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Estimates of nuclear DNA content in red algal lineages

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Abstract

Background and aims The red algae are an evolutionarily ancient group of predominantly marine organisms with an estimated 6000 species. Consensus higher-level molecular phylogenies support a basal split between the unicellular Cyanidiophytina and morphologically diverse Rhodophytina, the later subphylum containing most red algal species. The Rhodophytina is divided into six classes, of which five represent early diverging lineages of generally uninucleate species, whose evolutionary relationships are poorly resolved. The remaining species compose the large (27 currently recognized orders), morphologically diverse and typically multinucleate Florideophyceae. Nuclear DNA content estimates have been published for <1 % of the described red algae. The present investigation summarizes the state of our knowledge and expands our coverage of DNA content information from 196 isolates of red algae.

Methodology The DNA-localizing fluorochrome DAPI (4′,6-diamidino-2-phenylindole) and RBC (chicken erythrocytes) standards were used to estimate 2C values with static microspectrophotometry.

Principal results Nuclear DNA contents are reported for 196 isolates of red algae, almost doubling the number of estimates available for these organisms. Present results also confirm the reported DNA content range of 0.1–2.8 pg, with species of Ceramiales, Nemaliales and Palmariales containing apparently polyploid genomes with 2C = 2.8, 2.3 and 2.8 pg, respectively.

Conclusions Early diverging red algal lineages are characterized by relatively small 2C DNA contents while a wide range of 2C values is found within the derived Florideophyceae. An overall correlation between phylogenetic placement and 2C DNA content is not apparent; however, genome size data are available for only a small portion of red algae. Current data do support polyploidy and aneuploidy as pervasive features of red algal genome evolution.

Introduction

The Second Plant Genome Size Workshop and Discussion Meeting (hosted by the Royal Botanic Gardens, Kew, 8–12 September 2003) identified major gaps (systematic, regional and plant type) in our knowledge of plant DNA amounts (Bennett and Leitch 2005*a*, *b*). It was noted that no database was available for algae. This major gap was addressed with a compilation of genome size estimates for 247 species of macroscopic marine algae, including data for 95 isolates and species of red algae (Kapraun 2005). These data have been incorporated into a database of plant genome

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sizes (Kapraun *et al.* 2004) compiled and hosted by the Royal Botanic Gardens (RBG) Kew web page (http://data.kew.org/cvalues/). A subsequent investigation of green algae resulted in an expansion of coverage and characterization of the ancestral land plant flagellate genome (Kapraun 2007). More recently, efforts to expand coverage of DNA contents in brown algae were published (Phillips *et al.* 2011). This final report in the series summarizes nuclear DNA content data for red algae, both from our continuing investigations and from the literature. Results are updated at http://people.uncw.edu/kapraund/DNA (see links to 'Rhodophyta'). The present paper provides nuclear genome size estimates for 77 additional isolates of red algae and compiles all available data (196 species/isolates) into a single resource [see Additional Information]. Of this new list, 40 resulted from our ongoing research. Unicellular microalgae and freshwater red algae, which were previously under-represented (Kapraun 2005), are emphasized here.

Inclusion of published nuclear DNA content data for red algae in the present report was sometimes problematic. The Second Plant Genome Size Workshop and Discussion Meeting (Bennett and Leitch 2005*b*) 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 requires standard species different from those specified as appropriate for vascular plants (Doležel *et al.* 1998; Kapraun 2005). A comprehensive discussion on standard species and methods is included in the section 'Notes on Appendix I'.

The red algae (Rhodophyta) are predominantly marine organisms with >700 genera and 6000 species described in 38 orders (Guiry and Guiry 2011). The Rhodophyta are characterized by unstacked thylakoids in plastids, plastids containing the accessory pigments phycoerythrin, phycocyanin and allophycocyanin arranged in phycobilisomes, the lack of plastid endoplasmic reticulum, the presence of pit connections between cells in filamentous genera and the absence of flagellated cells in the life history (Woelkerling 1990). There are a variety of current higher-level classification schemes for red algae (Saunders and Hommersand 2004; Yoon *et al.* 2006; Guiry and Guiry 2011). Molecular analyses (Oliveira and Bhattacharya 2000; Yoon *et al.* 2002*a*, *b*, 2006) and organelle ultrastructure (Pueschel 1989; Scott and Broadwater 1990) support an early divergence for the Cyanidiales, which are resolved as a sister group to other red algae and classified as a separate subphylum (Cyanidiophytina). The remaining Rhodophyta are divided into six classes that are grouped as a single subphyllum (Yoon *et al.* 2006) or multiple subphyla (Saunders and Hommersand 2004; Guiry and Guiry 2011). Five of these classes, Porphyridiophyceae, Stylonematophyceae, Compsopogonophyceae, Rhodellophyceae and Bangiophyceae, are early diverging lineages of generally uninucleate species, whose evolutionary relationships are poorly resolved (Yoon *et al.* 2006; Verbruggen *et al.* 2010). These five classes represent about 1 % of the total number of described red algal species. The remaining species are typically multinucleate and classified within the Florideophyceae, a large class of 27 currently recognized orders falling within five subclasses represented by clades that terminate long, basally positioned branches in molecular phylogenies with specific synapomorphic pit plug characteristics (Saunders and Bailey 1997; Le Gall and Saunders 2007; Verbruggen *et al.* 2010).

The traditional view that the Acrochaetiales are the most primitive and the Ceramiales are the most highly derived of the florideophycidean red algal orders (Kylin 1956; Dixon 1973) is not supported by molecular data (e.g. Le Gall and Saunders 2007; Verbruggen *et al.* 2010). A more complex phylogenetic model is emerging for red algae, characterized by ancient lineages often terminating in modern radiations (Yoon *et al.* 2006; Le Gall and Saunders 2007; Verbruggen *et al.* 2010).

New availability of both a DNA C-values database (Kapraun *et al.* 2004) and consensus higher-level phylogenies has opened the way for determining evolutionary trends in DNA amounts for other red algae (Kapraun 2005). The present static microspectrophotometric investigation of additional species of red algae was initiated to determine the extent of nuclear DNA content variation, to identify any correlation between genome size and phylogenetic relationships, and to corroborate an alternation of haploid and diploid nuclear DNA contents in gametophyte and sporophyte tissue, respectively, of selected species.

Materials and methods

Species collection data and/or source of cultures for newly reported data are summarized at http://people.uncw.edu/kapraund/DNA (see links to 'Rhodophyta'). Algal material was fixed in Carnoy's solution (Kapraun 2005) and stored in 70 % ethanol at 4 °C. Selected specimens were rehydrated in water and softened in 5 % w/v ethylenediaminetetraacetic acid (Goff and Coleman 1986, 1987, 1990) for 12–48 h. Algal specimens were transferred to coverslips treated with subbing solution, and then air dried and stained with DAPI (4′,6-diamidino-2-phenylindole) (0.5 μg mL^{-1}) (Sigma

Chemical Co., St Louis, MO, USA) as previously described (Goff and Coleman 1986, 1987, 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.* (2004), 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, Ontario, Canada) (Gómez *et al.* 2010). For a comprehensive review of the theory and practice of DNA quantification by densitometry, see Hardie *et al.* (2002).

Nuclear DNA contents of algal specimens were estimated by comparing their I_f values with those of chicken erythrocytes (RBC) (Kapraun 1994; Kapraun and Dunwoody 2002). The rationale for accepting 2C DNA = 2.4 pg as the standard is included in 'Notes on Appendix I, Section (f)' [see Additional Information]. 4′,6-Diamidino-2-phenylindole 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 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 Rhodophyta indicate values in the range of 35–42 mol% G + C (Freshwater *et al.* 1990; Le Gall *et al.* 1993; Kapraun *et al.* 1993*a*, 1996). Members of the Rhodophyta 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 Rhodophyta include taxa with some or all of their cells being multinucleate or endopolyploid (Kapraun and Nguyen 1994; Kapraun 2005) as well as taxa that exhibit a nuclear 'incremental size decrease associated with a cascading down of DNA contents' (Kapraun 1994). Methodologies were developed for specific specimens to permit assignment of C level and interpretation of I_f data. However, 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.

Previously unpublished nuclear DNA content data in Appendix I 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 (pg) ± SD are available at http://people.uncw.edu/kapraun/DNA. Nuclear DNA content data are also 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

DNA content by group

Red algal DNA contents are presented following the two subphylum classification schemes of Yoon *et al.* (2006) and florideophycean classification of Saunders and Hommersand (2004) and Le Gall and Saunders (2007).

Cyanidiophyceae The Cyanidiophyceae are small, unicellular, anciently diverged red algae (Barbier *et al.* 2005; Coppin *et al.* 2005) whose evolutionary relationships remain a subject of controversy (Garbary *et al.* 1980; Gabrielson *et al.* 1985; Seckbach 1999; Müller *et al.* 2001*a*; Yoon *et al.* 2006). Molecular data support their placement as sister to the other Rhodophyta (Saunders and Hommersand 2004; Yoon *et al.* 2006), but the unique attributes of these organisms (Coppin *et al.* 2005) provide a sense that the cyanidiophytes are as distinct from other red algae as are phyla in the plant and animal kingdoms relative to one another (Saunders and Hommersand 2004; Yoon *et al.* 2004, 2006). Putative synapomorphies of the cyanidiophytes include a blue-green colour resulting from the presence of α-chlorophyll and C-phycoerythrin, complete lack of the red phycoerythrins (De Luca *et al.* 1978) and an ability to inhabit hot, acidic waters (acidothermophilic) (Seckbach 1999). The cyanidiophytes are reported to have the smallest known genomes of any phototrophic eukaryotes (Matsuzaki *et al.* 2004). Pulse-field gel electrophoresis (PFGE) and Feulgen microspectrophotometry with *Saccharomyces cerevisiae* Meyen *ex* Reess standard have yielded 1C nuclear genome size estimates of 10–16 Mbp (Suzuki *et al.* 1992; Maleszka 1993; Matsuzaki *et al.* 2004; Barbier *et al.* 2005) and $1.35–2.25 \times 10^{-2}$ pg (Muravenko *et al.* 2001) in eight isolates and species of these extremophile algae [see Additional Information]. The size of the nuclear genome in *Cyanidioschyzon* reported in the last decade has doubled as a result of progress in measuring techniques (Matsuzaki *et al.* 2004). It is assumed that these recent values are more accurate. Consequently, earlier nuclear genome size estimates listed here should be treated with caution.

Porphyridiophyceae, Stylonematophyceae, Compsopogonophyceae and Rhodellophyceae These classes were traditionally included with the Bangiophyceae in a group variously classified as a subphylum or subclass. Early molecular studies indicated that this was a polyphyletic grouping of distinct lineages (e.g. Freshwater *et al.* 1994; Müller *et al.* 2001*b*), and recent studies have assigned these lineages to separate classes (e.g. Saunders and Hommersand 2004; Yoon *et al.* 2006). The Porphyridiophyceae consists solely of unicellular forms including *Porphyridium* and *Flintiella*, while unicellular and pseudofilamentous taxa such as *Rhodosorus*, *Stylonema* and *Goniotrichopsis* make up the Stylonematophyceae (West *et al.* 2005; Yoon *et al.* 2006). The Compsopogonophyceae includes taxa of various morphologies that have been treated as separate families (Rintoul *et al.* 1999) or orders (Silva *et al.* 1996), but which form a monophyletic lineage in most analyses (e.g. Yoon *et al.* 2006; Verbruggen *et al.* 2010). The Rhodellophyceae is another group of primarily unicells such as *Dixoniella* and *Rhodella*. Relationships among these lineages are similar in the concatenated multilocus DNA sequence analyses of Yoon *et al.* (2006) and Verbruggen *et al.* (2010), but these relationships are poorly supported.

Few nuclear DNA content estimates are available for members of these early diverging lineages, and the current values are all relatively small (Fig. 1). Two species exemplifying these low values are *Compsopogon caeruleus* (Balbis ex C. Agardh) Montagne (Compsopogonales, Compsopogonophyceae) with a 2C DNA content of 0.2 pg and a reported chromosome complement of $1n = 7 \pm 1$ (Nichols 1964), and *Erythrotrichia carnea* (Dillwyn) J. Agardh (Erythropeltidales, Compsopogonophyceae) with a 2C DNA content of 0.7 pg. These data are consistent with a basal (ancestral) red algal genome characterized both by small genome sizes and small chromosome complements. In addition, the small range of the nuclear DNA content values in these early diverging lineages (0.1–0.7; Fig. 1) suggests that the long evolutionary separation of these lineages was not accompanied by substantial changes in DNA content [see Additional Information].

Bangiophyceae This class, as presently understood is monophyletic and includes 15 currently recognized extant genera (some still unnamed) (Sutherland *et al.* 2011). The chief characteristic used to separate the familiar genera *Bangia* and *Porphyra*, e.g. filament vs. blade, lacks taxonomic significance as these morphologies arose independently several times throughout the evolutionary diversification of the Bangiales (Oliveira *et al.* 1995; Müller *et al.* 2001*a*, *b*; Broom *et al.* 2004; Jones *et al.* 2004; Milstein and de Oliveira 2005). Molecular data do not support the distinction of *Bangia* and *Porphyra* as monophyletic genera, and analyses of these data have resulted in the transfer of a majority of species previously placed in *Porphyra*, including species of commercial value, to new genera (e.g. Nelson *et al.* 2006; Sutherland *et al.* 2011). DNA sequence analyses also suggest that the simple morphology of these organisms obscures significant levels of genetic diversity, including the presence of morphologically cryptic species (Klein *et al.* 2003; Müller *et al.* 2003; Nelson *et al.* 2003; Sutherland *et al.* 2011). Recently, two species of *Porphyra* were transferred to two new genera, *Pyrophyllon* and *Chlidophyllon*, which are included in the Erythropeltidales (Compsopogonophyceae) (Nelson *et al.* 2003). In addition to clarifying taxonomic classifications and identifying cryptic species, molecular data have been useful in recognizing the conspecific status of some widely distributed species (Broom *et al.* 2002; Neefus and Brodie 2009), including *Pyropia suborbiculata* (Kjellm.) J.E. Sutherl., H.G. Choi, M.S. Hwang *et* W.A. Nelson and *Pyropia elongata* (Kylin) Neefus *et* J. Brodie, which we investigated previously as *Porphyra carolinensis* Coll *et* J. Cox and *Porphyra rosengurtii* Coll *et* J. Cox, respectively (Kapraun and Freshwater 1987; Kapraun *et al.* 1991; Kapraun 2005). Transfer of the *Pyropia spiralis* (E.C. Oliveira *et* Coll) M.C. Oliveira, D. Milstein *et* E.C. Oliveira variety previously studied as *Porphyra spiralis* var. *amplifolia* E.C. Oliveira *et* Coll is needed after the generic reclassification of Sutherland *et al.* (2011) and is effected here:

Pyropia spiralis (E.C. Oliveira *et* Coll) M.C. Oliveira, D. Milstein *et* E.C. Oliveira var. *amplifolia* (E.C. Oliveira *et* Coll) Freshwater *et* Kapraun comb. nov.

Basionym: *Porphyra spiralis* var. *amplifolia* E.C. Oliveira and Coll (1975: p. 196, Figs 3, 10).

In the eight isolates of *Bangia*, *Porphyra* and *Pyropia* investigated, neither estimates of 2C nuclear DNA contents, which range from 0.6 to 1.2 pg, nor published chromosome complements, which range from $1n = 3–5$, appear to be genus specific (Kapraun 2005). The representation of these species and isolates in current phylogenetic studies (e.g. Sutherland *et al.* 2011) is insufficient to determine whether there is any relationship between nuclear DNA content and evolutionary patterns of the various Bangiophyceae lineages.

Florideophyceae—Nemaliophycidae The Florideophyceae includes five currently recognized subclasses (Saunders and Hommersand 2004; Le Gall and Saunders 2007). DNA content estimates are available for representatives of the Nemaliophycidae, Corallinophycidae and

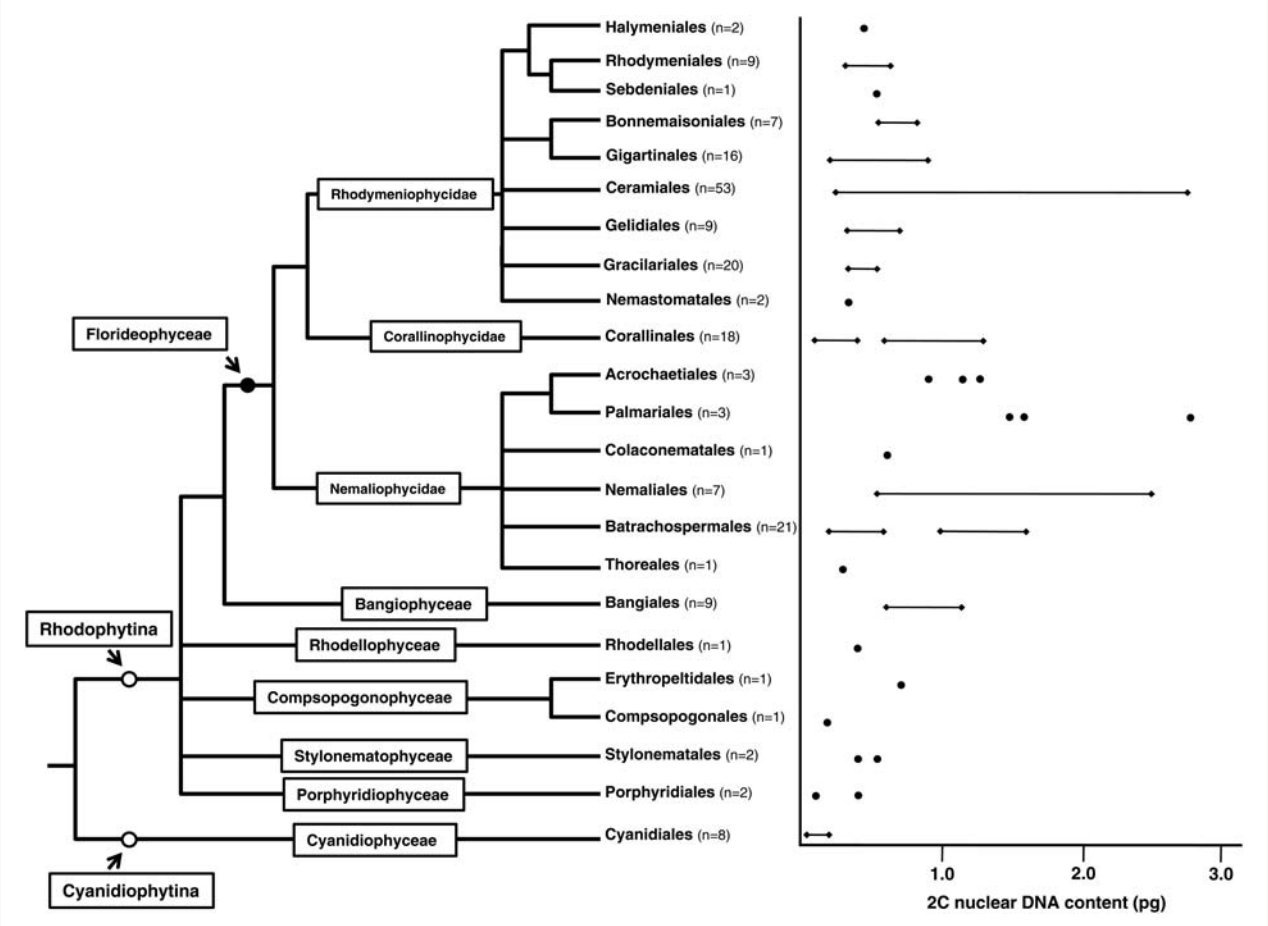

Fig. 1 Estimated 2C nuclear DNA contents superimposed on a consensus red algal phylogeny. Phylogeny based on the analyses of Yoon *et al.* (2006), Le Gall and Saunders (2007) and Verbruggen *et al.* (2010) with unsupported nodes in these analyses collapsed to polytomies. Branch labels follow the two-subphylum classification of Yoon *et al.* (2006) and florideophycean classification of Saunders and Hommersand (2004) and Le Gall and Saunders (2007). Dots represent individual DNA content estimates; lines represent the range of values for multiple species.

Rhodymeniophycidae. The remaining two subclasses, Ahnfeltiophycidae and Hildenbrandiophycidae, while evolutionarily distinct, include only a small number of species. Genome size estimates are available for six of the nine currently recognized Nemaliophycidae orders, and the group is characterized by a nuclear 2C DNA content range of 0.2–2.8 pg (Fig. 1).

Acrochaetiales, Palmariales, Colaconematales, Nemaliales. Molecular systematic investigations have resolved a close relationship among members of the Acrochaetiales, Palmariales, Colaconematales and Nemaliales (Saunders *et al.* 1995; Harper and Saunders 2002; Huisman *et al.* 2004), which are considered to represent early lineages of florideophytes. The transfer of *Rhodothamniella floridula* (Dillwyn) Feldmann from the Acrochaetiales (Saunders *et al.* 1995) and segregation of the Colaconematales (Harper and Saunders 2002) has resulted in a monophyletic Acrochaetiales that is sister to the Palmariales (e.g. Clayden and Saunders 2010). Although the relationships of the Colaconematales and Nemaliales are poorly resolved in large red algal phylogenies (Le Gall and Saunders 2007; Verbruggen *et al.* 2010), the more specific analysis of Clayden and Saunders (2010) indicates a sister relationship between these orders.

Nuclear DNA content data have been published for only one species of the Colaconematales, *Colaconema daviesii*, with 2C DNA = 0.6 pg, and are limited to two species of *Audouinella* in the Acrochaetiales [see Additional Information]. Data available for species of the Nemaliales suggest that this order is characterized by one of the

largest ranges of DNA contents (2C = 0.5–2.5 pg) of any of the florideophytes (Fig. 1). The Palmariales, as presently delimited (Clayden and Saunders 2010), includes three genera for which nuclear DNA content estimates are available: *Devaleraea* (Guiry 1982), *Palmaria* (Guiry 1974) and *Rhodothamniella* (Saunders *et al.* 1995). A 2C range of 1.5–2.8 pg gives the Palmariales the largest mean nuclear DNA size in the florideophytes.

Batrachospermales/Thoreales. Until recently, freshwater red algae belonging to the genera *Thorea* and *Nemalionopsis* were included in the order Batrachospermales (Kumano 2002). Small subunit ribosomal DNA and *rbc*L sequence analyses indicate that the Thoreaceae has been misclassified in the Batrachospermales and should be placed in its own order, the Thoreales (Müller *et al.* 2002). Species in this order are characterized by having freshwater representatives with multiaxial gametophytes, a uniaxial chantransia stage and pit plugs with two cap layers, the outer one of which is usually plate like. Nuclear DNA content estimate data are available for only one species in this order, *Thorea riekei*, with 2C = 0.28 pg [see Additional Information]. The Batrachospermales is distinguished from other freshwater rhodophyte orders based on a heterotrichous life history phase, lack of tetraspore production, and a two-layered pit plug, the outer layer of which is domed (Vis *et al.* 1998).

The Batrachospermales and the Thoreales are of particular interest as they are exclusively freshwater (Vis and Sheath 1997) in the Nemaliophycidae clade, which includes several additional orders that are primarily or at least partially freshwater: Acrochaetiales, Balbiniales, Balliales (Harper and Saunders 2001; Müller *et al.* 2001*a*, *b*; Le Gall and Saunders 2007). As members of the distantly related Compsopogonales (Compsopogonophyceae) are primarily freshwater as well (Sheath 1984; Müller *et al.* 2002), it is likely that adaptation to freshwater habitats involved multiple, independent events in the evolution of red algae.

The genus *Batrachospermum* appears to be polyphyletic, comprising many morphologically similar but distantly related taxa (e.g. Chiasson *et al.* 2007; Kapraun *et al.* 2007). Species of *Batrachospermum*, *Sirodotia* and *Tuomeya* (Batrachospermaceae) investigated in the present study have 2C nuclear DNA contents of about 0.2–0.6 pg, while species of *Lemanea* and *Paralemanea* (Lemaneaceae) have noticeably larger 2C genome sizes of 1.0–1.6 pg [see Additional Information]. Results of this study suggest a possible correlation between polyploidy and the expression of the *Batrachospermum* or *Lemanea* morphological phenotypes.

Published karyological studies for Batrachospermaceae indicate that most species have chromosome numbers in the range of $1n = 3–5$ or 10–12, while Lemaneaceae species have chromosome complements of $1n = 15–20$ (Kapraun *et al.* 2007). Both the larger genome sizes and chromosome complements in *Lemanea* and *Paralemanea* are consistent with polyploidy events in their common ancestry.

A unique pattern of somatic meiosis has been described in members of this order associated with development of haploid gametophytes from vegetative branches of the microscopic, diploid sporophyte phase (Necchi and Carmona 2002). The sporophyte phase has been described variously as '*Chantransia*' (Chiasson *et al.* 2005), *Audouinella* (Necchi and Zucchi 1997) and, possibly, *Balliopsis* (Saunders and Necchi 2002). Support for this life history comes from both cytological (von Stosch and Theil 1979; Necchi 1987) and microspectrophotometry (Sheath *et al.* 1994, 1996) investigations. In the present study, DAPI and microspectrophotometry demonstrated in isolates of three species (*Batrachospermum gelatinosum*, *Batrachospermum vagum* and *Lemanea torulosa*) I_f (fluorescence) levels in 2C nuclei in presumptive gametophytes that closely approximate 50 % of the 4C values in presumptive sporophytes.

Florideophyceae—Corallinophycidae Past molecular systematic investigations resolved the Corallinales as a lineage within the larger group of taxa that share the presence of pit plugs with two cap layers and were classified as the Nemaliophycidae (Saunders and Bailey 1997; Harper and Saunders 2002; Saunders and Hommersand 2004). The recent multigene study of Le Gall and Saunders (2007) demonstrated that the Corallinales and Rhodogorgonales represented a separate evolutionary lineage from the Nemaliophycidae, and established the Corallinophycidae. The analyses of Verbruggen *et al.* (2010) supported this classification and the inclusion of the Sporolithales in this subclass as suggested by Le Gall and Saunders (2007).

Nuclear DNA content data are only available from species in the Corallinales where 2C DNA contents range from 0.1 to 1.3 pg (Fig. 1) [see Additional Information]. Coralline algae can be divided into two types: geniculate (with alternating calcified internodes and uncalcified nodes) and non-geniculate (which usually grow as crusts) (e.g. 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, 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 (0.1–1.0 pg) (Kapraun 2005). Analysis of additional species will be required to determine whether these observations reflect a sampling artefact.

Florideophyceae—Rhodymeniophycidae Ordinal classification within the Rhodymeniophycidae continues to be refined, in large part as a result of distinct evolutionary lineages being recognized within the large, polyphyletic Gigartinales (e.g. Withall and Saunders 2006). The subclass currently includes 12 orders (Saunders and Hommersand 2004; Guiry and Guiry 2011), with DNA content estimates available for species in nine of these (Fig. 1). While the overall 2C DNA content range of 0.2–2.8 pg is relatively wide, five of the orders (Gelidiales, Gigartinales, Gracilariales, Halymeniales and Rhodymeniales) have particularly narrow ranges of DNA contents [see Additional Information].

Gelidiales. The relatively narrow range of small DNA content values but substantial range of chromosome numbers (Kapraun and Bailey 1989; Freshwater 1993; Kapraun *et al.* 1993*b*, 1994), and the absence of a correlation between nuclear genome size and chromosome number suggest a significant role of aneuploidy in Gelidialean evolution (Kapraun and Dunwoody 2002). Analyses of DNA sequence data from a variety of loci have resulted in a consistent molecular phylogeny for the Gelidiales (e.g. Freshwater and Bailey 1998; Shimada *et al.* 1999; Thomas and Freshwater 2001; Tronchin and Freshwater 2007). This well-circumscribed order includes only a handful of genera, but is particularly species rich (e.g. Millar and Freshwater 2005), and it would be very interesting to explore the possible role of aneuploidy in their evolution by obtaining additional chromosome and genome size data for representative species.

Bonnemaisoniales. This order was separated from the Nemaliales on the basis of their then known alternation of generations (Feldmann and Feldmann 1942). It is now known that this life history pattern lacks taxonomic significance as some Nemaliales are heteromorphic and some Bonnemaisoniales are isomorphic (Womersley 1996). For example, *Bonnemaisonia asparagoides* (Woodward) C. Agardh is monoecious and has a direct life history with no tetrasporophyte, while *Bonnemaisonia clavata* G. Hamel is dioecious and has an alternation of heteromorphic generations with '*Hymenoclonium serpens*' representing the tetrasporophyte (Salvador Soler *et al.* 2008). The Bonnemaisoniales are currently recognized at the ordinal level on the morphological basis of their apical development pattern and direct development of the gonimoblast. Ultrastructural details of pit plugs and caps (Pueschel 1989) and plastids (Chihara and Yoshizaki 1972), as well as molecular studies (e.g. Saunders *et al.* 2004; Le Gall and Saunders 2007), appear to support retention of this order.

In a recent study (N. Salvador Soler, University of Barcelona, Barcelona, Spain, unpubl. res.) and the current study, nuclear DNA content data for '*Falkenbergia rufolanosa*', the diploid sporophyte phase of the heteromorphic species *Asparagopsis armata*, and for the isomorphic species *Delisea plumosa* and *Ptilonia willana*, suggest 2C values for members of this order of 0.5–0.6 pg. Nuclear DNA content data for both phases of the heteromorphic species in the Bonnemaisoniales are needed to confirm that ploidy level shifts (2*n*/4*n*) are associated with the gametophyte and sporophyte phases, respectively.

Ceramiales. The Ceramiales is the largest red algal order, with close to 400 genera and 1500 species described (Kraft and Woelkerling 1990; Schneider and Wynne 2007; Wynne and Schneider 2010). Genome size data are available for fewer than 2 % of these species [see Additional Information]. Members of this order have both the largest DNA contents and the greatest range of DNA content values (0.26–2.8 pg). Past molecular systematics investigations indicate that the traditional ceramialean families, Dasyaceae, Delesseriaceae and Rhodomelaceae, evolved from a paraphyletic Ceramiaceae (de Jong *et al.* 1998; Phillips 2000; Lin *et al.* 2001; Choi *et al.* 2002; Zuccarello *et al.* 2002; Barros-Barreto *et al.* 2006). Choi *et al.* (2008) proposed a new taxonomy for the Ceramiales that split the paraphyletic traditional Ceramiaceae into the Ceramiaceae *sensu stricto* and three new families, Callithamniaceae, Spyridiaceae and Wrangeliaceae (Fig. 2). 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 <1.0 pg as well as species with elevated (polyploid?) DNA contents (Fig. 2). The simplest explanation is that polyploidy, characterized by even number multiple increases 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, although analysis of

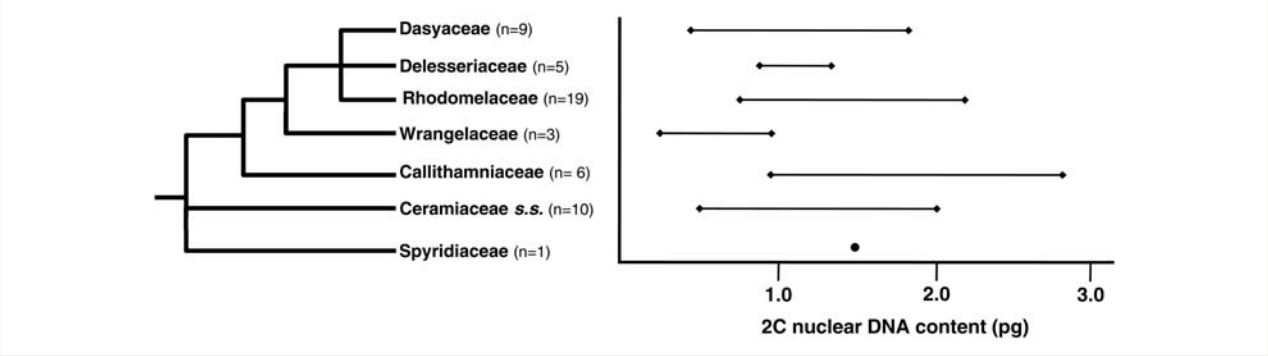

Fig. 2 Estimated 2C nuclear DNA contents superimposed on a family phylogeny for the Ceramiales. Phylogeny based on analyses of Choi *et al.* (2008) with unsupported nodes collapsed to polytomies. Dots represent individual DNA content estimates; lines represent the range of values for multiple species.

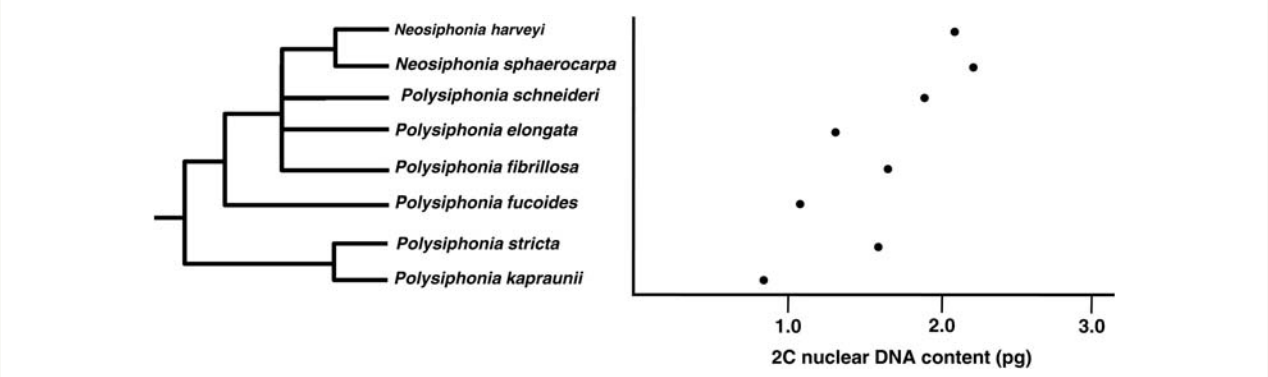

Fig. 3 Estimated 2C nuclear DNA contents superimposed on a phylogeny of *Neosiphonia* and *Polysiphonia* species. Phylogeny based on the analyses of Mamoozadeh and Freshwater (2011).

additional species will be required to eliminate the possibility that our observations reflect sampling error. 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 as described for species of the Rhodomelaceae genus *Polysiphonia* (Kapraun 1993*a*).

Molecular systematic investigations have demonstrated the paraphyly of *Polysiphonia sensu lato* (e.g. Choi and Kim 2001; Stuercke and Freshwater 2010; Mamoozadeh and Freshwater 2011), especially in relation to the recently described genus *Neosiphonia* (Kim and Lee 1999). The currently available data are insufficient to explore any relationships of genome size and species evolution (Fig. 3). However, now that a more accurate understanding of phylogenetic relationships is emerging, it would be of interest to determine whether nuclear genome sizes and chromosome complements have diagnostic value in delimiting the monophyletic species groups being revealed within *Polysiphonia sensu lato*.

Recent data suggest that Ceramiales are an ancient lineage relative to other Rhodymeniophycidae (Le Gall and Saunders 2007; Verbruggen *et al.* 2010), yet on average they have larger nuclear genome contents than most of the taxa that are believed to have diverged after them. Unless an assumption is made that the other taxa in the Rhodymeniophycidae lineage have experienced nuclear genome size decrease, an explanation is required to account for the larger genome sizes in the Ceramiales.

Although the existence of mechanisms for decreasing DNA amounts have 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 Rhodymeniophycidae 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.

Gigartinales. The Gigartinales is a large and diverse order (Fredericq *et al*. 1996; Hommersand *et al*. 1999; Tai *et al*. 2001; Saunders *et al*. 2004) including commercially important carrageenophytes such as *Eucheuma*, *Kappaphycus* and *Chondrus* (Craigie 1990; Kapraun 1999). Present results confirm previous studies (Kapraun *et al*. 1992; López-Bautista and Kapraun 1995; Kapraun and López-Bautista 1997), suggesting that members of this order are characterized by a wide range of chromosome numbers ($2n = 10–70$) and a narrow range of small nuclear DNA contents ($2C = 0.2–0.9$ pg) [see Additional Information]. The genome size (1C) of *Chondrus crispus* was estimated as 150 Mbp using flow cytometry of haploid nuclei (Le Gall *et al*. 1993), but recent complete sequencing of this genome indicates a size of only 105 Mbp (Collén 2011) concordant with previous estimates using static microspectrophotometry (Kapraun 2005). This relatively small size and the species' economic importance made *Chondrus* an ideal candidate among carrageenophytes for genome sequencing.

Halymeniales. The Halymeniales is a relatively large order of 270+ species classified in 26 currently recognized genera (Guiry and Guiry 2011). Currently, 2C DNA content data are only available for two species, *Grateloupia filicina* (Lamouroux) C. Agardh and *Halymenia floridana* J. Agardh, with both having identical values (Fig. 1) [see Additional Information].

Nemastomatales and Sebdeniales. Recent studies have reinstated the Nemastomatales and established the Sebdeniales for species previously part of the Gigartinales (Saunders and Kraft 2002; Withall and Saunders 2006). Although the orders are represented by relatively few species, molecular and morphological analyses reveal additional diversity (e.g. Schneider *et al*. 2006). Currently, estimates of 2C DNA content are only available for two *Predaea* (Nemastomatales) and one *Sebdenia* (Sebdeniales) species (Fig. 1) [see Additional Information].

Gracilariales. This order includes relatively few genera, but some of them, e.g., *Gracilaria*, are species rich (Fredericq and Hommersand 1990). No new nuclear DNA content estimates are available for this order, but previous data indicate that nuclear genome sizes are small (0.3–0.5 pg) (Kapraun 1993*b*, 2005). The Gracilariales, unlike the Gelidiales, is noted for genome size 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) exhibit some variation in both 2C DNA contents (0.3–0.5 pg) and $2n$ chromosome complements with values of $2n = 48$ and 64 reported.

Rhodymeniales. Nuclear DNA content estimates from previous (Kapraun 2005) and present studies are now available for nine species representing three families of the Rhodymeniales (Saunders *et al*. 1999) [see Additional Information]. This order, along with the Gelidiales and Gracilariales, has both a narrow range and a small mean nuclear genome size ($2C = 0.3–0.6$ pg).

Range of DNA contents

The size of the red algal genomes reported here and previously (Kapraun 2005) 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), but still have 1.5–2 times the estimated 15 000 genes per haploid genome required for development (Flavell, 1980). Similarly, the genome of the green alga *Volvox carteri* F. Stein, with 138 Mbp, has an estimated coding potential for 14 500 proteins (Prochnik *et al*. 2010). Even the smallest rhodophyte genome reported (e.g. $1C = 98$ Mb in *Compsopogon coerulus*), with its probable genomic redundancy (Kapraun 2005, 2007), has the genic capacity for morphologically complex development.

Polyploidy

Polyploidy has been reported widely in the Rhodophyta (Cole 1990; Kapraun 2005), especially in the Ceramiales, which have both the largest nuclear genomes and the highest chromosome numbers (Kapraun 1993*a*, 2005; Kapraun and Dunwoody 2002). For a recent review of

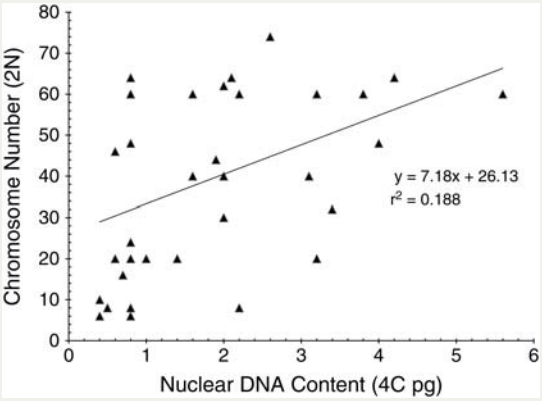

Fig. 4 Comparison of 4C nuclear DNA contents and 2*n* chromosome complements for 33 species of Florideophyceae. DNA content values from the present study and previously published data (Kapraun 2005; Kapraun *et al.* 2007; Salvador Soler *et al.* 2009), and 2*n* chromosome complements from Cole (1990) and Kapraun (2005).

concepts associated with adaptations and genetic variability associated with hybridization and polyploidy in algae, see Coyer *et al.* (2006). Comparison of 2*n* chromosome numbers and 2C nuclear DNA contents shows a poor relationship ($r^2 = 0.188$, Fig. 4), consistent with a high occurrence of aneuploidy, i.e. chromosomal fusion and/or fission events (Kapraun *et al.* 1993*b*; Kapraun 2005). Present results support previous suggestions (Kapraun 1989) that polyploidy and aneuploidy are pervasive features of red algal genomics. The extent of both species-level and intraplant ploidy-level variation (including endopolyploidy) remains to be determined (Goff and Coleman 1986, 1987), but represents an exciting area for future research.

Correlation between DNA content and phylogenetic placement

Although no correlation is apparent between phylogenetic placement and genome size, groups considered to be basal (Cyanidiophytina, Porphyridiophyceae, Stylonematophyceae, Compsopogonophyceae, Rhodellophyceae) generally have genome sizes $\leq$0.5 pg, while derived groups (Bangiophyceae, Florideophyceae) generally have genome sizes $\geq$0.5 pg, with values up to 2.8 pg reported. DNA contents may be diagnostic, synapomorphies in both the Corallinales and Batrachospermales. In the Corallinales (Kapraun 2005), 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 (0.1–1.0 pg), the overlap in these ranges is a result of single outlier species. Similarly, in the Batrachospermales, species of *Batrachospermum*, *Sirodotia* and *Tuomeya* have 2C nuclear DNA contents of 0.2–0.6 pg while species of *Lemanea* and *Paralemanea* have noticeably larger 2C genome sizes of 1.0–1.6 pg (Kapraun *et al.* 2007) [see Additional Information]. More definitive trends may be revealed as data for nuclear genome size and our understanding of red algal evolutionary relationships increase.

Correlation between DNA content and habitat

It is likely that adaptation to freshwater habitats involved multiple, independent events in the evolution of red algae. In the present study, no correlation between nuclear genome size and adaptation to freshwater habitats is apparent in the Compsopogonales, Thoreales and Batrachospermales.

Correlation between nuclear genome size and reproductive parameters

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: (i) increase in genome size was positively correlated with increase in carpospore volume; (ii) species with larger genome sizes produced fewer carpospores; and (iii) species that produced 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. 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).

Characteristics of an ancestral red algal genome

In the present study, cyanidophytes represent the earliest diverging red algal lineage and have reported genome sizes of 1C = 0.02–0.1 pg (1C = 10–55 Mbp) and 2*n* chromosome numbers between 4 and 20 [see Additional Information]. These genomic characteristics recommend this group for further investigations that could possibly help characterize the nuclear genome in unicellular organisms prior to the transition to multicellularity seen in other red algae. Among the basal Rhodophytina, members of the Compsopogonophyceae,

Porphyridiophyceae and Stylonematophyceae may represent appropriate candidates for investigations of nuclear genomes in extant, basal red algae. For example, *Compsopogon caeruleus* has a 2C DNA content of 0.25 pg (1C = 98 Mb) and a reported chromosome complement of $1n = 7 \pm 1$ (Nichols 1964). Small genome size and chromosome complements have been reported in *Porphyridium aerugineum* Geitler ($n = 2$) and *P. purpureum* (Bory de Saint-Vincent) K.M. Drew & R. Ross [as *P. cruentum* (S.F. Gray) Nägeli] ($n = 2$; 2C DNA content = 0.1 pg) (Sommerfeld and Nichols 1970), although it remains unclear whether these represent haploid or diploid values.

Candidates for genomic studies

DNA C-value remains a key character in biology, biodiversity and molecular investigations as genome size has many important practical implications (Bennett *et al.* 2000). Genome size directly influences the cost and difficulty of sequencing projects, and was a primary consideration in choosing subjects for early whole-genome analyses (Gregory 2001, 2005), including those of algae where small DNA content (haploid genomes ~100 Mbp) has been a major criterion (Peters *et al.* 2004; Waaland *et al.* 2004). Despite major improvements in sequencing cost and efficiency provided by current next-generation sequencing technology, genome size is still a consideration for coverage and *de novo* assembly. Many red algal species have haploid genomes in the range of 127–300 Mbp [see Additional Information], and the present study provides a list of target species with small genome sizes for whole-genome sequencing studies. Many of these species (e.g. Gelidiales and Gracilariales) are also amenable to culture and are of significant ecological and/or commercial importance (López-Bautista and Kapraun 1995; Kapraun and López-Bautista 1997; Kapraun 1999).

Conclusions and forward look

Early diverging red algal lineages are characterized by relatively small 2C DNA contents while a wide range of 2C values is found within the derived Florideophyceae. An overall correlation between phylogenetic placement and 2C DNA content is not apparent; however, genome size data are available for only a small portion of red algae. Current data do support polyploidy and aneuploidy as pervasive features of red algal genome evolution.

Red algae that warrant further investigation include the Nemaliales, Acrochaetiales and Colaconematales. Phylogenetic analyses indicate that these three orders are part of early diverging florideophycean lineages (e.g. Le Gall and Saunders 2007), are widely distributed and contain many genera that are species rich (Saunders *et al.* 1995; Harper and Saunders 1998), yet published information about their genome sizes is very limited. It would be of interest to determine whether the relatively wide range of DNA contents found in the Nemaliales occurs in these other related orders.

Another group of red algae that warrant attention is the Ceramiales, especially the Rhodomelaceae, which may include more species than all other red algae combined. Continuing molecular phylogenetic investigations provide us with evolutionary schemes (e.g. Martin-Lescanne *et al.* 2010; Mamoozadeh and Freshwater 2011) upon which genome size data can be superimposed to reveal the extent that speciation was accompanied by nuclear transformations.

Additional information

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

File 1. Appendix I—Chromosome numbers and nuclear DNA content estimates in isolates and species of red algae.

File 2. Notes on Appendix I.

File 3. Numbered references for chromosome complements and DNA content estimates in the Rhodophyta 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). D.W.F. (Center for Marine Science, University of North Carolina Wilmington, USA) and D.K. contributed algal cultures and/or field-collected materials, and prepared the manuscript for publication.

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Conflict of interest statement

None declared.

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