC GCCCCTCCCC T--------- CTC------- ---------- ---------- ---------- ----------
Sp. nana        AACTCCACTC GCCCCTCGTA TT-------- CTT------- ---------- ---------- ---------- ----------
Sp. racemosa    AACTCCACTC GCCCCTCCTC TT-------- CTT------- ---------- ---------- ---------- ----------
St. scoparium   AACTCCACTC GACCCGCCTC TTTTGTT--- ---------- ---------- ---------- ---------- ----------
H. filicina     ?????????? ?????????? ???CTTTAAC CTTTAAGATC TTGNCTTCNG GAATTCTGGG ACATNCTGGG AAGGGTNGGT

                        90        100        110        120        130        140        150        160
Sp. radicans    ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
Sp. caespitula  ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
Sp. nana        ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
Sp. racemosa    ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
St. scoparium   ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
H. filicina     ATCTCATNGG CCCTGTCTCT TTTGTGGTGT TGTGTCCTCN TTGTTTGGGA AGCCGCGTTT CTTTTTGNGT GGTTYCYTWT

                       170        180        190        200        210        220        230        240
Sp. radicans    ---------- ---------- ---------- --TTT----- ---------- ---------- ---------- ----------
Sp. caespitula  ---------- ---------- ---------- -GTC------ ---------- ---------- ---------- ----------
Sp. nana        ---------- ---CCTC--- ---------- -GTT------ ---------- ---------- ---------- ----------
Sp. racemosa    ---------- ----CT---- ---------- -G-------- ---------- ---------- ---------- ----------
St. scoparium   ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
H. filicina     TCTCTTTTCG TTTCCTCTCG GGGATATGGG GGTTTTGGGT TCCTTCTCGG AAGAGTTACT GGCGTGGTTT TTGCTCGAGA

                       250        260      ***** ********** ********** ********** ***    310        320
Sp. radicans    ---------- -GATTCATTT GAAAAGAAAG AAAGGGTTTG GCGGACTGTG CGTGTCCCGG CGC--CT--- ----------
Sp. caespitula  ---------- ---TAACG-- AC-GAGTCGG -GA-CGGG-- TCGGACTGTG CGTGTTCCGG CGC---T--- ----------
Sp. nana        ---------- ---------- -----GAAGA AGA-CGGGGG TCGGAATGTG CGTGTTCCGG AGC--CT--- ----------
Sp. racemosa    ---------- ---TAA-CTT -----GAAG- -GA-CGGGGG TCGGACTATG CGTGTTCCGG AGC---T--- ----------
St. scoparium   ---------- ---------- ----AGAGGA A----GGG-- TCGGACTCTG CGTGTTCCGG CGCATCTTTC GTACTCCAGT
H. filicina     AGAAGAAGAA TAATAAT--- AATAAGAAGA AGAGCGGGGG TCGGACTGTG CGTGTTCCGG C--------- ----------

                       330        340        350        360        370        380        390         **
Sp. radicans    ---------- ---------A A---TT---- -CGATG---- ---------- ---------- ---------- ------KCGC
Sp. caespitula  ---------- ---------G ATTTTT---- -CGAAACAA- ---------- ---------- ---------- ------GCGC
Sp. nana        ---------- ---------A N--CCT---- ---------- ---------- ---------- ---------- ------GCTC
Sp. racemosa    ---------- ---------T C--TCA---- -CGAA----- ---------- ---------- ---------- ------GCTC
St. scoparium   CAGCTTTTGT GTTAGCAATA ATTTTTTTTT ACGAAAGAGT TTATGTAATA CAGTACTGTT GGATGGTGTA AGAGCAGCGC
H. filicina     ----TTACTT GTTTGCTCAT CGAGCGAACG TC-AAAG--- ---------- ---------- ---------- ---------C
```

(continued)

(Fig. 4.2 continued)

```
                ********** ********** ********** ********** ********** ********** **     470        480
Sp. radicans    CGAGTGCACC CAAGCGTCTG GAAACCTCGT ---AAAGAAG AG-----CCT CTCTGCGTTT TCTCTTGTCT CGCT--GCCG
Sp. caespitula  CGAGTGCACC GAAGCGTCTG GAA--CTCGG ---AACGAAG -------CCT CT-------- TCTCTGCTCG ACCG----AC
Sp. nana        CGAGTGCACC GAAGCGCATG GAA--CTCGC ---AAAGAGA -G-----CCT CTC------- ACTGTT-G-G CTCG----AC
Sp. racemosa    CGAGTGCACC GAAGCATCTG GAA--CTCGC ---GAAGAGA AG-----CCT CTC------- ACTATTCG-G CTCG----AC
St. scoparium   CGAGTGCACC GRAGCGCCTT GTG--CTCGC ---AATGTAA GGAAAAGGCT GGCTCTCTAT TATGTCTCTT CGGG----AC
H. filicina     CGAGTGCACC GAAGCGCTTG GAG--CTCGC CGCAAGAGAA AGAAAAGCTT TTAGCTCTTT TTTTTCTTAC CTAGTAGCAC

                       490    ******* ********** ********** ********** ********** ********** **********
Sp. radicans    CGTTTMTAA- ---GCCGAAG CCCAAAGGAG AGAGTTGTTG ACGGTYTGTT GCACAACTCG CTTCTYTTTC GGCCCYYT-C
Sp. caespitula  C----AGGAC GG-GCCGAA- -CTAAAGGAG AGAGTTGTTG ACGGTT-GTT ACG--ACTCG CTCCTTT--C GGCCC-----
Sp. nana        -------GA- ---GCCGAA- -CTAAGGGAG AGAGTTGTTG ACGGTT-GTT ACG--ACTCG CTCCTT---C GGCGCAA--C
Sp. racemosa    -------GA- ---GCCGAA- -CTAAGGGAG AGAGTTGTTG ACGGTT-GTT ACG--ACTCG CTCCTT---C GGCGCCA--C
St. scoparium   ------GGA- ---TCGAGA- -CTACAGGAT CGAGTTGTTG ACGGCT-GTT GCACAACTCG GYCCTTCT-C GGCCCGGCGC
H. filicina     TCTTTGGGAC GGACTGAGA- -CCAAAGGGC AGAGTTGTTG ACGGTT-GTT ACACGACTCG GCCCTTTCTT GGCCCGGCGC

                *      570        580        590        600        610        620        630        640
Sp. radicans    G--------- ---------- ---------- ---------- ---------- -------AGG ---------- ----------
Sp. caespitula  ---------- ---------- ---------- ---------- ---------- -------AGC ---------- ----------
Sp. nana        ---------- ---------- ---------- ---------- ---------- -------AGA ---------- ----------
Sp. racemosa    ---------- ---------- ---------- ---------- ---------- -------AGA ---------- ----------
St. scoparium   GTCGGTATTT TGT--TTTAC TATTTA---- ---------- ---------- CCGGTGCAGA AGAAGAAGGA ----------
H. filicina     GTCGGTAGTG TGTGTGTTTC TTTTTTCTTT GAAAAGAGAA GACTGTACTG CCGGGGCAGA AGAAGAGGAA GTAAAACTGG

                       650        660        670        680        690        700        710        720
Sp. radicans    ---------- -------CGC GG-CGCGGTC TTGAGGTTTG AGTCGCTTTC TCTCTT---- --TTGCGAAA ----CCACGK
Sp. caespitula  ---------- --------GT CC-C-CGG-- --AAGACAAC ACGAAGAGGC GCTCTT---- --GTCCCGAG ----CCACGG
Sp. nana        ---------- -------GTT ---------T GT-------- ---GTGTTGT GCTCTTTGTT T-GT---GA- ----CCCCGG
Sp. racemosa    ---------- -------GTT AGTTGTG--T GT-----AGG TTAATTTYTT CTCCTC---- --GT---GA- ----CCACGG
St. scoparium   ---------- -----TCGTT AGTAGDGGCT GTTCTT---- ---GTTTTTY CCTATTATTG TCGT-C-GAA ----CCACGG
H. filicina     GTAGAAGAAG AAGAGTCGGG TGGATCTATT GT-CTTGCGG GGTGTTTTT- CCACTGTCTG TCCGTCTGTA GGTCCCATGT

                       730        740        750        760        770        780        790        800
Sp. radicans    CCG-TTGTTT GTGTTTTTCT TTCTTTCTTT CTTCCTTCCT TTCTTTCTTT CTTCAGAGAG AGAGAGAGAG AGAGATAGAG
Sp. caespitula  TCG-TTCGAC TTGT------ ---------- ---------- ---------- ---------- ---------- ----------
Sp. nana        CCGATTTTTG AAGAATCCG- ---------- ---------- ---------- ---------- ---------- ----------
Sp. racemosa    CCGATT---G AAAAATCCG- ---------- ---------- ---------- ---------- ---------- ----------
St. scoparium   TCG-TT---A AYAAA----- ---------- ---------- ---------- ---------- ---------- ----------
H. filicina     ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------

                       810        820        830        840
Sp. radicans    AAACAAGCAA GCAAACAAAC AAACAAACAA ACAAACCACT TTCGTT
Sp. caespitula  ---------- ---------- ----AAACAA AC---CCACT TTCGTT
Sp. nana        ---------- ---------- ---------A AC---CCACT TTCGTT
Sp. racemosa    ---------- ---------- ---------A AC---CCACT TTCGTT
St. scoparium   ---------- ---------- ---------A AC---CCACT TTCGTT
H. filicina     ---------- ---------- ---------A AC-------- --CGT-
```

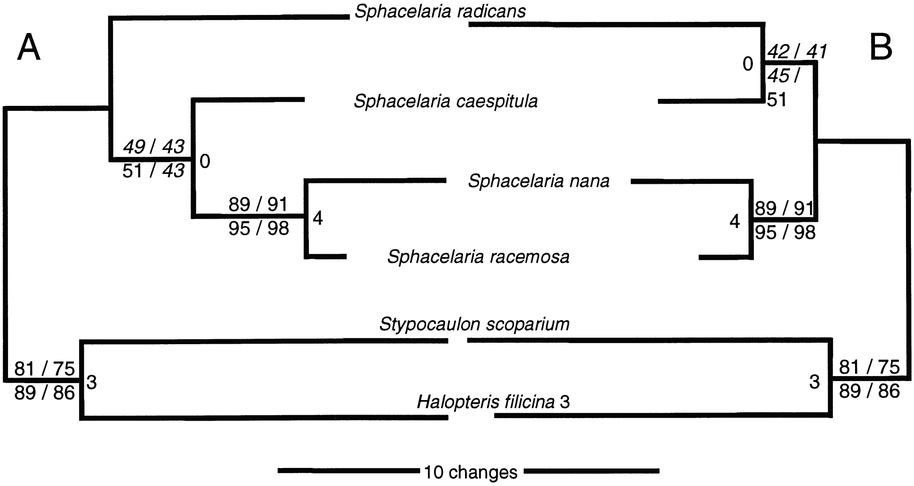


Fig. 4.3. ITS phylogeny of the Sphacelariales. Two MPTs (A and B) based on a MP analysis of combined ITS1 and ITS2 selections (indicated with * in Figs 4.1 and 4.2) excluding gapped positions (251 characters). Tree statistics are presented in Table 4.2. Branch lengths are proportional to the number of changes. Outgroup = *H. filicina* (isolate 3) and *S. scoparium*; bootstrap percentages are given above branches, MP left and ML right; parsimony jackknife percentages are given below branches, MP left and ML right; decay index values are given on the right (in A) or left (in B) of the nodes.

Sasaki *et al.* 2001), and 280–472 and 294–306 in the Scytothamnales (incl. *Asteronema ferruginea* [Harvey] Delépine *et* Asensi) (Müller *et al.* 1998, Peters and Clayton 1998). For the Tilopteridales only two ITS2 sequences are known (328–329 nt; Sasaki *et al.* 2001) and for the Dictyotales only one ITS1 sequence is known (481 nt; de Clerck 1998). The supposedly dictyotalean ITS1 and partial ITS2 sequences determined by Lee and King (1996) probably belong to epiphytic chlorophytes (de Clerck 1998).

The ITS sequences of the Sphacelariales are highly variable and large gaps were needed to construct an acceptable alignment. Still, only a small fraction of the ITSs could be aligned with confidence. In contrast, the coding parts that flank the ITSs were highly conservative and lack phylogenetic information at the intra-ordinal level of the Sphacelariales. The large gaps in the alignment often coincided with the presence of short tandem repeats in one or more of the sequences (Figs 4.1 and 4.2). The presence of these tandem repeats is likely due to slipped-strand mispairing events (also known as replication slippage) (Levinson and Gutman 1987, Li 1997). This process involves local denaturation and displacement of the double-stranded DNA followed by mispairing at the position of an existing short repeat. This leads to insertions or deletions of one or more short repeats. The process can be self-accelerating and thus short repeats can accumulate over time. Sequences like TTTCTTTTTTCTTT (position 577–590 in Fig. 4.2) and GAAAAGAGAAGA (position 591–602 in Fig. 4.2) in *H. filicina* may also have been caused by slipped-strand mispairing events, but through time they have been erased by other (point) mutations.

The ribosomal DNA cistron (= external transcribed spacer [5'-ETS] + SSU [18S] + ITS1 + 5.8S + ITS2 + LSU [26S] + 3'-ETS) of eukaryotic organisms is organised in long arrays of tandem repeats separated by intergenic spacers (IGS), often distributed over different chromosomes (Gerbi 1985). There are hundreds to thousands of cistron repeats per nucleus. These repeats may differ, as demonstrated by the intra-individual variation in the two cloned fragments of isolate 2 of *H. filicina* in the present study. However, it has also been demonstrated that these repeats are homogenised, a phenomenon called concerted evolution (Zimmer *et al.* 1980). The two most important mechanisms for the occurrence of concerted evolution are (non-reciprocal) gene conversion and unequal crossing-over (Li 1997). Gene conversion is a recombination process in which two sequences interact in such a way that one is converted by the other (non-reciprocal = one sequence is changed whereas the other is not). Unequal crossing-over creates a sequence duplication in one chromosome and a corresponding deletion in the other (reciprocal). In isolate 2 of *H. filicina* the ribosomal cistrons obviously have not been homogenised yet using the mechanisms of concerted evolution. In other brown algae intra-individual ITS variation has been reported for the Fucales (up to 2.6%) (Serrão *et al.* 1999) and the Laminariales (up to 4.6%) (Coyer *et al.* 2001). From the perspective of phylogeny reconstruction, the presence of multiple intra-individual ITS types is problematic. This is especially the case when hybridising species or recently diverged species are included that have not yet undergone lineage sorting. If only one type per individual or species is included in the analysis, then the estimated phylogeny will be potentially incorrect (Famà *et al.* 2000, Diekmann *et al.* 2001). This is probably not the case in the present study, because the observed interspecific variation was much higher than the observed intra-specific and intra-individual variation. Moreover, the intra-specific variation is mainly restricted to the simple repeat motifs, which are excluded from the phylogenetic analyses anyway.

Phylogeny

The ITS based trees could not be rooted with taxa outside the Sphacelariales as no ITS sequences were available for the closest relatives of the Sphacelariales: the Syringodermatales and the Onslowiaceae (Draisma and Prud'homme van Reine 2001/Ch. 7, Draisma *et al.* 2001/Ch. 2). However, they probably would have been unalignable with sphacelarialean ITS sequences, given the alignment problems encountered in this study. The sphacelarialean ITS1 sequences were certainly not alignable with the dictyotalean ITS1 sequence determined by de Clerck (1998).

All analyses show that the two stypocaulacean species, *H. filicina* and *S. scoparium*, are clearly separated from the remaining four sphacelarialean species. This is in complete agreement with the results obtained by Draisma *et al.* (2002/Ch. 3). In their study, which is based on *rbc*L sequences, it was also demonstrated that the *Sphacelaria* subgenus *Sphacelaria* is paraphyletic. The present ITS-based phylogenies support this conclusion. *Sphacelaria nana* is always sister to *Sphacelaria racemosa* (subgenus *Pseudochaetopteris*), and not to the other species of the subgenus *Sphacelaria* (*Sphacelaria caespitula* and *Sphacelaria radicans*).

Conclusion

ITS sequences are not suitable for reconstruction of the phylogenetic relationships at the genus level within the Sphacelariales. They are simply too variable. Making a satisfactory alignment was difficult even between species of the same genus, whereas the reliably alignable parts contained only a few informative sites. Nevertheless, earlier determined phylogenetic relationships based on *rbc*L were confirmed (Draisma *et al.* 2002/Ch. 3). ITS sequences may be suitable for phylogeny reconstructions of Sphacelariales from the sub-generic to the intra-specific (population) level.

Chapter 5

PROPAGULES AND MOLECULES DO NOT GO TOGETHER: PARAPHYLETIC SECTIONS AND CRYPTIC SPECIES IN THE *SPHACELARIA* SUBGENUS *PROPAGULIFERA* (PHAEOPHYCEAE) [1]

The *Propagulifera* comprise about 17–23 species which form a monophyletic subgenus within *Sphacelaria*. The traditional sections *Propagulifera*, *Furcigerae* and *Tribuloides* are defined by morphology of the propagules. The propagules in the section *Propagulifera* are characterised by an apical hair, those in *Furcigerae* by long cylindrical arms, and those in *Tribuloides* by a tribuliform shape with or without horns. The present study challenges the traditional circumscription of the sections. A phylogenetic analysis of the subgenus *Propagulifera*, using *rbc*L and RUBISCO-spacer sequences, revealed both paraphyly and the presence of cryptic species. Both sequences were analysed together and separately, with and without gaps, under maximum parsimony and maximum likelihood. The combined data set gave the best-supported phylogenetic trees in which three well-supported clades were recovered. The most basal clade was formed by *S. cirrosa* (*Propagulifera*) only, which was used as outgroup. A second clade consisted of *S. californica* (*Tribuloides*), which was basal within this clade, and *S. divaricata* and *S. rigidula* (both *Furcigerae*). The third clade consisted of the basal *S. tribuloides* (*Tribuloides*) and *S. yamadae* and *S. rigidula* (both *Furcigerae*). It is concluded that propagule morphologies have evolved multiple times within the sections and are, therefore, not phylogenetically informative within the subgenus. Well-supported genetic differences among the clades within the subgenus reveal cryptic species, i.e., *S. rigidula*. In the absence of reliable morphological differences it is recommended that the sections *Furcigerae* and *Tribuloides* either no longer be recognised or that they be merged into one section.

INTRODUCTION

The genus *Sphacelaria* Lyngbye 1818 (Sphacelariales, Phaeophyceae) is subdivided into 5–6 subgenera (Prud'homme van Reine 1993, Draisma *et al.* 2002 / Chapter 3). The subgenus *Propagulifera* is the most species-rich (17–23 species) and has the widest geographic distribution. Some species are cosmopolitan in tropical and temperate waters, while others are endemic to much smaller areas (Prud'homme van Reine 1993, Kitayama 1994, Draisma *et al.* 1998). The subgenus *Propagulifera* is especially characterised by specialised branchlets for vegetative propagation called

---

[1] *To be submitted as part of a larger study on the subgenus* Propagulifera *by Dr Y.-S. Keum (Seoul National University, South-Korea).*

propagules. Propagules can be formed in the diploid sporophyte phase of the life cycle as well as in the haploid gametophyte phase (van den Hoek and Flinterman 1968). Species delimitation within the subgenus *Propagulifera* is predominantly based on the distinct shape of the propagules (Kitayama 1994, Draisma *et al.* 1998). Diagnostic characters associated with the thallus include the diameter of the main axes, the number of cells that can be observed in lateral surface view of the main axes, the branching mode, and the shape and size of unilocular and plurilocular zoidangia (Prud'homme van Reine 1982, Kitayama 1994). The subgenus is subdivided into three sections (Prud'homme van Reine 1982) based on the shape of the propagules. The propagules in the section *Propagulifera* (2–3 species) have long, usually fusiform arms and usually a terminal hair. In the section *Furcigerae* (7–11 spp.) the propagules have long, cylindrical arms and are without a terminal hair. The propagules in the section *Tribuloides* (5–9 spp.) are characterised by a tribuliform shape (with or without conical horns) and by the absence of terminal hairs. This latter section is further subdivided into two series; *Tribuloides* (5–6 spp.) and *Humeratae* (3 spp.) (Prud'homme van Reine 1993). Tribuliform propagules can also develop arms (Prud'homme van Reine 1982; figs 439, 440, 451, 472, 506, 507), but usually only after they are shed, whereas the propagules of *Furcigerae* develop arms while they are still attached to the parent plant. So the development of arms in the section *Tribuloides* is in fact delayed. Ambiguities in the definition of propagule arms (section *Furcigerae*) and propagule horns (section *Tribuloides*) make it impossible to place some species in a section. Kitayama (1994), for example, noted that the propagules of *Sphacelaria cornuta* Sauvageau fit in the section *Furcigerae* as well as in the section *Tribuloides*. According to Kitayama (1994), the type of division of the subapical cell (the cell directly beneath the central lenticular apical cell) of the propagule may be more useful for the separation of these two sections. Propagules in the section *Furcigerae* show an unequal (or diagonal) division type of the subapical cell and in the section *Tribuloides* they show an equal (or perpendicular) division type. Draisma *et al.* (1998), however, noted that in some species both types of division can be observed, but stated that a majority-rule could be applied: in section *Tribuloides* the majority of the propagules shows an equal division type, in *Furcigerae* the majority shows an unequal division type. Consequently, Draisma *et al.* (1998) moved *Sphacelaria carolinensis* Trono from the section *Tribuloides* to the section *Furcigerae*, after having re-examined the type specimen.

Draisma *et al.* (2002 / Ch. 3) analysed phylogenetic relationships among species of Sphacelariales using DNA sequences of the plastid encoded large subunit of the RUBISCO-gene (*rbc*L). Their study included six species of the subgenus *Propagulifera* representing all sections and series. *Sphacelaria cirrosa*, the sole representative of the section *Propagulifera* and also the type of the subgenus, was basal with respect to the other five species. There was, however, no support for the monophyly of the section *Furcigerae* and of the section *Tribuloides*. Draisma *et al.* (2002 / Ch. 3) also included RUBISCO-spacer sequences in their analysis. For phylogenetic studies within the Sphacelariales these sequences turned out to be of minor importance because of alignment problems. Such problems were not encountered when only the *Propagulifera* were considered, making this marker a promising tool for revealing phylogenetic relationships within this subgenus. In the present study both *rbc*L and RUBISCO-spacer sequences were used in order to elucidate (1) the phylogenetic relationships between species belonging to the subgenus *Propagulifera* and (2) to test the supposed monophyletic status of the sections *Furcigerae* and *Tribuloides*.

MATERIALS AND METHODS

Taxon sampling, DNA extraction and amplification, cloning and plasmid isolation, DNA sequencing

Taxa are listed in Table 5.1. All steps from DNA extraction to DNA sequencing are described in Draisma *et al.* (2001 / Ch. 2).

Alignment and phylogenetic analysis

The RUBISCO-gene and -spacer sequences were aligned manually and edited using the BioEdit Sequence Alignment Editor (Hall 1999). Phylogenetic analyses were performed with PAUP* version 4.0b6 (PPC) (Swofford 1998) in which also pair-wise uncorrected ('p') distances were determined, treating gaps as missing data. All codon positions of *rbc*L were included in the phylogenetic analyses. Maximum parsimony (MP) analyses were done as exhaustive searches, treating gaps as missing data or as fifth base. For the Maximum Likelihood (ML) analyses the nucleotide substitution model to be used was first determined using MODELTEST version 2.1 (Posada and Crandall 1998). Subsequently ML analyses were done as heuristic searches with 100 random sequence additions.

Sphacelaria cirrosa was selected as outgroup. This species was sister to all other species of the subgenus *Propagulifera* (with high support) in the rooted phylogeny of the Sphacelariales in Draisma *et al.* (2002 / Ch. 3).

Decay analysis of MP trees was performed with AutoDecay version 4.0 (Erikson 1998). Bootstrapping (Felsenstein 1985) and parsimony jackknifing (Farris *et al.* 1996) was performed in PAUP* using 10 random sequence additions and 10,000 replicates. Character deletions per jackknife replicate were set at 37%. For ML bootstrapping only 1000 replicates were done with 5 random sequence additions per replicate.

Congruence of *rbc*L and RUBISCO-spacer sequences was evaluated using the partition homogeneity test (PHT) (Farris *et al.* 1995, Cunningham 1997) which is implemented in PAUP*. Constant characters were deleted prior to the analysis, the random sequence addition option (10 replicates) was used and 10,000 replicates were performed.

RESULTS

Alignment properties

No insertions/deletions were observed in the *rbc*L sequences, which made the alignment unambiguous. For most taxa 1375 nucleotides (nt), out of the potential 1467, were determined. For a few taxa only 1255 nt were determined (Table 5.1) due to the PCR amplification with the RbcL188F primer. The 1255 nt *rbc*L alignment was routinely used in the subsequent phylogenetic analyses. Both within *Sphacelaria*

Table 5.1. *Sphacelaria* specimens used in the present study. Type species of sections are indicated with '$^{(T)}$'. New sequences are indicated in bold. Species distributions taken from Prud'homme van Reine (1993) and Keum *et al.* (1999).

Species (ordered by section and series)	Collection site	Collector, year/ identifier	EMBL accession number		Geographic distribution of the species
			*rbc*L	RUBISCO-spacer	
section *Propagulifera*					
S. cirrosa (Roth) C. Agardh $^{(T)}$	Fife Ness, Scotland	S.G.A. Draisma, 1997	AJ287865 (1372 nt)	AJ287946 (255 nt)	temperate north Atlantic Ocean and temperate southern Australia
section *Furcigerae*					
S. divaricata Montagne	Tsuyazaki, Kyushu, Japan	S. Kawaguchi, 1989	AJ287889 (1374 nt)	AJ287949 (238 nt)	tropical and temperate Pacific Ocean
S. rigidula Kützing $^{(T)}$ 1	Galway, Ireland	W.F. Prud'homme van Reine, 1966	AJ287883 (1366 nt)	AJ287941 (252 nt)	cosmopolitan in tropical and temperate waters
2	Chaesuckang, South Korea	Y.-S. Keum, 1996	**AJ287888** (1255 nt)	**AJ287944** (252 nt)	
3	Muroran, Hokkaido, Japan	W.F. Prud'homme van Reine, 1993	**AJ287887** (1255 nt)	**AJ287942** (252 nt)	
5	Cape Verde	H. Pakker, 1991 / S.G. A.Draisma	**AJ287885** (1375 nt)	**AJ287941** (264 nt)	
S. yamadae Segawa	Ogi, Sado, Japan	T. Kitayama, 1990	AJ287890 (1375 nt)	AJ287950 (255 nt)	endemic to Japan and Korea
section *Tribuloides*					
series *Tribuloides*					
S. tribuloides $^{(T)}$ Meneghini 1	Sesoko, Okinawa, Japan	W.F. Prud'homme van Reine, 1993	AJ287891 (1375 nt)	AJ287947 (257 nt)	cosmopolitan in tropical and temperate waters
2	Jan Thiel, Curaçao	A.H. Engelen, 1999 / S.G.A. Draisma	**AJ287892** (1373 nt)	**AJ287948** (257 nt)	
series *Humeratae*					
S. californica Sauvageau *ex* Setchell *et* Gardner	Oiso, Awajishima, Japan	W.F. Prud'homme van Reine, 1993	AJ287893 (1255 nt)	AJ287951 (276 nt)	warm temperate boreal parts of the Pacific Ocean and Australia

rigidula isolates 1–3 and within the two isolates of *Sphacelaria tribuloides* differences at four positions were found. In the case of *S. rigidula*, two of these differences led to an amino acid substitution.

Alignment of the RUBISCO-spacer was more difficult and required 70 gapped positions (Fig. 5.1). Because the genes for both subunits of RUBISCO constitute a single operon in chromophytes and they are co-transcribed (Kostrzewa *et al.* 1990), a secondary structure of the RUBISCO-spacer can be expected. Inspection by eye revealed a palindromic region between positions 36 and 92 in the spacer sequence of *Sphacelaria californica*, which forms a stem-loop secondary structure. Within this region two tandem repeats of seven nt are present. This stem-loop could also be observed in the other taxa, though its integrity decreased gradually with the *Sphacelaria divaricata* sequence having hardly any secondary structure in this area (Fig. 5.1). All taxa showed a putative ribosomal binding site 11–15 nt upstream of the 3’-end of the spacer (Shine and Dalgarno 1974, 1975, Bailey and Andersen 1999). The spacers of *S. rigidula* 1 and 2 were identical, as were those of *S. tribuloides* 1 and 2. The spacer of *S. rigidula* 3 differed from *S. rigidula* 1 and 2 by only one substitution. The AT-content of the RUBISCO-spacers varied from 79–84%. EMBL accession numbers for both the *rbc*L and RUBISCO-spacer are given in Table 5.1.

Phylogenetic analysis

Comparing the *rbc*L and RUBISCO-spacer alignments with the PHT yielded a *P*-value of 0.375, indicating that the two subsets are not in conflict and can be combined. MP analyses were performed with the *rbc*L and the RUBISCO-spacer alignment separately as well as combined, including and excluding gapped positions, and treating gaps as missing data or as fifth base. All analyses resulted in one single most parsimonious tree (MPT). A summary of the MP analyses is presented in Table 5.2. The MPT that resulted from the analysis of the *rbc*L and spacer combined and treating gaps as missing data is shown in Figure 5.2, which is representative for all analyses which included *rbc*L. Analysis of the RUBISCO-spacer alone gave trees, which differed from the one given in Figure 5.2. If gaps were treated as fifth base, the *S. tribuloides* branches off from the *S. divaricata*/*S. rigidula* clade. In all other analyses of the spacer alone the *S. californica* becomes a sister clade to the clade with the two *S. tribuloides* sequences.

Table 5.3 summarises the four ML analyses performed and the invoked nucleotide substitution model. All these analyses resulted in trees that showed the same topology as the tree in Figure 5.2.

DISCUSSION

The phylogeny

The tree in Figure 5.2 is the best estimate of the *Propagulifera* phylogeny, because it is based on the highest number of informative characters and MP and ML analyses both resulted in the same tree topology when *rbc*L was included in the alignment. Bootstrap and parsimony jackknife percentages were higher for the analysis of the alignment of the *rbc*L + spacer combined than for the analysis of the alignments of

Fig. 5.1. Alignment of RUBISCO-spacer sequences in the *Sphacelaria* subgenus *Propagulifera*. Dots (.) indicate bases identical to the uppermost sequence. A double line indicates an inverted repeat in *S. californica*. Two 7 nt repeats herein are indicated in bold. Imperfect inverted repeats in *S. cirrosa* and in *S. rigidula* 5 are underlined with a single line. A putative ribosomal binding site at nt positions 272–6 is indicated in bold. Dashes (-) indicate gaps to restore alignment.

```
                            10         20         30         40         50         60         70         80
S. californica      CCAT-AAGCT -GATTGG-AT TATCAAATTT TTGTGTTTTT AAATATCATT AATCATTAAA TACTCTATTT AATGATTAAT
S. tribuloides 1&2  T...-..... -AT..A.-T. .......... ......C.C. .....GT.-- ----...... ....A..GC. .T.-------
S. rigidula 1&2     ..G.-..... -A..-..A.. ..A.CT.... ..TAA..... .....----- -----...TT .GTAT..... GG--------
S. rigidula 3       ..G.-..... -A..-..A.. ..A.CT.... ..TAA..... .....----- -----...TT .GTAT..... GG--------
S. rigidula 5       T...--..A. TA...A.-T. ..G.T..... ..C.A...C. .....GT.-- ----T..G.. .C..A..... ..A-------
S. divaricata       ....-..... -.......-. ....C.T... .GA....... .....----- ---------- ---.------ ----------
S. yamadae          ....--.--- ------.-T. ..G.T..... ..C.....C. .....GT.-- ----C..G.. ....A..... ..G-------
S. cirrosa          A...T..--- --------.. .C........ ....A...C. C....GG.-- ----...... ..A.A..G.. ...-------

                            90        100        110        120        130        140        150        160
S. californica      GATATTTAAA AATACTAACG CAATTAAATT TAA-TATTCA TTTTAGAGGT CTGTAAGAAA ATAATC-TTT TTGC---ATA
S. tribuloides 1&2  ------..G. .........A .......T.. ...-...... .......A.. .........G .G....-... ....---...
S. rigidula 1&2     ---.-..-G- ......CTAT TC.G...TC. .T.C...... .....A.T.. ..A....... ......-... ..A.---.G.
S. rigidula 3       ---.-..-G- ......CTAT TC.G...TC. .T.C...... .....A.T.. ..A....... ......-... ..A.---.G.
S. rigidula 5       ........G. ..CC....TA G......C.. ...-...... ....C..A.. T.....A..G .G...-T... ..A.---...
S. divaricata       ---TG..-G- ....T.TTAT TC.A...TA. .T.A...... ....G..... T.A....... ......-... ....---...
S. yamadae          ........G. ........TA G......C.. ...-...... ....C..A.. T.....A... ..TC.-T... ..A.---...
S. cirrosa          AC......G. ....T...TT TTT.GTTTA. ...T.....G ....T..TT. T.....T.CT ....G.T... ..AATTTTAT

                           170        180        190        200        210        220        230        240
S. californica      TTCAATTATC TAGAA-TGAA ACATGATTTT TATTTTAATC GTGTGACTAA TGGTCAAACA AAAAATTTAG TAAAATAAAA
S. tribuloides 1&2  .......... G.A..-.... .......... .......... ...CA..... .......... .......... ..........
S. rigidula 1&2     .-........ .....-.... ....A..... .......... ...CA....T .......... .......... ..........
S. rigidula 3       .-........ .....-.C.. ....A..... .......... ...CA....T .......... .......... ..........
S. rigidula 5       .........T ..A..-.... ....A..... .......... ....A..G.. .......... .......... .......G..
S. divaricata       .........T ...T.-.... ....A..... .......G.. ...CA....T .......... .......... ..........
S. yamadae          .......C.. ..A..-.... ....A..... .........A ...CA..A.C .......... .......... ..........
S. cirrosa          A.AC.A.TAT .....C.... ...AA..... ......C.AA ...CAGT... .A..T..... .......... ..G.......

                           250        260        270        280
S. californica      GATCATAGAT TTTAAGCATA T-ATAAATTA TAAGGATATC TTAATC
S. tribuloides 1&2  .......T.. .......... C-........ ........C. ......
S. rigidula 1&2     .......T.. ....G.T... .-........ .......... ......
S. rigidula 3       .......T.. ....G.T... .-........ .......... ......
S. rigidula 5       A......T.. .....AA... .T........ .......... ......
S. divaricata       .......T.. ....G..... .-........ .......... ......
S. yamadae          A......T.. ......A... .A........ .........T ......
S. cirrosa          .....G.T.. ....----.. C-..--.... ..G.A...C. .....A
```

Table 5.2. Summary of data properties and maximum parsimony analyses for different alignments. For all alignments ten taxa were used. All analyses resulted in a single most parsimonious tree (MPT). Gaps were treated as missing data, unless stated otherwise. CI / RI / RC = consistency index / retention index / rescaled consistency index of MPTs.

	Alignment		Tree			
Region(s)	Length	Number of variable (%)/ informative positions (%)	Length of MPT	CI / RI / RC	Topology	g_1[a]
*rbc*L + spacer	1541	254 (16%) / 123 (8%)	341	0.86 / 0.82 / 0.70	Fig. 5.2	-0.97
*rbc*L + spacer excl. gaps	1471	227 (15%) / 108 (7%)	303	0.84 / 0.80 / 0.68	as Fig. 5.2	-0.93
*rbc*L + spacer, gaps as 5th base	1541	297 (19%) / 147 (10%)	425	0.85 / 0.81 / 0.69	as Fig. 5.2	-0.95
*rbc*L	1255	138 (11%) / 65 (5%)	171	0.85 / 0.81 / 0.69	as Fig. 5.2	-0.94
spacer	286	116 (41%) / 58 (20%)	168	0.88 / 0.84 / 0.73	not shown	-0.97
spacer excl. gapped positions	216	89 (41%) / 43 (20%)	129	0.86 / 0.82 / 0.71	not shown	-0.89
spacer excl. inverted repeat	229	93 (41%) / 45 (19%)	136	0.86 / 0.82 / 0.70	not shown	-0.87
spacer, gaps as 5th base	286	159 (56%) / 77 (27%)	250	0.87 / 0.82 / 0.71	not shown	-0.94

[a] g_1 = skewness statistic.

Table 5.3. Summary of maximum likelihood analyses. For all alignments ten taxa were used. Gaps were treated as missing data. – lnL = – log likelihood.

Alignment			Tree	
Region(s)	Length	Selected model [a]	– lnL	Topology
*rbc*L + spacer	1541	TrN + G	3861.6	as Fig. 5.2
*rbc*L	1255	TrN + G	2688.1	as Fig. 5.2
spacer	286	K81uf + G	1037.1	as Fig. 5.2
spacer excl. gapped positions	216	K81uf + G	807.8	as Fig. 5.2

[a] TrN = Tamura & Nei 1993; K81uf = Kimura 1981 with unequal base frequencies; G = shape parameter of the gamma distribution.

the RUBISCO-spacer and *rbc*L separately. Alternative topologies found when analysing the RUBISCO-spacer on itself gave comparable high tree statistics (Table 5.2), but received significantly less support for most of the clades. Moreover, the *rbc*L alignment is unambiguous, unlike the RUBISCO-spacer alignment.

The fact that the current *Propagulifera* phylogeny is more resolved than in the study by Draisma *et al.* (2002 / Ch. 3) is probably caused by the inclusion *S. rigidula* 5 and not because the complete RUBISCO-spacer was used.

The RUBISCO-spacer

The palindromic region in the RUBISCO-spacer of *Sphacelaria californica* forms a stem-loop structure after transcription in which the nucleotides CTC form the loop. The tandem repeat within this region most likely is the result of a duplication event in one of the two stem regions and a subsequent compensation duplication in the other one. The function of the secondary structure is not clear. In the Phaeophyceae, as in all other chromophytes, the *rbc*L and *rbc*S are co-transcribed, which means that the RUBISCO-spacer has to be excised after transcription. As in the internal transcribed spacers of the ribosomal cistron, a particular folding pattern of the spacer could initiate this excision process. However, it seems unlikely that the observed stem-loop is a prerequisite for this process, because it is not (completely) present in the other *Propagulifera* taxa. Boczar *et al.* (1989), Hwang and Tabitas (1991), Fujiwara *et al.* (1993, 2001) also reported short palindromes in RUBISCO-spacers of other chromophytes. The function is also still unknown, but since similar palindromes were found in prokaryotes, these regions may have regulatory significance (Boczar *et al.* 1989).

Paraphyly

Kitayama (1994) performed a phylogenetic analysis of all eight species of *Propagulifera* occurring in Japan (representing the sections *Furcigerae* and *Tribuloides*), using thirteen morphological characters. *Sphacelaria plumigera* Holmes (not a member of the *Propagulifera*) was used as outgroup. Kitayama presented two

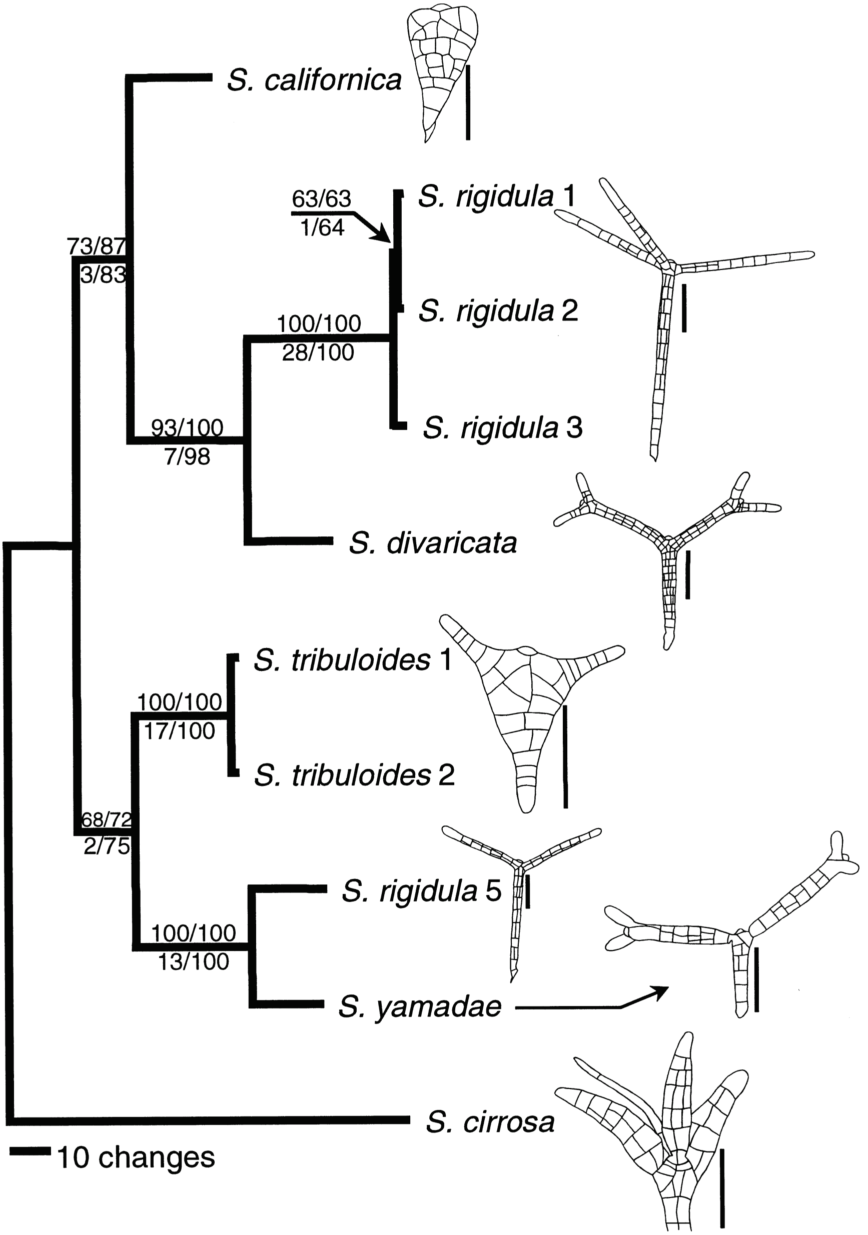


Fig. 5.2. Phylogeny of the *Sphacelaria* subgenus *Propagulifera* based on *rbc*L + RUBISCO-spacer sequences. Single most parsimonious tree. Tree statistics are presented in Table 5.2. Branch lengths are proportional to the number of nucleotide changes. Bootstrap percentages are given above branches, MP left and ML right; below branches the decay index values (left) and parsimony jackknife percentages (right) are given. Propagule morphology for each species is given next to taxon names with scale bars indicating 100 • m. These propagules were redrawn from Prud'homme van Reine 1982 (*S. cirrosa*, fig. 603, p. 233) and from Kitayama 1994 (*S. californica*, fig. 8A, p. 53; *S. divaricata*, fig. 35E, p. 88; *S. rigidula* fig. 24D, p. 76 [3 arms] and fig. 25A, p. 77 [2 arms, as *S. furcigera* holotype]; *S. tribuloides* fig. 14B, p. 62; *S. yamadae* fig. 50E, p. 107).

cladograms depicting alternative hypotheses, "using the principle of parsimony". However, the length of one tree was 18 steps, the length of the other tree was 21 steps. In the longest tree, the ingroup consisted of the monophyletic *Furcigerae* and the monophyletic *Tribuloides*. These two clades were defined by characteristics of the propagules. The shortest tree, however, differed from the longest tree in the position of *S. californica*, which was now basal to all other ingroup taxa. The separation of *S. californica* from the other *Propagulifera* was based on characters not related to the propagules.

In contrast to the morphology-based phylogeny of Kitayama (1994), the present molecular phylogeny clearly shows that the *Furcigerae* and *Tribuloides* are paraphyletic. There is no correlation between propagule morphology and the clade in which it occurs, indicating that this traditional morphological character is not phylogenetically informative within the *Propagulifera*. However, the driving selective forces behind the process of morphological convergence are not clear. Sequence divergences are relatively high (3–4% in the *rbc*L and 13–21% in the spacer).

Geographic disjunction

Two *S. tribuloides* specimens were compared from the Atlantic (Caribbean) and the Pacific (Japan). *Sphacelaria tribuloides* is a tropical to warm temperate species and cannot survive below 10 °C (Kitayama 1994). Therefore, the Atlantic and the Indo-Pacific populations must have been isolated from each other since the closure of the Isthmus of Panama 4–3 Ma ago (Lüning 1990, de Jong 1998), because seawater temperatures are below 10 °C around the southern and northern tips of the continents. However, the low genetic divergence between their *rbc*L genes (0.3%) and identical spacer sequences do not reflect such a long separation time. This is in contrast to comparable observations in another sphacelarialean species *Halopteris filicina* (Grateloup) Kützing (Draisma *et al.* 2002 / Ch. 3), where genetic divergence between an Atlantic and a Pacific isolate was 4.6% in *rbc*L. Invoking a human introduction for *S. tribuloides* in one of the ocean basins is a possible explanation, but there is no additional evidence to support this hypothesis.

Sphacelaria rigidula is clearly paraphyletic and can be found in each of the *Furcigerae* clades. Specimen 5 from the Cape Verde Islands is only very distantly related to the other specimens from Ireland (1), Korea (2) and Japan (3). The two entities obviously represent two separate cryptic species. Y.-S. Keum (personal communication) found specimens of both haplotypes in Korea, which means that the haplotype belonging to specimens 1, 2, and 3 (further referred to as type A) and to specimen 5 (further referred to as type B) are present in both the Atlantic and the Pacific Ocean. Y.-S. Keum (pers. comm.) also observed that the propagules of type A specimens are 2, 3 and/or 4-armed and that those of type B specimens never have more than 2 arms. Furthermore, in Korea specimens with bi-, tri- and quadri-furcate propagules occur intertidally and specimens with only bifurcate propagules occur subtidally (Y.-S. Keum pers. comm.). Re-examination of the *S. rigidula* voucher specimens and live cultures revealed that within type A, Specimen 1 did not have any propagules, Specimen 2 exclusively had propagules with two arms, and Specimen 3 had propagules with three or four arms. The microscopic voucher slide of Specimen 5 was badly preserved and showed only one single propagule with two arms and its live culture did not bear propagules at the moment of re-examination. The present results are not in conflict with the findings of Y.-S. Keum, but neither can these results confirm her conclusion.

Currently, morphological species boundaries of type A and type B cannot be defined. However, Y.-S. Keum was able to distinguish *S. rigidula* specimens with 2-armed propagules from specimens with 2–4 -armed propagules using morphological characters in a principle component analysis and a cluster analysis (Keum *et al.* 1997). Therefore, the search for diagnostic morphological characters of each haplotype may not be a lost cause. A combined molecular and morphological study is needed to assess the morphological limits of type A and type B. The single-stranded conformation polymorphism (SSCP) (Orita *et al.* 1989) may be a suitable method to determine the haplotypes, because it is easy to develop and less costly. It has been successfully applied for differentiating plastid haplotypes (RUBISCO-spacer variation) in red algae (Zuccarello *et al.* 1999a, b, 2000, 2001). Finally, it is recommended that morphological data from sporophytes be separated from morphological data from gametophytes. This is because a difference in robustness between sporophytes and gametophytes has been observed in specimens of *S. rigidula* (as *S. furcigera* Kützing) from The Netherlands (van den Hoek and Flinterman 1968).

Nomenclatural considerations

What species names should be attributed to the types A and B under the assumption that type B only has 2-armed propagules and type A has 2-, 3-, and/or 4-armed propagules? Kützing described *S. rigidula* in 1843 without propagules and in 1855 *S. furcigera* with propagules. However, Prud'homme van Reine (1982) observed 2-armed propagules in the type specimen of *S. rigidula* and consequently synonymised *Sphacelaria furcigera* Kützing 1855 with *S. rigidula* Kützing 1843. The type material of *S. rigidula* and *S. furcigera*, both collected in the tropical Red Sea, contains only few propagules, all with bifurcating propagules. In the future, the name *S. rigidula* should probably be used for the ones with strictly bifurcating propagules, presumably having a tropical to warm-temperate distribution. The names *Sphacelaria fusca* (Hudson) F.S. Gray 1821, *Sphacelaria variabilis* Sauvageau 1900 and *Sphacelaria subfusca* Setchell *et* N.L. Gardner 1924 are eligible for the species with bi-, tri- and quadri-furcating propagules. *Sphacelaria fusca* has priority over the other two names because it is the oldest name. Unfortunately, there is no good holotype material available for *S. fusca* or for *S. variabilis*. This is not an argument in favour of *S. subfusca* over *S. fusca* but simply an irreconcilable state of affairs. *Sphacelaria fusca* must be typified by the neotype proposed by de Haas-Niekerk (1965), which is different from the neotype proposed by Prud'homme van Reine (1982). More discussions about differences between *S. rigidula* (as *S. furcigera*) and *S. fusca* can be found in Irvine (1956), de Haas-Niekerk (1965), van den Hoek and Flinterman (1968), and Goodband (1971). The supposed difference between *S. rigidula* from temperate areas and *S. fusca* might be based on the difference in robustness of the sporophyte and gametophyte (Prud'homme van Reine 1982).

Conclusion

Within *Sphacelaria,* the presence of propagules is synapomorphic for the subgenus *Propagulifera* and defines it as a monophyletic group. Differences in propagule morphology are useful for identification of species (with the exception of the cryptic *S. rigidula*) but are not phylogenetically informative. It is, therefore, recommended that the sections *Furcigerae* and *Tribuloides* either no longer be recognised or that they be merged into one section.

Acknowledgements

This work was supported by grant No. 805-42.025 from the Foundation of Earth- and Life-Sciences (ALW), which is subsidised by the Netherlands Organisation for the Advancement of Research (NWO). The following persons are thanked for supplying algae: S. Kawaguchi, Y.-S. Keum, T. Kitayama, H. Pakker and H.J. van der Strate.

Chapter 6

THE SPECIES OF *SPHACELARIA* (SPHACELARIALES, PHAEOPHYCEAE) IN CHINA WITH A DESCRIPTION OF A NEW SPECIES [1]

This chapter deals with a study of a herbarium collection of marine *Sphacelaria* specimens, gathered from the Chinese coast. One freshwater *Sphacelaria* species and seven marine species had been previously recorded for China. However, of these seven marine species, three species are considered synonyms. According to our opinion only five marine *Sphacelaria* species had been previously recorded for China. In the Chinese herbarium collection examined seven marine species of *Sphacelaria* were found, *S. rigidula*, *S. tribuloides*, *S. novae-hollandiae*, *S. californica* and *S. carolinensis* being previously recorded in this region. The two newly recorded species are *Sphacelaria divaricata* and a new species, *S. tsengii*, that was found on subtidal rocks at two locations in southern China. *Sphacelaria tsengii* is characterised by long propagules with a tapering stalk and usually two tapering arms. According to this collection, *S. rigidula* and *S. tribuloides* are the dominant *Sphacelaria* species in respectively the north and the south of China. *Sphacelaria rigidula* and *S. divaricata* have a wide latitudinal range, while *S. californica* has only been found in Fujian and Zhejiang. The distribution of the other species is restricted to the south. Structural characters are discussed and the holotype of *S. carolinensis* is re-examined. It is suggested that *S. carolinensis* should be placed in the *Sphacelaria* section *Furcigerae* instead of in the section *Tribuloides*. A dichotomous identification key for the *Sphacelaria* species of China is given.

INTRODUCTION

The genus *Sphacelaria* was established by Lyngbye in 1818 (in Hornemann 1818). Since then it has been studied by various phycologists, especially Reinke (1890, 1891), Sauvageau (1900–1914), Prud'homme van Reine (1982) and Kitayama (1994). The genus is characterised by the hemiblastic branching mode for laterals and by parenchymatic secondary segments in its filaments (Prud'homme van Reine 1982). Worldwide over 100 species have been described, but many were put into synonymy (Prud'homme van Reine 1982). At present 46 species are recognised in the genus *Sphacelaria* which are listed in a table by Prud'homme van Reine (1993). Of these, 39 species are distributed over six subgenera, including six species with a still uncertain

---

[1] *Also published as:*
S.G.A. Draisma, Y.-S. Keum, W.F. Prud'homme van Reine & G.M. Lokhorst 1998. The species of *Sphacelaria* (Sphacelariales, Phaeophyceae) in China with a description of a new species. *Botanica Marina* 181–190.

taxonomic position. The remaining seven species are insufficiently known to place them in any of these subgenera. The subgenus *Propagulifera* Prud'homme van Reine 1982 incorporates the largest number of species (18) and also shows the widest geographic distribution of all subgenera in *Sphacelaria*. Some species within this subgenus are cosmopolitan and occur in tropical as well as in temperate waters, while others are restricted to much smaller distribution areas (Prud'homme van Reine 1993). The subgenus *Propagulifera* is especially characterised by the formation of propagules. Their shape plays a significant role in distinguishing species of this subgenus. Additional characters are the diameter of the main axes, the number of cells that can be observed in lateral surface view of the main axes, the branching mode, the shape and size of unilocular and of plurilocular zoidangia (Prud'homme van Reine 1982, Kitayama 1994). The subgenus *Propagulifera* is subdivided into three sections; section *Propagulifera*, section *Furcigerae* and section *Tribuloides* (Prud'homme van Reine 1982), the latter section is subdivided into the two series *Tribuloides* and *Humeratae* (Prud'homme van Reine 1993).

The present study was carried out on a herbarium collection of marine *Sphacelaria* specimens, collected along the Chinese coast. Eight species of *Sphacelaria* were previously recorded from China. Of these *S. fluviatilis*, a freshwater brown alga, was discovered by Jao (1943) in the Kialing River, Szechwan, west China and cannot be placed in one of the subgenera of the genus *Sphacelaria*. The other seven previously recorded *Sphacelaria* species all belong to the subgenus *Propagulifera*. Noda (1971) recorded *S. subfusca* Setchell *et* Gardner from north-eastern China, while Lu and Tseng (1980) recorded five species from the Xisha Islands (Paracel Islands) in the South China Sea: *S. tribuloides* Meneghini, *S. novae-hollandiae* Sonder, *S. furcigera* Kützing, *S. fusca* (Hudson) C. Agardh and *S. carolinensis* Trono. However, according to Prud'homme van Reine (1982) and Kitayama (1994) *S. furcigera* and *S. subfusca* are synonyms of *S. rigidula* Kützing and the taxonomic status of *S. fusca* is uncertain. *Sphacelaria fusca* only differs from *S. rigidula* in the diameter of its main axis, but in the distal parts these differences are often not discernible (Prud'homme van Reine 1982). Kitayama *et al.* (1995) have recently recorded *S. californica* Sauvageau *ex* Setchell *et* Gardner from the Zhoushan Islands in the Zhejiang Province.
According to our opinion only six *Sphacelaria* species have been recorded for China so far: *S. fluviatilis*, *S. tribuloides*, *S. novae-hollandiae*, *S. carolinensis*, *S. rigidula* and *S. californica*.

In the Chinese herbarium collection used for this study, one species cannot be assigned to any previously described *Sphacelaria* species and therefore it is described as a new species. Comparative structural analyses are provided and comments are given on the reliability of characters used to separate sections, series and species of the *Sphacelaria* subgenus *Propagulifera*. A dichotomous identification key for the *Sphacelaria* species of China is given.

MATERIALS AND METHODS

In total 372 *Sphacelaria* plants from the herbarium of the Institute of Oceanology, Chinese Academy of Sciences, Qingdao, China, were examined. Specimens were given a number that starts with the letters ‘AST’. The same abbreviation, however, refers to another herbarium (Holmgren *et al.* 1990). The algae were collected from intertidal and subtidal rocks or coral reefs as well as from other plants in the period between 1950 and 1992. After 1965, however, not many plants had been added. The locations from which the plants were collected are shown in Figure 6.1. For light microscopic observations, plants were soaked in a saturated solution of detergent to resume their original form. If no propagules were found, the material was identified as *Sphacelaria* spp. All plants with *Sphacelaria rigidula*-like propagules were identified as *S. rigidula* if trifurcate or quadrifurcate propagules were found and as *S. cf. rigidula* if only bifurcate propagules were found, because an unbranched bifurcate propagule can also be an immature propagule of *S. divaricata* Montagne or of *S. solitaria* (Pringsheim) Kylin.

Permanent microscope slides were made by mounting plant parts in 50 or 70% aqueous Karo corn syrup. Sets of permanent slides will be deposited in the *Nationaal Herbarium Nederland - Universiteit Leiden branch*, The Netherlands.

Fig. 6.1. Map of China, showing the coastal provinces and districts where *Sphacelaria* specimens were collected.

RESULTS

Seven *Sphacelaria* species were found in the Qingdao herbarium collection (Table 6.1). Occasionally two species were recognised in one sample. The occurrence of different individuals of the same species in one sample was not excluded, because in four samples unilocular and plurilocular zoidangia were found together. This was the case in *S. rigidula* specimens from Zhejiang (one collection, February '54), Qingdao (two collections, April '57 and '64) and Shandong (April '53). Four *S. rigidula* specimens from Shandong (April '51 and June '56, '57 and '63) and one *S. divaricata* specimen from Guangdong (March '56) had unilocular zoidangia. In one *S. rigidula* specimen from Shandong only plurilocular zoidangia were found (November '74).

Because of differences in the form of the propagules, *Sphacelaria tsengii* is described here as a new species. Details are shown in Figures 6.2–6.18. For the descriptive terminology used see Prud'homme van Reine (1982) and Kitayama (1994).

Sphacelaria tsengii S.G.A. Draisma, Y.-S. Keum, W.F. Prud'homme van Reine *et* G.M. Lokhorst, *sp. nov.*

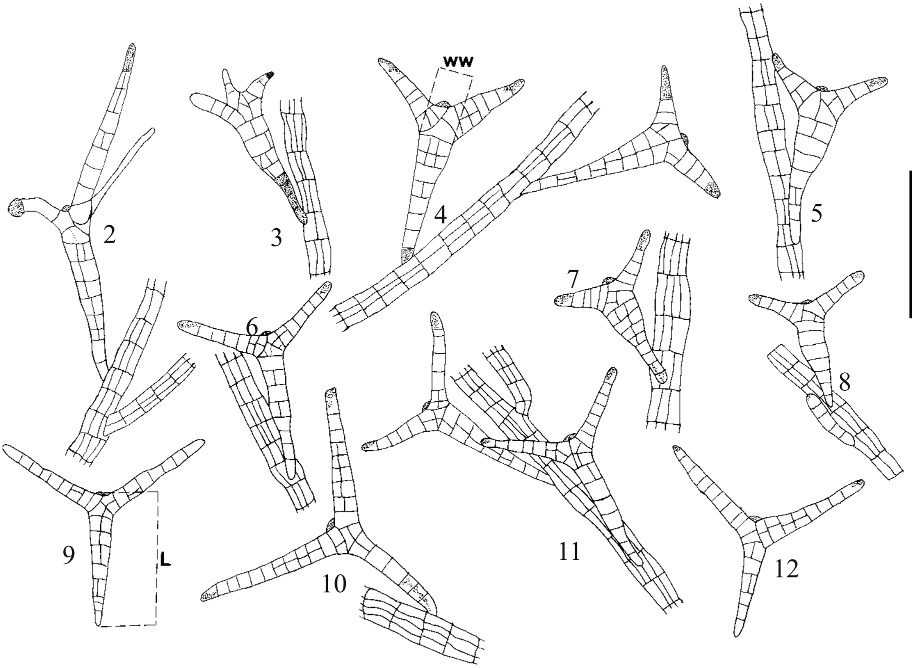
Diagnosis: *Sphacelaria caespites in saxis subliteralibus formans filamentis erectis cylindricis 30 ad 70 • m diametro, ramis lateralibus indeterminatis filamentis erectis similibus. Ramificatio irregularis. Diameter axicum principalium segmentis secundis 0.75– ad 1.15– plo longior. Segmentes parietibus longitudinalibus 2 ad 5. Propagula 160–(205–260)–380 • m longa, 40 ad 75 • m lata in centro, brachiis angustatis 2 (interdum 3).*

Plants form tufts on subtidal rocks, up to 1.5 cm high. Three types of holdfast elements can be present in one plant; polystromatic discs, stolons and rhizoids. Erect filaments are cylindrical, 30–70 • m in diameter. Lateral branches are indeterminate, not differing in morphology from erect filaments. Branching is irregular and more frequent in the upper parts of the plant. Secondary segments are 30–67 • m in length. Diameters of the main axes are 0.75–1.15 • the length of the secondary segments. The segments are radially divided; 2–5 longitudinal cell walls can be observed in lateral surface view. Secondary transverse cell walls are extremely scarce. Phaeophycean hairs are sometimes abundant and are 14–20 • m in diameter. Propagules are 160–380 • m in length (the most usual size range is 205–260 • m), 40–75 • m in width of waist. Stalks of the propagules taper towards their bases, from base to waist 6–8 secondary segments are discernible. Propagules have 2 (occasionally 3) tapering arms, each 4–8 secondary segments in length. Propagules are more frequent in the upper parts of filaments. Unilocular zoidangia occur in the lower parts of filaments, are spherical to oval in shape, 35–40 • m in length, 30–37 • m in width, and arise solitary on one-celled stalks. Plurilocular zoidangia have not been found.

Holotype: (AST 76-0283) Yaxian, Hainan Province, 18 February 1976, C.K. Tseng and T. Zhou. The holotype is preserved in the Herbarium of the Institute of Oceanology, Qingdao, China and isotypes in the *Nationaal Herbarium Nederland - Universiteit Leiden branch*, The Netherlands.

Table 6.1. List of Chinese *Sphacelaria* species found in the different provinces or districts, indicating their abundance. The first number between brackets behind the province/district name indicates the number of collections in that region. A second number between brackets indicates the number of specimens determined from these collections. The percentages given between brackets behind the number of species per province is proportional to the number of specimens found, not to the number of collections. Qingdao is an administrative part of Shandong. The Xisha Islands and the Nansha Islands are under administration of Guangdong.

	Taxa								
Province	*S. spp.*	*S. rigidula*	*S. cf. rigidula*	*S. divaricata*	*S. tsengii*	*S. carolinensis*	*S. tribuloides*	*S. novae-hollandiae*	*S. californica*
Liaoning (29)	4 (14 %)	23 (79 %)	2 (7 %)						
Shandong (100) (101)	3 (3 %)	97 (96 %)		1 (1 %)					
Qingdao (144)	21 (15 %)	114 (79 %)	9 (6 %)						
Zhejiang (16)		15 (94 %)		1 (6 %)					
Fujian (18)	3 (17 %)	9 (50 %)	3 (17 %)	2 (11 %)					1 (6 %)
Guangdong (17)	4 (24 %)	1 (6 %)		2 (12 %)	4 (24 %)		6 (36 %)		
Guangxi (6)							5 (83 %)	1 (17 %)	
Hainan (20) (22)	2 (9 %)	6 (27 %)			2 (9 %)		12 (55 %)		
Xisha Isl. (23) (27)	1 (4 %)	1 (4 %)				2 (7 %)	19 (70 %)	4 (15 %)	
Nansha Isl. (1) (2)		1 (50 %)					1 (50 %)		



Figs 6.2–12. *Sphacelaria tsengii* Draisma *et al.*, *sp. nov.* Various propagules from several collections; AST 55-1679 (Figs 4, 5, 7), AST 55-1862 (Fig. 3), AST 55-1901 (Fig. 10), AST 55-3308 (Fig. 2) and AST 76-0283 (Figs 6, 8, 11, 12).
L, length of a propagule; ww, width of waist of propagule. Scale bar represents 250 • m.

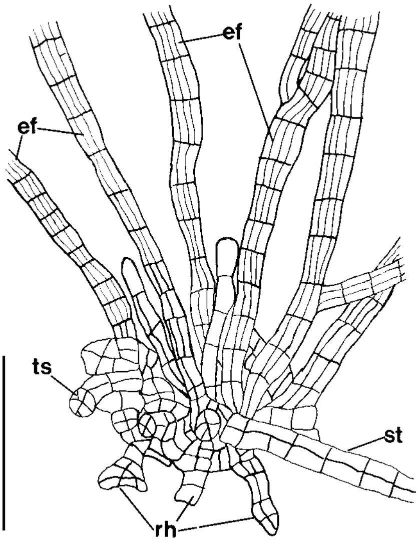


Fig 6.13. *Sphacelaria tsengii* Draisma *et al.*, *sp. nov.*, holdfast (AST 55-3308).
ef, erect filament; rh, rhizoid; st, stolon; ts, transverse section showing a radially divided segment. Scale bar represents 250 • m.

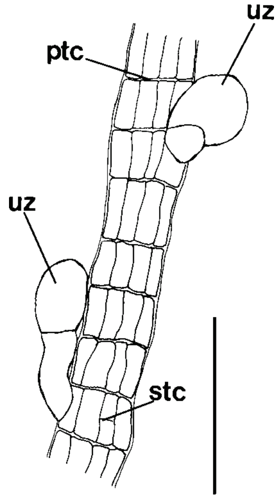


Fig 6.14. *Sphacelaria tsengii* Draisma *et al.*, *sp. nov.*, part of a main axis in the holotype (AST 76-0283) bearing two unilocular zoidangia. ptc, primary transverse cell wall; stc, secondary transverse cell wall; uz, unilocular zoidangia. Scale bar represents 100 • m.

Fig. 6.15. *Sphacelaria tsengii* Draisma *et al.*, *sp. nov.*, holotype (AST 76-0283); habit. Scale bar represents 1 cm.

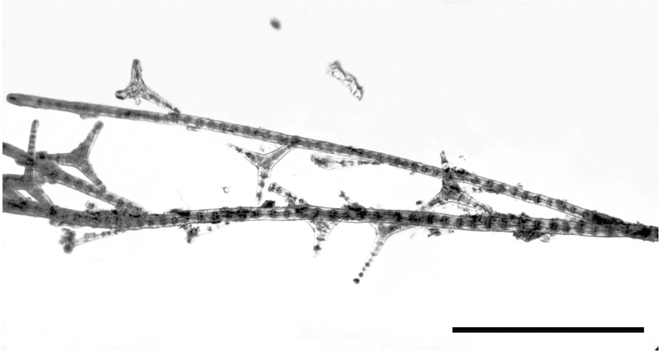


Fig. 6.16. *Sphacelaria tsengii* Draisma *et al.*, *sp. nov.*, holotype (AST 76-0283); propagules on filaments. Scale bar represents 500 • m.

The name *S. tsengii* honours Prof. Dr C.K. Tseng of the Institute of Oceanology in Qingdao, who is one of the main promoters of marine phycology in China and one of the collectors of the holotype specimen.

Paratypes: From Naozhoudao, Guangdong Province: AST 55-1679 (8 April 1955, C.F. Chang and F.L. Xu), AST 55-1862 (27 April 1955, S.D. Zheng), AST 55-1901 (28 April 1955, F.L. Xu) and AST 55-3308 (2 December 1955, S.D. Zheng). From Yaxian, Hainan Province: AST 62-2436 (20 March 1962, S.D. Zheng).

Geographical distribution: *S. tsengii* has been found in two localities in the south of China; Yaxian (Hainan) and Naozhoudao (Guangdong).

There is a distinct variation in the forms of the propagules of Sphacelaria *tsengii*. Some propagules are slender and have long arms (Figs 6.11 and 6.12), others show a somewhat thicker waist and short arms (Figs 6.5 and 6.7). In each of the samples AST 55-1862 and AST 55-3308 one three-armed propagule was found (Figs 6.2 and 6.3). In Naozhoudao four other *Sphacelaria* specimens were collected, three were identified as *S. tribuloides* and one did not have any propagules. In Yaxian seven other specimens were collected, five were identified as *S. tribuloides*, one as *S. rigidula* and one did not bear propagules.

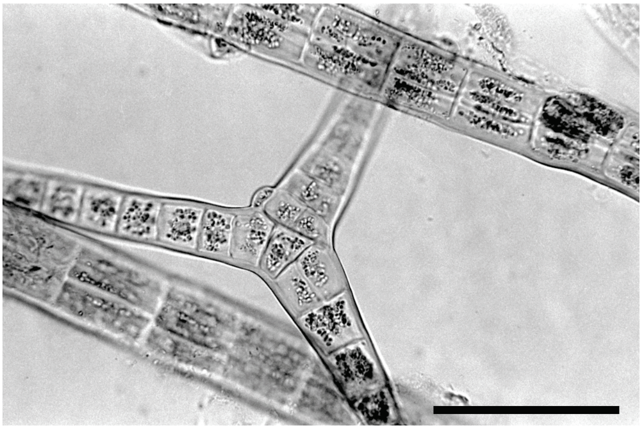


Fig. 6.17. *Sphacelaria tsengii* Draisma *et al.*, *sp. nov.*, holotype (AST 76-0283); propagule. Scale bar represents 100 • m.

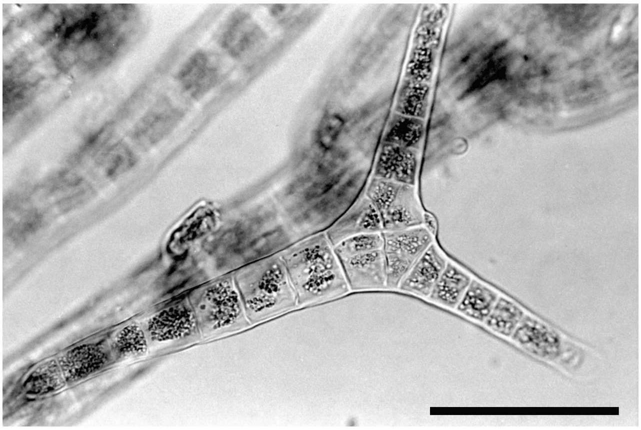


Fig. 6.18. *Sphacelaria tsengii* Draisma *et al.*, *sp. nov.*, holotype (AST 76-0283); propagule. Scale bar represents 100 • m.

In two samples from the Xisha Islands (AST 76-0624 from Zhongjiandao and AST 76-0804 from Jinyindao) that were initially identified as *S. tribuloides*, unattached propagules were found reminiscent of *S. carolinensis* (Fig. 6.19). These propagules, however, differ in detail from the branching mode of *S. carolinensis* as described by Trono (1971). In the type collection slide (BISH D21947.1) of *S. carolinensis* similar propagules occur as well and thus identification of these Chinese specimens is unambiguous.

A list of character states and ranges of measurements of diameters of main axes, length of propagules, etc., is given in Table 6.2.

In the Xisha Islands by far the most *Sphacelaria* specimens were collected from coral reefs. In the southernmost provinces Guangdong, Guangxi and Hainan most plants were collected from rocks. In the other provinces they were mainly collected from rocks and from *Sargassum thunbergii* (Mertens) O. Kuntze. Other substrates were *Sargassum confusum* C. Agardh (4•), *Sargassum pallidum* (Turner) C. Agardh

(1•), *Sargassum polycystum* C. Agardh (1•), *Sargassum* spp. (6•), *Chondria* spp. (1•), *Codium* spp. (1•), *Corallina* spp. (3•), *Dictyopteris divaricata* (Okamura) Okamura (1•), *Scytosiphon* spp. (1•), *Symphiocladia* spp. (1•), *Zostera* spp. (2•), an oyster (1•) and mud (1•).

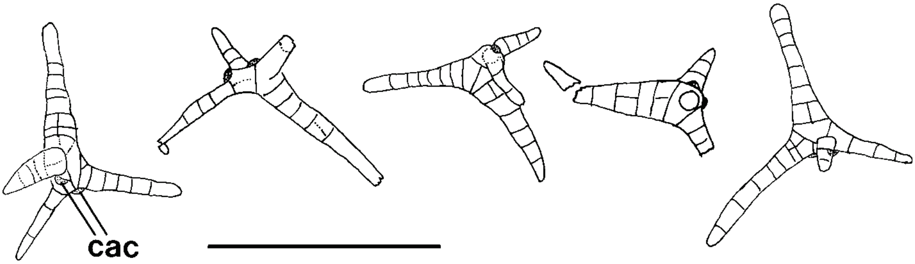


Fig. 6.19. *Sphacelaria carolinensis* Trono from the Xisha Islands. Several propagules showing an asymmetrical branching mode. Two from AST 76-0624 (left) and three from AST 76-0804 (right).
cac, central apical cell. Scale bar represents 250 • m.

DISCUSSION

Distribution

From the *Sphacelaria* species that were previously recorded from China, only *S. fluviatilis* was not found in the herbarium collection under study. This was not expected because only marine algae were collected. Two species were not recorded for China before, *viz. S. divaricata* and the newly described species *S. tsengii*.

Only *S. rigidula* was found along the entire Chinese coast from Liaoning in the north to the Xisha Islands in the south. Plants with unilocular or plurilocular zoidangia, however, were not found south of Zhejiang. *Sphacelaria rigidula* is the dominant *Sphacelaria* species from Liaoning to Fujian.

Guangdong is the most northern province where *S. tribuloides* occurs and from here to the south it is the dominant *Sphacelaria* species. That *S. tribuloides* has not been found north of Guangdong is peculiar since it has been found in Japan (Kitayama 1994) and Korea. The *S. tribuloides* from Korea recorded by Lee and Lee (1987) is actually *S. californica*. According to Kitayama (1994) *S. tribuloides* can not grow at less than 10 °C. It has only been found in the south of Japan; *viz.* on Kyushu Island and on Okinawa. In Korea it was found on Cheju Island, which is in the south (Keum, unpublished observation). Here the temperature of the surface water in January does not reach below 10 °C. In Liaoning and Shandong however, the sea water can get colder than 10 °C and that might be the reason why *S. tribuloides* does not occur in the northern provinces of China. But according to its temperature boundaries, *S. tribuloides* could be expected to be found in Zhejiang and Fujian. Insufficient collecting or a less suitable coastline may be the reason for its absence.

Table 6.2. Morphological characters of the Chinese species of *Sphacelaria* subgenus *Propagulifera*.

Character	Taxa *S. rigidula*	*S. divaricata*	*S. tsengii*	*S. carolinensis* [1]	*S. tribuloides*	*S. novae-hollandiae*	*S. californica* [2]
Erect filaments							
Branching mode	irregular [3]	irregular	irregular	?	irregular	irregular	partly irregular pinnate
Diameter of main axes (in • m)	30–70	30–45	30–70	?	40–80	45–65	25–50
Length of secondary segments (in • m)	25–60	30–35	30–65	?	30–45	40–70	25–55
Number of cells in surface lateral view	2–6	2–4	2–6	?	2–6	2–5	3–6
Secondary transverse cell walls	few	not found	extremely scarce	?	not found	not found	few
Propagules							
Length (in • m)	215–350	230–360	165–380	150–185	100–130	105–155	135–200
Width of waist (in • m)	25–40	30–40	40–75	30–45	40–60	50–70	50–70
Shape of arms	long and cylindrical	long and cylindrical	long or short tapering arms	long and short cylindrical or tapering arms	short conical	rudimentary	rudimentary
Branching of arms [4]	no	symmetrical	no	asymmetrical	no	no	no
Division of subapical primary segment	equal and unequal	unequal	equal and unequal	unequal	equal (occasionally unequal)	equal	equal
Unilocular zoidangia							
Length • width (in • m)	100–135 • 100–125	50–65 • 35–50 [5]	35–40 • 30–35 [5]	?	?	?	?

[1] Only propagules found in the present study.
[2] Observations from a single specimen only.
[3] One specimen (AST 57-2389 from Shandong) was pinnately branched.
[4] Branching in early mature stage of propagule. In *S. rigidula* propagules, arms branch later symmetrical or asymmetrical.
[5] Measurements from one specimen (*S. divaricata* AST 56-2970 and *S. tsengii* AST 76-0283) with probably young unilocular zoidangia.

Sphacelaria divaricata has a wide latitudinal range (from Shandong to Guangdong), though it is much less frequent than *S. rigidula*. *Sphacelaria novae-hollandiae*, *S. carolinensis* and *S. tsengii* seem to be of typical southern occurrence; they have not been recorded north of Guangdong. *Sphacelaria tsengii* is thought to be endemic for southern China and *S. carolinensis* has only been collected in the Caroline Islands (Federated States of Micronesia) (Trono 1971) and the Xisha Islands (Lu and Tseng 1980, this study).

Sphacelaria novae-hollandiae has a cosmopolitan distribution in tropical and warm temperate waters (Prud'homme van Reine 1993), but has nowhere in the world been recorded at a higher latitude than Guangdong. Of *S. californica*, only one specimen has been found in Fujian (this study), and one in Zhejiang (Kitayama *et al.* 1995), the only two records for China. Drifting *S. californica* plants have been found on the east coast of Korea by Boo and Choi (1986). Lee and Lee (1987) collected attached *S. californica* at Cheju Islands, which they identified as *S. tribuloides*. Attached *S. californica* plants have been collected on the east and south coast of Korea. In Japan it has been found on the islands Shikoku and Honshu (Kitayama 1994) and in the Sedo Inland Sea.

Status of *Sphacelaria californica vs. Sphacelaria novae-hollandiae*

Sphacelaria californica and *S. novae-hollandiae* resemble each other in having cymose unilocular sporangia and tribuliform propagules with occasional divided lateral apical cells (Kitayama *et al.* 1991). However, *S. californica* has irregular pinnate ramifications and rather frequent secondary transverse cell walls. The *S. californica* drawn by Abbott and Hollenberg (1976), on the other hand, shows very irregular pinnate branching and very few secondary transverse cell walls. The *S. californica* from Fujian was in some parts of the plant irregularly pinnately branched and had only few secondary transverse cell walls. A culture experiment by Kitayama *et al.* (1991) revealed that *S. californica* plants from Japan had many secondary transverse cell walls when exposed to 10 and 15 °C temperatures and very few of these when exposed to 20 °C. Since *S. californica* seems to be restricted to the north and *S. novae-hollandiae* to the south, these two species might be considered to represent two growth forms of a single species as Prud'homme van Reine already supposed in 1993. However, personal observations on herbarium specimens from the Herbarium of the University of Adelaide (ADU) that were previously identified as *S. novae-hollandiae* by Womersley (1987) revealed that *S. californica* and *S. novae-hollandiae* both occur in south Australia. The distribution of *S. californica* is restricted to the Pacific (Kitayama 1994). Keum *et al.* (1999) suggest that *S. californica* and *S. novae-hollandiae* can be separated by the way the lateral apical cells of the propagules are formed. In *S. californica* the cells establishing the shoulders of propagules are usually formed by oblique walls, while in *S. novae-hollandiae* these cells are formed by perpendicular walls and more divisions of the upper cells. However, the Chinese *S. novae-hollandiae* and *S. californica* specimens did not bear enough propagules to confirm this hypothesis.

Classification of *Sphacelaria tsengii*

Some of the propagules of the new species *S. tsengii* resemble the propagules of *S. nipponica* drawn by Kitayama (1994): *viz.* a thick waist and tapering horns that are shorter than the stalk. Other propagules are more similar to the propagules of *S. cornuta* Sauvageau: showing tapering horns of the same length as the stalk. The propagules of S. *tsengii*, however, are much longer than those of *S. cornuta* and the erect filaments of *S. tsengii* are much thicker. Likewise, erect filaments of *S. tsengii* are on the average thicker than those of *S. nipponica*. The propagules of *S. tsengii* do not have a constriction of the waist as in the propagules of *S. nipponica*, and secondary transverse cell walls are extremely scarce in *S. tsengii* while they often occur in *S. nipponica* (Kitayama 1994).

Sphacelaria nipponica and *S. cornuta* are both placed in the section *Tribuloides* because of the tribuliform shape of their propagules (Prud'homme van Reine 1993). However, the separation between the sections in the subgenus *Propagulifera* is not always clear. The section *Tribuloides* contains species with tribuliform propagules and the section *Furcigerae* contains species with slender propagules (Prud'homme van Reine 1993). This separation is rather artificial, since it is difficult to place species with intermediate propagule forms like *S. cornuta* and *S. nipponica* in one of these sections. For that reason Kitayama (1994) based the separation on the mode of division of subapical cells of the propagules. In the section *Tribuloides* subapical cells are supposed to divide primarily into two equal sized cells by a longitudinal cell wall which is perpendicular to the apical cell (equal or perpendicular division type, see Kitayama 1994 fig. 57A). In the section *Furcigerae* the initial cell is supposed to divide primarily into two unequal sized cells by a diagonal cell wall (unequal or diagonal division type, see Kitayama 1994 fig. 57B).

Sphacelaria tsengii bears propagules of which the external form also fits in both sections *Furcigerae* and *Tribuloides*. However, when the subapical cell division is considered, both the equal type (e.g. Figs 6.5, 6.8, and 6.18) as well as the unequal type (e.g. Figs 6.6, 6.7, 6.9, 6.10, 6.12, and 6.17) can be observed. Therefore, doubts are raised as to where to place *S. tsengii*. In the section *Furcigerae* or in the section *Tribuloides*? To solve this problem, propagules of living plants of *S. rigidula* and *S. yamadae* Segawa (section *Furcigerae*) and *S. tribuloides* (section *Tribuloides*) were examined for the subapical cell division type. Living plants were available in the extensive *Sphacelaria* culture collection in the *Nationaal Herbarium Nederland – Universiteit Leiden branch*. In the *S. rigidula* and *S. yamadae* specimens not only unequal division types were found but also equal division types (Figs 6.20 and 6.21), while in the *S. tribuloides* specimens not only the equal type was found, but also the unequal type (Fig. 6.22). According to Kitayama (1994) the equal type occurs occasionally in trifurcate propagules of *S. rigidula*, although in the present study also many bifurcate propagules of this equal type were observed. The majority of the propagules in this species, however, still is of the unequal type. In *S. yamadae* and *S. tribuloides* only few propagules are found that differed from the expected type (5%), suggesting that the proposed modes of division still may provide usable characters. However, caution must observed in using this character. Concerning *S. tsengii*, the majority of the propagules show an unequal division type and therefore it is suggested that it be placed in the section *Furcigerae*.

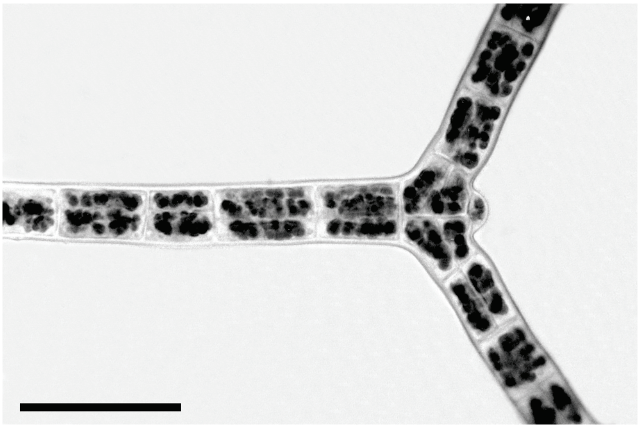


Fig. 6.20. *Sphacelaria rigidula* Kützing from Oshoro, Japan. Propagule with an equal division of the subapical primary segment.
Scale bar represents 50 • m.

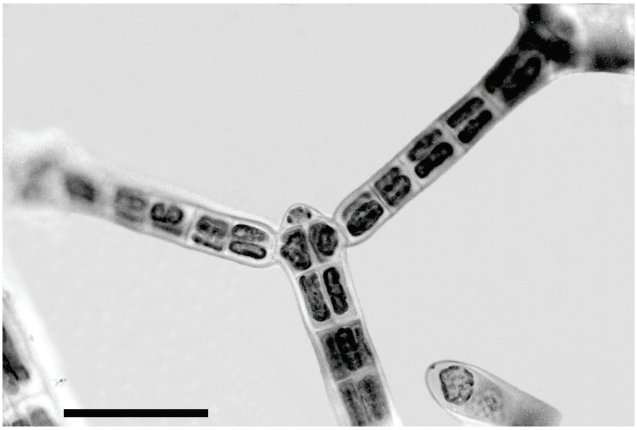


Fig. 6.21. *Sphacelaria yamadae* Segawa from Ogi, Japan. Propagule with an equal division of the subapical primary segment.
Scale bar represents 50 • m.

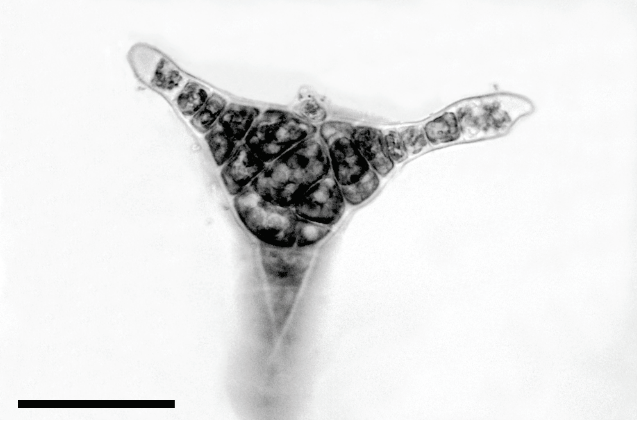
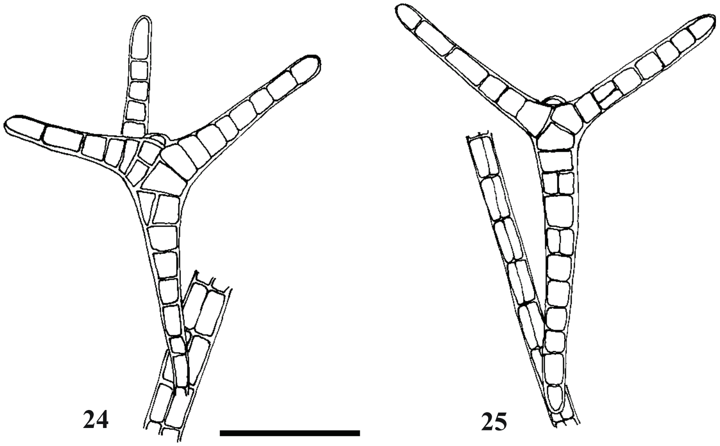


Fig. 6.22. *Sphacelaria tribuloides* Meneghini from Muroran, Japan. Propagule with an unequal division of the subapical primary segment.
Scale bar represents 50 • m.

Classification of *Sphacelaria carolinensis*

Sphacelaria carolinensis is usually placed in the section *Tribuloides* (Prud'homme van Reine 1993), but drawings by Trono (1971) and Lu and Tseng (1980) show that, according to the shape and the subapical cell division of the propagules, it fits better in the section *Furcigerae*. Almost all propagules of the type specimen of *S. carolinensis* (slide D21947.1, Herbarium of the Bernice P. Bishop Museum, Hawaii) show an unequal division type suggesting placement in the section *Furcigerae*. Trono (1971) mentions that in *S. carolinensis* propagules with three horns are of common occurrence. These so-called three-horned propagules are in fact asymmetrically branched two-horned propagules, in which one of the arms branches again immediately after formation (Fig. 6.23). These apparently trifurcate propagules thus show a branching mode similar to that in *S. solitaria* (see Kitayama 1994). In *S. solitaria*, however, the two secondary arms are only formed after the secondary segment of the primary arm (Keum *et al.* 2001), while in *S. carolinensis* they branch directly from the first subapical cell of the primary arm (Figs 6.19 and 6.23). In *S. carolinensis* the second branching is occasionally trifurcate. Based on these observations we suggest that Lu and Tseng (1980) have probably confused the figures of *S. fusca* and *S. carolinensis*, their figure 6 showing drawings of *S. carolinensis* instead of *S. fusca* (Fig. 6.24) and their figure 7 showing drawings of what might be *S. rigidula* instead of *S. carolinensis* (Fig. 6.25). The *S. fusca* and *S. carolinensis* specimens of Lu and Tseng (1980) are reported to be lost, except for sample AST 76-0846 which has been identified as containing *S. tribuloides* together with unattached propagules of *S. carolinensis*. The supposed drawing of *S. fusca* (Lu and Tseng 1980, fig. 6f), which is in fact *S. carolinensis*, is also used in Tseng (1983, pl. 102, fig. 3) and in Bing-Sheng (1989, fig. 25) to represent *S. fusca*. Moreover, in Tseng (1983) the figures of *S. tribuloides* and *S. novae-hollandiae* are interchanged.

Fig. 6.23. *Sphacelaria carolinensis* Trono. Propagule from the holotype (BISH, D21947.1) with an unequal division type. Scale bar represents 100 • m.



Figs 6.24–25. Redrawn from Lu and Tseng (1980; figs 6f and 7e). **Fig. 6.24.** *Sphacelaria fusca sensu* Lu and Tseng (1980), representing *S. carolinensis* according to the present authors. **Fig. 6.25.** *Sphacelaria carolinensis sensu* Lu and Tseng (1980), representing *S. rigidula* according to the present authors. Scale bar represents 100 • m.

KEY TO THE CHINESE SPECIES OF THE GENUS *SPHACELARIA*

1a. Plants 1–11 mm in height, epilithic in freshwater (or temporarily terrestrial); obovoid or subglobose propagation cells are directly attached to the filament or occasionally in clusters on a one-celled stalk; holdfast is not disc-shaped, but consists of stolons or rhizoids; erect filaments are irregularly branched, secondary segments are more than twice as long as the diameter of the filament, 2–4 cells can be observed in lateral surface view .... *S. fluviatilis* (*incertae sedis*)

b. Marine plants composed of a polystromatic or monostromatic disc, stolons or rhizoids, and incompletely pinnate or irregularly branched erect filaments; longitudinal segmentation is of the radial type; propagules are present; secondary transverse cell walls are usually scarce .... (subgenus *Propagulifera*) 2.

2a. Propagules are tribuliform and with or without obvious horns; divisions of propagule subapical segments are predominantly of the equal (perpendicular) type .... (section *Tribuloides*) 3.

b. Propagules with cylindrical or slender tapering stalk and arms; divisions of the propagule subapical segments are predominantly of the unequal (diagonal) type .... (section *Furcigerae*) 5.

3a. Propagules tribuliform, often with obvious horns; second divisions of the subapical primary segments are oblique to the first division, resulting in the formation of lateral apical cells .... (series *Tribuloides*) *S. tribuloides*

b. Tribuliform propagules without obvious horns; second divisions of the subapical primary segments are perpendicular to the first division, while the lateral apical cells only are formed after a third division . . . . (series *Humeratae*) 4.

4a. Branching of the plants is pinnate or partly pinnate. Secondary transverse cell walls can be observed in the filaments. Cells that establish the shoulders of the propagules are usually formed by a partitioning oblique to the cell wall that was formed during second division of the subapical primary segment *S. californica*

b. Branching of the plants is irregular. No secondary transverse cell walls occur in the filaments. Cells that establish the shoulders of the propagules are usually formed by a partitioning perpendicular to the wall that was formed during the second division of the subapical primary segment. This partitioning is often followed by more divisions of the upper cell . . . . *S. novae-hollandiae*

5a. Stalks of most propagules are obviously tapering from waist towards base; propagules with 2 (occasionally 3) branched or unbranched usually tapering arms . . . . 6.

b. Stalks of most propagules have more or less the same width along the entire length; propagules with 2, 3 or 4 branched or unbranched cylindrical arms . . . . 7.

6a. Diameters of the erect filaments are 18–26 • m; 2–3 cells per segment can be observed in lateral surface view; length of the propagules is 140–210 • m, the propagules have 2 arms that often branch asymmetrically . . . . . *S. carolinensis*

b. Diameters of the erect filaments are 30–70 • m; 2–5 cells per segment can be observed in lateral surface view; propagules with 2 (occasionally 3) long (sometimes short) tapering arms, the width of the waist differs between propagules and can vary from 40 to 75 • m; length of the propagules is 160–370 • m (most usual size range is 205–260 • m) . . . . *S. tsengii*

7a. Branching of the propagules is bifurcate, trifurcate or quadrifurcate; subapical cell divisions are in most cases of the unequal (diagonal) type, but also often of the equal (perpendicular) type . . . . *S. rigidula*

b. Propagules with 2 symmetrically branching (always bifurcate) slender arms. The arms may taper slightly . . . . *S. divaricata*

Acknowledgements

We wish to thank the Herbarium of the Institute of Oceanology, Qingdao, China for lending us their *Sphacelaria* collection and the Herbarium of the Bernice P. Bishop Museum, Hawaii for lending us the type specimen of *S. carolinensis*. Thanks are also due to Hans Nooteboom, who wrote the Latin diagnosis of *Sphacelaria tsengii sp. nov.* Further we are grateful to Ben Kieft who provided part of the photographic material and to Prof. Pieter Baas for critically reading a previous draft of this manuscript.

Chapter 7

ONSLOWIACEAE *FAM. NOV.* (PHAEOPHYCEAE)[1]

We propose the separation of *Onslowia* and *Verosphacela* from the Choristocarpaceae and from the Sphacelariales based on comparisons of DNA sequences of *rbc*L and nrRNA genes and morphological considerations. The new family Onslowiaceae is created to include these two genera. The families Choristocarpaceae and Onslowiaceae are considered *incertae sedis*.

The brown algal order Sphacelariales Migula 1909 traditionally includes three families, *viz.* the monotypic Cladostephaceae Oltmanns 1922, the Sphacelariaceae Decaisne *emend.* Oltmanns 1922 (two genera), and the Stypocaulaceae Oltmanns 1922 (five genera). Members of the Sphacelariales are characterised by growth from a prominent apical cell, heterotrichy and temporary darkening of cell walls when placed in sodium hypochlorite ('eau de Javel', bleaching liquid) (Reinke 1890). The chemical reactions that cause the cell walls to turn black in bleach are not known, but have exclusively been demonstrated in sphacelarialean species (Prud'homme van Reine 1978). The prominent apical cells divide transversely to form primary segments which subsequently divide in the same manner to form secondary segments.

Fritsch (1945) added a fourth family to the Sphacelariales, namely the Choristocarpaceae Kjellman 1891, which included only one species according to Fritsch: *Choristocarpus tenellus* (Kützing) G. Zanardini 1860. Kützing (1849, 1855) (as *Ectocarpus tenellus*) and Zanardini (1860) both described this species as having a dichotomous branching mode. However, none of the drawings in later publications showed dichotomous branching in *C. tenellus*, so that reexamination of the type specimen is desirable. Kjellman (1891) had previously included *Discosporangium mesarthrocarpum* (Meneghini) Hauck 1885 in the Choristocarpaceae, while Schmidt (1937) had used this species as basis for the monotypic Discosporangiales and the Discosporangiaceae. However, Fritsch (1945) considered the taxonomic position of *D. mesarthrocarpum* to be obscure and did not include it in the Choristocarpaceae.

Searles (in Searles and Leister 1980) described a new genus and species *Onslowia endophytica* and placed it in the Sphacelariaceae rather than in the Choristocarpaceae. Prud'homme van Reine (1982) removed the Choristocarpaceae and *O. endophytica* from the Sphacelariales because they do not show transverse division of subapical cells, nor do their cell walls blacken in bleach. Wynne (1982), however, continued to include the Choristocarpaceae and *O. endophytica* in the Sphacelariales. Henry (1987b)

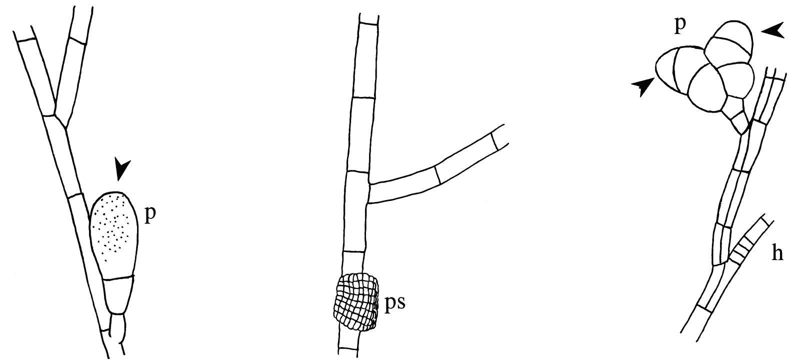
---

[1] *Also published as:*
S.G.A. Draisma & W.F. Prud'homme van Reine 2001. Onslowiaceae fam. nov. (Phaeophyceae). *Journal of Phycology* 37: 647–649.

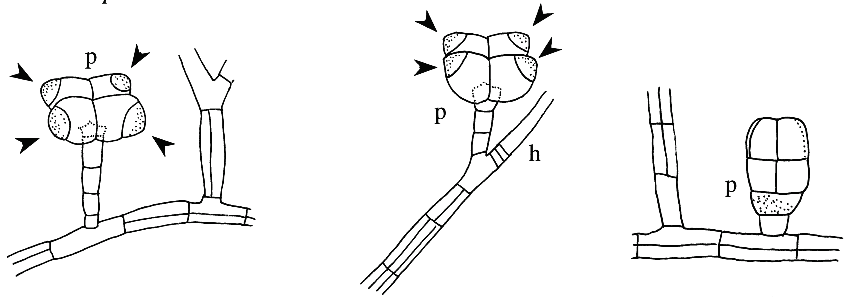
described a new species *Onslowia bahamensis* and a new monotypic genus with the species *Verosphacela ebrachia* and placed them together with *O. endophytica* in the Choristocarpaceae. Henry (1987b) also mentioned a probable additional species of Choristocarpaceae that has been illustrated as *Sphacelaria papilioniformis*, *sp. nov. ined.* without a description by Coppejans (1977, 1983). To Henry the systematic position of *D. mesarthrocarpum* again remained obscure. Nevertheless, from 1987 onward, most taxonomists have treated *D. mesarthrocarpum* as a member of the Choristocarpaceae, order Sphacelariales (e.g. Womersley 1987, Ribera *et al.* 1992, Haroun *et al.* 1993, Schneider and Searles 1998). Although skeptical, Prud'homme van Reine (1993) included the Choristocarpaceae (comprising the genera *Choristocarpus*, *Discosporangium*, *Onslowia* and *Verosphacela*) in the Sphacelariales in his review of the Sphacelariales of the world. This was mainly because no other place in the classification system of brown algae could be suggested with the result that he referred to the Sphacelariales *sensu stricto* (*s.s.*) and *sensu lato* (*s.l.*); the former excluding the Choristocarpaceae and the latter including it. De Reviers and Rousseau (1999) suggested that DNA sequences were needed in order to place enigmatic taxa such as the Choristocarpaceae and thus considered the family *incertae sedis*.

In a recent molecular phylogenetic study of the Phaeophyceae, Draisma *et al.* (2001 / Chapter 2) showed that the order Sphacelariales *s.l.* is paraphyletic. Using DNA sequences of both the large subunit of the chloroplast encoded RUBISCO-gene (*rbc*L) and partial small and large subunit of the nuclear RNA genes, they analysed all available sequences plus newly generated ones of the Phaeophyceae. Eight species of Sphacelariales were included representing all 4 families and 8 of the 12 genera. Results of that analysis revealed three clades in the *rbc*L-based tree. These included a clade containing the Sphacelariales *s.s.*, a clade containing *Onslowia endophytica* and *Verosphacela ebrachia*, and a clade containing *Choristocarpus tenellus* by itself. The third clade is located at the base of the rooted phaeophycean tree. The orders Dictyotales and Syringodermatales occurred in positions among the three clades of sphacelarialeans. In the rDNA-based tree *C. tenellus* was not separated from the Sphacelariales *s.s.*, but *O. endophytica* and *V. ebrachia* together unambiguously formed a distinct clade. These results provide unequivocal evidence that the genera *Onslowia* and *Verosphacela* do not belong to the Choristocarpaceae and neither do they belong to the Sphacelariales *s.s.* The molecular study of Draisma *et al.* (2001 / Ch. 2) did not include *Onslowia bahamensis* and *Discosporangium mesarthrocarpum*. In our opinion it is likely that *D. mesarthrocarpum* will form a fourth clade, based on the totally different structure of its plurilocular zoidangia. Until it can be demonstrated that *Discosporangium* and *Choristocarpus* do not form a monophyletic group, both genera are retained provisionally in the same family. *Choristocarpus tenellus* does not fit to the description of the Discosporangiales *sensu* Schmidt (1937), because no disk-shaped plurilocular zoidangia have been reported for *C. tenellus*. Here we propose a new family Onslowiaceae to include the genera *Onslowia* and *Verosphacela*, because the study by Draisma *et al.* (2001 / Ch. 2) showed that these genera are not monophyletic with the Choristocarpaceae. The Choristocarpaceae and Onslowiaceae remain *incertae sedis* until additional DNA sequences resolve their currently obscure systematic positions.

The Onslowiaceae differs from the Choristocarpaceae by the occasional occurrence of longitudinal cell walls in the filaments, whereas the filaments of *Choristocarpus* and *Discosporangium* are strictly uniseriate (Fig. 7.1). Furthermore, the propagules of *C. tenellus* do have a large conspicuous apical cell, whereas the propagules of the Onslowiaceae lack a large conspicuous apical cell (Fig. 7.1). Propagules have never been reported for *D. mesarthrocarpum*. The occurrence of longitudinal cell walls and propagules without a single conspicuous apical cell, suggests that *Sphacelaria papilioniformis sp. nov. ined.* as drawn by Coppejans (1977, 1983) might belong to the Onslowiaceae.



Choristocarpus tenellus *Discosporangium mesarthrocarpum* *Sphacelaria papilioniformis*



Onslowia endophytica *Onslowia bahamensis* *Verosphacela ebrachia*

Fig. 7.1. Diagrammatic representation of filaments of the species treated in this study showing branching, cell divisions and propagules, based on original drawings from Falkenberg (1878; *Discosporangium mesarthrocarpum*), Hauck (1885; *Choristocarpus tenellus* and *D. mesarthrocarpum*), Kuckuck (1895; *C. tenellus*), Fritsch (1945; *C. tenellus* and *D. mesarthrocarpum*), Searles and Leister (1980; *Onslowia endophytica*), Coppejans (1983; *C. tenellus*, *D. mesarthrocarpum* and *Sphacelaria papilioniformis*), Henry (1987a; *O. endophytica*, 1987b; *Onslowia bahamensis* and *Verosphacela ebrachia*), Womersley (1987; *D. mesarthrocarpum*), and Schneider and Searles (1998; *D. mesarthrocarpum*). Arrowheads indicate apical cells.
h, hair; p, propagule; ps, plurilocular zoidangium.

Onslowiaceae fam. nov.

Phaeophyceae marinae irregulariter ramosae filamentosae vitae rota isomorpha diplohaplontica epi- vel endophyticae, e cellulis apicalibus crescentes, cellulae subapicales sine divisione transversali, cellulae in filamentis semel vel bis parietibus longitudinalibus dividere possunt, propagulae sine cellula apicali grandi conspicua.

Irregular branched, filamentous, marine brown algae with an isomorphic diplohaplontic life cycle, growing epiphytic or endophytic. Growth by apical cells, but without transverse division of subapical cells. Cells in the filaments may each divide once or twice by longitudinal walls. Propagules without a large conspicuous apical cell.

Type genus: Onslowia Searles in Searles and Leister, *J. Phycol.* 16: 37. 1980.

Acknowledgements
We thank Dr J.-F. Veldkamp for providing the Latin diagnosis and Prof. Dr J.L. Olsen for critically reading a previous draft of this manuscript.

Chapter 8

THE PHAEOPHYCEAN *rbc*L-PHYLOGENY REVISITED [1]

INTRODUCTION

Almost simultaneously with the publication of Draisma *et al.* (2001/Chapter 2), Rousseau *et al.* (2001) published a parallel phylogeny of the Phaeophyceae based on the 3'-end of the small subunit (SSU or 18S) and the 5'-end of the large subunit (LSU or 26S) of the nuclear ribosomal cistron, in which all orders were represented. Taxa included by Rousseau *et al.* (2001) that were not included in the rDNA study of Chapter 2 were Ralfsiales, Sporochnales, Tilopteridales, and the genus *Bachelotia* (*incertae sedis*). However, they did not include the Choristocarpaceae and Onslowiaceae (both *incertae sedis*). Both studies supported the Dictyotales as the most basal brown algal order, followed by the Sphacelariales and Syringodermatales, and then a crown radiation of the remaining orders. Rousseau *et al.* (2001) also presented a phylogeny including seven brown algae based on the complete rDNA-LSU, but this did not result in a better resolved tree. The *rbc*L-based phylogeny of Draisma *et al.* (2001/Ch. 2), however, did give additional support for the sister-group relationship of the Ectocarpales and Laminariales. From this, one could expect that an analysis of *rbc*L sequences with a broader taxon sampling is able to assess more sister-group relationships within the crown radiation.

Since the publication of Draisma *et al.* (2001/Ch. 2), more than 60 additional phaeophycean *rbc*L sequences have been released through the EMBL/GenBank/DDBJ databases. These include 14 species of Scytosiphonales (currently included in the Ectocarpales) published by Kogame *et al.* (1999); sequences for Desmarestiales, Sporochnales, Tilopteridales, and all families of the Laminariales *sensu lato* (*s.l.*) published by Kawai and Sasaki (2000), Kawai *et al.* (2001a), and Sasaki *et al.* (2001); and several species belonging to the Ectocarpales *s.l.* and Scytothamnales, and the enigmatic genera *Asterocladon* and *Asteronema* (Peters and Ramírez 2001).

This chapter provides an update of the *rbc*L-based phylogeny using all currently available *rbc*L sequences and using members of the sister-classes Tribophyceae and Phaeothamniophyceae as outgroups. Kawai *et al.* (2001b) have recently proposed a new heterokont class Schizocladiophyceae and suggested that its single species, the

---

[1] *The* rbc*L-phylogeny is incorporated in a review on the recent advances in the phylogenetic research in brown algae which will be published in a special volume of* European Journal of Phycology *sponsored by the* British Phycological Society *to celebrate its jubilee. Submitted as:* A.F. Peters, R.L. Fletcher & S.G.A. Draisma. Evolution and taxonomy in the Phaeophyceae: effects of the molecular age on brown algal systematics.

new marine filamentous alga *Schizocladia ischiensis*, has a closer phylogenetic affinity with the Phaeophyceae than any other known taxa. However, Schizocladiophyceae and *S. ischiensis* have not yet been validly published and the *rbc*L sequence has not yet been released, which made it impossible to include this new species in the present update.

THE UPDATED *rbc*L-PHYLOGENY

The updated alignment of *rbc*L sequences includes 145 sequences of which 15 are outgroups from the heterokont classes Tribophyceae ($n = 9$) and Phaeothamniophyceae ($n = 6$). All taxa are presented in Figure 8.1 along with their EMBL/GenBank/DDBJ accession numbers. Taxonomic authorities are not given (except for taxa that are discussed in the text), but can be traced back with the help of the accession numbers. The new *rbc*L alignment covered 1399 nucleotides (nt) of the 1467 nt long phaeophycean *rbc*L-gene. Of the 1399 characters, 792 were variable and 623 were phylogenetically informative. There are still no *rbc*L sequences available for three orders, i.e., Ascoseirales, Cutleriales, and Ralfsiales (*nomen nudum*).

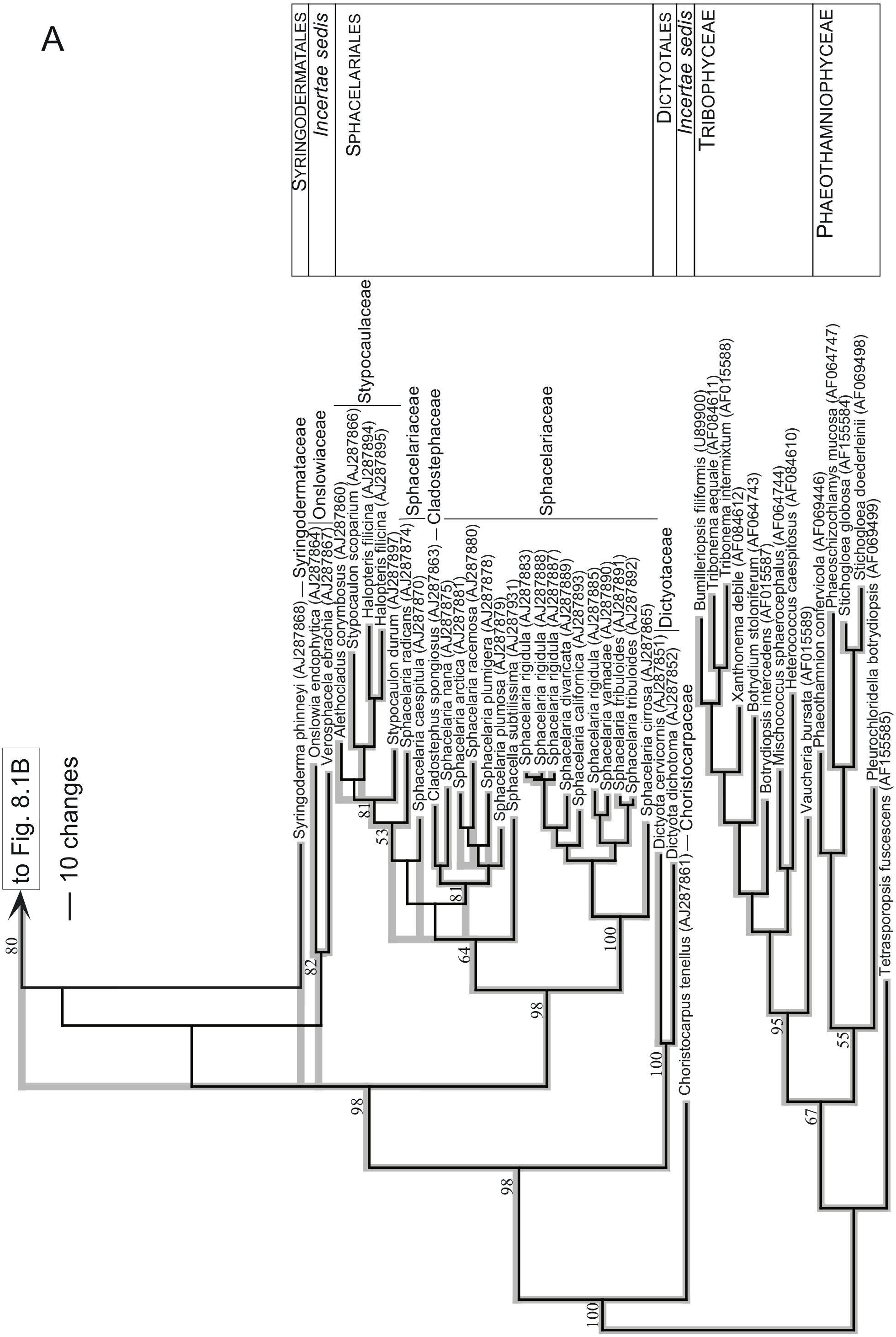
Maximum parsimony (MP) analysis of all three codon positions was performed using PAUP* version 4.0b4a (Swofford 1998) under a heuristic search, using the tree bi-section reconnection branch-swapping algorithm, with 100 random sequence additions. A maximum likelihood analysis was not possible due to the large size of the data set. Bootstrapping (Felsenstein 1985) was performed in PAUP* using 5 random sequence additions, 500 replicates, and maxtrees was set at 200. MP analysis resulted in 2,046 most parsimonious trees (MPTs) of 5,763 steps (consistency index = 0.23; retention index = 0.71; rescaled consistency index = 0.16). One of the MPTs is shown in Figure 8.1 and has the same topology as the 50% majority-rule consensus tree The family and ordinal affiliations of taxa follow the current classification. Most MPTs differed from each other within the crown of the Ectocarpales (i.e., Chordariaceae). Branches that collapsed in the strict consensus tree are marked in Figure 8.1.

CONCLUSIONS

The updated *rbc*L-based tree of the Phaeophyceae (Fig. 8.1) contained more clades with bootstrap support than the rDNA-based tree in Rousseau *et al.* (2001). The close relationship of the Laminariales, Ectocarpales, and *Asterocladon* + *Asteronema rhodochortonoides* (Børgesen) Müller *et* Parodi was not supported in their rDNA phylogeny. The rDNA data also failed to support the close relationship of the Fucales, Tilopteridales, Halosiphonaceae, and Phyllariaceae. Despite the poorer resolution of the rDNA data, the new *rbc*L phylogeny is consistent with the rDNA study of Rousseau *et al.* (2001), i.e., there are no contradicting sister-group relationships with bootstrap support. Their study also revealed the paraphyly of *Asteronema*: *A. rhodochortonoides* is closely related with *Asterocladon*, and *A. ferruginea* (Harvey) Delépine *et* Asensi with the Scytothamnales.

Choristocarpus tenellus (Kützing) Zanardini is the most basal brown alga followed by the Dictyotales. The sister-group relationships between the next three clades to branch-off, i.e., the Sphacelariales, Syringodermatales, and Onslowiaceae, were not resolved. The branching order in the crown of the Phaeophyceae tree was also not resolved (Fig. 8.1B). However, additional support that the Desmarestiales, Scytothamnales, and Sporochnales are the most basal orders within this crown, comes from the RUBISCO-spacer (see Box 8.1). A close relationship between the Desmarestiales and Sporochnales was expected (see also Fig. 1.1) and supports earlier work by Clayton (1981) that considered them as a single order.

Results of the newest analysis are basically confirmatory and do not indicate any significant shifts in ordinal-level sister-groups. Perhaps the most significant conclusion is that in order of resolving power *rbc*L still performs the best, followed by the rDNA-LSU and the rDNA-SSU. A partition homogeneity test (see Chapter 2) was not performed between *rbc*L and the rDNA-SSU and -LSU so it is not known whether or not a combined analysis would enhance the results further. Given the nearly complete taxon sampling at the ordinal level, it is doubtful that major sister-group relationships will change. However, within orders, notably the Dictyotales, Syringodermatales, Sporochnales, and Fucales, much more extensive taxon sampling is still highly desirable.


A
to Fig. 8.1B
— 10 changes
SYRINGODERMATALES
Incertae sedis
SPHACELARIALES
DICTYOTALES
Incertae sedis
TRIBOPHYCEAE
PHAEOTHAMNIOPHYCEAE
Syringoderma phinneyi (AJ287868) — Syringodermataceae
Onslowia endophytica (AJ287864)
Verosphacela ebrachia (AJ287867)
Onslowiaceae
Alethocladus corymbosus (AJ287860)
Stypocaulon scoparium (AJ287866)
Halopteris filicina (AJ287894)
Halopteris filicina (AJ287895)
Stypocaulaceae
Stypocaulon durum (AJ287897)
Sphacelaria radicans (AJ287874)
Sphacelaria caespitula (AJ287870)
Sphacelariaceae
Cladostephus spongiosus (AJ287863) — Cladostephaceae
Sphacelaria nana (AJ287875)
Sphacelaria arctica (AJ287881)
Sphacelaria racemosa (AJ287880)
Sphacelaria plumigera (AJ287878)
Sphacelaria plumosa (AJ287879)
Sphacella subtilissima (AJ287931)
Sphacelariaceae
Sphacelaria rigidula (AJ287883)
Sphacelaria rigidula (AJ287888)
Sphacelaria rigidula (AJ287887)
Sphacelaria divaricata (AJ287889)
Sphacelaria californica (AJ287893)
Sphacelaria rigidula (AJ287885)
Sphacelaria yamadae (AJ287890)
Sphacelaria tribuloides (AJ287891)
Sphacelaria tribuloides (AJ287892)
Sphacelaria cirrosa (AJ287865)
Dictyota cervicornis (AJ287851)
Dictyota dichotoma (AJ287852)
Dictyotaceae
Choristocarpus tenellus (AJ287861) — Choristocarpaceae
Bumilleriopsis filiformis (U89900)
Tribonema aequale (AF084611)
Tribonema intermixtum (AF015588)
Xanthonema debile (AF084612)
Botrydium stoloniferum (AF064743)
Botrydiopsis intercedens (AF015587)
Mischococcus sphaerocephalus (AF064744)
Heterococcus caespitosus (AF084610)
Vaucheria bursata (AF015589)
Phaeothamnion confervicola (AF069446)
Phaeoschizochlamys mucosa (AF064747)
Stichogloea globosa (AF155584)
Stichogloea doederleinii (AF069498)
Pleurochloridella botrydiopsis (AF069499)
Tetrasporopsis fuscescens (AF155585)
80
82
81
53
81
64
100
98
98
98
100
100
95
55
67
100

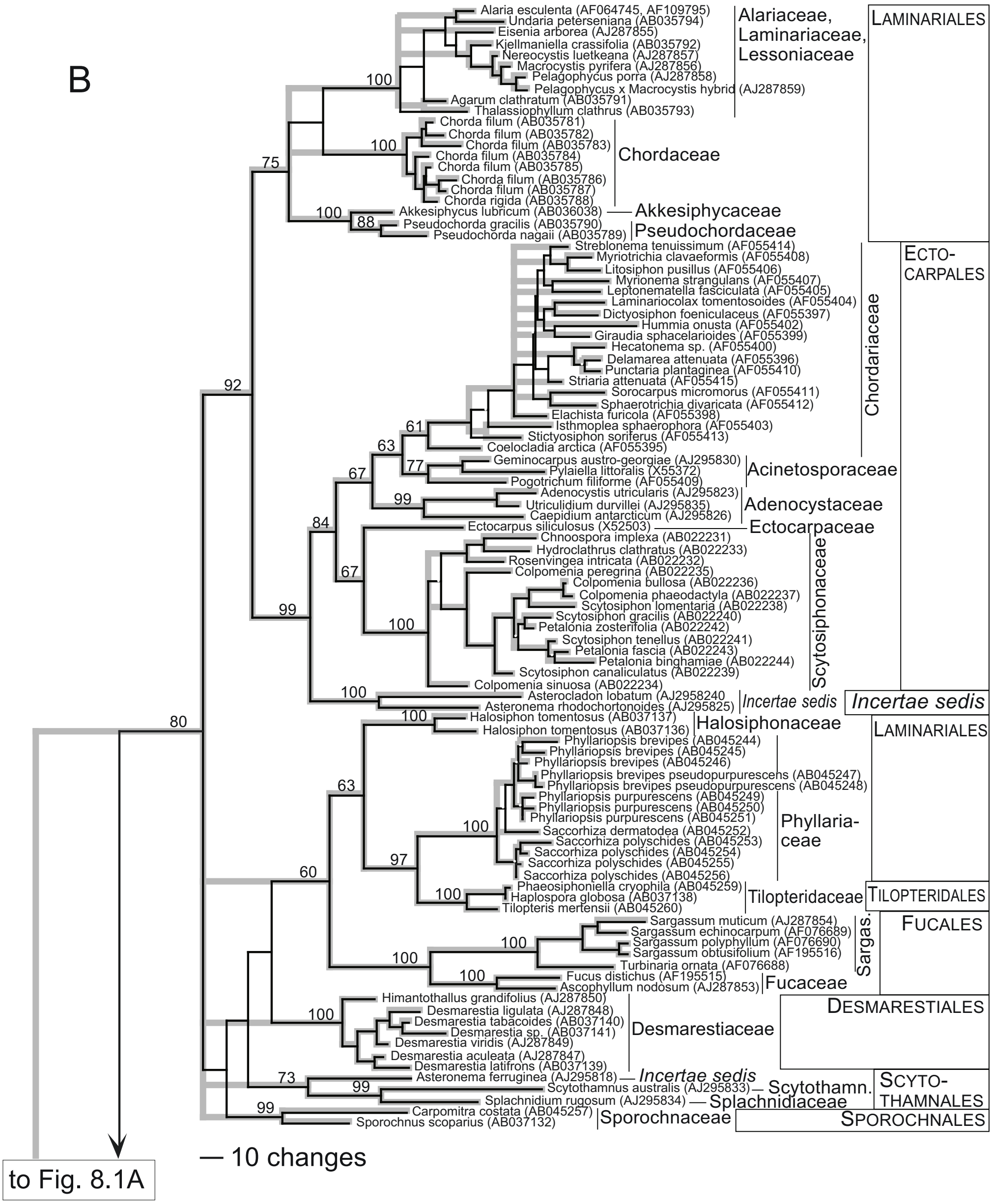


Fig. 8.1. *Rbc*L phylogeny of the Phaeophyceae. One of 2,046 MPTs (length = 5,763) with the same topology as the 50% majority-rule consensus tree. Figure A (opposite page) is the basal part of the tree including outgroup taxa (Tribophyceae and Phaeothamniophyceae) and Figure B (above) is the crown part of the tree. EMBL accession numbers are given in brackets for each taxon. Branch lengths are proportional to the number of changes (please note that the scales of Figures A and B differ slightly). The nodes that collapse in the strict consensus tree are indicated with white diamonds (•). The underlying backbone tree (grey) is the 50% bootstrap tree. Actual bootstrap percentages (above branches) within the Phaeophyceae are only given at the ordinal and the family level, because of space limitation, with the exception of the Sphacelariales where the status of the families is currently unclear (Draisma *et al.* 2002). Sargas. = Sargassaceae; Scytothamn. = Scytothamnaceae.

Box 8.1

A SPACER ODDITY

Although *rbc*L was able to resolve many relationships within the brown algae, the base of the crown clade (Fig. 8.1B) is still unresolved. Unfortunately, the RUBISCO-spacer is not a suitable marker for ordinal level comparisons due to its extensive length variation which makes it unalignable (Draisma *et al.* 2002/Ch. 3). However, inspection of RUBISCO-spacer sequences might still reveal some synapomorphies. Therefore, the EMBL database was screened for RUBISCO-spacer sequences of heterokont algae. The RUBISCO-spacer could only be aligned among different heterokont algae at the conservative 3'-end.

The partial RUBISCO-spacer alignment is summarised in Figure Box 8.1. As shown, seven of the ten crown orders have three extra nucleotides at the 3'-end. Although a tempting synapomorphy, the absence of the 3-bps in the Desmarestiales, Scytothamnales and Sporochnales is ambiguous. It may suggest that these three lineages are the basal taxa of the crown or simply that there was a secondary loss. Earlier speculations by Draisma *et al.* (2002/Ch. 3) that suggested a correlation between spacer length and evolutionary position are unfounded based on comparison with unpublished tribophycean sequences. The RUBISCO-spacer length of the basal tribophycean *Vaucheria* sp. is 115 nt and in other members of the Tribophyceae it ranges roughly from 100–200 nt (R.A. Andersen personal communication). RUBISCO-spacer lengths may, however, have increased independently in the Phaeophyceae and Tribophyceae.

Explanation of the superscripts in Figure Box 8.1 on the opposite page.

[a] Some unpublished RUBISCO-spacer sequences were included in this alignment. These unpublished sequences were obtained as described in Draisma *et al.* (2001/Ch. 2) and the EMBL accession numbers are AJ287907 (*Ascophyllum nodosum*), AJ287901 (*Desmarestia aculeata* [partial]), AJ287902 (*Desmarestia ligulata*), AJ287903 (*Desmarestia viridis*), AJ287909 (*Eisenia arborea*), AJ287904 (*Himantothallus grandifolius*), AJ287910 (*Macrocystis pyrifera*), AJ287911 (*Nereocystis luetkeana*), AJ287912 (*Pelagophycus porra*), and AJ287908 (*Sargassum muticum* [partial]). Collection data and taxonomic authorities can be found in Draisma *et al.* (2001/Ch. 2) where identical species names represent the same individuals as listed here.

[b] *Asteronema rhodochortonoides*.

[c] Including *Asteronema ferruginea*.

(Box 8.1 continued)

PHAEOPHYCEAE

Laminariales *s.s.* (*n*=28)	aTTTATCTTT-AAGGAATatTTGAATAGTG	crown taxa
Ectocarpales (*n*=19)	ATTTATCTCC-AAGGAATATTTGAATAGTG	
A. rhodochortonoides (*n*=1) [b]	ATTTATCTCC-AAGGACTATTTGAAGAGTA	
Halosiphonaceae (*n*=2)	ATTTATCTTC-AAGGGATATTTGAATAGCG	
Phyllariaceae (*n*=13)	AYTTATATCT-AAGAAATATTTGAATAGTT	
Tilopteridales (*n*=3)	ATYTATCTCC-TAAGAATATTTGAATAGTG	
Fucales (*n*=35)	ATTTAYATTAAATAMAATAMTTGAAGAGTR	
Desmarestiales (*n*=7)	ATATATCTTC-AAGGAGTATTTGAATA---	
Scytothamnales (*n*=3) [c]	ATTTMTATYY-ARRGRATATTTGAATA---	
Sporochnales (*n*=2)	ATTTATCTTT-AAGGAATATTTGAATA---	
Syringodermatales (*n*=1)	ATATATTTAT-AAGGGATATTCGATTA---	basal taxa
Onslowiaceae (*n*=2)	ATRTaTWTTA-AAGGARTAWYTRAAYW---	
Sphacelariales (*n*=25)	WWWWAAWTWT-AAGGAWWWSTTAAATA---	
Dictyotales (*n*=2)	TTWTWACTTT-AWGGAGYAtTTRAARY---	
Choristocarpaceae (*n*=1)	TAATATAATT-AAGGAGTATTTGAATA---	

BACILLARIOPHYCEAE

pennate diatoms (*n*=3)	AAAAAAATTA-AAGGAGTATTTGAATA---
centric diatoms (*n*=3)	ATAHAMATWW-AAGGAGTATTTGAATA---

BOLIDOPHYCEAE

Bolidomonas (*n*=3)	ATTAMTWATT-AAGGAGTATTtGAATA---

PELAGOPHYCEAE

Aureococcus (*n*=3)	TAATAAACTT-AAGGAGTA-TTGAAGA---
Aureoumbra (*n*=14)	AAATAAAAAC-AAGGAGTA-TTGAAGA---

RAPHIDOPHYCEAE

Heterosigma (*n*=1)	CTATATAATA-TAGGAGCATTTGAATA---

Fig. Box 8.1. Alignment of partial RUBISCO-spacer sequences [a] (3'-end) of heterokont algae. Sequences shown are majority-rule (>80%) consensus sequences. IUPAC-IUB codes were applied when there was no 80% majority for a nucleotide . Dashes (-) indicate gaps to restore alignment. Nucleotide symbols in small case indicate that there was a base in some taxa and a gap in other taxa. *n* = number of taxa; crown taxa = taxa situated in the crown part of the *rbc*L-tree (Fig. 8.1B); basal taxa = taxa situated in the basal part of the *rbc*L-tree (Fig. 8.1A).

Chapter 9

GENERAL DISCUSSION

TOWARDS A NEW CLASSIFICATION OF THE PHAEOPHYCEAE

From cladogram to classification

So far, phycological systematists have been reluctant to propose major nomenclatural changes in the classification of the brown algae based on their molecular phylogenies because of inadequate taxon sampling and concern for instability. However, de Reviers and Rousseau (1999; table 9, pp. 170–171) have made a first attempt by recognising 13 orders subdivided into 52 families of which three remained undescribed. They only recognised monophyletic groups based on DNA sequence data and retained traditional groups for which no sequence data were available. Taxa that could not be included in one of the 13 traditional orders were placed in the category *incertae sedis* (i.e., without an ordinal affiliation). This category comprised ten families of which five remained undescribed. De Reviers and Rousseau (1999) did not propose the creation of new orders to accommodate these unclassified families. New circumscriptions since de Reviers and Rousseau (1999) include the Akkesiphycaceae Kawai *et* Sasaki (2000), Halosiphonaceae Christensen *ex* Kawai *et* Sasaki (2000), and Onslowiaceae Draisma *et* Prud'homme van Reine (2001/Ch. 7). Peters and Ramírez (2001) proposed to reduce the number of families in the Ectocarpales (*s.l.*) from 23 (de Reviers and Rousseau 1999) to five.

I concur with the need for a new classification of the brown algae in which only monophyletic groups are recognised. Although formal nomenclatural changes will not be proposed in this thesis, Table 9.1 summarises the needed changes based on the *rbc*L analysis update in Figure 8.1 (Chapter 8) and the results of previous molecular and morphological studies. Brown algal groups that are not monophyletic or not included in Figure 8.1 are discussed below.

The Choristocarpaceae, the Onslowiaceae, and their former 'residence' within the Sphacelariales

Until recently, the members of the Onslowiaceae were included in the Choristocarpaceae and this family was included in the Sphacelariales (Draisma and Prud'homme van Reine 2001). However, results of the *rbc*L phylogeny suggest that the families Choristocarpaceae and Onslowiaceae do not belong to the Sphacelariales and should actually be given ordinal status. Currently, the Choristocarpaceae also include *Discosporangium mesarthrocarpum* (Meneghini) Hauck, although many authors consider it only distantly related to *Choristocarpus tenellus* (Prud'homme van Reine 1993, Draisma and Prud'homme van Reine 2001/Ch. 7). It could be proposed that the monotypic genus *Discosporangium* be removed from the Choristocarpaceae based on the

Table 9.1. Scheme for a new ordinal and familial classification of the Phaeophyceae.

Orders	**Families**
Ascoseirales Petrov (1963, 1964) *emend.* Moe *et* Henry 1982	**Ascoseiraceae** Skottsberg 1907
A new order has to be created for the proposed new families (right).	**A new family** has to be created for *Asterocladon*. **A new family** and a new genus have to be created for *Asteronema rhodochortonoides* and *Asteronema breviarticulatum*.
A new order has to be created for *Bachelotia*.	**A new family** has to be created for *Bachelotia*.
A new order has to be created for Choristocarpaceae.	**Choristocarpaceae** Kjellman 1891
Cutleriales Bessey 1907	**Cutleriaceae** Hauck 1883–1885
Desmarestiales Setchell *et* Gardner 1925	**Arthrocladiaceae** Chauvin 1842 **Desmarestiaceae** (Thuret) Kjellman 1880
Dictyotales Bory 1828	**Dictyotaceae** Lamouroux *ex* Dumortier 1822 **Dictyotopsidaceae** Allender 1980 **Scoresbyellaceae** Womersley 1987
Discosporangiales O.C. Smith 1937	**Discosporangiaceae** O.C. Smith 1937
Ectocarpales Bessey 1907 (incl. Chordariales Setchell *et* Gardner 1925, Dictyosiphonales Setchell *et* Gardner 1925, Punctariales Kylin 1933, and Scytosiphonales Feldmann 1949)	**Acinetosporaceae** Hamel *ex* J. Feldmann 1937 **Adenocystaceae** Rousseau, de Reviers, Leclerc, Asensi *et* Delépine 2000 **Chordariaceae** Greville 1830 **Ectocarpaceae** C. Agardh 1828 **Scytosiphonaceae** Farlow 1881
Fucales Kylin 1917 (incl. Durvillaeales Petrov 1965, and Notheiales Womersley 1987)	**A new family** has to be created for *Bifurcariopsis*[a] **Durvillaeaceae** (Oltmanns) De Toni 1891 **Fucaceae** Adanson 1763 **Himanthaliaceae** (Kjellman) De Toni 1891 **Hormosiraceae** Fritsch 1945 **Notheiaceae** O.C. Schmidt 1938 **Sargassaceae** Kützing *emend.* De Toni 1895 (incl. Cystoseiraceae De Toni 1891) **Seirococcaceae** Nizamuddin 1987 **A new family** has to be created for *Xiphophora*[a]
A new order has to be created for Halosiphonaceae.	**Halosiphonaceae** T. Christensen (1962) *ex* Kawai *et* Sasaki 2000
Laminariales Migula 1908	**Akkesiphycaceae** Kawai *et* Sasaki 2000 **Chordaceae** Dumortier 1822 **Laminariaceae** Bory 1827 (incl. Alariaceae Setchell *et* Gardner 1925, and Lessoniaceae Setchell *et* Gardner 1925) **Pseudochordaceae** Kawai *et* Kurogi 1985
A new order has to be created for Onslowiaceae.	**Onslowiaceae** Draisma *et* Prud'homme van Reine 2001
A new order has to be created for Phyllariaceae.	**Phyllariaceae** Hamel *ex* Petrov 1974

(continued)

(Table 9.1 continued)

Ralfsiales Y. Nakamura (1972). A Latin description has to be written.	**Ralfsiaceae** Farlow 1881 (incl. Lithodermataceae Hauck 1883, Nemodermataceae J. Feldmann 1937, and Stragulariaceae Strömfelt 1886)
Scytothamnales A.F. Peters *et* M.N. Clayton 1998	**Scytothamnaceae** Setchell *et* Gardner 1925 **Splachnidiaceae** Mitchell *et* Whitting 1892 **A new family** has to be created for *Asteronema*.
Sphacelariales Migula 1908 [b]	**Sphacelariaceae** Decaisne 1842 **Stypocaulaceae** Oltmanns 1922
Sporochnales Sauvageau 1926	**Sporochnaceae** Greville 1830
Syringodermatales E. C. Henry 1984	**Syringodermataceae** E.C. Henry 1984
Tilopteridales Bessey 1907	**Tilopteridaceae** Kjellman 1890
Incertae sedis	**Heterochordariaceae** Setchell *et* Gardner 1925

[a] According to de Reviers and Rousseau (1999).

[b] The two families may have to be merged into one; Sphacelariaceae (Draisma *et al.* 2002/Ch. 3).

different morphology of its plurilocular zoidangia. At present, however, there are still no DNA sequence data available to support this (Draisma and Prud'homme van Reine 2001/Ch. 7). For *Discosporangium* the family name Discosporangiaceae and the order name Discosporangiales are available (Schmidt 1937).

The familial subdivision of the Sphacelariales is currently under discussion and it will probably be reduced to a single family (Draisma *et al.* 2002/Ch. 3). The study by Draisma *et al.* (2001/Ch. 3) and Figure 8.1 support different basal sphacelarialean clades. In any case, the monotypic Cladostephaceae (including *Cladostephus spongiosus* [Hudson] C. Agardh) can be merged with the Sphacelariaceae. Whether or not the Stypocaulaceae will stand the test of time remains to be seen. Considering the amount of genetic variation within the Sphacelariales (compared to that in other orders) and the relatively basal position of the Sphacelariales within the Phaeophyceae, it is also justifiable to recognise two or more newly defined families. For example, the subgenus *Propagulifera* could be given family status.

The kelps

The Laminariales (kelps) are clearly polyphyletic. The Halosiphonaceae and the Phyllariaceae should be removed from the Laminariales and each family should be placed in a new order. Together with the Tilopteridales, the Halosiphonaceae and Phyllariaceae form a monophyletic group. The distributional range of the members of these three clades is restricted to relatively cold water regions of the north Atlantic and Mediterranean (Sasaki *et al.* 2001).

Although de Reviers and Rousseau (1999) and Rousseau *et al.* (2001) suggested that the Laminariales *s.s.* should be restricted to the ALL-complex (i.e., Alariaceae, Laminariaceae, and Lessoniaceae), the tree in Figure 8.1 supports inclusion of the Akkesiphycaceae, the Chordaceae, and the Pseudochordaceae. The families of the ALL-complex are themselves not monophyletic as has already been demonstrated by

Druehl *et al.* (1997), Boo *et al.* (1999a, b), Boo and Yoon (2000), and Yoon *et al.* (2001). In the latter two publications it is suggested that the ALL-complex be downgraded to family status or that eight independent families are erected, however, no decision is being made. By merging the Alariaceae and the Lessoniaceae into the Laminariaceae, the intra-familial genetic variation is comparable to that of other families.

Taxa with a stellate chloroplast arrangement

Asterocladon lobatum D.G. Müller, E.R. Parodi *et* A.F. Peters, *Asteronema australe* R. Delépine *et* A. Asensi, *Asteronema breviarticulatum* (J. Agardh) L.C. Ouriques *et* Z.L. Bouzon, *Asteronema ferruginea* (Harvey) R. Delépine *et* A. Asensi, *Asteronema rhodochortonoides* (Børgesen) D.G. Müller *et* E.R. Parodi, *Bachelotia antillarum* (Grunow) Gerloff, *Scytothamnus australis* (J. Agardh) Hooker *et* Harvey, *Scytothamnus fasciculatus* (Hooker *et* Harvey) Cotton, *Scytothamnus hirsutus* Skottsberg, *Splachnidium rugosum* (Linnaeus) Greville, and *Stereocladon rugulosus* (Bory de Saint-Vincent) Hariot are all characterised by a stellate chloroplast configuration with a central pyrenoid, a phenomenon unknown in any other brown alga (Müller *et al.* 1998). The latter three genera belong to the Scytothamnales and their cells contain one single deeply lobed chloroplast embracing a central pyrenoid (Peters and Clayton 1998). The former three genera contain several ribbon-shaped or elongate disk-shaped chloroplasts, each with a terminal or a lateral pyrenoid (Magne 1976, Delépine *et al.* 1976, Ouriques and Bouzon 2000). These pyrenoids form a compact body in the centre of the cell, in which the individual components are separated by narrow cytoplasmatic gaps (Müller and Parodi 1994).

Most authors consider *Bachelotia antillarum* only distantly related to the other taxa with stellate chloroplast arrangements (Delépine *et al.* 1976, Asensi *et al.* 1977, Müller and Parodi 1994). The initial growth of the erect filaments of *B. antillarum* is apical, but later growth is localised to a permanent intercalary region, which additionally produces intercalary reproductive structures (Blomquist 1958, Price 1973). In *Asterocladon*, *Asteronema*, and the Scytothamnales reproductive structures are sessile or stalked and growth is predominantly apical, except in *A. breviarticulatum* where growth only occurs by means of an intercalary meristem (Ouriques and Bouzon 2000).

The tree in Figure 8.1 clearly shows that *Asteronema* is polyphyletic. *Asteronema ferruginea* is closely related to the Scytothamnales and *Asteronema rhodochortonoides* is sister to *Asterocladon lobatum*. Together the latter two species are sister to the Ectocarpales. This is also supported by the 3'-end of the RUBISCO-spacer (Box 8.1, Ch. 8). The type species of *Asteronema* is *A. australe*, which is morphologically very similar to *A. ferruginea*. In fact, Delépine and Asensi (1975), Womersley (1987), and Ricker (1987) suspected that these two species might be identical and represent a single species. Therefore, the name *Asteronema* is reserved for *A. australe* and *A. ferruginea* and the genus *Asteronema* should be included in the Scytothamnales under a new family name. An increased length of the RUBISCO-spacer may be a synapomorphy of the Scytothamnales including *A. ferruginea* (Peters and Ramírez 2001). Besides the Scytothamnales, RUBISCO-spacers longer than 300 base pairs have only been observed in species belonging to the Sphacelariales (Draisma *et al.* 2002/Ch. 3). The pyrenoids of *Asteronema australe* and the traditional Scytothamnales are traversed by tubular invaginations of chloroplast endoplasmatic reticulum (Delépine *et al.* 1976, Asensi *et al.* 1977, Müller and Parodi 1994, Peters and Clayton 1998). This could be another synapomorphy of the Scytothamnales in a new classification. No invaginations were observed in *Asteronema rhodochortonoides*, *A. breviarticulatum*, and *Bachelotia antillarum* (Asensi *et al.* 1977, Müller and Parodi 1994, Ouriques and Bouzon 2000). Whether *Asteronema ferruginea* and *Asterocladon* have invaginations remains to be examined (Müller *et al.* 1998). Müller *et al.* (1998) did not mention the occurrence of invaginations in *Asterocladon*, but these could not be observed on their electron micrographs. Asensi *et al.* (1977) also reported the presence of a pyrenoid with invaginations in *Petroderma maculiforme* (Wollny) Kuckuck (Ralfsiales) which makes this species of interest for future molecular studies. Whereas the single plastid of the traditional Scytothamnales is axial, in *P. maculiforme* it is parietal.

Asteronema rhodochortonoides differs from *A. australe* and *A. ferruginea* in the absence of unilocular sporangia (Müller and Parodi 1994, Kogame *et al.* 2001). Unilocular sporangia have neither been observed in *Asterocladon lobatum* and *Asteronema breviarticulatum* (Müller *et al.* 1998, Ouriques and Bouzon 2000). Absence of unilocular sporangia and absence of pyrenoid invaginations suggest that *A. breviarticulatum* is more closely related to *A. rhodochortonoides* than to *A. australe* and *A. ferruginea*. For *A. rhodochortonoides* and *A. breviarticulatum* a new genus name and possibly a new family name should be created. This new family should be included in a new order together with *Asterocladon*, for which a new family name should be created. Members of the Scytothamnales (including *Asteronema australe* and *A. ferruginea*) are restricted to the cold waters of the Southern Hemisphere with the exception of *Scytothamnus fasciculatus* which is also present in the cold-temperate north-east Pacific (Müller *et al.* 1998). *Asterocladon*, *Asteronema rhodochortonoides* and *A. breviarticulatum* show a tropical to warm-temperate distribution (Müller *et al.* 1998, Ouriques and Bouzon 2000, Kogame *et al.* 2001).

For *Bachelotia antillarum*, a monotypic new family and a monotypic new order could be proposed as well, based on its unique morphology and its separate position in the rDNA-based phylogenetic tree (Rousseau *et al.* 2001) and in the *rbc*L-based phylogeny presented by Siemer (1998). The *rbc*L sequence of *B. antillarum* has not yet been released and thus could not be included in the phylogeny of Figure 8.1. However, in the *rbc*L study by Siemer (1998) this species formed a lineage by itself. In that study members of the Tribophyceae were used as outgroup, whereas the brown algae of the ingroup represented the Fucales, Desmarestiales, Tilopteridales, Laminariales, and Ectocarpales (*s.l.*). The latter two orders were sister to each other. Therefore, it can be concluded that *B. antillarum* is not closely related to *Asterocladon* and *A. rhodochortonoides* which were not included in the study by Siemer (1998), but are sister to the Ectocarpales in the tree of Figure 8.1.

Orders not represented in the updated rbcL-phylogeny

Representatives of the Ascoseirales, Cutleriales, and Ralfsiales were not included in the present update, because *rbc*L sequences are not available. These orders are distinct from other brown algal orders based on morphology and the rDNA phylogeny of Rousseau *et al.* (2001). In that phylogeny the Ascoseirales, represented by its single species *Ascoseira mirabilis* Skottsberg, formed a distinct lineage and so did the Cutleriales, represented by *Cutleria multifida* (J.E. Smith) Greville. However, the status of the Ralfsiales remains complicated.

The order Ralfsiales was established on the basis of the genus *Ralfsia* by Nakamura (1972), who failed to provide a Latin description. It has not yet been nomenclaturally validated, but is currently in use (Silva and de Reviers 2000). Nakamura (1972) recognised three families in the order, i.e., Lithodermataceae, Nemodermataceae, and Ralfsiaceae, separating them on the basis of the position of the reproductive organs. De Reviers and Rousseau (1999) only recognised the Ralfsiaceae in the Ralfsiales and placed the monotypic Heterochordariaceae (containing *Analipus japonicus* [Harvey] Wynne) *incertae sedis*. They considered it the best solution to place the algae with a single plate-like plastid without a pyrenoid in the Ralfsiales *sensu* Nakamura, since it was clear that these algae could no longer be placed in the Ectocarpales and as no clear relationships with other brown algae are known. In the rDNA-based study by Rousseau *et al.* (2001), the Ralfsiales were represented by *Nemoderma tingitanum* Schousboe *in* Bornet and it formed a separate lineage. However, *N. tingitanum* contains several scattered discoid plastids with several pyrenoids (Parente *et al.* 2000) and without pyrenoids according to Kawai (1992). De Reviers and Rousseau (1999) did not place *N. tingitanum incertae sedis*, but retained it in the Ralfsiaceae, order Ralfsiales. Rousseau *et al.* (2001) treated *N. tingitanum* as belonging to Ralfsiaceae, but did not discuss its systematic position.

At this moment the Ralfsiales are best retained, although the ordinal delimitation is still not clear. *Rbc*L sequences of the type genus *Ralfsia* are needed before validating Nakamura's Ralfsiales. It remains to be seen whether or not the Heterochordariaceae should be included in the order and whether or not the Nemodermataceae should be re-instated.

Calling the class to order

A new phylogenetic classification of the brown algae, based on the recognition of monophyletic groups would comprise twenty orders. A scheme for a new classification is shown in Table 9.1. It is recommended not to take final action until *rbc*L sequences of *Bachelotia antillarum* (B.L. Siemer), the outgroup taxon *Schizocladia ischiensis* (H. Kawai) and those of a few more taxa have been formally analysed. Attempts to sequence *rbc*L of *Ascoseira mirabilis* Skottsberg and *Ralfsia* sp. are currently being made (B. de Reviers personal communication), as well as of some freshwater taxa (pers. comm. A.F. Peters, H. Kawai, J. Wehr). It is not likely that *rbc*L sequences of many other taxa will soon be determined. However, this will not hinder the proposal of a new classification of the Phaeophyceae including circumscriptions of new orders and families.

An important point in the newly proposed classification is that while being phylogenetic, it also fits the traditional Linnaean hierarchy and follows the rules of the *International Code of Botanical Nomenclature* (Greuter 2000). Recently, a phylogenetic code of biological nomenclature has been proposed called PhyloCode (Forey 2001). The PhyloCode is not yet operational but can be downloaded from the web at www.ohio.edu/PhyloCode. It is designed to provide rules for the naming of clades across all of biology. Phylogenetic nomenclature starts from the premise that there should be congruence between phylogenetic hypotheses and nomenclature. It is designed so that it can be used concurrently with the existing codes based on Linnaean nomenclature. When the PhyloCode becomes more widely accepted, it can be applied to the Phaeophyceae. An advantage of the PhyloCode is that taxa do not necessarily have to be based on apomorphies (character-defined), but can also be node-based or stem-based (taxon-defined).

PHAEOPHYCEAN PHYLOGENETIC HISTORY IN THE FUTURE

More taxa and more genes

Challenges in the coming decade will be on lower taxonomic ranks and broader taxonomic sampling within these ranks. These efforts will help to break up some of the long branches leading to poorly sampled groups (Dictyotales, Fucales, Sporochnales, Syringodermatales). However, even with the most comprehensive taxon sampling, *rbc*L alone will not be informative enough to resolve all brown algal inter-ordinal relationships. Additional molecular markers are needed. A mitochondrial (mt) encoded gene is of interest because the reliability of a phylogenetic hypothesis increases when trees that are based on genes from different organelles are congruent. These mtDNA sequences could be analysed independently from the chloroplast and nuclear markers that have been used so far. At present, however, it is not clear which additional mt genes might be suitable.

Of the brown algal mitochondrial genes sequenced so far (*atp*6, *atp*8, *cox*1, *cox*3, *lrn* [coding for 23S rRNA], *nad*3, *nad*6, *nad*7, *nad*11, *rpl*31, *rps*10, *rps*14, *srn* [coding for 16S rRNA], *trn*K, *trn*P, *trn*Y), most is known about the *cox*I gene which codes for cytochrome *c* oxydase subunit I (Ehara *et al.* 1998, 1999). Complete and partial brown algal *cox*I sequences are available for 4 species of Ectocarpales, 3 species of Laminariales and for 6 tribophycean outgroups. I compared 12 *cox*I sequences (1059 nt) with 12 *rbc*L sequences (1399 nt) from species representing the same genera and in 7 cases including the same species. Pair-wise distances for phaeophycean *cox*I were more than twice as high as pairwise distances between phaeophycean *rbc*L (Table 9.2). This is remarkable, because plant mitochondrial genes are generally considered to evolve about three times slower than plant chloroplast genes (Page and Holmes 1998). The fraction of informative sites for respectively the *cox*I and the *rbc*L alignment including the outgroup (excluding outgroup in brackets) is 38% (16%) and 24% (9%) for all three codon positions, 14% (4%) and 9% (3%) for first + second codon positions only, and 84% (40%) and 54% (22%) for third codon positions only. In contrast to the *rbc*L alignment, however, MP bootstrap resampling (not shown) of the *cox*I analysis could not recover the monophyly of the Ectocarpales and Laminariales, despite the higher number of informative sites in *cox*I (398 against 337 in *rbc*L). In the outgroup only one clade had bootstrap support in the *cox*I analysis against four in the *rbc*L analysis. A Partition Homogeneity Test (PHT) (see Chapter 2) indicated that the two alignments were incongruent and should not be combined in one analysis. PHTs of only the first and second codon positions and of the amino acid translation, on the other hand, indicated congruence between *cox*I and *rbc*L. The level of homoplasy (saturation) at third codon positions is probably too high in *cox*I. Therefore, I do not recommend this gene for phylogeny reconstruction of the Phaeophyceae, although

Table 9.2. Uncorrected ('p') distance matrix for *cox*I (lower left) and *rbc*L (upper right) sequences of phaeophycean and tribophycean taxa. Only genus names are given. *Cox*I and *rbc*L sequences are in five cases not represented by the same species. The first three taxa belong to the Ectocarpales, the next three to the Laminariales (Phaeophyceae), and the last six to the Tribophyceae. EMBL accession numbers of *cox*I are given in the left column, those of *rbc*L in the upper row.

	Colpomenia AB022236	*Ectocarpus* X52503	*Pylaiella* X55372	*Chorda* AB035787	*Pseudochorda* AB035789	*Undaria* AB035794	*Botrydiopsis* AF015587	*Botrydium* AF064743	*Heterococcus* AF084610	*Mischococus* AF064744	*Tribonema* AF084611	*Vaucheria* AF015589
Colpomenia AF037995	–	0.07	0.08	0.09	0.10	0.10	0.18	0.18	0.19	0.18	0.19	0.18
Ectocarpus AF037994	0.14	–	0.08	0.10	0.10	0.10	0.18	0.18	0.18	0.18	0.19	0.17
Pylaiella Z72500	0.16	0.17	–	0.11	0.10	0.11	0.17	0.18	0.17	0.17	0.18	0.18
Chorda AF037991	0.20	0.18	0.21	–	0.08	0.08	0.18	0.18	0.19	0.18	0.20	0.18
Pseudochorda AF037992	0.18	0.17	0.19	0.20	–	0.08	0.17	0.16	0.16	0.16	0.18	0.17
Undaria AF037993	0.16	0.15	0.17	0.19	0.16	–	0.17	0.17	0.17	0.17	0.18	0.17
Botrydiopsis AB000203	0.25	0.25	0.24	0.23	0.24	0.24	–	0.08	0.12	0.08	0.08	0.11
Botrydium AB000204	0.22	0.23	0.25	0.24	0.24	0.22	0.18	–	0.12	0.09	0.08	0.12
Heterococcus AB000206	0.26	0.27	0.27	0.27	0.27	0.28	0.20	0.21	–	0.10	0.13	0.14
Mischococcus AB000208	0.22	0.23	0.24	0.23	0.24	0.23	0.19	0.17	0.21	–	0.09	0.12
Tribonema AB000211	0.25	0.24	0.26	0.24	0.26	0.25	0.21	0.20	0.24	0.20	–	0.12
Vaucheria AB000212	0.23	0.24	0.26	0.24	0.25	0.24	0.21	0.20	0.23	0.20	0.22	–

*cox*I might do better when more basal brown algal taxa are included in the analysis. *Cox*I sequences of outgroup taxa might simply be too distant from those of the most advanced brown algae Laminariales and Ectocarpales.

Another promising approach to ordinal level phylogeny is the use of complete mitochondrial genome sequences. At present complete chondriomes have been determined for *Pylaiella littoralis* (58,507 nt; acc. nr. AJ277126) (Oudot-Le Secq *et al.* 2001) and for *Laminaria digitata* (Hudson) J.V. Lamouroux (38,007 nt; M.-P. Oudot-Le Secq pers. comm.). Sequencing of chondriomes for *Ectocarpus*, *Sphacelaria*, *Fucus*, and *Dictyota* are currently in progress (J.L. Olsen, W.T. Stam pers. comm.). Inclusion of the chondriomes of other heterokonts is also of interest. These are available for the bicosoecid *Cafeteria roenbergensis* (43,159 nt; acc. nr. AF193903), the oomycete *Phytophtora infestans* (36,753 nt; U17009), and the chrysophyceans *Chrysodidymus synuroideus* (34,119 nt; AF222718) and *Ochromonas danica* (41,035 nt; AF287134).

Age of the Phaeophyceae based on molecular clocks

Issues around molecular clocks and their calibration remain contentious (reviewed in Hillis *et al.* 1996). Nevertheless, estimates continue to be made for those organisms possessing a good fossil record. Medlin *et al.* (1997) estimated the earliest probable origin of the Phaeophyceae at 155 million years (Ma) ago on the basis of small subunit rRNA gene sequence divergences. They calibrated their molecular clock by regressing branch lengths from diatoms (with a fossil record) from a linearised Neighbor-Joining tree against first appearance dates and subsequently extrapolated these regressions to the brown algal lineage. Extrapolation of regressions to provide speculative starting points for age estimations implies that relative rates of base substitution in the brown algae must be comparable to those of the diatoms. Because substitution rate is assumed to be correlated with many physiological and life history factors such as generation time, temperature, metabolic rates, differences in DNA repair efficiency, size, free radical formation (Martin and Palumbi 1993, Kooistra and Medlin 1996), comparisons between these disparate lineages is dubious. The only calcified phaeophyceans are found in the genus *Padina* (Dictyotales), but no fossils are known. The oldest putative brown algal fossils belong to the Laminariales and Fucales and date only to very recent Miocene deposits (ca. 7–16 Ma ago) (Parker and Dawson 1965, van den Hoek *et al.* 1995). Earlier attempts to calibrate a molecular clock for the Laminariales suggested a divergence time at about 15–22 Ma ago (Druehl and Saunders 1992, Saunders and Druehl 1992). At a lower taxonomic level, Stam *et al.* (1988) suggested that radiation within the genus *Laminaria* occurred 15–20 Ma ago, on the basis of single-copy DNA-DNA hybridisations.

The phaeophycean taxa in the study by Medlin *et al.* (1997) all belonged to the brown algal crown clade (Fig. 8.1B, Ch. 8). Inclusion of taxa belonging to the basal Phaeophyceae (Fig. 8.1A) might result in a somewhat older estimation. Calibration of an *rbc*L clock is currently also possible. Mann *et al.* (2001) reported the construction of a data-base with *rbc*L sequences from >100 diatom taxa. Re-estimations based on more genes is desirable, because Medlin *et al.* (1997) came to different estimates of emergence time of the diatoms based on nuclear (18S rRNA) and plastid (16S rRNA, *tuf*A, *rbc*L) DNA sequences, varying from 135–249 Ma. The great disparity of estimates demonstrates the unreliability of currently available molecular clock estimates.

FUTURE STUDIES WITHIN THE SPHACELARIALES

Phylogeny of Sphacelariales

The *rbc*L-based phylogenies in Draisma *et al.* (2002; fig. 2/Ch. 3; Fig. 3.2) and in Figure 8.1 are, currently, the best estimates of Sphacelariales phylogeny available. In both Figures 3.2 and 8.1, resolution at the base of the order is poor. According to these trees, either the Stypocaulaceae, *Sphacelaria caespitula*, *Sphacelaria radicans*, or the *Propagulifera* form the most basal clade of Sphacelariales. Inclusion of *rbc*L sequences of additional genera and subgenera in the analysis might result in a better resolved tree with a well-supported basal clade or it may result in even more basal candidates.

An understanding of sister-group relationships would help to obtain a better resolved phylogeny of the Sphacelariales. At present, it is not clear whether the Onslowiaceae and/or Syringodermatales branch-off before or after the Sphacelariales, or that one or both of them is/are the sister-clade of the Sphacelariales (see Figs 2.1–5, 3.2, and 8.1A). Therefore, not only *rbc*L sequences of more species of Syringodermatales and Onslowiaceae are desirable, but also a broader sampling of the Dictyotales (16 genera) which are certainly basal to the Sphacelariales. These efforts, however, will only be of use if *rbc*L displays enough resolving power. At present it is not clear what other sequences might be suitable. The ribosomal cistron is certainly unsuitable. The genes themselves are too conservative (Draisma *et al.* 2001/Ch. 2) and the internal transcribed spacers (ITSs) are paradoxically too variable (Ch. 4). Even though an analysis of conservative parts of the ITS sequences (Ch. 4) confirmed the paraphyly of the subgenus *Sphacelaria* as demonstrated by the *rbc*L analysis (Ch. 3) the utility of ITS is inadequate. Likewise, explorations within the subgenus *Propagulifera* and using the complete RUBISCO-spacer sequences (Ch. 5) revealed paraphyly of the sections of this subgenus and a cryptic species but is still unlikely to provide enough information on the larger sampling scale. Until new markers can be explored, it seems best to remain focused on *rbc*L for a little longer, while improving taxon sampling.

An area of particular attention is the Southern Hemisphere. *Sphacelaria* subgenera *Reinkea* and *Bracteata* (the latter with one Northern Hemisphere member) and the stypocaulacean genera *Phloiocaulon* and *Ptilopogon* are of particular interest. Of course the type species *Sphacelaria reticulata* is also of major interest, but it is not likely that this species will be found again (Draisma *et al.* 2002/Ch. 3). *Sphacelaria reticulata* is either extinct or it may represent an aberrant growth form of another sphacelarialean species (Prud'homme van Reine 1982). *Rbc*L sequences of the freshwater species *Sphacelaria fluviatilis* Jao and *Sphacelaria lacustris* Schloesser *et* Blum will certainly provide an answer to whether these taxa indeed belong to the Sphacelariales or not. Attempts are currently being made to sequence 18S and *rbc*L of these two taxa (J. Wehr pers. comm.). Finally, I recommend that *rbc*L sequences be determined for sphacelarialean taxa with a disjunct distribution in Europe and southern Australia in order to find out whether the representatives of both hemispheres are indeed conspecific. Taxa of Sphacelariales recorded from the north Atlantic and the south coast of Australia include *Cladostephus spongiosus* (Hudson) C. Agardh, *Sphacelaria cirrosa* (Roth) C. Agardh, and *Sphacella subtilissima* Reinke (Prud'homme van Reine 1993).

Intra-specific/population studies

Much is to be learned from genetic studies at the intra-specific or population level. A more extensive taxon sampling of some species should reveal whether cosmopolitan species really exist. *Sphacelaria rigidula* represents two cryptic species (type A and B), both represented in the Atlantic and the Pacific (Chapter 5). Sequences of *rbc*L and adjacent spacer, however, did not differ between the Pacific and Atlantic isolates of type A. Atlantic and Pacific isolates of *Sphacelaria tribuloides* also showed identical *rbc*L and spacer sequences (Ch. 5). A broader sampling is recommended for this species and sequencing could include ITS sequences, instead of only the RUBISCO-spacer. In contrast to *S. rigidula* (type A) and *S. tribuloides*, *rbc*L sequences of Atlantic and Pacific *Halopteris filicina* differed conspicuously (Draisma *et al.* 2002/Ch. 3). A more extensive sampling of this taxon in both ocean basins might give more insight in the divergence of species. Genetic variation within Atlantic and Pacific populations could be determined on the basis of ITS sequences (Ch. 4). The determination of the extent of sexual reproduction in *H. filicina* in the field is of interest and what its effect may be on population structure. The intra-individual ITS variation demonstrated in Chapter 4, might be a result of prolongued periods without sexual reproduction.

The present study does not provide sequence data of morphological variants of species. Morpho-types of several presently recognised species have been attributed to distinct species in the past, because the different morphologies were considered to be the result of phenotypic plasticity (Prud'homme van Reine 1982). DNA sequences of different morpho-types of a species (e.g., in *Sphacelaria cirrosa* or *Cladostephus spongiosus*) are desirable in order to find out if the lumping of morphological variants was justified. Culture experiments can also be of help to determine the morphological plasticity of a species.

In conclusion

Previous brown algal classifications (Kylin 1933, Papenfuss 1951, Scagel 1966, Wynne and Loiseaux 1976, van den Hoek *et al.* 1995) differed in the relative importance that was arbitrarily assigned to the various characters (i.e., isomorphic/heteromorphic life cycle, haplostichous/polystichous growth, [an]isogamy/oogamy). This thesis demonstrates that DNA sequence data are indispensable for phylogenetic reconstruction of a group like the Phaeophyceae, which represents extreme morphological plasticity. Likewise, molecular data are indispensable when the phylogenetic relationships of 'low-morphology' organisms like the Sphacelariales are explored. On the other hand, new phylogenies based on molecular data also stimulate research on non-molecular characters, as incongruities of the old classification become evident. Taxon sampling for non-molecular characters like pheromones or ultrastructural features, is currently more fragmentary than for DNA sequences. Therefore, comprehensive studies of non-molecular characters are still desirable in order to test evolutionary hypotheses inferred from the molecular data. Taken together these data sets provide reciprocity that will allow us to understand structural evolutionary change.

REFERENCES

Abbott, I.A. & Hollenberg, G.J. [Eds] 1976. *Marine Algae of California*. Stanford Univ. Press, CA, U.S.A. 827 pp.

Abe, K. 1935. Zur Kenntniss der Entwicklungsgeschichte von *Heterochordaria*, *Scytosiphon*, und *Sorocarpus*. *Sc. Rep. Tohuku Imp. Univ., Ser. 4, Biology* 9:329–337.

Abe, K. 1936. Kernphasenwechsel von *Heterochordaria abietana*. *Sc. Rep. Tohuku Imp. Univ., Ser. 4, Biology* 11:239–241.

Agardh, C.A. 1817. *Synopsis Algarum Scandinaviae*. xxv pp.

Andersen, R.A., Potter, D., Bidigare, R.R., Latasa, M., Rowan, K. & O'Kelly, C.J. 1998. Characterization and phylogenetic position of the enigmatic golden alga *Phaeothamnion confervicola*: Ultrastructure, pigment composition and partial ssu rDNA sequence. *J. Phycol.* 34:286–298.

Asensi, A., Delépine, R. & Guglielmi, G. 1977. Nouvelles observations sur l'ultrastructure du plastidome des Phéophycées. *Bull. Soc. Phycol. Fr.* 22:192–205.

Avise, J.C. 1994. *Molecular Markers, Natural History, and Evolution*, Chapman & Hall, New York, NY, U.S.A.

Bailey, J.C. & Freshwater, D.W. 1997. Molecular systematics of the Gelidiales: inferences from separate and combined analyses of plastid *rbc*L and nuclear SSU gene sequences. *Eur. J. Phycol.* 32:343–352.

Bailey, J.C. & Andersen, R.A. 1999. Analysis of clonal cultures of the brown tide algae *Aureococcus* and *Aureoumbra* (Pelagophyceae) using 18S rRNA, *rbc*L, and RUBISCO spacer sequences. *J. Phycol.* 35:570–574.

Bailey, J.C., Bidigare, R.R., Christensen, S.J. & Andersen, R.A. 1998. Phaeothamniophyceae *classis nova*: A new lineage of Chromophytes based upon photosynthetic pigments, *rbc*L sequence analysis and ultrastructure. *Protist* 149:245–263.

Bakker, F.T., Olsen, J.L. & Stam, W.T. 1995. Global phylogeography in the cosmopolitan species *Cladophora vagabunda* (Chlorophyta) based on nuclear rDNA ITS sequences. *Eur. J. Phycol.* 30:197–208.

Belkhiri, A., Buchko, J. & Klasson, G.R. 1992. The ribosomal RNA gene in *Pythium* species: two different genomic locations. *Mol. Biol. Evol.* 9:1089–1102.

Ben Ali, A., Wuyts, J., Wachter, R. de, Meyer, A. & Peer, Y. van de 1999. Construction of a variability map for eukaryotic large subunit ribosomal RNA. *Nucl. Acids Res.* 27: 2825–2831.

Ben Ali, A., Baere, R. de, Auwera, G. van der, Wachter, R. de & Peer, Y. van de 2001. Phylogenetic relationships among algae based on complete large–subunit rRNA sequences. *Int. J. Syst. Evol. Microbiol.* 51:737–749.

Bhattacharya, D. & Medlin, L. 1995. The phylogeny of plastids: A review based on comparisons of small–subunit ribosomal RNA coding regions. *J. Phycol.* 31:489–498.

Bhattacharya, D., Medlin, L., Wainright, P.O., Ariztia, E.V., Bibeau, C., Stickel, S.K. & Sogin, M.L. 1992. Algae containing chlorophylls *a* + *c* are paraphyletic: molecular evolutionary analysis of the Chromophyta. *Evolution* 46:1801–1817.

Bing-Sheng, X. [Ed.] 1989. *Cryptogamic Flora of the Yangtze Delta and Adjacent Regions*. (in Chinese). Shanghai Scientific & Technical Publishers, China. 573 pp., 28 plates.

Blomquist, H. L. 1958. The taxonomy and chromatophores of *Pylaiella antillarum* (Grunow) De Toni. *J. Elisha Mitchell scient. Soc.* 74:25–30.

Boczar, B.A., Delaney, T.P. & Cattolico, R.A. 1989. Gene for the ribulose-1,5-bisphosphate carboxylase small subunit protein of the marine chromophyte *Olisthodiscus luteus* is similar to that of a chemoautotrophic bacterium. *Proc. Natl. Acad. Sc. U.S.A.* 86:4996–4999.

Bold, H.C. & Wynne, M.J. 1985. *Introduction to the Algae, Structure and Reproduction*. Prentice-Hall Inc., Englewood Cliffs, U.K. xi + 706 pp.

Boo, S.M. & Choi, Y.T. 1986. Two species of *Sphacelaria* new to Korea. *Korean J. Phycol.* 1:95–101.

Boo, S.M. & Yoon, H.S. 2000. Molecular relationships of giant kelp (Phaeophyceae). *Algae* 15:13–16.

Boo, S.M., Lee, W.J., Yoon, H.S., Kato, A. & Kawai, H. 1999a. Molecular phylogeny of the Laminariales (Phaeophyceae) inferred from small subunit ribosomal DNA sequences. *Phycol. Res.* 47:109–114.

Boo, S.M., Yoon, H.S. & Lee, J.Y. 1999b. Phylogenetic relationships of the Alariaceae, Laminariaceae, and Lessoniaceae (Phaeophyta) inferred from nuclear rDNA ITS and RUBISCO spacer sequences. *J. Phycol.* 35 (suppl.):5–6.

Bremer, K. 1988. The limits of amino–acid sequence data in angiosperm phylogenetic reconstruction. *Evolution* 42:795–803.

Burkhardt, E. & Peters, A.F. 1998. Molecular evidence from nrDNA ITS sequences that *Laminariocolax* (Phaeophyceae, Ectocarpales *sensu lato*) is a worldwide clade of closely related kelp endophytes. *J. Phycol.* 34:682–691.

Cavalier-Smith, T. 1998. A revised six-kingdom system of life. *Biol. Rev. Cambridge Philos. Soc.* 73:203–266

Cavalli-Sforza, L.L. & Edwards, A.W.F. 1967. Phylogenetic analysis: Models and estimation procedures. *Evolution* 32:550–570 & *Am. J. Hum. Genet.* 19:233–257.

Chesnick, J.M., Morden, C.W. & Schmieg, A.M. 1996. Identity of the endosymbiont of *Peridinium foliaceum* (Pyrrophyta): Analysis of the *rbc*LS operon. *J. Phycol.* 32:850–857.

Clayton, M.N. 1981. Phaeophyta. *In* Clayton, M.N. & King, R.J. [Eds] *Marine Botany: An Australian Perspective.* Longman Cheshire, Melbourne, Australia. pp. 104–137.

Clayton, M.N. 1984. Evolution of the Phaeophyta with particular reference to the Fucales. *Progr. Phycol. Res.* 3:11–46.

Clayton, M.N. 1986. Culture studies on the life histories of *Scytothamnus australis* and *Scytothamnus fasciculatus* (Phaeophyta) with electron microscope observations on sporogenesis and gametogenesis. *Br. Phycol. J.* 21:371–386.

Clerck, O. de 1998. *A Revision of the Genus* Dictyota *Lamouroux (Phaeophyta) in the Indian Ocean*. Ph.D. thesis University of Ghent, Belgium. 356 pp.

Colijn, F. & Hoek, C. van den 1971. The life history of *Sphacelaria furcigera* Kütz (*Phaeophyceae*), II. *Nova Hedwigia* 21:899–922.

Coppejans, E. 1977. *Bijdrage tot de Studie van de Wierpopulaties (Chlorophyceae, Phaeophyceae, Rhodophyceae) van het Fotofiel Infralittoraal in het Noordwestelijk Mediterraan Bekken. Part I (text) & Part II (iconography)*. Ph.D. thesis University of Ghent, Belgium. 243 pp., 296 plates.

Coppejans, E. 1983. Iconographie d'algues Méditerranéennes. *Bibliotheca Phycologica* 63:i–xxviii, 317 plates.

Coyer, J.A., Robertson, D.L. & Alberte, R.S. 1994. Genetic variability within a population and between diploid/haploid tissue of *Macrocystis pyrifera* (Phaeophyceae). *J. Phycol.* 30:545–552.

Coyer, J.A., Smith, G.J. & Andersen, R.A. 2001. Evolution of *Macrocystis* spp. (Phaeophyceae) as determined by ITS1 and ITS2 sequences. *J. Phycol.* 37:574–585.

Cunningham, C.W. 1997. Can three incongruence tests predict when data should be combined? *Mol. Biol. Evol.* 14:733–740.

Daugbjerg, N. & Andersen, R.A. 1997. A molecular phylogeny of the heterokont algae based on analyses of chloroplast-encoded *rbc*L sequence data. *J. Phycol.* 33:1031–1041.

Daugbjerg, N. & Guillou, L. 2001. Phylogenetic analyses of Bolidophyceae (Heterokontophyta) using *rbc*L gene sequences support their sister group relationship to diatoms. *Phycologia* 40:153–161.

Delépine, R. & Asensi, A. 1975. *Asteronema* nov. gen. nouveau genre de Phéophycée australe. *Bull. Soc. bot. Fr.* 122:295–304.

Delépine, R., Asensi, A. & Guglielmi, G. 1976. Nouveaux types d'ultrastructure plastidiale chez Phéophycées. *Phycologia* 15:425–434.

Diekmann, O.E., Bak R.P.M., Stam, W.T. & Olsen, J.L. 2001. Molecular genetic evidence for probable reticulate speciation in the coral genus *Madracis* from a Caribbean fringing reef slope. *Mar. Biol.* 139:221–233.

Donoghue, M.J. & Sanderson, M.J. 1992. The suitability of molecular and morphological evidence in reconstructing plant phylogeny. *In* Soltis, P.S., Soltis, D.E. & Doyle, J. J. [Eds] *Molecular Systematics of Plants*, Chapman & Hall, New York, NY, U.S.A., pp. 340–368.

Dop, A.J. 1979. *Porterinema fluviatile* (Porter) Waern (Phaeophyceae) in The Netherlands. *Act. Bot. Neerl.* 28:449–458.

Draisma, S.G.A. & Prud'homme van Reine, W.F. 2001. Onslowiaceae *fam. nov.* (Phaeophyceae). *J. Phycol.* 37:647–649.

Draisma, S.G.A., Keum, Y.-S., Prud'homme van Reine, W.F. & Lokhorst, G.M. 1998. The species of *Sphacelaria* (Sphacelariales, Phaeophyceae) in China with a description of a new species. *Bot. Mar.* 41:181–190.

Draisma, S.G.A., Prud'homme van Reine, W.F., Stam, W.T. & Olsen, J.L. 2001. A reassessment of phylogenetic relationships within the Phaeophyceae based on RUBISCO large subunit and ribosomal DNA sequences. *J. Phycol.* 37:586–603.

Draisma, S.G.A., Olsen, J.L., Stam, W.T. & Prud'homme van Reine, W.F. 2002. Phylogenetic relationships within the Sphacelariales (Phaeophyceae): *rbc*L, RUBISCO-spacer and morphology. *Eur. J. Phycol.* 37:(in press).

Druehl, L.D. & Saunders, G.W. 1992. Molecular explorations in kelp evolution. *Progr. Phycol. Res.* 8:47–83.

Druehl, L.D., Mayes, C., Tan, I.H. & Saunders, G.W. 1997. Molecular and morphological phylogenies of kelp and associated brown algae. *In* Bhattacharya, D. [Ed.] *Origins of Algae and Their Plastids*, Springer-Verlag, Vienna, Austria. pp. 221–235. [Also edited as suppl. 11 of *Plant Syst. Evol.*].

Eernisse, D.J. & Kluge, A. 1993. Taxonomic congruence versus total evidence, and amniote phylogeny inferred from fossils, molecules and morphology. *Mol. Biol. Evol.* 10:1170–1195.

Ehara M., Watanabe K.I., Kawai H., Inagaki Y., Hayashi-Ishimaru Y. & Ohama T. 1998. Distribution of the mitochondrial deviant genetic code AUA for methionine in heterokont algae. *J. Phycol.* 34:1005–1008.

Ehara, M., Kitayama, T., Watanabe, K. I., Inagaki, Y., Hayashi-Ishimaru, Y. & Ohama, T. 1999. Comprehensive molecular phylogenetic analysis of a heterokont alga (NIES 548) using genes from all three cellular compartments. *Phycol. Res.* 47:225–231.

Eichenberger, W., Araki, S. & Müller, D.G. 1993. Betaine lipids and phospholipids in brown algae. *Phytochemistry* 34:1323–1333.

Erikson, T. 1998. AutoDecay ver. 4.0 (program distributed by the author). Department of Botany, Stockholm University, Stockholm, Sweden.

Ernst-Schwarzenbach, M. 1957. Zur Kenntnis der Fortpflanzungsmodi der Braunalge *Halopteris filicina* (Gratel.) Kütz. *Publ. Staz. Zool. Napoli* 29:347–388.

Falkenberg, P. 1878. Über *Discosporangium*, eines neues Phaeosporeen–Genus. *Mitt. Zool. Stat. Neapel* 1:54–66.

Famà, P., Olsen, J.L., Stam, W.T. & Procaccini, G. 2000. High levels of intra- and inter-individual polymorphism in the rDNA ITS1 of *Caulerpa racemosa* (Chlorophyta). *Eur. J. Phycol.* 35:349–356.

Farris, J.S. 1970. Methods for computing Wagner trees. *Syst. Zool.* 34:21–34.

Farris, J.S. 1997. Xac (Computer program and manual distributed by the author). Molekylärsystematiska laboratoriet, Naturhistoriska riksmuseet, Stockholm, Sweden.

Farris, J.S., Källersjö, M., Kluge, A.G. & Bult, C. 1995. Testing significance of incongruence. *Cladistics* 10:315–319.

Farris, J.S., Albert, V.A., Källersjö, M., Lipscomb, D. & Kluge, A.G. 1996. Parsimony jackknifing outperforms neighbor-joining. *Cladistics* 12:99–124.

Felsenstein, J. 1981a. Evolutionary trees from DNA sequences: A maximum likelihood approach. *J. Mol. Evol.* 17:368–376.

Felsenstein, J. 1981b. Evolutionary trees from gene frequencies and quantitative characters: finding maximum likelihood estimates. *Evolution* 35:1229–1242.

Felsenstein, J. 1985. Confidence limits on phylogenies: an approach using the bootstrap. *Evolution* 39:783–791.

Forey, P.L. 2001. The PhyloCode: description and commentary. *Bull. Zool. Nomencl.* 58:81–96.

Fritsch, F.E. 1945. *The Structure and Reproduction of the Algae*, II. Cambridge University Press, Cambridge, U.K. 939 pp.

Fujiwara, S., Iwahashi, H., Someya, J. & Nishikawa, S. 1993. Structure and cotranscription of the plastid-encoded *rbc*L and *rbc*S genes of *Pleurochrysis carterae* (Prymnesiophyta). *J. Phycol.* 29:347–355.

Fujiwara, S., Tsuzuki, M., Kawachi, M., Minaka, N. & Inouye, I. 2001. Molecular phylogeny of the Haptophyta based on the *rbc*L gene and sequence variation in the spacer region of the RUBISCO operon. *J. Phycol.* 37:121–129.

Gerbi, S.A. 1985. Evolution of ribosomal DNA. *In* MacIntyre, R.J. [Ed.] *Molecular Evolutionary Genetics*, Plenum Press, New York, NY, U.S.A. pp. 419–517.

Gerloff, J. 1967. Eine neue Phaeophycee aus dem Süsswasser: *Pseudobodanella peterfii* nov. gen. et nov. spec. *Rev. Roum. Biol.–Bot.* 12:27–35.

Gibson, M. 1994. Reproduction in *Cladostephus spongiosus* in southern Australia (Sphacelariales, Phaeophyceae). *Phycologia* 33:378–383.

Goodband, S.J. 1971. The taxonomy of *Sphacelaria cirrosa* (Roth) Ag., *Sphacelaria fusca* (Huds.) Ag., and *Sphacelaria furcigera* (Kütz.) Sauv. A simple statistical approach. *Ann. Bot.* 35:957–980.

Graham, L.E. & Wilcox, L.W. 2000. *Algae*. Prentice Hall, Inc., Upper Saddle River, NJ, U.S.A. 700 pp.

Gray, S.F. 1821. *A Natural Arrangement of British Plants, ..., I.* London, U.K. xxviii + 824 pp.

Greuter, W. [Ed.] 2000. *International Code of Botanical Nomenclature (Saint Louis Code)*. Koeltz Scientific Books, Königstein, Germany. 474 pp.

Haas-Niekerk, T. de 1965. The genus *Sphacelaria* Lyngbye (*Phaeophyceae*) in the Netherlands. *Blumea* 13:145–161.

Hall, T.A. 1999. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucl. Acids. Symp. Ser.* 41:95–98.

Hanyuda, T., Arai, S. & Ueda, K. 2000. Variability in the *rbc*L introns of caulerpalean algae (Chlorophyta, Ulvophyceae). *J. Plant Res.* 113:403–413.

Haroun, R.J., Prud'homme van Reine, W.F., Müller, D.G., Serrão, E. & Herrera, R. 1993. Deep-water macroalgae from the Canary Islands: new records and biogeographical relationships. *Helgol. Meeresunters.* 47:125–143.

Hauck, F. 1885. Die Meeresalgen Deutschlands und Österreichs. *In* Rabenhorst, L. [Ed.]. *Kryptogamen-Flora von Deutschland, Österreich und der Schweiz*, Leipzig, Germany. Aufl. 2, 2. xviii + 576 pp.

Hennig, W. 1950. *Grundzüge einer Theorie der Phylogenetischen Systematik*, Deutscher Zentralverlag, Berlin, Germany.

Hennig, W. 1966. *Phylogenetic Systematics*, University of Illinois Press, Urbana, IL, U.S.A.

Henry, E.C. 1984. Syringodermatales ord. nov. and *Syringoderma floridana* sp. nov. (Phaeophyceae). *Phycologia* 23:419–426.

Henry, E.C. 1987a. The life history of *Onslowia endophytica* (Sphacelariales, Phaeophyceae) in culture. *Phycologia* 26:175–181.

Henry, E.C. 1987b. Morphology and life histories of *Onslowia bahamensis* sp. nov. and *Verosphacela ebrachia* gen. et sp. nov., with a reassessment of the Choristocarpaceae (Sphacelariales, Phaeophyceae). *Phycologia* 26:182–191.

Henry, E.C. & Müller, D.G. 1983. Studies on the life history of *Syringoderma phinneyi* sp. nov. (Phaeophyceae). *Phycologia* 22:387–393.

Hillis, D.M. & Bull, J.J. 1993. An empirical test of bootstrapping as a method for assessing confidence in phylogenetic studies. *Syst. Biol.* 42:182–192.

Hillis, D.M. & Huelsenbeck, J.P. 1992. Signal, noise, and reliability in molecular phylogenetic analyses. *J. Heredity* 83:189–195.

Hillis, D.M., Moritz, C. & Mable, B.K. 1996a. *Molecular Systematics*, Sinauer Associates, Inc., Sunderland, MA, U.S.A. 655 pp.

Hillis, D.M., Mable, B.K. & Moritz, C. 1996b. Applications of molecular systematics. *In* Hillis, D.M., Moritz, C. & Mable, B.K. [Eds] *Molecular Systematics*, Sinauer Associates, Inc., Sunderland, MA, U.S.A., pp. 515–543.

Hoek, C. van den & Flinterman, A. 1968. The life-history of *Sphacelaria furcigera* Kütz. (Phaeophyceae). *Blumea* 16:193–242.

Hoek, C. van den & Jahns, H.M. 1978. *Algen: Einführung in die Phykologie.* Georg Thieme Verlag, Stuttgart, Germany. 481 pp.

Hoek, C. van den, Mann, D.G. & Jahns, H.M. 1995. *Algae: An Introduction to Phycology*. University Press, Cambridge, U.K. 623 pp.

Holmgren, P.K., Holmgren, N.H. & Barnet, L.C. 1990. *Index Herbariorum. Part I: The Herbaria of the World.* 8th ed. Int. Assoc. for Plant Taxonomy, New York Botanical Garden, New York, U.S.A. 693 pp.

Holmquist, R. 1983. Transitions and transversions in evolutionary descent: an approach to understanding. *J. Mol. Evol.* 19:134–144.

Hommersand, M.H., Frederique, S. & Freshwater, D.W. 1994. Phylogenetic systematics and biogeography of the Gigartinaceae (Gigartinales, Rhodophyta) based on sequence analysis of *rbc*L. *Bot. Mar.* 37:193–203.

Hooper, R.G., Henry, E.C. & Kuhlenkamp, R. 1988. *Phaeosiphoniella cryophyla* gen. et sp. nov., a third member of the Tilopteridales. *Phycologia* 27:395–404.

Hoot, S.B., Kadereit, J.W., Blattner, F.R., Jork, K.B., Schwarzbach, A.E. & Crane, P.R. 1997. Data congruence and phylogeny of the Papaveraceae *s.l.* based on four data sets: *atp*B and *rbc*L sequences, *trn*K restriction sites, and morphological characters. *Syst. Bot.* 22:575–590.

Horiguchi, T. & Yoshida, T. 1998. The phylogenetic affinities of *Myagropsis myagroides* (Fucales, Phaeophyceae) as determined from 18S rDNA sequences. *Phycologia* 37:237–245.

Hornemann, J.V. [Ed.] 1818. *Flora Danica* 9 (27). Claudius Philibertus, Copenhagen, Denmark.

Hugall, A. Stanton, J. & Moritz, C. 1999. Reticulate evolution and the origins of ribosomal internal transcribed spacers diversity in apomictic *Meloidogyne*. *Mol. Biol. Evol.* 16:157–164.

Hwang, S.R. & Tabita, F.R. 1991. Cotranscription, deduced primary structure, and expression of the chloroplast-encoded *rbc*L and *rbc*S genes of the marine diatom *Cylindrotheca* sp. strain N1. *J. Biol. Chem.* 266:6271–6279.

Irvine, D.E.G. 1956. Notes on British species of the genus *Sphacelaria* Lyngb. *Trans. Proc. bot. Soc. Edinb.* 37:24–45.

IUPAC-IUB Commission on Biological Nomenclature 1968. A one-letter notation for amino acid sequences. Tentative rules. *J. Biol. Chem.* 243:3557–3559.

Jao, C.C. 1943. Studies on the fresh–water algae of China. XI. *Sphacelaria fluviatilis*, a new fresh-water brown alga. *Sinensia* 14:151–154.

Johnson, L.A. & Soltis, D.E. 1998. Assessing congruence: Emperical examples from molecular data. *In* Soltis, D.E., Soltis, P.S. & Doyle, J.J. [Eds] *Molecular Systematics of Plants II, DNA Sequencing*. Kluwer Academic Publishers, Boston/Dordrecht/London. pp. 297–348.

Jong, Y.S.D.M. de 1998. *Systematic, Phylogenetic and Biogeographic Studies of Atlantic Seaweeds.* Ph.D. thesis Leiden University, The Netherlands, 206 pp.

Källersjö, M., Farris, J.S., Chase, M.W., Bremer, B., Fay, M., Humphries, C., Pedersen, G., Seberg, O. & Bremer, K. 1998. Simultaneous parsimony jackknife analysis of 2538 *rbc*L DNA sequences reveals support for major clades of green plants, and plants, seed plants and flowering plants. *Plant Syst. Evol.* 213:259–287.

Källersjö, M., Albert, V.A. & Farris, J.S. 1999. Homoplasy *Increases* Phylogenetic Structure. *Cladistics* 15:91–93.

Kawai, H. 1992. A summary of the morphology of chloroplast and flagellated cells in the Phaeophyceae. *Kor. J. Phycol.* 7:33–43.

Kawai, H. & Prud'homme van Reine, W. F. 1998. Life history of Japanese *Stypocaulon durum* (Sphacelariales, Phaeophyceae). *Phycol. Res.* 46:263–270.

Kawai, H. & Sasaki, H. 2000. Molecular phylogeny of the brown algal genera *Akkesiphycus* and *Halosiphon* (Laminariales), resulting in the circumscription of the new families Akkesiphycaceae and Halosiphonaceae. *Phycologia* 39:416–428.

Kawai, H., Muto, H., Fujii, T. & Kato, A. 1995. A linked 5S rRNA gene in Scytosiphon lomentaria. *J. Phycol.* 31:306–311.

Kawai, H., Sasaki, H., Maeda, Y. & Arai, S. 2001a. Morphology, life history and molecular phylogeny of *Chorda rigida* sp. nov. (Laminariales, Phaeophyceae) from the Sea of Japan and the genetic diversity of *Chorda filum. J. Phycol.* 37:130–142.

Kawai, H., Maeba, S. & Sasaki, H. 2001b. *Schizocladia ischiensis*: a new filamentous marine chromophyte, and proposal of a new class Schizocladiophyceae. *Phycologia* 40 (suppl.):6–7.

Kellogg, E.A. & Juliano, N.D. 1997. The structure and function of RuBisCO and their implications for systematic studies. *Am. J. Bot.* 84:413–428.

Keum, Y.-S., Oh, Y.S. & Lee, I.K. 1995. Morphology and life history of *Halopteris filicina* (Sphacelariales, Phaeophyceae) from Korea. *Phycol. Res.* 43:137–144.

Keum, Y.-S., Prud'homme van Reine, W.F. & Lee, I.K. 1997. A taxonomic study of *Sphacelaria* subgenus *Propagulifera* (Sphacelariaceae, Phaeophyta). *Phycologia* 36 (suppl.):49.

Keum, Y.-S., Oak, J.H., Boo, S.M., Prud'homme van Reine, W.F. & Lee, I.K. 1999. *Sphacelaria californica* and *S. novae-hollandiae*, two related species of the genus *Sphacelaria* (Sphacelariales, Phaeophyta). *Algae* 14:23–35.

Keum, Y.-S., Oak, J.H, Prud'homme van Reine, W.F. & Lee, I.K. 2001. Two species of *Sphacelaria* (Sphacelariales, Phaeophyceae), *S. solitaria* (Pringsheim) Kylin and *S. recurva* sp. nov. from Korea. *Bot. Mar.* 44:267–275.

Kimura, M. 1980. A simple method for estimating evolutionary rate of base substitutions through comparative studies of nucleotide sequences. *J. Mol. Evol.* 16:111–120.

Kimura, M. 1981. Estimation of evolutionary distances between homologous nucleotide sequences. *Proc. Natl. Acad. Sci. U.S.A.* 78:454–458.

Kishino, H. & Hasegawa, M. 1989. Evaluation of the maximum likelihood estimate of the evolutionary tree topologies from DNA sequence data, and the branching order in Hominoidea. *J. Mol. Evol.* 29:170–179.

Kitayama, T. 1994. A taxonomic study of the Japanese *Sphacelaria* (Sphacelariales, Phaeophyceae). *Bull. Nat. Sc. Mus. Ser. B (Bot.)* 20:37–141.

Kitayama, T., Kawai, H. & Yoshida, T. 1991. Morphological observations on *Sphacelaria californica* Sauvageau *ex* Setchel *et* Gardner (Sphacelariales, Phaeophyceae), new to Japan. *Jpn. J. Phycol.* 39:189–193.

Kitayama, T., Dai, H.-P. & Han, J.-X. 1995. Brown algae from Zhoushan Islands, Zhejiang Province, China. *Bull. Nat. Sc. Mus. Ser. B (Bot.)* 21:169–178.

Kjellman, F.R. 1891. Choristocarpaceae. pp. 190–192. *In* Engler, A. & Prantl, K. [Eds] *Die Natürlichen Pflanzenfamilien nebst ihren Gattungen und wichtigeren Arten insbesondere den Nutzpflanzen, ... Ed. 1, I(2)*, Wilhelm Engelmann Verlag, Leipzig, Germany. 588 pp. [Publication of the volume: 1897]

Klinger, T. 1993. The persistence of haplodiploidy in algae. *Trends Ecol. Evol.* 8:256–258.

Kogame, K. & Masuda, M. 2001. Crustose sporophytes of *Colpomenia bullosa* (Scytosiphonaceae, Phaeophyceae) in nature. *Cryptogamie, Algol.* 22:201–208.

Kogame, K., Horiguchi, T. & Masuda, M. 1999. Phylogeny of the order Scytosiphonales (Phaeophyceae) based on DNA sequences of *rbc*L, partial *rbc*S, and partial LSU nrDNA. *Phycologia* 38:496–502.

Kogame, K., Uwai, S. & Kawaguchi, S. 2001. First record of *Asteronema rhodochortonoides* (Phaeophyceae) for the Pacific Ocean. *Phycol. Res.* 49:281–284.

Kooistra, W.H.C.F. & Medlin, L.K. 1996. Evolution of the diatoms (Bacillariophyta) IV. Reconstruction of their age from small subunit rRNA coding regions and the fossil record. *Mol. Phylogen. Evol.* 6:391–407.

Kooistra, W.H.C.F., Stam, W.T., Olsen, J.L. & Hoek, C. van den 1992. Biogeography of *Cladophoropsis membranacea* (Chlorophyta) based on comparisons of nuclear rDNA ITS sequences. *J. Phycol.* 28:660–668.

Kostrzewa, M., Valentin, K., Maid, U., Radetzky, R. & Zetsche, K. 1990. Structure of the rubisco operon from the multicellular red alga *Antithamnion spec. Curr. Genet.* 18:465–469.

Kraan, S. & Guiry, M.D. 2000a. Sexual hybridization experiments and phylogenetic relationships as inferred from RuBisCO spacer sequences in the genus *Alaria* (Phaeophyceae). *J. Phyc.* 35:190–198.

Krishnamurthy, V. 1992. Latin diagnoses of three new species of marine algae from India. *Seaweed Research and Utilisation* 14:135–136.

Krishnamurthy, V. & Baluswami, M. 1988. A new species of *Sphacelaria* Lyngbye from South India. *Seaweed Research and Utilisation* 11:67–69.

Kuckuck, P. 1895. Über Schwärmsporenbildung bei den Tilopterideen und über *Choristocarpus tenellus* (Kütz.) Zan. *Jahrb. Wiss. Bot.* 28:290–322.

Kuckuck, P. 1912. Beiträge zur Kenntnis der Meeresalgen. 10. Neue Untersuchungen über *Nemoderma* Schousboe. *Wiss. Meeresunters. Abt. Helgoland, N. F.* 5:117–152.

Kuckuck, P. 1929. *Fragmente einer Monographie der Phaeosporen*. Nienburg, W. [Ed.], Special reprint from *Wiss. Meeresunters., Abt. Helgoland* 17(4), Ad. Littman, Oldenburg, Germany. 93 pp.

Kuhlenkamp, R. & Müller, D.G. 1985. Culture studies on the life history of *Haplospora globosa* and *Tilopteris mertensii* (Tilopteridales, Phaeophyceae). *Br. Phycol. J.* 20:301–312.

Kützing, F.T. 1843. *Phycologia Generalis*. Leipzig, Germany. xxxii + 459 pp., 80 plates.

Kützing, F.T. 1849. *Species Algarum*. F. A. Brockhaus, Leipzig, Germany. 922 pp.

Kützing, F.T. 1855. *Tabulae Phycologicae oder Abbildungen der Tange V. Band*. Nordhausen, Germany. 30 pp., 100 plates.

Kylin, H. 1933. Über die Entwicklungsgeschichte der Phaeophyceen.*Lunds Universitets Årsskrift, Ny Földj*, Adv. 2, Band 29, 102 pp.

Lane, C.E., Mayes, C. & Saunders, G.W. 2001. Using additional genes to shed light on the unresolved issues in kelp (Laminariales; Phaeophyceae) systematics. *Phycologia* 40 (suppl.): 6.

Leclerc, M. C., Barriel, M., Lecointre, G. & Reviers, B. de 1998. Low divergence in rDNA ITS sequences among five species of *Fucus* (Phaeophyceae) suggests a very recent radiation. *J. Mol. Evol.* 46:115–120.

Lee, W.J. & King, R.J. 1996. The molecular characteristics of five genera of Dictyotaceae (Phaeophyta) from Australia: based on DNA sequences of nuclear rDNA Internal Transcribed Spacer (ITS) and 5.8S. *Algae* 11:381–388.

Lee, Y.P. and I.K. Lee. 1987. Notes on *Sphacelaria* (Phaeophyta) from Cheju Island. (in Korean). *Korean J. Bot.* 30:311–321.

Lee, Y.K., Yoon, H.S., Motomura, T., Kim, Y.J. & Boo, S.M. 1999. Phylogenetic relationships between *Pelvetia* and *Pelvetiopsis* (Fucaceae, Phaeophyta) inferred from sequences of the RuBisCo spacer region. *Eur. J. Phycol.* 34:205–211.

Leipe, D.D., Wainright, P.O., Gunderson, J.H., Porter, D., Patterson, D.J., Valois, F., Himmerich, S. & Sogin, M.L. (1994). The stramenopiles from a molecular perspective: 16S–like rRNA sequences from *Labyrinthuloides minuta* and *Cafeteria roenbergensis*. *Phycologia* 33:369–377.

Levinson, G. & Gutman, G.A. 1987. Slipped–strand mispairing: a major mechanism for DNA sequence evolution. *Mol. Biol. Evol.* 4:203–221.

Li, W.-H. 1997. *Molecular Evolution*. Sinauer Associates, Inc., Sunderland, MASS, U.S.A. 487 pp.

Lim, B.-L., Kawai, H., Hori, H. & Osawa, S. 1986. Molecular evolution of 5S ribosomal RNA from red and brown algae. *Jpn. J. Genet.* 61:169–176.

Lu, B.R. & Tseng, C.K. 1980. Studies on some brown algae from the Xisha Islands, Guangdong Province, China. I. (in Chinese). *Studia Marina Sinica* 17:21–35.

Lüning, K. 1990. *Seaweeds. Their Environment, Biogeography, and Ecophysiology*. John Wiley & Sons, New York, U.S.A. 527 pp.

Lyngbye, H.C. 1818. *Sphacelaria reticulata*. t. 1600. *In* Hornemann, J.V. [Ed.] *Flora Danica* 9 (27). Copenhagen, Denmark. plates 1561–1620.

Lyngbye, H.C. 1819. *Tentamen Hydrophytologiae Danicae Continens Omnia Hydrophyta Cryptogama Daniae, Holsatiae, Faeroae, Islandae, Groenlandiae Hucusque Cognita, Systematice Disposita, Descripta et Iconibus Illustrata, Adjectis Simul Speciebus Norvegicis*. Copenhagen, Denmark. 248 pp., LXX tab.

Maddison, W.P. & Maddison, D.R. 1992. *MacClade, Analysis of Phylogeny and Character Evolution, version 3*. Sinauer Associates, Inc., Sunderland, MA, U.S.A.

Magne, F. 1976. Quelques caractères cytologique particuliers du *Bachelotia antillarum* (Phéophycées, Ectocarpales). *Phycologia* 15:309–319.

Malta, E.-j., Draisma, S.G.A., Kamermans, P. 1999. Free-floating Ulva in the southwest Netherlands: species or morphotypes? A morphological, molecular and ecological comparison. *Eur. J. Phycol.* 34 :443–454.

Mann, D.G., Simpson, G.E., Sluiman, H.J. & Möller, M. 2001. *Rbc*L gene tree of diatoms: A second large data-set for phylogenetic reconstruction. *Phycologia* 40 (suppl.):1–2.

Martin, A.P. & Palumbi, S.R. 1993. Body size, metabolic rate, generation time, and the molecular clock. *Proc. Natl. Acad. Sci. USA* 90:4087–4091.

Mathias, W.T. 1935. *Halopteris filicina* Kütz. The cytology of the reproductive organs. *Publs. Hartley Bot. Labs Liverpool Univ.* 13:24–28.

McCourt, R.M., Karol, K.G., Kaplan, S. & Hoshaw, R.W. 1995. Using *rbc*L sequences to test hypotheses of chloroplast and thallus evolution in conjugating green algae (Zygnematales, Charophyceae). *J. Phycol.* 31:989–995.

Medlin, L.K., Kooistra, W.H.C.F., Potter, D., Saunders, G.W. & Andersen, R.A. 1997. Phylogenetic relationships of the ‘golden algae’ (haptophytes, heterokont chromophytes) and their plastids. *In* Bhattacharya, D. [Ed.] *Origins of Algae and Their Plastids*, Springer-Verlag, Vienna, Austria. pp. 187–219. [Also edited as suppl. 11 of *Plant Syst. Evol.*].

Meusnier, I., Olsen, J.L., Stam, W.T., Destombe, C., & Valero, M. 2001. Phylogenetic analyses of *Caulerpa taxifolia* (Chlorophyta) and of its associated bacterial microflora provide clues to the origin of the Mediterranean introduction. *Mol. Ecol.* 10:931–946.

Mickevitch, M.F. & Farris, J.S. 1981. The implications of congruence in *Menidia*. *Syst. Zool.* 30:351–370.

Migula, W. 1909. *Kryptogamen-Flora von Deutschland, Deutsch-Österreich und der Schweiz. Bd II, Algen. 2. Theil.* Verlag Friedriech von Zezschwitz. Gera, Germany. 383 pp.

Moritz, C. & Hillis, D.M. 1996. Molecular systematics: context and controversies. *In* Hillis, D. M., Moritz, C. & Mable, B.K. [Eds] *Molecular Systematics*, Sinauer Associates, Inc., Sunderland, MA, U.S.A. pp. 1–12.

Müller, D.G. & Parodi, E.R. 1994. *Asteronema rhodochortonoides* nov. comb. (Ectocarpales, Phaeophyceae) – a newly recognized taxon with stellate chloroplast arrangement. *Phycologia* 33:471–474.

Müller, D.G., Parodi, E.R. & Peters, A.F. 1998. *Asterocladon lobatum gen. et sp. nov.*, a new brown alga with stellate chloroplast arrangement, and its systematic position judged from nuclear rDNA sequences. *Phycologia* 37:425–432.

Nakamura, Y. 1972. A proposal on the classification of the Phaeophyta. *In* Abbott, I.A. & Kurogi, M. [Eds] *Contributions to the Systematics of Benthic Marine Algae of the North Pacific*, Japanese Society of Phycology, Kobe, Japan. pp.147–155, 3 figs.

Nelson, W.A. 1982. A critical review of the Ralfsiales, Ralfsiaceae and the taxonomic position of *Analipus japonicus* (Harv.) Wynne (Phaeophyta). *Br. Phycol. J.* 17:311–320.

Newton, L. 1931. *A Handbook of the British Seaweeds*. British Museum (Natural History), London, U.K. 478 pp.

Noda, M. 1971. I. Algae of the North-Eastern China and Korea. pp. 1383–1601. *In* Noda, M. [Ed.] *Flora of the North-Eastern Province (Manchuria) of China* Part IV. 1613 pp.

Novaczek, I., Breeman, A.M. & Hoek, C. van den 1989. Thermal tolerance of *Stypocaulon scoparium* (Phaeophyta, Sphacelariales) from eastern and western shores of the North Atlantic Ocean. *Helgoländer Meeresunters.* 43:183–193.

Nozaki, H., Ito, M., Sano, R., Uchiba, H., Watanabe, M.M., Takahashi, H. & Kurowai, T. 1997. Phylogenetic analysis of *Yamagishiella* and *Platydorina* (Volvocaceae, Chlorophyta) based on *rbc*L gene sequences. *J. Phycol.* 33:272–278.

O’Donnell, E.H.J. 1954. The structure and taxonomic position of the brown alga *Microzonia*. *Am. J. Bot.* 41:380–384.

Olsen, J.L., Valero, M., Meusnier, I., Boele-Bos, S. & Stam, W.T. 1998. Mediterranean *Caulerpa taxifolia* and *C. mexicana* (Chlorophyta) are not conspecific. *J. Phycol.* 34:850–856.

Oltmanns, F. 1922. *Morphologie und Biologie der Algen,* II Band. Jena, Germany. 439 pp.

Oppen, M.J.H. van 1995. *Tracking Trails by Cracking Codes: Molecular Biogeography and Evolution of Benthic Cold-Water Seaweeds*. Ph.D. thesis University of Groningen, The Netherlands. 167 pp.

Oppen, M.J.H. van, Olsen, J.L., Stam, W.T., Hoek, C. van den & Wiencke, C. 1993. Arctic-Antarctic disjunctions in the benthic seaweeds *Acrosiphonia arcta* (Chlorophyta) and *Desmarestia viridis/willii* (Phaeophyta) are of recent origin. *Mar. Biol.* 115: 381–386.

Oppen, M.J.H. van, Draisma, S.G.A., Olsen, J.L. & Stam, W.T. 1995. Multiple trans-Arctic passages in the red alga *Phycodrys rubens*: evidence from nuclear rDNA ITS sequences. *Mar. Biol.* 123:179–188.

Orita, M., Suzuki, M., Sekiya, T. & Hayashi, K. 1989. Rapid and sensitive detection of point mutations and DNA polymorphisms using the polymerase chain reaction. *Genomics* 5:874–879.

Oudot-Le Secq, M.-P., Fontaine, J.-M., Rousvoal, S., Kloareg, B. & Loiseaux-de Goër, S. 2001. The complete sequence of a brown algal mitochondrial genome, the ectocarpale *Pylaiella littoralis* (L.) Kjelm. *J. Mol. Evol.* 53:80–88.

Ouriques, L.C. & Bouzon, Z.L. 2000. Stellate chloroplast organization in *Asteronema breviarticulatum* comb. nov. (Ectocarpales, Phaeophyta). *Phycologia* 39:267–271.

Page, R.D.M. & Holmes, E.C. [Eds] 1998. *Molecular Evolution: A Phylogenetic Approach*. Blackwell Science Ltd, London, U.K. 346 pp.

Papenfuss, G.F. 1951. Problems in the classification of the marine algae. *Svensk Botanisk Tidskrift utgifven af svenska botaniska föreningen* 45:4–11.

Parente, M.I., Fletcher, R.L. & Neto, A.I. 2000. New records of brown algae (Phaeophyta) from the Azores. *Hydrobiologia* 440:153–157.

Parker, B.C. & Dawson, E.Y. 1965. Non–calcareous marine algae from California Miocene deposits. *Nova Hedwigia* 10:273–295 + 21 plates.

Peer, Y. van de, Jansen, J., Rijk, P. de & Wachter, R. de 1997. Database on the structure of small ribosomal subunit RNA. *Nucl. Acids Res.* 25:111–116.

Peer, Y. van de, Wachter, R. de 1997. Evolutionary relationships among the eukaryotic crown taxa taking into account site-to-site rate variation in 18S rRNA. *J. Mol. Evol.* 45:619–630.

Peters, A.F. 1998. Ribosomal DNA sequences support taxonomic separation of the two species of *Chorda*: reinstatement of *Halosiphon tomentosus* (Lyngbye) Jaasund (Phaeophyceae, Laminariales). *Eur. J. Phycol.* 33:65–71.

Peters, A.F. & Burkhardt, E. 1998. Systematic position of the kelp endophyte *Laminarionema elsbetiae* (Ectocarpales *sensu lato*, Phaeophyceae) inferred from nuclear ribosomal DNA sequences. *Phycologia* 37:114–120.

Peters, A. F. & Clayton, M. N. 1998. Molecular and morphological investigations of three brown algal genera with stellate plastids: evidence for Scytothamnales ord. nov. (Phaeophyceae). *Phycologia* 37:106–113.

Peters, A. F. & Ramírez, M. E. 2001. Molecular phylogeny of small brown algae, with special reference to the systematic position of *Caepidium antarcticum* (Adenocystaceae, Ectocarpales). *Cryptogamie, Algol.* 22:187–200.

Peters, A. F., Oppen, M. J. H. van, Wiencke, C., Stam, W. T. & Olsen, J. L. 1997. Phylogeny and historical ecology of the Desmarestiaceae (Phaeophyceae) support a southern hemisphere origin. *J. Phycol.* 33:294–309.

Peters, A. F., Ramírez, M. E. & Rülke, A. 2000. The phylogenetic position of the subantarctic marine macroalga *Desmarestia chordalis* (Phaeophyceae) inferred from nuclear ribosomal ITS sequences. *Polar Biology* 23:95–99.

Phillips, N.E. 1998. *Molecular Phylogenetic Analysis of the Pan-Pacific Genus* Sargassum *(Fucales, Phaeophyceae)*. Ph.D. thesis University of Hawaii, U.S.A.

Posada, D. & Crandall, K.A. 1998. MODELTEST: testing the model of DNA substitution. *Bioinformatics* 14:817–818.

Potter, D., Saunders, G.W. & Andersen, R.A. 1997. Phylogenetic relationships of the Raphidophyceae and Xanthophyceae as inferred from nucleotide sequences of the 18S ribosomal RNA gene. *Am. J. Bot.* 84:966–972.

Price, I.R. & Ducker, S.C. 1966. The life history of the brown alga *Splachnidium rugosum*. *Phycologia* 5:261–272.

Price, S.M. 1973. Studies on *Bachelotia* (*Pilayella*?) *antillarum*. I. The occurrence of plurilocular sporangia in culture. *Br. phycol. J.* 8:21–29.

Prud'homme van Reine, W.F. 1978. Criteria used in systematic studies in the Sphacelariales. *In* Irvine, D.E.G. & Price, J.H. [Eds] *Modern approaches to the taxonomy of red and brown algae*, Academic Press, London, U.K. pp. 301–323.

Prud'homme van Reine, W.F. 1982. A taxonomic revision of the European Sphacelariaceae (Sphacelariales, Phaeophyceae). *Leiden Bot. Ser.* 6:1–193.

Prud'homme van Reine, W.F. 1991. *Stypocaulon* and *Halopteris* (Stypocaulaceae, Sphacelariales, Phaeophycophyta), reasons to separate these genera again. *J. Phycol.* 27 (Suppl.):60.

Prud'homme van Reine, W.F. 1993. Sphacelariales (Phaeophyceae) of the world, a new synthesis. *Korean J. Phycol.* 8:145–160.

Pueschel, C.M. & Stein, J.R. 1983. Ultrastructure of a freshwater brown alga from western Canada. *J. Phycol.* 19:209–215.

Quijada, A., Liston, A., Robinson, W. & Alvarez-Buylla, E. 1997. The ribosomal ITS region as a marker to detect hybridization in pines. *Mol. Ecol.* 6:995–996.

Reinke, J. 1890. Übersicht der bisher bekannten Sphacelariaceen. *Ber. dt. bot. Ges.* 8:201–215.

Reinke, J. 1891. Beiträge zur vergleichenden Anatomie und Morphologie der Sphacelariaceen. *Biblthca bot.* 23:1–40.

Reviers, B. de & Rousseau, F. 1999. Towards a new classification of the brown algae. *Progr. Phycol. Res.* 13:107–201.

Ricker, R.W. [Ed.] 1987. *Taxonomy and Biogeography of Macquarie Island Seaweeds*. British Museum (Natural History), London, U.K. 344 pp.

Ribera, M.A., Gomez Garreta, A., Gallardo, T., Cormaci, M., Furnari, G. & Giaccone, G. 1992. Check-list of Mediterranean seaweeds. I. Fucophyceae (Warming, 1884). *Bot. Mar.* 35:109–130.

Rijk, P. de, Robbrecht, E., Hoog, S. de, Caers, A., Peer, Y. van de & Wachter, R. de 1999. Database on the structure of large subunit ribosomal RNA. *Nucl. Acids Res.* 27:174–178.

Rodríguez, F., Oliver, J.F., Márin, A. & Medina, J.R. 1990. The general stochastic model of nucleotide substitutions. *J. Theor. Biol.* 142:485–501.

Rousseau, F., Leclerc, M.-C. & Reviers, B. de 1997. Molecular phylogeny of European Fucales (Phaeophyceae) based on partial large-subunit rDNA sequence comparison. *Phycologia* 36:438–446.

Rousseau, F. & Reviers, B. de 1999a. Phylogenetic relationships within Fucales (Phaeophyceae) based on combined partial SSU + LSU rDNA sequence data. *Eur. J. Phycol.* 34:53–64.

Rousseau, F. & Reviers, B. de 1999b. Circumscription of the order Ectocarpales (Phaeophyceae): bibliographical synthesis and molecular evidence. *Cryptogamie, Algol.* 20:5–18.

Rousseau, F., Reviers, B. de, Leclerc, M.-C., Asensi, A. & Delépine, R. 2000. Adenocystaceae fam. nov. (Phaeophyceae) based on morphological and molecular evidence. *Eur. J. Phycol.* 35:35–43.

Rousseau, F., Burrowes, R., Peters, A.F., Kuhlenkamp, R. & Reviers, B. de 2001. A comprehensive phylogeny of the Phaeophyceae based on nrDNA sequences resolves the earliest divergences. *C. R. Acad. Sci. Paris, Sciences de la Vie* 324:1–15.

Russell, G. & Fletcher, R.L. 1975. A numerical taxonomic study of the British Phaeophyta. *J. Mar. Biol. Ass. U.K.* 55:763–783.

Saitou, N. & Nei, M. 1987. The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Mol. Biol. Evol.* 4:406–425.

Sang, T., Crawford, D.J. & Stuessy, T.F. 1995. Documentation of reticulate evolution in Peonies (*Peonia*) using internal transcribed spacer sequences of nuclear ribosomal DNA: implications for biogeography and concerted evolution. *Proc. Natl. Acad. Sci. USA* 92:6813–6817.

Sarich, V.M. & Wilson, A.C. 1967. Immunological time scale for hominoid evolution. *Science* 158:1200–1203.

Sasaki, H., Flores-Moya, A., Henry, E.C., Müller, D.G. & Kawai, H. 2001. Molecular phylogeny of Phyllariaceae, Halosiphonaceae and Tilopteridales (Phaeophyceae). *Phycologia* 40:123–134.

Saunders, G.W. & Druehl, L.D. 1992. Nucleotide sequences of the small-subunit ribosomal RNA genes from selected Laminariales (Phaeophyta): Implications for kelp evolution. *J. Phycol.* 28:544–549.

Saunders, G.W. & Druehl, L.D. 1993. Nucleotide sequences of the internal transcribed spacers and 5.8S rRNA genes from *Alaria marginata* and *Postelsia palmaeformis* (Phaeophyta: Laminariales). *Mar. Biol.* 115:347–352.

Saunders, G.W. & Kraft, G.T. 1995. The phylogenetic affinities of *Notheia anomala* (Fucales, Phaeophyceae) as determined from partial small–subunit rRNA gene sequences. *Phycologia* 34:383–389.

Saunders, G.W. & Kraft, G.T. 1997. A molecular perspective on red algal evolution: focus on the *Florideophycidae*. *In* Bhattacharya, D. [Ed.] *Origins of Algae and Their Plastids*, Springer-Verlag, Vienna, Austria. pp. 115–138. [Also edited as suppl. 11 of *Plant Syst. Evol.*].

Saunders, G.W., Hill, D.R.A. & Tyler, P.A. 1997a. Phylogenetic affinities of *Chrysonephele palustris* (Chrysophyceae) based on inferred nuclear small-subunit ribosomal RNA sequence. *J. Phycol.* 33:132–134.

Saunders, G.W., Potter, D. & Andersen, R.A. 1997b. Phylogenetic affinities of the Sarcinochrysidales and Chrysomeridales (Heterokonta) based on analysis of molecular and combined data. *J. Phycol.* 33:310–318.

Sauvageau, C. 1900–1914. Remarques sur les Sphacélariacées. Published 1900: *J. Bot., Paris* 14; 1901: *J. Bot., Paris* 15; 1902: *J. Bot., Paris* 16; 1903: *J. Bot., Paris* 17; 1904: *J. Bot., Paris* 18 and published separately; 1914: published separately.

Scagel, R.F. 1966. The Phaeophyceae in perspective. *Oceanogr. Mar. Biol. Annu. Rev.* 4:123–194.

Schloesser, R.E. & Blum, J.L. 1980. *Sphacelaria lacustris* sp. nov., a freshwater brown alga from Lake Michigan. *J. Phycol.* 16:201–207.

Schmidt, O. C. 1937. Choristocarpaceen und Discosporangiaceen. *Hedwigia* 77:1–4.

Schneider, C.W. & Searles, R.B. 1998. Notes on the marine algae of the Bermudas. 3. *Avrainvillea sylvearleae*, *Discosporangium mesarthrocarpum*, and *Peyssonnelia valentinii*. *J. Phycol.* 34:180–188.

Searles, R.B. & Leister, G.L. 1980. North Carolina marine algae. IX. *Onslowia endophytica* gen. et sp. nov. (Phaeophyta, Sphacelariales) and notes on other new records for North Carolina. *J. Phycol.* 16:35–40.

Serrão, E.A., Alice, L.A. & Brawley, S.H. 1999. Evolution of the Fucaceae (Phaeophyceae) inferred from nrDNA-ITS. *J. Phycol.* 35:382–394.

Setchell, W.A. & Gardner, N.L. 1924. Expedition of the California Academy of Sciences to the Gulf of California in 1921. The marine algae. *Proc. Calif. Acad. Sci.* ser. 4, 22:695–949.

Shine, J. & Dalgarno, L. 1974. The 3'-terminal sequence of *E. coli* 16S rRNA: complementarity to nonsense triplets and ribosomal binding sites. *Proc. Natl. Acad. Sci. U.S.A.* 71:1342–1346.

Shine, J. & Dalgarno, L. 1975. Determinant of cistron specificity in bacterial ribosomes. *Nature* 254:34–38.

Siemer, B.L. 1998. *Molecular Phylogenetic Studies in the Simple Brown Algae – Ectocarpales, Chordariales, Dictyosiphonales, Scytosiphonales and Tilopteridales*. Ph.D. thesis University of Copenhagen, Denmark, 138 pp.

Siemer, B.L., Stam, W.T., Olsen, J.L. & Pedersen, P.M. 1998. Phylogenetic relationships of the brown algal orders Ectocarpales, Chordariales, Dictyosiphonales, and Tilopteridales (Phaeophyceae) based on rubisco large subunit and spacer sequences. *J. Phycol.* 34:1038–1048.

Silva, P.C. 1980. Remarks on algal nomenclature VI. *Taxon* 29:121–145.

Silva, P.C. & Reviers, B. de 2000. Ordinal names in the Phaeophyceae. *Cryptogamie Algologie* 21:49–58.

Silva, P.C., Basson, P.W. & Moe, R.L. 1996. *Catalogue of the Benthic Marine Algae of the Indian Ocean*. University of California Publications in Botany, volume 79, University of California Press, Berkeley/Los Angeles/London. pp. [i]–xiv, 1–1259.

Slowinski, J.B. & Page, D.M. 1999. How should species phylogenies be inferred from sequence data? *Syst. Biol.* 48:814–825.

Sokal, R.R. & Sneath, P.H.A. 1963. *Principles of Numerical Taxonomy*, Freeman, San Francisco, CA, U.S.A.

Soltis, D.E. & Soltis, P.S. 1998. Choosing an approach and an appropriate gene for phylogenetic analysis. *In* Soltis, D.E., Soltis, P.S. & Doyle, J.J. [Eds] *Molecular Systematics of Plants II. DNA Sequencing*, Kluwer Academic Publishers, Dordrecht, The Netherlands. pp. 1–42.

Stache-Crain, B., Müller, D.G. & Goff, L.J. 1997. Molecular systematics of *Ectocarpus* and *Kuckuckia* (Ectocarpales, Phaeophyceae) inferred from phylogenetic analysis of nuclear- and plastid-encoded DNA sequences. *J. Phycol.* 33:152–168.

Stam, W.T., Bot, P.V.M., Boele-Bos, S.A., Rooij, J.M. van & Hoek, C. van den 1988. Single-copy DNA-DNA hybridizations among five species of *Laminaria* (Phaeophyceae): phylogenetic and biogeographic implications. *Helgol. Meeresunters.* 42:251–267.

Swofford, D.L. 1991. When are phylogeny estimates from molecular and morphological data incongruent? *In* Miyamote, M.M. & Cracraft, J. [Eds] *Phylogenetic Analysis of DNA Sequences*, Oxford University Press, New York, NY, U.S.A., pp. 185–197.

Swofford, D.L. 1998. PAUP* (Phylogenetic Analysis Using Parsimony), Version 4.0b1a. Sinauer, Sunderland, MA, U.S.A.

Swofford, D.L. & Olsen, G.J. 1990. Phylogeny reconstruction. *In* Hillis, D.M. & Moritz, C. [Eds] *Molecular Systematics*, Sinauer Associates, Inc., Sunderland, MA, U.S.A. pp. 411–502.

Tajima, F. & Nei, M. 1984. Estimation of evolutionary distance between nucleotide sequences. *Mol. Biol. Evol.* 1:269–285.

Tamura, K. & Nei, M. 1993. Estimation of the number of nucleotide substitutions in the control region of mitochondrial DNA in humans and chimpanzees. *Molec. Biol. Evol.* 10:512–526.

Tan, I.H. & Druehl, L.D. 1993. Phylogeny of the northeast Pacific brown algal (Phaeophycean) orders as inferred from 18S rDNA gene sequences. *Hydrobiologia* 260/261:699–704.

Tan, I.H. & Druehl, L.D. 1994. A molecular analysis of *Analipus* and *Ralfsia* (Phaeophyceae) suggests the order Ectocarpales is polyphyletic. *J. Phycol.* 30:721–729.

Tan, I.H. & Druehl, L.D. 1996. A ribosomal DNA phylogeny supports the close evolutionary relationships among the Sporochnales, Desmarestiales, and Laminariales (Phaeophyceae). *J. Phycol.* 32:112–118.

Trono, G., Jr. 1971. Some new species of marine benthic algae from the Caroline Islands, Western–Central Pacific. *Micronesia* 7: 45–77.

Tseng, C.K. [Ed.] 1983. *Common Seaweeds of China*. Science Press, Beijing, China. 316 pp.

Uwai, S., Kogame, K. & Masuda, M. 2001. Reassessment of the taxonomic status of *Elachista tenuis* and related species (Elachistaceae, Phaeophyceae), based on culture studies and molecular phylogenetic analyses. *Eur. J. Phycol.* 36:103–111.

Valentin, K. & Zetsche, K. 1989. The genes of both subunits of ribulose-1,5-bisphosphate carboxylase constitute an operon on the plastome of a red alga. *Curr. Genet.* 16:203–209.

Valero, M., Richerd, S., Perrot, V. & Destombe, C. 1992. Evolution of alternation of haploid and diploid phases in life cycles. *Trends Ecol. Evol.* 7:25–29.

Wehr, J.D. & Stein, J.R. 1985. Studies on the biogeography and ecology of the freshwater phaeophycean alga *Heribaudiella fluviatilis*. *J. Phycol.* 12:81–93.

Wendel, J.F. & Doyle, J.J. 1998. Phylogenetic incongruence: Window into genome history and molecular evolution. *In* Soltis, D.E., Soltis, P.S. & Doyle, J.J. [Eds] *Molecular Systematics of Plants II, DNA Sequencing*. Kluwer Academic Publishers, Boston/Dordrecht/London. pp. 265–296.

Wendel, J.F., Schnabel, A. & Seelanan, T. 1995. Bidirectional interlocus concerted evolution following allopolyploid speciation in cotton (*Gossypium*). *Proc. Natl. Acad. Sci. USA* 92:280–284.

White, T.J., Bruns, T.D., Lee, S.B. & Taylor, J.W. 1990. Amplification and direct sequencing of fungal ribosomal genes for phylogenetics. *In* Innis, M.A., Gelfland, D.H., Sminskey, J.J. & White, T.J. [Eds] *PCR Protocols, a Guide to Methods and Applications*. Academic Press, Inc., San Diego, CA, U.S.A. pp. 315–322.

Wiley, E.O., Siegel-Causey, D., Brooks, D.R. & Funk, V.A. 1991. *The Complete Cladist: a Primer of Phylogenetic Procedures*, University of Kansas Museum of Natural History, Lawrence, KA, U.S.A. 158 pp.

Womersley, H.B.S. 1987. *The Marine Benthic Flora of Southern Australia. Part II*. South Australian Government Printing Division, Adelaide, Australia. 484 pp.

Wynne, M.J. 1982. Phaeophyceae. *In* Parker, S. P. [Ed.] *Synopsis and Classification of Living Organisms*. McGraw-Hill, New York, U.S.A. pp. 115–127.

Wynne, M.J. & Loiseaux, S. 1976. Phycological Reviews 5. Recent advances in life history studies of the Phaeophyta. *Phycologia* 15:435–452.

Yoon, H.S. & Boo, S.M. 1999. Phylogeny of Alariaceae (Phaeophyta) with special reference to *Undaria* based on sequences of the RuBisCo spacer region. *Hydrobiologia* 398/399:47–55.

Yoon, H.S., Lee, J.Y., Boo, S.M. & Bhattacharya, D. 2001. Phylogeny of Alariaceae, Laminariaceae, and Lessoniaceae (Phaeophyceae) based on plastid-encoded RuBisCo spacer and nuclear-encoded ITS sequence comparisons. *Molec. Phylog. Evol.* 21:231–243.

Yotsukura, N., Denboh, T., Motomura, T., Horiguchi, T., Coleman, A.W. & Ichimura, T. 1999. Little divergence in ribosomal DNA internal transcribed spacer -1 and -2 sequences among non-digitate species of *Laminaria* (Phaeophyceae) from Hokkaido, Japan. *Phycol. Res.* 47:71–80.

Zanardini, G. 1860. *Iconographia Phycologica Adriatica ossia Scelta di Ficee Nuove o più Rare del Mare Adriatico Vol. I*. G. Antonelli, Venice, Italy. 175 + VIII pp.

Zimmer, E.A., Martin, S.L., Beverley, S.M., Kan, Y.W. & Wilson, A.C. 1980. Rapid duplication and loss of genes coding for *a* chains of hemoglobin. *Proc. Natl. Acad. Sci. USA* 77:2158–2162.

Zuccarello, G.C., West, J.A., Karsten, U. & King, R.J. 1999a. Molecular relationships within *Bostrychia tenuissima* (Rhodomelaceae, Rhodophyta). *Phycol. Res.* 47:81–85.

Zuccarello, G.C., West, J.A., Kamiya, M. & King, R.J. 1999b. A rapid method to score plastid haplotypes in red seaweeds and its use in determining parental inheritance of plastids in the red alga *Bostrychia* (Ceramiales). *Hydrobiologia* 401:207–214.

Zuccarello, G.C., Bartlett, J. & Yeates, P.H. 2000. Differentiation of *Caloglossa leprieurii* (Rhodophyta) populations in northern and eastern Australia using plastid haplotypes. *Eur. J. Phycol.* 35:357–363.

Zuccarello, G.C., Yeates, P.H., Wright, J.T. & Bartlett, J. 2001. Population structure and physiological differentiation of haplotypes of *Caloglossa leprieurii* (Rhodophyta) in a mangrove intertidal zone. *J. Phycol.* 37:235–244.

Zuckerkandl, E. & Pauling, L. 1965. Evolutionary divergence and convergence in proteins. *In* Bryson, V. and Vogel, H.J. [Eds] *Evolving Genes and Proteins*. Academic Press, New York, U.S.A. pp. 97–166.

SUMMARY

The Phaeophyceae or brown algae form a class of multicellular heterokont algae. As a group they play a key role in structuring subtidal marine communities throughout temperate and tropical zones. Kelp forests (Laminariales) contain the giants of the algal world with thalli reaching >60 m. Fucoids (e.g. wracks) play dominant inter- or subtidal roles by forming large monostands. The class of brown algae includes about 13–17 orders, including the Sphacelariales. Because of the enormous morphological diversity, combined with a variety of ultrastructural and cytological characters, the evolutionary relationships among orders have long been a source of speculation. This is the basis for the present research. Chapter 1 provides an introduction to the brown algae and the hierarchical approach adopted in this thesis.

Historically, phycological systematists have hypothesised many phylogenies ranging from the Ectocarpales being considered most primitive/ancestral based on their simple filamentous construction, to the Fucales being hypothesised the most advanced/recent because of their oogamous, diplontic life history. With the advent of formal phylogenetic methods in the 1970s and 80s and comparative DNA sequence data in the 1990s, opportunities for more accurate investigation became possible. Previous molecular phylogenetic studies (Table 2.1) have utilised rDNA 18S sequence data and an exemplar approach to taxon sampling. In a number of cases, consolidation of data sets has remained problematic because the sequences were incomplete with different regions missing from different data sets. With the recent availability of automated sequencing and additional sequences, the time was ripe to completely re-evaluate phylogenetic relationships within the brown algae including the position and putative monophyly of the Sphacelariales.

The Sphacelariales are characterised by apical growth, parenchymatous tissues and a black staining response following the application of household bleach. Filaments form mats and tufts which may be epilithic or epiphytic. Though a cosmopolitan group, species diversity seems to be highest along the temperate coasts of western Europe and southern Australia-New Zealand. Tropical and (Ant)arctic species diversity seems to be low. The degree to which this reflects sampling bias or a true assessment is unclear.

The traditional four families are primarily based on differences in branching mode. The Cladostephaceae are monotypic, the Sphacelariaceae have two genera, the Stypocaulaceae have 5 genera, and the Choristocarpaceae have four genera. The Sphacelariaceae are the most speciose with >40 species out of a total for the order of >60. Further taxonomic details are provided in order to set the stage for the new analyses.

Phylogenetic relationships within the Phaeophyceae are assessed in Chapter 2 using nuclear encoded partial 18S + 26S rDNA sequences and chloroplast encoded *rbc*L sequences (coding for the large subunit of the RUBISCO-gene). Both the rDNA and *rbc*L data showed the Dictyotales, Sphacelariales and Syringodermatales to be the most basal brown algal orders and the Sphacelariales to be non-monophyletic (Figs 2.2 and 2.4). Furthermore, both data sets confirmed earlier suggestions that the Chorda-

riales, Dictyosiphonales (incl. Punctariales), and Scytosiphonales should be merged with the Ectocarpales. The *rbc*L tree showed additional support for the sister-group relationship of the Laminariales and the Ectocarpales *sensu lato*. See also Chapter 8.

Chapter 3 focuses on phylogenetic relationships within the Sphacelariales *sensu stricto* (i.e., a monophyletic group containing the Cladostephaceae, Sphacelariaceae, and Stypocaulaceae). Both molecular and morphological data were analysed. The morphological data set included 23 characters, the molecular data set *rbc*L + partial RUBISCO-spacer sequences. Taxon sampling at the genus level was 75%, at the species level ± 30%. The 19 species were all collected from the Northern Hemisphere with one exception. Analysis of the morphological data set resulted in an almost completely unresolved tree (Fig. 3.3). The only supported clade consisted of the *Sphacelaria* subgenus *Propagulifera*. Maximum parsimony analysis of *rbc*L sequences revealed four basal clades with moderate to high support (Fig. 3.2): (1) Stypocaulaceae, (2) *Sphacelaria radicans*, (3) *Sphacelaria caespitula*, and (4) all other included taxa; (*Sphacella* + *Sphacelaria* subgenus *Propagulifera*) and (Cladostephaceae + *Sphacelaria nana* + *Sphacelaria* subgenera *Battersia* and *Pseudochaetopteris*). The phylogenetic relationships between these groups could not be established unambiguously. There was no enhancement in the resolution of the trees when the RUBISCO-spacer sequences were analysed on their own or in combination with the *rbc*L sequences. Subsequently, morphological characters were mapped onto the *rbc*L tree in order to identify diagnostic or phylogenetically informative characters (Fig. 3.5). The presence of propagules with a lenticular central apical cell or a terminal hair defines the *Propagulifera*; and a strict acroblastic branching mode and axillary zoidangia define the Stypocaulaceae. The remaining characters have been gained or lost multiple times. Four options for a new classification within the order including nomenclatural changes are discussed (Fig. 3.6). It is concluded that none of the options will lead to greater clarity either with respect to identification or classification.

In Chapter 4 the suitability of the internal transcribed spacers (ITS1 and ITS2) of the ribosomal cistron for phylogenetic studies in the Sphacelariales is explored. ITS sequences were determined for six species, representing all four clades mentioned in the previous paragraph (Ch. 3). The ITSs were extremely variable and very difficult to align due to length differences, large indels and long stretches of repeat motifs (Figs 4.1, 4.2). Furthermore, intra-specific and intra-individual ITS variation was observed. These results pretty well confirm that ITS sequences will not be suitable for phylogenetic reconstructions within the Sphacelariales at the generic level. However, ITS sequences may be suitable for phylogenetic studies in certain species of Sphacelariales from the subgeneric to the intra-specific level. If intra-individual variation can be shown to be less than inter-individual variation some interesting haplotypic distributions may be revealed that would shed light on how populations disperse or differentiate.

In Chapter 5 phylogenetic relationships within the most species-rich *Sphacelaria* subgenus, i.e., *Propagulifera*, are investigated. The tropical to temperate *Propagulifera* are characterised by the occurrence of propagules: specialised branchlets for vegetative propagation. The traditional sections *Propagulifera*, *Furcigerae* and *Tribuloides* are defined on the basis of morphology of the propagules. Results show that these characters are not reliable. A phylogenetic analysis, using *rbc*L and complete RUBISCO-spacer sequences, revealed both paraphyly and the presence of cryptic species. Three well-supported clades were recovered (Fig. 5.1). The most basal clade was formed by *S. cirrosa* (section *Propagulifera*) only, which was used as outgroup. A second clade consisted of *S. californica* (*Tribuloides*), which was basal within this clade, and *S. divaricata* and *S. rigidula* (both *Furcigerae*). The third clade consisted of the basal *S. tribuloides* (*Tribuloides*) and *S. yamadae* and *S. rigidula* (both *Furcigerae*). It is concluded that propagule morphologies have evolved multiple times within the sections and are, therefore, not phylogenetically informative within the subgenus. Well-supported genetic differences among the clades within the subgenus reveal cryptic species, i.e., *S. rigidula*. In the absence of reliable morphological differences it is recommended that the sections *Furcigerae* and *Tribuloides* either no longer be recognised or that they be merged into one section.

Chapter 6 reports on a study of a herbarium collection of marine *Sphacelaria* specimens collected along the entire Chinese coast, including islands in the South China Sea. Seven species of *Sphacelaria* were found, all belonging to the subgenus *Propagulifera*: *S. rigidula*, *S. tribuloides*, *S. novae-hollandiae*, *S. californica* and *S. carolinensis* being previously recorded in this region. The two newly recorded species are *S. divaricata* and a new species, *S. tsengii*. *Sphacelaria tsengii* is characterised by long propagules with a tapering stalk and two tapering arms (Figs 6.4 to 6.12). The holotype of *S. carolinensis* was also re-examined, revealing an asymmetric branching mode that had not been described before (Fig. 6.23). Finally, a dichotomous identification key for the *Sphacelaria* species of China is given.

In Chapter 7 the separation of the genera *Onslowia* and *Verosphacela* from the Choristocarpaceae and from the Sphacelariales is proposed based on the results of Chapter 2 and morphological considerations. The new family Onslowiaceae is created to include these two genera.

Chapter 8 provides an update of the *rbc*L-based phaeophycean phylogeny of Chapter 2, including many new sequences and representing all, but three, brown algal orders. According to this phylogeny (Fig. 8.1), the Halosiphonaceae and Phyllariaceae do not belong to the Laminariales. These two families are more closely related to the Tilopteridales and Fucales. The genus *Asteronema* is not monophyletic either: *A. rhodochortonoides* is sister to *Asterocladon* and together they are sister to the Ectocarpales *s.l.*, whereas *A. ferruginea* is sister to the Scytothamnales.

Phylogenetic hypotheses about brown algae have long been dominated by the concept that life history pattern and thallus construction are of fundamental importance for the inference of the evolutionary history. The past years of DNA sequence studies have shown that this view is incorrect: the classical phaeophycean phylogenetic tree has been turned upside down. The Dictyotales and Sphacelariales (both until recently considered advanced groups) are the basal orders and consequently an apical meristem, an isomorphic life history, and cells with numerous lens-shaped chloroplasts without pyrenoids should be regarded as ancestral characters in brown algae. The oogamous diplontic Fucales, previously considered to be either primitive or advanced, branch-off intermediate. Since molecular studies demonstrated a low degree of genetic diversity in the brown algae compared to red algae, it has been hypothesised that the brown algae are a relatively young group originating in the Mesozoic (Triassic-Jurassic-Cretaceous).

In Chapter 9 the updated *rbc*L phylogeny (Fig. 8.1) is used as a basis for a new phylogeny-based classification scheme for the Phaeophyceae and results from rDNA-based studies and morphology are included in the discussion. A new classification (Table 9.1) would comprise 20 orders, including 6 new ones (for *Asterocladon* + *Asteronema rhodochortonoides* + *A. breviarticulatum*, *Bachelotia*, Choristocarpaceae, Halosiphonaceae, Onslowiaceae, and Phyllariaceae).

The following suggestions are made for future research in the Phaeophyceae and the Sphacelariales. At the ordinal level, the 'brown tree' needs intensive taxon sampling in unsampled (Ascoseirales, Cutleriales, Ralfsiales) and poorly sampled (Dictyotales, Fucales, Sporochnales, Syringodermatales) orders. *Rbc*L provides better resolution than nuclear ribosomal genes. If a decision between the two must be made, *rbc*L is preferred. Additional genes, both nuclear and mitochondrial, need to be sought. Within the Sphacelariales, the focus needs to shift to better biogeographic coverage, specifically in the Southern Hemisphere.

In conclusion, this thesis, in combination with previous monographs of the European (Prud'homme van Reine 1982) and Japanese (Kitayama 1994) Sphacelariaceae and the Australian Sphacelariales (Womersley 1987), represents one of the most comprehensive accounts of any phaeophycean order. This in turn provides a unique opportunity to investigate phylogeographic patterns and species radiations within particular sub-lineages. The cosmopolitan abundance of the Sphacelariales and the disjunctions within the genus *Sphacelaria* provide interesting cases for investigating recolonisation pathways in temperate latitudes and vicariant events in tropical latitudes.

GEARFETTING

De Phaeophyceae of brúnwieren foarmje in klasse fan mearsellige wieren hearrend ta de divyzje Heterokontophyta (goud-brúne wieren) wêrfan de oare klassen hast inkeld en allinne ienselllich binne. Brúnwieren nimme in wichtich plak yn yn'e maryne libbensmienskippen fan'e matige en tropyske kriten. Yn kelpwâlden (oarder Laminariales) fynt men de reuzen fan'e wierenwrâld dy't mear as 60 meter grut wurde kinne. De Fucales (û.o. Beiwier, Gnodzewier, Blaeswier) kinne tige grutte iensoartige fjilden foarmje yn'e tijsône of ûnder de leechwetterstreek. De klasse fan'e brúnwieren is ûnderferdield yn sa'n 13 oant 17 oarders, wêrûnder de Sphacelariales. Oer de evolúsjonêre sibskippen tusken de brúnwieroarders is lange tiid spekulearre, fanwegen it ôfgryslike ferskaat oan foarmen yn kombinaasje mei in fariëteit fan ultrastrukturele en sytologyske skaeimerken. Dit is de basis foar dit proefskrift. Haadstik 1 jout in ynlieding oer de brúnwieren en it hiërargyske oanpakken dat yn it ûndersyk tapast is.

Wierologen hawwe foarhinne in protte brúnwierfylogenieën (= sibskipbeammen) ornearre. Guon hâlden de triedfoarmige Ectocarpales foar de primityfste brúnwierskift, wylst oaren ornearen dat de Fucales mei harren diplonte libbenssyklus de susterskift fan alle oare brúnwieren binne. Krekter ûndersyk waard mooglik mei it opkommen fan formele fylogenetyske metoades yn'e jieren 70 en 80 (fan'e 20ste ieu) en de mooglikheid ta it ferlykjen fan DNA-sekwinsjes yn'e jieren 90. Eardere molekulêre fylogenetyske stúdzjes (Tabel 2.1) hawwe benammen gebrûkmakke fan DNA-sekwinsjes fan de lytse subienheid (SSU) fan it ribosomale DNA (rDNA) en faak waard eltse brúnwieroarder mar troch ien soarte fertsjinwurdige. It kombinearjen fan de gegevens fan ûnderskate stúdzjes waard wol gauris dreger makke trochdat sekwinsjes faak net folslein wiene en ûngelikense stikken DNA brûkt binne. Mei de resinte mooglikheid ta automatysk sekwinsjen en de beskikberens fan meardere sekwinsjes wie de tiid ryp om de fylogenetyske sibskippen binnen de Phaeophyceae en de posysje fan'e Sphacelariales en harren ornearre monofyly (= ôfstamming yn'e mande) opnij te hifkjen.

De Sphacelariales wurde karakterisearre troch waaksdom fan út in topsel, parengymatysk weefsel (= seldielingen yn alle flakken) en tydlik swartkleurjen as reaksje op in behanneling mei oanlinge bleekwetter. De planten foarmje matten en túfkes op stiennen en/of oare wieren. Alhoewol't se in wrâldwiide fersprieding fertoane, is it ferskaat oan soarten it heechst lâns de matige kusten fan west-Europa en it súden fan Australië en Nij-Seelân. It ferskaat yn'e tropen en by de poalen is nei alle gedachten leech. Hoefier dit wier sa is of dat it in artefakt is fan in legere meunstertichtens yn dizze kriten, is net rjocht dúdlik.

De tradysjonele yndieling yn fjour famyljes is benammen basearre op ferskillen yn'e wize fan útspranteljen. De Cladostephaceae bestean út mar ien soarte, de Sphacelariaceae bestean út twa slachten, de Stypocaulaceae hawwe fiif slachten en de Choristocarpaceae fjour. De Sphacelariaceae binne it soartenrykst mei mear as 40 soarten op in gehiel fan mear as 60 foar de hiele oarder. Fierders wurde taksonomyske fine puntsjes jûn as ynlieding op'e nije analyzes.

Fylogenetyske sibskippen binnen de Phaeophyceae wurde rekonstruearre yn Haadstik 2 gebrûkmeitsjend fan yn'e chloroplast koadearre *rbc*L-sekwinsjes (koadearjend foar de grutte subienheid [LSU] fan't RUBISCO-gen) en fan kearnkoadearre dielen SSU + LSU rDNA-sekwinsjes. Sawol de rDNA- as de *rbc*L-gegevens toanden oan dat de Dictyotales, Sphacelariales en Syringodermatales de meast basale brúnwieroarders binne en dat de Sphacelariales net monofyletysk binne (Figueren 2.2 en 2.4). Fierders bekrêftigen allebeide data-sets eardere stúdzjes wêryn suggerearre waard dat de Chordariales, Dictyosiphonales (Punctariales ynbegrepen) en Scytosiphonales opnommen soene moatte wurde yn'e Ectocarpales *sensu lato*. Sjoch ek Haadstik 8.

Haadstik 3 is talein op'e fylogenetyske sibskippen binnen de Sphacelariales *sensu stricto* (in monofyletyske skift besteande út de Cladostephaceae, Sphacelariaceae en Stypocaulaceae). Sawol molekulêre as ek morfologyske data waarden analisearre. De morfologyske dataset bestie út 23 skaeimerken, de molekulêre dataset út *rbc*L-sekwinsjes en it lêste diel fan'e RUBISCO-*spacer*. Taksonmeunstering op't slachtenivo wie 75% en op't soartenivo ± 30%. De 19 soarten binne, útsein ien, allegearre sammele op it noardlik healrûn. Út de analyze fan'e morfologyske dataset kaam in hast folslein ûnoploste beam fuort (Fig. 3.3). De iennichste skift dy't stipe waard wie it *Sphacelaria*-ûnderslachte *Propagulifera*. Maksimum parsimony-analyze fan *rbc*L-sekwinsjes leverde fjour basale skiften op (Fig. 3.2): (1) Stypocaulaceae, (2) *Sphacelaria radicans*, (3) *Sphacelaria caespitula* en (4) alle oare analisearre taksa; (*Sphacella* + *Sphacelaria*-ûnderslachte *Propagulifera*) en (Cladostephaceae + *Sphacelaria nana* + *Sphacelaria*-ûnderslachten *Battersia* en *Pseudochaetopteris*). De fylogenetyske sibskippen tusken dizze fjour skiften koene net mei wissens fêststeld wurde. Analyze fan'e dielen fan'e RUBISCO-*spacer*-sekwinsjes allinne of yn kombinaasje mei *rbc*L-sekwinsjes joech gjin better oploste beammen. Fierders waarden de morfologyske skaeimerken projektearre op'e *rbc*L-beam om sa diagnostyske of fylogenetysk ynformative skaeimerken te identifisearjen (Fig. 3.5). It ûnderslachte *Propagulifera* wurdt definiearre troch it hawwen fan propagula (spesjaal foar de fegetative fuortplanting foarme tûkjes) mei in linsfoarmige sintrale topsel of in einhier. De Stypocaulaceae wurde definiearre troch de konsekwinte akroblastyske (sjoch Fig. 3.1) wize fan útspranteljen en de posysje fan fuortplantingsstruktueren yn'e hernes fan sydtûken. De oare skaeimerken binne meardere kearen ûntstien of ferlern gien. Fjour opsjes foar in nije yndieling binnen de oarder mei tabehearrende nomenklatueryske feroarings wurde bediskusjearre (Fig. 3.6). Net ien fan'e opsjes liedt ta mear dúdlikens wat de identifikaasje of klassifikaasje oanbelanget.

Yn Haadstik 4 is de geskiktheid fan'e *internal transcribed spacers* (ITS1 en ITS2) fan't ribosomale sistron foar fylogenetyske stúdzjes oan de Sphacelariales ûndersocht. ITS-sekwinsjes binne bepaald foar seis soarten, dy't de fjour skiften fertsjinwurdigje dy't yn'e foarige paragraaf neamd waarden (H. 3). De ITS'en wiene tige fariabel en dreech mei inoar yn oerienstimming te bringen (*alignen*) as gefolch fan lingteferskillen, grutte ynsersjes en/of deleesjes en lange stikken mei *repeat motifs* (stikjes herhelle sekwinsje) (Figueren 4.1 en 4.2). Der waard ek yntra-spesifyke en yntra-yndividuele fariaasje fûn. Dizze úkomsten meitsje klear dat ITS-sekwinsjes net geskikt binne foar fylogenetyske rekonstruksjes fan'e Sphacelariales op't slachtenivo. ITS-sekwinsjes binne miskien wol geskikt foar fylogenetyske stúdzjes fan bepaalde Sphacelarialessoarten op't nivo fan ûnderslachte en it yntra-spesifyke nivo. Bywannear't oantoand wurde kin dat yntra-yndividuele fariaasje leger is as de fariaasje tusken yndividuën, kinne in mannich ynteressante genotypyske ferspriedingen ûntbleatse wurde, itjinge ús mear ynsjoch jaan kin oer hoe't populaasjes harren ferspriede of differinsjearre.

Yn Haadstik 5 binne fylogenetyske sibskippen binnen it soartenrykste *Sphacelaria*-ûnderslachte *Propagulifera* ûndersocht. De tropyske oant matige *Propagulifera* wurde karakterisearre troch it hawwen fan propagula. De tradysjonele seksjes *Propagulifera*, *Furcigerae* en *Tribuloides* binne definiearre op basis fan'e morfology fan'e propagula. De útkomsten toane oan dat dizze skaeimerken net fertroud binne. In fylogenetyske analyze fan *rbc*L- en folsleine RUBISCO-*spacer*-sekwinsjes ûntbleatet sawol parafyly (gjin monofyly) as kryptyske soarten (d.w.s. morfologysk net te ûnderskieden). Der waarden trije goed stype skiften fûn (Fig. 5.1). De meast basale skift waard foarme troch *S. cirrosa* (seksje *Propagulifera*) allinne, dy't brûkt waard as bûtenskift. In twadde skift bestie út *S. californica* (*Tribuloides*), dy't basaal wie binnen dizze skift, *S. divaricata* en *S. rigidula* (beiden *Furcigerae*). De tredde skift bestie út de basale *S. tribuloides* (*Tribuloides*) en fierders *S. yamadae* en *S. rigidula* (beiden *Furcigerae*). Der wurdt konkludearre dat de ferskillende foarmen fan propagula meardere kearen binnen de seksjes evoluearre binne en dêrom net fylogenetysk ynformatyf binne binnen it ûnderslachte. Goed stype genetyske ferskillen tusken de skiften binnen it ûnderslachte ûntbleatsje kryptyske soarten, te witten *S. rigidula*. Fanwegen de ôfwêzigens fan fertroude morfologyske ferskillen wurdt foarslein om de seksjes *Furcigerae* en *Tribuloides* net mear te erkennen of om se meiïnoar yn ien seksje te pleatsen.

Haadstik 6 berjochtet oer in stúdzje fan in herbariumkolleksje fan maryne *Sphacelaria*-eksimplaren dy't sammele binne lâns de hiele Sjineeske kust en atollen yn'e Súd-Sjineeske See. Der waarden sân soarten *Sphacelaria* fûn, allegearre hearrend ta it ûnderslachte *Propagulifera*: *S. rigidula*, *S. tribuloides*, *S. novae-hollandiae*, *S. californica* en *S. carolinensis* binne earder fermeld foar dit gebiet. Twa soarten binne nea earder fermeld foar Sjina, te witten *S. divaricata* en in nije soarte, *S. tsengii*. *Sphacelaria tsengii* wurdt karakterisearre troch lange propagula mei in tarinnende stâle en twa tarinnende earms (Figueren 6.4–6.12). It holotype (it eksimplaar op basis wêrfan de soarte beskreaun is) fan *S. carolinensis* is ek opnij besjoen en de propagula blykten asymmetrysk út te spranteljen, itjinge noch nea earder beskreaun wie (Fig. 6.23). Ta beslút wurdt in dichotome identifikaasjekaai foar de *Sphacelaria*-soarten fan Sjina presintearre.

Yn Haadstik 7 wurdt de ôfsplitsing fan de slachten *Onslowia* en *Verosphacela* fan'e Choristocarpaceae en fan'e Sphacelariales foarslein op basis fan'e úkomsten fan Haadstik 2 en morfologyske omtinkens. De nije famylje Onslowiaceae is oprjochte foar dizze twa slachten.

Haadstik 8 jout in aktualisearre ferzje fan'e op *rbc*L basearre brúnwierfylogeny fan Haadstik 2 mei in soad nije sekwinsjes en wêryn alle brúnwieroarders, útsein trije, fertsjinwurdige binne. Neffens dizze fylogeny (Fig. 8.1) hearre de Halosiphonaceae en Phyllariaceae net ta de Laminariales. Dizze twa famyljes binne nauwer besibbe oan'e Tilopteridales en Fucales. It slachte *Asteronema* is ek net monofyletysk: *A. rhodochortonoides* is besibbe oan it slachte *Asterocladon* en tegearre binne se de susterskift fan'e Ectocarpales *s.l.*, wylst *A. ferruginea* besibbe is oan de Scytothamnales.

De hearskjende ideeën oer de fylogeny fan'e brúnwieren binne lange tiid dominearre west troch it idee dat it slach libbenssyklus en de talluskonstruksje fan fundaminteel belang binne foar it bepalen fan'e evolúsjonêre sibskippen. Ûndersyk fan DNA-sekwinsjes yn’e ôfrûne jieren hat oantoand dat dit byld net korrekt is: de tradysjonele brúnwierbeam is op’e kop setten. De Dictyotales en Sphacelariales (beiden oant foar koarten beskoud as avensearre skiften) binne de meast basale oarders en sadwaande moatte yn’e brúnwieren topselwaaksdom, in isomorfe libbenssyklus en sellen mei machtige linsfoarmige chloroplasten sûnder pyrenoïden beskoud wurde as primitive skaeimerken. De oögame diplonte Fucales, foarhinne beskoud as itsij primityf, itsij avensearre, sprantelje earne healwei de brúnwierbeam út. Om’t molekulêre stúdzjes oantoand hawwe dat’t genetysk ferskaat yn brúnwieren leech is neffens de readwieren, hat men oannommen dat de brúnwieren in relatyf jonge skift binne dy’t ûngefear op itselde stuit as de blomplanten ûntstien is (sa’n 150 miljoen jier lyn).

Yn Haadstik 9 is de aktualisearre *rbc*L-fylogeny fan Haadstik 8 brûkt as basis foar in nij fylogeny-basearre klassifikaasjeskema foar de Phaeophyceae. De resultaten fan rDNA-stúdzjes en morfology binne meinommen yn it besprek. In nije klassifikaasje (Tabel 9.1) soe út 20 oarders bestean, wêrfan seis nije (foar *Asterocladon* + *Asteronema rhodochortonoides* + *A. breviarticulatum*, *Bachelotia*, Choristocarpaceae, Halosiphonaceae, Onslowiaceae, en Phyllariaceae).

De folgjende suggestjes wurde dien foar takomstig ûndersyk oan de Phaeophyceae en de Sphacelariales. Op oardernivo is der ferlet fan in wiidweidigere taksonmeunstering fan ûnmeunsterde (Ascoseirales, Cutleriales, Ralfsiales) en amper meunsterde (Dictyotales, Fucales, Sporochnales, Syringodermatales) oarders. *Rbc*L leveret in bettere resolúsje op as de ribosomale genen út de kearn. Bywannear’t der tusken de twa keazen wurde moat, fertsjinnet *rbc*L de foarkar. Der moat nei ekstra genen socht wurde, sawoll mitochondriële as kearngenen. Binnen de Sphacelariales moat it swiertepunt ferskowe nei in bettere geografyske dekking, benammen op it súdlik healrûn.

By eintsjebeslút fertsjinwurdiget dit proefskrift, yn kombinaasje mei eardere monografyën fan Europeeske (Prud’homme van Reine 1982) en Japanske (Kitayama 1994) Sphacelariaceae en Australyske Sphacelariales (Womersley 1987), ien fan’e meast wiidfiemjende ferslachlizzingen fan in brúnwieroarder. Dit jout wer in unyke gelegenheid om fylogeografyske patroanen en soarteradiaasjes binnen bepaalde subskiften te ûndersykjen. De wrâldwiide ferspriedingfan’e Sphacelariales en de disjunksjes binnen it slachte *Sphacelaria* jouwe ynteressante mooglikheden om werkolonisaasjepaden yn matige kriten en fikariânsje-barrens yn tropyske kriten te bestudearjen.

SAMENVATTING

De Phaeophyceae of bruinwieren vormen een klasse van meercellige algen behorende tot de divisie Heterokontophyta (goud-bruine algen) waarvan de overige klassen bijna uitsluitend eencellig zijn. Bruinwieren nemen een belangrijke plaats in in de mariene levensgemeenschappen van de gematigde en tropische streken. In kelpwouden (orde Laminariales) vindt men de reuzen van de algenwereld welke meer dan 60 meter lang kunnen worden. De Fucales (o.a. Bessenwier, Knotswier, Blaaswier) kunnen uitgestrekte eensoortige velden vormen in de getijdezone of onder de laagwaterlijn. De klasse van de bruinwieren is onderverdeeld in zo'n 13 à 17 orden, waaronder de Sphacelariales. Over de evolutionaire verwantschappen tussen de bruinwierorden is lange tijd gespeculeerd, vanwege de enorme diversiteit aan vormen in combinatie met een variëteit van ultrastructurele en cytologische kenmerken. Dit is de basis voor dit proefschrift. Hoofdstuk 1 verschaft een inleiding over de bruinwieren en de hiërarchische benadering die in het onderzoek is toegepast.

Algologische systematici hebben voorheen vele bruinwierfylogenieën (= verwantschapsbomen) verondersteld. Sommigen beschouwden de draadvormige Ectocarpales als primitiefste bruinwiergroep, terwijl anderen veronderstelden dat de Fucales met hun diplonte levencyclus de zustergroep van alle andere bruinwieren zijn. Nauwkeuriger onderzoek werd mogelijk met de opkomst van formele fylogenetische methoden in de jaren 70 en 80 (van de 20ste eeuw) en de mogelijkheid tot het vergelijken van DNA-sequenties in de jaren 90. Eerdere moleculaire fylogenetische studies (Tabel 2.1) hebben voornamelijk gebruik gemaakt van DNA-sequenties van de kleine subeenheid (SSU) van het ribosomaal DNA (rDNA) en vaak werd iedere bruinwierorde slechts door één soort vertegenwoordigd. Het combineren van de gegevens van verschillende studies werd vaak bemoeilijkt doordat sequenties vaak onvolledig waren en verschillende stukken DNA gebruikt zijn. Met de recentelijke mogelijkheid tot automatisch sequencen en de beschikbaarheid van meerdere sequenties was de tijd rijp om de fylogenetische verwantschappen binnen de Phaeophyceae opnieuw te evalueren en de positie van de Sphacelariales en hun veronderstelde monofylie (= gezamenlijke afstamming) te bepalen.

De Sphacelariales worden gekenmerkt door groei vanuit een topcel, parenchymatisch weefsel (= celdelingen in alle vlakken) en tijdelijke zwartkleuring als reaktie op een behandeling met verdund bleekwater. De planten vormen matten en toefjes op stenen en/of andere wieren. Hoewel ze een wereldwijde verspreiding vertonen, is de diversiteit aan soorten het hoogst langs de gematigde kusten van west-Europa en het zuiden van Australië en Nieuw-Zeeland. De diversiteit in de tropen en bij de polen is waarschijnlijk laag. In hoeverre dit werkelijk zo is of een weergave is van een lagere monsterdichtheid in deze streken, is niet geheel duidelijk.

De traditionele indeling in vier families is voornamelijk gebaseerd op verschillen in de wijze van vertakking. De Cladostephaceae omvatten maar één soort, de Sphacelariaceae bestaan uit twee geslachten, de Stypocaulaceae hebben vijf geslachten en de Choristocarpaceae vier. De Sphacelariaceae zijn het soortenrijkst met meer dan 40 soorten op een totaal van meer dan 60 voor de hele orde. Verdere taxonomische details worden verschaft als inleiding van de nieuwe analyses.

Fylogenetische verwantschappen binnen de Phaeophyceae worden gereconstrueerd in Hoofdstuk 2 gebruikmakend van in de chloroplast gecodeerde *rbc*L-sequenties (coderend voor de grote subeenheid [LSU] van het RUBISCO-gen) en van kerngecodeerde gedeeltelijke SSU + LSU rDNA-sequenties. Zowel de rDNA- als de *rbc*L-gegevens toonden aan dat de Dictyotales, Sphacelariales en Syringodermatales de meest basale bruinwierorden zijn en dat de Sphacelariales niet monofyletisch zijn (Figuren 2.2 en 2.4). Voorts bevestigden beide data-sets eerdere studies waarin werd gesuggereerd dat de Chordariales, Dictyosiphonales (incl. Punctariales) en Scytosiphonales zouden moeten worden opgenomen in de Ectocarpales *sensu lato*. Zie ook Hoofdstuk 8.

Hoofdstuk 3 is gericht op de fylogenetische verwantschappen binnen de Sphacelariales *sensu stricto* (een monofyletische groep bestaande uit de Cladostephaceae, Sphacelariaceae en Stypocaulaceae). Zowel moleculaire als ook morfologische gegevens werden geanalyseerd. De morfologische data-set bestond uit 23 kenmerken, de moleculaire data-set uit *rbc*L en gedeeltelijke RUBISCO-*spacer*-sequenties. Taxonbemonstering op het geslachtsniveau was 75% en op het soortsniveau ± 30%. De 19 soorten zijn allemaal verzameld op het noordelijke halfrond met uitzondering van één. Analyse van de morfologische data-set resulteerde in een bijna volledig onopgeloste boom (Fig. 3.3). De enige groep die ondersteund werd was het *Sphacelaria*-ondergeslacht *Propagulifera*. Maximum parsimonie-analyse van *rbc*L-sequenties leverde vier basale groepen op (Fig. 3.2): (1) Stypocaulaceae, (2) *Sphacelaria radicans*, (3) *Sphacelaria caespitula* en (4) alle andere geanalyseerde taxa; (*Sphacella* + *Sphacelaria*-ondergeslacht *Propagulifera*) en (Cladostephaceae + *Sphacelaria nana* + *Sphacelaria*-ondergeslachten *Battersia* en *Pseudochaetopteris*). De fylogenetische verwantschappen tussen deze vier groepen konden niet onomstotelijk worden vastgesteld. Analyse van de gedeeltelijke RUBISCO-*spacer*-sequenties op zichzelf of in combinatie met *rbc*L-sequenties resulteerde niet in beter opgeloste bomen. Vervolgens werden de morfologische kenmerken geprojecteerd op de *rbc*L-boom om zo diagnostische of fylogenetisch informatieve kenmerken te identificeren (Fig. 3.5). Het ondergeslacht *Propagulifera* wordt gedefinieerd door het hebben van propagula (speciaal voor de vegetatieve voortplanting gevormde takjes) met een lensvormige centrale topcel of een eindhaar. De Stypocaulaceae worden gedefinieerd door de consequente acroblastische (zie Fig. 3.1) manier van vertakken en de positie van voortplantingsstructuren in de oksels van zijtakken. De overige kenmerken zijn meerdere malen ontstaan of verloren gegaan. Vier opties voor een nieuwe indeling binnen de orde met bijbehorende nomenclatorische veranderingen worden bediscussieerd (Fig. 3.6). Geen van de opties leidt tot meer duidelijkheid wat betreft identificatie of klassifikatie.

In Hoofdstuk 4 is de geschiktheid van de *internal transcribed spacers* (ITS1 en ITS2) van het ribosomale cistron voor fylogenetische studies aan de Sphacelariales onderzocht. ITS-sequenties zijn bepaald voor zes soorten, die de vier groepen vertegenwoordigen die in de vorige paragraaf genoemd werden (H. 3). De ITS'en waren uiterst variabel en zeer moeilijk met elkaar in overeenstemming te brengen (*alignen*) als gevolg van lengte-verschillen, grote inserties en/of deleties en lange stukken met *repeat motifs* (stukjes herhaalde sequentie) (Figuren 4.1 en 4.2). Er werd ook intra-specifieke en intra-individuele variatie gevonden. Deze resultaten maken duidelijk dat ITS-sequenties niet geschikt zijn voor fylogenetische reconstructies van de Sphacelariales op geslachtsniveau. ITS-sequenties zijn mogelijk wel geschikt voor fylogenetische studies van bepaalde Sphacelariales-soorten op het niveau van ondergeslacht en het intra-specifieke niveau. Indien aangetoond kan worden dat intra-individuele variatie lager is dan de variatie tussen individuën, kunnen enkele interessante genotypische verspreidingen onthuld worden, hetgeen ons meer inzicht kan geven over hoe populaties zich verspreiden of differentiëren.

In Hoofdstuk 5 zijn fylogentische verwantschappen binnen het soortenrijkste *Sphacelaria*-ondergeslacht *Propagulifera* onderzocht. De tropische tot gematigde *Propagulifera* worden gekenmerkt door het hebben van propagula. De traditionele secties *Propagulifera*, *Furcigerae* en *Tribuloides* zijn gedefinieerd op basis van de morfologie van de propagula. De resultaten tonen aan dat deze kenmerken niet betrouwbaar zijn. Een fylogenetische analyse van *rbc*L- en complete RUBISCO-*spacer*-sequenties onthulde zowel parafylie (geen monofylie) als cryptische soorten (d.w.z. morfologisch niet te onderscheiden). Er werden drie goed ondersteunde groepen gevonden (Fig. 5.1). De meest basale groep werd gevormd door *S. cirrosa* (sectie *Propagulifera*) alleen, welke gebruikt werd als buitengroep. Een tweede groep bestond uit *S. californica* (*Tribuloides*), welke basaal was binnen deze groep, *S. divaricata* en *S. rigidula* (beide *Furcigerae*). De derde groep bestond uit de basale *S. tribuloides* (*Tribuloides*) en verder *S. yamadae* en *S. rigidula* (beide *Furcigerae*). Er wordt geconcludeerd dat de verschillende vormen van propagula meerdere malen binnen de secties zijn geëvolueerd en daarom niet fylogenetisch informatief zijn binnen het ondergeslacht. Goed ondersteunde genetische verschillen tussen de groepen binnen het ondergeslacht onthullen cryptische soorten, te weten *S. rigidula*. Vanwege de afwezigheid van betrouwbare morfologische verschillen wordt voorgesteld om de secties *Furcigerae* en *Tribuloides* niet meer te erkennen of om ze samen te laten gaan in één sectie.

Hoofdstuk 6 behelst een studie van een herbariumcollectie van mariene *Sphacelaria*-exemplaren die verzameld zijn langs de gehele Chinese kust en atollen in de Zuid-Chinese Zee. Er werden zeven soorten *Sphacelaria* gevonden, allen behorend tot het ondergeslacht *Propagulifera*: *S. rigidula*, *S. tribuloides*, *S. novae-hollandiae*, *S. californica* en *S. carolinensis* zijn eerder vermeld voor dit gebied. Twee soorten zijn niet eerder vermeld voor China, te weten *S. divaricata* en een nieuwe soort, *S. tsengii*. *Sphacelaria tsengii* wordt gekenmerkt door lange propagula met een taps toelopende steel en twee taps toelopende armen (Figuren 6.4–6.12). Het holotype (het exemplaar op basis waarvan de soort beschreven is) van *S. carolinensis* is ook opnieuw bekeken en de propagula bleken asymmetrisch te vertakken, hetgeen nog niet eerder was beschreven (Fig. 6.23). Tot slot wordt een dichotome identificatiesleutel voor de *Sphacelaria*-soorten van China gepresenteerd.

In Hoofdstuk 7 wordt de afsplitsing van de geslachten *Onslowia* en *Verosphacela* van de Choristocarpaceae en van de Sphacelariales voorgesteld op basis van de resultaten van Hoofdstuk 2 en morfologische overwegingen. De nieuwe familie Onslowiaceae wordt voorgesteld voor deze twee geslachten.

Hoofdstuk 8 verschaft een geactualiseerde versie van de op *rbc*L gebaseerde bruinwierfylogenie van hoofdstuk 2 met veel nieuwe sequenties en waarin alle bruinwierorden, op drie na, vertegenwoordigd zijn. Volgens deze fylogenie (Fig. 8.1) behoren de

Halosiphonaceae en Phyllariaceae niet tot de Laminariales. Deze twee families zijn nauwer verwant met de Tilopteridales en Fucales. Het geslacht *Asteronema* is ook niet monofyletisch: *A. rhodochortonoides* is verwant aan het geslacht *Asterocladon* en samen zijn zij de zustergroep van de Ectocarpales *s.l.*, terwijl *A. ferruginea* verwant is aan de Scytothamnales.

De heersende ideeën over de fylogenie van de bruinwieren zijn lang gedomineerd door het idee dat het type levenscyclus en de thallusconstructie van fundamenteel belang zijn voor het bepalen van de evolutionaire verwantschappen. Onderzoek van DNA-sequenties in de afgelopen jaren heeft aangetoond dat dit beeld niet correct is: de traditionele bruinwierboom is op zijn kop gezet. De Dictyotales en Sphacelariales (beide tot voor kort beschouwd als geavanceerde groepen) zijn de meest basale ordes en derhalve moeten in de bruinwieren topcelgroei, een isomorfe levenscyclus en cellen met talrijke lensvormige chloroplasten zonder pyrenoïden beschouwd worden als primitieve kenmerken. De oögame diplonte Fucales, voorheen beschouwd als hetzij primitief, hetzij geavanceerd, takken ergens halverwege de bruinwierboom af. Omdat moleculaire studies aangetoond hebben dat de genetische diversiteit in bruinwieren laag is vergeleken met roodwieren, heeft men aangenomen dat de bruinwieren een relatief jonge groep zijn die ongeveer tegelijkertijd als de bloemplanten zijn ontstaan (zo'n 150 miljoen jaar geleden).

In Hoofdstuk 9 is de geactualiseerde *rbc*L-fylogenie van Hoofdstuk 8 gebruikt als basis voor een nieuw fylogenie-gebaseerd klassifikatieschema voor de Phaeophyceae. De resultaten van rDNA-studies en morfologie zijn meegenomen in de discussie. Een nieuwe klassifikatie (Tabel 9.1) zou 20 orden omvatten, waarvan zes nieuwe (voor *Asterocladon* + *Asteronema rhodochortonoides* en *A. breviarticulatum*, *Bachelotia*, Choristocarpaceae, Halosiphonaceae, Onslowiaceae, en Phyllariaceae).

De volgende suggesties worden gedaan voor toekomstig onderzoek aan de Phaeophyceae en de Sphacelariales. Op orde-niveau is er een uitgebreidere taxonbemonstering nodig van onbemonsterde (Ascoseirales, Cutleriales, Ralfsiales) en schamel bemonsterde (Dictyotales, Fucales, Sporochnales, Syringodermatales) orden. *Rbc*L levert een betere resolutie op dan de ribosomale genen uit de kern. Indien er tussen de twee moet worden gekozen, verdient *rbc*L de voorkeur. Er dient naar extra genen gezocht te worden, zowel mitochondriële als kerngenen. Binnen de Sphacelariales moet het zwaartepunt verschuiven naar een betere geografische dekking, met name op het zuidelijk halfrond.

Tot besluit vertegenwoordigt dit proefschrift, in combinatie met eerdere monografieën van Europese (Prud'homme van Reine 1982) en Japanse (Kitayama 1994) Sphacelariaceae en Australische Sphacelariales (Womersley 1987), één van de meestomvattende studies van een bruinwierorde. Dit verschaft weer een unieke gelegenheid om fylogeografische patronen en soortsradiaties binnen bepaalde subgroepen te onderzoeken. De wereldwijde verspreiding van de Sphacelariales en de disjuncties binnen het geslacht *Sphacelaria* bieden interessante mogelijkheden om herkolonisatieroutes in gematigde streken en vicariantie-gebeurtenissen in tropische streken te bestuderen.

AAN DIT PROEFSCHRIFT WERKTEN MEE

Akira Peters: Stuurde mij *Syringoderma phinneyi*; ik heb door hem geëxtraheerd Desmarestiales-DNA dat nog in de vriezer in Haren lag, gebruikt.
ALW: zie NWO.
Anonieme reviewers: Gaven aanwijzingen ter verbetering van de hoofdstukken 2, 3, 6 en 7.
Arjen Speksnijder: Organiseerde speciaal voor mij een privé mini-workshop *Adobe PageMaker*.
Aschwin Engelen: zie Han van der Strate.
Ben Kieft: Schoot Fig. 6.15 en digitaliseerde alle foto's en ook een aantal figuren.
Bert Lokhorst: Is co-auteur van de gepubliceerde versie van hoofdstuk 6; las menig hoofdstuk door voor het de deur uitging.
Bertie Joan van Heuven: Leerde mij microscopische preparaten fotograferen en films ontwikkelen en afdrukken; zorgde voor voedingssupplementen voor de algen; verzorgde jarenlang cultures.
Bruno de Reviers: Doet ook onderzoek naar de fylogenie van bruinwieren en discussieerde daar met mij over; stuurde me een nog niet gepubliceerd artikel.
Connie Baak: Was mijn *PageMaker-helpdesk*
David Patterson: Was onze gastheer op het *Gatty Marine Laboratory* in St. Andrews, Schotland.
Dieter Müller: Stuurde mij de *Sphacella subtilissima*-culture.
Ding Hao: Hielp mij een in het Chinees geschreven artikel enigszins te begrijpen.
Engel Vrieling: Gooide een monstertje van een culture onder de electronen-microscoop en bevestigde aldus het vermoeden van een contaminatie met een kiezelwier.
Han van der Strate: Ontdekte een takje bruinwier in één van zijn door Aschwin op Curaçao verzamelde plukken *Cladophoropsis,* dit takje bleek *Sphacelaria tribuloides*.
Hans Kruijer: Leerde mij hippelen en de fi-fi-transformatie.
Hans Nooteboom: Voorzag de nieuwe soort *Sphacelaria tsengii* van een Latijnse diagnose.
Herre Stegenga: Stelde een luisterend oor ter beschikking als ik mijn hart vol zeewier eens uit wilde storten, een onderwerp dat bij veel landgenoten helaas nog op veel onbegrip stuit.
Jan van der Kuur: Verbeterde de Friestalige samenvatting.
Jan-Frits Veldkamp: Voorzag de nieuwe familie *Onslowiaceae* van een Latijnse diagnose.
Jeanine Olsen: Voorzag eerdere versies van alle hoofdstukken van commentaar; co-auteur van de gepubliceerde versies van de hoofdstukken 2 en 3 en hopelijk 4.
Jim Coyer: Stelde DNA beschikbaar van Laminariales.
Lisette de Senerpont Domis: Is één mijner paranimfen en tevens *'bitching buddy';* las uit collegialiteit of interesse ook wel eens een versie van een hoofdstuk door; pikte mijn Leidse werkkamer in toen ik in Groningen zat, waardoor ik bij terugkomst gedoemd was om mijn laatste jaren tussen de schimmels in de verste uithoek van het Van Steenisgebouw te slijten.
Léon Raijmann: Bekostigde uit eigen zak een expeditie naar Sardinië om daar voor mij *Discosporangium mesarthrocarpum* te zoeken en vervolgens niet vond.
Loes Venekamp: Leerde mij zeewater halen in de Rijkswaterstaat-vleugel.
Madeleine van Oppen:Extraheerde DNA uit *Desmarestia* en liet dat achter in de Harense vriezer.
Mari Källersjö: Verzorgde samen met Steve Farris de *parsimony-jackknife*-analyse van hoofdstuk 2 en gaf er commentaar bij.
Mary Gibson: Verzamelde Sphacelariales in Australië en stuurde deze naar mij (ik heb er helaas geen bruikbaar DNA uit kunnen extraheren).
NWO: Zorgde voor de financiële kant van het onderzoek.
Olivier de Clerck: Stuurde mij *Dictyota*-DNA.
Onno Diekmann: Is mijn andere paranimf.
Pieter Baas: Las eerdere versies van hoofdstukken door en paaide de reintegratie-consulent.
René Haanstra: Leerde mij de ABI-377 automatische sequencer bedienen.
SIR: Betaalde een veldtrip naar Schotland en een congresbezoek in Italië.
Stella Boele-Bos: Keek over mijn schouder mee toen ik de CsCl-DNA-extractie-methode leerde.

Willem Prud'homme van Reine: Schreef het project-voorstel; verzamelde het grootste deel van de gebruikte algen; voorzag eerdere versies van hoofdstukken van commentaar; co-auteur van de gepubliceerde versies van de hoofdstukken 2, 3, (4), 6 en 7; vergezelde mij op een verzamelreis naar Schotland en leerde mij algen in het veld te herkennen.

Wim Star: Verzorgde jarenlang de cultures.

Wytze Stam: Had als co-promotor een belangrijk aandeel in vele hoofdstukken; co-auteur van de gepubliceerde versies van de hoofdstukken 2 en 3 (en 4).

Yde de Jong: Deelde met mij een werkkamer tijdens mijn eerste Leidse periode en maakte mij een beetje wegwijs op het onderzoeksinstituut dat toen nog Rijksherbarium/Hortus Botanicus heette.

Yeon-Shim Keum: Determineerde een deel van de Chinese *Sphacelaria*'s; co-auteur van de gepubliceerde versie van hoofdstuk 6; discussieerde via e-mail over de Sphacelariaceae.

Dit proefschrift werd tegengewerkt door tal van mensen en andere organismen die ik hier niet allemaal bij naam zal noemen. Zij zorgden de afgelopen zes jaar voor de nodige afleiding en hebben alszodanig misschien indirekt toch bijgedragen aan de uiteindelijke afronding van dit proefschrift. Voorbeelden zijn (voorzover niet reeds bovengenoemd): mijn ouders (Lenie en Frans), mijn broer Renzo en zijn gezin, de rest van de familie, Arjan Stroo, Mike Blueberry, Calamarianen, Creusa Hittipeuw, Dirk en Desiree Tweekeerbellen, Erik-jan Malta, Ferry Slik en Sumi Yuami, Foppe de Haan *cum suis*, G.R.C.'ers, Inez Flameling, James Bond, L.S.D.'ers, Magengoth, Marieke van der Werf, Mebrahtu, medewerkers van de vakgroep Mariene Biologie in Haren, de Molukse witbuikzeearend, de Neushoorns van Chitwan, N.H.N.'ers, Peter Boelen, de Piranhas van de Pantanal, Raoul Haegens, de Staartmezen in de boom tegenover mijn raam, Tries Razak, U2, en de Zeekatten van de Oosterschelde.

Allen worden hartelijk bedankt voor hun bijdrage.

Stefan(o)

CURRICULUM VITAE

Stefano Giovanni Angelo Draisma (Stefan) werd geboren op 24 februari 1970 in Sneek /Snits. In 1988 slaagde hij voor het eindexamen atheneum aan de Rijksscholengemeenschap *Magister Alvinus* te Sneek. In augustus van datzelfde jaar begon hij met de studie Biologie aan de Rijksuniversiteit Groningen. In het tweede jaar koos hij voor de afstudeerrichting Mariene Biologie. De doctoraalfase werd ingevuld met drie doctoraalonderwerpen en twee literatuurstudies (scriptie en colloquium). Bij de afdeling Populatie-genetica werd onder begeleiding van Albert Kamping onderzoek gedaan naar de geschiktheid van vissen-eieren en -larven van de cichlide *Aequidens rivulatus* voor populatie-genetisch onderzoek met behulp van allozym-electroforese. Aan de Universiteit van Kreta in Heraklion (Griekenland) werd onder begeleiding van •••••••• •••••• een groeicurve bepaald van de Mediterrane zaagbaars (*Serranus hepatus*) op basis van het tellen van jaarringen op de schubben. Bij de vakgroep Mariene Biologie werd onder begeleiding van Madeleine van Oppen onderzoek gedaan naar het mogelijke bestaan van saliniteits-oecotypen van het roodwier *Phycodrys rubens* door middel van DNA-sequentie-analyse. De doctoraalscriptie had als onderwerp het gebruik van moleculaire technieken voor de analyse van genetische variatie in de cichliden van de oost-Afrikaanse grote meren en werd beoordeeld door Wilke van Delden (Populatie-genetica). Het colloquium ging over de structuur en evolutie van mitochondrieel DNA in mariene dieren en werd beordeeld door Jeanine Olsen (Mariene Biologie). De studie Biologie werd in augustus 1994 afgerond. Van april 1995 tot april 1996 vervulde hij zijn vervangende dienstplicht als onderzoeker bij het Nederlands Instituut voor Oecologisch Onderzoek (NIOO) in Yerseke (Centrum voor Estuariene en Mariene Oecosystemen) en Nieuwersluis (Centrum voor Limnologie). Hij deed daar onderzoek naar het voorkomen van verschillende soorten of morfotypen Zeesla (*Ulva spp.*) in het Veerse Meer. In april 1996 kreeg hij een aanstelling als Onderzoeker-in-Opleiding bij de Stichting Levenswetenschappen (SLW) van de Nederlandse Organisatie voor Wetenschappelijk Onderzoek (NWO) met als standplaatsen Leiden en Haren (Gn). De onderzoeksopdracht was het bepalen van de evolutionaire verwantschappen binnen de bruinwierorde Sphacelariales en het bepalen van hun positie binnen de bruinwieren. Het eerste jaar werd uitgevoerd aan het onderzoeksinstituut Rijksherbarium/Hortus Botanicus (RHHB). Tegenwoordig is het herbariumgedeelte opgegaan in het Nationaal Herbarium Nederland. Het tweede en derde jaar werden uitgevoerd bij de afdeling Mariene Biologie op het Biologisch Centrum van de Rijksuniversiteit Groningen te Haren. In het vierde jaar keerde hij terug naar Leiden. Dit proefschrift is het schriftelijke verslag van dit onderzoek met Pieter Baas en Jeanine Olsen als promotores en Willem Prud'homme van Reine en Wytze Stam als co-promotores.

Doctoraalverslagen

Draisma, S.G.A. 1992. Gebruik van visse-eieren en larven voor populatie-genetisch onderzoek m.b.v. enzymelectroforese: een voorstudie voor toekomstig papegaaivis-onderzoek a.d.h.v. de cichlide *Aequidens rivulatus*. Doctoraalverslag Rijksuniversiteit Groningen, 59 p.

Draisma, S.G.A. 1992. Age Determination and Growth of *Serranus hepatus* L., 1758 from the Cretan Continental Shelf. Doctoraalverslag Rijksuniversiteit Groningen, 20 p.

Draisma, S.G.A. 1993. Molecular Approaches to the Analysis of Genetic Variation in the Cichlidae (Teleostei) of the East African Great Lakes. Doctoraalscriptie Rijksuniversiteit Groningen, 30 p.

Draisma, S.G.A. 1994. Rubisco Spacer and ITS Sequences Do Not Support the Existence of Salinity Ecotypes in the Red Alga *Phycodrys rubens*. Doctoraalverslag Rijksuniversiteit Groningen, 28 p.

Publikaties

Draisma, S.G.A. & W.F. Prud'homme van Reine 2001. Onslowiaceae *fam. nov.* (Phaeophyceae). *Journal of Phycology* 37 (4): 647–649. [Hoofdstuk 7 van dit proefschrift]

Draisma S.G.A., Y.-S. Keum, W.F. Prud'homme van Reine & G.M. Lokhorst 1998. The Species of *Sphacelaria* (Sphacelariales, Phaeophyceae) of China with a Description of a New Species. *Botanica Marina* 41: 181–190. [Hoofdstuk 6 van dit proefschrift]

Draisma, S.G.A., W.F. Prud'homme van Reine, W.T. Stam & J.L. Olsen 2001. A Reassessment of Phylogenetic Relationships within the Phaeophyceae Based on RUBISCO Large Subunit and Ribosomal DNA Sequences. *Journal of Phycology* 37 (4): 586–603. [Hoofdstuk 2 van dit proefschrift]

Draisma, S.G.A., J.L. Olsen, W.T. Stam & W.F. Prud'homme van Reine 2002. Phylogenetic Relationships within the Sphacelariales (Phaeophyceae): *rbc*L, RUBISCO Spacer and Morphology. *European Journal of Phycology* 37: in druk. [Hoofdstuk 3 van dit proefschrift]

Draisma, S.G.A., W.F. Prud'homme van Reine, J.L. Olsen & W.T. Stam. The Internal Transcribed Spacers of the Ribosomal Cistron Are Unsuitable for Phylogenetic Studies in the Sphacelariales (Phaeophyceae). Ingezonden naar *Cryptogamie Algologie*. [Hoofdstuk 4 van dit proefschrift]

Malta, E.-j., S.G.A. Draisma & P. Kamermans 1999. Free-Floating *Ulva* in the Southwest Netherlands: Species or Morphotypes? A Morphological, Molecular and Ecological Comparison. *European Journal of Phycology* 34 (4): 443–454. [Tevens gepubliceerd in *Macroalgal Mats in a Eutrophic Lagoon: Dynamics and Control Mechanisms*, E.-j. Malta 2000. Proefschrift Katholieke Universiteit Nijmegen, p. 51–65.]

Oppen, M.J.H. van, S.G.A. Draisma, J.L. Olsen & W.T. Stam 1995. Arctic Passages in the Red Alga *Phycodrys rubens*: Evidence from Nuclear rDNA ITS Sequences. *Marine Biology* 123: 179–188. [Tevens gepubliceerd in *Tracking Trails by Cracking Codes: Molecular Biogeography and Evolution of Benthic Cold-Water Seaweeds*, M.J.H. van Oppen 1995. Proefschrift Rijksuniversiteit Groningen, p. 87–102.]

Peters, A.F., R.L. Fletcher & S.G.A. Draisma. Evolution and taxonomy in the Phaeophyceae: effects of the molecular age on brown algal systematics. Ingezonden naar *European Journal of Phycology*.

Proceedings en *Abstracts*

Draisma, S.G.A. 1996. A Comparison of Sequence Variation in Nuclear rDNA Gene and Spacer Regions of Morphological *Ulva* Species (Chlorophyta) from the Veerse Meer Lagoon (The Netherlands). In *Eutrophicaton and Macrophytes. Synthesis Report and Proceedings of the Second EUMAC Workshop held in Sète 29 February – 3 March 1996*. J.W. Rijstenbil, P. Kamermans & P.H. Nienhuis [Eds]. NIOO-CEMO, Yerseke. p. 344–359.

Draisma, S.G.A., Y.-S. Keum & W.F. Prud'homme van Reine 1997. The Species of *Sphacelaria* (Sphacelariales, Phaeophyceae) in China with a Description of a New Species. *Acta Botanica Neerlandica* 46: 430–431.

Draisma, S.G.A., Y.-S. Keum, W.F. Prud'homme van Reine & G.M. Lokhorst 1997. The Species of *Sphacelaria* (Sphacelariales, Phaeophyceae) in China with a Description of a New Species. *Phycologia* 36 (suppl.): 26–27.

Draisma, S.G.A., J.L. Olsen, W.T. Stam & W.F. Prud'homme van Reine 1999. Phylogenetic Studies in the Sphacelariales (Phaeophyceae). In *2nd European Phycological Congress; Book of Abstracts*. Montecatini Terme, Italië. p. 48.

Draisma, S.G.A., J.L. Olsen, W.T. Stam & W.F. Prud'homme van Reine 2001. Phylogeny of the Phaeophyceae Based on *rbc*L Sequences. In *7th International Phycological Congress; Programme*. Thessaloniki, Griekenland. *Abstract* nummer 446 op een los inlegvel.

Malta, E.-j., S.G.A. Draisma, H. Stegenga, G. Crielaard & R. Rottier 1997. Succession of *Ulva* Blooms: A Morphological, Molecular and Ecophysiological Comparison of Closely Related Species. *Phycologia* 36 (suppl.): 69.

WORLDMAP showing the topology of the geographical names in this thesis.


Alaria esculenta (AF064745, AF109795)
Undaria peterseniana (AB035794)
Eisenia arborea (AJ287855)
Kjellmaniella crassifolia (AB035792)
Nereocystis luetkeana (AJ287857)
Macrocystis pyrifera (AJ287856)
Pelagophycus porra (AJ287858)
Pelagophycus x Macrocystis hybrid (AJ287859)
Agarum clathratum (AB035791)
Thalassiophyllum clathrus (AB035793)
Chorda filum (AB035781)
Chorda filum (AB035782)
Chorda filum (AB035783)
Chorda filum (AB035784)
Chorda filum (AB035785)
Chorda filum (AB035786)
Chorda filum (AB035787)
Chorda rigida (AB035788)
Akkesiphycus lubricum (AB036038)
Pseudochorda gracilis (AB035790)
Pseudochorda nagaii (AB035789)
Streblonema tenuissimum (AF055414)
Myriotrichia clavaeformis (AF055408)
Litosiphon pusillus (AF055406)
Myrionema strangulans (AF055407)
Leptonematella fasciculata (AF055405)
Laminariocolax tomentosoides (AF055404)
Dictyosiphon foeniculaceus (AF055397)
Hummia onusta (AF055402)
Giraudia sphacelarioides (AF055399)
Hecatonema sp. (AF055400)
Delamarea attenuata (AF055396)
Punctaria plantaginea (AF055410)
Striaria attenuata (AF055415)
Sorocarpus micromorus (AF055411)
Sphaerotrichia divaricata (AF055412)
Elachista furicola (AF055398)
Isthmoplea sphaerophora (AF055403)
Stictyosiphon soriferus (AF055413)
Coelocladia arctica (AF055395)
Geminocarpus austro-georgiae (AJ295830)
Pylaiella littoralis (X55372)
Pogotrichum filiforme (AF055409)
Adenocystis utricularis (AJ295823)
Utriculidium durvillei (AJ295835)
Caepidium antarcticum (AJ295826)
Ectocarpus siliculosus (X52503)
Chnoospora implexa (AB022231)
Hydroclathrus clathratus (AB022233)
Rosenvingea intricata (AB022232)
Colpomenia peregrina (AB022235)
Colpomenia bullosa (AB022236)
Colpomenia phaeodactyla (AB022237)
Scytosiphon lomentaria (AB022238)
Scytosiphon gracilis (AB022240)
Petalonia zosterifolia (AB022242)
Scytosiphon tenellus (AB022241)
Petalonia fascia (AB022243)
Petalonia binghamiae (AB022244)
Scytosiphon canaliculatus (AB022239)
Colpomenia sinuosa (AB022234)
Asterocladon lobatum (AJ2958240
Asteronema rhodochortonoides (AJ295825)
Halosiphon tomentosus (AB037137)
Halosiphon tomentosus (AB037136)
Phyllariopsis brevipes (AB045244)
Phyllariopsis brevipes (AB045245)
Phyllariopsis brevipes (AB045246)
Phyllariopsis brevipes pseudopurpurescens (AB045247)
Phyllariopsis brevipes pseudopurpurescens (AB045248)
Phyllariopsis purpurescens (AB045249)
Phyllariopsis purpurescens (AB045250)
Phyllariopsis purpurescens (AB045251)
Saccorhiza dermatodea (AB045252)
Saccorhiza polyschides (AB045253)
Saccorhiza polyschides (AB045254)
Saccorhiza polyschides (AB045255)
Saccorhiza polyschides (AB045256)
Phaeosiphoniella cryophila (AB045259)
Haplospora globosa (AB037138)
Tilopteris mertensii (AB045260)
Sargassum muticum (AJ287854)
Sargassum echinocarpum (AF076689)
Sargassum polyphyllum (AF076690)
Sargassum obtusifolium (AF195516)
Turbinaria ornata (AF076688)
Fucus distichus (AF195515)
Ascophyllum nodosum (AJ287853)
Himantothallus grandifolius (AJ287850)
Desmarestia ligulata (AJ287848)
Desmarestia tabacoides (AB037140)
Desmarestia sp. (AB037141)
Desmarestia viridis (AJ287849)
Desmarestia aculeata (AJ287847)
Desmarestia latifrons (AB037139)
Asteronema ferruginea (AJ295818)
Scytothamnus australis (AJ295833)
Splachnidium rugosum (AJ295834)
Carpomitra costata (AB045257)
Sporochnus scoparius (AB037132)
Syringoderma phinneyi (AJ287868)
Onslowia endophytica (AJ287864)
Verosphacela ebrachia (AJ287867)
Alethocladus corymbosus (AJ287860)
Stypocaulon scoparium (AJ287866)
Halopteris filicina (AJ287894)
Halopteris filicina (AJ287895)
Stypocaulon durum (AJ287897)
Sphacelaria radicans (AJ287874)
Sphacelaria caespitula (AJ287870)
Cladostephus spongiosus (AJ287863)
Sphacelaria nana (AJ287875)
Sphacelaria arctica (AJ287881)
Sphacelaria racemosa (AJ287880)
Sphacelaria plumigera (AJ287878)
Sphacelaria plumosa (AJ287879)
Sphacella subtilissima (AJ287931)
Sphacelaria rigidula (AJ287883)
Sphacelaria rigidula (AJ287888)
Sphacelaria rigidula (AJ287887)
Sphacelaria divaricata (AJ287889)
Sphacelaria californica (AJ287893)
Sphacelaria rigidula (AJ287885)
Sphacelaria yamadae (AJ287890)
Sphacelaria tribuloides (AJ287891)
Sphacelaria tribuloides (AJ287892)
Sphacelaria cirrosa (AJ287865)
Dictyota cervicornis (AJ287851)
Dictyota dichotoma (AJ287852)
Choristocarpus tenellus (AJ287861)
Bumilleriopsis filiformis (U89900)
Tribonema aequale (AF084611)
Tribonema intermixtum (AF015588)
Xanthonema debile (AF084612)
Botrydium stoloniferum (AF064743)
Botrydiopsis intercedens (AF015587)
Mischococcus sphaerocephalus (AF064744)
Heterococcus caespitosus (AF084610)
Vaucheria bursata (AF015589)
Phaeothamnion confervicola (AF069446)
Phaeoschizochlamys mucosa (AF064747)
Stichogloea globosa (AF155584)
Stichogloea doederleinii (AF069498)
Pleurochloridella botrydiopsis (AF069499)
Tetrasporopsis fuscescens (AF155585)