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Nuclear DNA Content Estimates in Multicellular Green, Red and Brown Algae: Phylogenetic Considerations

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• *Background and Aims* Multicellular eukaryotic algae are phylogenetically disparate. Nuclear DNA content estimates have been published for fewer than 1 % of the described species of Chlorophyta, Phaeophyta and Rhodophyta. The present investigation aims to summarize the state of our knowledge and to add substantially to our database of C-values for theses algae.
• *Methods* The DNA-localizing fluorochrome DAPI (4′, 6-diamidino-2-phenylindole) and RBC (chicken erythrocyte) standard were used to estimate 2C values with static microspectrophotometry.
• *Key Results* 2C DNA contents for 85 species of Chlorophyta range from 0·2–6·1 pg, excluding the highly polyploidy Charales and Desmidiales with DNA contents of up to 39·2 and 20·7 pg, respectively. 2C DNA contents for 111 species of Rhodophyta range from 0·1–2·8 pg, and for 44 species of Phaeophyta range from 0·2–1·8 pg.
• *Conclusions* New availability of consensus higher-level molecular phylogenies provides a framework for viewing C-value data in a phylogenetic context. Both DNA content ranges and mean values are greater in taxa considered to be basal. It is proposed that the basal, ancestral genome in each algal group was quite small. Both mechanistic and ecological processes are discussed that could have produced the observed C-value ranges.

Key words: C-value enigma, Chlorophyta, DNA C-values, eukaryotic algae, nuclear genome size, Phaeophyta, Rhodophyta.

INTRODUCTION

About 20 years ago, publications began to appear encouraging the development of technologies for the genetic transformation of commercially important seaweeds into domesticated 'sea crops' (Zhao and Zhang, 1981; Tang, 1982; Zhang, 1983; Saga *et al*., 1986; Polne-Fuller and Gibor, 1987; Cheney, 1988*a*, *b*, 1990). For the most part, these investigations were based on the adoption of biotechnology procedures employed with flowering plants (Evans *et al*., 1981; Harms, 1983; Torrey, 1985). In seaweeds, initial problems in confirming heterokaryon formation (Fujita and Migita, 1987; Kapraun, 1989, 1990) and in defining parameters for protoplast and heterokaryon regeneration (Cheney *et al*., 1987; Polne-Fuller and Gibor, 1987; Xue-wu and Gordon, 1987; Cheney, 1988*a*, *b*; Kapraun and Sherman, 1989) seemed to be surmountable. It appeared that development of new genotypes for seaweed mariculture would inevitably track the progress reported by crop scientists. However, this was not to be, as the more or less routine genetic manipulations in crop plants, including production of somatic hybrids, were made possible by an extensive body of knowledge from decades of genetic research. In brief, applied phycology could mimic the technology of crop science, but it could not deliver somatic hybrids with stable, desired genomes. Some of us suspected that the successful application of biotechnology procedures to the domestication of seaweeds would require criteria for pre-screening candidate species (Dutcher *et al*., 1990; Kapraun and Dutcher, 1991). For most target taxa, only chromosome complement data were available, usually without any reference to their karyotype (Cole, 1990). The extent of polyploidy in target taxa and related species was generally unknown. No published data existed for nuclear DNA contents, nuclear G+C molecular percentages and genome complexity. Random manipulations based on chance were more likely to result in unstable constructs than in fortuitous combinations (van der Meer and Patwary, 1983; van der Meer, 1987, 1990; Zhang and van der Meer, 1988). Consequently, the most comprehensive program to date was initiated at the Center for Marine Science Research, University of North Carolina—Wilmington to provide the basic genetic data that were otherwise unavailable for seaweeds (Kapraun, 1999). Specifically, it was our intent to pre-screen target taxa for genetic manipulation by identifying potentially significant nucleotype parameters, including nuclear genome size, organization and complexity (extent of unique and repetitive nucleotide sequences), chromosome number, karyotype pattern and presence of polyploidy in closely related taxa.

Initially, efforts were focused on commercially important red seaweeds, including *Porphyra* (Kapraun *et al*., 1991; Dutcher and Kapraun, 1994); agarophytes including taxa of the Gracilariales (Dutcher *et al*., 1990; Kapraun and Dutcher, 1991; Kapraun, 1993*b*; Kapraun *et al*., 1993*a*, *b*, 1996; Lopez-Bautista and Kapraun, 1995) and the Gelidiales (Freshwater, 1993; Kapraun *et al*., 1993*a*, 1994) and selected carrageenophytes including *Eucheuma* and *Kappaphycus* (Kapraun and Lopez-Bautista, 1997), *Agardhiella* (Kapraun *et al*., 1992), *Gymnogongrus* (Kapraun *et al*., 1993*b*) and *Hypnea* (Kapraun *et al*., 1994). Eventually, these

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investigations were expanded beyond strictly applied research to include opportunistic studies of other available taxa (Kapraun, 1993*a*; Kapraun and Dunwoody, 2002).

The significance of nuclear genome size variation in seaweeds is best appreciated in the larger context of our emerging understanding of the role of the nucleotype on phenotypic expression (Wenzel and Hemleben, 1982; Bennett, 1985). Specifically, an up to 200 000-fold variation in nuclear DNA content (C-value) has been reported in eukaryotes (Gregory, 2001). Although little correlation generally exists between nuclear genome size and an organism's complexity (the C-value paradox; Thomas, 1971), there is substantial evidence that the nucleotype affects the phenotype in a non-genic manner in response to environmental demands (Bennett, 1972; Cavalier-Smith, 1978, 1985*a*, *b*; 2005; Ohri and Khoshoo, 1986). In both plants and animals (Bachmann *et al*., 1972; Grime and Mowforth, 1982; Price, 1988) genome size and cell size extend their influence to ecological selection types. Larger genome size is associated with *K*-selection that favours slower development, delayed reproduction and larger body size. Smaller genome size is associated with *r*-selection that favours rapid development, high population growth rate, early reproduction and small body size (Bennett, 1972, 1987; Cavalier-Smith, 1978, 1985*a*; Begon *et al*., 1990).

An appreciation began to develop that nuclear genome profile data acquired for target species associated with the commercial seaweed industry might have an equally valuable basic research application in promoting our understanding of nucleotype transformations that have accompanied evolution in the major groups of marine algae. For example, in multinucleate coenocytic green algae, very large nuclear genomes (2C DNA contents = 2·6–4·9 pg) have a role in maintaining nucleus/cytoplasm 'domains' (Kapraun and Nguyen, 1994). In the Dasycladales (e.g. *Acetabularia*), nuclear genome content data superimposed on a phylogeny of the group suggest that ancient polyploidy events accompanied major radiations in extant families (Kapraun and Buratti, 1998). In red algae, nuclear genome size was found to be positively correlated with both size and number of reproductive spores and with ecological considerations, including *K*- and *r*-selection (Kapraun and Dunwoody, 2002). In addition, 'basal' or ancestral groups of red algae appear to have somewhat larger nuclear genomes than do more recently derived taxonomic groups (Kapraun and Dunwoody, 2002).

There are no published nucleotype data for representatives of many major groups of the Chlorophyta (Kapraun, 1993*c*) and the Rhodophyta (Kapraun and Dunwoody, 2002). The present investigation expands our knowledge of both groups with numerous original DNA content estimates. Nucleotype data for brown algae appear to be restricted to three investigations treating a handful of species (Dalmon and Loiseaux, 1981; Stam *et al*., 1988; Le Gall *et al*., 1993). Consequently, we initiated a significant effort to obtain nuclear genome size data for representatives of the major orders of brown algae. The present paper includes DNA content values for 44 species and varieties of Phaeophyta, only five of which had been previously investigated.

Certainly, one of the greatest challenges of this paper is to discuss nuclear genome size variation and trends that apply to all of the major groups of multicellular eukaryotic algae. These photosynthetic organisms have little more in common than the name 'algae', which has greater ecological implications (aquatic habitat) than taxonomic significance (Fig. 1) as algae are only distantly related to each other, and to photosynthetic land plants (Van de Peer *et al*., 1996). Red and brown algae have plastids surrounded by four membranes and contain chlorophyll *a* and *c* (or phycobillins; Chapman *et al*., 1998). The Chlorophyta, including the Zygnematales, Desmidiales and the Charales in the charophycean lineage, are characterized by plastids with two membranes and contain chlorophyll *a* and *b* as in land plants (McFadden *et al*., 1994*a*, *b*). Although classical taxonomic schemes implied that morphologically simple green algae where probably ancestral to land plants (Bold and Wynne, 1985), it is now understood that they are sister clades, and probably share a common ancestor (Mishler *et al*., 1994; Kenrick and Crane, 1997). This paper will discuss each of the three major groups of multicellular algae separately, elaborating their distinctive features and summarizing their similarities. Nuclear DNA content data from the present investigation and from the literature are summarized in three Appendices. Excluded are the numerous groups of mostly unicellular, microalgae such as the familiar diatoms (Chrysophytes), green microalgae and Prasinophytes, and eukaryotic Cyanidiophyceae (red algae). For some of these groups, limited anecdotal information is included in the text to support discussions on nuclear genome sizes in ancestral and basal algal lineages.

MATERIALS AND METHODS

Algal material was fixed in Carnoy's solution and stored in 70 % ethanol at 4 °C. Preserved material was rehydrated in water and softened in 5 % w/v EDTA (Goff and Coleman, 1990) for between 30 min and 3 h. Algal specimens were transferred to cover slips treated with subbing solution, air-dried and stained with DAPI (0·5 μg mL^{-1} 4′, 6-diamidodino-2-phenylindole; Sigma Chemical Co., St. Louis, MO 63178) as previously described (Goff and Coleman, 1990; Kapraun and Nguyen, 1990). Detailed procedures for microspectrophotometry with DAPI and requirements for reproducible staining have been specified previously (Kapraun and Nguyen, 1990; Kapraun, 1994) using a protocol modified after Goff and Coleman (1990). Microspectrophotometric data for *Gallus* (chicken erythrocytes or RBC) with a DNA content of 2·4 pg (Clowes *et al*., 1983) were used to quantify mean fluorescence intensity (I_f) values for algal specimens (Kapraun, 1994). DAPI binds by a non-intercalative mechanism to adenine and thymine rich regions of DNA that contain at least four A–T base pairs (Portugal and Waring, 1988). Consequently, RBC are best used as a standard for estimating amounts of DNA when the A–T contents of both standard and experimental DNA are equivalent (Coleman *et al*., 1981). *Gallus* has a nuclear DNA base composition of 42–43 mol % (molecular percent) G + C (Marmur and Doty, 1962). Limited published data for algae indicate mean values of 43·5 mol % G + C ($n = 9$, range = 40–47 mol %)

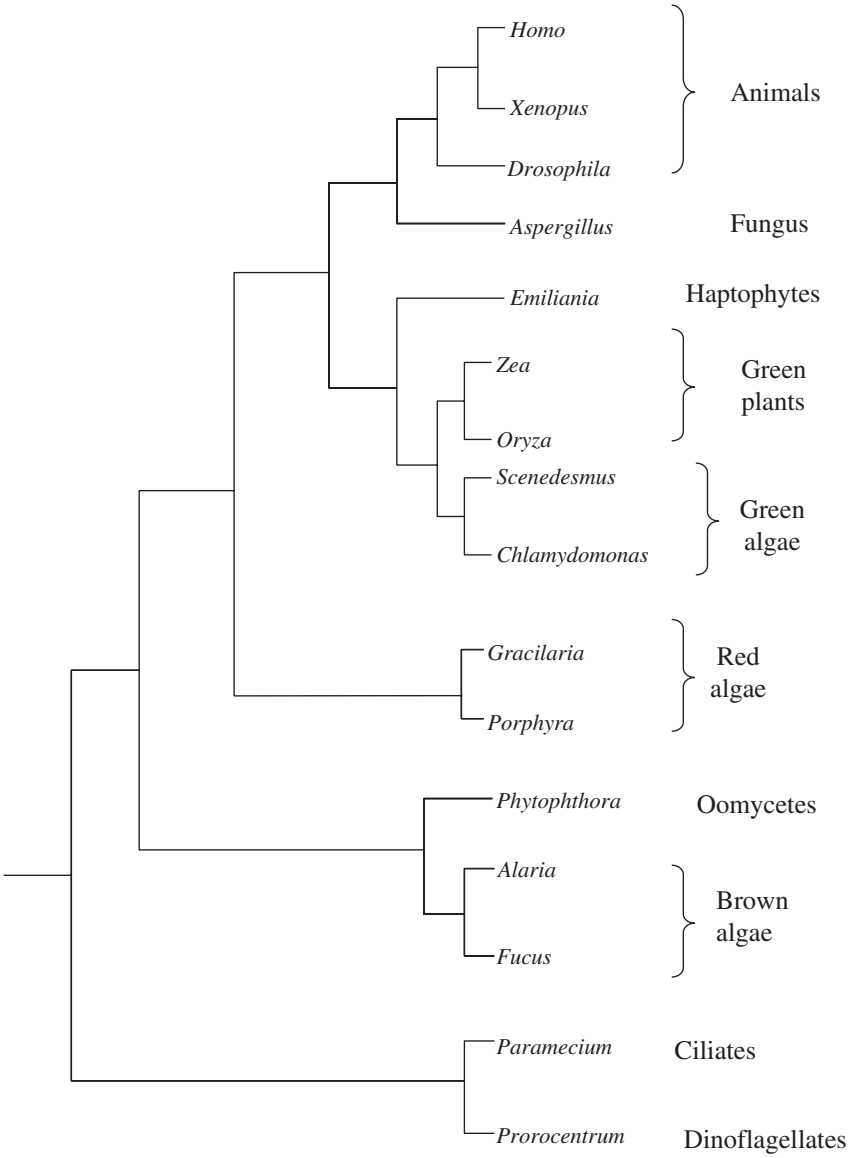

FIG. 1. Evolutionary tree constructed from a distance matrix of eukaryotic SSU rRNA sequences and based on 'substitution rate calibration'. Redrawn from Van der Peer *et al.* (1996).

for the Phaeophyta (Olsen *et al.*, 1987; Stam *et al.*, 1988; Le Gall *et al.*, 1993), 41·6 mol % G + C (n = 22, range = 28–49 mol %) for the Rhodophyta (Kapraun *et al.*, 1993*b*, *c*; Le Gall *et al.*, 1993), and 46·2 mol % (n = 22, range = 35–56 mol %) for the Chlorophyta (Olsen *et al.*, 1987; Freshwater *et al.*, 1990; Kooistra *et al.*, 1992; Le Gall *et al.*, 1993). Algae investigated in this study are assumed to have a similar range of base pair compositions, and linearity is accepted between DAPI-DNA binding in both RBC and algal samples (Le Gall *et al.*, 1993). Nuclear DNA contents were estimated by comparing the I_f values of the RBC standard and algal sample (Kapraun, 1994). All three algal groups contain taxa with some or all of their cells being multinucleate and often endopolyploid (Goff *et al.*, 1992; Kapraun and Nguyen, 1994; Garbary and Clarke, 2002; Kapraun and Dunwoody, 2002). In addition, both red algae (Goff and Coleman, 1986) and green algae (Kapraun, 1994) have taxa that exhibit a nuclear 'incremental size decrease associated with a cascading down of DNA contents'. Consequently, methodologies were developed specific for specimens of each algal group to permit assignment of C-level and interpretation of I_f data. Materials and methods, as well as information for collection locations, and data for number of algal nuclei examined in each sample and estimates of nuclear genome size (pg ± s.d.) are available at http://www.uncw.edu/people/kapraund/DNA.

RESULTS AND DISCUSSION

CHLOROPHYTA

The Division Chlorophyta contains the eukaryotic green algae, which possess chlorophylls *a* and *b*, as well as starch stored inside plastids with stacks of two to six thylakoids per

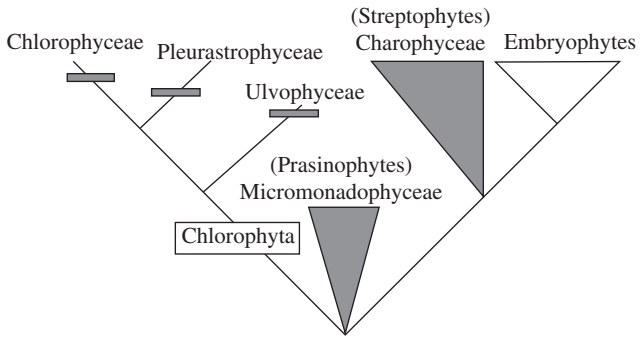

FIG. 2. Summary results of combined analysis using morphological, ultrastructural and large and small subunit rRNA gene sequences for the five classes of green algae and four lineages of embryophytes (liverworts, hornworts, mosses and tracheophytes). Redrawn from McCourt (1995).

band (Bold and Wynne, 1985). Approximately 425 genera and 6500 species have been described (Alexopoulos and Bold, 1967). Simplicity and antiquity of green algae have long been accepted as evidence of their apparent ancestry to land plants (McCourt, 1995). Recently, parsimony analysis of sequence data for the RuBisCO large subunit (*rbc*L) (McCourt, 1995; McCourt *et al*., 1996) and small-subunit (SSU) rRNA group I interons (Bhattacharya *et al*., 1994) contradict this view and present a compelling case that an ancient divergence separates green plants into two major monophyletic lineages: the Chlorophyta and the Streptophyta (McCourt, 1995; Karol *et al*., 2001). The Chlorophyta contain the classical 'green algae', primarily the Chlorophyceae and Ulvophyceae (Watanabe *et al*., 2001; Fig. 2). The Streptophyta includes the charophycean lineage comprised of five orders, along with bryophytes and tracheophytes (Mishler *et al*., 1994). Identification of the charophycean lineage as the sister group of land plants suggests that their common ancestor was a branched, filamentous organism with a haplontic life cycle and oogamous reproduction (Karol *et al*., 2001).

A third polyphyletic green plant lineage, at the base of the split of the Chlorophyta and the Streptophyta, includes the green alga *Mesostigma viride* (Turmel *et al*., 2002*a*). A significant body of research centered around *Mesostigma* is emerging that provides insights into the timing of events that restructured both mitochondrial (mtDNA) and chloroplast (cpDNA) genomes during the evolution of green algae (Turmel *et al*., 2002*b*) and the transition from charophytes to land plants (Turmel *et al*., 2002*a*). The exact placement of *Mesostigma* remains controversial as some phylogenetic analyses include this species with the Prasinophyceae (Lemieux *et al*., 2000; Turmel *et al*., 2002*b*) while others consider it to be basal in the charophycean lineage (Karol *et al*., 2001). Whatever the exact position of *Mesostigma*, there is no doubt that this alga belongs to a deeply diverging lineage because it represents the most basal branch in trees inferred from sequences of land plants and all five orders of charophytes (Karol *et al*., 2001).

A residium of related unicellular micromonadophytes (= Prasinophytes; Kantz *et al*., 1990; Steinkötter *et al*., 1994; Karol *et al*., 2001) is, likewise, associated with the Chlorophyta–Streptophyta divergence (Fig. 2). Nuclear genome size and organization remain largely unknown in the Prasinophytes. Pulse field gel electrophoresis of *Ostreococcus tauri* (Prasinophyceae) resulted in a nuclear genome size estimate of 10·20 mbp (Courties *et al*., 1998) or 0·1 pg using the expression 1 pg = 980 Mbp (Bennett *et al*., 2000). The minute size of this genome, one of the smallest among free-living eukaryotic organisms, is best appreciated by comparison with the chloroplast (cpDNA) genome size of 118 360 bp or 0·012 pg (Lemieux *et al*., 2000) reported in the closely related *Mesostigma viride*. It is assumed that this small nuclear genome size is evolutionarily derived rather than ancestral (Courties *et al*., 1998) as other members of the Mamiellaceae represent secondarily reduced forms (Daugbjerg *et al*., 1995). If such extreme reduction of nuclear genome size is typical of the micromonads, it may not be possible to reconstruct a hypothetical ancestral nuclear genome from extant species.

Charophycean algae

The charophycean lineage includes the Chlorokybales (Qiu and Palmer, 1999), Klebsormidiales (Karol *et al*., 2001), Conjugophyta (Zygnematales; Hoshaw *et al*., 1990), the Coleochaetales (Bhattacharya *et al*., 1994; McCourt, 1995) and the Charophyta (Surek *et al*., 1994; McCourt *et al*., 1996; Fig. 3).

Coleochaetales. Members of this small and obscure group are minute epiphytes on aquatic angiosperms and aquatic algae (Bold and Wynne, 1985). Feulgen microspectrophotometry was used to elucidate the life history of *Coleochaete scutata* (Hopkins and McBride, 1976). However, data were given as relative fluorescence units (rfu) without reference to a DNA standard. Apparently, there are no published estimates for nuclear DNA contents in any member of this order. Karyological studies of three *Coleochaete* speces have been published (Sarma, 1982). Reported chromosome complements of $1n = 24$, 36 and 42 suggest a polyploid sequence derived from a basic complement of $x = 12$. The reported chromosome complement of $n = 22$ in *Klebsormidium* (Chaudhary and Sarma, 1978) likewise is consistent with polyploidy.

Desmidiales and Zygnematales. The conjugating green algae or Zygnematales make up a widely distributed group of freshwater algae characterized by the lack of flagellated cells and reproduction by conjugation (Hoshaw *et al*., 1990). Most biologists are familiar with *Spirogyra* and its strikingly prominent ribbon-shaped spiral chloroplast (Bold and Wynne, 1985). The Zygnematales are among the most investigated green algae cytologically (Sarma, 1982). The lowest chromosome number of $n = 2$ is recorded in several species of *Spirogyra*. The group is known for extensive polyploidy with chromosome complements of $n = 30$, 60, 90 to 592 reported (Sarma, 1982). Presence of polycentric chromosomes in both filamentous and unicellular (desmid) forms is a unique feature of the group (King, 1960; Hoshaw and McCourt, 1988). Karyotype analyses indicate an extraordinary range in

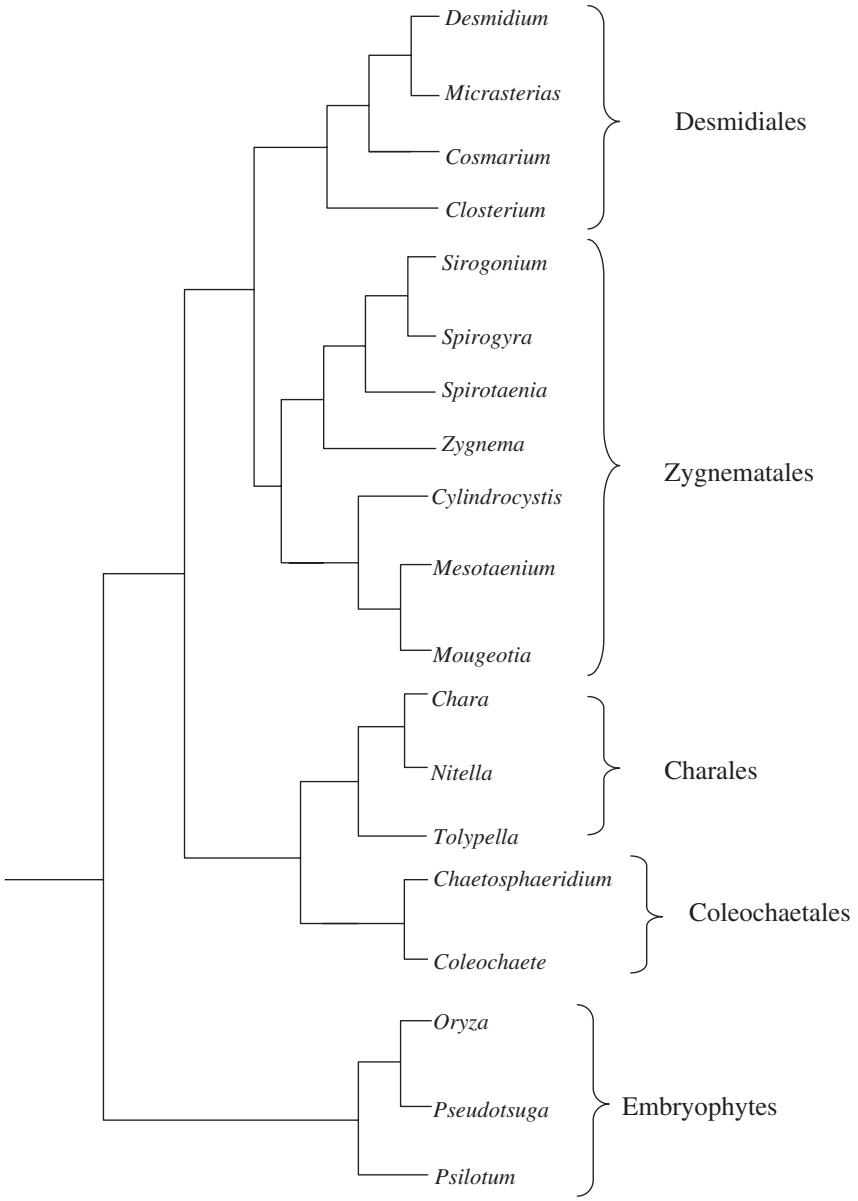

FIG. 3. Phylogram of conjugating green algae based on MP analysis of *rbc*L sequences. Redrawn from McCourt *et al*. (2000).

chromosome lengths as well, from 1–20 μm (King, 1960). DAPI microspectrophotometry was used to investigate a species complex in *Spirogyra* (Wang *et al*., 1986). Specimens identified as three separate species, based primarily on filament diameter and cell size, were determined to be polyploid races of a single species. Ploidal changes observed in both culture and field material was described as autopolyploidy, characterized by spontaneous even-number multiplication of the genome (Wang *et al*., 1986). Data were given in rfu and nuclear DNA contents were not quantified. In the present study, these same isolates (UTEX 2465 and 2466) were re-investigated and found to have essentially equivalent nuclear DNA amounts (Appendix I). Apparently, autopolyploid forms in these algae are unstable and can spontaneously revert to lower ploidy levels in culture.

The sole published estimate of nuclear DNA contents in the true desmids (Desmidiales) is for *Closterium* (2C = 2·7 pg; Hamada *et al*., 1985). Unpublished investigations in our laboratory of the filamentous Zygnematales (Purvis, 1998) and unicellular Desmidiales (Marlowe, 1998) are summarized in Appendix I. The 2C nuclear DNA contents in the Zygnematales ranged from 0·5–4·2 pg, and from 1·1–20·7 pg in the Desmidiales. Several desmids investigated had nuclei too large to be accommodated by the photometer aperture system and could easily have had nuclear DNA contents in excess of 4*x* specimens that were measured. Thus, nuclear DNA contents approaching 100 pg may occur in some desmids. In the desmids that permitted quantification, reported chromosome complements and nuclear DNA contents are highly correlated (r^2 = 0·7897), providing circumstantial evidence of

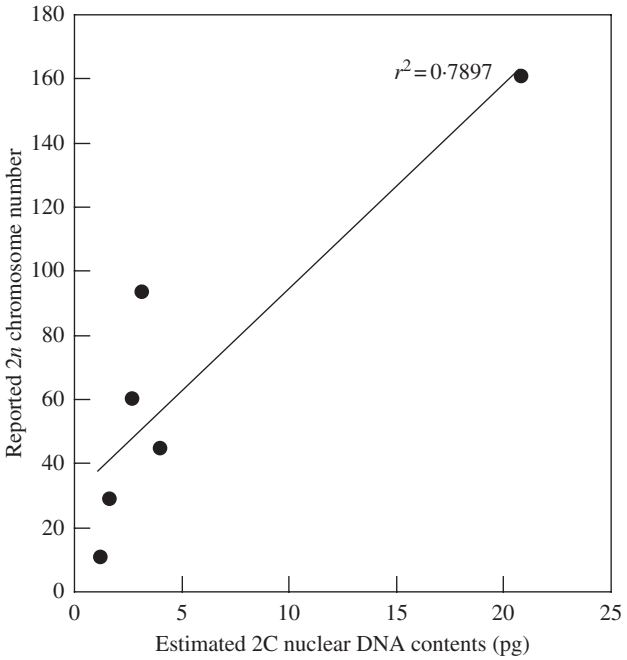

FIG. 4. Comparison of 2*n* chromosome complements and estimated 2C nuclear DNA contents in Desmids. See Appendix I for data sets.

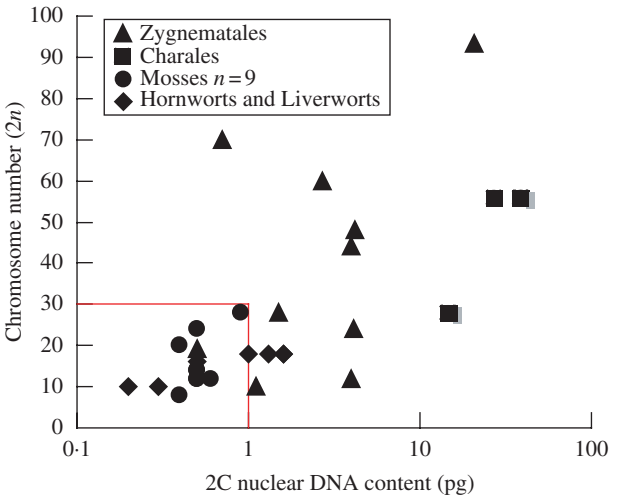

FIG. 5. Comparison of 2*n* chromosome complements and estimated 2C nuclear DNA contents in charophycean and embryophyte lineages. Data for hornworts, liverworts and mosses from Renzaglia *et al*. (1995). Data for Charales in Appendix I.

polyploidy in the group (Fig. 4). In contrast, no correlation was observed between nuclear DNA contents and reported chromosome complements for several filamentous Zygnemataceae (data not shown) as would be expected if higher chromosome numbers resulted primarily from duplication of chromosome fragments resulting from fusion and/or fission events associated with their polycentric centromeres.

Both the filamentous Zygnemataceae and the true desmids have undergone explosive speciation, resulting in thousands of described species (for example, see Prescott *et al*., 1972, 1977, 1981; Hoshaw and McCourt, 1988) on every continent except Antarctica. Now that a basic understanding of phylogenetic relationships is emerging for the Zygnematales (Surek *et al*., 1994; McCourt *et al*., 2000) and the Desmidiales (Denboh *et al*., 2001), it would be a matter of great interest to identify possible nucleotype transformations that have accompanied their speciation. Many of the Zygnemataceae appear to be characterized by polyploid 'species complexes' (Hoshaw and McCourt, 1988) and reported large cell sizes in many of the Desmidiales suggest that polyploidy in these uninucleate, unicellular organisms has produced some of the largest nuclear genome sizes known in plants.

Charales. The Charales, commonly known as stoneworts or brittleworts, flourish in fresh and brackish water habitats throughout the world (Bold and Wynne, 1985). Charophytes are prone to calcification and have left an abundant fossil record up to the Cretaceous, and perhaps beyond (Grambast, 1974; Feist *et al*., 2003). The order is well circumscribed and includes a mere handful of extant genera (McCourt *et al*., 1996), remnants of a once diverse, but now largely extinct group (Feist *et al*., 2003). The base chromosome number for *Chara* is $n = 7$ and in *Nitella* is $n = 3$. However, many species exhibit polyploidy, with chromosome complements up to $n = 70$ reported (Sarma, 1982). Published C-value data are limited to a single investigation of five species of *Chara* (Maszewski and Kolodziejczyk, 1991). Two of these species, with $2n = 28$, have 2C DNA contents of about 14 pg. Interestingly, while one of the species with a polyploid $2n = 56$ has the expected 2DNA content of 28 pg, the other two species with $2n = 56$ have 2C DNA contents of about 19 pg or three times the lowest value (Appendix I).

Comparative molecular data indicate that the charophycean green algae are a sister group and paraphyletic to land plants (Mishler *et al*., 1994; McCourt, 1995; McCourt *et al*., 2000). It is perhaps informative to compare the C-values of these green algal groups with those of the oldest group of land plants, the bryophytes (Kenrick and Crane, 1997). Unfortunately, data for the basal groups in the charophycean lineage (Chlorokybales and Klebsormidiales) are limited to chromosome numbers for *Klebsormidium* (Sarma, 1963). Published information for members of the Zygnematales and the Charales indicate that they can be characterized either by chromosome complements of more than $2n = 30$ or 2C nuclear DNA contents greater than 1 pg, or both (Fig. 5). Unfortunately, no DNA content estimates are available for any member of the Coleochaetales, but the smallest chromosome complements reported in the order, $2n = 44$ and 48, are consistent with polyploidy and a larger nuclear genome. In contrast, hornworts, liverworts and mosses, in general, have chromosome complements less than $2n = 30$ and/or 2C nuclear DNA contents less than 1 pg (Renzaglia *et al*., 1995; Voglmayr, 2000). Although greater values for both parameters are known in the bryophytes, they appear to be restricted to polyploid species and do not contradict the generalization. For example, more than 80 % of the nuclear DNA C-values in mosses were reported to occur in a narrow peak between 0·25–0·6 pg (Voglmayr, 2000). It has been suggested that the small DNA amounts and low C-value

variation are linked to the biflagellate nature of bryophyte sperm cells (Renzaglia *et al*., 1995). As nuclear genome size and sperm cell size are tightly correlated, and sperm cells are thought to drastically lose their motility with increasing size, a strong selection pressure against larger sperm, and therefore also against larger DNA amounts, is hypothesized (Voglmayr, 2000).

These observations gain additional significance in the context of the suggestion that the common ancestor of all angiosperms may have possessed a small genome (Leitch *et al*., 1998, 2005). Small genome size appears to be correlated with phenotypic characteristics such as rapid seedling establishment, short minimum generation times, reduced cost of reproduction, and an increased reproductive rate (Bennett, 1987; Midgley and Bond, 1991). Consequently, small genome size may permit greater evolutionary flexibility (Leitch *et al*., 1998) whereas larger size and amplification may lead to 'genomic obesity' (Bennetzen and Kellogg, 1997). It is to be wondered if the relatively large nuclear genomes found in the charophycean algae, perhaps appropriate in an ancient atmosphere with low amounts of oxygen and UV-absorbing ozone, rendered them unsuitable contenders for the colonization of land as atmospheric conditions improved (Graham, 1993).

Ulvophycean algae

The other major monophyletic lineage related to the charophycean algae discussed above contains the classical 'green algae', primarily the Chlorophyceae and Ulvophyceae (Watanabe *et al*., 2001). The Chlorophyceae apparently arose during the later stages of green algal evolution and are not a basal lineage (Watanabe *et al*., 2001). This group includes many of the familiar flagellates such as *Volvox* and *Chlamydomonas* and is characterized by the predominance of freshwater taxa. There are few published DNA content estimates for members of the Chlorophyceae. The pioneering investigation of Holm-Hansen (1969) which used 'fluorometric measurement', reported 2C = 0·6 pg for *Dunaliella tertiolecta*. However, no calibration standard was specified. Higashiyama and Yamada (1991) used pulse field electrophoresis to estimate a 2C genome size in *Chlorella* of 40 Mbp (or 0·04 pg using the expression 1 pg = 980 Mbp (Bennett *et al*., 2000). The Ulvophyceae are primarily marine species, most with larger and more complex morphologies than typically found in the Chlorophyceae. Molecular data support a model for the Ulvophyceae *sensu* Mattox and Stewart (1984) with two separate lineages: a clade including the Ulotrichales and Ulvales (Hayden and Waaland, 2002) and a clade with the Caulerpales, Cladophorales/Siphonocladales complex, Dasycladales and the Trentepohliales (Zechman *et al*., 1990; Hanyuda *et al*., 2002). Published information is available for all of the major groups of the Ulvophyceae, and significant new data are included in this study (Appendix I).

Ulvales. Recent phylogenetic investigations using chloroplast and nuclear DNA sequences have redefined the boundary between the Ulotrichales and Ulvales (Hayden and Waaland, 2002). Species of *Capsosiphon* and *Monostroma*, included in the Ulvales by Bliding (1963, 1968) appear to be more closely related to the Ulotrichales (Fig. 6). The amended order Ulvales is monophyletic, but the chief characteristic used to separate the familiar genera *Ulva* and *Enteromorpha*, i.e. blade vs. tubular thallus, lacks taxonomic significance (Hayden and Waaland, 2002). The *Ulva* and *Enteromorpha* morphologies apparently arose independently several times throughout the evolutionary diversification of the group, making distinctions between these two genera problematic (Tan *et al*., 1999; Shimada *et al*., 2003). Estimates of nuclear DNA contents for species of the Ulvales range from 2C = 0·2–1·1 pg (Appendix I). The absence of a correlation between nuclear genome size and chromosome number in these species (data not shown) suggests a significant role of aneuploidy in their evolution (Kapraun and Bailey, 1992).

Ulotrichales. The Ulotrichales as presently delimited has been expanded to include the Acrosiphoniaciae (*sensu* Kornmann and Sahling, 1977). Nuclear DNA content data, available for only three species of this large and diverse order, suggest that it is characterized by small 2C values of 0·5–0·9 pg (Appendix I). These relatively small genome sizes are complemented by relatively small chromosome numbers of $2n$ = 8–24 (Kapraun, 1993*c*).

Trentepohliales. The order Trentepohliales includes more than 60 species of subaerial and terrestrial green algae (Lopez-Bautista *et al*., 2000). Molecular investigations place members of this order with the second lineage of the Ulvophyceae (Mishler *et al*., 1994; Chapman *et al*., 1995), which are otherwise almost exclusively marine. Nuclear DNA content estimates of 2C = 1·1–4·1 pg and reported chromosome complements of $2n$ = 22–36 (Appendix I) are indicative of polyploidy (Lopez-Bautista *et al*., 2000). However, there is no apparent correlation between chromosome number and nuclear DNA content.

Dasycladales. The order Dasycladales includes extant tropical and subtropical benthic marine green algae and existed as long ago as the Cambrian (approx. 570 mya; Berger and Kaever, 1992). Members of the Dasycladales are unicells characterized by a highly differentiated cell body with radially disposed branches and a persistent primary nucleus (Spring *et al*., 1978). Detailed investigations of evolution in the order have benefited from the abundance of fossilized morphotypes, which record periodic radiations and extinctions (Olsen *et al*., 1994). Only 11 of 175 known fossil genera are extant, representing 38 species in two families: Dasycladaceae and Polyphysaceae (= Acetabulariaceae). The small number of extant genera permits characterization of the Dasycladales as living fossils. Monophyly of the Dasycladales is unchallenged and supported by morphological, ultrastructural, biochemical and DNA sequence data (O'Kelly and Floyd, 1984; Mishler *et al*., 1994; Watanabe *et al*., 2001; Zechman, 2003).

In the Dasycladales, estimated 2C DNA contents range from 0·7–3·7 pg (Appendix I). The smallest 2C DNA values occur in the basal (and primitive) genera *Bornetella* and *Cymopolia* (Fig. 7). The relatively larger DNA contents

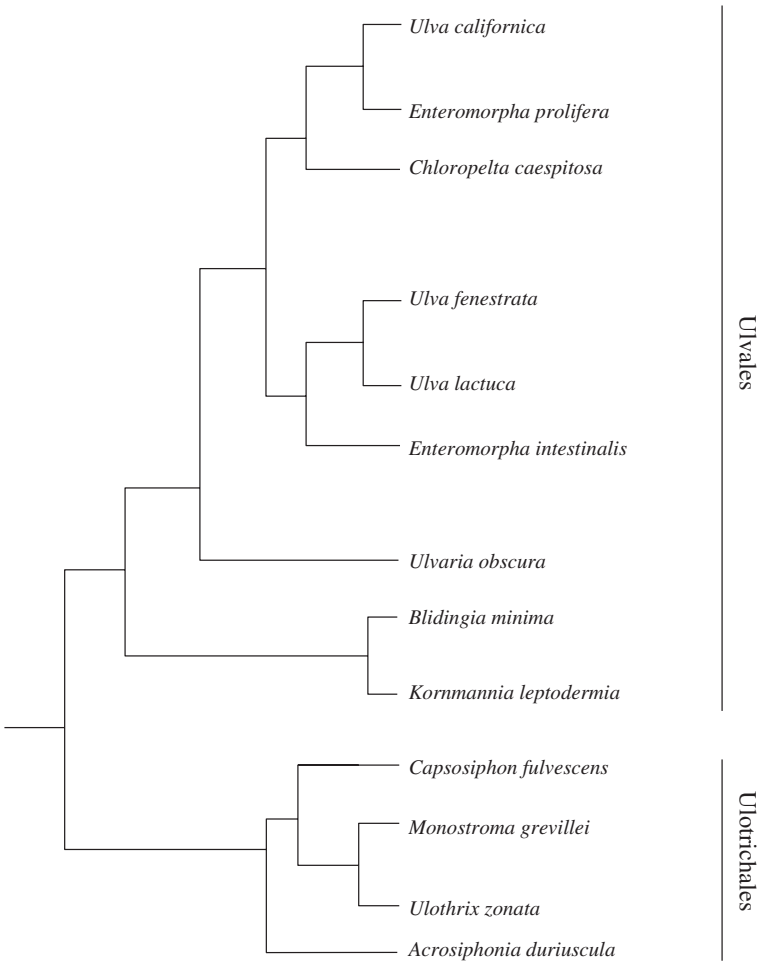

FIG. 6. Phylogenetic tree of the Ulvales and Ulotrichales inferred from 18S rDNA and *rbc*L sequence analysis. Redrawn from Hayden and Waaland (2002).

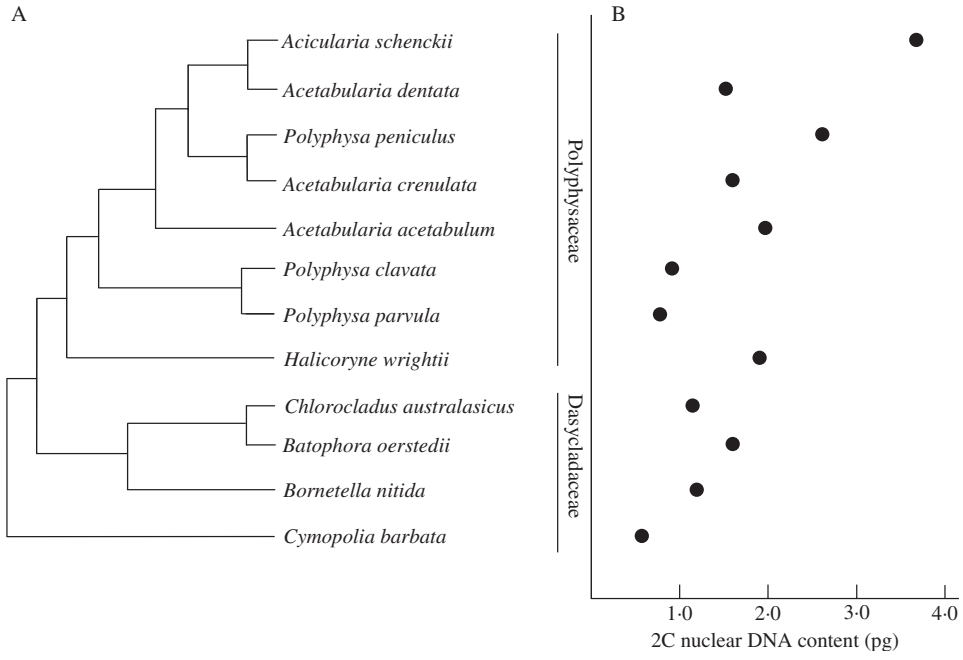

FIG. 7. (A) Consensus phylogeny for the Dasycladales from analyses of *rbc*L (Zechman, 2003), and (B) 18S rDNA (Berger *et al*., 2003) gene sequence data.

found in more recently evolved taxa almost certainly reflect a sequence of multiple polylploidy events. It is noteworthy that although the dasyclads are an ancient lineage, most extant species are recent, resulting from dramatic radiation events within the last 65 million years. In most taxa investigated, cyst volume was found to be inversely related to genome size (Kapraun and Buratti, 1998). The adaptive significance seems to be that small genome size and large cyst size result in the production of increased numbers of gametes per cyst.

Recent molecular investigations based on analyses of *rbc*L (Zechman, 2003) and 18SrDNA (Berger *et al*., 2003) sequence data indicate that reproductive cap morphotypes characteristic of *Polyphysa* and *Acetabularia* (Sawitzky *et al*., 1998) are polyphyletic. The revised and expanded circumscription of *Acetabularia* now includes *Polyphysa peniculus* and *Acicularia schenckii* (Berger *et al*., 2003). *Acetabularia* species are characterized by an earlier cap ray initiation relative to the formation of *corona superior* hairs compared with development in other members of the Polyphysaceae (Berger *et al*., 2003). It is noted with great interest that nuclear genome size is highly correlated with these developmental patterns. Specifically, all species of the expanded genus *Acetabularia* have 2–3 times the 2C DNA contents found in *Polyphysa clavata* and *Polyphysa parvula*, which are basal to other Polyphysaceae (Fig. 7). The strong correlation between cap morphotypes (Sawitzky *et al*., 1998) and cap morphogenesis (Kratz *et al*., 1998) and 'polyploid' nucleotypes in the Dasycladales implies a significant but poorly understood role for the nucleotype in gene expression (Gregory, 2001).

Caulerpales. Members of the Caulerpales (Codiales *sensu* Taylor, 1960) are multinucleate and coenocytic. Preliminary molecular data seem to support classical taxonomic treatments that separate the order into two groups (Zechman *et al*., 1990), one generally characterized by diplobiontic life histories and non-holocarpic production of gametes (e.g. *Bryopsis* and *Codium*), the other generally characterized by haplobiontic and diploid life histories and holocarpic production of gametes (e.g. *Caulerpa* and *Halimeda*; Kapraun, 1994). Nuclei with endopolyploid DNA contents have been reported in several caulerpalean algae, and a remarkable regular, incremental size decrease (cascading) in DNA contents of vegetative nuclei corresponding to values of 8C to 2C was observed in *Halimeda* (Kapraun, 1994). Estimates of 2C nuclear DNA contents range from 0·2–6·1 pg (Appendix I). The largest nuclear genome (2C = 6·1 pg) was observed in *Codium fragile* subsp. *tomentosoides* isolates from North Carolina. Originally endemic to Japan or the northwest Pacific (Goff *et al*., 1992), this invasive seaweed spread throughout the North Atlantic during the 20th century and became a nuisance species in some localities. It reportedly reproduces exclusively by parthenogenetic female gametes (Searles *et al*., 1984) and fragmentation (Fralick and Mathieson, 1973). Because of its mode of reproduction and unusually large nuclear genome, it is speculated that its success as a weed could be attributed, in part, to its behaviour as an autopolyploid apomict (Kapraun and Martin, 1987; Kapraun *et al*., 1988).

The large and diverse genus *Caulerpa* includes more than 75 described species, mostly from tropical shallow marine habitats (Price *et al*., 1998). Nuclear DNA contents published for four of these species are essentially identical (2C $\approx$ 0·2 pg). Now that a molecular phylogeny has been published (Famà *et al*., 2002), it would be a matter of great interest to determine if evolution in this group has been accompanied by transformations involving chromosome complements and nuclear DNA contents.

Recently, the genus *Caulerpa* attracted considerable media attention as species expanded their ranges into more temperate environments (Olsen *et al*., 1998). One of these, *C. taxifolia*, is especially aggressive (Meinesz *et al*., 1993; De Villèle and Verlaque, 1995). It has been variously labelled as a mutant or superstrain that may have resulted from autopolyploidy or hybridization. Although the mechanism of its origin remains speculative, gigantism, fast growth rates, low temperature tolerances and facultative apomixes make it a formidable competitor (Olsen, 1997). Based on previous experience with *Codium fragile*, it would be a matter of great interest to determine if invasive *C. taxifolia* likewise is characterized by an elevated nuclear DNA content and functions as a polyploid apomictic strain.

A recent molecular and morphological analysis of *Bryopsis* revealed the presence of four genetically distinct clades from the western Atlantic and Caribbean that appear to be either seasonally or geographically disjunct (Krellwitz *et al*., 2001). However, these genetic clades do not coincide with current morphological species concepts in the genus. It has been suggested that investigations based on misidentification of these polymorphic, poorly delimited species might account for the considerable variation in reported chromosome numbers, including $1n$ = 7, 8, 10, 12 and 14 (Kapraun, 1993*c*). Nuclear DNA estimates are available for only three *Bryopsis* species (Appendix I). In light of the investigation by Krellwitz *et al*. (2001), species assignment of these specimens, based solely on morphological features (Kapraun and Shipley, 1990), requires reconfirmation. It would be a matter of great interest to obtain both chromosome complement and nuclear genome size data for these molecularly delimited clades.

Cladophorales/Siphonocladales complex. Since nuclear volume is strongly correlated with cell size and cell cycle lengths in higher plants (Shuter *et al*., 1983) it is not surprising that these algae with their large, multinucleate cells and relatively long cell generation times have relatively large genomes (Kapraun and Nguyen, 1994). Many algae are characterized by an alternation of haploid gametophyte and diploid sporophyte generations. If the phases are isomorphic, a mechanism must be present to equilibrate the ratio between nuclear volume and cytoplasmic area to maintain a constant area of cytoplasmic domain per standardized nuclear DNA unit (Goff and Coleman, 1987, 1990). In members of the Cladophorales/Siphonoclades complex investigated, isomorphy is maintained by both increasing the number of nuclei per cell and increasing the ploidy level of nuclei (Kapraun and Nguyen, 1994).

The Cladophorales and Siphonocladales are a related patristic lineage sharing a gradation of 'architectural'

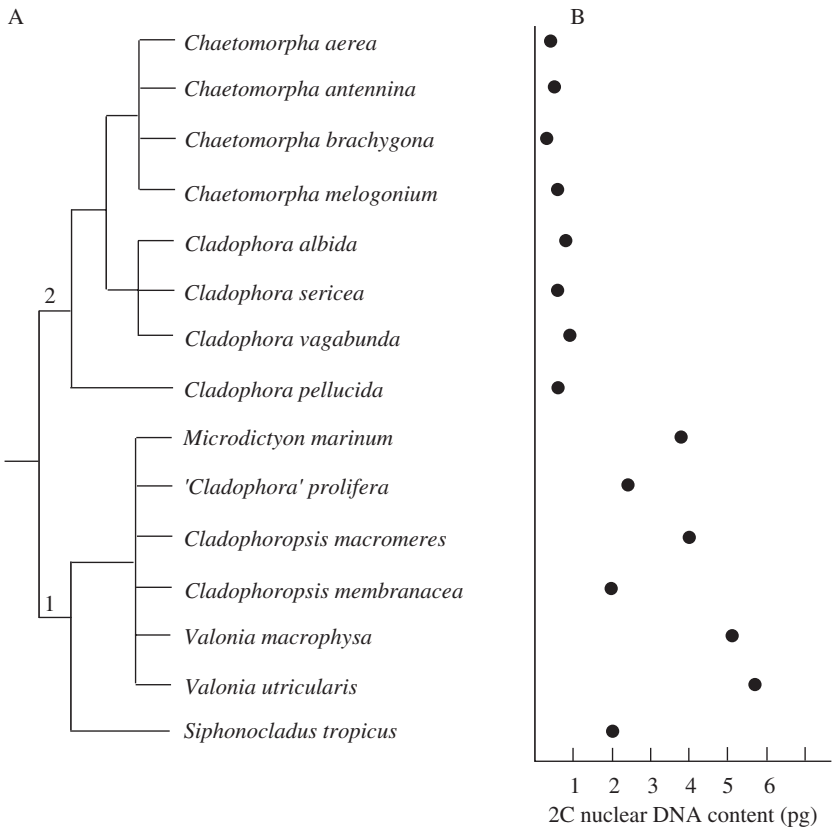

FIG. 8. (A) Phylogram based on 18S rRNA gene sequence analysis, and (B) nuclear DNA contents in members of the Cladophorales/Siphonocladales complex. Numbering (1 and 2) indicates major clades. Redrawn from Hanyuda *et al*. (2002).

morphological types (van den Hoek *et al*., 1988). Immunological distance estimates (Olsen-Stojkovich *et al*., 1986; van den Hoek *et al*., 1988) and cladistic analyses of nuclear encoded rDNA sequences (Zechman *et al*., 1990; Hanyuda *et al*., 2002) support a close relationship between the Cladophorales and Siphonocladales. Contemporary molecular studies support a phylogeny consisting of three well-supported clades: (1) species belonging to the cladophoracean genera *Chaetomorpha*, *Cladophora* and *Rhizoclonium*; (2) species belonging primarily to the Siphonocladales *sensu* Børgesen (1913); and (3) mostly freshwater species of cladophoracean genera, including *Pithophora* and *Wittrockiella* (Hanyuda *et al*., 2002). Confusingly, the genera *Chaetomorpha*, *Cladophora* and *Rhizoclonium* are polyphyletic, and their characteristic morphologies appear to have evolved several times, independently, in all three clades

Karyological studies indicate that species in this first clade, without exception, share a unique constellation of karyotype features including: (1) six basic chromosomes, three of which have median centromeres and three with submedian ones; and (2) almost universal polyploidy, resulting in chromosome complements in most species of $x = 12, 18, 24, 30, 36$, etc. (Wik-Sjöstedt, 1970; Kapraun and Gargiulo, 1987*a*, *b*). Species in the second clade have (1) various combinations of both metacentric and acrocentric chromosomes (Kapraun and Breden, 1988; Bodenbender and Schnetter, 1990; Kapraun and Nguyen, 1994); and (2) chromosome complements consistent with an aneuploid origin: $1n = 8$, 12, 14, 16, 18, and 20 (Kapraun, 1993*c*; Kapraun and Nguyen, 1994). Nuclear DNA content estimates indicate that members of clade II (Fig. 8) have relatively small genomes (2C = 0·2–0·7 pg) while members of clade I, including the bulk of the Siphonocladales, have much larger genomes of 2C = 2·0–5·7 pg (Appendix I). Although the cladophoracean morphotype appears to have evolved independently in all of the clades, the combination of karyotype pattern and nuclear genome size characteristic of the core clade of the Cladophorales appears to be unique and diagnostic (Fig. 9). It would be a matter of great interest to obtain karyotype and nuclear genome size estimates for representative members of all three clades to determine if these generalizations are universal in the Cladophorales/Siphonocladales complex.

Conclusions and directions for further study

We are not aware of published investigations of G + C mol % or reassociation kinetics for any charophyceaen algae. Consequently, their nucleotype characterization is restricted to chromosome complement, karyotype pattern and nuclear DNA content estimates. In general, charophycean algae have larger genomes (2·0–20·7 pg; Fig. 10) and larger chromosome complements ($1n = 2$–90 up to 592) than

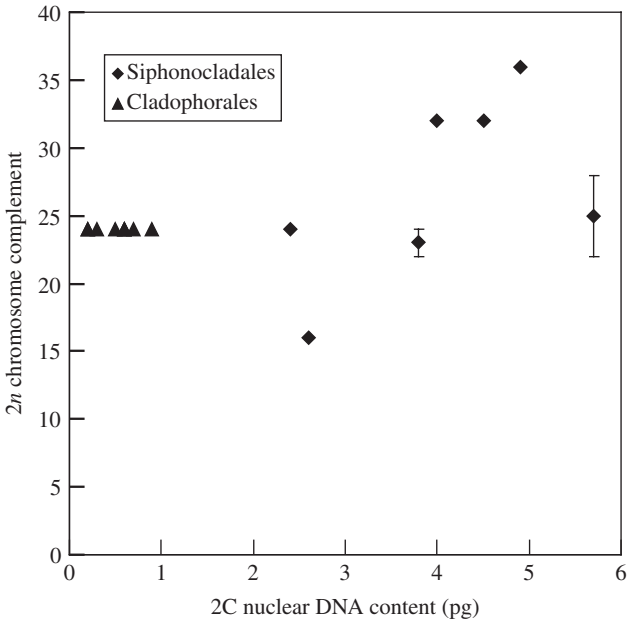

FIG. 9. Comparison of 2*n* chromosome complements and 2C nuclear DNA contents in members of the Cladophorales/Siphonocladales complex. See Appendix I for data sets.

do most ulvophycean algae. The two orders most studied, the Zygnematales and Charales, have unique karyotypes. The former is known for its large, polycentric chromosomes; the latter for long chromosomes (up to 12 μm) with a high heterochromatin content.

The unicellular Desmidiales, characterized by thousands of morphotypes, should be a target group for investigations of nuclear DNA content variation. Specifically, (1) reported large cell size could be compared with nuclear genome size, and (2) coincidence of elevated (polyploid) genome sizes with the number of described species per genus could be evaluated to determine if morphotypes delimited as species have primarily a genotypic or a nucleotypic basis.

The exact relationship of the Prasinophytes to land plants remains unclear (Qiu and Palmer, 1999) and the apparent miniaturization of their nuclear genomes may defeat attempts to use them as a model in reconstruction of land plant ancestral genomes (Cunningham *et al*., 1998; Oakley and Cunningham, 2000). Consequently, the basal groups in the charophycean lineage (Soltis *et al*., 1999), including the Chlorokybales, Klebsormidiales and Coleochaetales, may provide the best opportunity for gaining these insights, yet there are no published estimates of DNA contents in any member of these orders. Species of both *Coleochaete* and *Klebsormidium* are commonly investigated and are readily available to researchers. It should be a priority to obtain nuclear DNA content values for these green algae. The present investigation has noted that charophycean algae appear to be characterized either by chromosome complements and/or nuclear DNA contents greater than typically encountered in primitive land plants. It should be a priority to obtain data for many additional charophycean algae to evaluate this suspected relationship.

Finally, no published data are available for the flagellated unicellular and colonial Chlorophyceae, including the familiar *Chlamydomonas* and *Volvox*. It should be a priority to obtain nucleotype data for comparison with speciation patterns resolved in emerging molecular phylogenetic studies for these algae (e.g. Nozaki *et al*., 1995).

The present and previous investigations (Olsen *et al*., 1987; Bot *et al*., 1989*a*, *b*, 1990, 1991; Kooistra *et al*., 1992) permit some generalizations concerning nuclear genomes in the predominantly marine species of the Ulvophyceae:

(1) Chromosome numbers range from $1n = 5–12$ (excluding polyploid values), and both polyploidy and aneuploidy events appear to have accompanied speciation in specific groups. Comparison of 2*n* chromosome numbers and 2C nuclear DNA contents results in a low correlation of $r^2 = 0{\cdot}3177$ (Fig. 11), consistent with a high occurrence of aneuploidy, i.e. chromosomal fusion and/or fission events.
(2) Estimated 2C nuclear DNA contents range from 0·2–4·9 pg.
(3) G + C ranges from 35–56 mol %.
(4) Reassociation kinetics has identified the presence of highly repetitive, mid-repetitive and unique sequences in the few species investigated. These preliminary results indicate a predominance of unique and mid-repetitive sequences and a relatively small proportion of highly repetitive sequences. The findings are consistent with the suggestion that much of the reported variation in nuclear genome sizes may result from accumulation and/or deletion of non-genic, repetitive elements (Cavalier-Smith and Beaton, 1999).

PHAEOPHYTA

The brown algae or Phaeophyta are an essentially marine assemblage of more than 265 genera and 1500 species (Bold and Wynne, 1985). Nuclear genome size estimates in Appendix II include previously unpublished observations (Criswell, 1998) as well as data from the present study. The range of 2C nuclear genome sizes estimated for the Phaeophyta (0·2–1·8 pg) approximates one order of magnitude (Appendix II). The smallest mean 2C genome sizes were found in the Ectocarpales (0·2–0·9 pg) and the largest 2C genome sizes were found in the Sphacelariales (1·8 pg), Fucales (1·7 pg) and Laminariales (1·6 pg). Previous published information for genome sizes in the Phaeophyta based on data from reassociation kinetics (Stam *et al*., 1988) and quantitative staining with DAPI (Stache, 1991; Le Gall *et al*., 1993) for six species of Phaeophyta indicated haploid genome sizes range from 430–1550 Mb. These researchers published DNA content estimates of 0·45–1·6 pg using the expression $1 \text{ pg} = 0{\cdot}965 \times 10^9$ (Britten and Davidson, 1971). The currently accepted conversion factor of 1 pg = 980 Mb (Cavalier-Smith, 1985*a*) results in slightly smaller estimates of 0·44–1·58 pg.

Members of the Ectocarpales are notorious for development of polyploid populations, with ‘haploid, diploid and

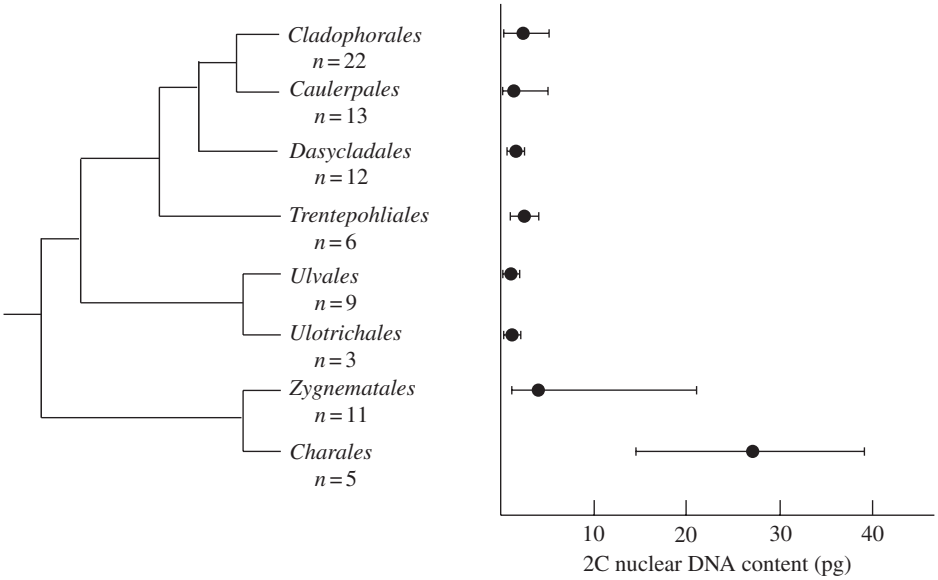

FIG. 10. Mean and range of 2C nuclear DNA contents for species representing eight orders of Chlorophyta included in Appendix I.

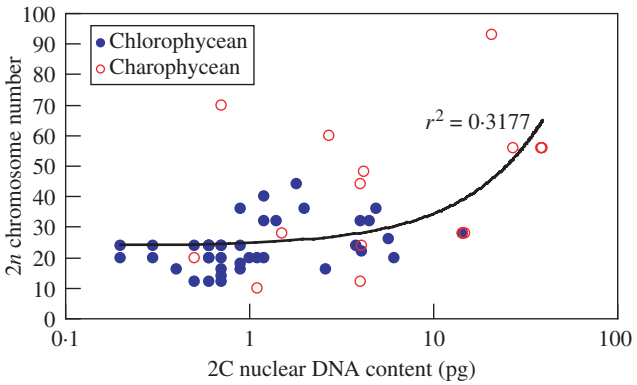

FIG. 11. Comparison of 2*n* chromosome complements and 2C nuclear DNA contents in species of Chlorophyta included in Appendix I.

tetraploid plants connected with each other in a complex system of meiosis, heteroblasty and spontaneous increase in chromosome numbers' (Müller, 1967, 1969, 1970, 1975, 1986). In the present study, the 2C nuclear genome size estimate of 0·50 pg for *Ectocarpus siliculosus* closely approximates previous estimates of 0·54 pg (as 524 Mb; Stache, 1990, 1991) and of 0·52 pg (as 500 Mb) for *Pilayella littoralis* (L.) Kjellman (Le Gall *et al*., 1993).

In the present study, 2C genome size estimates resulting from static microspectrophotometry generally approximate previously published estimates based on flow cytometry (Le Gall *et al*., 1993) for *Laminaria saccharina* and *L. digitata* (Appendix II). Both of these techniques appear to result in larger estimates than obtained by reassociation kinetics (Stam *et al*., 1988). In the present study, large nuclei were observed in older medullary cells of *L. saccharina*. However, these nuclei were too large to be accommodated by the aperture on the microspectrophotometry system, and their I_f could not be measured. Endopolyploid nuclei with DNA levels of 8C or greater have been reported in vegetative tissue of *Laminaria saccharina* and *Alaria esculenta* (Garbary and Clarke, 2002).

Our understanding of the classification and phylogeny of the Phaeophyta has undergone a marked change in the last decade (Peters and Müller, 1986; Peters, 1998; Peters and Clayton, 1998; Rousseau *et al*., 2001; Draisma *et al*, 2001). Traditional phylogenetic interpretations of classifications take progressive complexity and increasingly fixed or obligate life histories as evidence of evolutionary advancement (Siemer *et al*., 1998). In the brown algae, traditional phylogenetic schemes assigned an ancestral or basal position to the Ectocarpales (Papenfuss, 1951; van den Hoek *et al*., 1995) and assumed the Fucales to be the most recent, derived group. Contemporary DNA sequence data reveal a more complex pattern of phylogenetic relationships in the brown algae (Lee *et al*., 2003). Morphological grades of organization, modes of growth and type of life history have evolved and/or have been lost independently and repeatedly. Apparently, the Dictyotales and Sphacelariales are basal while the Ectocarpales (including the Scytosiphonales in Kogame *et al*., 1999) and the morphologically complex Laminariales are the most recent/derived group (Kawai and Sasaki, 2000; Stefano *et al*., 2001). Some refer to the Ectocarpales *sensu lato* as 'simple brown algae', thus avoiding the phylogenetic connotation of 'primitive' (Peters and Burkhardt, 1998). Interestingly, the Fucales occupy a phylogenetic position in the middle of the tree despite their suite of supposedly advanced characteristics including an oogamous, monophasic, diploid life history. It seems noteworthy that taxa included in the expanded circumscription of the order Ectocarpales (Kornmann and Sahling, 1977; Tan and Druehl, 1993; Druehl *et al*., 1997; Siemer *et al*., 1998) are characterized by having both smaller genome sizes and chromosome complements while the Dictyotales, Fucales and Sphacelariales have some of the largest nuclear genome sizes (Fig. 12) and chromosome complements. It has been suggested that algae with a large volume (plant size) at

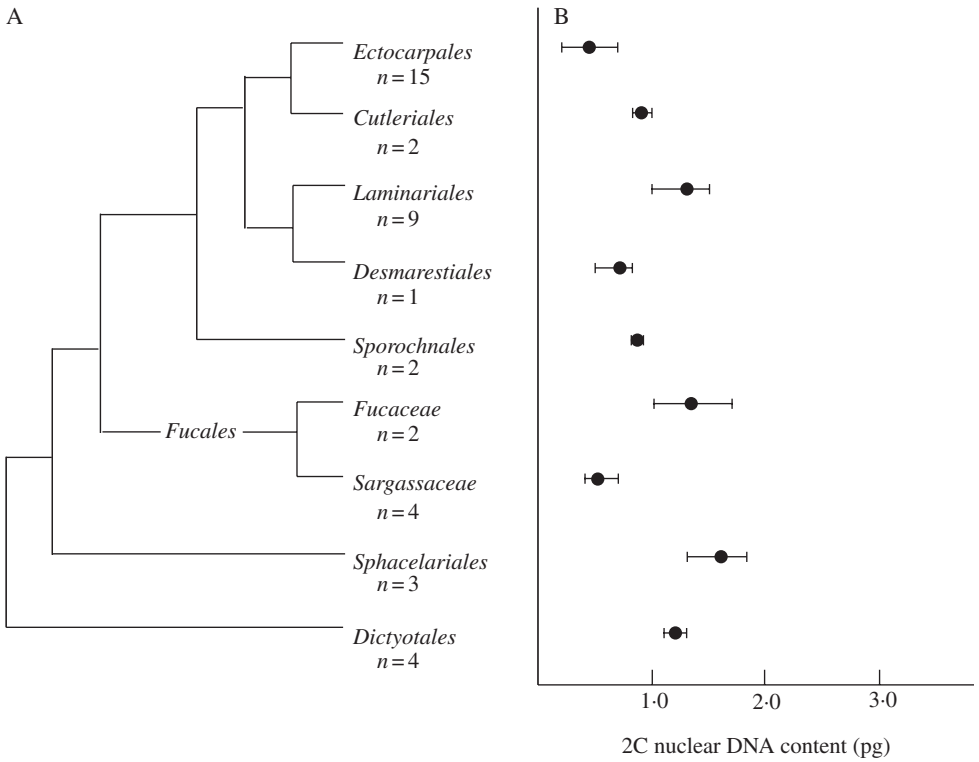

FIG. 12. (A) Molecular phylogeny, and (B) range of 2C nuclear DNA contents in the Phaeophyta. Redrawn from Draisma *et al*. (2001) and Rousseau *et al*. (2001).

maturity usually display anisogamy or oogamy, as expected if larger zygotes permit more rapid growth to these adult sizes (Madsen and Waller, 1983). Assuming a positive correlation between nuclear genome size and cell size, especially of female gametes and eggs, brown algae with larger plants at maturity would tend to have larger nuclear genomes. Present data are consistent with this analysis. Orders that are characterized by oogamy and are reported to have large female gametes (eggs), have the largest nuclear genomes observed regardless of their phylogenetic position (Fig. 12).

The Fucales constitute a large monophyletic order (Rousseau and Reviers, 1999*a*, *b*), and include about 40 of the approximately 265 genera reported in the Phaeophyta (Rousseau *et al*., 1997). The Fucales reportedly evolved and diversified in southern Australia (Clayton, 1988), but are now widely distributed throughout the world (Serrão *et al*., 1999). The order includes six families in two large, well-supported groups: Group I includes the Fucaceae and Hormosieraceae, among others, and Group II the Sargassaceae and Cystoseiraceae, among others (Rousseau and Reviers, 1999*a*, *b*; Rousseau *et al*., 2001). The Fucaceae in Group I have a bipolar distribution, with *Fucus*, *Ascophyllum* and *Pelvetia* being restricted to the North Atlantic and *Hormosira* and *Xiphophora* restricted to the southern hemisphere. Molecular data support a large divergence time between these northern and southern hemisphere taxa (Serrão *et al*., 1999). Unfortunately, there are no published C-value data for any southern hemisphere representatives, and data for only two species from the North Atlantic (Appendix II). It would be a matter of great interest to compare nucleotype data for these two well-circumscribed groups to determine what DNA content transformations may have followed their ancient divergence some 40 million years ago (Serrão *et al*., 1999).

Although most Fucales are restricted to cold-water environments, members of the Group II families, the Sargassaceae and Cystoseiraceae, have primarily tropical and warm temperate distributions (Bold and Wynne, 1985; Saunders and Kraft, 1995). Present data are insufficient to support any conclusions, but there is some indication that cold-water genera *Ascophyllum* and *Fucus* may have larger nuclear genomes than do the warm water genera *Sargassum* and *Turbinaria* (Fig. 12). Unfortunately, no data are available for any species of the Cystoseiraceae, which are other important Group II members.

Most orders of brown algae are reported to have basic chromosome numbers between 8–13 (Cole, 1967) with higher numbers for 1*n* chromosome complements resulting from polyploidy (whole-number multiples of a basic genome) (Lewis, 1996). If polyploidy has played a significant role in the evolution of the brown algae, then ancestral taxa could be expected to share a genome characterized by an ancestral chromosome complement and a 2C genome size. Published chromosome counts are available for 21 species of the brown algae (Lewis, 1996) included in the present study (Appendix II). Comparison of these 1*n* chromosome complements and estimated 2C genome sizes indicates a low correlation (r^2 = 0·2037; data not shown) indicative of significant aneuploidy processes (Kapraun, 1993*c*).

Conclusions and directions for further study

Phaeophyta that warrant further investigation include the Fucales as discussed above, and the Sphacelariales, which have the largest 2C nuclear genomes of all the brown algae investigated. Although this order is cosmopolitan in the world's oceans (Draisma *et al*., 2002), it is of particular interest because of the many species endemic to the Southern Hemisphere. As with the Fucales, geographic disjunction (northern vs. southern taxa) and habitat restriction (cold-water vs. temperate/tropical) almost certainly have resulted in nucleotype transformations.

Present and previous invesitigations permit some generalizations concerning nuclear genomes in the Phaeophyta:

(1) Chromosome numbers range from $1n = 4$–64, with 93 % of the species in the range of $n = 8$–32 (Lewis, 1996), and both polyploidy and aneuploidy events appear to have accompanied speciation in some taxonomic groups.
(2) Estimated 2C nuclear DNA contents range from 0·2–1·8 pg.
(3) G + C ranges from 28·6–49·7 mol % (Le Gall *et al*., 1993).
(4) Reassociation kinetics indentified the presence of highly repetitive, mid-repetitive and unique sequences in species of *Laminaria* (Stam *et al*., 1988).
(5) All of the brown algal orders investigated exhibit considerable variation in both chromosome numbers and nuclear genome sizes. Nuclear genome size and phylogenetic advancement are poorly correlated. However, orders that are characterized by oogamy (or pronounced anisogamy) and are reported to have large female gametes (eggs) have the largest nuclear genomes observed regardless of their phylogenetic position.

RHODOPHYTA

The red algae are predominantly marine organisms with more than 700 genera and 6000 species described in about two dozen orders (Chapman *et al*., 1998). Current classification schemes for the red algae based on molecular data (Freshwater *et al*., 1994; Saunders and Bailey, 1997; Harper and Saunders, 2001*a*, *b*) as well as organelle ultrastructure (Pueschel, 1989; Scott and Broadwater, 1990) recognize two subclasses: Bangiophycidae (which are generally uninucleate) with 3 or 4 orders, and Florideophycidae (which are typically multinucleate) with 14 orders (Woelkerling, 1990). The Bangiophycidae appears to be polyphyletic (Freshwater *et al*., 1994; Müller *et al*., 2001). The Florideophycidae form a monophyletic clade with most orders falling within two clades (Fig. 13) that terminate long branches of basal position and having specific synapomorphic pit plug characteristics. Group I orders have two pit plug cap layers and include the Acrochaetiales, Balbianiales, Balliales, Batrachospermales, Colaconematales, Corallinales, Nemaliales, Palmariales, Rhodogorgonales and Thoreales (Saunders and Bailey, 1997; Harper and Saunders, 2001*a*). Group II orders lack cap layers but possess a cap membrane and include the Bonnemaisoniales, Ceramiales, Gelidiales, Gigartinales, Gracilariales, Halymeniales, Plocamiales and Rhodymeniales (Freshwater *et al*., 1994; Ragan *et al*., 1994; Saunders and Bailey, 1997; Harper and Saunders, 2001*b*). The widely held traditional view that the Acrochaetiales are the most primitive and the Ceramiales the most highly derived of the florideophycidean red algal orders (Kylin, 1956; Dixon, 1973) is not supported by molecular data (Chapman *et al*., 1998). A more complex phylogenetic model is emerging for red algae characterized by ancient lineages often terminating in modern radiations (Saunders and Bailey, 1997).

The Bangiales and Compsopogonales are sister groups in the polyphyletic Bangiophycidae and are basal to, and more ancient than, any of the Florideophycidae (Freshwater *et al*., 1994; Oliveira *et al*., 1995). *Compsopogon coerulus* (Compsopoganales) has a 2C DNA content of 0·25 pg and a reported chromosome complement of $1n = 7 \pm 1$ (Nichols, 1964). Estimates of 2C nuclear DNA contents range from 0·6–1·2 pg for the seven isolates of *Bangia* and *Porphyra* (Bangiales) investigated (Fig. 13). Published chromosome complements for these isolates range from $1n = 3$–5 (Appendix III). These data are consistent with a basal (ancestral) red algal nucleotype characterized both by small genome sizes and small chromosome complements. Comparison of $2n$ chromosome complements with 2C nuclear geonome size estimates for species in the Bangiales indicates a poor correlation consistent with an aneuploid sequence (Kapraun *et al*., 1991). Regional isolates of *Porphyra leucosticta* and *P. spiralis* var. *amplifolia* exhibited different chromosome complements and/or estimates of nuclear DNA contents, underscoring the inherent difficulty in delineating species when substantial genotypic divergence (Lindstrom and Cole, 1992; Dutcher and Kapraun, 1994; Oliveira *et al*., 1995) is masked by narrow phenotypic expression (Kapraun *et al*., 1991; Chapman *et al*., 1998).

DNA amount data are available from five of the ten orders in Group I of the Florideophycidae, but four of these five orders are represented by only one to a few species (Fig. 13). The newly recognized order Colaconematales is apparently one of the more derived in this group (Harper and Saunders, 2002). In the present study, uninucleate vegetative cells of *Colaconema daviesii* were found to have 2C DNA contents of 0·6 pg. The Corallinales appear to be sister to other orders in Group I (Saunders and Bailey, 1997). Members of this order have 2C DNA contents of 0·1–1·3 pg (Appendix III). Coralline red algae can be divided into two types: geniculate (with alternating calcified internodes and uncalcified nodes) and non-geniculate (which usually grow as crusts) (Woelkerling *et al*., 1993). Recently, molecular studies demonstrated that genicula are non-homologous structures that evolved independently in several families (Bailey and Chapman, 1996, 1998). When DNA content data are superimposed on this molecular phylogeny (Fig. 14), it becomes apparent that geniculate clades are represented by species with larger nuclear genomes (0·6–1·3 pg) while non-geniculate clades contain species with relatively small nuclear genomes (0·1–c.0·4 pg). *Neogoniolithon spectabile* and *Titanoderma pustulatum,* non-geniculate species with 2C DNA contents of 0·8 and 1·0 pg, respectively, appear to be the sole exceptions to this generalization among the species investigated. The strong correlation between

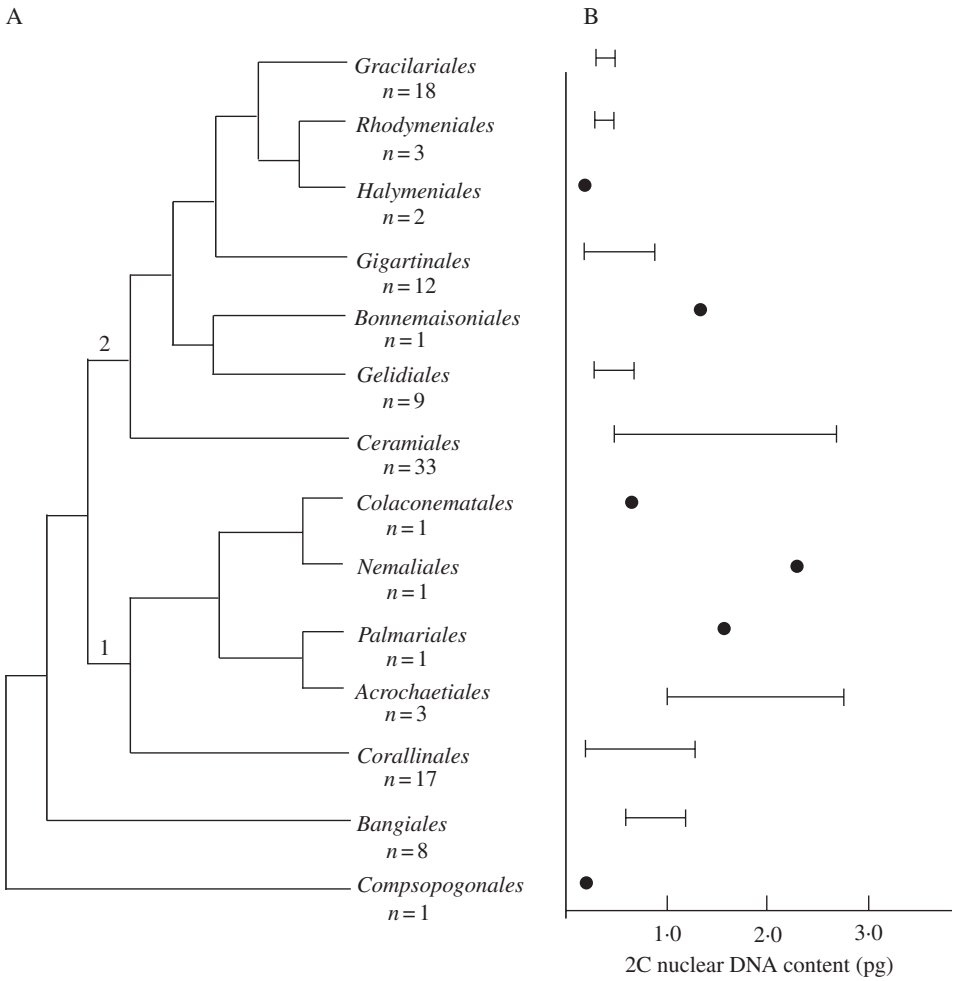

FIG. 13. (A) Combined analysis phylogenetic tree using morphological, ultrastructural and gene sequence data, and (B) range of 2C nuclear DNA contents in the Rhodohyta. Numbering (1 and 2) indicates major clades in the Florideophycidae. Redrawn from Freshwater *et al*. (1994), Saunders and Bailey (1997), de Jong *et al*. (1998), and Harper and Saunders (2001*a*, *b*, 2002).

the geniculate/nongeniculate morphotype and a 'polyploid' nucleotype is remarkable as it implies a significant role for the nucleotype (in addition to a substantial genotype role) in the expression of this morphotype.

Since coralline red algae deposit calcite in their cell walls, they are represented by an extensive fossil record (Wray, 1977). Crustose (non-geniculate) taxa, with a fossil record extending to the mid-Mesozoic and beyond, appear to be more ancient than articulate (geniculate) taxa, which experienced rapid and substantial radiation following the Cretaceous/Tertiary extinction (K/T event) (Adey and Johansen, 1972; Adey and Macintyre, 1973). If available molecular data have been correctly interpreted and geniculate taxa are polyphyletic and arose independently in several families (Bailey and Chapman, 1996, 1998), then multiple polyploidy events must have occurred independently in these several lineages following the K/T event (Fig. 15).

In a previous investigation of coralline green algae (i.e. Dasycladales), which also have an extensive fossil representation, it was noted that some genera experienced similar rapid and expansive speciation following the K/T event some 65 million years ago. Extant species are characterized by nuclear DNA contents that are 2 times the values found in taxa assumed to be ancestral or basal (Kapraun and Buratti, 1998). For the most part, genera with elevated nuclear DNA content values are more species-rich than are genera with smaller (ancestral) genome sizes. Again, we wonder at the constellation of environmental circumstances that appears to have rewarded nuclear genome size increase in a diversity of genotypes in the post-K/T marine environment. The classical explanation for success of diploidy and polyploidy relies on the protection it offers against expression of deleterious mutations, especially those that are particularly harmful (Perrot *et al*., 1991). It is tempting to speculate that the post-K/T marine environment may have included elevated UV radiation levels leading to increased DNA hazards.

Group II of the Florideophycidae is characterized by a nuclear 2C DNA content range of 0·2–2·8 pg (Appendix III). Five of these Orders (Gelidiales, Gigartinales, Gracilariales, Halymeniales and Rhodymeniales) have particularly

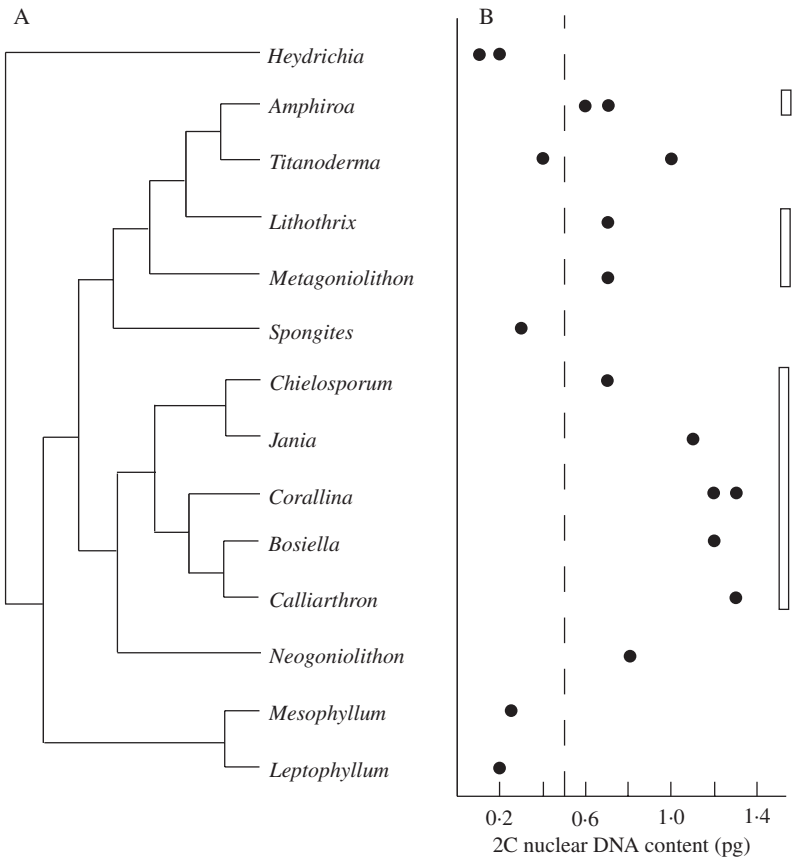

FIG. 14. (A) Molecular phylogeny, and (B) estimated 2C nuclear DNA contents and of the coralline red algae. Geniculate genera are indicated by open vertical bars. Note that genicula are non-homologous structures that evolved independently in multiple clades. Geniculate taxa are characterized by larger nuclear genomes (>0·6 pg) and crustose taxa are characterized by smaller (<0·6 pg) with the exception of *Neogoniolithon* and *Titanoderma*. Phylogeny redrawn from Bailey and Chapman (1998). DNA content data from J. C. Bailey and D. F. Kapraun (unpubl. res.).

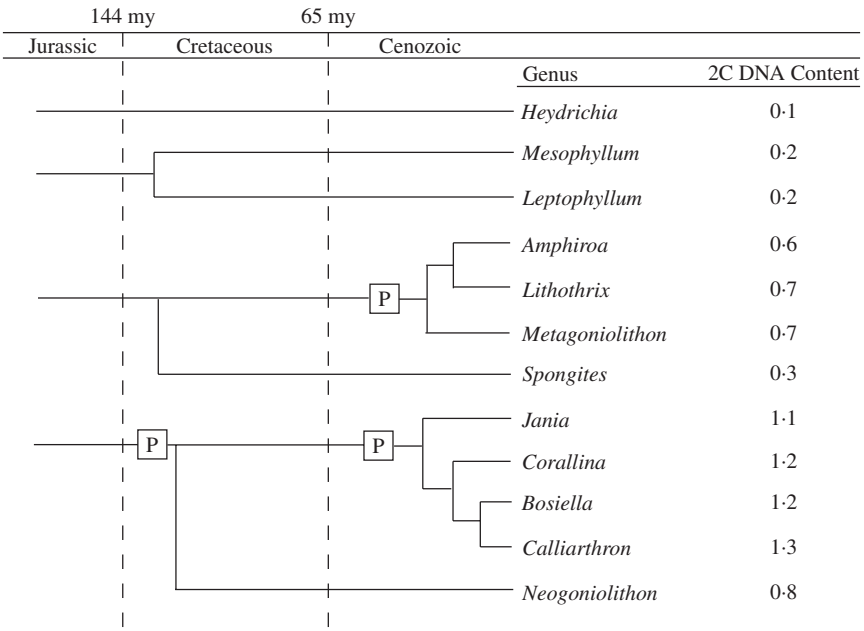

FIG. 15. Phylogeny for some Corallinales based on the fossil record (redrawn from Wray, 1977) and inferred from 18S rRNA gene sequence analysis (Bailey and Chapman, 1998; Bailey, 1999). Nuclear genome size estimates from J. C. Bailey and D. F. Kapraun (unpubl. res.). Proposed polyploidy events are indicated by [P]. Vertical dashed lines indicate 70 my (million year) intervals. Note proposed polyploidy events and subsequent radiation at the K/T boundary.

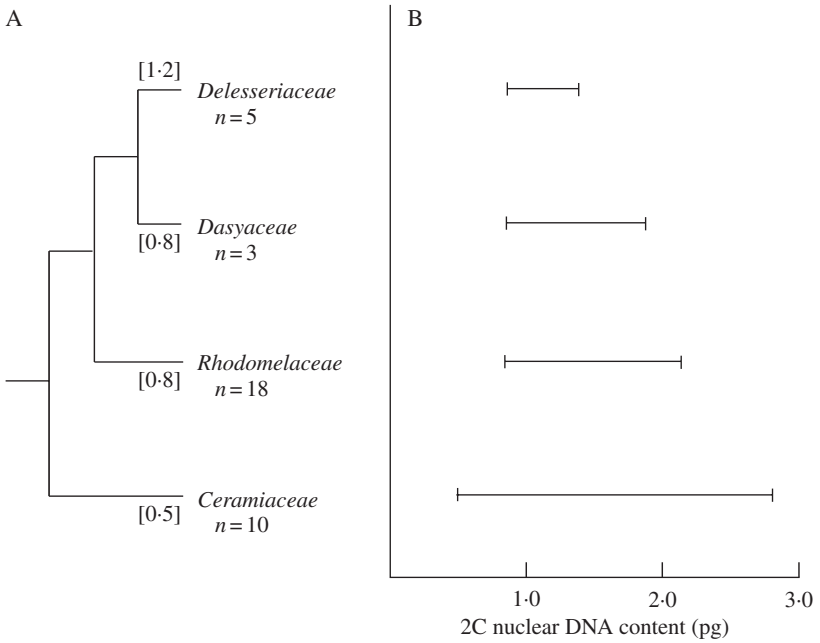

FIG. 16. (A) Molecular phylogeny, (B) and range of 2C nuclear DNA contents in the Ceramiales. Redrawn from de Jong *et al*. (1998), Phillips (2000) and Zuccarello *et al*. (2002). The figure in square brackets [ ] represents smallest DNA content (pg) observed in each family. *n* = number of species and isolates represented by data.

narrow ranges of DNA contents (data not shown). In the Gelidiales, the relatively narrow range of small DNA content values but substantial range of chromosome numbers (Appendix III), and the absence of a correlation between nuclear genome size and chromosome number suggests a significant role of aneuploidy in their evolution (Kapraun and Dunwoody, 2002). Analyses of rbcL and LSU gene sequence data have resulted in a molecular phylogeny for the Gelidiales (Freshwater and Bailey, 1998; Thomas and Freshwater, 2001). This well-circumscribed order includes only a handful of genera, but is particularly species-rich (Thomas and Freshwater, 2001). It would be a matter of great interest to obtain nucleotype data for additional representative species of this economically important group of agarophytes to determine the possible role of aneuploidy in their evolution.

The order Gracilariales, like the Gelidiales, includes just a handful of genera, but some of them, e.g. *Gracilaria*, are species-rich (Fredericq and Hommersand, 1990). Unlike the Gelidiales, the Gracilariales are noted for nucleotype constancy, with all species of *Gracilaria* investigated having identical 2C DNA contents of 0·4 pg and chromosome complements of $2n = 48$ (Kapraun and Dutcher, 1991; Kapraun, 1993*a*). Species of the closely related *Gracilariopsis* (Bird *et al*., 1994: Bellorin *et al*., 2002; Gurgel *et al*., 2003) similarly have constant 2C DNA contents (0·4 pg) and $2n$ chromosome complements ($2n = 64$).

The Gigartinales is a large and diverse order (Fredericq *et al*., 1996; Hommersand *et al*., 1999; Tai *et al*., 2001) including commercially important carrageenophytes such as *Eucheuma* and *Kappaphycus* (Craigie, 1990). Members of this order are characterized by a wide range of chromosome complements ($2n$ = 10–70) and a narrow range of small nuclear DNA contents (2C = 0·2–0·9 pg) (Appendix III).

The Ceramiales is the largest red algal order, with more than 325 genera and 1500 species described (Kraft and Woelkerling, 1990). Nucleotype data are available for fewer than 2 % of these species (Appendix III). Members of this order have both the largest DNA contents and the greatest range of DNA content values (0·5–2·8 pg). Recent molecular systematics investigations indicate that three families (Dasyaceae, Delesseriaceae and Rhodomelaceae) have evolved from the paraphyletic Ceramiaceae (de Jong *et al*., 1998; Phillips, 2000; Lin *et al*., 2001; Choi *et al*., 2002). Consistent with the molecular phylogeny, the smallest DNA value (2C = 0·5 pg) was found in *Ceramium* (Fig. 16). When nuclear DNA content data are superimposed on a consensus molecular phylogeny for the order, each family is seen to have at least one (ancestral?) species with a 2C DNA content of 0·8–1·2 pg as well as species with elevated (polyploid?) DNA contents (Fig. 16). The simplest explanation is that polyploidy, characterized by even-number multiple increase in chromosome complements as well as increase in nuclear genome size, accompanied speciation in each of these lineages. A strong correlation between chromosome complements and nuclear genome size in many Ceramiales investigated is consistent with this explanation. Conspicuous exceptions include *Acanthophora spicifera* with $2n = 64$ and 2C = 1·1 pg, and *Antithamnion villosum* with $2n = 48$ and 2C = 2·0 pg. Clearly, in some genera, polyploidy events were followed by chromosome reorganization, including fission/fusion processes ultimately resulting in aneuploidy (Fig. 17) as described for species of *Polysiphonia* (Kapraun, 1993*a*).

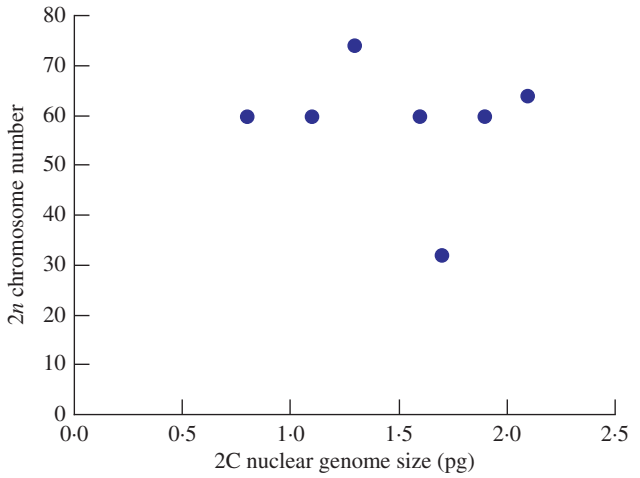

FIG. 17. Comparison of 2C DNA contents and 2*n* chromosome numbers for seven species and isolates of *Polysiphonia* (Ceramiales). Data from Kapraun (1979, 1993*b*) and Kapraun and Dunwoody (2002).

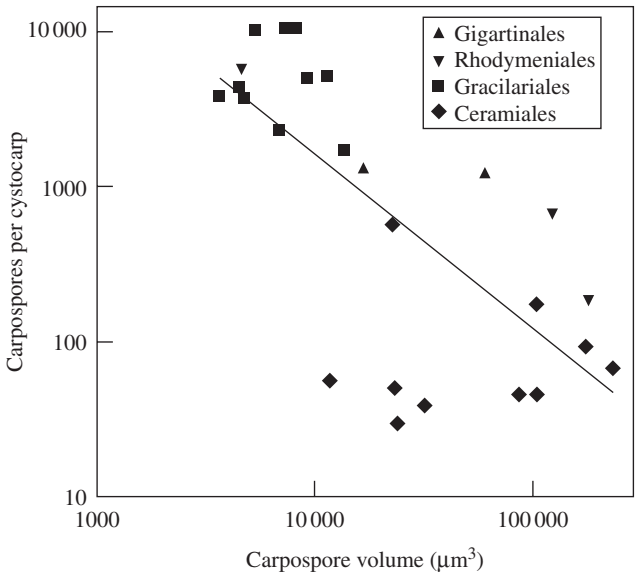

FIG. 18. Negative correlation of carpospore volume with the number of carpospores per cystocarp for four orders of Florideophycidae. Regression analysis of data for all orders, $r^2 = -0{\cdot}512$. Without data for the Ceramiales, $r^2 = 0{\cdot}719$.

The Ceramiales appear to be a basal and ancient lineage relative to other Group II Florideophicidae (Saunders and Bailey, 1997), yet on average have larger nuclear genome contents (2C = 3·5 pg) than do most of the taxa that are believed to have diverged after them (Fig. 13). Unless an assumption is made that the other taxa in the Florideophycean lineage have experienced nuclear genome size decrease, an explanation is required to account for the larger genome sizes in the Ceramiales. Two explanations seem worth considering: (1) a mechanistic model; and (2) an ecological model.

Although the existence of mechanisms for decreasing DNA amounts has been proposed (Wendel *et al*., 2002), it is more probable that polyploidy and transposable element amplification will result in genome size increase through time (Bennetzen, 2002), ultimately resulting in genomic 'obesity' (Bennetzen and Kellogg, 1997). Since the Ceramiales are arguably the oldest members of the Group II Florideophycean lineage, they would have accumulated the largest genomes and may have been subject to a predictable genomic expansion. Although data are severely limited, there appears to be a correlation between antiquity of these red algal lineages and their mean nuclear DNA contents (Fig. 13).

An ecological model suggests that the role of selective forces can be a significant factor in effecting genome size transformations. It can be argued that the single-cell stage is the most vulnerable period in any multicellular organism's life history. This is especially applicable for red algae, which uniquely lack flagellated (motile) cells in their life history. If the non-motile tetraspore and carpospore do not survive, the life history is not completed (Searles, 1980). The survivability of the single cell may depend, in part, on nuclear genome size (DNA content) (Destombe *et al*., 1992) because of its correlation with cell size (Swanson *et al*., 1991), nuclear volume, and cell cycle length (Price, 1988). The implication is that cell (spore) size may be indirectly adaptive. For example, larger spore size could reduce predation by zooplankton, promote rapid settlement, and accommodate greater energy reserves for increased initial growth after germination. But selective forces may be more directly related to genome size, such that cellular DNA content results from a compromise between two conflicting forces: smaller genomes increasing cellular growth rates and larger genomes increasing cell size (Parker *et al*., 1972).

Where there is a high degree of competition and fewer resources, larger cell sizes and slower rates of development are favoured. These parameters coincide with larger and smaller genomes, respectively, in both plants and animals (Grime and Mowforth, 1982; Price, 1988). The corresponding selection types have been designated *K* and *r*, where *K*-selection favours slower development, delayed reproduction, larger body size, and longer life span, and *r*-selection favours rapid development, high population growth rate, early reproduction, small body size, and short life span (Begon *et al*., 1990). On the basis of developmental rates, body size, and life span (cell longevity), *K*-selected species would tend to have larger reproductive cells in smaller numbers and *r*-selected species would tend to have smaller reproductive cells produced in large quantities (Madsen and Waller, 1983).

In a previous investigation of the relationship of nuclear genome size to reproductive cell parameters in the Rhodophyta (Kapraun and Dunwoody, 2002), three general trends regarding carpospore production were noted: (1) increase in genome size is positively correlated with increase in carpospore volume; (2) species with larger genome sizes produce fewer carpospores; and (3) species that produce larger carpospores produce fewer carpospores. Members of the Ceramiales, with their larger genome sizes, typically produce fewer, but larger carpospores and generally behave as predicted in a *K*-selection model (Fig. 18). In contrast, members of the Gelidiales, Gigartinales and Gracilariales, with their smaller genome sizes, typically produce large numbers of small carpospores as predicted

in an *r*-selected model (Kapraun and Dunwoody, 2002). The conspicuous limitation of this ecological model is that the Ceramiales generally produce small, structurally simple, short-lived plants (associated with *r*-selection), while the other orders generally produce large, structurally complex, long-lived plants (associated with *K*-selection).

Conclusions and directions for further study

Members of Group I red algae that warrant further investigation include the Nemaliales, Acrochaetiales and Colaconematales. These three orders are among the oldest of the florideophycean algae, are widely distributed, and contain many genera that are species-rich (Saunders *et al*., 1995; Harper and Saunders, 1998), yet published information for their nucleotypes is very limited. It is to be wondered if the relatively large DNA content of 2·3 pg in *Galaxaura* is representative of the Nemaliales.

A second group of red algae that warrant our attention is the Ceramiales. Continuing molecular phylogenetic investigations provide us with evolutionary schemes (de Jong *et al*., 1998; Phillips, 2000; Lin *et al*., 2001; Choi *et al*., 2002) upon which nucleotype data can be superimposed to reveal the extent that speciation was accompanied by nuclear transformations.

Present and previous invesitigations permit some generalizations concerning nuclear genomes in the Rhodophyta:

(1) Chromosome numbers range from $1n$ = 2–68 (−72) (Cole, 1990), and both polyploidy and aneuploidy events appear to have accompanied speciation in some taxonomic groups.
(2) Estimated 2C nuclear DNA contents range from 0·22–2·85 pg.
(3) G + C ranges from 28·6–49·7 mol % (Kapraun *et al*., 1993*b*; Le Gall *et al*., 1993).
(4) Reassociation kinetics indentified the presence of highly repetitive, mid-repetitive and unique sequences in species of Gracilariales and Gelidiales investigated (Kapraun *et al*., 1993*a*).
(5) Some red algal taxa such as the Gracilariales were found to have remarkably constant chromosome numbers and nuclear genome sizes, while other taxa such as species of the Gelidiales have considerable variation in both chromosome complements and karyotype patterns, and in nuclear genome sizes. Members of the Ceramiales are characterized by having both the largest nuclear genomes and the largest chromosome complements (Fig. 19).

GENERAL SUMMARY

Nuclear DNA content estimates for the Rhodophyta (2C = 0·2–2·8 pg), Chlorophyta (0·2–6·1 pg) and the Phaeophyta (2C = 0·2–1·8 pg) approximate an order of magnitude. DNA contents in the freshwater charophycean orders Charales and Zygnematales are significantly larger (39·2 and 20·7 pg, respectively). The size of these algal genomes is best appreciated when compared with the minimum amount of DNA estimated for specifying the mRNA sequences

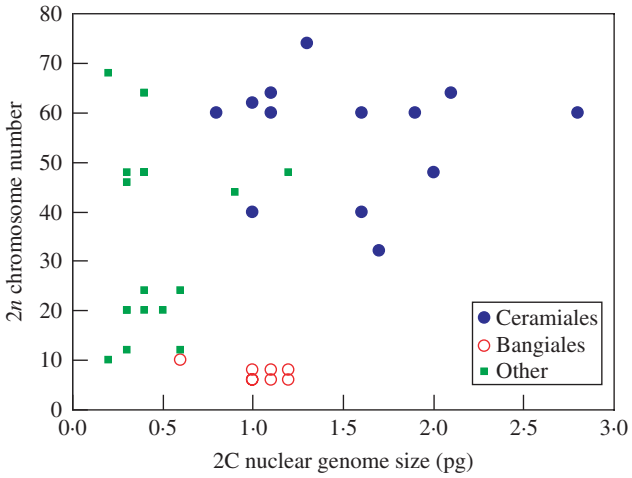

FIG. 19. Comparison of $2n$ chromosome complements and 2C nuclear DNA contents in the Rhodophyta. Data taken from Appendix III.

required for angiosperm development. Specifically, the genome of *Arabidopsis thaliana* (L.) Heynhold, with 0·16 pg = 157 Mb (Bennett *et al*., 2003) is one of the smallest found in angiosperms (Bennett and Smith, 1976) but still has 30 000 or twice the estimated 15 000 genes per haploid genome required for development (Flavell, 1980). Correlation between genome size and phylogeny is indicated in some of the algal groups investigated. Genome size appears to correspond more closely to specific reproductive and developmental parameters in all three algal groups.

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NOTES ON APPENDIXES I–III. CHROMOSOME NUMBERS AND NUCLEAR DNA CONTENT ESTIMATES IN SPECIES OF MACROSCOPIC ALGAE

(a) Orders are listed alphabetically. In all three major groups of algae, insights from continuing molecular phylogeny investigations impact on our understanding of the delineation and composition of taxa at all levels: orders, family and genus. An attempt has been made to assign genera to currently recognized families, but on-going molecular investigations have demonstrated that many of these families are not natural assemblages. Synonyms are provided in cases where chromosome complements and/or nuclear DNA content estimates were originally published under different genus and/or species epithets. Footnotes are

provided in the Appendixes for some of these examples. References within these footnotes are included in the general Literature Cited. References within the tables themselves are listed in a key below each individual Appendix.

(b) Most comprehensive lists of chromosome numbers have been published as haploid (1*n*) values for the Chlorophyta (Kapraun, 1993), Phaeophyta (Lewis, 1996) and the Rhodophyta (Cole, 1990). In the Appendixes, chromosome numbers are extrapolated from 1*n* numbers (and ranges of probable 1*n* numbers).

(c) Since most DNA amounts in the literature are given in picograms (pg), unless otherwise indicated Mbp values in the Appendixes are derived, using the expression 1 pg = 980 Mbp (Cavalier-Smith, 1985*a*; Bennett *et al*., 2000). DNA amounts originally published as megabase pairs (Mbp) are indicated with a dagger ([†]). These values were derived from reassociation kinetics (Olsen *et al*., 1987; Stam *et al*., 1988; Bot *et al*., 1989*a*, *b*, 1990, 1991; Kooistra *et al*., 1992;), with the sole exception of LeGall *et al*. (1993) who used ethidium bromide (Hoechst 33342) and mithramycin A, two fluorochromes specific for the bases A–T and G–C, respectively, with RBC standard and flow cytometry.

(d) Algal life histories typically are characterized by an alternation of haploid gametophyte and diploid sporophyte generations (Kapraun, 1993*c*; Kapraun and Dunwoody, 2002). Thus, DNA content (pg) measurements could be based on either or both 2C replicated haploid nuclei or 4C replicated diploid nuclei. In practice, most published DNA content (pg) values are for 2C diploid nuclei and most 1C and 4C values are extrapolated. In the Appendixes, the original published DNA content (pg) value for each species is indicated with an asterisk (*). In some samples, available specimens were not reproductive and ploidy level could not be determined with certainty. Assignment of DNA content to specific C-level for these isolates is speculative ([1]).

(e) Previously unpublished data are indicated as (unp). Information for collection locations, and data for number of algal nuclei examined in each sample and estimates of nuclear genome size (pg) ± s.d. are available at http://www.uncw.edu/people/kapraund/DNA. Nuclear DNA content estimates for members of the Desmidiales and Zygnematales are taken from Honors investigations by William Purvis and Mickie Marlowe.

(f) Standard species. The vast majority of nuclear DNA estimates for algae have used chicken red blood cells or erythrocytes (RBC) for a DNA standard and the published value of 2·4 pg accepted for the 4C DNA content of *Gallus gallus* (Clowes *et al*., 1983; Riechmann *et al*., 2000). Limitations of RBC as a standard for plant material has been discussed elsewhere (Johnston *et al*., 1999; Bennett *et al*., 2000). Mouse (*Mus*) sperm was used as a standard by Hamada *et al*. (1985), the fish *Betta splendens* was used as a standard by Spring *et al*. (1978) and *Allium cepa* was used by Maszewski and Kolodziejczyk (1991). Initial investigations in our laboratory utilized a standard line based on the fluorescence intensity of an alga with a known DNA content and an angiosperm: *Antirrhinum majus* L. (e.g. Kapraun and Shipley, 1990; Hinson and Kapraun, 1992; Kapraun and Bailey, 1992) or *Impatiens balsamina* L. (e.g. Kapraun and Shipley, 1990). Species used as a calibration standard for published algal nuclear DNA content estimates are listed in Table 1.

TABLE 1. *Species used as a callibration standard in Appendixes I, II and III*

Species	Reported 4C DNA content (pg)	Reference	Abbreviation used in Column 8 of Appendixes
Antirrhinum majus L.	3·2	Bennett and Smith, 1976; Kapraun and Shipley, 1990	Ant
Betta splendens	1·3	Shapiro, 1976; Spring *et al*., 1978	Betta
Gallus gallus	2·4	Clowes *et al*., 1983; Riechmann *et al*., 2000	Gallus
Impatiens balsamina L.	4·7	Bennett and Smith, 1976; Kapraun and Shipley, 1990	Imp
Mus	5	Shapiro, 1976; Hamada *et al*., 1985	Mus
Allium cepa	67	Maszewski and Kolodziejczyk, 1991; Bennett *et al*., 2000	Allium

(g) Methods. Both flow cytometry (FC) (Le Gall *et al*., 1993) and static cytometry or microspectrophotometry (MI) (Kapraun 1994; Kapraun and Buratti, 1998) have been shown to be reliable methods for quantification of nuclear DNA contents in green algae. Feulgen microdensitometry (Fe) was used by Maszewski and Kolodziejczyk (1991). Reassociation kinetics (RK) has been used successfully as well (Bot *et al*., 1989*a*, *b*, 1990, 1991; Kooistra *et al*., 1992; Olsen *et al*., 1987).

Several DNA-localizing fluorochromes have been used in published investigations. DAPI (4′, 6-diamidino-2-phenylindole) is certainly the most popular, especially in recent studies (Kapraun 1994; Kapraun and Buratti, 1998). Hydroethidine (H) (Kapraun and Bailey, 1992), ethidium bromide (EB) and mithramycin (Kapraun *et al*., 1988; Le Gall *et al*., 1993) and propidium iodide (PI) (Spring *et al*., 1978) were used in selected green algal investigations.

Recently, the Angiosperm Genome Size Workshop (Bennett *et al*., 2000) identified 'best practice' methodology for nuclear genome size estimation in plant tissues (for details and recommendations, see http://www.rbgkew.org.uk/cval/conference.html under Angiosperm Genome Size Discussion Meeting). Virtually none of the published genome size data for algae resulted from investigations adhering to all of the best practice recommendations. Even in cases where the preferred methodology of Feulgen microdensitometry was employed, researchers typically used animal (RBC) rather than plant (*Allium* or *Pisum*) standards. Consequently, all present and previously published data included in these Appendices should be considered accurate only to ±0·1 pg (Kapraun and Shipley, 1990; Hinson and Kapraun, 1991; Kapraun and Dutcher, 1991; Kapraun and Bailey, 1992).

APPENDIX I. CHROMOSOME NUMBER AND NUCLEAR DNA CONTENT IN SPECIES OF CHLOROPHYTA

A key to the references appears at the end of this Appendix.

Entry number	Species[a]	2*n*[b]	Original ref. for 2*n*	DNA amount				Original ref. for C-value[e]	Standard species[f]	Method[g]
				1C (Mbp)[c]	1C (pg)[d]	2C (pg)[d]	4C (pg)[d]			
	CAULERPALES									
	Bryopsidaceae									
1	*Bryopsis hypnoides* Lamouroux	20	25	490	0·5	1·0*	2·0	25	*Imp.*	MI:H
2	*Bryopsis pennata* Lamouroux	20	25	343	0·4	0·7*	1·4	25	*Imp.*	MI:H
3	*Bryopsis plumosa* (Hudson) C. Agardh	20	25	343	0·4	0·8*	1·6	unp	*Gallus*	MI:DAPI
4	*Derbesia marina* (Lyngbye) Solier	16	33	197	0·2	0·4	0·9*	unp	*Gallus*	MI:DAPI
5	*Derbesia tenuissima* (De Notaris) Crouan frat.	16	33	197	0·2	0·4	0·8*	unp	*Gallus*	MI:DAPI
6	*Ostrobium queketii* Bornet et Flahault				0·2	0·4	0·9*	unp	*Gallus*	MI:DAPI
7	*Pedobesia lamourouxii* (J. Agardh) Feldmann, Loseau, Codomier et Couté			196	0·2	0·4*	0·8	17	*Gallus*	MI:DAPI
8	*Trichosolen duchessangii* (J. Agardh) W. R. Taylor Caulerpaceae			98	0·1	0·2*	0·4	17	*Gallus*	MI:DAPI
9	*Caulerpa mexicana* Sonders *ex* Kützing			98	0·1	0·2*	0·4	17	*Gallus*	MI:DAPI
10	*Caulerpa paspaloides* (Bory) Greville			88	0·1	0·2*	0·4	17	*Gallus*	MI:DAPI
11	*Caulerpa prolifera* (Forsskål) Lamouroux			147	0·1	0·3*	0·6*	17	*Gallus*	MI:DAPI
12	*Caulerpa verticillata* J. Agardh Codiaceae			98	0·1	0·2*	0·4	17	*Gallus*	MI:DAPI
13	*Codium arabicum* Kützing			490	0·5	1·0*	2·0	26	*Gallus*	MI:DAPI
14	*Codium carolinianum* Searles			2695	2·7	5·5*	11·0	26	*Gallus*	MI:DAPI
15	*Codium decorticatum* (Woodward) Howe	20	26	588	0·6	1·2*	2·4*	26	*Gallus*	MI:DAPI
16	*Codium fragile* subsp. *tomentosoides (van Goor) P. C. Silva* Udotaceae	20	23	3430	3·5*	6·1*	14·2*	26	*Gallus*	MI:DAPI
17	*Halimeda macrophysa* Askanasy			1470	1·5*	3·1*	6·4*	17	*Gallus*	MI:DAPI
	CHARALES									
	Characeae									
18	*Chara aspera* Detharding *ex* Willdenow	28	32	7056	7·2*	14·4	28·8	32	*Allium*	Fe
19	*Chara contraria* Kützing	56	32	19208	19·6*	39·2	78·4	32	*Allium*	Fe
20	*Chara fragilis* Desvaux	56	32	18914	19·3*	38·6	77·2	32	*Allium*	Fe
21	*Chara tomentosa* Linnaeus	28	32	7252	7·4*	14·8	29·6	32	*Allium*	Fe
22	*Chara vulgaris* Linnaeus	56	32	13230	13·5*	27·0	54·0	32	*Allium*	Fe
	CLADOPHORALES/SIPHONOCLADALES COMPLEX[1]									
23	*Anadyomene stellata* (Wulfen) C. Agardh	16	10	1470	1·5*	[(1)]2·6*	5·2	24	*Gallus*	MI:DAPI
24	*Boergesenia forbesii* (Harvey) J. Feldmann	36	37	2646	2·7*	4·9*	9·5*	24	*Gallus*	MI:DAPI
25	*Boodlea composita* (Harvey) Brand	22–24	2	1862	1·9	[(1)]3·8*	7·6	24	*Gallus*	MI:DAPI
26	*Chaetomorpha aerea* (Dillwyn) Kützing	24	12	98	0·1	0·2*	0·4	12	*Ant.*	MI:H
27	*Chaetomorpha antennina* (Bory) Kützing	24	12	245	0·3	0·5*	1·0	12	*Ant.*	MI:H
28	*Chaetomorpha brachygona* Harvey	24	12	147	0·1	0·3*	0·6	12	*Ant.*	MI:H
29	*Chaetomorpha gracilis* Kützing					1·4	2·8*	unp	*Gallus*	MI:DAPI
30	*Chaetomorpha melagonium* (Weber *et* Mohr) Kützing	24	2	284	0·3	0·6*	1·2	12	*Ant.*	MI:H
31a	*Cladophora albida* (Hudson) Kützing	24	21	372	0·4	0·7–0·8*	1·6	4		RK
31b	*C. albida*			345	0·4	0·7	1·4*	12	*Ant.*	MI:H
32	*Cladophora laetevirens* (Dillwyn) Kützing	24	22	294	0·3*	0·6	1·2	5		RK
33	*Cladophora pellucida* (Hudson) Kützing			490	0·5*	1·0	2·0	6		RK
34	*Cladophora rupestres (*L.) Kützing	24	42	294	0·3	0·6	1·2	3		RK

APPENDIX I. (*continued*)

Entry number	Species[a]	2*n*[b]	Original ref. for 2*n*	DNA amount 1C (Mbp)[c]	1C (pg)[d]	2C (pg)[d]	4C (pg)[d]	Original ref. for C-value[e]	Standard species[f]	Method[g]
35	*Cladophora prolifera* (Roth) Kützing			1127	1·2	2·4	4·8	unp	*Gallus*	MI:DAPI
36	*Cladophora sericea* (Hudson) Kützing	24	42	294	0·3*	0·6	1·2	3		RK
37	*Cladophora vagabunda* (L.) van den Hoek	24	21	392	0·4 *	0·9	1·8	5		RK
38	*Cladophoropsis macromeres* W. R. Taylor	32	24	421	2·0	4·0*	8·4	24	*Gallus*	MI:DAPI
39a	*Cladophoropsis membranacea* (C. Agardh) Børgesen	32	24	1960	2·1*	4·5*	9·0*	24	*Gallus*	MI:DAPI
39b	*C. membranacea*			933†	1·0*	2·0	4·0	29		RK
40a	*Dictyosphaeria cavernosa* Børgesen			1127	1·2	2·3*	4·3*	34	*Gallus*	MI:DAPI
40b	*D. cavernosa*			1764	1·8*	3·6	7·2	32		RK
41	*Dictyosphaeria ocellata* (Howe) Olsen-Stojkovich			2524	2·6	(1)5·1	10·3*	24	*Gallus*	MI:DAPI
42	*Microdictyon marinum* (Børgesen) P. C. Silva			1960	2·0*	3·8*	8·6*	24	*Gallus*	MI:DAPI
43	*Siphonocladus tropicus* (P. Crouan *et* H. Crouan *ex Maze et Schramm) J. Agardh*			1078	1·1*	2·0 *	4·4*	24	*Gallus*	MI:DAPI
44	*Valonia macrophysa* Kützing			2450	2·5	(1)5·1	10·2*	24	*Gallus*	MI:DAPI
45	*Valonia utricularis* (Roth) C. Agardh	22–28	1	2793	2·9	(1)5·7	11·4*	24	*Gallus*	MI:DAPI
46	*Valonia ventricosa* (J. Agardh) Olsen *et* West			2058	2·1	(1)4·2	8·4*	24	*Gallus*	MI:DAPI
	DASYCLADALES[2]									
	Dasycladaceae									
47	*Batophora oerstedii* J. Agardh	32	36	686	0·7	1·4*	2·8	19	*Gallus*	MI:DAPI
48	*Bornetella nitida* (Harvey) Munier-Chalmas			588	0·6	1·2*	2·4	19	*Gallus*	MI:DAPI
49	*Bornetella sphaerica* (Zanardini) Solms-Laubach			588	0·6	1·2*	2·4	19	*Gallus*	MI:DAPI
50	*Chlorocladus australaxicus Sonder*			588	0·6	1·2*	2·4	unp	*Gallus*	MI:DAPI
51	*Cymopolia barbata* (L.) Lamouroux	14	41	343	0·4	0·7*	1·4	19	*Gallus*	MI:DAPI
52	*Halicoryne wrightii* Harvey			931	1·0	1·9*	3·8	19	*Gallus*	MI:DAPI
53	*Neomeris annulata* Dickie			588	0·6	1·2*	2·4	19	*Gallus*	MI:DAPI
54	*Neomeris van bosseae* Howe (= Acetabulariaceae)	40	16	588	0·6	1·2*	2·4	19	*Gallus*	MI:DAPI
55	*Acetabularia acetabulum* (L.) P. C. Silva [as A. mediterranea Lamouroux]	40–48	38	882	0·9	1·8*	3·6	37	*Betta*	FC:PI
56a	*Acetabularia crenulata* Lamouroux			882	0·9	1·8*	3·6	19	*Gallus*	MI:DAPI
56b	*Acetabularia crenulata*			784	0·8	1·7*	3·4	unp	*Gallus*	MI:DAPI
57	*Acetabularia dentata* Solms-Laubach			784	0·8	1·6*	3·2	19	*Gallus*	MI:DAPI
58	*Acetabularia major* Martens			1176	1·2	2·4*	4·8	19	*Gallus*	MI:DAPI
59	*Acicularia schenckii* (Mobius) Solms-Laubach			1764	1·8	3·7*	7·4	unp	*Gallus*	MI:DAPI
60	*Polyphysa clavata* (Yamada) Schnetter et Bula-Meyer			490	0·5	1·0*	2·0	19	*Gallus*	MI:DAPI
61	*Polyphysa parvula* (Solms-Lauback) Schnetter *et* Bula-Meyer [as Acetabularia moebii Solms-Laubach]	36	16	441	0·5	0·9*	1·8	19	*Gallus*	MI:DAPI
62	*Polyphysa peniculus* (R. Brown *ex* Turner) C. Agardh			1274	1·3	2·7*	5·4	unp	*Gallus*	MI:DAPI
	DESMIDIALES[3]									
	Closteriaceae									
63	*Closterium ehrenbergii* Meneghini *ex* Ralfs	60	11	1323	1·4*	2·7*	5·4	11	*Mus*	MI:DAPI
	Desmidiaceae									
64	*Cosmarium cucumis* Corda	44	28	1960	2·0	4·0*	8·0	unp	*Gallus*	MI:DAPI
65	*Cosmarium subcostatum* Nordstedt	10	9	539	0·6	1·1*	2·2	unp	*Gallus*	MI:DAPI
66	*Desmidium swartzii* (C. Agardh) C. Agardh *ex* Ralfs	28	28	735	0·8	1·5*	3·0	unp	*Gallus*	MI:DAPI
67	*Micrasterias americana* Ehrenberg *ex* Ralfs	93	7	10143	10·4(1)	20·7*	41·4	unp	*Gallus*	MI:DAPI
68	*Micrasterias rotata* Ralfs	160	27	1470	1·5	3·0*	5·3*	unp	*Gallus*	MI:DAPI

	TRENTEPOHLIALES									
	Trentepohliaceae									
69	*Cephaleuros parasiticus* Karsten			1911	2·0	3·9*	7·2	31	*Gallus*	MI:DAPI
70	*Cephaleuros virescens* Kunze in Fries	36	14	980	1·0	2·0*	4·0	31	*Gallus*	MI:DAPI
71	*Physolinum monile* (De Wildeman) Printz	22	8	2009	2·1	4·1*	8·2	31	*Gallus*	MI:DAPI
72	*Trentepohlia arborum* (Agardh) Hariot			1470	1·5	3·0*	6·0	31	*Gallus*	MI:DAPI
73	*Trentepohlia aurea* (L.) Martens	32,34	39	588	0·6	1·2*	2·4	31	*Gallus*	MI:DAPI
74	*Trentepohlia odorata* (Wiggers) Wittrock			539	0·6	1·1*	2·2	31	*Gallus*	MI:DAPI
	ULOTRICHALES[4]									
	Acrosiphoniaceae									
75	*Spongomorpha arcta* (Dillwyn) Kützing	12		304	0·3	0·6*	1·2	unp	*Gallus*	
	Monostromaceae									
76	*Monostroma grevillei* (Thuret) Wittrock	12		255	0·3	0·5*	1·0	unp	*Gallus*	
	Ulotrichaceae									
77	*Ulothrix flacca* (Dillwyn) Thuret *in* Le Jolis	18		441	0·5	0·9*	1·8	unp	*Gallus*	
	ULVALES[5]									
	Incertae sedis									
78	*Blidingia marginata* (J. Agardh) P. Dangeard	16	18	372	0·4	0·7*	1·4	18	*Ant.*	MI:H
79	*Blidingia minima* (Nägeli *ex* Kützing) Kylin	16	18	441	0·4	0·9*	1·8	18	*Ant.*	MI:H
	Ulvaceae									
80	*Enteromorpha compressa* (Linnaeus) Greville	20	21	120†	0·1*	0·2	0·4	30	*Gallus*	FC:EB
81	*Enteromorpha linza* (Linnaeus) J. Agardh	20	20	294	0·3	0·6*	1·2	18	*Ant.*	MI:H
82	*Enteromorpha prolifera* (O.F. Miller) J. Agardh	20	15	588	0·6	1·1*	2·2	18	*Ant.*	MI:H
83	*Ulva curvata* (Kützing) DeToni	24	18	343	0·4	0·7*	1·4	18	*Ant.*	MI:H
84	*Ulva fasciata* Delile	20	18	294	0·3	0·6*	1·2	18	*Ant.*	MI:H
85	*Ulva rigida* C. Agardh [as U. lactuca Linnaeus var. rigida (C. Agardh) LeJolis]	20	30	150†	0·16*	0·3	0·6	18	*Gallus*	FC:EB
86	*Ulvaria oxysperma* (Kützing) Bliding [as Gayralia oxysperma (Kützing) Vinogradova]	12	20	343	0·3	0·7	1·4	unp	*Gallus*	MI:DAPI
	ZYGNEMATALES									
	Zygnemataceae									
87	*Sirogonium strictum* (J. E. Smith) Kützing	48	40	2058	2·1	4·2*	8·4	unp	*Gallus*	MI:DAPI
88a	*Spirogyra communis* (Hassall) Kützing UTEX 2465	24	13	2009	2·1	4·1*	8·2	unp	*Gallus*	MI:DAPI
88b	*Spirogyra communis* (Hassall) Kützing UTEX 2466	12	13	1960	2·0	4·0*	8·0	unp	*Gallus*	MI:DAPI
89	*Zygnema circumcarcinatum* Czurda	c.19	35	245	0·3	0·5*	1·0	unp	*Gallus*	MI:DAPI
90	*Zygnema cylindricum* Transeau	V70	35	343	0·4	0·7*	1·4	unp	*Gallus*	MI:DAPI

[1] Molecular data clearly demonstrate that classifications of the genus *Cladophora* should be revised (Hanyuda *et al*., 2002). Circumscription of families in this complex will require sequence data for additional cladophoralean algae.

[2] Recent molecular investigations indicate that genera of the Dasycladaceae are well delineated, but this does not hold true for genera of the Polyphysaceae (=Acetabulariaceae). 18S rDNA data support transfer of *Acicularia schenckii* and *Polyphysa peniculus* to the genus *Acetabularia* (Berger *et al*., 2003).The familiar binomials are retained here for convenience until a complete taxonomic revision of the Dasycladales is available.

[3] Traditional taxonomic lists often grouped all conjugating green algae within one order, the Zygnematales (Conjugales) (Bold and Wynne, 1985). Results of recent molecular studies support recognition of two orders, the Desmidiales and the Zygnematales (McCourt *et al*., 2000).

[4] Recent phylogenetic investigations have redefined the boundary between the Ulotrichales and Ulvales. Species of *Monostroma* appear to be more closely related to the Ulotrichales than to the Ulvales (Hayden and Waaland, 2002). No contemporary characterization of families is available for this newly circumscribed order.

[5] Characters used to separate the genera *Ulva* and *Enteromorpha* lack taxonomic significance (Tan *et al*., 1999; Shimada *et al*., 2003). The familiar binomials have been retained here in the absence of formal reassignment of species (Hayden and Waaland, 2003). Placement of *Blidingia* in the Ulvales remains uncertain (*Insertae sedis*) as no representatives of this genus were included in recent molecular investigations.

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APPENDIX II. CHROMOSOME NUMBER AND NUCLEAR DNA CONTENT IN SPECIES OF PHAEOPHYTA

A key to the references appears at the end of this Appendix.

Entry number	Species[a]	2*n*[b]	Original reference for 2*n*	DNA amount				Original ref. for C-value[e]	Standard species[f]	Method[g]
				1C (Mbp)[c]	1C (pg)[d]	2C (pg)[d]	4C (pg)[d]			
	CUTLERIALES									
	Cutleriaceae									
1	*Cutleria hancockii* Dawson			392	0·4	0·8*	1·6	unp	Gallus	MI:DAPI
2	*Cutleria multifida* (Smith) Greville	50	10	490	0·5	1·0*	2·0	unp	Gallus	MI:DAPI
	DESMARESTIALES									
	Arthrocladiaceae									
3a	*Arthrocladia villosa* (Hudson) Duby	46–54	14	245	0·2	0·5*	1·0	unp	Gallus	MI:DAPI
3b	*A. villosa* Desmarestiaceae			270	0·3	0·5	1·1*	unp	Gallus	MI:DAPI
4	*Desmarestia aculeata* (Linnaeus) Lamouroux			392	0·4	0·8	1·6*	unp	Gallus	MI:DAPI
5	*Desmarestia viridis* (O. F. Muller) Lamouroux	c.44	16	416	0·4	0·8	1·7*	unp	Gallus	MI:DAPI
	DICTYOTALES									
	Dictyotaceae									
6	*Dictyota menstrualis* (Hoyt) Schnetter, Horning *et Weber-Peukert* (= *Dictyota dichotoma (Hudson) Lamouroux*)	32	18	539	0·5	1·1	2·2*	unp	Gallus	MI:DAPI
7	*Dictyopteris polypodioides* (De Candolle) Lamouroux (= Dictyopteris membranacea (Stackhouse) Batters)	28–32	6	637	0·6	1·3*	2·6	unp	Gallus	MI:DAPI
8	*Padina durvillaei* Bory			637	0·7	1·3*	2·6	unp	Gallus	MI:DAPI
9	*Padina gymnospora* (Kützing) Sonder			882	0·9	1·8	3·5*	unp	Gallus	MI:DAPI
10	*Padina jamaicensis* (Collins) Papenfuss			539	0·5	1·1	2·2*	unp	Gallus	MI:DAPI
11	*Padina japonica* Yamada			588	0·6	1·2	2·4*	unp	Gallus	MI:DAPI
									Gallus	MI:DAPI
	ECTOCARPALES[1]									
12	*Acinetospora crinita* (Harvey) Kornmann	47	13	245	0·3	0·5	1·0*	unp	Gallus	MI:DAPI
13	*Cladosiphon occidentalis* Kylin			172	0·2	0·3	0·7*	unp	Gallus	MI:DAPI
14	*Colpomenia sinuosa* (Mertens *ex* Roth) Derbès *et* Solier			294	0·3	0·6*	1·2	unp	Gallus	MI:DAPI
15	*Colpomenia phaeodactyla* (Dawson) Norris *et* Wynne			245	0·2	0·5*	1·0	unp	Gallus	MI:DAPI
16a	*Ectocarpus siliculosus* (Dillwyn) Lyngbye	42–50	19	245	0·2	0·5*	1·0	unp	Gallus	MI:DAPI
16b	*E. siliculosus*						1·0*	unp	Gallus	MI:DAPI
17a	*Hinksia irregularis* (Kützing) Amsler (= Ectocarpus irregularis Kützing)			98	0·1	0·2*	0·4	unp	Gallus	MI:DAPI
17b	*H. irregularis*			98	0·1	0·2	0·4*	unp	Gallus	MI:DAPI
18a	*Hinksia mitchelliae* (Harvey) Silva in Silva, Meñez et Moe (= Giffordia mitchelliae (Harvey) Hamel)	36–44	12	343	0·3	0·7*	1·4	unp	Gallus	MI:DAPI
18b	*H. mitchelliae*			441	0·4	0·8	1·6*	unp	Gallus	MI:DAPI
19	*Hummia onusta* (Kützing) Fiore	26	5	294	0·3	0·6	1·1*	unp	Gallus	MI:DAPI

APPENDIX II. (*continued*)

Entry number	Species[a]	2*n*[b]	Original reference for 2*n*	DNA amount 1C (Mbp)[c]	1C (pg)[d]	2C (pg)[d]	4C (pg)[d]	Original ref. for C-value[e]	Standard species[f]	Method[g]
20	*Hydroclathrus clathratus* (C. Agardh) Howe			441	0·4	0·9*	1·8	unp	Gallus	MI:DAPI
21	*Petalonia fascia* (O. F. Müller) Küntze			245	0·2	0·5*	1·0	unp	Gallus	MI:DAPI
22	*Pilayella littoralis* (Linnaeus) Kjellman			500†	0·3	0·52*	1·0	8	Gallus	FC:EB
23	*Punctaria tennuissima* (C. Agardh) Greville (= Desmotrichum undulatum (J. Agardh) Reinke)			98	0·1	0·2	0·45*	unp	Gallus	MI:DAPI
24	*Rosenvingea orientalis* (J. Agardh) Børgesen			196	0·2	0·4*	0·8	unp	Gallus	MI:DAPI
25	*Scytosiphon lomentaria* (Lyngbye) C. Agardh	44	7	245	0·2	0·5*	1·0	unp	Gallus	MI:DAPI
26a	*Stilophora rhizodes* (Turner) J. Agardh	28–32	17	98	0·1	0·2*	0·4	unp	Gallus	MI:DAPI
26b	*S. rhizodes*			98	0·1	0·2	0·3*	unp	Gallus	MI:DAPI
27	*Striaria attenuata* (C. Agardh) Greville	20	1	147	0·1	0·3	0·6*	unp	Gallus	MI:DAPI
	FUCALES									
	Fucaceae									
28	*Ascophyllum nodosum* (Linnaeus) Le Jolis	64	9	784	0·8	1·7	3·3*	unp	Gallus	MI:DAPI
29	*Fucus vesiculosus* Linnaeus Sargassaceae	64	2	529	0·5	1·1	2·2*	unp	Gallus	MI:DAPI
30	*Sargassum echinocarpum* J.Agardh			319	0·3	0·7	1·3*	unp	Gallus	MI:DAPI
31	*Sargassum filipendula* C. Agardh			196	0·2	0·4	0·8*	unp	Gallus	MI:DAPI
32	*Sargassum fluitans* Børgesen			196	0·2	0·4	0·8*	unp	Gallus	MI:DAPI
33	*Turbinaria ornata* (Turner) J. Agardh			196	0·2	0·4	0·8*	unp	Gallus	MI:DAPI
	LAMINARIALES									
	Alariaceae									
34	*Alaria esculenta* (Linnaeus) Greville	56	4	686	0·7	1·2	2·5*	unp	Gallus	MI:DAPI
35	*Ecklonia radiata* (C.Agardh) J. Agardh			588	0·6	1·3	2·6*	unp	Gallus	MI:DAPI
36	*Undaria pinnatifida* (Harvey) Suringar Laminariaceae			580†	0·6	1·3*	2·6	8	Gallus	FC:EB
37	*Agarum clathratum* Dumortier	c.44	16	588	0·6	1·2	2·0*	unp	Gallus	MI:DAPI
38a	*Laminaria digitata* (Hudson) Lamouroux	62	4	686	0·7	1·4	2·7*	unp	Gallus	MI:DAPI
38b	*L. digitata*			640†	0·7	1·4*	2·8	8	Gallus	FC:EB
38c	*L. digitata*			490	0·5*	1·0	2·0	20		RK
39a	*Laminaria saccharina* (Linnaeus) Greville	62	3	588	0·6	1·3*	2·6	unp	Gallus	MI:DAPI
39b	*L. saccharina*			720†	0·8	1·6*	3·2	8	Gallus	FC:EB
	SPHACELARIALES									
40	*Sphacelaria rigidula* Kützing	50–60	21	882	0·9	1·8*	3·6	unp	Gallus	MI:DAPI
41	*Sphacelaria sp.*			1550†	0·8	1·7*	3·4	8	Gallus	FC:EB
42	*Sphacelaria sp. 4315*			882	0·9*	1·8	3·6	unp	Gallus	MI:DAPI
	SPOROCHNALES									
43	*Carpomitra costata* (Stackhouse) Batters	c.30	11	392	0·4	0·8	1·6*	unp	Gallus	MI:DAPI
44	*Perithalia caudata* (Labillardière) Womersley	31–43	15	466	0·5	0·9	1·9	unp	Gallus	MI:DAPI

[1] The Ectocarpales, Scytosiphonales, Chordariales and Dictyosiphonales are paraphyletic with respect to each other, forming a highly interwoven clade (Siemer *et al*., 1998; Kogame *et al*., 1999). Recently, a formal circumscription of these orders into the Ectocarpales *sensu lato* was proposed (Rousseau and Reviers, 1999a; Rousseau *et al*., 2001) which left many taxa in strange alliances or as outliers (Draisma *et al*., 2001).In the present study, taxa were not assigned to specific familes as phylogenetic relationships in this order remain unresolved.

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APPENDIX III. CHROMOSOME NUMBER AND NUCLEAR DNA CONTENT IN SPECIES OF RHODOPHYTA

A key to the references appears at the end of this Appendix.

Entry number	Species[a]	$2n$[b]	Original ref. for $2n$	DNA amount				Original ref. for C-value[e]	Standard species[f]	Method[g]
				C (Mbp)[c]	1C (pg)[d]	2C (pg)[d]	4C (pg)[d]			
	ACROCHAETIALES[1]									
	Acrochaetiaceae									
1	*Audouinella botryocarpa* (Harvey) Woelkerling			588	0·6[(1)]	1·3*	2·6	unp	Gallus	MI:DAPI
2	*Audouinella thuretii* (Bornet) Woelkerling Rhodothamniellaceae			588	0·6*[(1)]	1·2	2·4	unp	Gallus	MI:DAPI
3	*Rhodothamniella floridula* (Dillwyn) J. Feldmann in Christensen (=Audouinella floridula (Dillwyn) Woelkerling)			1176	1·4[(1)]	2·8*	5·6	unp	Gallus	MI:DAPI
	BANGIALES									
	Bangiaceae									
4	*Bangia atropurpurea* (Roth) C. Agardh	6	36	490	0·5	1·0*	2·0	unp	Gallus	H
5	*Erythrotrichia carnea* (Dillwyn) J. Agardh			295	0·3	0·7*	1·4	unp	Gallus	MI:DAPI
6	*Porphyra carolinensis* Coll *et* Cox	8	16	490	0·5*	1·1	2·2	21	Ant	H
7a	*Porphyra leucosticta* Thuret *in* Le Jolis (NC)	8	16	480	0·5*	1·0	2·0	21	Ant	H
7b	*P. leucosticta* (TX)	6	16	490	0·5*	1·1	2·2	21	Ant	H
8	*Porphyra purpurea* (Roth) C. Agardh	10	16	270†	0·3*	0·6	1·2	26	Gallus	FC:EB
9	*Porphyra rosengurtii* Coll *et Cox*	6	16	490	0·5*	1·0	2·0	21	Ant	H
10a	*Porphyra spiralis var. amplifolia* Oliveira Filho *et* Coll	8	16	588	0·6*	1·2*	2·4	21	Ant	H
10b	*P. spiralis* var. *amplifolia* Oliveira Filho et Coll	6	16	588	0·6*	1·2*	2·4	21	Ant	H
	BONNEMAISONIALES									
	Bonnemaisoniaceae									
11	*Bonnemaisonia hamifera* Hariot	>40	28	588	0·6	1·3*	2·6	unp	Gallus	MI:DAPI
	CERAMIALES									
	Ceramiaceae									
12	*Aglaothamnion boergesenia* (Aponte *et* Ballantine) L'Ardy-Halos et Rueness (as Callithamnion byssoides Arnott ex Harvey)	60	9	1372	1·4	2·8*	5·6	14	Gallus	MI:DAPI
13	*Anotrichium multiramosum* (Setchell and Gardner) Baldock			394	0·4[(1)]	0·8	1·5*	unp	Gallus	MI:DAPI
14	*Antithamnion villosum* (Kützing) Athanasiadis in Maggs *et Hommersand (as Antithamnion cruciatum (C. Agardh) Nägeli)*	48	11	980	1·0	2·0*	4·0	14	Gallus	MI:DAPI
15	*Centroceras clavulatum* (C. Agardh in Kunth) Montagne in Durieu de Maisonneuve			588	0·6[(1)]	1·2*	2·4	unp	Gallus	MI:DAPI
16	*Ceramium cimbricum* H. Petersen			392	0·4[(1)]	0·8	1·6*	unp	Gallus	MI:DAPI
17	*Ceramium strictum* Harvey			245	0·3	0·5*	1·0*	unp	Gallus	MI:DAPI
18	*Crouania attenuata* (C. Agardh) J. Agardh			392	0·4[(1)]	0·9*	1·8	unp	Gallus	MI:DAPI
19	*Crouania pleonospora* W. Taylor			931	0·9[(1)]	1·8*	3·3*	unp	Gallus	MI:DAPI
20	*Spyridia filamentosa* (Wulfen) Harvey in Hooker			833	0·8[(1)]	1·5*	3·0	unp	Gallus	MI:DAPI
21	*Wrangelia penicillata* (C. Agardh) C. Agardh Dasyaceae			931	0·9[(1)]	1·8	3·6*	unp	Gallus	MI:DAPI
22	*Dasya baillouviana* (S. G. Gmelin) Montagne	40	34	490	0·5	1·0*	2·0	14	Gallus	MI:DAPI
23	*Dasya ocellata* (Grateloup) Harvey in Hooker			931	0·9[(1)]	1·8	3·5*	unp	Gallus	MI:DAPI
24	*Heterosiphonia gibbesii* (Harvey) Falkenberg Delesseriaceae			394	0·4[(1)]	0·8*	1·6	unp	Gallus	MI:DAPI
25	*Caloglossa leprieurii* (Montagne) J. Agardh			590	0·6	1·2*	2·4*	unp	Gallus	MI:DAPI
26	*Calonitophyllum medium* (Hoyt) Aregood			690	0·7[(1)]	1·4	2·9*	unp	Gallus	MI:DAPI
27	*Grinnellia americana* (C. Agardh) Harvey			588	0·6[(1)]	1·2	2·4*	unp	Gallus	MI:DAPI
28	*Hypoglossum tenuifolium* (Harvey) J. Agardh			586	0·7[(1)]	1·4	2·9*	unp	Gallus	MI:DAPI
29	*Martensia fragilis* Harvey Rhodomelaceae			394	0·4[(1)]	0·9*	1·8	unp	Gallus	MI:DAPI

30	*Acanthophora spicifera* (Vahl) Børgesen	64	6	490	0·5	1·1*	2·1*	unp	Gallus	MI:DAPI
31	*Bostrychia moritziana* (Sonders) J. Agardh			690	0·7(1)	1·3	2·7*	unp	Gallus	MI:DAPI
32	*Bostrychia radicans* (Montagne) Montagne			931	0·9(1)	1·8	3·5*	unp	Gallus	MI:DAPI
33	*Bryothamnion seaforthii* (Turner) Kützing			490	0·5(1)	1·0*	2·0	unp	Gallus	MI:DAPI
34	*Chondria dasyphylla* (Woodward) C. Agardh	62	32	490	0·5	1·0*	2·0	14	Gallus	MI:DAPI
35	*Chondria littoralis* Harvey			490	0·5	1·1*	2·2	14	Gallus	MI:DAPI
36	*Laurencia papillosa* (C. Agardh) Greville	40	38	833	0·8(1)	1·6*	3·1*	unp	Gallus	MI:DAPI
37	*Murrayella periclados* (C. Agardh) Schmitz			586	0·7(1)	1·4	2·8*	unp	Gallus	MI:DAPI
38	*Polysiphonia boldii* Wynne *et* Edwards			833	0·8	1·7*	3·4	13	Gallus	MI:DAPI
39	*Polysiphonia denudata* (Dillwyn) Greville *ex* Harvey in Hooker	60	13	931	0·9	1·9*	3·8	13	Gallus	MI:DAPI
40	*Polysiphonia elongata* (Hudson) Sprengel	74	1	637	0·7	1·3*	2·6	13	Gallus	MI:DAPI
41	*Polysiphonia harveyi* Bailey	64	10	1029	1·1	2·1*	4·2	13	Gallus	MI:DAPI
42	*Polysiphonia nigrescens* (Hudson) Greville	60	1	539	0·6	1·1*	2·2	13	Gallus	MI:DAPI
43	*Polysiphonia opaca* (C. Agardh) Moris *et* De Notaris	60	1	784	0·8	1·6*	3·2	13	Gallus	MI:DAPI
44	*Polysiphonia sphaerocarpa* Børgesen			539	1·1	2·2*	4·4	13	Gallus	MI:DAPI
45a	*Polysiphonia urceolata* Lightfoot *ex* Dillwyn (NC)	60	13	392	0·4	0·8*	1·6	13	Gallus	MI:DAPI
45b	*P. urceolata* Lightfoot *ex* Dillwyn(NO)			784	0·8	1·6*	3·2	13	Gallus	MI:DAPI
46	*Polysiphonia violacea* (Roth) Sprengel	32	33	833	0·8	1·7*	3·4	13	Gallus	MI:DAPI
	COLACONEMATALES[1]									
	Colaconemataceae									
47	*Colaconema daviesii* (Dillwyn) Stegenga (= Audouinella daviesii (Dillwyn) Woelkerling)			294	0·3(1)	0·6*	1·2	unp	Gallus	MI:DAPI
	COMPSOPOGONALES[2]									
	Compsopogonaceae									
48	*Compsopogon coerulus* (C. Agardh) Montagne	c.14	30	98	0·1	0·2	0·4	unp	Gallus	MI:DAPI
	CORALLINALES									
	Corallinaceae									
49	*Amphiroa beauvoisii* Lamouroux			343	0·3	0·7*	1·4	2	Gallus	MI:DAPI
50	*Amphiroa zonata* Yendo			294	0·3	0·6*	1·2	2	Gallus	MI:DAPI
51	*Bossiella orbigniana ssp. dichotoma* (Manza) Johansen			588	0·6	1·2*	1·4	2	Gallus	MI:DAPI
52	*Calliarthron tuberculosum* (Postels *et* Ruprecht) Dawson			637	0·6	1·3*	1·6	2	Gallus	MI:DAPI
53	*Cheilosporum sagittatum* (Lamouroux) Areschoug			343	0·3	0·7*	1·4	2	Gallus	MI:DAPI
54	*Corallina officinalis* Linnaeus	48	36	588	0·6	1·2*	2·4	2	Gallus	MI:DAPI
55	*Corallina vancouveriensis* Yendo			637	0·6	1·3*	2·6	2	Gallus	MI:DAPI
56	*Heydrichia wolkerlingii* Townsend, Chamberlain *et Woelkerling*			69	0·1	0·1*	0·2	2	Gallus	MI:DAPI
57	*Heydrichia sp.*			98	0·1	0·2*	0·4	2	Gallus	MI:DAPI
58	*Jania adhaerens* Lamouroux			539	0·6	1·1*	2·2	2	Gallus	MI:DAPI
59	*Leptophytum ferox* (Foslie) Chamberlain *et* Keats			98	0·1	0·2*	0·4	2	Gallus	MI:DAPI
60	*Lithothrix aspergillum* Gray			343	0·3	0·7*	1·4	2	Gallus	MI:DAPI
61	*Mesophyllum discrepans* Foslie			118	0·1	0·2*	0·4	2	Gallus	MI:DAPI
62	*Metagoniolithon radiatum* (Lamarck) Ducker			343	0·3	0·7*	1·4	2	Gallus	MI:DAPI
63	*Neogoniolithon spectabile* (Foslie) Setchell *et* Mason			394	0·4(1)	0·8	1·5*	*	Gallus	MI:DAPI
64	*Spongites yendoi* (Foslie) Chamberlain			147	0·1	0·3*	0·6	2	Gallus	MI:DAPI
65	*Titanoderma polycephalum* Foslie			196	0·2	0·4*	0·8	2	Gallus	MI:DAPI
66	*Titanoderma pustulatum* (Lamouroux) Näg*eli*			490	0·5	1·0*	2·0	2	Gallus	MI:DAPI
	GELIDIALES									
	Gelidiaceae									
67	*Gelidiella acerosa* (Forsskål) Feldmann et Hamel	12	23	147	0·2	0·3*	0·6*	23	Gallus	MI:DAPI
68	*Gelidium americanum* (Taylor) Santelices	24	19	294	0·3	0·6*	1·1	7	Gallus	MI:DAPI
69	*Gelidium coulteri* Harvey			245	0·3	0·5*	0·9*	7	Gallus	MI:DAPI
70	*Gelidium crinale* (Turner) Lamouroux (as Gelidium pusillum (Stackhouse) Le Jolis)			294	0·3	0·65*	1·2*	7	Gallus	MI:DAPI
71	*Gelidium floridanum* W. R. Taylor	12	19	294	0·3	0·6*	1·1*	7	Gallus	MI:DAPI

APPENDIX III. (*continued*)

Entry number	Species[a]	2*n*[b]	Original ref. for 2*n*	DNA amount 1C (Mbp)[c]	1C (pg)[d]	2C (pg)[d]	4C (pg)[d]	Original ref. for C-value[e]	Standard species[f]	Method[g]
72	*Gelidium robustum* (Gardner) Hollenberg *et* Abbott			294	0·3	0·6*	1·2*	7	Gallus	MI:DAPI
73	*Gelidium serrulatum* J. Agardh	20	19	196	0·2	0·4*	0·8*	7	Gallus	MI:DAPI
74	*Pterocladiella capillacea* (S. G. Gmellin) Santecles et Hommersand (as Pterocladia capillacea (S. G. Gmellin) Bornet et Thuret)	20	19	245	0·3	0·5*	1·0*	19	Gallus	MI:DAPI
75	*Pterocladiella melanoidea* (Schousboe *et* Bornet) Santelices *et* Hommersand			343	0·3	0·7*	1·2*	7	Gallus	MI:DAPI
	GIGARTINALES[3]									
	Solieriaceae									
76	*Agardhiella subulata* (C. Agardh) Kraft et Wynne	44	20	441	0·4	0·9*	1·9*	20	Gallus	MI:DAPI
77	*Eucheuma denticulatum* (N. L. Burman) Collins *et* Hervey	20	17	147	0·1	0·3*	0·6	17	Gallus	MI:DAPI
78	*Eucheuma isiforme* (C. Agardh) J. Agardh			196	0·2	0·4*	0·8	17	Gallus	MI:DAPI
79a	*Kappaphycus alvarezii* (Doty) Doty	20	17	147	0·1	0·3*	0·5*	17	Gallus	MI:DAPI
79b	*K. alvarezii*			196†	0·2	0·4*	0·8	26	Gallus	FC:EB
80	*Kappaphycus striatum* (Schmitz) Doty			196	0·2	0·4*	0·8	17	Gallus	MI:DAPI
81	*Soliera filiformis* (Kützing) Gabrielson Gigartinaceae			197	0·2(1)	0·4*	0·8	unp	Gallus	MI:DAPI
82a	*Chondrus crispus* Stackhouse	64–70	8	98	0·1	0·2*	0·5*	unp	Gallus	MI:DAPI
82b	*C. crispus* Dumontiaceae			98	0·1	0·2*	0·4	26	Gallus	FC:EB
83	*Dumontia contorta* (S. G. Gmelin) Ruprecht Phyllophoraceae	22–24	28	196	0·2(1)	0·4*	0·8	unp	Gallus	MI:DAPI
84	*Ahnfeltiopsis concinna* (J. Agardh) P. C. Silva et DeCew			147	0·2(1)	0·3 *	0·6	unp	Gallus	MI:DAPI
85	*Gymnogongrus griffithsiae* (Turner) Martius Hypneaceae	46	22	147	0·1	0·3*	0·6	22	Gallus	MI:DAPI
86	*Hypnea musciformis* (Wulfen in Jacquin) Lamouroux	10	18	147	0·1	0·2*	0·4	18	Gallus	MI:DAPI
	GRACILARIALES									
	Gracilariaceae									
87	*Gracilaria arcuata* Zanardini			186	0·2	0·4*	0·8*	24	Gallus	MI:DAPI
88	*Gracilaria blodgettii* Harvey			186	0·2	0·4*	0·8*	27	Gallus	MI:DAPI
89	*Gracilaria caudata* J. Agardh			196	0·2	0·4*	0·8	27	Gallus	MI:DAPI
90	*Gracilaria cervicornis* (Turner) J. Agardh			196	0·2	0·4*	0·8	27	Gallus	MI:DAPI
91	*Gracilaria divaricata* Harvey			196	0·2	0·4*	0·8	27	Gallus	MI:DAPI
92	*Gracilaria eucheumoides* Harvey			206	0·2	0·4*	0·8	24	Gallus	MI:DAPI
93	*Gracilaria firma* Zhang *et* Xia			196	0·2	0·4*	0·8	24	Gallus	MI:DAPI
94	*Gracilaria flabelliforme* P. Crouan *et* H. Crouan *ex Schramm et Maze*	48	12	191	0·2	0·4*	0·8	12	Gallus	MI:DAPI
95	*Gracilaria mammillaris* (Montagne) M. A. Howe	48	12	196	0·2	0·4*	0·8	12	Gallus	MI:DAPI
96	*Gracilaria pacifica* Abbott	48	4,12	196	0·2	0·4*	0·8	12	Gallus	MI:DAPI
97	*Gracilaria salicornia* (C. Agardh) E. Y. Dawson			191	0·2	0·4*	0·8*	24	Gallus	MI:DAPI
98	*Gracilaria tikvahiae* McLachlan	48	29	191	0·2	0·4*	0·8	12	Gallus	MI:DAPI
99	*Gracilaria verrucosa* (Hudson) Papenfuss	48	3	147	0·2	0·3*	0·6	12	Gallus	MI:DAPI
100	*Gracilaria sp. (NC)*			196	0·2	0·4	0·8*	unp	Gallus	MI:DAPI
101	*Gracilariopsis bailinae* Zhang *et* Xia			196	0·2	0·4*	0·8*	24	Gallus	MI:DAPI
102	*Gracilariopsis carolinensis* Liao *et* Hommersand (as Gracilariopsis lemaneiformis (Bory) Dawson, Acleto et Foldvik)	64	12	196	0·2	0·4*	0·8	12	Gallus	MI:DAPI
103	*Gracilariopsis tenuifrons* (Bird *et* Oliveira) Fredericq et Hommersand	64	12	196	0·2	0·4*	0·8	12	Gallus	MI:DAPI
104	*Hydropuntia cornea* (J. Agardh) Wynne			245	0·2	0·5*	1·0	12	Gallus	MI:DAPI
105	*Hydropuntia dentata* (J. Agardh) Wynne			196	0·2	0·4*	0·8	12	Gallus	MI:DAPI
106	*Hydropuntia fastigiata* (Zhang *et* Xia) Wynne			196	0·2	0·4*	0·8	24	Gallus	MI:DAPI
	HALYMENIALES[3]									

	Halymeniaceae									
107	*Grateloupia filicina* (Lamuroux) C. Agardh			196	0·2*(1)	0·4	0·8	unp	Gallus	MI:DAPI
108	*Halymenia floridana* J. Agardh			196	0·2*(1)	0·4	0·8	unp	Gallus	MI:DAPI
	NEMALIALES									
	Galaxauraceae									
109	*Galaxaura rugosa* (Ellis *et* Solander) Lamouroux			1084	1·1(1)	2·3	4·6	unp	Gallus	MI:DAPI
	PALMARIALES									
	Palmariaceae									
110	*Palmaria palmata* (Linnaeus) Kuntze	38–48	37	794	0·8(1)	1·6*	3·3*	unp	Gallus	MI:DAPI
	RHODYMENIALES									
	Rhodymeniaceae									
111	*Champia parvula* (C. Agardh) Harvey	24	14	196	0·2	0·4*	0·8	14	Gallus	MI:DAPI
112	*Lomentaria baileyana* (Harvey) Farlow	20	14	196	0·2	0·3*	0·6	14	Gallus	MI:DAPI
113	*Rhodymenia pseudopalmata* (Lamouroux) Silva	20	14	294	0·3	0·5*	1·0	14	Gallus	MI:DAPI

[1] Recent molecular investigations have demonstrated that algae previously referred to as 'acrochaetoid' are not a natural assemblage, but include filamentous forms allied with at least three groups in the Bangiophycideae: 1) the Batrachospermales and sister groups (Pueschel *et al.*, 2000), the Acrochaetales (Saunders *et al.*, 1995; Harper and Saunders, 1998) which are allied with the Palmariales (Figure 13), and the newly recognized Colaconematales (Harper and Saunders, 2002) which are weakly allied with the Nemaliales.

[2] The relationship of the Compsopogonales to other Bangiophycidae remains under investigation (Harper and Saunders, 1998; Vis *et al.*, 1998; Müller *et al.*, 2002).

[3] Kraft and Robbins (1985) proposed merging the Cryptonemiales and Gigartinales. Neither the traditional classification (Kylin, 1956) nor the proposed merger is supported by *rbc*L data (Freshwater *et al.*, 1994). Instead, most families previously included in the Cryptonemiales form a monophyletic clade within the Gigartinales. The central family of the former order Cryptonemiales, Halymeniaceae, is now treated as an order (Huisman *et al.*, 2003).

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