ORIGINAL ARTICLE

Temporal variations of vegetative features, sex ratios and reproductive phenology in a *Dictyota dichotoma* (Dictyotales, Phaeophyceae) population of Argentina

M. Cecilia Gauna · Eduardo J. Cáceres · Elisa R. Parodi

Abstract This paper addresses the phenology of a *Dictyota dichotoma* population from the North Patagonian coasts of Argentina. The morphology of the individuals was characterized, and analyses of the temporal variations of vegetative features, diploid and haploid life cycle generations and sex ratios are provided. Individuals, represented by growing sporophytes and gametophytes, occurred simultaneously throughout the year. Morphological variables showed temporal variation, except the width and height of medullary cells, which did not vary between seasons. All vegetative variables were significantly correlated with daylength. Besides, frond length, frond dry mass and apical and basal branching angles were significantly correlated with seawater temperatures. Vegetative thalli were less abundant than haploid and diploid thalli. Sporophytes were less abundant than male and female gametophytes. Male gametophytes dominated in May, August, October and January, and female gametophytes were more abundant in September, November, December, February and March. The formation of female gametangia showed a significant correlation with daylength, and the highest number of gametangia was registered in spring. In general, the male/female sex ratio varied between 1:2 and 1:1. Apical regions were more fertile than basal regions. Our data about frequency in the formation of reproductive structures and male/female ratios are the first recorded in the *Dictyota* genus and thus could not be compared with populations from other regions of the world. Significant morphological variation was observed in thalli of both life cycle generations, regarding length and dry mass, number of primary branches and branching basal angle. In general, all variables analyzed varied seasonally except cortical cell width.

Keywords *Dictyota dichotoma* · Morphology · North Patagonian Atlantic coasts · Phenology · Seasonal variation · Sex ratios

M. C. Gauna (✉) · E. R. Parodi
Laboratorio de Ecología Acuática, Departamento de Biología, Bioquímica y Farmacia, Universidad Nacional del Sur, San Juan 670, B8000FTN Bahía Blanca, Argentina
e-mail: cgauna@criba.edu.ar

M. C. Gauna · E. R. Parodi
CONICET-CCTBBca, Laboratorio GIBEA, Instituto Argentino de Oceanografía (I.A.D.O.), Camino Carrindanga 7.5 km, B8000FWB Bahía Blanca, Argentina

E. J. Cáceres
Laboratorio de Ficología y Micología, Departamento de Biología, Bioquímica y Farmacia, Universidad Nacional del Sur-CIC, San Juan 670, B8000FTN Bahía Blanca, Argentina

Introduction

Species of the genus *Dictyota* Lamouroux are common elements of tropical, subtropical and temperate marine floras, indicating that they can colonize various different niches (Lüning 1990; De Clerck and Coppejans 1999) and retain significant biomass under high grazing pressure (Hay et al. 1987; Herren et al. 2006; Wiesemeier et al. 2007). This aptitude is mainly due to their efficient system of chemical defense and their capacity to propagate successfully by fragmentation. Also *Dictyota* populations apparently survive less favorable seasons as small germlings that form new populations when favorable conditions return (van den Hoek et al. 1995; Richardson 1979). In general, in annual species, most of the biomass is allocated to

reproductive structures, which in adverse seasons survive either as a reduced form or as spores or crust-like disks when spores have germinated (Lüning 1990).

It was often reported that seasonal variation in growth and reproduction of benthic marine macroalgae is usually attributed to seasonal changes in major environmental factors such as temperature, light intensity, nutrients, water movement and tide levels (Lüning and tom Diek 1989; Lüning 1990; Santelices 1990). Several *Dictyota* species have been the subject of phenological studies in many regions of the world (Peckol 1982; King and Farrant 1987; Phillips 1988; Hwang et al. 2005; Tronholm et al. 2008), but to date, there has been no report on seasonality of any *Dictyota* species in the Southern Atlantic Ocean. Also only few morphological studies exist, with an emphasis on Brazilian *Dictyota* species (Guimaraens and Coutinho 1996; De Castro Nunes and De Paula 2001).

Particularly along the North Patagonian coasts from Argentina several *Dictyota* species have been reported (Asensi 1966; Piriz et al. 2003; Boraso and Zaixo 2012). *Dictyota* acquire also ecological importance along Argentinean coasts as one of the most abundant genera in diverse regions (Casas et al. 2004).

The *Dictyota* specimens studied in this work strongly resembles morphologically to *Dictyota dichotoma* (Hudson) J.V. Lamouroux. Moreover, a number of recently sequenced *Dictyota* specimens from Argentina leave little doubt that the *Dictyota* Argentinian populations are conspecific with the European *D. dichotoma*. (Dr. O. De Clerck, pers. com.). Consequently, the identity of the present material as *D. dichotoma* is plausible.

To date, no studies have been published on ecological or phenological issues of any *Dictyota* populations of Argentina. Therefore, the general aim of this study is to morphologically characterize a population of *D. dichotoma* from the temperate locality Las Grutas, Río Negro, Argentina. Particular goals were to carry out a detailed account of vegetative and reproductive morphologies with analyses of the temporal morphological plasticity, rates of diploid/haploid and male/female individuals and the reproductive efficiencies of two distinct fronds' regions. Also an important goal was to provide phenological data on the population dynamics.

Materials and methods

Study site

We studied a *D. dichotoma* population at Las Grutas Beach (40°48′S, 64°48′W), located in the NW corner of the San Matías Gulf, Argentina (Fig. 1). The shore is

Fig. 1 Sampling site. **1** Location of the study site at Las Grutas, San Matías Gulf, Río Negro Province, Argentina

characterized by a moderate degree of wave exposure and a large tidal range, with high tides reaching a maximum height of 9 m. Surface seawater temperatures were measured, and the daylength was registered during the study period.

Field survey and morphological analysis

Specimens of *D. dichotoma* were collected monthly by SCUBA diving from the subtidal zone, from April 2009 to March 2010. Each month, 60 entire attached individuals were collected haphazardly in the same study place. Each selected frond was cut with scissors precisely above the holdfast. All specimens were squeezed by hand to remove excess seawater and subsequently transported to the laboratory in plastic bags. Samples were stored overnight in a refrigerator. The following day, each specimen was washed thoroughly with seawater to remove sand.

Dry mass was estimated on a monthly basis by selecting 20 individuals and drying them for 48 h at 70 °C. The length of each individual was measured, and the reproductive stage (non-fertile thalli, sporophytes, male and female gametophytes) was recorded. Small sections were selected at random (ca. 3 mm × 3 mm), to be examined under the light microscope for morphological analyses and descriptive purposes (Tronholm et al. 2008). Vegetative and reproductive characters used in this study are listed in Table 1. Micrographs were taken using a Nikon Eclipse TE 300 (Nikon, Tokyo, Japan) microscope with photo camera Nikon FDX 35.

For scanning electron microscopy (SEM), portions of *D. dichotoma* fronds were fixed in 2.5 % glutaraldehyde-seawater at 5 °C, buffered with cacodylate (pH 7.2) for 2 h. They were mounted on slides covered with 0.5 % poly-D-lysine and dehydrated in a graded acetone series. Samples were critical point dried during 1 h, coated with gold using a Sputter Coater 9100 mod. III and observed with a Leo Evo 40 scanning electron microscope.

Table 1 Quantitative variables used in the morphometric analysis

Vegetative characters	Reproductive characters
Thallus length (cm)	Sporangia diameter (μm)
Thallus dry mass (g)	Stalk cell height of sporangia (μm)
Branches from main axes (number)	Oogonial sori length (μm)
Interdichotomies length (mm)	Oogonial sori width (μm)
Interdichotomies width (mm)	Oogonia per sorus (number)
Branching angle, apical parts (°)	Antheridial sori length (μm)
Branching angle, basal parts (°)	Antheridial sori width (μm)
Cortical cell length (μm)	Antheridial row width (μm)
Cortical cell width (μm)	Antheridial row length (μm)
Cortical cell height (μm)	Antheridial tiers (number)
Medullary cell length (μm)	Antheridial stalk cell height (μm)
Medullary cell width (μm)	
Medullary cell height (μm)	

Reproductive efficiency and sex ratio estimation

The sex ratio (total males/total females) was estimated. To calculate reproductive efficiency, small reproductive areas (ca. 2 mm × 2 mm = 4 mm^2) were monthly removed from apical and basal regions of 20 different individuals of each type (female/male gametophytes and sporophytes). The reproductive capacity was established by counting reproductive structures on fronds under microscope.

Statistical analyses

Each data set was examined for variance of homogeneity using Barlett′s test and transformed if necessary to meet parametric requirements. To study the effects of seasons on vegetative characteristics, data from 3 month were pooled (autumn: March, April and May; winter: June, July and August, spring: September, October and November and summer: December, January and February). A one-way analysis of variance (ANOVA) was performed to evaluate the differences in reproductive efficiency in apical and basal zones of fronds and to evaluate differences between formation of male and female gametophytes and seasons. A two-way analysis of variance model was employed to analyze the combined effect of seasons and life stages on morphological characteristics. The Student–Newman–Keuls' (SNK) multiple comparison tests were used in cases of significant differences, to facilitate the interpretation of the ANOVA results by differentiating subsets of means.

Pearson's correlation analyses were carried out to determine relationships of the morphological characteristics (Table 1) with seawater temperature and daylength ($\alpha = 0.05$). All statistical tests and analyses were performed using Xlstat version 2012.6.01 for Windrows 7 Home Basic (2009).
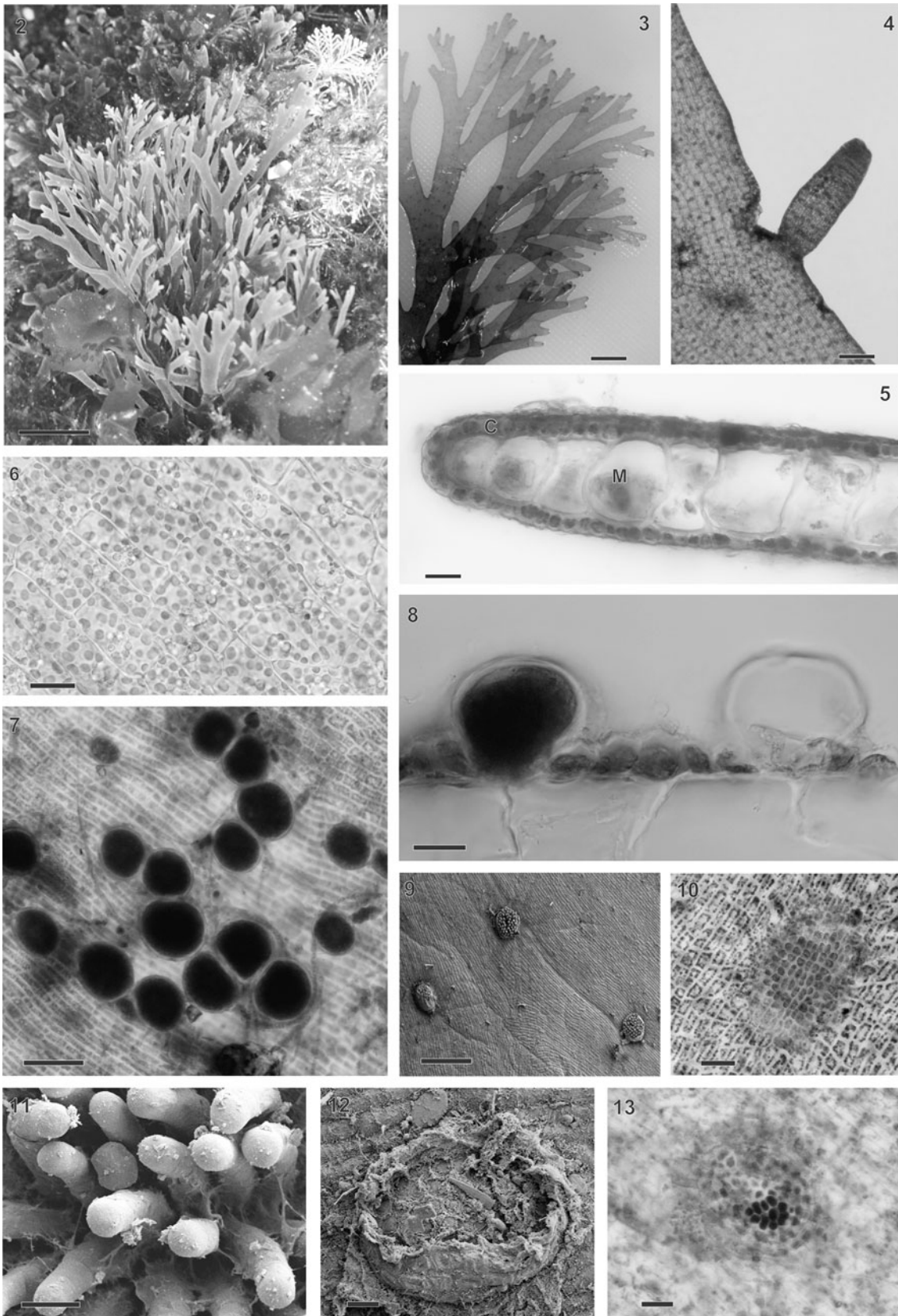
Results

Vegetative morphological characteristics

Thalli were (1.7) 7–8 (19.3) cm long (Fig. 2), with main axis (4) 8.2–8.8 (17) times branched (Fig. 3). Branching angles are (7) 31–35 (81) degrees in apical portions and (10) 56–62 (135) degrees in basal segments. Interdichotomies were (3) 6.9–7.7 (19) mm in length and (0.1) 1.5–1.7 (5) mm width. Thallus margins and surfaces were smooth, although occasionally, in basal regions, proliferations perpendicular to surface were observed (Fig. 4). Cortical cells (Fig. 5) were rectangular in surface view, (12.5) 39–42 (72.5) μm in length and (7.5) 13–13.7 (30) μm width (Fig. 6), and square in transverse section, (5) 13–14 (45) μm height. Medullar cells were rectangular in both surface view and transverse section, (20) 67–73 (130) μm in length, (17.5) 41–44 (105) μm width and (20) 44–49 (97.5) μm height (Fig. 5).

Reproductive morphological characteristics

Sporangia were scattered on both sporophyte thallus surfaces (Fig. 7) and (30) 59–61 (200) μm in diameter (Fig. 8), born on single stalk cells (5) 15–20 (28) μm height. Antheridial sori were ellipsoidal in surface view, (30) 92–108 (227.5) μm width and (25) 97–112 (170) μm length (Figs. 9, 10), each one surrounded by a ring of pigmented paraphyses. Antheridia were arranged in regular rows, each row with a width of (7) 12–18 (25) μm and a length of (50) 99–227 (430) μm length and. Each antheridium consisted of (5) 30–42 (55) tiers (Fig. 11) and was born on a single stalk cell 15–20 μm in height. Paraphyses persisted as a ring after the release of antherozoids (Fig. 12). Oogonial sori were round to oval in surface view

◄ **Figs. 2–13** *Dictyota dichotoma*. Vegetative and reproductive structures. **2** Habit of a thallus in nature. **3** Detail of terminal portions showing dichotomous branching, rounded apices and smooth margins. **4** Detail of a perpendicular proliferation arising from the thallus surface associated with tissue rupture. **5** Transverse section of a thallus showing the unilayered cortex (*C*) and the medulla (*M*). **6** Detail of the thallus in surface view showing rectangular cortical cells with numerous discoid chloroplast. **7** Detail of a sporophyte in surface view to see dark brown, subspherical sporangia. **8** Detail of two sporangia, one of them empty, in a transverse section of a sporophyte. **9** Scanning electron micrograph of a male gametophyte in surface view showing three mature antheridial sori. **10** Detail of a mature antheridial sorus. Antheridia were arranged in regular rows. **11.** Detail of antheridia under scanning electron microscope. **12** An antheridial sorus after gametes release; a ring of paraphyses persists **13.** Oogonial sorus. *Scale bars*: Fig. 2, 3 cm; Figs. 3, 4, 50 mm; Fig. 5, 30 μm; Fig. 6, 15 μm; Fig. 7, 60 μm; Fig. 8, 40 μm; Fig. 9, 200 μm; Fig. 10, 100 μm; Figs. 11, 12, 20 μm; Fig. 13, 150 μm

(Fig. 13), (45) 145–280 (320) μm long and (37.5) 92–120 (230) μm wide, with 15–25 oogonia per sorus.

Environmental parameters and temporal variation in vegetative variables

Thalli were observed throughout the year. Their morphology showed significant seasonal variation, being them longer during spring–summer than during autumn–winter ($F_{3,226} = 77.808$, $p < 0.0001$) (Figs. 14, 15). The largest thalli were observed in December and January (10.62 ± 2.21, 10.67 ± 2.71 cm, respectively) and the smallest in June and July (4.59 ± 1.49, 4.58 ± 1.43 cm, respectively). Frond length showed a positive correlation with daylength (Pearson's correlation: $R = 0.721$, $p < 0.0001$) (Fig. 14). Surface seawater temperature also showed an increase during the summer, but the relationship of frond length with seawater temperature does not fit as well as with daylength (Fig. 14). However, among seawater temperature and frond length, a significant correlation was observed (Pearson's correlation: $R = 0.591$, $p < 0.0001$).

Dry mass also varied seasonally ($F_{3,226} = 40.826$, $p < 0.001$). The lowest biomass was observed in winter, coinciding with shorter daylength (Fig. 15). There was also a significant correlation between both variables (Pearson's correlation: $R = 0.180$, $p = 0.006$). Dry mass also correlated highly significant with seawater surface temperature (Pearson's correlation: $R = 0.457$, $p < 0.0001$) and frond length (Pearson's correlation: $R = 0.357$, $p < 0.0001$).
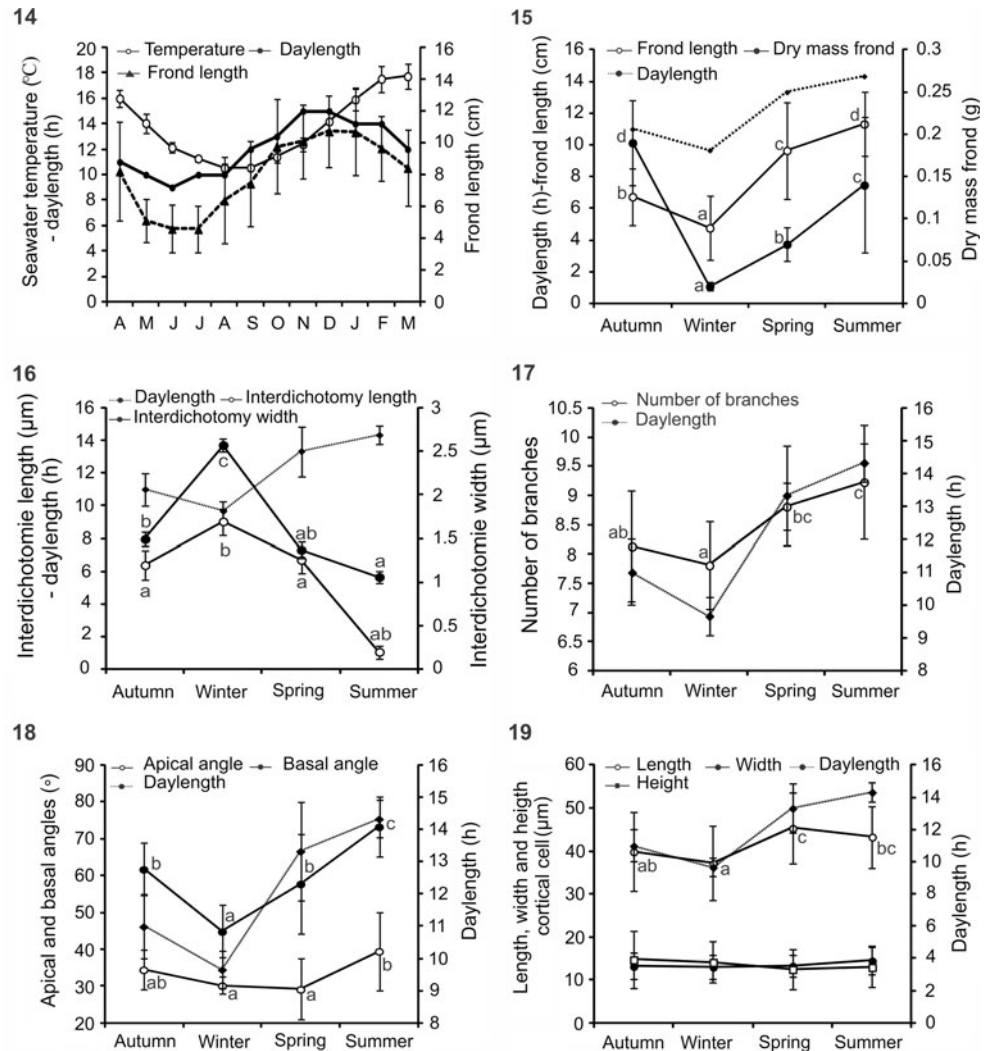
Length and width of apical interdichotomies showed the same behavior throughout the year (Fig. 16). Between seasons interdichotomy length varied significantly ($F_{3,225} = 4.636$; $p < 0.05$). Interdichotomies were longest in winter, while they considerably shortened in summer (Fig. 16). Moreover, interdichotomies were wider in winter and significantly narrower in summer ($F_{3,225} = 26.785$;

$p < 0.0001$) (Fig. 16). This variable presented negative significant correlations with daylength (Pearson's correlation: $R = -0.489$; $p < 0.0001$), frond length (Pearson's correlation: $R = -0.308$, $p < 0.0001$) and frond dry mass (Pearson's correlation: $R = -0.182$, $p = 0.006$). These results indicated that shorter and lighter thalli presented wider interdichotomies. Otherwise, there was no significant correlation between interdichotomy length and frond length (Pearson's correlation: $R = -0.079$, $p = 0.233$). Furthermore, a significant negative correlation between interdichotomy width and frond dry mass was registered (Pearson's correlation: $R = -0.182$, $p = 0.006$), indicating that heavier thalli presented narrower interdichotomies.

The number of primary branches originated from main axis showed significant seasonal variation ($F_{3,226} = 5.56$; $p < 0.001$) (Fig. 17). During winter, fronds were less branched compared to those observed in spring–summer (Fig. 17). There were significant correlations between the number of branches and both frond length and daylength (Pearson's correlations: $R = 0.264$, $p < 0.0001$; $R = 0.248$; $p = 0.00015$, respectively). In contrast, no significant correlation was observed between frond dry mass and primary branching (Pearson's correlation: $R = 0.013$, $p = 0.850$). Moreover, a significant negative correlation was registered between the number of primary branches and interdichotomy width (Pearson's correlation: $R = -0.149$, $p = 0.024$), suggesting that more branched thalli presented narrower interdichotomies.

A seasonal variation of branching angles was observed, with higher values in summer and lower ones in winter–spring (apical angle: $F_{3,226} = 4.943$, $p = 0.002$; basal angle: $F_{3,226} = 10.662$, $p < 0.0001$) (Fig. 18). There were significant positive correlations between both positional angles with seawater surface temperature, daylength, frond length and dry mass (Pearson' correlations to apical angle, $R = 0.260$, $p < 0.0001$; $R = 0.165$, $p = 0.012$; $R = 0.186$, $p = 0.005$; $R = 0.324$, $p < 0.0001$, respectively, and Pearson's correlation to basal angle, $R = 0.332$, $p < 0.0001$; $R = 0.334$, $p < 0.0001$; $R = 0.303$, $p < 0.0001$, $R = 0.143$, $p = 0.031$, respectively). Furthermore, a significant negative correlation between both apical and basal branching angles and interdichotomie width was also observed (Pearson's correlations, $R = -0.176$, $p = 0.008$; $R = -0.134$, $p = 0.043$, respectively), thus fronds with larger branching angles presented narrower interdichotomies. A highly significant correlation between the number of branches and the basal branching angle was detected (Pearson's correlation: $R = 0.241$, $p = 0.0002$), suggesting that thalli with larger basal angles carry more primary branches. A significant positive correlation between the number of primary branches and interdichotomy width appeared (Pearson's correlation: $R = 0.164$, $p = 0.013$). A significant positive correlation between apical and basal angles existed

Figs. 14–19 *Dictyota dichotoma.* Temporal variation of vegetative parameters (mean ± SD). **14** Variation of frond length, daylength and surface water temperature monitored during April 2009 and March 2010. **15** Seasonal variation of frond length, frond dry mass and daylength. **16** Seasonal variations of length and width interdichotomies and daylength. **17** Seasonal variation of primary branches occurrence, main axes and daylength. **18** Seasonal variation of apical and basal branching and daylength. **19** Seasonal variation of length, width and height of cortical cells and daylength. In Figs. 15, 16, 17, 18 and 19, significant differences between seasons (α < 0.05) are indicated by *different letters* (SNK-tests)

(Pearson's correlation: $R = 0.164$, $p = 0.013$), by which it is expected to find in a same individual broad or narrow apical angles when it had broad or narrow basal angles, respectively.

Regarding cortical cells in both superficial views and transverse sections, it was observed that only cellular lengths varied seasonally ($F_{3,226} = 5.747$, $p = 0.001$), with cells longer in spring–summer and shorter in winter (Fig. 19). Both width and height of cortical cells were statistically unchanged during the seasons, ($F_{3,226} = 0.497$, $p = 0.685$; $F_{3,226} = 2.218$, $p = 0.087$, respectively) (Fig. 19). Correlation analysis indicated weak positive significant correlations between cortical cell length, on the one hand, and daylength and frond length, on the other hand (Pearson's correlations: $R = 0.268$, $p < 0.0001$; $R = 0.264$, $p < 0.0001$, respectively). Therefore, the longest thalli with the longest cortical cells may be expected on the longest days of the year. An interesting negative correlation was observed between the cortical cell length

and the interdichotomy width (Pearson's correlation: $R = -0.203$, $p = 0.002$). Therefore, in narrower interdichotomies, longer cortical cells are expected. A significant correlation was also observed between the cortical cell lengths and the branching basal angles (Pearson's correlation: $R = 0.135$, $p = 0.041$).

The cortical cell width was also significantly correlated with daylengths (Pearson's correlation: $R = 0.151$, $p = 0.023$); thus, cortical frond cell was wider in spring–summer. In contrast, cortical cell height was negatively correlated with daylengths (Pearson's correlation: $R = -0.136$, $p = 0.039$), which indicated that during spring–summer thalli would be thinner, owing to lower cellular height.

Length, width and height of medullar cells varied significantly between seasons (length: $F_{3,226} = 15.604$, $p < 0.0001$; width: $F_{3,226} = 3.170$, $p = 0.025$; height: $F_{3,226} = 4.785$, $p = 0.003$) (Fig. 20). The cellular length was greater in summer and lower in winter, and cells were

wider in spring and narrower in autumn, whereas cellular height behaved differently and was lower in summer (Fig. 20). Medullar cell length showed a significant positive correlation with seawater surface temperature, daylength, frond length, cortical cell length and the medullar cell width (Pearson's correlation: $R = 0.219$, $p = 0.001$; $R = 0.376$, $p < 0.0001$; $R = 0.260$, $p < 0.0001$; $R = 0.372$, $p < 0.0001$, $R = 0.183$, $p = 0.005$, respectively). These results suggest that thalli tend to be longer during spring–summer (the time with higher seawater temperature and longer days), with both larger cortical and medullar cells. The medullar cell width only showed a significant correlation with medullar cell length and cortical cell length (Pearson's correlations: $R = 0.183$, $p = 0.005$; $R = 0.234$, $p = 0.0003$, respectively). Medullar cell height was correlated significantly with seawater surface temperature and daylength (Pearson's correlation: $R = -0.184$, $p = 0.005$, $R = -0.183$, $p = 0.005$, respectively). Thus, in nature, thinner thalli may be expected during spring–summer than in autumn–winter.
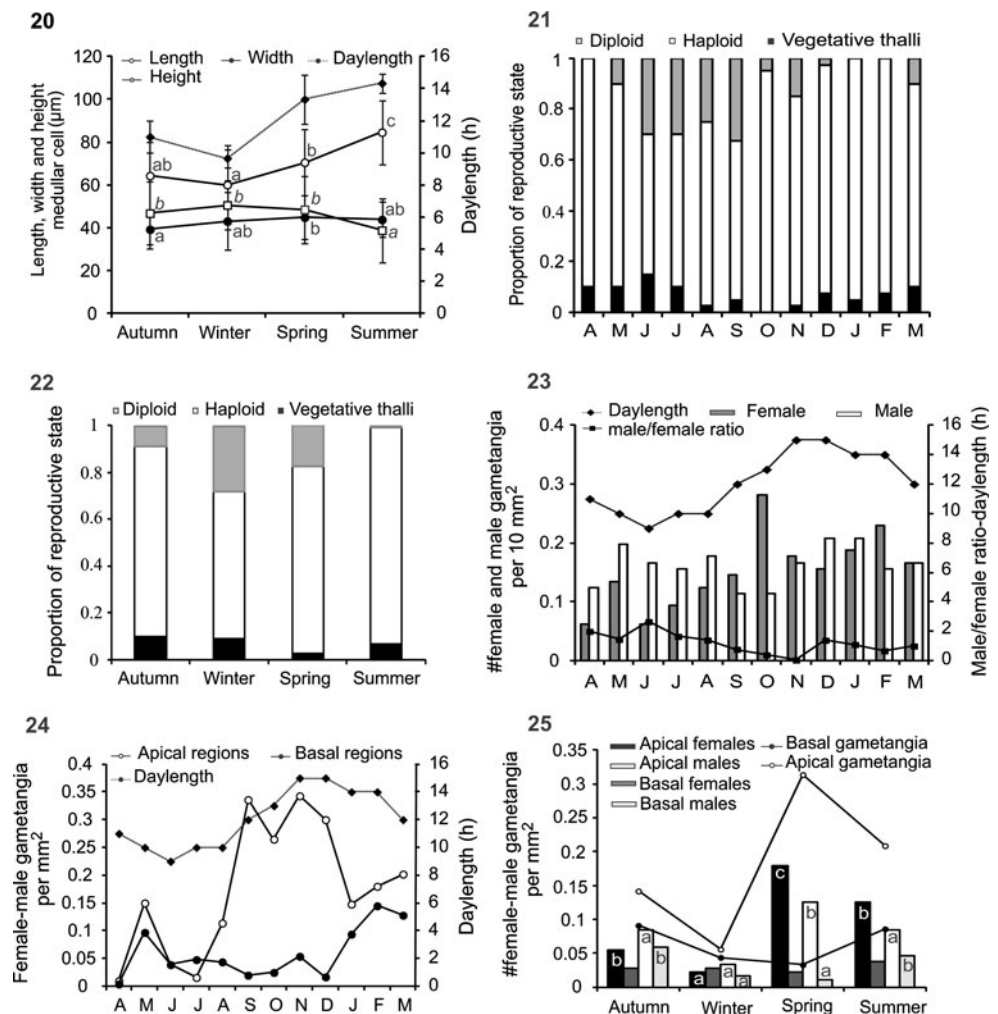
Reproductive phenology

Prevalence of vegetative individuals

The prevalence ratio of vegetative and reproductive thalli presented always low values (Fig. 21). Moreover, it was not constant throughout the year being the highest proportions observed from March to June (Fig. 21). Seasonally, vegetative thalli always had a low prevalence, reaching minimum values in spring (Fig. 22).

Fertile individuals' proportion and sex ratio

The proportions of haploid and diploid fertile individuals observed throughout the year are given in Figs. 21 and 22. The highest proportions of haploid thalli were observed in January–February and April, coinciding with the absence of diploid thalli (Fig. 21). On the other hand, the highest proportions of diploid thalli were observed during the coldest months, i.e., June, July, August and September

Figs. 20–25 *Dictyota dichotoma*. Temporal variation of vegetative and reproduction parameters. **20** Seasonal variation of length, width and height of medullar cells and daylength. Significant differences between seasons ($\alpha < 0.05$) are indicated by *different letters* (SNK-tests). **21** Monthly variation of diploid, haploid and vegetative thalli. **22** Seasonal variation of diploid, haploid and vegetative thalli. **23** Monthly variation of female and male gametangia/10 mm^2 of examined algal tissue, male/female ratio and daylength. **24** Monthly variation of female and male gametangia formed per mm^2 of examined algal tissue and daylength. **25** Seasonal variation of female and male gametangia/mm^2 of examined algal tissue and total apical and basal gametangia/mm^2 examined algal tissue. Significant differences between seasons ($\alpha < 0.05$) are indicated by *different letters* (SNK-tests)

(Fig. 21). Seasonally, the highest proportions of haploid individuals were observed in summer and the minimum in winter, whereas the maximal presence of diploid individuals occurred in winter with the minimum in summer (Fig. 22).

Regarding male/female sex ratios, we observed values of 2:1 from April to August, values of 1:2 and 1:3 in September/October and 1:1 ratios in the rest of the months (Fig. 23). Seasonally, significant differences were detected ($F_{3,8} = 1.241$, $p = 0.357$), with the highest values found in autumn/winter.

Regarding the number of male and female gametangia/ 10 mm^2 of examined algal tissue, it was observed that male gametangia dominated from April to August and in December/January (Fig. 23). Female gametangia were most abundant from September to November and in February (Fig. 23). A significant correlation was observed between the number of female gametangia and daylength (Pearson's correlation: $R = 0.682$, $p = 0.015$). On the other hand, no significant correlation between the numbers of female and male gametangia and male/female sex ratios with seawater temperature was observed.

In gametophytes, the apical zones were more fertile than the basal zones ($F_{1,22} = 9.934$, $p < 0.005$), with exception of the months of June and July (Fig. 24). Furthermore, a significant correlation was detected between the density of gametangia in apical zones and daylength (Pearson's correlation: $R = 0.723$, $p = 0.008$), indicating that on longer days, these regions were more fertile (Fig. 24). Moreover, a significant correlation was observed between the number of gametangia per mm^2 of basal thallus regions and the surface water temperature (Pearson's correlation: $R = 0.652$, $p = 0.022$), indicating that at low temperatures, the number of gametangia in basal areas was scarce.

Regarding the number of sporangia per mm^2 of algal tissue, there were no significant differences between apical and basal regions ($F_{1,22} = 0.015$, $p > 0.904$). Seasonally, a high number of sporangia were recorded in autumn–winter.

Overall, no significant differences were detected between the number of female and male gametangia in apical regions ($F_{1,22} = 0.027$, $p > 0.870$) or basal regions ($F_{1,22} = 0.154$, $p > 0.698$). However, significant seasonal differences were observed between the numbers of female gametangia arising from apical regions ($F_{3,108} = 18.374$, $p < 0.0001$) (Fig. 25). The maximum number of female gametangia per mm^2 algal tissue was recorded in spring and the lowest in winter (Fig. 25). On the contrary, the number female gametangia formed in basal regions showed no significant differences between seasons ($F_{3,108} = 2.449$, $p > 0.068$) (Fig. 25).

In male gametophytes, the density of gametangia showed significant seasonal differences both in apical and basal thallus regions (Apical: $F_{3,81} = 7.907$, $p < 0.0001$; basal: $F_{3,81} = 6.374$, $p < 0.001$) (Fig. 25). In apical zones,

most male gametangia were produced in spring, whereas in basal zones, the maximum number was recorded in autumn and summer (Fig. 25).

Temporal variation between different life cycle stages

Among different life cycle stages, significant variations were observed in the following variables: frond length, frond dry mass, number of primary branches and branching basal angle (Table 2). Regarding frond length, sporophytes were the shorter, while male and female gametophytes were the longest. Sporophytes presented also the lower dry mass, followed by both non-fertile thalli and male gametophytes, whereas female gametophytes were the individuals with higher dry mass (Table 2).

Male gametophytes showed a higher number of primary branches, compared to the other three stages (Table 2). Considering the variable branching basal angles, both male and female gametophytes showed greater angulations as compared to sporophytes and non-fertile thalli. All variables analyzed varied seasonally, except cortical cell width that was stable between seasons (Table 2). Regarding interaction between stages per season factors for different variables, in all cases, they showed no significant F values (Table 2).

Discussion

Taxonomic commentary

Members of Dictyotales are defined by differences in external morphology and vegetative and reproductive anatomy, which may constitute unreliable taxonomical parameters since most of them show extensive variation (Phillips 1997; De Clerck and Coppejans 2003). Particularly in *Dictyota*, species delimitation is mainly based on quantitative features, i.e., interdichotomies sizes, branching angles, sizes of cortical and medullary cells, presence or absence of teeth or outgrowths from margin or thallus surface and the size of reproductive structures (Weber-Peukert 1985; Hörnig et al. 1992a, b; De Clerck 2003; Tronholm et al. 2013). In species as *Dictyota robusta* J. Agardh, *Dictyota suhrii* G. Murray, *Dictyota radicans* Harvey and *Dictyota crenulata* J. Agardh, medulla variations were reported (De Clerck 2003; Altamirano-Cerecedo and Riosmena-Rodríguez 2007). In our study, only uni-layered medulla was present in all extensions of thalli and proliferations.

The application of DNA sequencing has considerably altered the general ideas about algal diversity at the species-level. Several studies have revealed cryptic species within morphologically defined species, falsifying the

Table 2 *Dictyota dichotoma*. Effects of stages and seasons on vegetative characteristics

Parameter	Factor	df	MS	F	p	SNK
Length frond (cm)	Stage	3	0.950	8.273	**<0.0001**	a)sp; b)m, f; ab)nf
	Season	3	8.933	77.808	**<0.0001**	a)w; b)au; c)s; d)su
	Season × stage	8	0.087	0.757	0.641	
Dry mass frond (g)	Stage	3	7.419	7.991	**<0.0001**	a)sp; b)f; ab)m, nf
	Season	3	37.903	40.826	**<0.001**	a)w, s; b)su, au
	Season × stage	8	0.565	0.609	0.770	
Interdichotomie width (μm)	Stage	3	0.259	2.158	0.094	
	Season	3	3.217	26.785	**<0.0001**	a)s; ab)w, au; b)su
	Season × stage	8	0.050	0.419	0.909	
Interdichotomie length (μm)	Stage	3	0.110	0.749	0.524	
	Season	3	0.684	4.638	**0.04**	a)au, s; b)su, w
	Season × stage	8	0.237	1.609	0.123	
Number of branches	Stage	3	0.187	2.955	**0.033**	a)sp, f, nf; b) m
	Season	3	0.352	5.560	**0.001**	a)w; ab)au, s; b)su
	Season × stage	8	0.063	1.000	0.437	
Apical angle (°)	Stage	3	0.115	0.637	0.592	
	Season	3	0.893	4.943	**0.002**	a)w, s; ab)au; b)su
	Season × stage	8	0.127	0.702	0.689	
Basal angle (°)	Stage	3	10.248	4.492	**0.004**	a)sp, nf; b)m, f
	Season	3	24.326	10.662	**<0.0001**	a)w; ab)s; b)au; c)su
	Season × stage	8	2.737	1.199	0.300	
Cortical cell length (μm)	Stage	3	118.218	0.957	0.414	
	Season	3	709.56	5.747	**0.001**	a)w; ab)au; b)su; c)s
	Season × stage	8	73.64	0.596	0.780	
Cortical cell width (μm)	Stage	3	0.639	1.341	0.262	
	Season	3	0.237	0.497	0.685	
	Season × stage	8	0.394	0.826	0.580	
Cortical cell height (μm)	Stage	3	0.197	1.510	0.213	
	Season	3	0.290	2.218	0.087	
	Season × stage	8	0.130	0.999	0.438	
Medullar cell length (μm)	Stage	3	0.581	0.371	0.774	
	Season	3	24.456	15.604	**<0.0001**	a)w; ab)au; bc)su; c)s
	Season × stage	8	1.821	1.162	0.324	
Medullar cell width (μm)	Stage	3	0.036	0.073	0.974	
	Season	3	1.573	3.170	**0.025**	a)w; ab)au; bc)su; c)s
	Season × stage	8	0.233	0.470	0.876	
Medullar cell height (μm)	Stage	3	0.378	2.612	0.052	
	Season	3	0.692	4.785	**0.003**	a)w; ab)au, su; c)s
	Season × stage	8	0.136	0.937	0.487	

Two-way model I ANOVA. Monthly sampling during April 2009–March 2010; *df* degrees of freedom, *MS* mean square, *F* ANOVA *F*-statistic, *p* probability; significant effects in bold type. Significant differences between stages and seasons (α < 0.05) are indicated by different letters (SNK-test)

Seasons' abbreviations: *au* autumn, *w* winter, *s* spring, *su* summer. Stages' abbreviations: *sp* sporophytes, *m* male gametophyte, *f* female gametophyte, *nf* non-fertile

assumption that speciation events coincide with any morphological differentiation (Tronholm et al. 2010a). Therefore, molecular work is currently conducted by us to precisely corroborate the identity of the present population and to evaluate the possibility that it could represent a cryptic species (Marine macroalgae collections in north Patagonian coasts to obtain DNA barcodes: iBol Project, CONICET).

Nevertheless, although we assume that molecular information is of paramount importance for correct and complete identifications of populations, we emphasize also the importance of enhancing morphological and phenological data in representatives of marine benthic flora as is the particular case of the genus *Dictyota*, especially in regions of the world poorly studied.

Life cycle

Species in the order Dictyotales basically have an isomorphic diploid–haploid life cycle (van den Hoek et al. 1995). In the present *Dictyota* population from Argentina, male and female gametophytes, sporophytes and non-fertile specimens could be found in any season. The same observations were recorded in one *D. dichotoma* population from Spain (Tronholm et al. 2010b). In both populations, sterile specimens were present in very low numbers. On the contrary, in a Canary Islands population of *D. dichotoma* (Tronholm et al. 2008), all thalli were absent during September–October.

Gametophytes were always more abundant in Argentina suggesting a relevant role of sexual reproduction in the population dynamics. In contrast, in the population from Spain (Tronholm et al. 2010b), fertile sporophytes were constantly more numerous. In a population of *D. dichotoma* at Punta del Hidalgo, Spain (Tronholm et al. 2008), sporophytes also dominated every month, which was interpreted by the author as a support for the hypothesis that reproduction in all dictyotalean species takes place almost entirely by means of sporangia, with sexual reproduction only occasional (Allender 1977; King and Farrant 1987; Phillips 1988; Hwang et al. 2005; Tronholm et al. 2008).

In several populations of *Dictyota* and akin genera around the world, the occurrence of a complete life cycle is not common. Hwang et al. (2005) found in *D. dichotoma* sensu Okamura from Korea that both sexual and asexual reproduction occurred only on the south coast, while a dominant sexual life cycle came about along the west coast and an exclusive asexual life cycle took place along the east coast, where only sporophytes existed and male or female gametophytes were apparently absent. These authors also observed asexual reproduction through in situ germination of monospores on parental thalli, which generated propagules that were released by abscission, resulting in a great amount of both free-living and attached thalli, the latter with a single stalk cell.

Ateweberhan et al. (2005) never observed gametophytes in a population of *Canistrocarpus cervicornis* (Kützing) De Paula & De Clerck. These authors suggested that this absence is attributable to the fact that the population asexually persisted by direct development of apomeiotic tetraspores into new sporophytes. This type of reproduction has been also previously reported in other Dictyotales by Gaillard (1972).

Temporal variation in vegetative and reproductive variables and reproductive phenology

In the present population, the different vegetative and reproductive parameters were strongly seasonal. Peaks were observed during spring–summer in frond length, frond dry mass, number of primary branches and length of cortical and medullar cells. Also during spring–summer, a maximum in reproductive capacity was observed, which was measured by the number of gametophytes per algal tissue examined. Previous works in other macroalgal communities in Argentina, also demonstrated that algal biomass peaks occur in spring–summer (Piriz et al. 2003). The Mediterranean population of *D. dichotoma* (Tronholm et al. 2008) also presented the longest and more branched thalli in spring–summer, as well as the longest and narrowest interdichotomies and the lowest apical and basal branching angles. On the contrary, in a *Canistrocarpus cervicornis* population from the Red Sea (Ateweberhan et al. 2005), peaks in thallus length, biomass and reproduction were detected during the cold season, while macrothalli were completely absent in the hot season.

Tronholm et al. (2008) reported that *D. dichotoma* from Punta del Hidalgo survives in autumn as microscopic resting stages and considered that this fact can be correlated with the temperature ranges observed along the warm temperate north-eastern Atlantic region. Thus, these authors suggested that the main factor controlling thallus growth was seawater temperature. In the present study, we found a correlation of temporally variable vegetative traits with water temperature but also, in many cases, we found them better correlated with daylengths.

The highest reproductive rate was observed mainly in November, coinciding with larger thalli and the seasonally increase in daylength. Sporophytes were formed mainly in winter, when water temperature was low and days were short. The fronds apical regions were the most reproductive in the *Dictyota* population here studied. Male and female gametophytes formed mostly apical gametangia during spring and early summer, possibly owing to favorable light conditions. To date, our data about the frequency in the formation of reproductive structures and male/female ratios are the first recorded for a member of the genus *Dictyota*, and thus, they could not be compared with populations from other regions of the world.

To our knowledge, no comprehensive quantitative studies of the reproductive capacity have so far been realized in *Dictyota*. Only for *D. dichotoma*, the relative proportion of gametophytes, sporophytes and non-fertile thalli has been reported (Hwang et al. 2005; Tronholm

et al. 2008). The striking lack of data about algal sex ratios may be due in part to the difficulties of distinguishing males from females, especially among spores and in pre-reproductive material. In general, 1:1 ratios were the most frequently observed in the here studied *Dictyota* population from Argentina but, when this was not the case, female gametophytes were more common than male ones.

De Wreede and Klinger (1988) signaled the temperature range as a general key factor for the determination of the sex ratio in macroalgae. It was reported that in *Laminaria religiosa Miyabe*, a 1:1 gametophytes ratio occurred at optimum growth temperatures (10–15 °C), but proportionately fewer males were present at both higher and lower temperatures (Funano 1983). In contrast, in our study, we detected no correlation between sex ratios and water temperatures.

References

Allender BM (1977) Ecological experimentation with the generations of *Padina japonica* Yamada (Dictyotales, Phaeophyceae). J Exp Mar Biol 26:225–234

Altamirano-Cerecedo MC, Riosmena-Rodríguez R (2007) Vegetative and reproductive variability of *Dictyota crenulata* (Phaeophyta: Dictyotales) along the Central and Southwestern Gulf of California. Mexico. Pac Sci 61(4):575–586

Asensi AO (1966) Guía para reconocer los géneros de algas pardas de la Argentina. Contribución del Instituto Antártico Argentino, Argentina, pp 1–51

Ateweberhan M, Bruggermann JH, Breeman AM (2005) Seasonal patterns of biomass, growth and reproduction in *Dictyota cervicornis* and *Stoechospermum polypodioides* (Dictyotales, Phaeophyta) on a shallow reef flat in the southern Red Sea (Eritrea). Bot Mar 48:8–17

Boraso AL, Zaixo E (2012) Atlas de Sensibilidad Ambiental de la Costa y el Mar Argentino. Inventarios y distribución de flora y fauna. Available from: www.atlas.ambiente.gov.ar

Casas G, Scrosati R, Piriz ML (2004) The invasive kelp Undaria pinnatifida (Phaeophyceae, Laminariales) reduces native seaweeds diversity in Nuevo Gulf (Patagonia, Argentina). Biol Inv 6:11–416

De Castro Nunes JM, De Paula EJ (2001) O gênero *Dictyota* Lamouroux (Dictyotaceae-Phaeophyta) no litoral do Estado da Bahia, Brasil. Acta Bot Malac 26:5–18

De Clerck O (2003) The genus *Dictyota* (Dictyotales, Phaeophyta) in the Indian Ocean. Opera Botanica Belgica, National Botanic Garden of Belgium, Belgium, pp 1–205

De Clerck O, Coppejans E (1999) Two new species of *Dictyota* (Dictyotales, Phaeophyta) from the Indo-Malayan region. Phycologia 38:184–194

De Clerck O, Coppejans E (2003) Morphology and systematic of two aberrant species of *Dictyota* (Dictyotaceae, Phaeophyta), including a discussion on the generic boundaries in the tribe Dictyoteae. In: Chapman ARO, Anderson RJ, Vreel VJ, Davison IR (eds) Proceedings of 17th Int. Seaweed Symp. Cape Town, Oxford University Press, New York, pp 275–284

De Wreede RE, Klinger T (1988) Reproductive strategies in algae. In: Lovett Doust J, Lovett Doust L (eds) Plant reproductive ecology. Oxford University Press, New York, pp 267–284

Funano T (1983) The ecology of *Laminaria religiosa* Miyabe. I. The life history and alternation of nuclear phases of *Laminaria religiosa*, and the physiological ecology of the gametophytes and the embryonal sporophytes. Hokusui-Shiho 25:61–109

Gaillard J (1972) Quelques remarques sur le cycle reproducteur des Dictyotales et sur ses variations. Bull Soc Bot Fr Mém. pp. 145–150

Guimaraens M, Coutinho R (1996) Spatial and temporal variation of benthic marine algae at the Cabo Frio upwelling region, Rio de Janeiro, Brazil. Aquat Bot 52:283–299

Hay ME, Duffy JE, Pfister CA, Fenical W (1987) Chemical defense against different marine herbivores: are amphipods insect equivalents? Ecology 68:1567–1568

Herren LW, Walters LJ, Beach KS (2006) Fragment generation, survival, and attachment of *Dictyota* spp. at Conch Reef in the Florida Keys, USA. Coral Reefs 25:287–295

Hörnig I, Schnetter R, Prud'homme van Reine WF (1992a) The genus *Dictyota* (Phaephyceae) in the North Atlantic. I. A new generic concept and new species. With contribution from Coppenjans, E, Aschenbach-Wege K, Over JM. Nova Hedwigia 54:45–62

Hörnig I, Schnetter R, Prud'homme van Reine WF (1992b) The genus *Dictyota* (Phaeophyceae) in the North Atlantic. II, Key to the species. Nova Hedwigia 54:397–402

Hwang IK, Kim HS, Lee WJ (2005) Polymorphism in the brown alga *Dictyota dichotoma* (Dictyotales, Phaeophyceae) from Korea. Mar Biol 147:999–1015

King RJ, Farrant PA (1987) The phenology of the Dictyotales (Phaeophyceae) at a sheltered locality in Sydney Harbour, New South Wales, Australia. Bot Mar 30:341–350

Lüning K (1990) Seaweeds: their environment, biogeography and ecophysiology. Wiley-Interscience, New York, USA, pp 1–527

Lüning K, tom Diek I (1989) Environmental triggers in algal seasonality. Bot Mar 32:389–397

Peckol O (1982) Seasonal occurrence and reproduction of some marine algae of the continental Shelf, North Carolina. Bot Mar 25:185–190

Phillips JA (1988) Reproduction in Southern Australian species of the Dictyotales (Phaeophyta). Bot Mar 31:437–445

Phillips JA (1997) Genus and species concept son *Zonaria* and *Homoestrichus* (Dictyotales, Phaeophyceae), including the description of *Exallosorus gen. nov*. Eur J Phycol 32:303–311

Piriz ML, Eyras MC, Rotagno CM (2003) Changes in biomass and botanical composition of beachcoast seaweeds in a disturbed coastal area from Argentine Patagonia. J Appl Phycol 15:67–74

Richardson JP (1979) Overwintering of *Dictyota dichotoma* (Phaeophyceae) near its northern distribution limit on the east coast of North America. J Phycol 15:22–26

Santelices B (1990) Patterns of reproduction, dispersal and recruitment in seaweeds. Oceanogr Mar Biol Annu Rev 28:177–276

Tronholm A, Sansón M, Afonso-Carrillo J, De Clerck O (2008) Distinctive morphological features, life-cycle phases and seasonal variations in subtropical populations of *Dictyota dichotoma* (Dictyotales, Phaeophyceae). Bot Mar 51:132–144

Tronholm A, Sansón M, Afonso-Carrillo J, Verbruggen H, De Clerck O (2010a) Niche partitioning and the coexistence of two cryptic *Dictyota* (Dictyotales, Phaeophyceae) species from the Canary Islands. J Phycol 46(6):1075–1187

Tronholm A, Steen F, Tyberghein L, Leliaert F, Verbruggen H, Ribera Siguan MA, De Clerck O (2010b) Species delimitation, taxonomy, and biogeography of *Dictyota* in Europe (Dictyotales, Phaeophyceae). J Phycol 46(6):1301–13021

Tronholm A, Afonso-Carrillo J, Sansón M, Leliaert F, Fernández-García C, De Clerck O (2013) Taxonomy of *Dictyota ciliata-crenulata* complex (Dictyotales, Phaeophyceae). Phycologia 52:171–181

van den Hoek C, Mann DG, Jahns HM (1995) Algae: and introduction to phycology. Cambridge University Press, Cambridge, pp 1–627

Weber-Peukert G (1985) Ontogenetische, aütokologische und taxonomische Untersuchungen an ausgewählten Arten der Gattung *Dictyota* (Dictyotales, Phaeophyceae). Nova Hedwigia 42:123–149

Wiesemeier T, Hay M, Pohnert G (2007) The potential role of wound-activated volatile release in the chemical defense of the brown alga *Dictyota dichotoma*: blend recognition by marine herbivores. Aquat Sci 69:403–412