Nuclear DNA Content Variation in Life History Phases of the Bonnemasoniaceae (Rhodophyta)

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Abstract

Nuclear DNA content in gametophytes and sporophytes or the prostrate phases of the following species of Bonnemasoniaceae (Asparagopsis armata, Asparagopsis taxiformis, Bonnemaisonia asparagoides, Bonnemaisonia clavata and Bonnemaisonia hamifera) were estimated by image analysis and static microspectrophotometry using the DNA-localizing fluorochrome DAPI (4’, 6-diamidino-2-phenylindole, dilactate) and the chicken erythrocytes standard. These estimates expand on the Kew database of DNA nuclear content. DNA content values for 1C nuclei in the gametophytes (spERMAtia and vegetative cells) range from 0.5 pg to 0.8 pg, and for 2C nuclei in the sporophytes or the prostrate phases range from 1.15–1.7 pg. Although only the 2C and 4C values were observed in the sporophyte or the prostrate phase, in the vegetative cells of the gametophyte the values oscillated from 1C to 4C, showing the possible start of endopolyploidy. The results confirm the alternation of nuclear phases in these Bonnemasoniaceae species, in those that have tetrasporogenesis, as well as those that have somatic meiosis. The availability of a consensus phylogenetic tree for Bonnemasoniaceae has opened the way to determine evolutionary trends in DNA contents. Both the estimated genome sizes and the published chromosome numbers for Bonnemasoniaceae suggest a narrow range of values consistent with the conservation of an ancestral genome.

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Introduction

The marine red algal genera Asparagopsis and Bonnemaisonia (Bonnemaisoniales) have been the subject of numerous life history studies [1,2,3,4,5,6], invasive species ecology [7,8,9], phylogeography [10,11,12] and potential applications of their bioactive metabolites [13,14]. Despite continuing interest in members of this order, modern molecular techniques are only now beginning to overcome a history of pervasive taxonomic and nomenclatural confusion [8,15,6]. Although the Bonnemaisoniales was separated from the Nemaliales on the basis of their then known alternation of generations [1], it is now understood that this life history pattern lacks taxonomic significance and many orders of red algae are heterogeneous with regard to life history [16]. The distinction of these two orders is now generally recognized on the basis of sexual reproduction and cystocarp development [17], ultrastructural details of pit plugs and plastids [18,19] as well as molecular studies [20,21].

The Bonnemaisoniales, as originally proposed [1], is characterized by a heteromorphic life history. The Asparagopsis genus has a much branched erect gametophyte, and a tufted sporophyte (“Falkenbergia” stage) with polysiphonous axes. The Bonnemaisonia genus also has a much branched erect gametophyte, and a mycorropic and prostrate sporophyte, (“Hymenoclonium” stage), or filamentous and tufted (“Tradescantia” stage). According to Dixon [22], the information available for members of these taxa indicates both a ‘Bonnemaisonia’-type life history as well as a direct development of gametophytes from vegetative branches of the assumed diploid sporophyte with an absence of tetrasporogenesis [23,24,25,26]. In addition, in Bonnemaisonia asparagoides (Woodward) C. Agardh and Bonnemaisonia clavata Hamel somatic meiosis has been described [4,6] as reported in the ‘Lemanea’-type life history [27]. Despite the numerous studies carried out on the life history of the Bonnemaisoniales, the sequence of nuclear phases has been demonstrated only in B. asparagoides and B. clavata [6].

Microspectrophotometry with the DNA-localizing fluorochrome DAPI (4’, 6-diamidino-2-phenylindole, dilactate) was used successfully to demonstrate an alternation of ploidy levels associated with meiosis and sexual reproduction in red algae [28,29]. Among these algae there are members of Batrachospermales and Thorecales which have a ‘Lemanea’-type life history [30,27], such as some Bonnemaisonia species.

The present research of nuclear DNA contents in Bonnemaisoniaceae was initiated to determine the extent of nuclear DNA content variation, to identify any correlation between genome size and phylogeny, and to corroborate an alternation of haploid and diploid nuclear DNA contents in gametophyte and sporophyte/prostrate phases, respectively.

Materials and Methods

Source of specimens

“The locations for plant collections in this study were not privately-owned or protected in any way, so no specific
permissions were required for these locations/activities; also none of the species used in this study involve endangered or protected species”.

Five species of Bonnemaisoniaceae were collected from the Mediterranean [Aiguafreda and Llançà (Girona), Porto Colom (Majorca)] and Atlantic [Cabo Cruz (A Coruña), Zumaya (Gipuzcoa)] coasts of Spain: *Bonnemaisonia hamifera* Harriot (including *Treillitella intricata* Batters), *B. asparagoides*, *B. clavata*, *Asparagopsis armata* Harvey (including *Falkenbergia rufolamina* (Harvey) F. Schmitz) and *Asparagopsis taxiformis* (Delile) Trevisan (including *Falkenbergia hillebrandii* (Borner) Falkenberg) (Table 1). Due to the difficulty in obtaining ‘Hymenoclonium’ phases of *B. clavata* and *B. asparagoides*, these phases were cultured in the laboratory from carpospores which produced gametophytes [15].

**Nuclear DNA content estimates**

Algal specimens were fixed in Carnoy’s solution (3:1 95% ethanol: glacial acetic acid) and stored in 70% ethanol at 4°C [28]. Preserved material was rehydrated in water and softened in 5% w/v EDTA for 12 h [31]. Algal material was squashed, transferred to cover slips treated with subbing solution, air dried and stained with DAPI (0.5 μg mL⁻¹) (Sigma Chemical Co., St. Louis, MO 63178) as previously described [31,32]. Nuclear DNA content estimates based on microspectrophotometry with DAPI followed procedures specified previously [31,32] using a protocol modified after Goff & Coleman [31]. This method was carried out at the University of North Carolina Wilmington. Nuclear DNA content estimates based on image analysis of DAPI-stained specimens followed a procedure modified from Kapraun & Dunwoody [33] and Choi et al. [34] using a Cooled CCD Miramars RTE 782-Y high performance digital camera placed on a Leica DMRB fluorescence microscope and subsequently analyzed using MetaMorph software (Molecular Devices, Toronto, Canada). This method was carried out at the University of Barcelona. Fluorescence intensity (I₉) values were obtained from image analysis and microspectrophotometry for algal specimens [35,33].

DAPI binds by a non-intercalative mechanism to adenine and thymine rich regions of DNA which contain at least four A-T base pairs [36]. Chicken erythrocytes (RBC) with a DNA content of 2.4 pg [37] were used as standard to quantify nuclear DNA contents. 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 [38]. Chicken has a nuclear DNA base composition of 42–43 mol % G and experimental DNA are equivalent [38]. Chicken DNA content of 2C mean values of all the species studied [2C = 1.2 pg], while *A. armata* and *A. taxiformis* present the lowest 2C mean values of all the species studied [2C = 1.3 pg]. Regarding *B. hamifera*, the 2C mean value (2C = 1.4 pg) is between the two groups. These results agree with the unclear taxonomic position of this species, previously considered within the *Asparagopsis* genus [6]. The only data on DNA nuclear content of Bonnemaisoniaceae published in picograms corresponds to *B. hamifera* with values of 2C = 1.3 pg [28], coinciding with our results. The DNA range observed in the gametophytes (1G–4C) agrees with the range indicated by Salvador et al. [6] for *B. asparagoides* and *B. clavata*. However, for the sporophytes or the prostrate phases, the same authors give a range of 2C to 8C.

On the other hand, keeping in mind the 1C value of the spermatia, it can be affirmed that the nuclear genome size of *B. clavata* from Mediterranean (Girona) and Atlantic (A Coruña) coasts did not show any differences.

The presence of 4C nuclei in the Bonnemaisoniaceae gametophytes suggests the possible start of an endopolyploidy process both in vegetative cells and in carpospores. These results agree with those of Salvador et al. [6] in *B. asparagoides* and *B. clavata* that showed a high endopolyploidy level in the axial cells of the gametophytes, as well as in the carpospores. These authors give DNA values for the carpospores of up to 32C in *B. asparagoides* and up to 16C in *B. clavata*.

**Molecular phylogeny and patterns of genome size variation**

A phylogenetic hypothesis for Bonnemaisoniaceae [20,8,21,6] provides a picture of nuclear genome size evolution among these taxa (Fig. 1). Southern hemisphere genera *Delseia* and *Ptilonia* are a
sister group to a Bonnemaisonia-Asparagopsis clade according to the phylogenetic tree resulting from SSU analysis [8]. Results of the present study indicate 1C nuclear genome sizes in Bonnemaisoniaceae (0.5–0.8 pg) within similar range observed in other members of the Florideophycidae [28].

Table 1. Nuclear DNA content of Bonnemaisoniales. Data standardized to the DNA level of chicken erythrocytes (RBC = 2.4 pg).

| Species                  | Location      | Date          | Phase | Cell Type | N° of Nuclei examined | 1C         | 2C         | 4C         | Method |
|--------------------------|---------------|---------------|-------|-----------|-----------------------|------------|------------|------------|--------|
| Asparagopsis armata      | Llançà        | 04/02/2007    | G     | C         | 11                    | 3.2 ± 0.6  | IA         |
|                          |               |               |       | V         | 137                   | 1.7 ± 0.2  | IA         |
|                          |               |               |       | Sp        | 51                    | 1.8 ± 0.3  | IA         |
|                          |               |               |       | S         | 123                   | 0.65 ± 0.1 | M          |
|                          |               |               |       | S         | 157                   | 1.6 ± 0.1  | M          |
|                          |               |               |       | V         | 14                    | 2.7 ± 0.4  | M          |
| Asparagopsis taxiformis  | Porto Colom   | 06/05/2007    | G     | V         | 63                    | 1.8 ± 0.2  | IA         |
|                          |               |               |       | G         | 17                    | 2.4 ± 0.4  | IA         |
|                          |               |               |       | G         | 10                    | 2.9 ± 0.4  | M          |
|                          |               |               |       | G         | 160                   | 0.7 ± 0.2  | M          |
|                          |               |               |       | G         | 29                    | 0.65 ± 0.1 | M          |
|                          |               |               |       | G         | 67                    | 1.7 ± 0.1  | IA         |
|                          |               |               |       | G         | 4                     | 1.6 ± 0.1  | M          |
|                          |               |               |       | S         | 102                   | 1.7 ± 0.3  | M          |
| Bonnemaisonia asparagoidea | Aiguafreda  | 30/05/2007    | G     | V         | 51                    | 0.6 ± 0.1  | M          |
|                          |               |               |       | G         | 46                    | 0.6 ± 0.2  | IA         |
|                          |               |               |       | G         | 21                    | 0.5 ± 0.1  | M          |
|                          |               |               |       | G         | 12                    | 0.6 ± 0.2  | IA         |
|                          |               |               |       | G         | 30                    | 1.1 ± 0.2  | M          |
|                          |               |               |       | G         | 34                    | 1.45 ± 0.2 | IA         |
|                          |               |               |       | G         | 5                     | 2.3 ± 0.2  | IA         |
|                          | culture       |               |       | G         | 29                    | 2.2 ± 0.1  | IA         |
|                          | culture       |               |       | G         | 99                    | 1.65 ± 0.2 | IA         |
|                          | culture       |               |       | S         | 168                   | 1.15 ± 0.2 | M          |
|                          | culture       |               |       | S         | 183                   | 2.25 ± 0.1 | IA         |
| Bonnemaisonia clavata    | Cabo Cruz     | 13/06/2006    | G     | Sp        | 51                    | 0.7 ± 0.1  | M          |
|                          |               |               |       | G         | 13                    | 1.1 ± 0.1  | M          |
|                          | Aiguafreda    | 30/05/2007    | G     | V         | 8                     | 0.6 ± 0.2  | IA         |
|                          |               |               |       | G         | 25                    | 1.6 ± 0.1  | IA         |
|                          |               |               |       | G         | 83                    | 0.6 ± 0.2  | IA         |
|                          |               |               |       | G         | 71                    | 2.2 ± 0.2  | IA         |
|                          | culture       |               |       | G         | 84                    | 1.6 ± 0.1  | IA         |
|                          | culture       |               |       | S         | 87                    | 1.2 ± 0.2  | M          |
|                          | culture       |               |       | S         | 183                   | 2.2 ± 0.2  | IA         |
| Bonnemaisonia hamifera   | Zumaya        | 07/10/2006    | G     | V         | 104                   | 0.8 ± 0.2  | M          |
|                          |               |               |       | G         | 89                    | 1.5 ± 0.3  | M          |
|                          |               |               |       | G         | 16                    | 2.7 ± 0.7  | M          |
|                          |               |               |       | S         | 172                   | 3.5 ± 0.2  | IA         |
|                          |               |               |       | S         | 110                   | 3.2 ± 0.6  | M          |
| Delisea plumosa          | New Zealand   |               | G     | V         | 47                    | 1.0 ± 0.2  | M          |
| Ptilonia willana         | New Zealand   |               | G     | V         | 165                   | 0.6 ± 0.1  | M          |

Unpublished data from Kapraun.

G = gametophe, S = sporophytic/prostrate phases, C = carpospora, V = vegetative cell, Sp = spermatia, IA = image analysis, M = microspectrophotometry.

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Asparagopsis armata and A. taxiformis have become widely distributed in Europe as an alien introduction [7,49,9] and fit the definition of a marine invader [50]. The highest 2C levels observed in their respective tetrasporophytes could be related with the fact that in Asparagopsis this phase ("Falkenbergia" type) is the most resilient [10] and the primary means of dispersal [8].

Karyological studies limited to three species, Asparagopsis armata (n = ca. 20), Bonnemaisonia asparagoides (n = ca. 18, n = ca. 20, n = ca. 30) and Bonnemaisonia hamifera (n = 20–25) [51], show that the n chromosome number is variable. Reported chromosome complements of n = 10 in A. armata [52] should be reinvestigated. In red algae, the hypothesised basal (ancestral) nucleotype is characterized both by small genome sizes and small chromosome complements [28]. Chromosome complements greater than n = 10 probably reflect ancestral polyploidy events [51,29]. Due to the variation of the chromosome number and nuclear DNA content estimates in the present study, we can suggest one or more instances of aneuploidy following an ancestral polyploidy event [53,54,55] as a possibility.

Nuclear DNA content variation associated with a diplobiontic life history

Considerable life history variations have been reported in species of Bonnemaisoniales [22,4,56]. Culture studies suggest intraspecific variability in the development and life history of Delisea pulchra (Greville) Montagne [57]. In Atractophora and Naccaria, gametophytes develop directly from the prostrate protonemal stage produced from carpospores [25]. Asparagopsis taxiformis and A. armata have an alternation of generations with a ‘Falkenbergia’ tetrasporophyte [1,2,5]. Bonnemaisonia hamifera alternates with a ‘Trailliella’ tetrasporophyte [26] and Bonnemaisonia geniculata Gardner is reported to have a different type of tetrasporophyte [3]. In contrast, recent research of B. asparagoides and B. clavata confirms direct development of gametophytes from the prostrate ‘Hymenoclonium’ phase following vegetative meiosis [6].

The DNA-localizing fluorochrome DAPI and microspectrophotometry have been used to demonstrate variations in nuclear DNA levels consistent with an alternation of haploid and diploid phases in red algae associated with a sexual life cycle [33,40,29]. In the present study, no evidence of tetrasporogenesis was observed in either collected or cultured material. However, in comparing the mean values obtained between phases, the gametophytes showed a 1C range of 0.6–0.8 pg whereas their prostrate/sporophytic phases (‘Falkenbergia’, ‘Hymenoclonium’ and ‘Trailliella’) had a 2C range of 1.2–1.7 pg (Table 2). In addition, the 1C values observed in the gametophytes are corroborated by the 1C value observed in the spermatia (0.5–0.7 pg). These results confirm the alternation of haploid and diploid phases suggested by culture studies [2,23,26,4,6], but not clearly demonstrated by previous cytological research [58,59,4] in the Bonnemaisoniales.

### Table 2. Nuclear DNA means content of Bonnemaisoniales. 1C values (spermatia) and 2C values (sporophytic/prostrate phases).

| Species                  | Reproductive Phase | Nuclear Genome Size (pg) |
|-------------------------|--------------------|-------------------------|
|                         |                    | 1C                  | 2C                  |
| Asparagopsis armata     | G                  | 0.7±0.1              | 1.6±0.1             |
| (= ‘Falkenbergia’ phase)| S                  |                       |                     |
| Asparagopsis taxiformis | G                  | 0.7±0.2              | 1.7±0.3             |
| (= ‘Falkenbergia’ phase)| S                  |                       |                     |
| Bonnemaisonia hamifera  | G                  |                       | 1.4±0.2             |
| (= ‘Trailliella’ phase) | S                  |                       |                     |
| Bonnemaisonia asparagoides| G                | 0.6±0.2              | 1.2±0.2             |
| (= ‘Hymenoclonium’ phase)| S              |                       |                     |
| Bonnemaisonia clavata   | G                  | 0.7±0.3              | 1.2±0.2             |
| (= ‘Hymenoclonium’ phase)| S               |                       |                     |

G = gametophyte, S = sporophytic/prostrate phase.

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Figure 1. Nuclear DNA contents in picograms (pg) superimposed on a consensus molecular phylogenetic tree for Bonnemaisoniales on the basis of supported clades in published phylogenies [20,8,21,6]. ○ 1C nuclear DNA contents. ● 2C nuclear DNA contents.
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Therefore, in addition to contributing the nuclear DNA content values of 7 Bonnemaisoniaceae species, this study confirms the alternation of nuclear phases and reports significant information about the understanding of the life histories of this group, where several variations have been described.

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Author Contributions

Conceived and designed the experiments: NSS AGG MARS DFK. Performed the experiments: NSS AGG. Analyzed the data: NSS MARS. Contributed reagents/materials/analysis tools: NSS AGG. Wrote the paper: NSS AGG MARS DFK.

References

1. Feldmann J, Feldmann G (1942) Recherches sur les Bonnemaisoniacees et leur alternances de generations. Annales des Sciences Naturelles, Botanique, serie 11, 3, 75–179.
2. Chihara M (1961) Life cycle of the bonnemaisoniaceous alga in Japan (1) Reports of the Tokyo Kyoku Daigaku, Section B 10: 121–133.
3. Shevlin DE, Polanshek AR (1978) Life history of Bonnemaisonia gracilaulata (Rhodophyta): a laboratory and field study. Journal of Phycology 14: 202–209.
4. Rueness J, Åsen PA (1982) Field and culture observations on the life history of Bonnemaisonia asparagoides (Chordophyceae). C. Ag. (Rhodophyta) from Norway. Botanica Marina 25: 377–387.
5. Bounin DR, Hawkes MW (1987) Systematics and life histories of New Zealand Bonnemaisoniaceae (Bonnemaisoniaceae, Rhodophyta): I. The genus Apooglossis. New Zealand Journal of Botany 25: 577–590.
6. Salvador N, Gómez Garreta A, Ribera Siguan MA (2008) Characterization of several variations have been described.
7. Hawkins WA (1994) Introduction of marine benthic algae into Atlantic European waters. In: Introduced Species in European Coastal Waters (eds Bidmead CF, Nolan C), pp. 32–36. European Commission, Luxembourg.
8. Ni Chuailláin FN, Magge CA, Saunders GW, Guiry MD (2004) The invasive genus Apooglossis (Bonnemaisoniaceae, Rhodophyta): molecular systematics, morphology, and ecophylogeny of Falklandian isolates. Journal of Phycology 40: 1112–1126.
9. Alkamarino M, Andräkis N, Carmona R, de la Rosa J, Barbosa AM, et al. (2009) A multidisciplinary project to study the invasion of Apooglossis taxiformis in western Mediterranean Sea. Phycologia 48 (Supplement): 1–5.
10. Salvador N, Gómez Garreta A, Ribera Siguan MA (2007) Characterization of two frequently confused species, Bonnemaisonia clavata and Bonnemaisonia clavata var. clavata (Bonnemaisoniaceae, Rhodophyta): one of the Mediterranean red alga Apooglossis armata. Planta Media 67: 301–305.
11. Salvador N, Procaccini G, Kooistra WHCF (2004) Apooglossis taxiformis and Apooglossis armata (Bonnemaisoniaceae, Rhodophyta): genetic and morphological identification of Mediterranean populations. European Journal of Phycology 39: 273–283.
12. Salvador N, Procaccini G, Magge C, Kooistra WHCF (2007) Phylogeography of the invasive seaweed Apooglossis (Bonnemaisoniaceae, Rhodophyta) reveals cryptic diversity. Molecular Ecology 16: 2295–2299.
13. Sherwood AR (2008) Phylogeography of Apooglossis taxiformis (Bonnemaisoniaceae, Rhodophyta) in the Hawaiian Islands: two mtDNA markers support three separate introductions. Phycologia 47: 79–88.
14. Haslin C, Lahaye M, Pellegrini M, Chermann JC (2001) In vitro anti-HIV activity of sulfated cell-wall polysaccharides from gametophytic and tetrasporic stages of the Mediterranean red alga Apooglossis armata. Planta Medica 67: 301–305.
15. Salvador N, Gómez Garreta A, Ribera Siguan MA (2008) Characterization of two frequently confused species, Bonnemaisonia asparagoides and Bonnemaisonia clavata (Bonnemaisoniaceae, Rhodophyta), on the basis of morphological and molecular evidence. Phycologia 47: 177–190.
16. Garbary DJ, Gabrielton PW (1990) Taxonomy and evolution. In: Biology of the Red Algae (eds Cole KM, Sheath RG), pp. 305–345. New York, Cambridge University Press.
17. Hommersand MH, Frederiq S (1998) Sexual reproduction and cystocarp development. In: Biology of the Red Algae. (eds Cole KM, Sheath RG), pp. 305–345. New York, Cambridge University Press.
18. Chihara M, Yoshizaki M (1972) Anatomical and reproductive features of Poltia ulada (Rhodophyta, Bonnemaisoniaceae). Phycologia 17: 382–387.
19. Paeschel CM (1989) An expanded survey of the ultrastructure of red algal pit plugs. Journal of Phycology 25: 625–636.
20. Freshwater DW, Frederiq S, Butler BS, Hommersand MH, Chase MW (1994) A gene phylogeny of the red algae (Rhodophyta) based on plastid rbcL. Proceedings of the National Academy of Sciences of the United States of America 91: 7281–7285.
21. Le Gall L, Saunders GW (2007) A nuclear phylogeny of the Florideophyceae (Rhodophyta) inferred from combined EF2, small subunit and large subunit ribosomal DNA: Establishing the new red algal subclass Corallinophycidae. Molecular Phylogenetics and Evolution 43: 1118–1130.
22. Dixon PS (1982) Life histories in the Florideophyceae with particular reference to Nemalessa sensu lato. Botanica Marina 25: 611–621.
46. Gregory TR (2005) The C-value enigma in plants and animals: a review of parallels and an appeal for partnership. Annals of Botany 95: 133–146.
47. Gall J (1981) Chromosome structure and the C-value paradox. Journal of Cell Biology 91: 133–145.
48. Goff LJ, Coleman AW (1984) Elucidation of fertilization and development in a red alga by quantitative DNA microspectrophotometry. Developmental Biology 102: 173–194.
49. Ribera Siguan MA (2002) Review of non-native marine plants in the Mediterranean Sea. In Invasive Aquatic Species of Europe: Distribution, Impacts and Management (eds Leppokoski E, Gollasch S, Olenin S) Dordrecht, The Netherlands, Kluwer Academic Publishers. pp. 291–310.
50. Cronk QCB, Fuller JL (1995) Plant Invaders. London, Chapman and Hall.
51. Cole K (1990) Chromosomes. In: Biology of the Red Algae. (eds Cole KM, Sheath RG), pp. 73–101. New York, Cambridge University Press.
52. Svedelius N (1933) On the development of *Asparagopsis armata* Harv. and *Bonnemaisonia asparagoides* (Woodw.) Ag. Nova Acta Regiae Society Sciences Upsala 9: 1–61.
53. Kapraun DF, Bailey JC (1992) Karyology and cytophotometric estimation of nuclear DNA variation in seven species of Ulvales (Chlorophyta). Japanese Journal of Phyiology 40: 1526.
54. Kapraun DF, Buratti JR (1996) Evolution of genome size in the Dasycladales (Chlorophyta) as determined by DAPI cytophotometry. Phycologia 37: 176–183.
55. Kapraun DF (1993) Karyology of marine green algae. Phycologia 32: 1–21.
56. Havkes MW (1990) Reproductive Strategies. In: Biology of the Red Algae. (eds Cole KM, Sheath RG), pp. 435–476. New York, Cambridge University Press.
57. Bonin DR, Havkes MW (1980) Systematics and life histories of New Zealand Bonnemaisoniaceae (Bonnemaisoniaceae, Rhodophyta): II. The genus *Delisea*. New Zealand Journal of Botany 26: 619–632.
58. Magne F (1960) Sur le lieu de la méiose chez le *Bonnemaisonia asparagoides* (Woodw.) C. Ag. Comptes Rendus Hebdomadaires des Sciences de l’Académie des Sciences, Paris, série D 230: 2742–2744.
59. Magne F (1964) Recherches caryologiques chez les Floridées (Rhodophycees). Cahiers de Biologie Marine 5: 461–671.