



Estimates of nuclear DNA content in 98 species of brown algae (Phaeophyta)

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Abstract

Background and aims

Brown algae are critical components of marine ecosystems around the world. However, the genome of only one species of the class has so far been sequenced. This contrasts with numerous sequences available for model organisms such as higher plants, flies or worms. The present communication expands our coverage of DNA content information to 98 species of brown algae with a view to facilitating further genomic investigations of the class.

Methodology

The DNA-localizing fluorochrome DAPI (4',6-diamidino-2-phenylindole) and the red blood cell (chicken erythrocyte) standard were used to estimate 2C values by static microspectrophotometry.

Principal results

2C DNA contents are reported for 98 species of brown algae, almost doubling the number of estimates available for the class. The present results also expand the reported DNA content range to 0.2–3.6 pg, with several species of Fucales and Laminariales containing apparent polyploid genomes with 2C = 1.8–3.6 pg.

Conclusions

The data provide DNA content values for 12 of the 19 recognized orders of brown algae spanning the breadth of the class. Despite earlier contentions concerning DNA content and the presence of oogamy, the present results do not support a correlation between phylogenetic placement and genome size. The closest sister groups to the brown algae have genome sizes on the order of 0.3 pg (e.g. Schizocladiophyceae), suggesting that this may be the ancestral genome size. However, DNA content ranges widely across the class.

Introduction

During the Second Plant Genome Size Workshop and Discussion Meeting [Royal Botanic Gardens (RBG), Kew, 8–12 September 2003], major gaps (systematic, regional

and plant type) in our knowledge of plant DNA amounts were identified (Bennett and Leitch 2005). Significantly, it was noted that no database was available for the algae. This shortcoming was soon addressed with a compilation of genome size estimates for 247 species

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of red, green and brown macroscopic algae (Kapraun 2005). At a subsequent workshop entitled 'Genome size: a research discipline in development', held in conjunction with the XVII International Botanical Congress (July 2005), green algae, especially streptophytes, were identified as critical in efforts to reconstruct a hypothetical ancestral nuclear genome for the green algal ancestor basal to land plants (Leitch *et al.* 1998; Delwiche *et al.* 2002; Pryer *et al.* 2002; Greilhuber *et al.* 2006). We immediately initiated an investigation that expanded coverage for lineages of green algae by 72–157, and this resulted in the characterization of the ancestral land plant flagellate genome (Kapraun 2007). These data (Kapraun 2005, 2007) are now incorporated into a database of plant genome sizes (Kapraun *et al.* 2004; Bennett and Leitch 2005; Gregory *et al.* 2007) compiled and hosted by the RBG Kew web page (<http://data.kew.org/cvalues/>).

The present communication concerns our recent efforts to expand our coverage of DNA contents in brown algae which are being increasingly nominated as candidates for genomic investigations (e.g. Peters *et al.* 2004; Waaland *et al.* 2004; Phillips *et al.* 2008a). Recently, criteria for genomics investigations of several macro-algal candidates have been proposed (Peters *et al.* 2004; Waaland *et al.* 2004). Low DNA content (genomes ~100 Mb) has been a major criterion in the selection of algae for genomic and genetic analyses (Peters *et al.* 2004; Waaland *et al.* 2004), including when employing bacterial artificial chromosomes cloning technology as used for large-scale physical mapping and genomic sequencing (Wang *et al.* 2005). To date, macro-algal (multicellular) species nominated as candidates have genomes in the range of 127–300 Mb (Waaland *et al.* 2004). Remarkably, these nominations have been made on the basis of data limited to about a dozen species and isolates (Peters *et al.* 2004; Waaland *et al.* 2004).

DNA C-value remains a key character in biology, biodiversity and molecular investigations as genome size has many important and practical implications (Cavalier-Smith 1985; Bennett *et al.* 2000; Leitch and Leitch 2008). For example, genome size directly influences the cost and difficulty of sequencing projects, and is therefore a primary consideration in choosing future sequencing subjects (Gregory 2005a, b; Gregory *et al.* 2007) as species with large DNA amounts or genome sizes make such genome projects prohibitively expensive (Fay *et al.* 2005).

The present study increases, by an order of magnitude, the number of brown algal taxa for which DNA content data are available. In addition, it expands the list of target brown algal species with appropriately small

genome sizes, and identifies taxa that have genome sizes too large for most projects even though they may meet many other criteria for genomics investigations (Waaland *et al.* 2004). However, as sequencing costs decrease with new technologies (e.g. 454 or Illumina), even these projects may become feasible.

The availability of a DNA C-values database and a consensus higher-level phylogenetic tree for green algae has opened the way for determining evolutionary trends in DNA amounts for the chlorophytes and streptophytes (Kapraun 2005, 2007; López-Bautista *et al.* 2006). Unfortunately, in brown algae, a well-resolved higher-level phylogeny remains elusive despite recent advances (e.g. Phillips *et al.* 2008b; Silberfeld *et al.* 2010). In the last decade, DNA sequence data, especially from studies utilizing ribosomal DNA (rDNA) such as the 28S (LSU) or *rbcL*, have shown that classic brown algal phylogenies based on a sequence of simple/primitive to complex/advanced were more apparent than real (e.g. Rousseau and De Reviers 1999; Draisma *et al.* 2001; Phillips *et al.* 2008b). However, earlier studies were characterized by poor resolution in the branching order among the many groups, especially basal and crown group lineages. This was probably due to the low resolving power of the genes employed, use of an insufficient amount of data and/or the rapidity of the radiation event for the class (Draisma *et al.* 2001; Rousseau *et al.* 2001).

A comprehensive phylogeny of the Phaeophyta (Phillips *et al.* 2008b), developed from two-gene (*rbcL* and LSU rDNA) sequence analyses, resolves several monophyletic early lineages: (i) *Choristocarpus* (Disco-sporangiales), a distant sister to the remaining brown algal taxa as proposed earlier (Burrowes *et al.* 2003; Draisma *et al.* 2003; Cho *et al.* 2004), (ii) *Ishige* (Ishigeales) as proposed previously (Cho *et al.* 2004), (iii) *Heribaudiella/Bodanella* (McCauley and Wehr 2007) and (iv) Desmarestiales at the base of the crown group (BACR) (Phillips *et al.* 2008b). The remaining brown algae can be delineated into two groups: (i) an early diverging set of basal lineages including the Syringodermatales, Sphacelariales, Dictyotales, Onslowiales, Phaeostrophionaceae/*Bodanella* clade and (ii) the BACR of remaining orders (Phillips *et al.* 2008b). This work was recently taken a step further by the multigene efforts of Silberfeld *et al.* (2010) which confirmed the early insights into evolutionary patterns and increased resolution in the BACR. Yet in spite of this progress, many relationships along the backbone of the brown algal tree remain unresolved. However, with at least this preliminary pattern of brown algal evolution in place, it is now possible to suggest correlations between nuclear DNA content variation and evolution among the brown algae.

The present paper expands coverage for species and isolates of brown algae from 54 to 98, almost doubling published data (Kapraun 2005). Of this total, 16 resulted from our ongoing research and from recent investigations of members of the Dictyotales and Fucales from the coasts of Spain (Gómez Garreta *et al.* 2010, 2011).

Materials and methods

Source of specimens

Collection data and/or source of cultures are summarized at <http://people.uncw.edu/kapraund/DNA/> (see links to Table 2 Phaeophyta).

Laminariales data were obtained from gametophytes in the culture collection of R. Lewis or from collections in nature made by N. Phillips along the Sonoma coast of northern California. Additional specimens originated from the Provasoli-Guillard National Center for Culture of Marine Phytoplankton (CCMP), the Kobe University Macro-Algal Culture Collection (KU-MACC) and the culture collection of N. Phillips.

On the coast of Spain, 16 taxa of Fucales (Fucaceae and Sargassaceae) and seven taxa of Dictyotales were collected from the Mediterranean [Calella and Cadaqués (Girona)] and Atlantic [A Coruña (Galicia), Ondarreta and Zumaya (Guipúzcoa)] (see links to Table 1 at <http://gargoyle.arcadia.edu/biology/phillips2010.htm>).

Assignment of ploidy level

Assignment of estimated nuclear DNA contents to specific C-values in the present study is presumptive in that no karyological investigations were conducted on the algal samples used for nuclear DNA content estimates.

Nuclear DNA content estimates

Algal material was fixed in Carnoy's solution (Kapraun 2005) and in Methacarn (methanol–Carnoy) to avoid reported staining inhibition associated with intracellular phenolic compounds (Puchtler *et al.* 1970a, b). Samples were stored in 70 % ethanol at 4 °C; selected specimens were rehydrated in water and softened in 5 % w/v EDTA (Goff and Coleman 1990) for 12–48 h. Algal specimens were transferred to cover slips treated with subbing solution, and then air dried and stained with DAPI (0.5 µg/mL 4',6-diamidino-2-phenylindole) (Sigma Chemical Co., St Louis, MO, USA) as previously described in detail (Goff and Coleman 1990; Kapraun and Nguyen 1994). Nuclear DNA contents were based on estimates from both microspectrophotometry and image analysis.

Microspectrophotometry with DAPI followed procedures published previously (Kapraun and Nguyen 1994; Kapraun *et al.* 2007) using a protocol modified

after Goff and Coleman (1990). Nuclear DNA content estimates based on image analysis of DAPI-stained specimens followed a procedure modified from Kapraun and Dunwoody (2002) and Choi *et al.* (1994), using a Cooled CCD Miramax RTE 782-Y high-performance digital camera placed on a Leica DMRB fluorescence microscope and analysed with MetaMorph software (Molecular Devices, Toronto, Canada) (Gómez Garreta *et al.* 2010, 2011). For a recent, comprehensive review of theory and practice of DNA quantification by densitometry, see Hardie *et al.* (2002) or Greilhuber (2008).

Nuclear DNA contents of algal specimens were estimated by comparing their I_f values with those of chicken erythrocytes (red blood cells; RBC); (Kapraun 1994, Kapraun and Dunwoody 2002). See Appendix IA, Section (f) of Additional information, 'Standard Species', for the rationale in accepting 2C DNA = 2.4 pg as the standard. 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, chicken erythrocytes (RBC) can be used directly as standards for determining amounts of DNA only when the A–T contents of both standard and experimental DNA are equivalent (Coleman *et al.* 1981). Chicken has a nuclear DNA base composition of 42–43 mol% G + C (Marmur and Doty 1962). Limited published data for the Phaeophyta indicate values in the range of 38–43 mol% G + C (Stam *et al.* 1988; Le Gall *et al.*, 1993). Members of the Phaeophyta investigated in this study are assumed to have a similar range of base pair compositions, and the linearity is accepted between DAPI–DNA binding in both RBC and algal samples (Le Gall *et al.* 1993).

The Second Plant Genome Size workshop and Discussion Meeting (Bennett *et al.* 2000; Bennett and Leitch 2005) identified 'best practice' methodology for nuclear genome size estimation in plant tissues. Virtually none of the published genome size data for algae resulted from investigations adhering to all of the best practice recommendations, primarily because measurement of the relatively small algal nuclear genomes (Kapraun 2005, 2007) requires standard species different from those specified as appropriate for vascular plants (Doležel *et al.* 1998). Consequently, all present and previously published data should be considered accurate only to ± 0.1 pg (Kapraun 2005).

Previously unpublished nuclear DNA content data in Appendix IB of Additional information are indicated by (°). Supplementary materials and methods, information for collection locations, and data for number of algal nuclei examined in each sample and estimates of nuclear genome size (picograms) \pm SD are available at

<http://www.uncw.edu/people/kapraun/DNA> or <http://gargoyle.arcadia.edu/biology/phillips2010.htm> (see Table 2 Phaeophyta, Appendix IB Phaeophyta of Additional information). Nuclear DNA content data for these and other brown algae are incorporated into a database of plant genome sizes (Kapraun 2005; Gregory et al. 2007) hosted by the RBG Kew web page (<http://data.kew.org/cvalues/>).

Results and discussion

Range of DNA contents

Comparison of I_f values for species of phaeophytes with chicken erythrocytes (RBCs) permitted estimation of nuclear DNA contents for 98 species of brown algae, almost doubling the number of estimates available for the class. Previously, a DNA content range of $2C = 0.2$ – 1.8 pg was reported (Kapraun 2005). The present results, which expand the reported DNA content range upward to 3.6 pg, approximate one order of magnitude (Appendix I of Additional information). The smallest mean $2C$ genome sizes were found in the Ectocarpales (0.2 – 1.0 pg) and the Phaeostrophionaceae/*Bodanella* complex (0.2 – 0.6 pg) while the largest $2C$ genome sizes were found in the Fucales (0.4 – 3.6 pg), Laminariales (0.6 – 3.2 pg), Dictyotales (0.7 – 1.8 pg) and Discosporangiales (2.3 pg). Larger genome sizes (≥ 2.0 pg) reported in the Fucales and Laminariales almost certainly represent polyploid values (Kapraun 2005). By comparison, these estimates for phaeophytes closely approximate previously published DNA content estimates for both the Chlorophyta ($2C = 0.2$ – 6.1 pg) (Kapraun 2005, 2007) and the Rhodophyta ($2C = 0.2$ – 2.8 pg) (Kapraun 2005; Kapraun et al. 2007).

The size of the larger phaeophyte genomes is best appreciated when compared with the minimum amount of DNA estimated for specifying the mRNA sequences required for angiosperm development. Specifically, the genomes of *Genlisea margaretae* Hutchinson and *Arabidopsis thaliana* (L.) Heynhold, with $2C = 126$ and 314 Mb, respectively (Riechmann et al. 2000; Bennett et al. 2003; Greilhuber et al. 2006), are among the smallest found in angiosperms (Bennett and Smith 1976), with *A. thaliana* having 1.5 – $2\times$ the estimated $15\,000$ genes per haploid genome required for development (Flavell 1980). Even the smallest phaeophyte genomes reported (e.g. $1C = 98$ Mb in *Hinksia irregularis*, *Punctaria tenuissima* and *Stilophora rhizodes*), with their probable genomic redundancy (Kapraun 2005, 2007), have the genic capacity for morphologically complex development.

Candidates for genomic studies

The results of the present study reveal many macro-algal (multicellular) species of brown algae with genome sizes comparable to those of species previously nominated or used as candidates for genomic studies (i.e. 127 – 300 Mbp) (Waaland et al. 2004; Phillips et al. 2008a). For example, some isolates of *Laminaria saccharina* (Garbary and Clarke 2002) (Laminariales) and several species of Sargassaceae (Gómez Garreta et al. 2010) have small ($1C = 196$ – 319 Mb) genomes (Appendix IB of Additional information), are amenable to culture and are of significant ecological and/or commercial importance. Previously, attention was called to the need to redirect basic algal research toward economically important species (Kapraun 1999).

Polyploidy

Polyploidy has been reported widely in the Phaeophyta (Kapraun 2005), especially in the Laminariales (Lewis et al. 1993; Lewis 1996; Garbary and Clarke 2002), Ectocarpales (Müller 1967, 1970), Fucales (Yabu and Yasui 1983; Lewis 1996; Coyer et al. 2006; Gómez Garreta et al. 2010) and Dictyotales (Gómez Garreta et al. 2011). For a recent review of concepts associated with adaptations and genetic variability associated with hybridization and polyploidy in brown algae, see Coyer et al. (2006). The present results support previous suggestions that polyploidy is a pervasive feature of brown algal genomics. The extent of both species-level and intraplant ploidy level variation (including endopolyploidy) remains to be determined, but represents an exciting area for future research (Coyer et al. 2006).

Correlation between DNA content and phylogenetic placement

Although no correlation is apparent between phylogenetic placement (Fig. 1) and genome size, in both the Fucales and Dictyotales, DNA contents may be diagnostic, representing synapomorphies. Most members of the Fucales are characterized by discrete ranges of $2C$ nuclear genome size values of 1.1 – 2.2 pg, while most members of the Sargassaceae are characterized by discrete values of 0.4 – 0.8 pg (Gómez Garreta et al. 2010). Members of the Dictyotales are characterized by discrete ranges of $2C$ nuclear genome sizes: species of *Dictyota* have a range of 0.6 – 0.8 pg while other genera have a range of 1.0 – 1.7 pg (Gómez Garreta et al. 2011).

Previously, it was suggested that orders characterized by oogamy (Dictyotales and Fucales) or a pronounced anisogamy (Sphacelariales) and having large female gametes (eggs) have the largest nuclear genomes observed regardless of their phylogenetic position. The present results make this generalization less clear as

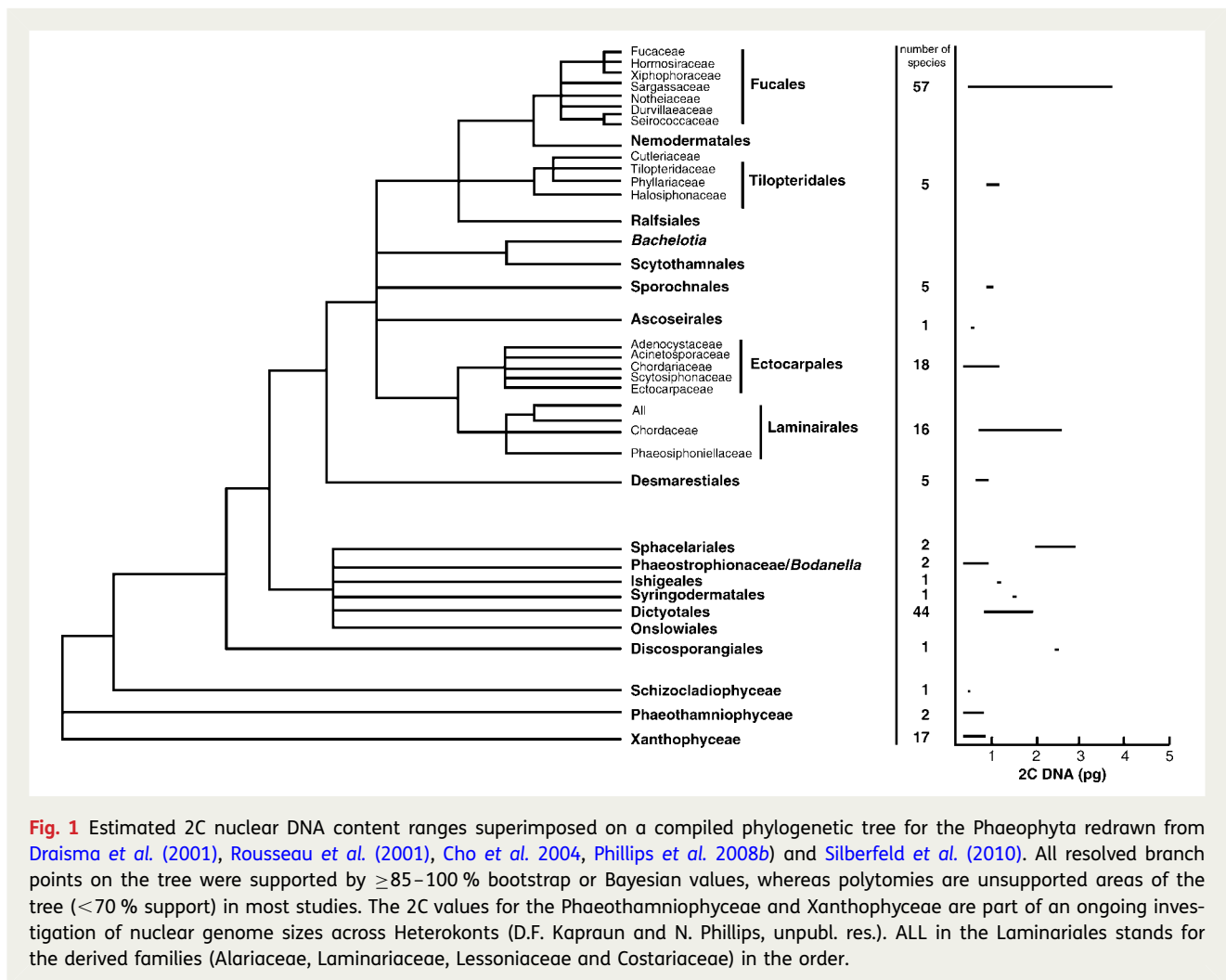


Fig. 1 Estimated 2C nuclear DNA content ranges superimposed on a compiled phylogenetic tree for the Phaeophyta redrawn from Draisma et al. (2001), Rousseau et al. (2001), Cho et al. 2004, Phillips et al. 2008b) and Silberfeld et al. (2010). All resolved branch points on the tree were supported by ≥ 85 –100 % bootstrap or Bayesian values, whereas polytomies are unsupported areas of the tree (< 70 % support) in most studies. The 2C values for the Phaeothamniophyceae and Xanthophyceae are part of an ongoing investigation of nuclear genome sizes across Heterokonts (D.F. Kapraun and N. Phillips, unpubl. res.). ALL in the Laminariales stands for the derived families (Alariaceae, Laminariaceae, Lessoniaceae and Costariaceae) in the order.

the large genome ($2C = 2.3$ pg) in *Choristocarpus tenellus* (Discosporangiales) is comparable to those reported in the oogamous Dictyotales and Fucales (Fig. 1).

Characterization of an ancestral brown algal genome

In the present study, *Schizocladia ischiensis* is the closest sister group to the brown algae and has a small genome ($2C = 0.3$ pg). Unfortunately, neither chromosome numbers nor karyotype data are available for this species or any of the basal phaeophytes (Phillips et al. 2008b). Most orders of brown algae are reported to have basic chromosome numbers between 8 and 13 (Cole 1967; Lewis 1996). A basic chromosome number of 4 has been reported for the Dictyotales, since haploid chromosome numbers of 12 and 16 are common (Hörnig et al. 1992). If the small genome size

of *Schizocladia* is found to be complemented by a small chromosome number (e.g. $n = 4$), then it would be an appropriate candidate for investigations of an extant closely related sister group to the brown algae and possibly provide a window into the transition to multicellularity seen in the brown algae.

Conclusions and forward look

These data provide DNA content values for 12 of the 19 recognized orders of brown algae spanning the breadth of the class. The present results do not support a correlation between phylogenetic placement and genome size in the brown algae. The closest sister groups to the brown algae have genome sizes on the order of 0.3 pg (e.g. Schizocladiphyceae), suggesting that this may be the ancestral genome size of the class.

A number of phaeophytes warrant further study, including several of the basal lineages with small genome sizes and interesting evolutionary histories like the Phaeostrophionaceae or Dictyotales, and the ecologically and economically important Fucales and Laminariales (Fig. 1) for which published DNA content data are surprisingly limited or absent. The Fucales include seven families with many ecologically important genera with interesting biogeographic patterns. For instance, *Fucus*, *Ascophyllum* and *Pelvetia* are restricted to the North Atlantic while *Hormosira* and *Xiphophora* are restricted to the southern hemisphere. Recently, the number of nuclear DNA content estimates for species of *Fucus*, *Ascophyllum* and *Pelvetia* has more than doubled (Gómez Garreta et al. 2010). However, no C-value data for any southern hemisphere Fucales have been published.

The Laminariales as recently redefined includes 22 genera and four families (Lane et al. 2006). In temperate regions, many of these genera form expansive kelp forests representing some of the most productive marine ecosystems. Additionally, many of the Laminariales are commercially important, forming the basis of the alginic acid food additive industry (e.g. Andersen 1992). In terms of genome sizes, the kelps have some of the largest genomes reported in the phaeophytes (Appendix IB of Additional information and Fig. 1). Further study is warranted to explore the nature of the large genome sizes, to aid in hybridization experiments and in efforts to domesticate target species for mariculture.

Additional information

The following Additional information is available in the online version of this article –

File 1: Appendix IA includes notes on chromosome numbers and nuclear DNA content estimates in isolates and species of brown algae.

File 2: Appendix IB (Table 1) includes the number of algal nuclei examined in each sample and nuclear genome size estimates (picograms) \pm SD (see links to Table 1 on <http://www.uncw.edu/people/kapraun/DNA> or <http://gargoyle.arcadia.edu/biology/phillips2010.htm>).

File 3: Appendix IC includes numbered references for chromosome complements and DNA values in the Phaeophyta cited in Appendix I.

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Contributions by the authors

All microspectrophotometry was conducted by D.K. (University of North Carolina Wilmington, USA), while image analysis data were obtained by A.G.G., M.A.R.S., J.L.R. and N.S.S. (Universitat de Barcelona, Spain). All authors contributed algal cultures and/or field-collected materials. D.K. and N.P. prepared the manuscript for publication.

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Conflicts of interest statement

None declared.

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