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NICHE PARTITIONING AND THE COEXISTENCE OF TWO CRYPTIC *DICTYOTA* (DICTYOTALES, PHAEOPHYCEAE) SPECIES FROM THE CANARY ISLANDS[1]

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Coexistence in a homogeneous environment requires species to specialize in distinct niches. Sympatry of cryptic species is of special interest to both ecologists and evolutionary biologists because the mechanisms that facilitate their persistent coexistence are obscure. In this study, we report on two sympatric *Dictyota* species, *D. dichotoma* (Huds.) J. V. Lamour. and the newly described species *D. cymatophila* sp. nov., from the Canary Islands. Gene sequence data (*rbc*L, *psb*A, *nad*1, *cox*1, *cox*3, and LSU rDNA) demonstrate that *D. dichotoma* and *D. cymatophila* do not represent sister species. Rather, *D. cymatophila* and *D. dichotoma* have converged on a nearly identical morphology, only to be distinguished with detailed morphometric observations. Both species co-occur in eulittoral pools and the shallow subtidal in Tenerife. Even though *D. cymatophila* was more dominant in wave-exposed places and *D. dichotoma* in less exposed areas, the spatial distribution of both species overlapped in intermediate habitats. The species display radically different phenologies. *D. dichotoma* reached its highest density in winter and early spring and disappeared nearly completely in autumn, while *D. cymatophila* dominated the study site from July until November. The timing of gamete release also differs between both species, *D. dichotoma* releasing gametes twice every lunar cycle, while the release of gametes in *D. cymatophila* occurred roughly every other day.

Key index words: **Canary Islands; cryptic species; *Dictyota cymatophila; Dictyota dichotoma;* niche partitioning; phenology; reproductive ecology**

Abbreviations: **AIC, Akaike information criterion; ANOSIM, analyses of similarity; BI, Bayesian inference; BIC, Bayesian information criterion; GTR, general time reversible; ML, maximum likelihood; nMDS, nonmetric multidimensional scaling; SIMPER, similarity percentage analyses**

The advent of DNA sequencing two decades ago has considerably altered our ideas about algal species-level diversity. A plethora of studies has revealed cryptic or sibling species within morphologically defined species, falsifying the assumption that speciation events always coincide with any noticeable morphological differentiation. As aptly stated by Saunders and Lemkuhl (2005), species do not evolve specifically to render their identification easier for scientists. In many cases, the respective cryptic species are confined to discrete nonoverlapping geographic regions. However, cryptic species are also shown to occur in close sympatry. Studies on mangrove algae reveal that individuals of *Bostrychia*, conforming to a single morphospecies but belonging to separate noninterbreeding genealogical lineages, may grow completely intermingled (Zuccarello and West 2003).

The coexistence of species that are morphologically identical challenges community ecological theories that predict that two organisms competing for the same resource cannot stably coexist if other ecological factors are constant (Gause 1932). So, how similar can species be before they can no longer coexist? According to Gause's law of competitive exclusion, one of the species will outcompete the other, leading to local extinction or an evolutionary or behavioral shift toward a different ecological niche. The resulting partitioning of niches can occur in several ways: classical resource partitioning and temporal and spatial niche partitioning (Chesson 2000, Amarasekare 2003, Noda 2009).

Competitive coexistence is best studied in animal communities, where coexisting species mostly exhibit differences in diet and foraging patterns. It is now widely accepted that plants also compete for natural resources (Silvertown 2004). However, niche differences among plants and algae have not been characterized in the same amount of detail as in animal systems. The high species richness encountered in plant communities combined with an apparent lack of niche differences between the composing species has led certain authors to challenge the classical theories based on the Lotka–Volterra

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competition model. In its most extreme form, the unified neutral theory, the importance of niche differences between competing species is discarded altogether (Hubbell 2001, Volkov et al. 2003).

Algae have long served as model systems to study competitive coexistence in rocky coastal habitats (Dayton 1971, Connell 1972, Noda 2009). Several studies have indicated that the response of sympatric species to environmental gradients and grazers differs and that this facilitates coexistence. Differences are obvious in cases where the co-occurring species are either morphologically or ecologically dissimilar (Littler and Littler 1980). In the case of cryptic or pseudocryptic species (i.e., species that can be identified by means of key morphological characters, which are established after detailed comparisons of morphological and nonmorphological features; Sáez and Lozano 2005), however, it is all but clear whether genetic differentiation necessarily coincides with ecological differentiation.

In this study, we address coexistence and potential niche differentiation in the brown algal genus *Dictyota*. *Dictyota* species are very common in intertidal and shallow subtidal habitats along rocky coasts worldwide. Although present in cold-temperate waters, the genus reaches its highest diversity in tropical and warm-temperate environments where multiple species often coexist and reach high densities (Herren et al. 2006, Sotka and Hay 2009). Identification of *Dictyota* species is generally considered difficult due to high levels of morphological plasticity combined with the absence of clear diagnostic characters separating many species (Schnetter et al. 1987, De Clerck and Coppejans 1999, De Clerck 2003, Tronholm et al. 2008). Gene sequence data have greatly aided species delineation and resulted in the detection of multiple cryptic entities in *Dictyota* (Lee and Bae 2002, Hoshina et al. 2004, Hwang et al. 2005, Tronholm et al. 2008).

Recent fieldwork and morphological examination of *D. dichotoma* populations raised the suspicion that pseudocryptic diversity may be present on the Canary Islands' rocky shores. The principal goal of this study was to investigate the possibility that multiple species are present using analyses of DNA sequence data sets. Following the observation of two distinct genetic entities (see Results), we set out to (i) evaluate whether morphological differences exist using a morphometric and multivariate approach; (ii) investigate potential niche differentiation by studying fine-scale distribution patterns, phenology, and gamete release periodicity; and (iii) make appropriate taxonomic changes. The obtained results can serve to study the generality of Gause's law for rocky shore algae.

MATERIALS AND METHODS

Sampling, density estimation, and specimen preservation. Specimens were collected at Punta del Hidalgo, a rocky shore in the north of Tenerife, Canary Islands (28°35′ N, 16°20′ W). The *Dictyota* population was monitored on a monthly basis from April 2003 to March 2004 (see Tronholm et al. 2008), from three different habitats (semiexposed eulittoral pools, semiexposed shallow sublittoral, and exposed lower eulittoral), and genotyped using partial *psb*A sequences. Total density and the respective proportions of the different life-cycle phases were estimated in situ counting all specimens from a eulittoral area of about 100 m^2. Sporophytes and male and female gametophytes were distinguished by the distinctive pattern of arrangement of sporangia, antheridial sori, and oogonial sori, respectively (see Fig. 7).

For morphometric analyses and descriptive purposes, a minimum of 10 individuals were randomly collected each month and preserved in a 4% formalin/seawater solution. For molecular analyses, specimens were collected from the study site as well as other localities in the Canary Islands and dried in silica gel (see Table S1 in the supplementary material). All liquid-preserved and voucher specimens are deposited in TFC (Departamento de Biología Vegetal, Universidad de La Laguna, Canary Islands, Spain). Silica samples and DNA extracts are preserved at Ghent University (Ghent, Belgium).

Morphological analyses. For morphometric examination and descriptive purposes, 119 randomly collected specimens from different seasons and life-cycle phases were studied. The 33 vegetative and reproductive characters used in this study are listed in Table 1. The branching angle was measured in the apical and median-proximal parts of the thallus. The length and width of cortical and medullary cells were measured in surface view at a branch situated well below the apex, while

Table 1. Quantitative variables used in the morphometric analysis.

Vegetative characters	Reproductive characters	
Thallus length (cm)	Sporangia	Male gametophytes
Number of branches from main axes	Diameter (μm)	Sori width (μm)
Interdichotomies length (mm)*	Stalk cell height (μm)	Sori length (μm)
Interdichotomies width (mm)*	Female gametophytes	Rows of antheridia (number)
Apical width (mm)*	Sori width (μm)	Antheridia row length (μm)
Branching angle, apical parts (°)*	Sori length (μm)	Antheridia row width (μm)
Branching angle, middle-basal parts (°)*	Oogonia per sorus (number)	Central antheridia length (μm)
Cortical cell length (μm)*	Central oogonia height (μm)	Central antheridia width (μm)
Cortical cell width (μm)*	Central oogonia diameter (μm)	Antheridia height (μm)
Cortical cell height (μm)*	Oogonia stalk cell height (μm)	Antheridia stalk cell height (μm)
Medullary cell length (μm)*		Paraphyses height (μm)
Medullary cell width (μm)*		Tiers (number)
Medullary cell height (μm)*		Loculi per tier (number)

*Variables used in multivariate analyses.

their height was measured in transverse section. Mature parts of each individual were selected for reproductive features. The number, width and length of rows of antheridia, length and width of central antheridia and number of loculi per tier, number of oogonia per sorus, and diameters of central oogonia were obtained in surface view. The height of paraphyses surrounding male sori, antheridia, oogonia, and stalk cells, as well as number of tiers per antheridia were measured in transverse section. For each character, minimum and maximum sizes and the 95 percentile were determined. Micrographs were taken using a Nikon Coolpix 4600 (Nikon, Tokyo, Japan), and drawings were made using a camera lucida, attached to a ZEISS standard microscope (Zeiss, Berlin, Germany).

Molecular phylogenetics. Total genomic DNA was extracted using a standard cetyltrimethylammonium bromide (CTAB)–extraction method and subsequent purification with a Wizard® DNA Clean-Up System (Promega Inc., Madison, WI, USA) as outlined in De Clerck et al. (2006). The plastid encoded *psb*A (PSII reaction center protein D1) and *rbc*L (RUBISCO LSU) genes were amplified and complemented with nuclear ribosomal LSU rDNA sequences following Hwang et al. (2009). In addition, mitochondrial *cox*1, *cox*3, and *nad*1 genes were amplified and sequenced according to Tronholm et al. (2010). Specimens used in the molecular analyses are listed in Table S1.

A first alignment based on the *psb*A gene containing 45 sequences—40 of *Dictyota* and five of outgroup genera—was used to investigate species boundaries. The protein coding sequences were aligned by eye using MEGA 4 (Kumar et al. 2008). Alignment of the partial LSU sequences follows De Clerck et al. (2006). The *psb*A alignment was analyzed under the maximum-likelihood (ML) criterion using PhyML (Guindon and Gascuel 2003). MrBayes 3.1.2 (Ronquist and Huelsenbeck 2003) was used for Bayesian phylogenetic inference (BI). The model of nucleotide substitution for ML was selected with Modeltest 3.7 (Posada and Crandall 1998) according to the Akaike information criterion (AIC) (Posada and Buckley 2004). A general time reversible (GTR) + I + G model was used to analyze the data set under ML with parameters estimated by PhyML. Bayesian analyses also used a GTR substitution model with rate heterogeneity among sites. Two runs of four incrementally heated chains were run in parallel. The chains were run for 2 million generations, with a sample frequency of 1,000. MrBayes' default priors, proposal probabilities, and other settings were used. Convergence of the runs was assessed by visual examination of parameter traces and marginal densities using Tracer v.1.4. (Rambaut and Drummond 2007).

The species definitions provided by the *psb*A analyses facilitated inferring a better-resolved species phylogeny using a multimarker approach. The alignment consisted of six genes (*rbc*L, *psb*A, *nad*1, *cox*1, *cox*3, and LSU rDNA) for a single representative of each species suggested by the *psb*A analyses (Table S2 in the supplementary material). After inferring phylogenies of each of the six genes to verify compatibility, the genes were concatenated. To accommodate the complex patterns of molecular evolution that may exist in multimarker data sets, we evaluated which partitioning strategy and models of sequence evolution provided the best fit to the data. This model selection procedure used the Bayesian information criterion (BIC). The guide tree used during the entire model selection procedure was obtained by ML analysis of the unpartitioned concatenated alignment with PhyML, using a JC + Γ_8 model. All subsequent likelihood optimizations and BIC calculations were carried out with Treefinder (Jobb et al. 2004). We evaluated seven potentially suitable partitioning strategies and six models of sequence evolution, which are listed in Table S3 (see the supplementary material). The partitioning strategy plus model combination that received the lowest BIC score was used in the phylogenetic analyses. This was a composite model containing seven partitions (LSU rDNA, three codon positions of plastid genes, three codon positions of mitochondrial genes), with completely unlinked GTR + Γ_8 models for all partitions. ML searches were carried out with Treefinder, a program that allows tree searches under partitioned models. Because this program uses fast tree search heuristics, we started tree searches from 100 different starting trees to avoid local optima. Each of the starting trees was generated by performing 20 random nearest-neighbor interchanges on the guide tree used for model selection. Branch support was calculated by nonparametric bootstrapping (1,000 replicates), and it was considered high when values were close or equal to 100. Bayesian phylogenetic inference was carried out with MrBayes 3.1.2, using default priors. Two parallel runs, each consisting of four incrementally heated chains, were run for 5 million generations, sampling every 1,000 generations. Convergence of log likelihoods and parameter values was assessed in Tracer v1.4. A burn-in sample of 1,000 trees was removed before constructing the majority-rule consensus tree.

Multivariate statistical analyses. Multivariate methods were used to test whether morphological (morphometric data), ecological (degree of exposure), or phenological (seasons) differences could be detected between both entities. Data from 119 individuals and 11 vegetative characters (Table 1, characters indicated by asterisks) were examined using multivariate statistics (Clarke 1993). As no strong skewness or outliers could be detected in draftsman plots (i.e., pair-wise scatter plots between all variables), we did not transform the data. Normalization was carried out to weigh all variables equally. A resemblance matrix, based on Euclidean distances, was subjected to nonmetric multidimensional scaling (nMDS), where the samples (individuals) were coded for two factors, species and habitat. Two-way crossed analyses of similarity (ANOSIM) were conducted to ascertain whether morphology differed significantly among species, among habitats, among seasons, and/or combination of two of the factors (species-season; species-habitat), in each case removing the effect of the other factor. The factors included in the ANOSIM analyses were as follows: species, with two levels (*D. dichotoma* and *D. cymatophila*) (see below); habitat, with three levels (semiexposed shallow sublittoral, semiexposed eulittoral pools, and exposed lower eulittoral); and season, with four levels (spring, summer, autumn, and winter). For ANOSIM tests, the null hypothesis (no among-group differences) can be rejected for *P*-values <0.05. A similarity percentage analysis (SIMPER) was performed to identify which variables were contributing most to the species or exposure degree differences. Variables detected by SIMPER analysis as contributing most were graphically represented in the same nMDS by superimposing circles of increasing size to identify morphological disparity in these variables. A nonparametric form of Mantel test (RELATE) was used to test for significance of correlations between resemblance matrices, with the Spearman rank correlation as the test statistic. The BIOENV procedure was applied to find a small set of morphometric variables that were as effective as a larger set in discriminating both species. All multivariate statistical analyses and routines were carried out using the PRIMER v.6 software package (Clarke and Gorley 2006).

Reproduction periodicity. To analyze the periodicity in gamete release, 18 female gametophytes were collected in early summer, when the proportion of gametophytes is nearly 50% of the population. Thalli were transported minimizing manipulation and were maintained in filtered seawater in the laboratory under approximately the same conditions as in the field (natural light and 20°C–22°C) on a north-facing windowsill during 55 d (from 11 July to 3 September 2008). Egg release was monitored on a daily basis. Following egg release, thalli were transferred to new flasks. The number of eggs released

per day was estimated by counting six replicates of 3 mL of homogenized medium. Specimens were kept in the laboratory until no more crops of new sori developed.

RESULTS

Molecular analysis. The *psb*A alignment, used for species delimitation, consisted of 850 nt and 45 specimens belonging to 23 species. ML analysis and Bayesian inference resulted in nearly identical trees (Fig. S1 in the supplementary material). Species generally receive high support, but relationships among them are unresolved in the *psb*A tree. Specimens collected at the study site are resolved in two different clades, providing strong evidence for the presence of two species. A first clade is identified as *D. dichotoma* as confirmed by inclusion of a reference sequence of the neotype of *D. dichotoma* (GU255542; see Tronholm et al. 2010). The second clade, which we will describe below as the new species *D. cymatophila*, comes out sister to *D. mertensii*, a Caribbean species. The concatenated six-gene alignment consisted of 5,314 nt and 23 species and was 87% filled (see Table S2). The species trees inferred with ML and BI were fully congruent (Fig. 1). Nearly all clades, except for the deepest branches in the genus *Dictyota*, receive high or very high support. It is evident that *D. dichotoma* is only distantly related to *D. cymatophila*, with which it grows in close sympatry at the study site.

Spatial distribution. Specimens of *D. dichotoma* and *D. cymatophila* grow on rocks in the infralittoral fringe together with *Cystoseira abies-marina* (S. G. Gmelin) C. Agardh, *Taonia atomaria* (Woodw.) J. Agardh, and *Sargassum* spp. *D. dichotoma* and *D. cymatophila* exhibit differences in habitat preference. *D. cymatophila* is more abundant in exposed areas compared to *D. dichotoma* and therefore dominates the lower eulittoral pools exposed to swell (Fig. 2). The less exposed pools are dominated by *D. dichotoma*, where *D. cymatophila* is only occasionally found. Intermediate pools show a more balanced presence of both species.

Temporal distribution. The phenology of the species was monitored year-round. *D. dichotoma* and *D. cymatophila* exhibit clear trends with distinct peaks and resting periods (Fig. 3). *D. cymatophila* showed the highest abundance in late summer and autumn reaching 26–32 individuals $\cdot$ m^{-2}, and the lowest values in spring. *D. dichotoma*, on the other hand, reaches its highest abundance in spring and disappears nearly completely in autumn. Sporophytes and gametophytes of both sexes, and nonfertile specimens of both species can usually be found in any season. The

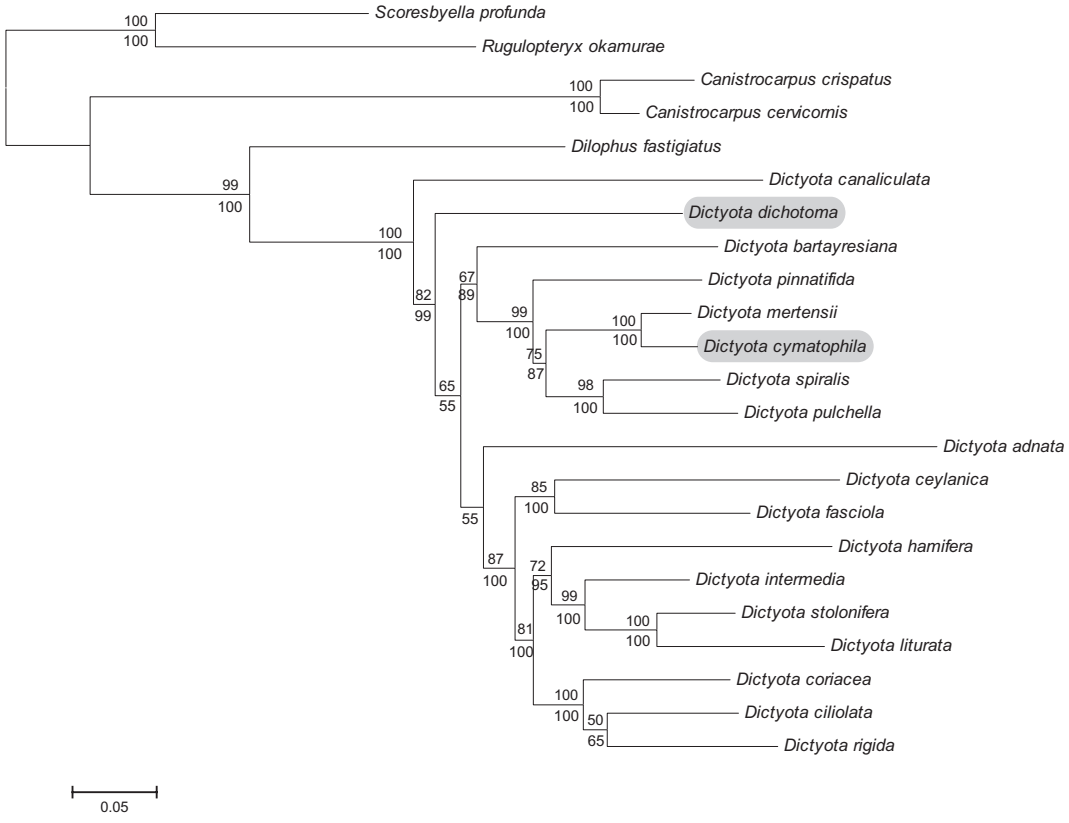

FIG. 1. Phylogenetic hypothesis (−lnL = 26544.32) obtained by maximum-likelihood (ML) inference of a data set containing six genes (partial LSU rDNA, *rbc*L, *psb*A, *cox*1, *cox*3, and *nad*1). Numbers above the nodes indicate ML bootstrap values, and those below the nodes indicate posterior probabilities; values less than, respectively, 50 and 0.7 are not shown.

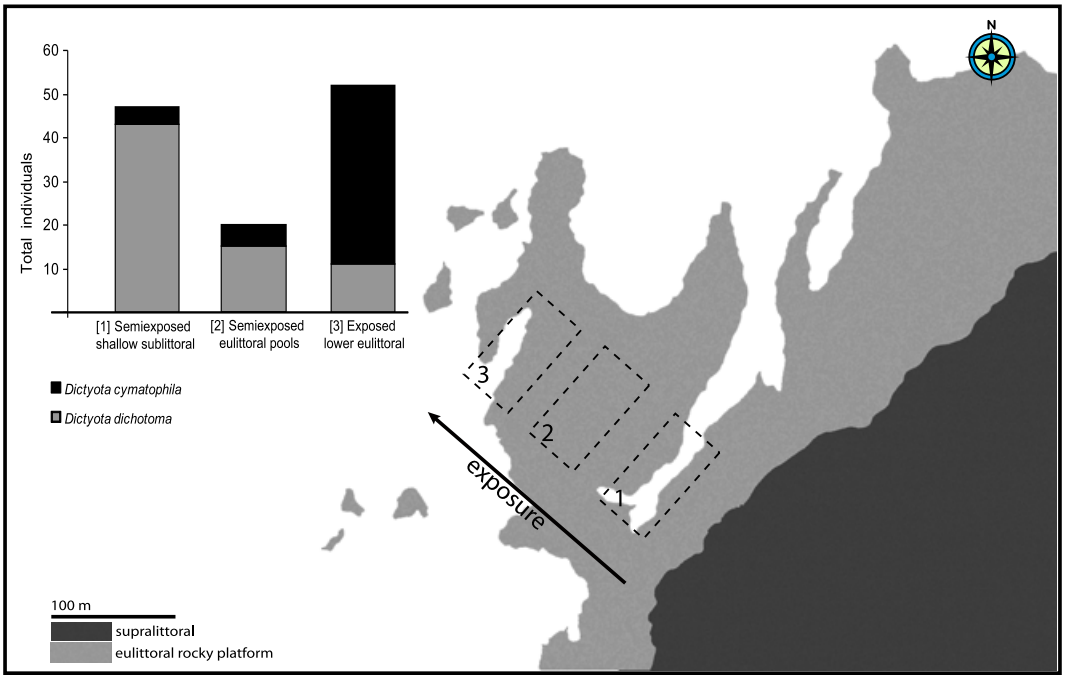

FIG. 2. Study site, Punta del Hidalgo (Tenerife), with indication of the sampling locations and the ecological preference of *Dictyota dichotoma* and *Dictyota cymatophila,* respectively, based on 119 specimens.

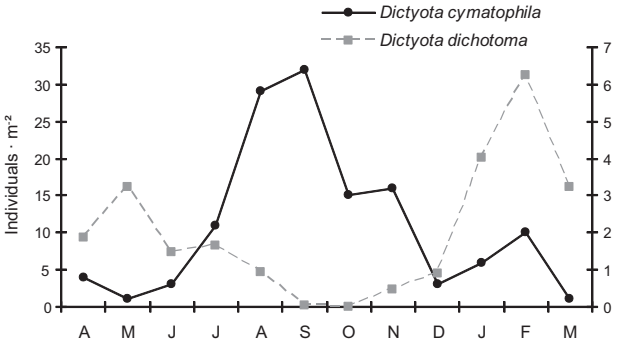

FIG. 3. Temporal variations in density through an annual cycle (April 2003 to March 2004) of *Dictyota cymatophila* (left ordinate) and *Dictyota dichotoma* (right ordinate).

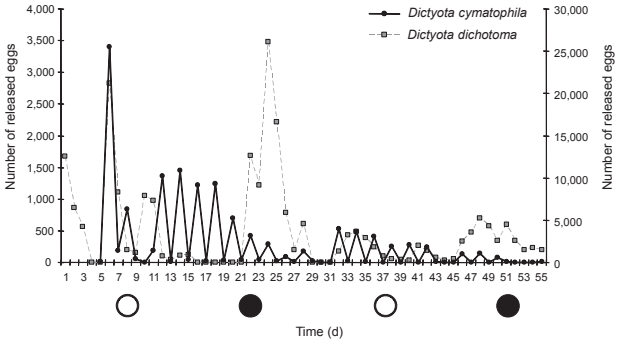

FIG. 4. Comparative egg release periodicity between *Dictyota dichotoma* (left ordinate) and *Dictyota cymatophila* (right ordinate) maintained in the laboratory under natural light and temperature conditions during 55 d (11 July to 3 September 2008).

number of gametophytes, however, was low year-round: maximum density was 5 individuals · m^{-2} in July for *D. cymatophila*, and 0.7 individuals · m^{-2} in March for *D. dichotoma*. Populations were always dominated by fertile sporophytes regardless of the season. Sterile specimens were present in very low numbers only.

Periodicity in gamete release. Both species release female gametes at the break of day, but noticeable differences in the periodicity of release were observed (Fig. 4). In *D. dichotoma*, egg release occurred at fortnightly intervals (especially perceptible during the first 30 d of the experiment). Egg release correlated with the lunar cycle, the first eggs being released around full and new moon or 1–2 d prior. Release lasted about 5–6 d, with a peak during which about half of all mature eggs were released around the second or third day. By contrast, eggs of *D. cymatophila* were discharged in continuous pulses every other day without evidence of a relation to the lunar cycle.

Morphological differentiation. The two-dimensional nMDS ordination based on the Euclidean distance matrix of 119 specimens of *D. dichotoma* and *D. cymatophila*, and 11 vegetative characters are shown in Figure 5. A gradient on the morphological parameters of the *Dictyota* specimens could be differentiated in this plot using species and habitat as factors. The specimens representing *D. cymatophila* lay to the right of those of *D. dichotoma*. Most points

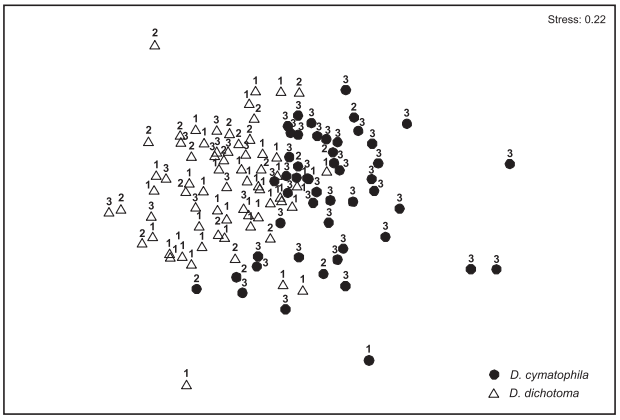

FIG. 5. Two-dimensional nonmetric MDS ordination for 119 specimens of *Dictyota dichotoma* (triangle) and *Dictyota cymatophila* (circle) based on normalized morphometric variables and Euclidean distance matrix, from three different habitats: 1, semiexposed shallow sublittoral; 2, semiexposed eulittoral pools; 3, exposed lower eulittoral.

for sites representing habitat type 3 (exposed lower eulittoral) also lay to the right of those for habitat 2 (semiexposed eulittoral pools) and 1 (semiexposed shallow sublittoral).

Two-way crossed ANOSIM demonstrated that morphological parameters differed significantly between species and seasons (both $P < 0.001$), with the *R*-statistic being greater for species (0.530) than season (0.207). The *R*-statistics for seasonal pair-wise comparisons demonstrated that measurements of the individuals from autumn were the most distinct, with the highest *R* values for autumn versus the other seasons (autumn vs. spring: 0.485; autumn vs. summer: 0.294; autumn vs. winter: 0.200) and being greater than between any other two seasons. A two-way crossed ANOSIM test for differences between species and habitat confirmed that there were significant differences between both species ($R = 0.369$, $P < 0.001$); however, differences between habitat groups could not be detected.

The SIMPER procedure showed that the length and width of medullary cells, the width of cortical cells, the middle-basal branching angle, the apical branching angle, and the height of medullary cells were the morphological variables that (in this order of relevance) contributed most to the differentiation between both species (Table 2). The extent to which the relative magnitudes of each of these six variables vary among the 119 specimens is reflected by the differences in the relative size of the circles superimposed on the points representing those specimens on the MDS ordination plot (Fig. S2 in the supplementary material). The subset of morphometric variables most closely matching was that constituted only by the length of the medullary cells (ρ=0.305, $P < 0.001$). This variable best explained the differences between samples, since it ''captured'' the pattern of *Dictyota* individuals, with a correlation value of 0.588. Widths of medullary and cortical cells were the next variables included in the consecutive subsets with a correlation value > 0.5.

TABLE 2. Results of similarity percentage analysis (SIMPER) test on percentage contributions of variables to determine significant differences between *Dictyota dichotoma* and *Dictyota cymatophila*. Variables are ordered in decreasing percent contribution. Sq Dist./SD = squared distance/standard deviation; Contrib. % = contribution; Cum. % = cumulative percentage. List of variables has been truncated after cumulative percentage exceeded 60%.

Variables	Sq Dist./SD	Contrib. %	Cum. %
Medullary cells length	0.99	13.09	13.09
Medullary cells width	0.61	10.69	23.79
Cortical cells width	0.70	10.07	33.85
Middle-basal angle	0.77	9.20	43.05
Apical angle	0.81	9.13	52.18
Medullary cells height	0.61	8.46	60.64

Taxonomy. Based on the presented results, which highlight genetic as well as phenological differentiation between both lineages, we here provide a formal description of the undescribed lineage.

Dictyota cymatophila Tronholm, M. Sansón et Afonso-Carr. **sp. nov.** (Figs. 6 and 7).

Planta (4.3–)8.4–8.9(–15.4) cm longa, erecta, rigida, crispata, in spiram. Color fuscus, cum fascis transversis iridescens. Segmenta (2–)7.9–8.4(–22) mm longa et (1.5–)3.4–3.5(–7) mm lata. Apices rotundati vel acuti, (0.25–)1.0–1.1(–2.5) mm lati. Ramificatio subdichotoma. Anguli superni acuti, (6–)14–16(–51)°, et latioris medio inferaque segmenta, (11–)49–53(–147)°. Marginibus et paginae undulatae, curvatae et rugosae. Cortex monostromaticus, cellulis (17–)43–45(–83) μm longis, (10–)18–19(–48) μm latis et (10–)16–17(–31) μm altis. Medulla monostromatica, cellulis (75–)202–212(–420) μm longis, (30–)71–74(–135) μm latis et (48–)85–89(–174) μm altis. Sporangia in pagina dispersa vel aggregata in soris aut solis, (57–)102–105(–145) μm diametra, cellula basali simplicia (7–)14–15(–31) μm alta. Antheridia in ellipsoidam soris dispositiis, (270–)410–445(–600) μm longis et (180–)294–321(–480) μm latis, soris (1–)2(–3) anulus maculus paraphysium circumcincti, antheridia (40–)65–71(–95) μm altis, cellula basali simplicia (7–)12–14(–19) μm alta. Paraphysium unicellularum (60–)78–84(–119) μm altis. Oogonia in oval sori disposita, (180–)341–366(–525) μm longis et (120–)269–292(–420) μm latis, cum (15–)31–35(–58) oogonia per sorus. Matura oogonia (40–)62–67(–93) μm diametra, (57–)91–97(–126) μm altis, cellula basali simplicia (10–)16–17(–24) μm alta.

Thallus (4.3–)8.4–8.9(–15.4) cm long, erect, stiff, crisp, spirally twisted. Color pale brown, iridescent transverse banding pattern. Branches (2–)7.9–8.4(–22) mm long and (1.5–)3.4–3.5(–7) mm wide. Apices rounded to acute, (0.25–)1.0–1.1(–2.5) mm wide. Branching subdichotomous. Angles acute toward

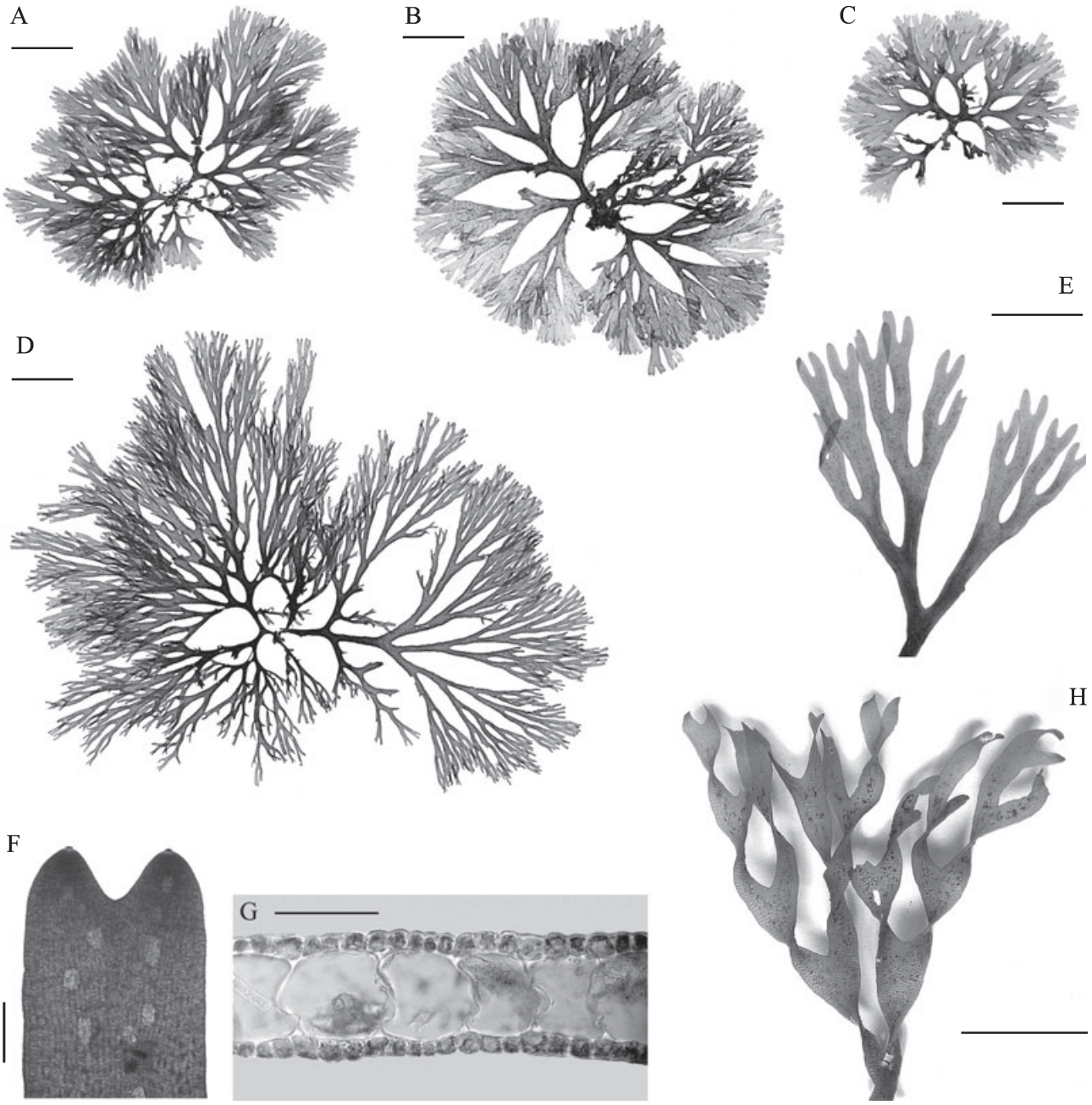

FIG. 6. (A–H) External morphology and anatomy of *Dictyota cymatophila*. (A) Habit of a sporophyte (TFC Phyc 14319). Scale bar, 2 cm. (B) Habit of a male gametophyte (Holotype TFC Phyc 14267). Scale bar, 2 cm. (C) Habit of a small female gametophyte (TFC Phyc 14322). Scale bar, 2 cm. (D) Habit of a large female gametophyte (TFC Phyc 14282). Scale bar, 2 cm. (E) Detail of terminal portion of a pressed sporophyte showing subdichotomous branching and undulate margins (TFC Phyc 14323). Scale bar, 1 cm. (F) Detail of rounded to acute apices showing protruding apical cells (TFC Phyc 14336). Scale bar, 500 μm. (G) Transverse section of a thallus with unilayered cortex and medulla (TFC Phyc 14338). Scale bar, 100 μm. (H) Detail of the spirally twisted habit of a liquid-preserved sporophyte (TFC Phyc 14336). Scale bar, 1 cm.

apical segments, (6–)14–16(–51)°, and broader in middle and basal segments, (11–)49–53(–147)°. Margins and surface undulate, curved and rugose. Cortex unilayered, cells (17–)43–45(–83) μm long, (10–)18–19(–48) μm wide, and (10–)16–17(–31) μm high. Medulla unilayered, cells (75–)202–212 (–420) μm long, (30–)71–74(–135) μm wide, and (48–)85–89(–174) μm high. Sporangia scattered in blocklike patches or single in thallus surface, (57–)102–105(–145) μm in diameter, borne on a single stalk cell (7–)14–15(–31) μm high. Antheridia grouped in ellipsoidal sori, (270–)410–445(–600) μm long and (180–)294–321(–480) μm wide, surrounded by (1–)2(–3) rings of pigmented paraphyses,

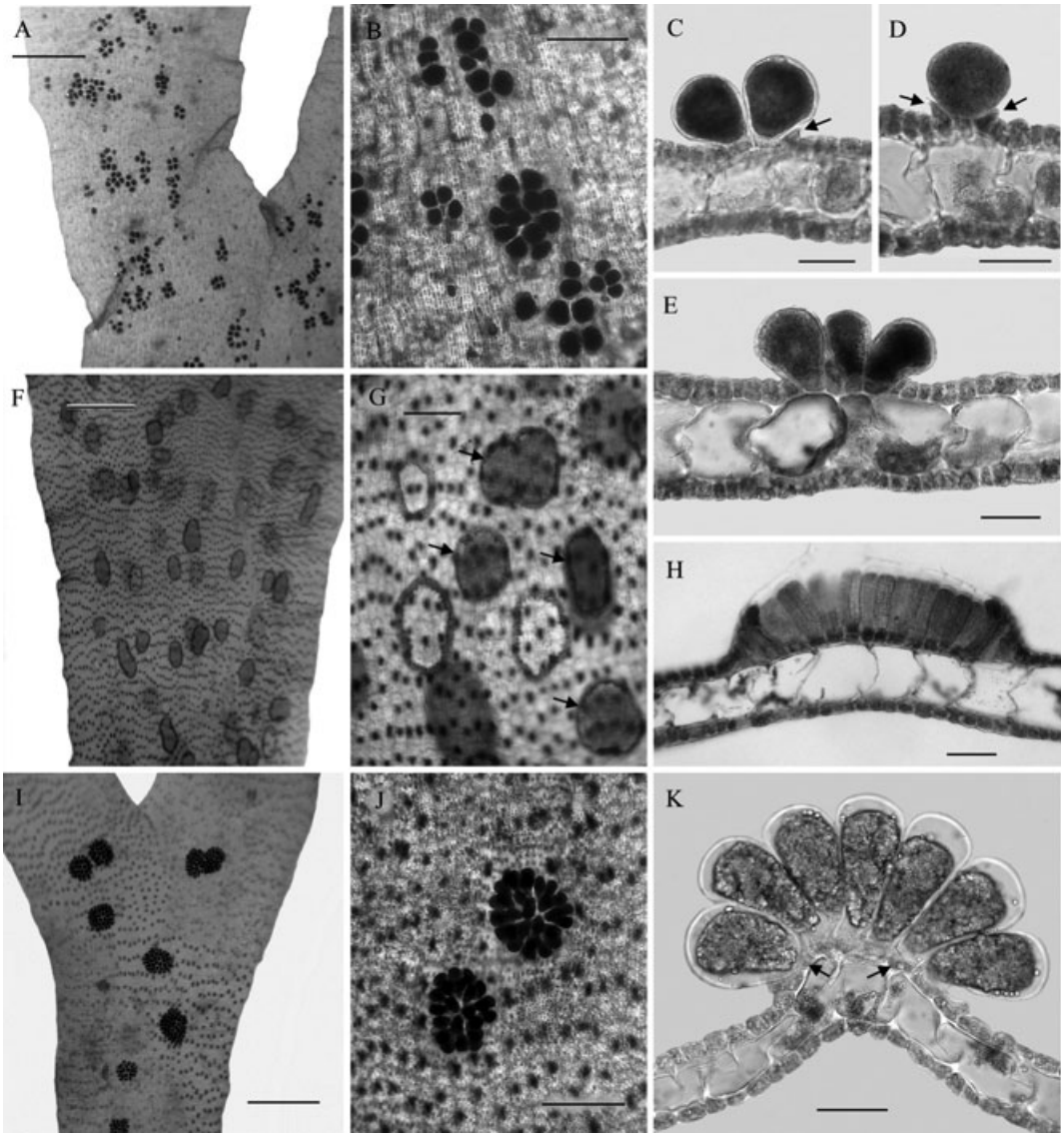

FIG. 7. (A–K) Reproductive morphology of *Dictyota cymatophila*. (A) Sporophyte with sporangia scattered over the surface or grouped in blocklike patches (Isotype TFC Phyc 14269). Scale bar, 1 mm. (B) Detail of aggregated sporangia in surface view (TFC Phyc 14269). Scale bar, 300 μm. (C–E) Transverse sections of a thallus showing single to aggregated sporangia. Note the immature sporangia adjacent to mature sporangia resembling an involucrum (arrows) (TFC Phyc 14269). Scale bars, 50 μm. (F) Surface view of a male gametophyte showing the arrangement of antheridial sori (Isotype TFC Phyc 14272). Scale bar, 1 mm. (G) Detail of mature antheridial sori (arrows) and empty sori in which only the ring of rows of pigmented paraphyses persists (Isotype TFC Phyc 14272). Scale bar, 300 μm. (H) Transverse section of an antheridial sorus with subcylindrical antheridia surrounded by unicellular paraphyses (Isotype TFC Phyc 14272). Scale bar, 50 μm. (I) Surface view of a female gametophyte showing the arrangement of oogonial sori (Isotype TFC Phyc 14275). Scale bar, 1 mm. (J) Detail of mature oogonial sori (Isotype TFC Phyc 14275). Scale bar, 300 μm. (K) Transverse section of an oogonial sorus with subclavate to pyriform oogonia, borne on a single stalk cell (arrows) (Isotype TFC Phyc 14275). Scale bar, 50 μm.

antheridia (40–)65–71(–95) μm high, borne on a single stalk cell, (7–)12–14(–19) μm high. Paraphyses unicellular (60–)78–84(–119) μm high. Oogonia grouped in oval sori, (180–)341–366 (–525) μm long and (120–)269–292(–420) μm wide, with (15–)31–35(–58) oogonia per sorus. Mature oogonia (40–)62–67(–93) μm in diameter, (57–) 91–97(–126) μm high, borne on a single stalk cell (10–)16–17(–24) μm high.

Etymology: kyma, root *kymat* (Greek) = ''wave,'' referring to the habitat of this species on rocks where the waves often break; *phila* (Greek) = ''preferring this environment.''

Holotype: TFC Phyc 14267, D403, male gametophyte, collected by A. Tronholm at Punta del Hidalgo (28°35′ N, 16°20′ W), northern Tenerife, Canary Islands, Spain, exposed lower eulittoral, on 30 August 2007.

Isotypes: TFC Phyc 14268 to 14271, sporophytes; TFC Phyc 14272 to 14274, male gametophytes; TFC Phyc 14275 to 14277, female gametophytes. Isotypes in GENT and L.

Other specimens examined: Canary Islands: Tenerife, Punta del Hidalgo (A. Tronholm, April 2003–March 2004 TFC Phyc 14278–14327; sporophytes, male and female gametophytes; A. Tronholm, 10 April 2007, TFC Phyc 14328, D306, sterile; A. Tronholm, 11 July 2007, TFC Phyc 14330, D399, sporophyte; A. Tronholm, 15 July 2008, TFC Phyc 14336, sporophyte; A. Tronholm, 30 August 2007, TFC Phyc 14338, female; A. Tronholm, 15 July 2008, TFC Phyc 14336, sporophyte; A. Tronholm, 31 July 2008, TFC Phyc 14337, female), El Médano (A. Tronholm, 31 August 2007, TFC Phyc 14334, D405, sporophyte; TFC Phyc 14335, D406, sporophyte); Gran Canaria, Faro de Maspalomas (A. Tronholm, 17 August 2007, TFC Phyc 14331, D392, female; TFC Phyc 14332, D393, female), El Berriel (A. Tronholm, 18 August 2007, TFC Phyc 14333, D397, sporophyte).

Distribution: So far, the species is only known from the Canary Islands.

Habit and vegetative features: The thallus is completely erect, (4.3–)8.4–8.9(–15.4) cm long, with stiff and crisp axes that are spirally twisted and somewhat harsh to the touch. Plants are attached to the substratum by basal rhizoids that usually form a discoid holdfast. Color in vivo is pale brown, often with a transverse banding pattern of slightly iridescent zones, constituted by bright yellowish dots corresponding to medullary cell content. Dry specimens retain the color, although banding pattern is not visible. The width of the axes is homogeneous throughout the thallus, branches are (2–)7.9–8.4(–22) mm long and (1.5–)3.4–3.5(–7) mm wide. Apices are rounded to acute, (0.25–)1.0–1.1(–2.5) mm wide, and the apical cell protruding. Branching is subdichotomous, with more or less distinct main axes that branch (7–)12–13(–17) times. The branching angles are acute toward the apical segments, (6–)14–16(–51)°, whereas they are broader in the middle and basal segments, (11–)49–53(–147)°. The surface and margins are undulate, sinusoidally curved and rough, occasionally with proliferations arising perpendicularly from the surface near the base of the thallus (occasionally bearing reproductive structures). Small triangular teeth can be observed on the margins but are rare. Hair tufts are common, except on the margins. The cortex is unilayered, consisting of elongate cortical cells, (17–)43–45(–83) μm long and (10–)18–19(–48) μm wide. Cortical cells are isodiametric in transverse section, (10–)16–17(–31) μm high, with numerous discoid plasts arranged toward the cell periphery. The medulla is unilayered, although a local duplication of medullary cells near the margins and in damaged parts can be occasionally observed. Medullary cells are hyaline, (75–)202–212(–420) μm long, (30–)71–74(–135) μm wide, and (48–)85–89(–174) μm high. Thickenings of the medullary cell walls are absent (Fig. 6).

Reproductive features: Sporophytes bear sporangia scattered on both thallus surfaces, in blocklike patches or single, mainly arranged on the central part of the thallus and lacking toward the base (Fig. 7, A and B). Sporangia are dark brown, pyriform to subspherical, (57–)102–105(–145) μm in diameter, which are borne on a single stalk cell (7–)14–15(–31) μm high, rarely up to four stalk cells. Immature sporangia, arrested in their development, adjacent to mature sporangia may resemble an involucrum (Fig. 7, C–E). Mature divided tetrasporangia have been observed but are rare.

Male gametophytes form whitish sori of antheridia, dispersed over the whole thallus surface, often merging with adjacent sori to form large, irregular patches (Fig. 7F). Sori are ellipsoidal in surface view, (270–)410–445(–600) μm long and (180–)294–321(–480) μm wide, each surrounded by a ring of (1–)2(–3) rows of pigmented paraphyses (the innermost ring darker and more developed than the subsequent) (Fig. 7G). Antheridia are arranged in (7–)11–12(–19) regular rows, the central mature antheridia are nearly isodiametric in surface view, (17–)24–26(–43) μm long and (12–)23–25(–33) μm wide, with (4–)24–30(–64) loculi per tier. In transverse section, antheridia are subcylindrical (40–)65–71(–95) μm high, composed of (10–)16–18(–28) tiers. Each antheridium is borne on a single stalk cell, (7–)12–14(–19) μm high (Fig. 7H). Paraphyses are unicellular, subclavate (60–)78–84(–119) μm high, and persist as a ring after the sperms are released and antheridia are detached.

Oogonia are grouped in dark brown sori spread over the whole thallus surface (Fig. 7I). Sori are oval in surface view, (180–)341–366(–525) μm long and (120–)269–292(–420) μm wide, with (15–)31–35(–58) oogonia per sorus (Fig. 7J). Mature oogonia are (40–)62–67(–93) μm in diameter. In transverse section, oogonia are subclavate to pyriform, (57–)91–97(–126) μm high, each borne on a single stalk cell, (10–)16–17(–24) μm high (Fig. 7K).

DISCUSSION

Our data clearly demonstrate the presence of two quasi-identical *Dictyota* species, *D. dichotoma* and *D. cymatophila*, in the Canary Islands. The species belong to different lineages of the genus *Dictyota*, with *D. cymatophila* being most closely related to *D. mertensii* from the Caribbean Sea, and *D. dichotoma* representing an early diverging lineage in the genus that is not closely related to any other species. Even though very similar in overall appearance, morphometric analyses reveal subtle but significant differences between the two species in the sizes of medullary and cortical cells as well as branching angles of the axes. *D. cymatophila* showed larger and higher medullary cells, and wider medullary and

cortical cells than *D. dichotoma*. On the other hand, *D. dichotoma* showed broader apical and middle-basal branching angles than *D. cymatophila*, as is illustrated in Figure 8. These results corroborate the importance of cell size measurements and their ratios as diagnostic characters for *Dictyota* species (cf. Weber-Peukert 1985, Schnetter et al. 1987, Hörnig et al. 1992a,b, De Clerck 2003).

The morphological similarity between *D. dichotoma* and *D. cymatophila* would qualify them as pseudocryptic (Sáez et al. 2003). The presence of hidden diversity in the genus *Dictyota* is not surprising. Examples of cryptic and pseudocryptic diversity are rife, especially in the marine environment where speciation processes are less likely to be coupled to morphology than to other phenotypic aspects (Knowlton 1993). Sibling species may occupy nonoverlapping geographic areas, being confined to different ocean basins (e.g., *Halimeda*; Kooistra et al. 2002, Verbruggen et al. 2009) or stratigraphically (Rocap et al. 2003, Rodríguez et al. 2005). In many cases, however, there is at least a partial overlap in the distribution of the species. Ecological competition theory predicts at least some differentiation between the respective competitors (Silvertown 2004). Studies focusing on coexisting cryptic microalgae have repeatedly highlighted different preferences or tolerances with respect to small-scale spatial and temporal environmental variation. Especially, physiological differentiation related to photoacclimation or salinity tolerance appears to be important (Moore et al. 1998, Foulon et al. 2008, Quijano-Scheggia et al. 2009, Vanelslander et al. 2009). Comparatively few studies, however, have addressed the mechanisms that facilitate the coexistence of cryptic macroalgal species. Physiological differentiation among cryptic lineages that live in close sympatry has been demonstrated in the mangrove- and salt-marsh-associated red algae *Bostrychia* and *Caloglossa* (Karsten et al. 1993, Zuccarello et al. 2001, Zuccarello and West 2003). Physiological differentiation (e.g., growth rates, photoacclimation, grazing resistance) was not assessed during the present study, but the two species display subtle ecological differences that may facilitate their coexistence. Even though *D. cymatophila* and *D. dichotoma* coexist in the same habitats, *D. cymatophila* has a preference for wave-exposed habitats, while *D. dichotoma* dominates less-exposed areas in the intertidal and shallow sublittoral. In areas of intermediate exposure, both species live in close sympatry. Spatial partitioning at small scales appears to be a common mechanism allowing the coexistence of competing species in the marine environment (e.g., benthic fish assemblages; Wellenreuther et al. 2007). Microhabitat differentiation has long been accepted as an important mechanism for the coexistence of algae also (Noda 2009). Classic studies (e.g., Dayton 1971) have shown that coexistence may be achieved by slight shifts in habitat use, thereby allowing the survival of competing species in the same area. More recently, Benzie et al. (2000) describes ecological differentiation between several *Sargassum* species in the Great Barrier Reef, where pseudocryptic species occupy distinct niches with respect to depth and exposure.

Another aspect of niche differentiation that became apparent in this study is the temporal shift between the two species. The species display a markedly different phenology, with peaks in abundance (density) of one species that coincide with temporal absence of the other, and vice versa. For years, *D. dichotoma* was considered a species that was present throughout the year in the Canary Islands. Only recently, Tronholm et al. (2008) demonstrated that genuine *D. dichotoma* disappears almost completely in autumn, surviving as microscopic resting stages. The disappearance of *D. dichotoma*, however, is masked by the presence of *D. cymatophila*, which reaches maximum abundance in this period when

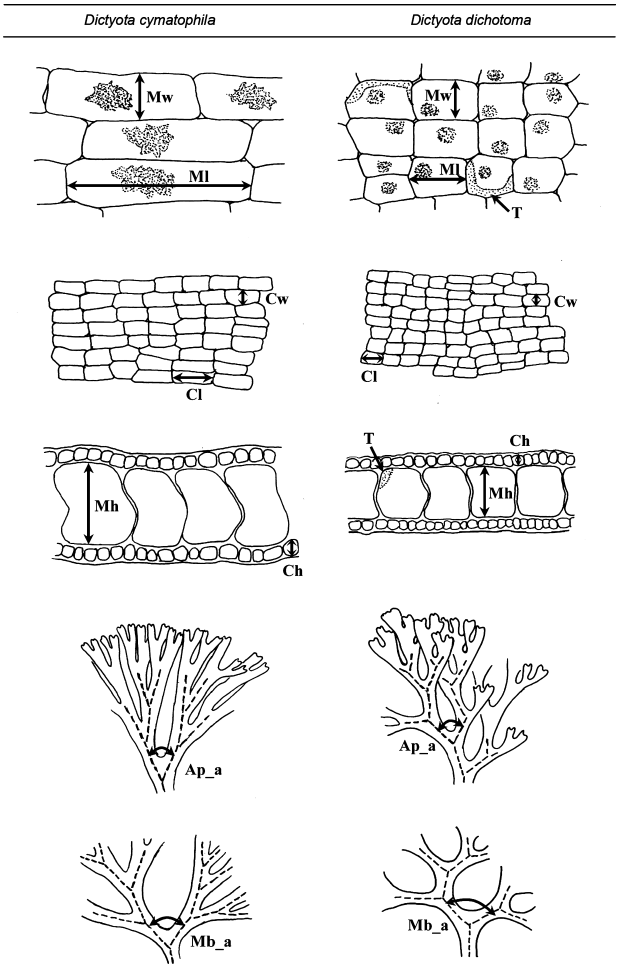

FIG. 8. Diagnostic characters differentiating *Dictyota cymatophila* and *Dictyota dichotoma*. Ml, medullary cell length; Mw, medullary cell width; T, medullary cell thickening; Cl, cortical cell length; Cw, cortical cell width in surface view; Mh, medullary cell height; Ch, cortical cell height in transverse section; Ap_a, apical angle; Mb_a, middle-basal angle.

surface seawater temperatures reach their maximum values (21°C–22°C; Barton et al. 1998). *D. dichotoma* shows its maximum abundance in late winter when surface seawater temperatures reach their minimum values (17°C–18°C; Barton et al. 1998). During this cold period, the *D. cymatophila* population declines severely. Given the present distribution of *D. dichotoma,* this phenology is somehow expected. *D. dichotoma* is a warm-temperate species, which survives the coldest season along the northeast Atlantic coast as microscopic resting stages. In the southern reaches of its distribution, the coldest season coincides with maximum population density, and the species overcomes the less favorable conditions of the warmest season (autumn) as cryptic microthalli (Tronholm et al. 2008). *D. cymatophila*, on the other hand, is more likely to be a (sub)tropical species, given its close relationship with the Caribbean *D. mertensii* and the fact that the coldest months constitute the unfavorable season for this species. Temporal differentiation between *D. dichotoma* and *D. cymatophila* is probably a consequence of interspecific competition and a secondary effect of temperature as limiting factor and not exclusively due to one of these aspects.

An additional matter one has to consider when related species live in sympatry is avoidance of hybrid formation. Especially in the marine environment where fertilization is commonly external, there is ample opportunity for interspecific hybrid formation. To avoid hybridization, organisms have evolved a whole suite of pre- and postzygotic isolation mechanisms, among which temporal barriers of reproductive isolation (Coyne and Orr 2004). Many algae have well-defined periods of reproduction and shed their gametes in the water at different times (Clifton and Clifton 1999, Santelices 2002, Pearson and Serrao 2006). This periodicity has been widely related to the lunar cycle. Periodic release of gametes has been studied extensively in *Dictyota*. Culture studies (Müller 1962, Kumke 1973) as well as in situ observations (Williams 1905, Lewis 1910, Hoyt 1927) revealed that *D. dichotoma* releases gametes twice every lunar cycle. This study confirms this periodic release pattern in *D. dichotoma* with oogonia being released close to full and new moon. In contrast, *D. cymatophila* showed a very different release pattern with oogonia being discharged every other day. Although not studied in detail during the present study, these markedly different release patterns are reflected in the way gametangial sori are formed and mature on the plants. In *D. dichotoma* synchronous release of oogonia coincides with simultaneous maturation of the sori. Usually two crops at different maturity stage can be observed on the female gametophytes, a mature crop to be released the following new or full moon and a young crop that will be released later. In *D. cymatophila*, oogonial sori of variable maturity stages can be observed at all times. The gamete release patterns of both species are not entirely exclusive, leaving the opportunity for interspecific hybridization. It would, therefore, be worth investigating the fine-scale timing of gamete release in situ and the potential of hybrid formation by means of crossing experiments using laboratory cultures.

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Supplementary Material

The following supplementary material is available for this article:

Figure S1. Maximum likelihood phylogeny of *psb*A sequences, rooted with *Scoresbyella profunda* and related genera of the Dictyoteae (Ln = −3590.25). Node support values are given at each ramification of the tree (ML bootstrap above and BI support below the branches). Base frequencies are A = 0.25, C = 0.18, G = 0.20, T = 0.36. The substitution rates are A-C = 0.161, A-G = 2.416, A-T = 2.343, C-G = 0.104, C-T = 9.254, G-T = 1. The shape parameter of the gamma distribution among site rate heterogeneity is 0.777.

Figure S2. Two-dimensional non-metric MDS ordination (the same of MDS plot of Fig. 22) with superimposed circles of increasing size (the larger the circle, the greater the value) representing, respectively, increasing of medullary cells length (μm), medullary cells width (μm), cortical cells width (μm), middle-basal angle (°), apical angle (°) and medullary cells height (μm), of the studied specimens of *Dictyota cymatophila* and *Dictyota dichotoma* to identify morphological disparity in these variables (stress = 0.22).

Table S1. Specimens used in this study for the molecular analyses with indication of collecting data.

Table S2. GenBank accession numbers of the sequences used in the concatenated alignment, including strain numbers and sequence length.

Table S3. Partitioning strategies, corresponding nucleotide substitution models, and their respective likelihood and Bayesian information criterion (BIC) values. Partitioning the data set by rDNA (LSU), chloroplast, mitochondrial DNA, and by codon positions yielded the lowest BIC value (bold) and was hence selected for further analyses.

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