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ARTICLE

Nuclear DNA content in *Gelidium chilense* (Gelidiales, Rhodophyta) from the Chilean coast

Contenido de ADN nuclear en *Gelidium chilense* (Gelidiales, Rhodophyta) de la costa chilena

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Resumen.- Durante los últimos años se ha producido un notable progreso en el número de registros sobre el tamaño del genoma para el grupo de las plantas. Sin embargo, todavía se requiere más información. Concretamente, en el caso de las algas rojas (Rhodophyta), de las ~7.000 especies descritas hasta la fecha, sólo existen datos para 196 (~3%). Esta investigación representa la primera estimación del tamaño del genoma del alga roja endémica del Pacífico Sudeste *Gelidium chilense*, proporcionando además características nucleares de la especie tales como tamaño y número por célula. Los contenidos de ADN nuclear fueron estimados a partir de las observaciones realizadas en 153 núcleos teñidos con DAPI. Las células de *G. chilense* mostraron una variación del contenido de ADN nuclear intraplanta de 0,2-4,0 pg. En total, 6 niveles de ploidía fueron observados en esta especie. El nivel 1C fue observado solo en las células corticales mientras que el mayor nivel de ploidía (16C) fue observado en los tetrasporangios. Los valores obtenidos en los tetrasporangios indicaron que el tamaño del genoma aumenta durante la tetrasporogenesis mediante endopoliploidía (desde 4C a 16C). Por otra parte, el menor nivel de ploidía observado en las tetrasporas correspondió al 3C, lo que confirma la hipótesis de que la meiosis no ocurre en los esporangios de *G. chilense*. Este trabajo de investigación contribuye al conocimiento de las estrategias reproductivas relacionadas con el ciclo biológico en las especies del orden Gelidiales.

Palabras clave: Contenido de ADN nuclear, endoreduplicación, *Gelidium*, ciclo biológico, poliploidía

Abstract.- There has been progress in novel additions of algal data to the Plant DNA C-values database during recent years; however more information is still required. Specifically, in the case of red algae (Rhodophyta), from ~7000 species described up to date, DNA C-values for only 196 species have been incorporated (~3%). This research represents the first estimation of genome size for the Southeast Pacific endemic red alga *Gelidium chilense* and provides nuclear features such as number per cell and size. Nuclear DNA content estimates were obtained from measurements of 153 DAPI-stained nuclei. The cells of *G. chilense* showed intra-plant variation with DNA content values ranging from 0.2-4.0 pg and a total of 6 ploidy levels were found. The lowest level (1C) was observed only in outer cortical cells whereas tetrasporangia displayed the highest levels (16C). The nuclear DNA contents obtained in tetrasporangia indicated that the genome size increases during tetrasporogenesis by endopolyploidy (from 4C to 16C). In addition, the minimum value observed in tetraspores corresponds to a 3C. Our results confirm the hypothesis that meiosis does not occur within the sporangia in *G. chilense*. This study contributes to knowledge of reproductive strategies related with the life history of Gelidiales.

Key words: DNA content, endoreduplication, *Gelidium*, life history, polyploidy

INTRODUCTION

Seaweeds are key components of coastal ecosystems and are economically important as food and as a source of gelling agents because of their polysaccharide content. However, genomic information such as genome size and whole genome sequences for these organisms is scarce (Kapraun 2005, Kapraun & Freshwater 2012). Therefore, many of the molecular mechanisms related to their life history or others traits remain unresolved (Nakamura *et al*. 2013). Algal genes and genome knowledge is crucial for the understanding of the evolution of the photosynthetic life in general. Furthermore, genomic data are important to ensure a sustainable aquaculture of macroalgae (Browdy *et al*. 2012).

The amount of nuclear DNA in a cell is usually referred as the genome size or C-value. This represents multiples of the minimum amounts of DNA corresponding to the non-replicated haploid chromosome complement (Greilhuber *et al*. 2005). Interest in this genomic feature began during the late 1940s when researchers started to measure and compare DNA amounts within and between plants and animals (Swift 1950, García *et al*. 2013). Although the first C-values estimates were obtained using tedious and complicated chemical extraction methods, new techniques (Feulgen microdensitometry, flow cytometry and DNA image cytometry) have made estimating DNA amounts easier and faster[1]. Interest in such data, as well as, the number of newly estimated C-values published has increased in recent years (Bennett & Leitch 2011).

Information about C-values is used in a wide range of biological fields (*e.g.*, see Bennett *et al*. 2000 or Bennett & Leitch 2011), and genomic information for algae has provided a wealth of information about the unicellular origin of higher plants and evolution of photosynthetic eukaryotes (Bowler & Allen 2007, Tirichine & Bowler 2011). Indeed, the data are being used for the duration of cell cycle (Francis *et al*. 2008), seed size and mass (Beaulieu *et al*. 2007), plant growth form and distribution (Ohri 2005), leaf cell size and stomatal density (Beaulieu *et al*. 2008, Hodgson *et al*. 2010), patterns of invasiveness (Kubešová *et al*. 2010, Lavergne *et al*. 2010), patterns of genome size evolution (Beaulieu *et al*. 2010, Leitch *et al*. 2010, Leitch & Leitch 2013), and large scale comparative analyses (Levin *et al*. 2005). Moreover, knowledge of nuclear DNA content has practical implications, such as estimating the cost and time for whole genome sequencing projects (Kelly *et al*. 2012) and selecting protocols for DNA fingerprinting studies (Garner 2002).

Concerning Plant DNA C-values[2] land plants are one of the best-studied groups, and estimates for over 8,500 plant species have been described to date. However, the database only includes data for 253 algal species from Chlorophyta, Phaeophyceae and Rhodophyta, representing less than 2% of the described species. In addition, C-values for South American plants are scarce and to date the Chilean Plants Cytogenetic Database[3] includes none algal data. The only study including a Chilean macroalgae to our knowledge was that of Badilla *et al*. (2008), in which they described chromosome numbers and mean values of nuclear DNA fluorescence from different morphotypes of *Pyropia columbina* (Montagne) W.A. Nelson.

Gelidium J.V. Lamouroux, comprised of ca. 125 species, is the largest genus within the Gelidiales (Guiry & Guiry 2014). Some species are valuable economic resources with diverse uses as: food, agar, biofuel and paper pulp (Jeon *et al*. 2005, Seo *et al*. 2010). *Gelidium chilense* (Montagne) Santelices & Montalva is a turf forming alga, endemic to Chile and southern Peru (Hoffmann & Santelices 1997). It is the most common *Gelidium* species in the middle intertidal and shallow subtidal habitats of Central Chile (Santelices *et al*. 1981) and is an economically and ecologically important macroalgae harvested for agar extraction (Santelices 1986). DNA contents have been estimated for only six species of *Gelidium* (Freshwater 1993) and none from the Southeast Pacific. The aim of this paper is to provide the first estimation of genome size for *G. chilense* and determine if meiosis is present in the life history.

Materials and methods

Algal material

Tetrasporic specimens of *Gelidium chilense* were collected from Cocholgüe, Biobío Region, Chile (36°35'38.41''S, 72°58'43.85''W) in June 2014. Samples were collected during low tide at rocky platforms and intertidal pools. Algal material was preserved in Carnoy's fixative (3:1 of 95% ethanol-glacial acetic acid) and stored in 70% ethanol at 4°C (Kapraun 2005). Voucher specimens were deposited at the BCN-Phyc. Herbarium (Documentation Center of Plant Biodiversity, University of Barcelona, Spain).

Microfluorometric analysis

Samples were rehydrated in water and softened in 5% w/v EDTA for 96 h. Algal material was squashed and transferred to coverslips treated with subbing solution and then air dried and stained with 0.5 µg mL^{-1} 4'-6-diamidino-2-phenylindole (DAPI; Sigma Chemical Co., St. Louis, Missouri, USA). Nuclear DNA content estimates based on image analysis of DAPI-stained specimens followed a procedure modified from Kapraun & 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 subsequently analyzed using MetaMorph software (Molecular Devices, Toronto, Canada). The nuclear DNA content parameters of Total Area (relative fluorescence area, in μm^2) and Total Intensity (in relative fluorescence units, rfu) were estimated from microspectrophotometry and image analysis. According to Varela-Álvarez *et al*. (2012), microspectrophotometry followed by image analyses allows the user to observe and differentiate every single data unit obtained. Nuclei from diverse regions of

[1]<http://data.kew.org/cvalues/>
[2]<http://data.kew.org/cvalues/>
[3]<http://www.chileanpcd.com>

the thallus (cortex, medulla) can be identified and checked by optical microscopy before the fluorescence microscope, thus this technique is more rigorous despite having the drawback of being slower than flow cytometry.

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 & Waring 1988). Chicken erythrocytes (RBC) with a DNA content of 2.4 picograms (pg) were used as a standard to quantify nuclear DNA contents (Clowes *et al*. 1983). RBC can be used directly as a standard 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 & Doty 1962). Kapraun *et al*. (1993) determined the % G + C for three Gelidiales species and obtained values that ranged between 35- 42%. This published data indicate similar mean mol % values for algae and linearity is presumed between DAPI-DNA binding in both RBC and algal samples (Le Gall *et al*. 1993).

Nuclear DNA contents were estimated by comparing the total intensity of fluorescence (rfu) values of the RBC standard and algal samples (Kapraun & Nguyen 1994). However, it was not possible to measure the total intensity of the tetraspores nuclear content due to its interference with the auto-fluorescence associated to their cell walls and intracellular granules (Goff & Coleman 1986). Therefore, to observe their nuclei it was necessary to overexpose the cells to the microscope light and to consider in this case the nuclear area. Consequently, the tetraspores nuclear DNA content was estimated by comparing their total area with the RBC standard, as proven useful in previous studies (Salvador *et al*. 2009, Bothwell *et al*. 2010, Varela-Alvarez *et al*. 2012).

Measurements of reproductive cells are considered the best way to determine the numerical relationship between rfu and C-values (Goff & Coleman 1990). Mitotic figures in dividing somatic cells were measured to determine the 1C level in this study because of auto-fluorescence interference in *G. chilense* tetrasporangia.

Nuclear DNA content data obtained herein will be incorporated into the database of plant genome sizes (Kapraun 2005, Gregory *et al*. 2007) compiled and hosted by the Royal Botanic Gardens (RBG) Kew web page.

Data analyses

Data were grouped into different categories of ploidy levels according to the frequency distribution of nuclear DNA contents obtained in the histograms for each specimen or thallus portion. The highest peak in each histogram is established as the G1 (unreplicated nuclei) in the corresponding ploidy level, with the following minor peak with twice the DNA amount as G2 (replicated nuclei). Therefore, data are sorted into groups corresponding to the ploidy levels[4]. Means and standard deviations were calculated for each group.

Results

A total of 580 nuclei (algae and standard together) were localized and measured. 118 nuclei were measured from vegetative thalli portions and 35 from fertile tetrasporic specimens of *G. chilense*. Nuclear DNA content reflects the position of a cell within a cell cycle, and the C-values inferred from the nuclear relative fluorescent units (rfu) measurements represented G_1, S and G_2 phases of the cell population examined. The G_1 and G_2 peaks were represented by a Gaussian function and their C-values were associated to the different ploidy levels specified in Table 1.

DNA content values ranged from 0.2-4.0 pg in cells of *G. chilense* (Table 1, Figs. 1 and 2). Four ploidy levels were determined in vegetative cells (Table 1, Fig. 1). The first peak in the histogram corresponds to the 1C ploidy level, and included

Table 1. Nuclear DNA content with corresponding C levels in different cell types of *Gelidium chilense* / Contenido de ADN nuclear y valores C en diferentes tipos de células de *Gelidium chilense*

	DNA content (pg)		Ploidy level	Cell types	n
	mean	sd			
Vegetative cells					
	0.23	0.04	1C	O	23
	0.4	0.09	2C	O I	56
	0.71	0.11	4C	I M	38
	1.4	-	8C	M	1
Reproductive cells					
	0.6[(a)]	-	3C	Ts	3
	1.1	0.3	4C	Ta	22
	2.3	0.3	8C	Ta	9
	4	-	16C	Ta	1

O, outer cortical cells; I, inner cortical cells; M, medullary cells; Ts, tetraspores; Ta, tetrasporangia; pg, picograms; n, number of nuclei analyzed; [(a)], value obtained by nuclear area

[4]<http://olomouc.ueb.cas.cz/book/analysis-endopolyploidy>

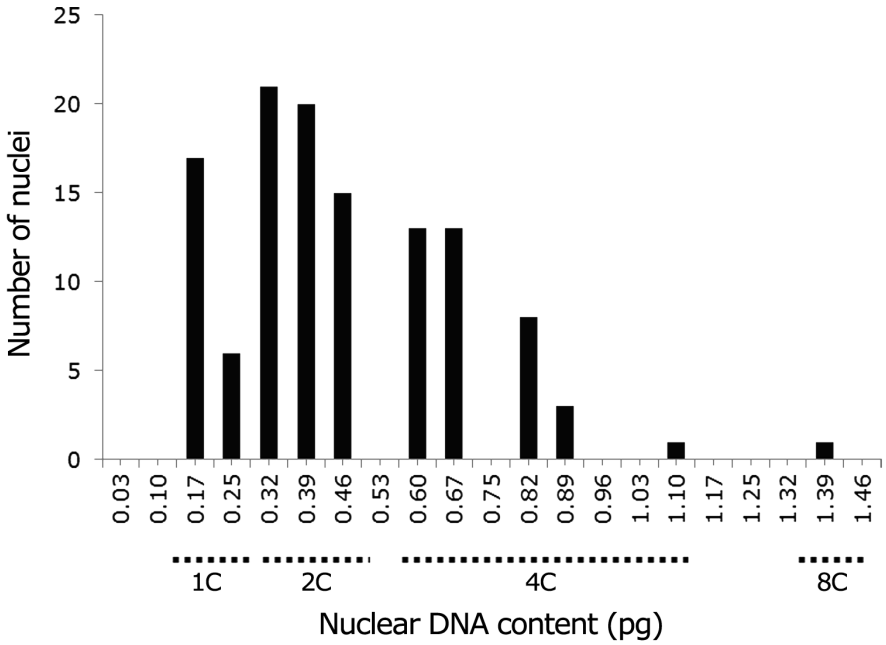

Figure 1. Frequency distribution of nuclear DNA contents and ploidy levels measured from DAPI-stained DNA for vegetative cells of *Gelidium chilense* / Distribución de frecuencias del contenido de ADN nuclear y niveles de ploidía obtenidos en células vegetativas de *Gelidium chilense* teñidas con DAPI

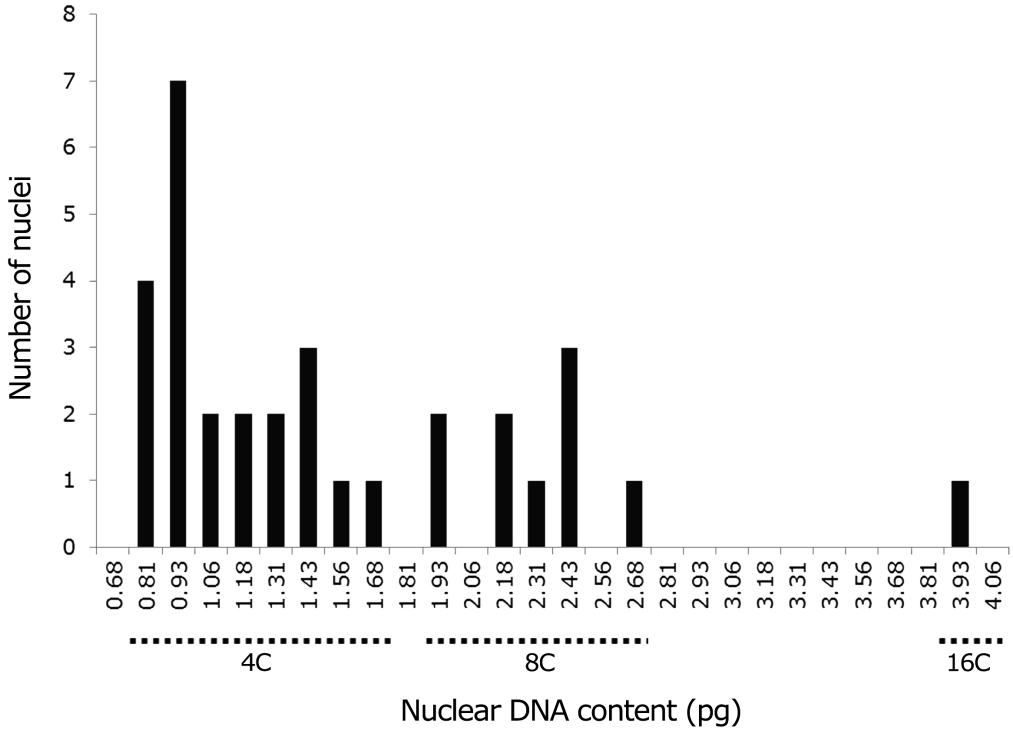

Figure 2. Frequency distribution of nuclear DNA contents and ploidy levels measured from DAPI-stained DNA for tetrasporangia cells of *Gelidium chilense* / Distribución de frecuencias del contenido de ADN nuclear y niveles de ploidía obtenidos en tetrasporangios de *Gelidium chilense* teñidos con DAPI

the nuclei of the outer cortical cells in G1 phase. The second peak corresponds to the 2C level, which included outer cortical nuclei in G2 phase and also inner cortical cell nuclei in G1 phase. The third peak corresponds to the 4C level integrated by inner cortical cell nuclei in G2 phase and the G1 medullary cell nuclei. A minor peak was evident at an intensity corresponding to the G2-phase nuclei of the medullary cells. The cortex was composed by uninucleate outer and inner cortical cells and trinucleate cells were present in the medulla (Fig. 3).

The mean of the nuclear DNA contents corresponding to the 1C-8C ploidy levels were established from the measurements of the vegetative cells, whereas the values of 3C and 16C levels were established from the measurements of reproductive cells (Table 1, Fig. 2). The 4C and 8C ploidy levels were observed in both vegetative and reproductive cells. Ploidy levels overlapped between different cell types such as inner cortical and medullary cells (Table 1). The 1C ploidy level was determined in mitotic figures of dividing outer cortical cells (Fig. 4).

Both tetrasporangia and tetraspores were measured in reproductive cells (Figs. 5 and 6). These cells increased their size as well as their nuclear DNA content during tetrasporogenesis. Thus, a wide range of values were obtained in tetrasporangia (Table 1). The lowest ploidy level estimated for tetraspores of *G. chilense* was 3C.

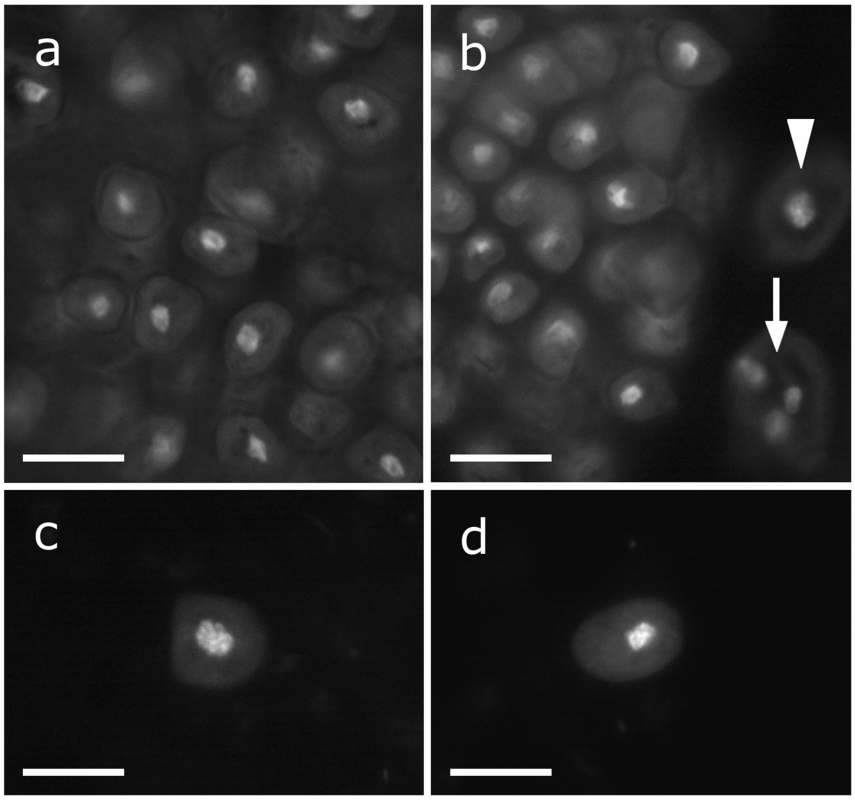

Figure 3. Cells of *Gelidium chilense* fixed in Carnoy´s and stained with DAPI. a) Uninucleate outer cortical cells. b) Uninucleate inner cortical (arrowhead) and trinucleate medullary (arrow) cells. c) Inner cortical cells in G2 phase (4C). d) Inner cortical cells in G2 phase (4C). Scale bars represent: a-d, 5 μm / Células de *Gelidium chilense* fijadas en Carnoy y teñidas con DAPI. a) Células corticales externas uninucleadas. b) Células corticales internas uninucleadas (punta de flecha) y células medulares trinucleadas (flecha). c) Célula cortical interna en fase G2 (4C). d) Célula cortical interna en fase G2 (4C). Escala: a-d, 5 μm

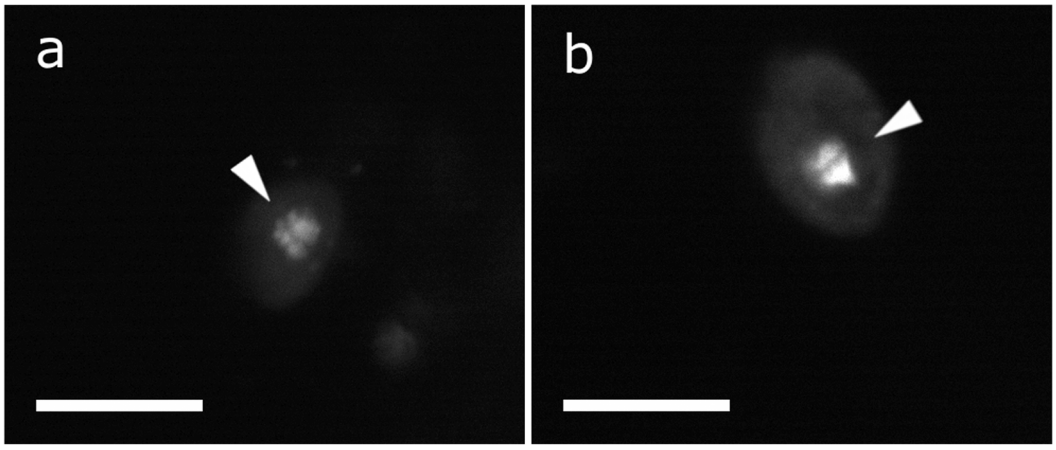

Figure 4. a-b. Mitotic figures of dividing cortical cells of *Gelidium chilense* stained with DAPI. Scale bars represent: a-b, 5 μm / a-b. Figuras mitóticas de células corticales en división celular observadas en *Gelidium chilense* y teñidas con DAPI. Escala: a-b, 5 μm

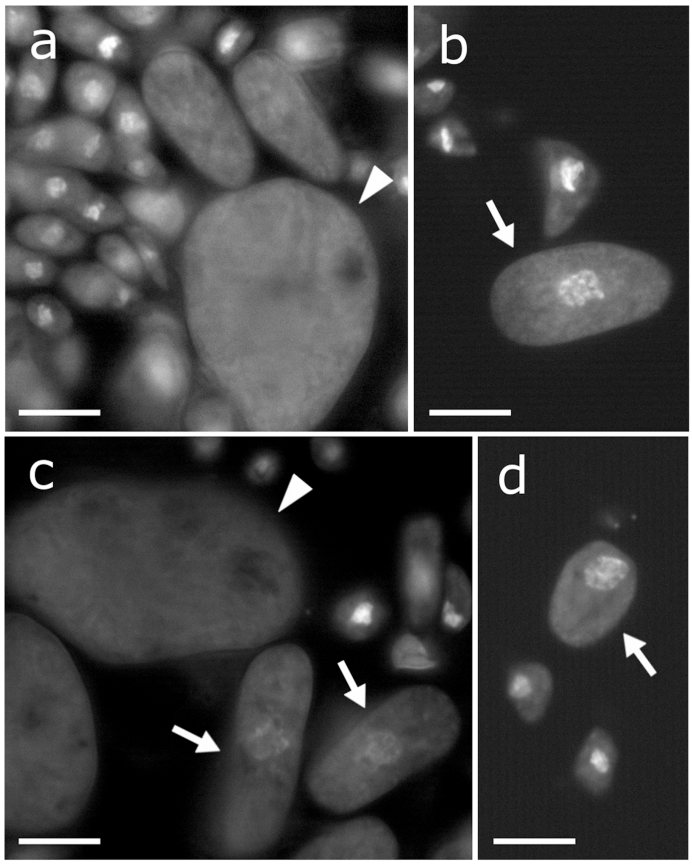

Figure 5. a-d. Inmature (arrows) and mature tetrasporangia (arrowhead) and cortical cells of *Gelidium chilense* fixed in Carnoy´s and stained with DAPI. Scale bars represent: a-d, 5 μm / a-d. Tetrasporangios inmaduros (flechas) y maduros (punta de flecha), y células corticales de *Gelidium chilense* fijadas en Carnoy y teñidas con DAPI. Escala: a-d, 5 μm

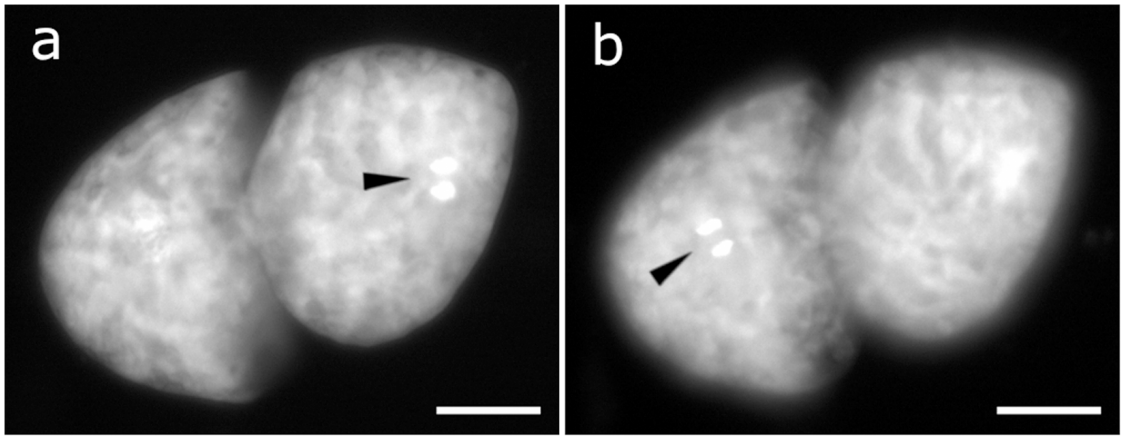

Figure 6. a-b. Overexposed nuclei (arrowhead) of tetraspores. Scale bars represent: a-b, 5 µm / a-b. Núcleos de tetrasporas (punta de flecha) sobreexpuestos a la luz. Escala: a-b, 5 µm

Discussion

Nuclear pattern

Previous studies have revealed that red algae are considerably diverse in their nuclear cytology (Goff & Coleman 1986, 1990; Kapraun & Dunwoody 2002, Gómez-Garreta *et al*. 2010, Kapraun & Freshwater 2012, Varela-Alvarez *et al*. 2012). According to Goff & Coleman (1990), the different nuclear patterns in Florideophyceae play an important role in cell differentiation, branching and final thallus morphology.

A wide intraplant variation in DNA contents was observed in vegetative (1C-8C) and reproductive cells (3C-16C) of *G. chilense*. In vegetative cells, the variation of DNA amounts also includes polyploidy from uninucleate outer cortical (1C-2C) to the uninucleate inner cortical (2C-4C) to medullary cells (4C-8C). In addition, the medullary cells display both polyploidy and polygenomy since they are multinucleate (Fig. 3). Our observations indicated that *G. chilense* displayed, from medulla to cortex, a process of 'incremental size decrease associated with a cascading down of DNA contents' that has been described previously in both red (Goff & Colemann 1986) and green algae (Kapraun 1994).

According to Goff & Coleman (1990), although homologous somatic cells in isomorphic gametophytes and sporophytes should theoretically differ two-fold in their DNA content and cell volume, in some Florideophyceae the DNA content measurements in non-apical vegetative cells show only small differences between these stages. The authors explain that this is possible because 'the total DNA content of the cell is not a function of its generation, but of cell and nuclear size' (Goff & Coleman 1990, p. 69). This is confirmed by the unexpected 1C ploidy level observed in the small cortical cells of the sporophytic samples examined.

Gelidium chilense possess cruciate tetrasporangia of 10 µm diameter and 30 µm length (Hoffmann & Santelices 1997), a large size compared to the cortical cells that produce them. Our microspectrofluorometric measurements indicated that during tetrasporogenesis the tetrasporangia increased their size as well as their nuclear DNA content (from 4C to 16C). A strong and positive correlation between nuclear DNA content and cell dimensions has been described previously in red algae (Goff & Coleman 1990, Kapraun & Dunwoody 2002), green algae (Kapraun & Nguyen 1994) as well as in higher plants (Shuter *et al*. 1983). In addition, our observations indicate that tetrasporangia development is followed by nuclear endopolyploidy. This is in agreement with the theory that cell differentiation in plant species may be accompanied by endopolyploidization via either endomitosis or endoreduplication (Levin 2002, Bothwell *et al*. 2010).

Examples of endopolyploidy in reproductive cells have been observed before in both Rhodophyta and Phaeophyceae. An endoreduplication process can occur after or before sporangial production in the kelp *Alaria esculenta* (L.) Greville (Garbary & Clarke 2002), and partheno-sporophytes derived from haploid filaments of the brown alga *Ectocarpus* are able to produce meiospores via endoreduplication (Bothwell 2010). A

similar phenomenon was observed in the red alga *Bonnemaisonia* during carposporangia production and values up to 6C in *B. clavata* G. Hamel and 8C in *B. asparagoides* (Woodward) C. Agardh were recorded for those cells (Salvador *et al*. 2009).

DNA CONTENT

The 2C DNA content values observed in *G. chilense* cells (0.4 pg) were similar to those of *G. serrulatum* J. Agardh (Freshwater 1993). This result was also in agreement with the narrow range of DNA content values (2C= 0.42 - 0.68 pg) compiled by Kapraun (2005) for several Gelidiales species.

The DNA content values obtained in the tetraspores (0.6 pg) were twice the values listed by Kapraun (2005) in some Gelidiales (0.2-0.3 pg) and also higher than the 1C values obtained from the cortical cells of *G. chilense*. These DNA contents suggest that the values obtained in tetraspores might correspond to a 3C ploidy level. These results are congruent with the hypothesis based on studies of British species that life histories in *Gelidium* may be highly variable and meiosis may not occur in all sporangia (Dixon 1961). This may explain the differences in the ratio of life history stages for some *Gelidium* species in Spain (Polifrone *et al*. 2012) and in *G. chilense* and *Gelidium lingulatum* Kützing in central Chile, where the biomass of sexual thalli throughout the year is less than 10% of the fertile biomass (Montalva & Santelices 1981). The same hypothesis was suggested by Ponce-Márquez *et al*. (2009) in their cytogenetic study of *Gelidium sclerophyllum* W. R. Taylor, but they were unable to count the chromosomes in sporangia.

Both the large size of *G. chilense* tetrasporangia and the absence of meiosis in its life history could be adaptive strategies, the former to increase the survival of reproductive cells and the latter to produce new diploid tetrasporic thalli. In agreement with Kapraun (2005) and Destombe *et al*. (1992) the implication is that large spores have several advantages such as reduced predation by zooplankton, more rapid settlement and greater energy reserves for initial growth after germination.

The main conclusions of this study are: 1) the intraplant variation of DNA contents observed in vegetative cells originates from a process of 'incremental size decrease associated with a cascading down of DNA contents' from the multinucleate medullary cells to uninucleate cortical cells, 2) that the differentiation from small cortical cells to large tetrasporangia in *G. chilense* occurs by means of polyploidy during sporogenesis, and 3) that in the specimens of *G. chilense* examined herein the sporogenesis was not accompanied with meiosis.

This study is also the first report of DNA C-values from an endemic Chilean red alga. Additional studies on the nuclear DNA content of Chilean *Gelidium* species will increase the DNA C-values database and help to understand the life history of this economically important group of agarophytes.

Finally, detailed cytological studies are important for clarifying important features of reproductive structures and life histories of algae as well as associating the intraplant variation of the nuclear DNA contents with their morphology.

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