Intraindividual variation in nuclear DNA content in *Durvillaea antarctica* (Chamisso) Hariot, *Macrocystis pyrifera* (Linnaeus) C. Agardh and *Lessonia spicata* (Suhr) Santelices (Phaeophyceae)

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ABSTRACT
Macrocystis C. Agardh, *Durvillaea* Bory and *Lessonia* Bory are three brown seaweeds genera of commercial importance for Chilean fishermen. *Macrocystis pyrifera* (Linnaeus) C. Agardh (Laminariales, Laminariaceae) is one of the world's most ecologically and economically important seaweed collected in Chile for alginate extraction; and as food for abalone aquaculture, *Lessonia spicata* (Suhr) Santelices (Laminariales, Lessoniaceae) represents more than 70% of the total brown seaweeds annual landing in Chile; and *Durvillaea antarctica* (Chamisso) Hariot (Fucales, Durvillaeaceae) is consumed as food, being considered very healthy because of its iodine content. Despite the economic importance of these species their nuclear DNA content is unknown until this moment. The present research was initiated to determine the nuclear DNA content and the intraindividual ploidy level variation in these seaweeds. The DNA-localizing fluorochrome DAPI (4',6-diamidino-2-phenylindole) and red blood cell (chicken erythrocyte) standard were used to estimate the nuclear DNA contents by image analysis. *Durvillaea antarctica* presented lower 2C DNA (1.2 pg) content and narrower range of DNA contents (1C-2C) than the Laminariales, which showed higher 2C DNA contents (1.4 -1.5 pg) and a wider range of ploidy level, achieving up to 4C in *L. spicata* and up to 8C in *M. pyrifera.* This higher ploidy levels observed would be related with the more complex cortical organization in the Laminariales than *D. antarctica*. 1C nuclei were only found in mature antheridia (*D. antarctica*) and sporangia (*M. pyrifera* and *L. spicata*). The 2C values observed for the taxa assessed were in the range of other previously published Fucales and Laminariales.

KEY WORDS
C-value,
DNA content,
Fucales,
Laminariales,
polyploidy.

RÉSUMÉ
Variation intra-individuelle du contenu en ADN nucléaire de Durvillaea antarctica*,* Macrocystis pyrifera *et* Lessonia spicata *(Phaeophyceae).*
Macrocystis C. Agardh, *Durvillaea* Bory et *Lessonia* Bory sont trois genres d'algues brunes d'importance commerciale pour les pêcheurs chiliens. *Macrocystis pyrifera* (Linnaeus) C. Agardh (Laminariales, Laminariaceae) est, parmi les algues récoltées au Chili, l'une des plus importantes au monde sur les plans écologique et économique ; comme aliment pour l'aquaculture de l'ormeau, *Lessonia spicata* (Suhr) Santelices (Laminariales, Lessoniaceae) représente plus de 70 % du total annuel des algues brunes débarquées au Chili ; et *Durvillaea antarctica* (Chamisso) Hariot (Fucales, Durvillaeaceae) est consommée comme aliment, car elle est considérée comme très saine en raison de sa teneur en iode. Malgré l'importance économique de ces espèces, leur contenu en ADN nucléaire est resté inconnu jusqu'à présent. L'objectif de la présente étude est de déterminer le contenu en ADN nucléaire et la variation du niveau de ploïdie intra-individuelle chez ces algues. Le fluorochrome DAPI (4',6-diamidino-2-phenylindole) permettant la localisation de l'ADN a été utilisé, suite à un étalonnage avec des érythrocytes de poulet, pour estimer par analyse d'image leur contenu en ADN nucléaire. *Durvillaea antarctica* présentait un contenu inférieur en ADN 2C (1,2 pg) et une gamme plus étroite de contenu en ADN (1C-2C) que les Laminariales, qui présentaient des teneurs en ADN 2C plus élevées (1,4 à 1,5 pg) et une gamme plus étendue de taux de ploïdie, atteignant jusqu'à 4C chez *L. spicata* et jusqu'à 8C chez *M. pyrifera*. Les niveaux de ploïdie plus élevés observés seraient liés à l'organisation corticale plus complexe des Laminariales relativement à celle de *D. antarctica*. Les noyaux 1C n'ont été trouvés que dans des anthéridies matures (*D. antarctica*) et des sporanges (*M. pyrifera* et *L. spicata*). Les valeurs de 2C observées pour les taxons évalués se situaient dans la plage des autres Fucales et Laminariales publiées antérieurement.

MOTS CLÉS
C-value,
contenu en ADN,
Fucales,
Laminariales,
polyploïdie.

INTRODUCTION

The kelp fishery in Chile lands up to 300 000 dry tons annually and the industry has an economic value exceeding *c.* US $60 million (Vásquez 2008; Vásquez *et al.* 2012). According to Vásquez *et al.* (2012) the three brown seaweeds genera of commercial importance for Chilean fishery are *Macrocystis* C. Agardh (Laminariales, Laminariaceae), *Durvillaea* Bory (Fucales, Durvillaeaceae) and *Lessonia* Bory (Laminariales, Lessoniaceae). *Macrocystis* is one of the world's most ecologically and economically important taxa (Demes *et al.* 2009). Several studies about marine ecological interrelationships have identified *Macrocystis pyrifera* (Linnaeus) C. Agardh as an important refuge environment from herbivory, for nurseries of invertebrates and fish (Macchiavello *et al.* 2010). In Chile, it has been exploited along the Northern and central zones being used as a foodstuff in the cultivation of abalone (*Haliotis* spp.) and exported, ground up, for the extraction of alginic acid (Vásquez 2008). *Lessonia spicata* (Suhr) Santelices represents more than 70% of the total brown seaweed annual landing in Chile (Vásquez *et al.* 2012) and *Durvillaea antarctica* (Chamisso) Hariot is culturally recognized as a food named *cochayuyo* that also has high nutritional value due to its essential nutrients content (Hoffmann & Santelices 1997). Although, there is a national and international demand for brown seaweeds, it is not satisfied by algal species like *Laminaria* J.V. Lamouroux*, Durvillaea, Ecklonia* Hornemann*, Sargassum* C. Agardh and *Lessonia* (Alveal *et al.* 1990).

The genome size or C-value is the amount of nuclear DNA in a cell which represents multiples of the minimum amounts of DNA corresponding to the non-replicated haploid chromosome complement (Greilhuber *et al.* 2005). Increasing interest in such data is evidenced by the number of newly estimated C-values published in recent years (Bennett & Leitch 2011) due to the nuclear DNA content is used in a wide range of biological fields as ecological or environmental indicators (Kubešová *et al.* 2010) as well as predictors of phenotypic characters at cell, tissue or organism level (Beaulieu *et al.* 2008; Hodgson *et al.* 2010). Moreover, these data have been related to patterns of both invasiveness (Lavergne *et al.* 2010) and evolution (Leitch & Leitch 2013), inclusive in the macroalgae (Kapraun 2005; Phillips *et al.* 2011). According to Browdy *et al.* (2012), genomic data are important to ensure a sustainable aquaculture of macroalgae.

The first compilation of genome size estimates in algae was addressed by Kapraun (2005) with 245 species of red, brown and green macroalgae. Later, these data were incorporated into the database of plant genome size hosted by the Royal Botanic Gardens Kew web page (http://data.kew.org/cvalues/). To date, the latest contributions of new estimates of nuclear DNA contents for the brown algae were provided by Gómez Garreta *et al.* (2010), Phillips *et al.* (2011), Ribera Siguan *et al.* (2011) and Martin Martin *et al.* 2016 who included 19, 98, 17 and 5 additional taxa, respectively. Recently, Sjøtun *et al.* (2017) identified large variation in genome size within populations of the genus Fucus Linnaeus. All these previous studies highlighted the absence of published DNA content data of the ecologically and economically important Fucales and Laminariales. In addition, no C-value data for any southern hemisphere Fucales are available (Phillips *et al.* 2011).

The nuclear DNA variation within individuals has been studied in a variety of plant groups (Biradar & Rayburn 1993). In the algae it was initiated by Goff & Coleman (1984) who not only mapped the DNA content within individuals but

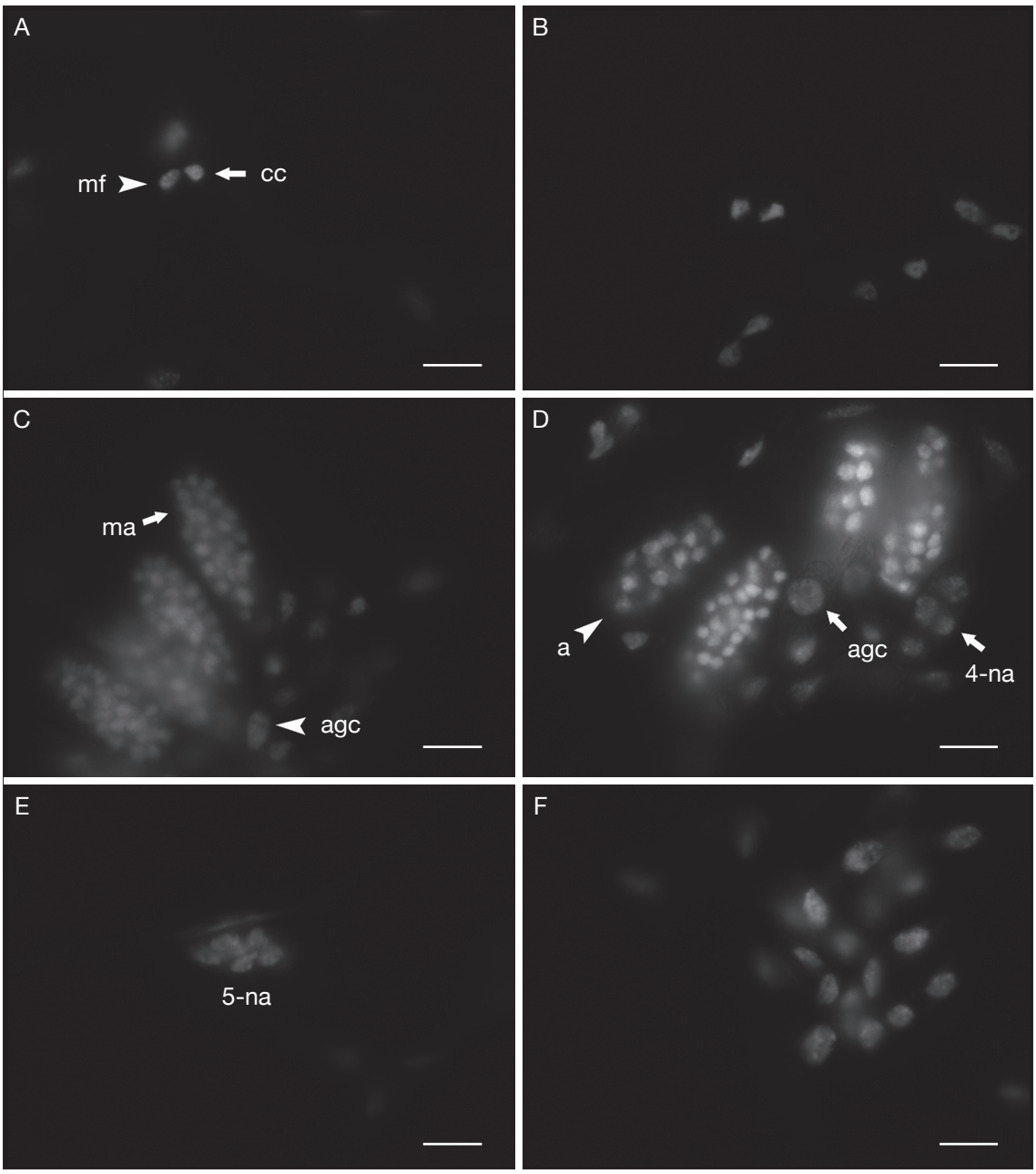

FIG. 1. — Cells of *Durvillaea antarctica* (Chamisso) Hariot stained with DAPI: **A**, mitotic figure (**mf**) of dividing cortical cells (**cc**); **B**, uninucleate cortical cells; **C**, **D**, mature antheridia (**ma**), antheridia germinative cells (**agc**), antheridium (**a**) and four-nucleate antheridium (**4-na**); **E**, five-nucleate antheridium (**5-na**); **F**, antheridia germinative cells. Scale bars: A-D, 5 µm.

throughout the life history of a single species. Afterwards, the same authors conclude their extensive research with the description of both major nuclear patterns associated with DNA intraindividual variation in the red algae and phenomena associated with the DNA dynamics such as polyploidy and polygenomy (Goff & Coleman 1990).

Several studies have reported polyploidy in the Laminariales (Lewis 1996; Phillips *et al.* 2011) and Fucales (Coyer *et al.* 2006; Gómez Garreta *et al.* 2010; Sjøtun *et al.* 2017). According to Kapraun (2005) its larger genome sizes (2C ≥ 2.0 pg) almost certainly are related with this phenomenon. Müller *et al.* (2016) reported one ploidy variant in their gametophyte cultures and Coyer *et al.* (2006) identified polyploid *Fucus* species adapted to salt marshes.

On the other hand, the measurement of the nuclear DNA level provides also key information for a better understanding of the life history in the macroalgae. In this sense Müller *et al.* (2016) reported sex specific polyteny in *Macrocystis* gametophyte cultures, observing in female gametophytes approximately double the DNA content than in male gametophytes.

The estimation of the nuclear DNA content provides useful information not only to understand the complex life histories but morphologies of the red macroalgae (Goff & Coleman 1990; Salvador *et al.* 2009). Taking into account the pres-

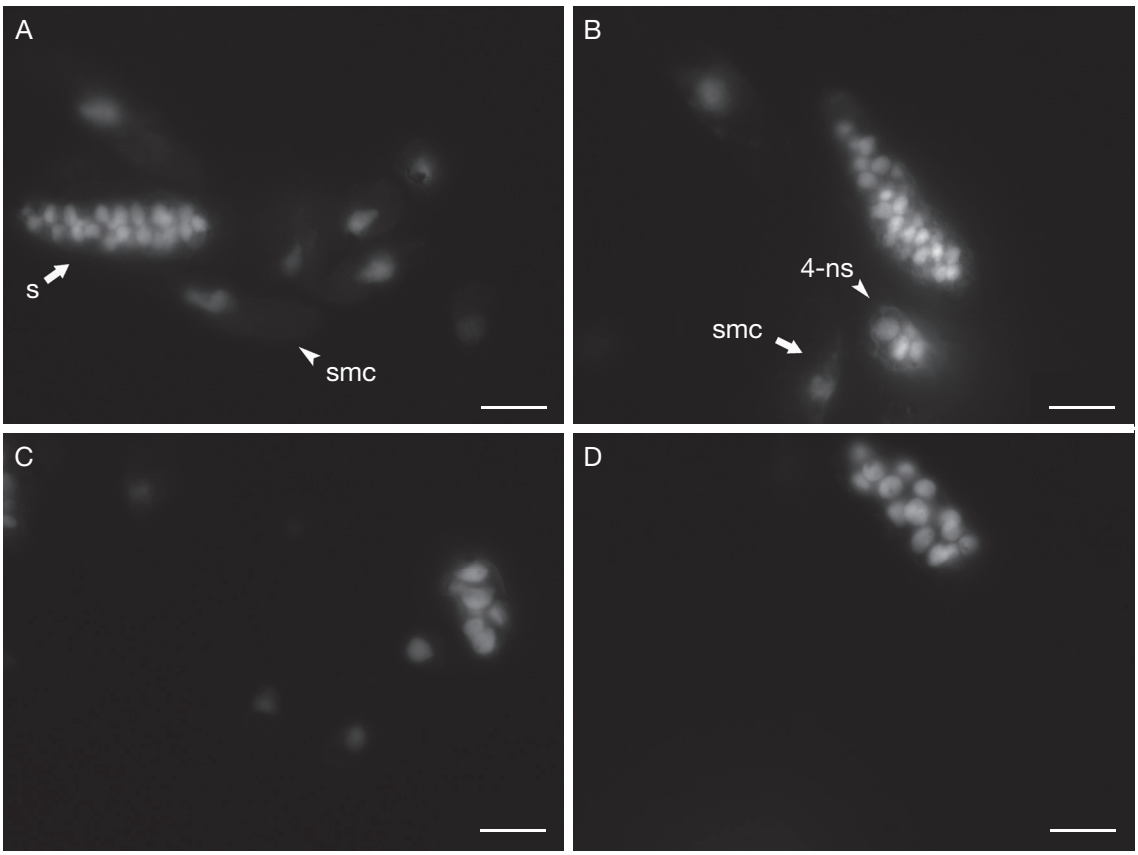

Fig. 2. — Developmental stages of sporangia in *Lessonia spicata* (Suhr) Santelices stained with DAPI: **A**, sporangia (**s**) and sporangial mother cells (**smc**); **B**, four-nucleate sporangium (**4-ns**) and sporangial mother cells (**smc**); **C**, **D**, different developmental stages of the sporangia. Scale bars A-D, 5 µm.

ence of DNA intraindividual variation in the macroalgae, the DNA quantification from various tissues is relevant for the interpretation of DNA content measurements in macroalgae in this type of studies. In this sense, endopolyploid nuclei (8C) were reported in vegetative tissue of *Saccharina latissima* (Linnaeus) C.E. Lane, C. Mayes, Druehl & G.W. Saunders (as *Laminaria saccharina* (Linnaeus) Lamouroux) and *Alaria esculenta* (Linnaeus) Greville (Garbary & Clarke, 2002). Later, Kapraun (2005) observed large nuclei in older medullary cells of S. *latissima* (as *L. saccharina*) which were not possible to quantify. Taking into account the wide range of cell sizes in individual thalli of many brown algae, it has not been studied in the context of nuclear endopolyploidy.

The present investigation was initiated to determine the nuclear DNA content and variation of the level of intraindividual ploidy of *M. pyrifera*, *L. spicata* and *D. antarctica*, three marine species of the southern hemisphere. Despite the economic importance of these species, their nuclear DNA content had not been estimated so far.

MATERIAL AND METHODS

Algal material

Reproductive specimens of *Durvillaea antarctica*, *Lessonia spicata* and *Macrocystis pyrifera* 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. Fertile fragments were cut off from each plant and 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 72 h. Algal material was squashed and transferred to coverslips treated with subbing solution and then air dried and stained with 0.5 µg mL of DAPI (4'-6-diamidino-2-phenylindole; 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 consequently analyzed using MetaMorph software (Molecular Devices, Toronto, Canada). The total intensity (in relative fluorescence units, rfu) was estimated from 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

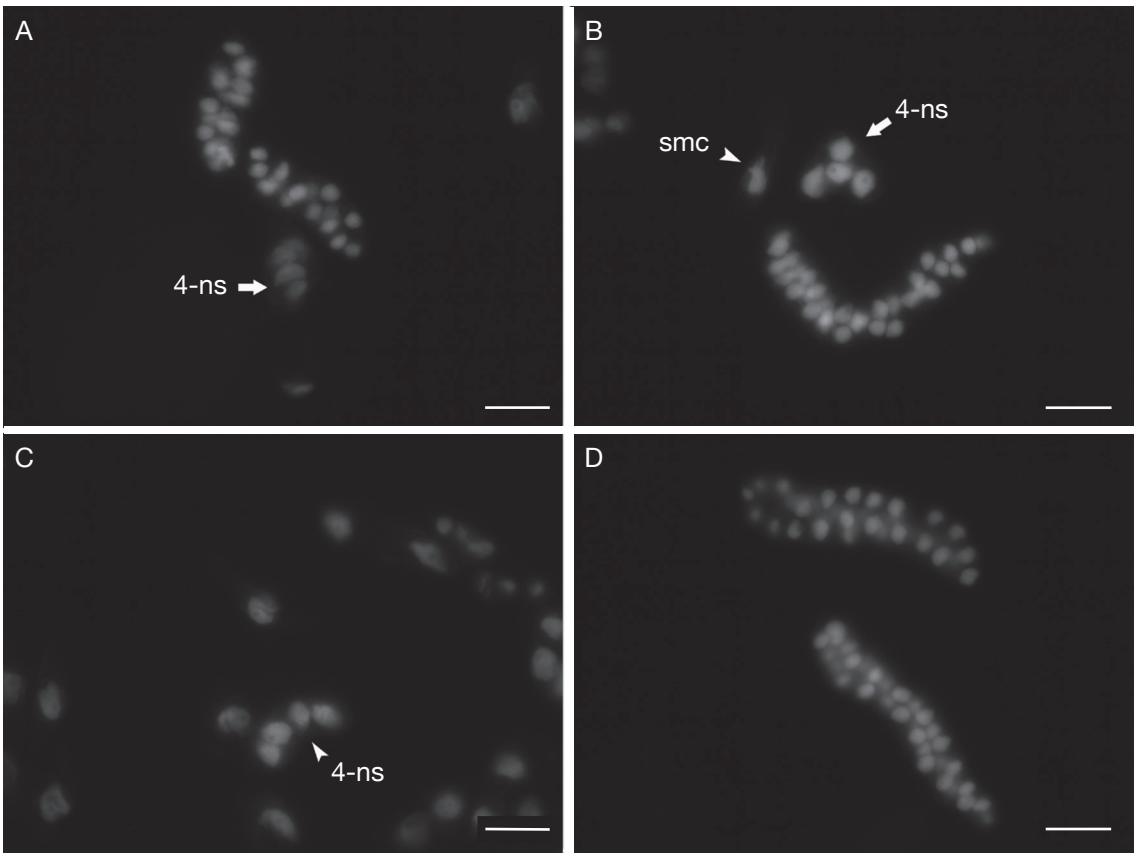

Fig. 3. — Developmental stages of sporangia in *Macrocystis pyrifera* (Linnaeus) C. Agardh stained with DAPI: **A-C**, four-nucleate sporangium (**4-ns**) and sporangial mother cells (**smc**); **D**, mature sporangia. Scale bars: A-D, 5 µm.

obtained. Nuclei from diverse regions of 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). A nuclear DNA base composition of 42-43 mol % G+C was determined in the chicken by Marmur & Doty (1962) and of 53 % in the *Ectocarpus siliculosus* by Cock *et al.* (2010). These published data indicate similar mean mol % values 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). 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 G1, S and G2 phases of the cell population examined. Measurements of reproductive cells are considered the best way to determine the numerical relationship between rfu and C-values (Goff & Coleman 1990). In addition, mitotic figures in dividing somatic cells were measured to confirm the 1C level.

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.

RESULTS AND DISCUSSION

A total of 777 nuclei (algae and standard together) were localized and measured. *Durvillaea antarctica* showed the lowest nuclear DNA content (2C = 1.2 pg) as well as the narrowest range of ploidy level (1C-2C) in comparison with the Laminariales analyzed taxa (Table 1). This data represents the first C-value for a southern hemisphere representative of Fucales. *Lessonia spicata* and *Macrocystis pyrifera* exhibited the highest nuclear DNA contents (2C = 1.4-1.5 pg) and ploidy levels, achieving up to 4C and 8C in *L. spicata* and *M. pyrifera*, respectively (Table 1). The data reported here are in agreement with those observed by Phillips *et al.* (2011), being the 2C values observed inside the range of DNA content values of the Fucales and Laminariales published by these authors.

TABLE 1. — Nuclear DNA content with corresponding C levels in different cell types of the species examined. Abbreviations: **A**, antheridia; **P**, paraphyses; **C**, cortical cells; **S**, sporangia; cells; **pg**, picograms; **Me**, meristoderm; **n**, number of nuclei analyzed.

	DNA content (pg)				
	mean	sd	Ploidy level	Cell types	n
Durvillaea	0.6	0.1	1C	A	28
antarctica	1.2	0.2	2C	A	25
(Chamisso)	1.2	0.2	2C	P	67
Hariot	1.2	0.2	2C	C	118
Lessonia	0.8	0.1	1C	S	23
spicata	0.9	0.2	1C	Me	3
(Suhr)	1.5	0.2	2C	Me	3
Santelices	–	–	1C-4C	C	26
Macrocystis	0.7	0.1	1C	S	112
pyrifera	1.4	0.2	2C	C	70
(Linnaeus) C. Agardh	–	–	2C-8C	C	178

Vegetative and reproductive cells were analyzed separately. All the cells observed in the studied taxa were uninucleate. A similar DNA content (2C = 1.2) among different cell types of *D. antarctica* was observed (Table 1). Concerning cortical cells, this fact could be related with the uniform cell size of this tissue that Collantes *et al.* (2002) described as several rows of uniform cells arranged neatly. In contrast, a major variation in ploidy levels in the cortex of the kelp fronds was observed (Table 1), due to its major range in cell size in comparison with *D. antarctica*. Similarly, Garbary & Clarke (2002) suggested that all multicellular plant groups having a major range in cell size show higher variation in nuclear DNA amount. Even more, according to several authors the nuclear DNA levels correlate positively with cell size (Kapraun 2005; Beaulieu *et al.* 2008; Hodgson *et al.* 2010; Katagiri *et al.* 2016), facilitating the association of C levels with different cell types. Nevertheless, in *L. spicata* and *M. pyrifera*, the wide ranges of ploidy levels observed in their cortical cells were not apparently related with the cell size, making it difficult the calculation of the mean C values in this cell type (Table 1). Furthermore, some cortical cells of *L. spicata* exhibited the same low values (1C-2C) observed in the meristoderm cells (Table 1). Garbary & Clarke (2002) obtained similar results in *Saccharina latissima* (as *Laminaria saccharina*) explaining this fact by the presence of some cortical cells that change their vacuolar volume while preserving a constant cytoplasmic volume. Previous studies (Davies *et al.* 1973; Chung *et al.* 1987) confirm the low vacuolar volume in meristoderm cells of *Laminaria* in comparison with their higher volume in cortical cells, which are highly vacuolated too. Recently, the observations of Katagiri *et al.* (2016) suggested that the cell type is a factor to consider in the relationship between nuclear DNA content and cell size.

Regarding the reproductive cells, they were clearly identified in different developmental stages (Figs 1-3). 1C values were observed in mature antheridia and sporangia of studied species (Table 1; Figs 1-3), but neither in the four-nucleate antheridia of *D. antarctica* (Figs. 1D-F) nor in this stage of the sporangia of the Laminariales (Figs 2B; 3A-C) as would be expected after meiosis. Similarly, the observations of Collantes *et al.* (2002) on the four-nucleate antheridia showed bigger nuclei indicating higher DNA contents than a 1C value, thus unexpected values for post-meiotic nuclei. Even more, in the following divisions of Laminariales sporangia and antheridia of *D. antarctica* the nuclear DNA content decrease considerably. These observations are consistent with those observed by Garbary & Clarke (2002) in *Alaria esculenta*, who detailed the nuclear ploidy changes during sporogenesis. Following meiosis they observed 8C nuclei instead the 1C expected in the four-nucleate stage and reductions in DNA content in the following mitotic divisions until the nuclei reach the 2C level instead the DNA synthesis before the mitosis to maintain the 1C level expected. Several authors confirmed the presence of meiosis in the first division of the sporangia in other brown algae (Robinson & Cole 1971; Loiseaux 1973; Katsaros & Galatis 1986; Motomura 1993) and others identified the haploid chromosome complement at the four-nucleate sporangia of some *Alaria* and *Laminaria* species (Magne 1952; Robinson & Cole 1971). For this reason Garbary & Clarke (2002) suggested that no 1C nuclei are observed in this stage of the sporogenesis by its extremely rapid endoreduplication. By means of this process, the cell cycle skips the mitotic phase increasing the ploidy level and resulting in polyploid cells (Katagiri *et al.* 2016). According to the detailed study about gametogenesis, fertility and embryogenesis by Collantes *et al.* (2002) the germinative cells of antheridia are originated by cellular differentiation of the cortical cells. In our study the germinative cells of *D. antarctica* (Fig. 1D-F) showed higher nuclei than those observed in their cortical cells with 2C values (Fig. 1A-B) suggesting that this cell differentiation could imply endoreduplication. This process is believed to contribute to the activation of metabolism for cell growth and differentiation (Katagiri *et al.* 2016).

Examples of endopolyploidy in reproductive cells have been observed before in both Phaeophyceae and Rhodophyta. Bothwell *et al.* (2010) observed partheno-sporophytes derived from haploid filaments of the brown alga *Ectocarpus* which produces meiospores via endoreduplication. Oppliger *et al.* (2014) observed unreduced (2N) spores which formed phenotipically normal gametophytes formed in part by automixis. In the Rhodophyta, Salvador *et al.* (2009) observed an endoreduplication process in the carposporangia production achieving values up to 6C in *Bonnemaisonia clavata* G. Hamel and 8C in *Bonnemaisonia asparagoides* (Woodward) C. Agardh. A similar pattern of endoreduplication was observed in the sporogenesis of the red alga *Gelidium chilense* (Montagne) Santelices & Montalva (Salvador *et al.* 2016), in this case the sporangia achieved values from 4C to 16C.

CONCLUSIONS

The main conclusions of this study are: 1) Both nuclear DNA contents and ploidy levels in Laminariales examined here were higher than in *D. antarctica*; 2) 1C values were observed in

mature antheridia and sporangia of studied species but no in their four-nucleate stage as it would be expected after a meiosis; 3) Meiosis was not observed in the sporogenesis of *L. spicata* and *M. pyrifera* or the male gametogenesis of *D. antarctica*; 4) Endoreduplication was observed during the development of the reproductive cells from its differentiation from meristoderm or cortical cells; and 5) Higher ploidy values (4C, 8C) were related to variation in cell sizes in the cortical organization of the Laminariales.

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