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SPECIES DELIMITATION, TAXONOMY, AND BIOGEOGRAPHY OF *DICTYOTA* IN EUROPE (DICTYOTALES, PHAEOPHYCEAE)[1]

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Taxonomy of the brown algal genus *Dictyota* has a long and troubled history. Our inability to distinguish morphological plasticity from fixed diagnostic traits that separate the various species has severely confounded species delineation. From continental Europe, more than 60 species and intraspecific taxa have been described over the last two centuries. Using a molecular approach, we addressed the diversity of the genus in European waters and made necessary taxonomic changes. A densely sampled DNA data set demonstrated the presence of six evolutionarily significant units (ESUs): *Dictyota dichotoma* (Huds.) J. V. Lamour., *D. fasciola* (Roth) J. V. Lamour., *D. implexa* J. V. Lamour., *D. mediterranea* (Schiffn.) G. Furnari, *D. spiralis* Mont., and the newly described *D. cyanoloma* sp. nov., which was previously reported as *D. ciliolata* from the Mediterranean Sea. Species distributions, based on DNA-confirmed occurrence records, indicate that all species are geographically confined to the NE Atlantic Ocean with the exception of *D. dichotoma* and *D. implexa,* which also occur in South Africa and Bermuda, respectively. To investigate potential hybridization between *D. dichotoma* and *D. implexa*, which were previously shown to be sexually compatible in culture, we compiled and analyzed sets of mitochondrial, plastid, and nuclear markers to detect putative hybrids or introgression in natural populations. Failure to detect natural hybrids indicates that effective pre- and postzygotic isolation mechanisms are at play in natural populations and supports the by-product hypothesis of reproductive isolation.

Key index words: **biogeography; *Dictyota*; Dictyotales; diversity; molecular phylogenetics; taxonomy**

Abbreviations: **AIC, Akaike information criterion; BI, Bayesian inference; BIC, Bayesian information criterion; GTR, general time reversible; ML, maximum likelihood**

Species of the genus *Dictyota* J. V. Lamour., along with other Dictyotales, are key components of many coastal ecosystems (Lüning 1990). Due to their efficient chemical defense systems, which involve various diterpenes as well as gaseous volatiles (Hay et al. 1987, Wiesemeier et al. 2007), and their ability to propagate successfully by fragmentation (Herren et al. 2006), the species are able to maintain a significant biomass under high grazing pressure and therefore have an important role in the structuring of benthic communities in tropical and temperate ecosystems. Despite their prevalence and ecological importance, species-level taxonomy is long and troubled.

Species are routinely used as fundamental units of ecological surveys, conservation biology, biogeography, and macroevolution, yet the empirical delimitation of species can pose serious difficulties (Wiens 1999, Agapow et al. 2004, Sites and Marshall 2004). Defining species boundaries is inherently linked to the species concept applied. Despite extensive disputes over species concepts, most biologists agree that species are lineages, and what previous authors

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have generally disagreed about are the best criteria for recognizing these lineages (Mayden 1997, De Queiroz 1998). As with most algal groups, the morphological species concept has dominated systematics in *Dictyota.* Species are recognized by discontinuities in morphological characters, but our inability to distinguish morphological plasticity from diagnostic traits that separate the various species has severely confounded species delimitation. The lack of understanding of the variability of morphological characters has led to an erroneous taxonomy whereby specimens on the fringes of the morphological spectrum have habitually been described as different species (De Clerck and Coppejans 1999) or where different species were lumped into a single entity on the basis of superficial similarity. From continental Europe, more than 60 species and intraspecific taxa have been described over the last two centuries (Hörnig and Schnetter 1988, De Clerck 2003). It is generally accepted that this number is a gross overestimation of the real taxonomic diversity caused by misinterpretation of morphological plasticity. There is currently no consensus as to how many species are present. Along the Atlantic coast of mainland Europe, reports are limited to two species: *D. dichotoma,* a common species ranging as far north as southern Norway (Hoek 1982), and *D. spiralis,* which is probably common along the Portuguese coast but known only from scattered localities in more northern reaches (Newton 1931, Gayral 1966, Ardré 1970, Hardy and Guiry 2003, Araujo et al. 2009). The Mediterranean Sea is thought to harbor a much higher diversity than the Atlantic coast, but there is no consensus on how many species are present there.

In an attempt to clarify the taxonomy of *Dictyota* in the North Atlantic, Schnetter and coworkers employed a biological species concept in combination with karyological observations and morphological analyses. Schnetter et al. (1987) and Hörnig and Schnetter (1988) recognized three Mediterranean species, *D. fasciola, D. spiralis,* and the morphologically extremely variable *D. dichotoma.* Slender growth forms of the latter species were named *D. dichotoma* var. *intricata* (C. Agardh) Grev. While there was relatively little doubt about the identities of *D. fasciola* and *D. spiralis,* the studies of Schnetter and coworkers applied a very broad concept of *D. dichotoma.* Previously, phycologists had recognized several species in the *D. dichotoma* complex, *D. divaricata* J. V. Lamour., *D. linearis* (C. Agardh) Grev., and *D. pusilla* J. V. Lamour. being the most commonly applied names. Chromosome counts and crossing experiments revealed a haploid chromosome number of $n = 16$ and full interfertility for all European specimens of the *D. dichotoma* complex. The authors also elevated the western Atlantic *D. dichotoma* var. *menstrualis* Hoyt to species rank (Schnetter et al. 1987) because it is characterized by a different chromosome number and does not cross with *D. dichotoma.* Caribbean representatives of the *D. dichotoma* complex were segregated and described as *D. pulchella* Hörnig and Schnetter (1988). Even though this tropical species shares its chromosome number with *D. dichotoma,* it appears to be partially reproductively isolated, the sporophyte generation resulting from reciprocal crosses being unable to produce viable tetraspores.

The application of a biological species concept in *Dictyota* yielded an orderly and simple classification. However, the ability of different strains to produce viable offspring under artificial conditions, though meaningful in itself, does not necessarily reflect natural conditions. It is obvious that the evolution of reproductive isolation is far more complex than gametic compatibility alone (Coyne and Orr 2004). Various pre- and postzygotic reproductive barriers may interact to reduce or completely prevent the formation of hybrid offspring between two sexually compatible species. It is therefore of interest to test if the observed gametic compatibility of the *D. dichotoma* complex extends to the formation of hybrids in natural populations also. The detection of hybrids or introgression in natural populations has become feasible by applying molecular techniques. Differential inheritance patterns of DNA from different genomic compartments offer an effective tool to detect putative hybrids or the lack thereof (Coyer et al. 2002a, Fraser et al. 2009, Niwa et al. 2009, Tellier et al. 2009). In brown algae, mtDNA appears to be maternally inherited (Motomura 1990, Coyer et al. 2002b, Peters et al. 2004, Kato et al. 2006), be it that paternal leakage has recently been demonstrated in a hybrid zone between two closely related *Fucus* species (Hoarau et al. 2009). The inheritance pattern of chloroplast DNA is likely correlated with the morphological differentiation of male and female gametes. Inheritance is biparental in isogamous species (Peters et al. 2004, Kato et al. 2006); all oogamous brown algae examined to date inherit chloroplasts maternally. It is therefore assumed that in *Dictyota* chloroplasts are maternally transmitted.

Besides the conceptual issues related to delimiting species, several problems regarding the diversity of *Dictyota* in Europe persist. First, the presence of only three species along the coasts of mainland Europe and the Mediterranean Sea was never unanimously accepted. Taxa such as *D. linearis* and *D. mediterranea,* which were considered synonyms of, respectively, *D. dichotoma* and *D. fasciola* by Hörnig et al. (1992a,b), continue to be recognized by several authors (Furnari et al. 1999, Pena Martín et al. 2004, Rull Lluch et al. 2005, Serio et al. 2006). Second, the geographic distribution of the various species remains uncertain. Literature reports suggest that most species possess a nearly global distribution. Hörnig et al. (1992a) and De Clerck (2003) expressed doubt about the widespread nature of most species, but these claims have never been tested with DNA sequence data. Third, recent reports of alien species, *Rugulopteryx okamurae* (formerly *D. okamurae*; Verlaque et al. 2009) and *D. ciliolata* Sond. ex Kütz. (Rull Lluch et al. 2007), call for a thorough reinvestigation of the European diversity, which may have been subject to cryptic invasions.

In this study, we used a molecular approach to define ESUs of European *Dictyota*. We aimed to (i) reassess species boundaries and make necessary taxonomic changes, (ii) define species distributions within a European context and on a global scale based on DNA-confirmed occurrence records, (iii) investigate the congruence between the biological species concept and our molecular species delineation approach, and (iv) evaluate the potential of morphological characters to define species boundaries. We used a densely sampled DNA data set (*psb*A) of nearly 400 sequences to identify ESUs. Phylogenetic relationships were interpreted using a multigene data set (six genes) containing a single representative per species. Potential natural hybridization was investigated by comparison of the genetic signatures, derived from chloroplast (*psb*A), mitochondrial (*cox*1), and nuclear markers (LSU rDNA).

MATERIALS AND METHODS

Dictyota specimens were collected throughout European seas and preserved in silica gel and/or pressed on herbarium sheets. Voucher specimens are deposited in GENT (Ghent University, Belgium), TFC (Universidad de La Laguna, Canary Islands, Spain), and BCN-Phyc (Centre de Documentació de Biodiversitat Vegetal, Universitat de Barcelona, Spain) herbaria. 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). Plastid-encoded PSII reaction center protein D1 (*psb*A) and RUBISCO LSU (*rbc*L), mitochondrial-encoded cytochrome oxydase subunit 1 and 3 (*cox*1, *cox*3) and NADH dehydrogenase subunit 1 (*nad*1), and partial nuclear ribosomal LSU DNA genes were amplified following De Clerck et al. (2006), Hwang et al. (2009), and Silberfeld et al. (2010). Mitochondrial *cox*1, *cox*3, and *nad*1 genes were amplified and sequenced using the following primers: cox1F = TCAACAAATCATAAAGATATTGG, cox1R = ACTTCTGGATGTCCA AAAAAYCA, cox3F = CATCGCCACCCATTTCATTT, cox3R = CATCGACAAAATGCCAATACCA, nad1F = CGTAAAATTATGGCTTCTATTCA, nad1R = AAAGGTAAAAAACATTTCCAACC. The protein coding sequences were aligned by eye. Alignment of the partial LSU followed De Clerck et al. (2006).

The genetic divergence displayed by the *psb*A gene was used to assess the diversity of the lineages occurring in continental Europe. In addition to sequences obtained from European specimens, we included sequences from a broader geographic area and a representative set of non-European species (Table S1 in the supplementary material). Special attention was paid to include species from the temperate western Pacific Ocean, a notorious source of introduced algae in European waters. The *psb*A data set was analyzed using the neighbor-joining criterion in MEGA 4.0 (Kumar et al. 2008). A Kimura-2-parameter model (K2P) was used for the reconstruction. Branch support was assessed using nonparametric bootstrapping (1,000 replicates). ESUs, which have been proved to generally correspond to species (i.e., independently evolving lineages), were delineated as clusters of sequences showing little intracluster sequence divergence, preceded by a relatively long and highly supported branch following Verbruggen et al. (2007). The number of haplotypes and segregating sites (S) were computed for each ESU. Mean nucleotide diversity π (Nei and Li 1979) was calculated for each lineage using Arlequin v. 3.1 (Excoffier et al. 2005).

Because *psb*A by itself does not contain sufficient phylogenetic information to resolve some deeper nodes in the *Dictyota* tree (see Results), a species phylogeny was inferred from a multigene alignment consisting of a single representative of each species (*rbc*L, *psb*A, *nad*1, *cox*1, *cox*3, and LSU rDNA) (Tables S2 and S3 in the supplementary material). A suitable partitioning strategy and suitable models of sequence evolution were selected using the Bayesian information criterion (BIC) (Table S4 in the supplementary material). The guide tree used during the entire procedure was obtained by maximum-likelihood (ML) analysis of the unpartitioned concatenated alignment with PhyML, using a JC + Γ_8 model (Guindon and Gascuel 2003). All subsequent likelihood optimizations and BIC calculations were carried out with Treefinder (Jobb et al. 2004). The partitioning strategy plus model combination that received the lowest BIC score was used in the phylogenetic analyses. The model selection procedure proposed seven partitions: LSU nrDNA (one partition), and plastid and mitochondrial genes were partitioned according to codon position (2 × 3 partitions). General time reversible (GTR) + Γ_8 was the optimal model for all partitions. An ML analysis was carried out using Treefinder. Bayesian phylogenetic inference (BI) was carried out with MrBayes 3.1.2 (Ronquist and Huelsenbeck 2003). Two parallel runs, each consisting of four incrementally heated chains, were run for 5 million generations, using default priors and chain temperature settings. The cold chain was sampled every 1,000 generations. Convergence of log-likelihoods and parameter values was assessed in Tracer v.1.4. (Rambaut and Drummond 2007). A burn-in sample of 1,000 trees was removed before constructing the majority-rule consensus tree.

Due to the possibility that different morphological entities within the *D. dichotoma* complex are reproductively compatible, we designed our study to allow detection of natural hybrids using a combination of markers from the three different genome compartments: mitochondria (*cox*1), plastid (*psb*A), and nuclear LSU rDNA. Congruence between the genes was studied using a subset of 57 specimens indicated by asterisks in Table S1. Haplotype networks of the three genes were constructed using the statistical parsimony algorithm as implemented in TCS v.1.21 (Clement et al. 2000) with gaps treated as a fifth state for the LSU rDNA gene.

For descriptive purposes, a selection of thalli of each European species was scored for 14 vegetative characters. In addition, sporophytes were scored for two additional characters, and gametophytes for 10 (male) and six (female) characters. For each specimen, thallus length, number of branches arising from main axes, lengths and widths of branches, and apical width were measured. The angle between two branches was measured in the median and proximal parts of the thallus. Lengths and widths of cortical and medullary cells were measured in surface view from a branch situated well below the apex, while their heights were measured in transverse section. Mature parts of each individual were selected for reproductive features. Number, widths, and lengths of rows of antheridia; lengths and widths of central antheridia and loculi per tier; number of oogonia per sorus; and diameters of central oogonia were obtained in surface view. Measurements are presented as 95% confidence limits flanked by minimum and maximum values.

RESULTS

Species diversity. The *psb*A alignment used for ESU delimitation contained 396 sequences of European origin, 23 sequences of non-European species, and five sequences of outgroup taxa. Due to missing data at the 3′ and 5′ ends, only 822 bp of the 954 bp alignment were analyzed. European *Dictyota* specimens clustered in six clades, which we consider to be ESUs and equate to the species *D. dichotoma*, *D. fasciola*, *D. implexa*, *D. mediterranea*, *D. spiralis*, and a new species that we describe below as *D. cyanoloma* (Fig. 1). The branches preceding the ESUs were markedly longer than within-ESU branch lengths, and bootstrap support values for these branches ranged from 91 to 100. Interspecific divergence within the ingroup, measured as uncorrected *p* distances, ranged from 2% to 8.1% with 95% of the values contained within a 3.1% to 6.8% interval. Within-ESU divergence was very low with 95% of the values equal to or lower than 0.8% in *D. cyanoloma*, *D. dichotoma*, *D. mediterranea*, and *D. fasciola* (Table 1). Nucleotide diversity in these taxa ranged from 0.000 to 0.002. *D. implexa* and *D. spiralis* exhibited higher intra-ESU divergence values, the P_{95} values being 1.4% and 1.7%. The *D. implexa* clade was subdivided into two subclades with low bootstrap support (BS 51 and 61). The *D. spiralis* clade consisted of a mixture of moderately to highly supported clades and grades, with varying numbers of specimens assigned to them. The higher divergence within the *D. implexa* and *D. spiralis* clade

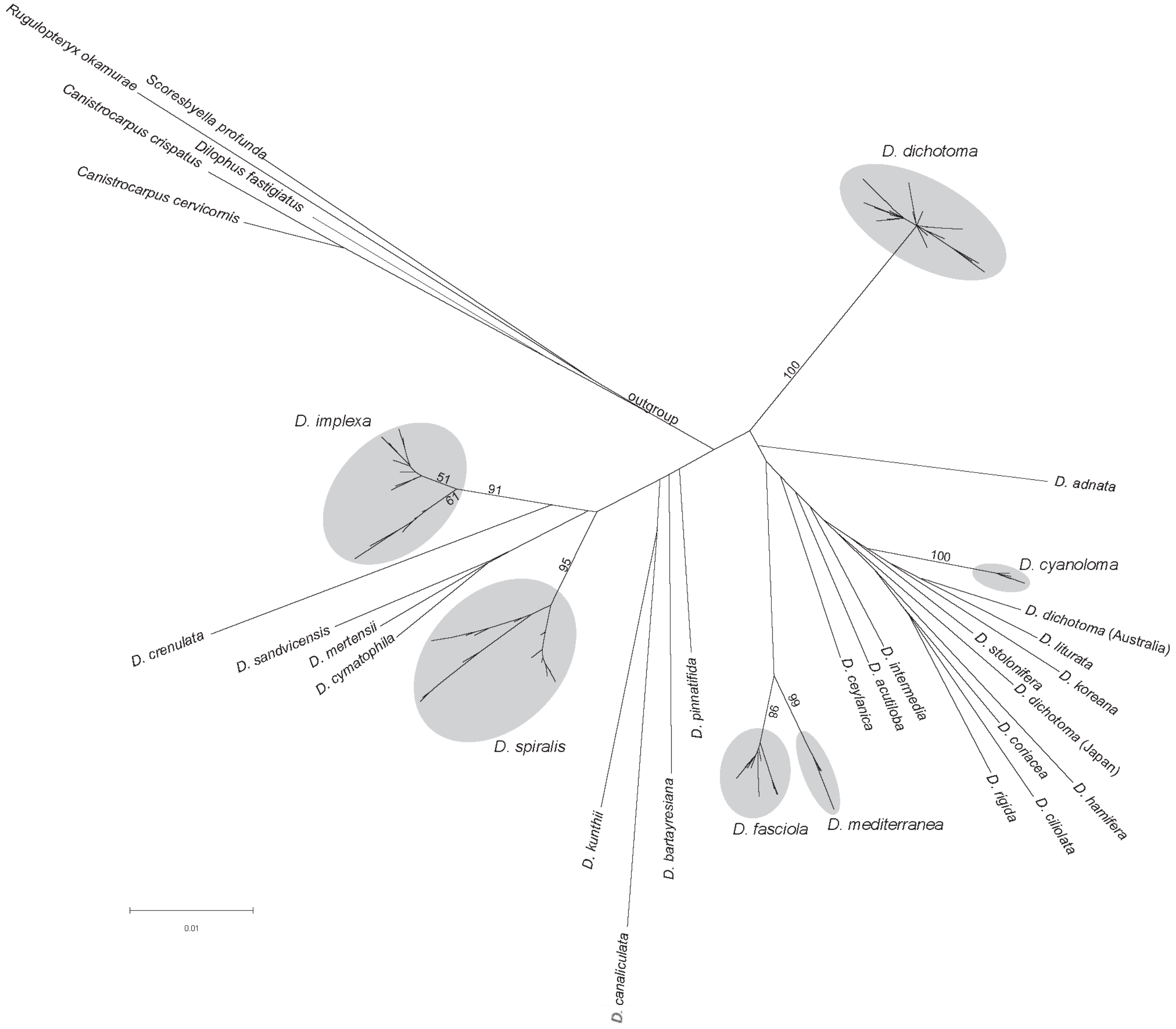

FIG. 1. Genetic diversity of European *Dictyota* based on *psb*A sequences as estimated by the neighbor-joining algorithm (K2P-model). European evolutionarily significant units (ESUs) are indicated in gray. Bootstrap values (1,000 replicates) are presented for the clades containing European specimens only.

TABLE 1. Plastid DNA (*psb*A) sequence variation in the European *Dictyota* species.

Taxon	*n*	*K*	*S*	P_{95}	π
Dictyota cyanoloma	12	1	0	0	0.000000 ± 0.000000
Dictyota dichotoma	194	15	18	0.8	0.001025 ± 0.000833
Dictyota fasciola	30	10	11	0.6	0.002116 ± 0.001438
Dictyota implexa	60	12	17	1.4	0.002984 ± 0.001847
Dictyota mediterranea	6	2	1	0.5	0.000442 ± 0.000582
Dictyota spiralis	45	11	22	1.7	0.006479 ± 0.000583

n, number of sequences; *K*, number of haplotypes; *S*, number of segregating sites; P_{95}, uncorrected *p* distances for intraspecific divergence; π, nucleotide diversity.

resulted in higher nucleotide diversity, 0.003 and 0.006, respectively. *D. fasciola* was somewhat intermediate with low intra-ESU divergence (P_{95}: 0–0.6%), but relatively high nucleotide diversity (π: 0.002).

Species distributions. Our intensive sampling in European waters indicates that most ESUs are geographically confined (Fig. 2). Along northern European coasts, only *D. dichotoma* and *D. spiralis* are present. The latter was infrequently sampled from two localities in northern Spain and Brittany. *D. dichotoma* is by far the most widely distributed species. Helgoland and Taynish (Scotland) represent the most northerly sampled localities in our data set, but the species is believed to occur as far north as southern Norway (Fro Islands, north of Trondheimsfjord; J. Rueness pers. comm.). Defining a southern boundary for *D. dichotoma* appears more difficult. This study confirms the widespread nature of *D. dichotoma* in the Canary Islands, but the species was not collected during a recent expedition to the Cape Verde Islands. In addition, gene sequences demonstrated the presence of genuine *D. dichotoma* from the temperate coasts of South Africa. Specimens attributed to *D. dichotoma* from the western Pacific Ocean (AY748319, Japan; AY528443, Australia) do not belong to *D. dichotoma.* All other taxa occur in the Mediterranean Sea, Macaronesia, and southern Portugal, except for *D. mediterranea,* which is exclusively Mediterranean. *D. fasciola* and *D. spiralis* were not collected in the Azores, but this could be a result of limited sampling. Interestingly, *D. cyanoloma,* which was only recently recorded from a few localities along the Mediterranean Sea coast of northern Spain (Rull Lluch et al. 2007, as *D. ciliolata*), appears to be widely distributed in the Mediterranean Sea, southern Portugal, and the Macaronesian Islands. A single sequence from Bermuda demonstrates that *D. implexa* also occurs in the western Atlantic Ocean.

Phylogeny. A concatenated alignment of six genes consisted of 29 species and 5,324 nt (LSU rDNA = 1,166 bp; *psb*A = 894 bp; *rbc*L = 1,188; *cox*1 = 654 bp; *cox*3 = 666 bp; *nad*1 = 756 bp). The matrix was 82% filled (Table S3). The ML and BI trees were highly congruent, differing only in the position of some unsupported clades (Fig. 3). Although our extensive gene sampling resulted in many clades with excellent support, the backbone of the genus remains poorly supported. European *Dictyota* do not form a monophyletic assemblage. Instead, they are grouped in five clades: *D. dichotoma, D. implexa, D. spiralis, D. cyanoloma,* and a clade uniting *D. mediterranea* and *D. fasciola.*

Congruence of nuclear and cytoplasmic markers. To investigate potential hybridization between *D. dichotoma* and *D. implexa,* we compiled and analyzed sets of mitochondrial, plastid, and nuclear markers. The mitochondrial *cox*1 alignment consisted of 500 nt, of which 74 (14.8%) were polymorphic. No insertions or deletions were observed. This variability translated into 12 mitochondrial haplotypes (Fig. 4), which could be subdivided into two groups of eight and four haplotypes, respectively, separated by 55 substitutions. The plastid *psb*A alignment consisted of 500 nt, of which 36 (7.2%) were polymorphic. No insertions or deletions were observed. The resulting 10 plastid haplotypes clustered into two groups of five haplotypes separated from each other by 19 substitutions. The nuclear LSU rDNA alignment consisted of 696 nt, of which 30 (4.3%) were polymorphic. The resulting nine ribotypes clustered into two groups of four and five ribotypes separated by 22 substitutions.

The three markers yielded congruent results: haplotypes showed a clear separation between *D. dichotoma* and *D. implexa* with no indication for hybridization. Whereas intraspecific haplotypes were separated by a small number of mutations, both species were clearly distinct and separated by a large number of mutations. Cytoplasmic incongruence whereby mitochondrial haplotypes typical of one species associated with chloroplast haplotypes of another species were not observed (Table 2). Likewise, the ribotypes always segregated with mitochondrial and chloroplast haplotypes characteristic of the same species.

Morphology and taxonomy. We refer to Table 3 for a morphological comparison of the various lineages identified in the phylogenetic analyses. Taxonomic and nomenclatural implications are detailed for each species separately below.

Dictyota cyanoloma Tronholm, De Clerck, Gómez Garreta et Rull Lluch **sp. nov.** (Fig. 5, A–M; Table 3).

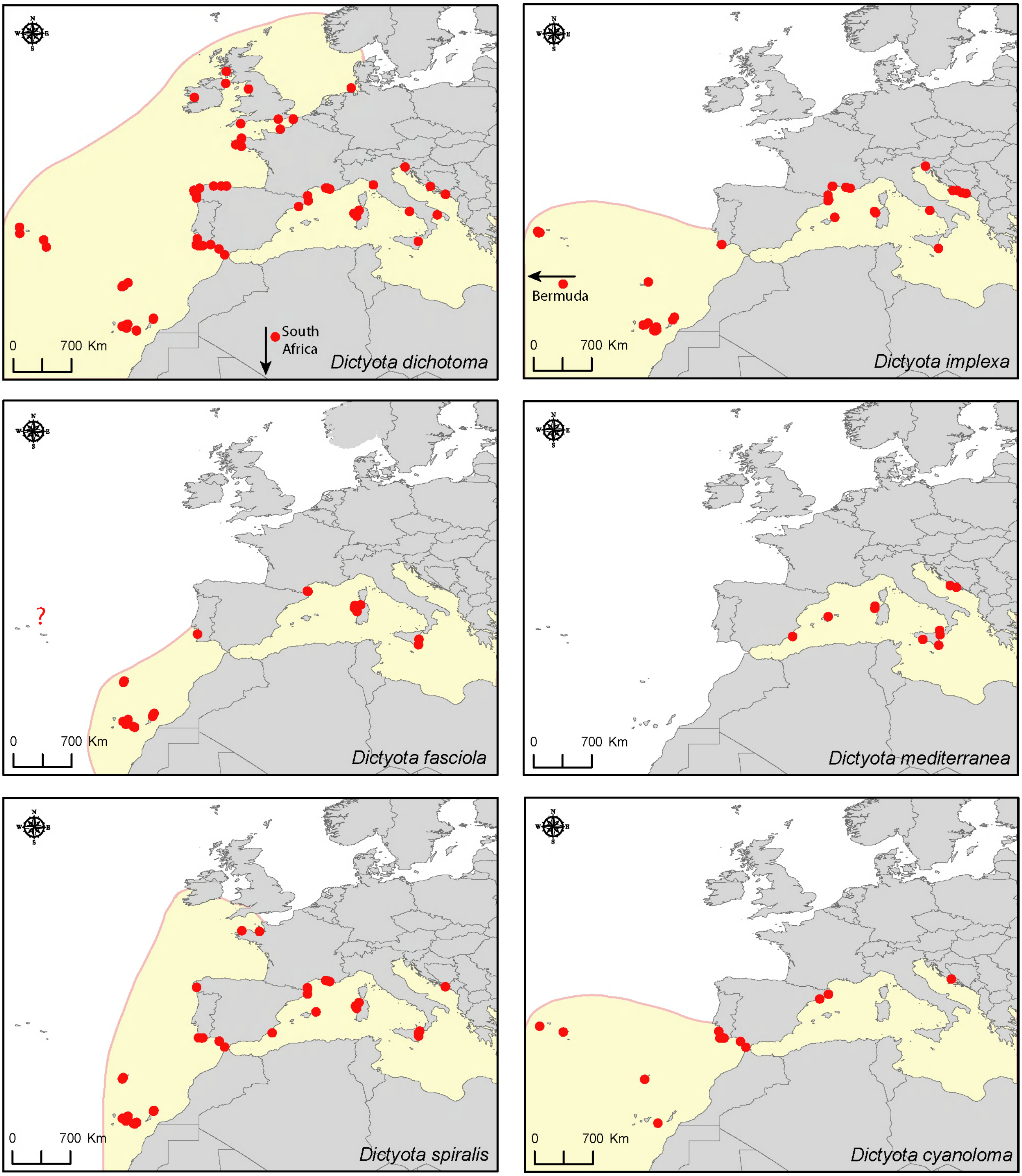

FIG. 2. Distribution maps of European *Dictyota* species. Dots represent DNA-confirmed distribution records; shaded areas indicate the estimated distribution range.

Recently, Rull Lluch et al. (2007) reported *D. ciliolata* from the western Mediterranean Sea. Because the species had not been previously recorded in the Mediterranean Sea, even in well-sampled localities, the authors raised the possibility that the species had been recently introduced to the area. Our phylogenetic analyses demonstrated that despite morphological similarity, these Mediterranean specimens are not closely related to *D. ciliolata*. In addition, the species is much more

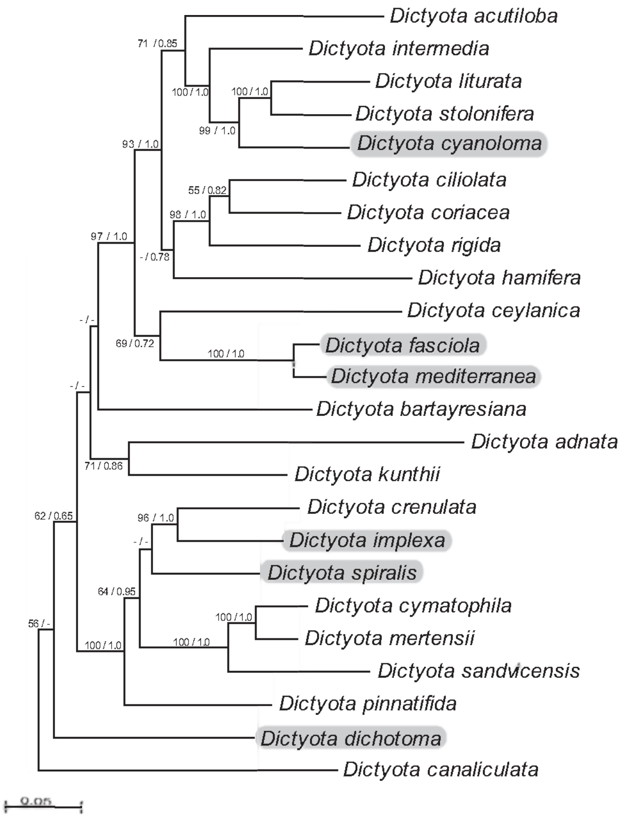

FIG. 3. Phylogenetic hypothesis (–lnL = 31283.8) obtained by maximum-likelihood inference of a data set containing six genes (partial LSU rDNA, *rbc*L, *psb*A, *cox*1, *cox*3, and *nad*1). Numbers at the nodes indicate ML bootstrap values followed by posterior probabilities; values below, respectively, 50 and 0.7 are not shown. Outgroups (*Canistrocarpus*, *Dilophus fastigiatus*, *Rugulopteryx*, *Scoresbyella*) have been pruned from the tree.

widely distributed than hitherto imagined. Our studies considerably expand the known distribution of this species to the Adriatic Sea, southern Portugal, Canary Islands, Madeira, and the Azores. Comparative sequence analyses suggest that the species does not occur outside this distribution range and that it is not introduced. Because this species does not correspond to the type material of any *Dictyota* species of Mediterranean or Macaronesian origin, we describe it as a new species.

Latin description: Planta (3.4–)7.6–8.2(–18) cm longa, erecta. Color atrofuscus, marginibus caeruleus iridescens. Segmenta (4–)10.3–10.7(–45) mm longa et (2.4–)5.4–5.5(–13) mm lata. Apices rotundati vel obtusi, (1–)1.9–2(–4) mm lati. Ramificatio dichotoma. Anguli superni acuti, (13–)33–35(–68)°, et latioris medio inferaque segmenta, (27–)49–51(–81)°. Marginibus laevis, interdum vel plerumque dentatis. Cortex monostromaticus, cellulis (21–)34–35(–55) μm longis, (10–)16–17(–24) μm latis et (14–)18–19(–26) μm altis. Medulla monostromatica, cellulis (75–)121–126(–195) μm longis, (55–)66–68(–105) μm latis et (55–)72–75(–102) μm altis. Sporangia in pagina dispersa solis, (52–)82–87(–107) μm diametra, cellula basali simplicia (7–)10–11(–14) μm alta. Antheridia in ellipsoidam soris dispositiis, (300–)535–610(–855) μm longis et (180–)246–274(–390) μm latis, soris (1–)2(–3) anulus maculus paraphysium circumcincti. Oogonia in oval sori disposita, (225–)319–345(–525) μm longis et (120–)223–239(300) μm latis, cum (31–)54–60(–101) oogonia per sorus. Matura oogonia (24–)39–41(–57) μm diametra, (64–)73–75(–86) μm altis, cellula basali simplicia (10–)16–17(–21) μm alta.

Thallus (3.4–)7.6–8.2(–18) cm long, erect, attached to the substrate by stupose and velvety rhizoids. Color in situ dark brown with blue iridescent margins in fertile specimens and entirely blue iridescent in nonfertile individuals. Interdichotomies (4–)10.3–10.7(–45) mm long and (2.4–)5.4–5.5(–13) mm wide. Apices rounded to obtuse, (1–)1.9–2(–4) mm wide. Branching dichotomously, (4–)8–9(–11) times branched. Angles acute toward apical segments, (13–)33–35(–68)°, and broader in middle and basal segments, (27–)49–51(–81)°. Smooth margins, occasionally to frequently with small teeth that become leaflike proliferations. Cortex unilayered, cells (21–)34–35(–55) μm long, (10–)16–17(–24) μm wide, and (14–)18–19(–26) μm high. Medulla unilayered, cells (75–)121–126(–195) μm long, (55–)66–68(–105) μm wide, and (55–)72–75(–102) μm high. Reproductive structures located in the central part of the thallus leaving a clear sterile area near the margins. Sporangia scattered single in thallus surface, (52–)82–87(–107) μm in diameter, borne on a single stalk cell (7–)10–11(–14) μm high. Antheridia grouped in ellipsoidal sori, (300–)535–610(–855) μm long and (180–)246–274(–390) μm wide, surrounded by (1–)2(–3) rings of pigmented paraphyses. Oogonia grouped in oval sori, (225–)319–345(–525) μm long and (120–)223–239(300) μm wide, with (31–)54–60(–101) oogonia per sorus. Mature oogonia (24–)39–41(–57) μm in diameter, (64–)73–75(–86) μm high, borne on a single stalk cell (10–)16–17(–21) μm high.

Etymology: cyano, root *cyanos* (Greek) = blue color; *loma* (Greek) = fringe, border.

Holotype: BCN-Phyc 5892, Port de Palamós, Girona, Spain, Joana Aragay, 11 February 2009, on hard substrata, −0.2 m, sporophyte. Isotypes BCN-Phyc 5893 in TFC and GENT.

Other specimens examined: Spain: Barcelona, harbor (J. Rull, 24 March 2005, BCN-Phyc 1599, sporophyte), ibidem (J. Aragay, 26 May 2009, BCN-Phyc 2786, sporophyte), Barcelona, Sitges (E. Ballesteros, June 2004, BCN-Phyc 1601, sporophyte); Canary Islands: Gran Canaria, Zoco del Negro (A. Tronholm, 29 March 2008, TFC Phyc 14431, D502, female gametophyte), El Hierro, Puerto de La Restinga (J. M. Landeira, 4 June 2009, TFC Phyc 14435, TFC Phyc 14439, TFC Phyc 14440, sporophytes; TFC Phyc 14436, TFC Phyc 14437, TFC Phyc 14438, TFC Phyc 14441, TFC Phyc 14442, female gametophytes); Portugal: Algarve, Portimão, Praia

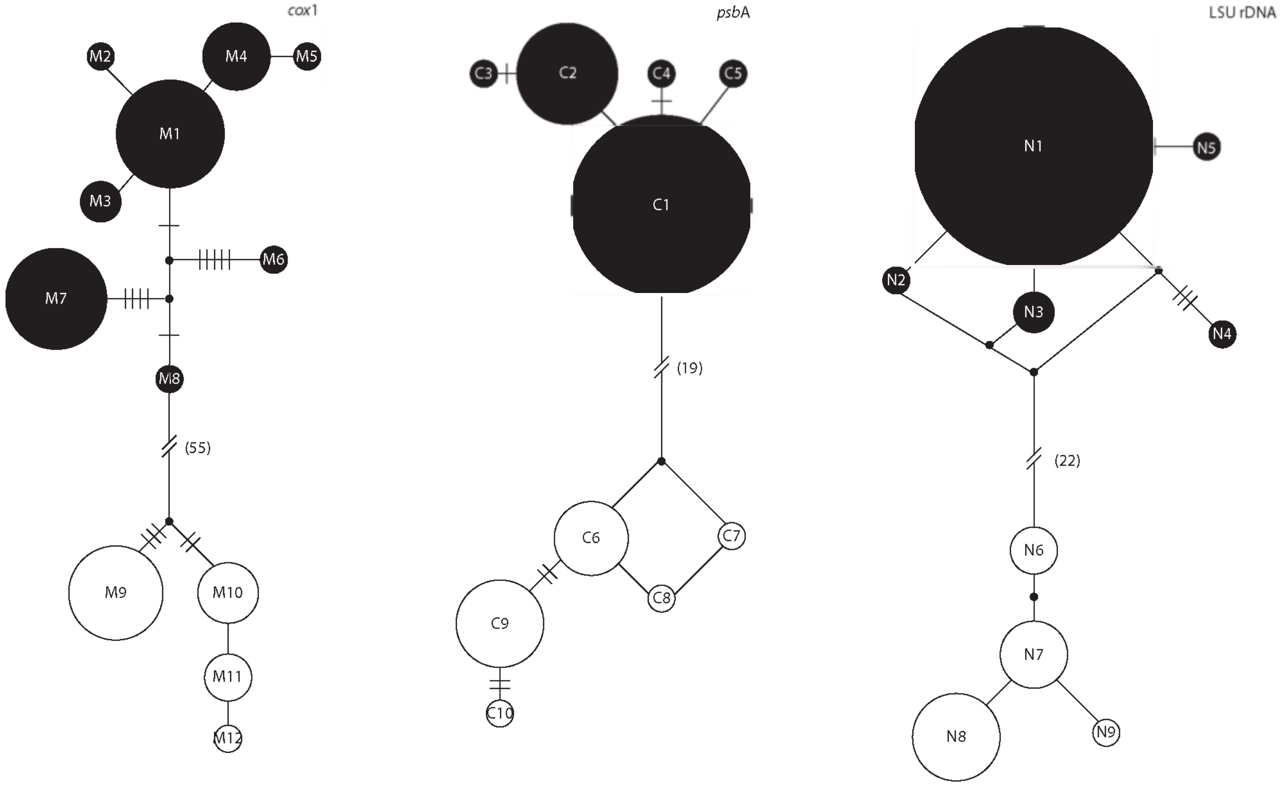

FIG. 4. Statistical parsimony networks of mitochondrial (*cox*1), chloroplast (*psb*A), and nuclear ribosomal (LSU) haplotypes. Each circle represents a haplotype. The size of the circle is proportional to the number of sampled individuals with a given haplotype. White circles correspond to *Dictyota implexa*, black circles to *Dictyota dichotoma*. Each line between haplotypes and/or bars represents one mutation step. Missing haplotypes, indicated by small black circles, were either not sampled or extinct.

da Rocha (A. Tronholm, 4 February 2008, TFC Phyc 14432, D544, sporophyte), Carvoeiro, A Boneca (K. Pauly, 22 July 2008, FAO006, D617, sporophyte); Azores: São Miguel, São Roque (Wallenstein, Terra, Santos and Torrão, 22 May 2006, AZB SMG-06-58, D971, male gametophyte), Pico, Santa Barbara (Amaral, Álvaro and Couto, 11 July 2007, Pix07637, D712, sterile); Madeira Island, Rais Magos (E. Coppejans and O. De Clerck, 15 May 2006, GENT HEC15777, D236, female gametophyte).

The bluish iridescent margin of *D. cyanoloma* is by far the most diagnostic character of the new species (Fig. 5A). Its external morphology is very similar to certain growth forms of *D. dichotoma* and *D. ciliolata*. Originally, Rull Lluch et al. (2007) had misidentified *D. cyanoloma* specimens from Spain as *D. ciliolata*. Both species have a stupose base and velvety rhizoid in the extreme basal parts of the thallus, and the tendency to develop dentate margins. The latter character, however, poses difficulties since degree of dentation is rather variable both in *D. ciliolata* and *D. cyanoloma*. The iridescence, however, unequivocally differentiates both species in situ. If present, iridescence in *D. ciliolata* is yellow greenish and follows a transverse banding pattern, while in *D. cyanoloma* iridescence is bright bluish and restricted to the margins in fertile specimens.

Dictyota dichotoma (Hudson) J. V. Lamouroux 1809: 42 (Fig. 6, A–F; Table 3).

D. dichotoma is the type species of the genus. The species was originally described as *Ulva dichotoma* Hudson (1762) from Walney Island, Cumbria, England. Efforts to trace authentic collections were unsuccessful. Hudson's algal collection is believed to have been largely destroyed by a fire at his house in 1783 (Dixon 1959). Additional attempts to locate authentic material in BM (including the Sloane Herbarium) were equally unsuccessful. In our analyses, we included material collected at the type locality (Walney Island, Barrow-in-Furness, England, O. De Clerck and G. Zuccarello, 3 June 2008, GENT ODC1689), which is here designated as the neotype (Fig. 6A). De Clerck (2003) gives a comprehensive list of homotypic and likely heterotypic synonyms of *D. dichotoma*.

D. dichotoma displays an extremely wide range of variation in external morphology (Fig. 6, A–F), and its anatomic and reproductive features do not allow differentiating it from the majority of *Dictyota* species. The morphological variation may seem spatially or geographically structured to some degree (Tronholm et al. 2008), but very often, one will observe growth forms spanning the entire morphological spectrum growing side by side. Hörnig and Schnetter (1988) segregated the variety *D. dichotoma* var.

TABLE 2. Association between cytoplasmic and nuclear sequences in *Dictyota dichotoma* and *Dictyota implexa*. The number given for each genetic combination corresponds to the number of individuals bearing this genotype. The name of the samples analyzed for each combination is indicated in Table S1 (in the supplementary material) with (*).

	*cox*1 (mitochondria)										
*psb*A (plastid)	M1	M2	M3	M4	M5	M6	M7	M8	M9	M10	M11
C1	13		3		1	1					
C2				6			12				
C3								1			
C4				1							
C5		1									
C6									9		
C7									1		
C8									1		
C9										5	4
C10										1	

	Partial LSU rDNA (nuclear)								
Cytoplasmic	N1	N2	N3	N4	N5	N6	N7	N8	N9
M1C1	10	3							
M2C5	1								
M3C1	3								
M4C2	5			1					
M4C4	1								
M5C1	1								
M6C1	1								
M7C2	10		1		1				
M8C3	1								
M9C6						1	1		7
M9C7						1			
M9C8									1
M10C9							3	1	1
M10C10							1		
M11C9						2	2		
M12C9									1

intricata (C. Agardh) Grev. from *D. dichotoma* proper, the former being characterized by narrow axes especially in the distal portions of the thallus. It should be noted, however, that these varieties could not be distinguished using the genetic markers applied in this study or with highly variable mitochondrial spacer sequences designed for phylogeographic purposes (F. Steen and O. De Clerck, unpubl. data). Furthermore, all possible morphological intergrades exist, ranging from broad to nearly filamentous thalli, rendering the formal distinction at the intraspecific level highly arbitrary, and we therefore advise against their use.

D. dichotoma is a common species of eulittoral pools and the shallow subtidal in the NE Atlantic during spring and summer. At higher latitudes, *D. dichotoma* largely disappears in late autumn, surviving the unfavorable winter period as cryptic microthalli (germlings, rhizoidal filaments) embedded in the substrate. In the southern ranges of its distribution, *D. dichotoma* displays an opposite seasonality, winter being the favorable season and exhibiting a brief resting period in autumn (Tronholm et al. 2008).

Dictyota fasciola (Roth) J. V. Lamouroux 1809: 43–44 (Fig. 7, A–G; Table 3).

D. fasciola is based on *Fucus fasciola* Roth (1797: 146, pl. VII: fig. 1). A type locality was not specified but is likely to have been the Mediterranean. Roth's specimens were part of the algal collections in the Berlin Herbarium, which was completely destroyed in 1943. De Clerck (2003) selected the drawing that accompanied the original description as a lectotype. Fortunately, there is little confusion regarding the delineation of *D. fasciola*. Specimens can be readily identified by their yellowish color, stoloniferous base, slender straps with narrow branching angles, acute apices, and a multilayered medulla in the basal parts of the thallus. For future reference, we select the following specimen as epitype: GENT ODC 1057 (O. De Clerck, 24 May 2005, Cap du Troc, Banyuls, Languedoc-Roussillon, France) (Fig. 7A).

Dictyota implexa (Desfontaines) J. V. Lamouroux 1809: 43 (Fig. 8, A–E; Table 3).

Our analyses reveal that the *D. dichotoma* complex comprises two distinct lineages in the Mediterranean Sea and Macaronesia. There is no problem with adopting the name *D. dichotoma* for one of these lineages given the type locality of the latter, but finding the correct name for the remaining

TABLE 3. Morphological comparative Table of the European *Dictyota* species.

	D. cyanoloma	*D. dichotoma*	*D. fasciola*	*D. implexa*	*D. mediterranea*	*D. spiralis*
Thallus length (cm)	(3.4–)7.6–8.2(–18)	(3.7–)8.7–9.4(–18.6)	(3.4–)6.1–7.5(–13.5)	(3.6–)6.7–8.7(–13.6)	3–10	(4–)7.4–8.4(–13.5)
Branches from main axes	(4–)8–9(–11)	(6–)13–14(–25)	(3–)4–5(–7)	(8–)8–9(–12)	3–7	(3–)4–5(–6)
Texture	Supple	Supple, occasionally stiff base	Supple	Supple	Stiff	Supple
Habit	Erect (stupose and velvety rhizoids)	Erect	Erect (stoloniferous fibers)	Erect	Erect	Erect (stoloniferous fibers)
Iridescence	Blue iridescent (only margins when fertile)	Blue-green iridescent surface	Absent	Green-yellow light iridescent margins	Absent	Green-yellow light iridescent margins
Apices						
Shape	Rounded to obtuse	Rounded to obtuse	Acute	Rounded	Acute	Rounded
Width (mm)	(1–)1.9–2(–4)	(0.25–)1.1–1.2(–3)	(0.3–)0.6–0.7(–1)	(0.1–)0.4–0.5(–1.5)	(0.2–)0.3–0.4 (–0.5)	(1–)1.9–2(3.5)
Dentation	Occasionally to frequently (small triangular teeth becoming leaflike proliferations)	Absent	Absent	Absent	Absent	Absent
Surface	Smooth	Smooth, occasionally basal proliferations	Smooth, often many germinations	Smooth	Smooth	Smooth
Interdichotomies						
Length (mm)	(4–)10.3–10.7(–45)	(3–)7.5–7.8(–15)	(2–)12.4–13.5(–42)	(4–)8–8.7(–15)	(6–)11.8–13.6 (–26)	(4–)14.7–15.7(–42)
Width (mm)	(2.4–)5.4–5.5(–13)	(1.5–)3.2–3.3(–6)	(0.5–)1.2–1.3(–2.5)	(0.2–)0.8–1(–2.5)	(0.5–)0.7–0.8 (–1)	(1.5–)3.2–3.3(–6)
Branching angle						
Apical (°)	(13–)33–35(–68)	(6–)19–21(–45)	(18–)29–30(–50)	(8–)15–19(–45)	(25–)35–40(–45)	(10–)20–22(–48)
Middle-basal (°)	(27–)49–51(–81)	(27–)58–61(–130)	(8–)30–34(–67)	(17–)42–49(–73)	(23–)30–36(–50)	(16–)33–35(–50)
Cortical cells						
Length (μm)	(21–)34–35(–55)	(16–)40–42(–69)	(29–)46–49(–110)	(29–)51–55(–88)	(31–)41–45(–68)	(24–)42–44(–73)
Width (μm)	(10–)16–17(–24)	(7–)15–16(–31)	(10–)16–17(–25)	(12–)17–19(–36)	(13–)21–23(–26)	(12–)17–18(–33)
Height (μm)	(14–)18–19(–26)	(9–)16–17(–29)	(14–)21–22(–31)	(12–)18–19(–29)	(24–)26–27(–31)	(14–)18–19(–24)
Medullary cells						
Layers	Single	Single	Multilayered near the base	Single	Multilayered	Multilayered near the base and the margins
Thickenings	Absent	Present, molding-like	Absent	Absent	Absent	Present, lomentaceus
Length (μm)	(75–)121–126(–195)	(47–)93–96(–190)	(100–)154–159(–225)	(88–)174–177(–335)	(105–)134–148 (–200)	(80–)145–150(–225)
Width (μm)	(55–)66–68(–105)	(45–)60–62(–105)	(60–)76–79(–120)	(26–)62–63(–114)	(45–)50–54(–75)	(60–)74–77(–120)
Height (μm)	(55–)72–75(–102)	(42–)80–82(–136)	(60–)90–95(–143)	(57–)126–141(–214)	(31–)43–47(–57)	(71–)89–91(–107)
Sporangia						
Arrangement	Solitary	Solitary	Solitary	Solitary or grouped	Solitary	Blocklike patches
Diameter (μm)	(52–)82–87(–107)	(52–)112–115(–160)	(71–)91–95(–155)	(76–)95–99(–131)	(170–)181–188 (–200)	(60–)117–122(–152)
Stalk cell height (μm)	(7–)10–11(–14)	(9–)17–18(–33)	(10–)16–17(–24)	(10–)14–15(–24)	No data	(12–)18–19(–24)

Table 3. Continued.

	D. cyanoloma	*D. dichotoma*	*D. fasciola*	*D. implexa*	*D. mediterranea*	*D. spiralis*
Male gametophytes			No data		No data	
Sori length (µm)	(300–)535–610(–855)	(270–)477–510(–720)		(315–)374–391(–450)		(345–)400–415(–465)
Sori width (µm)	(180–)246–274(–390)	(195–)296–319(–570)		(225–)258–273(–315)		(225–)297–321(–435)
Rows of antheridia (number)	No data	(5–)10–11(–21)		(7–)9–10(–12)		(6–)9–10(–14)
Central antheridia length (µm)	No data	(19–)31–34(–64)		(21–)23–24(–26)		(24–)29–31(–41)
Central antheridia width (µm)	No data	(16)25–28(–38)		(21–)24–25(–26)		(24–)30–32(–38)
Antheridia height (µm)	No data	(52–)83–88(–136)		(71–)78–81(–88)		(71–)92–99(–126)
Antheridia stalk cell height (µm)	No data	(9–)15–16(–24)		(12–)14–16(–24)		(17–)19–20(–21)
Paraphyses height (µm)	No data	(64–)90–95(–124)		(48–)62–67(–86)		(88–)112–116(–126)
Loculi/tier (number)	No data	(4–)33–40(–112)		(20–)26–29(–36)		(28–)42–48(–64)
Tiers/antheridia (number)	No data	(4–)20–24(–36)		(16–)28–31(–41)		(14–)20–22(–25)
Female gametophytes			No data		No data	
Sori length (µm)	(225–)319–345(–525)	(180–)320–347(–540)		(255–)322–338(–390)		(240–)310–335(–420)
Sori width (µm)	(120–)223–239(300)	(90–)227–248(–405)		(180–)255–276(–300)		(195–)216–228(–300)
Oogonia/sorus (number)	(31–)54–60(–101)	(9–)32–36(–60)		(30–)36–40(–65)		(22–)25–27(–35)
Central oogonia diameter (µm)	(24–)39–41(–57)	(26–)55–61(–93)		(64–)77–80(–90)		(48–)60–63(–74)
Central oogonia height (µm)	(64–)73–75(–86)	(30–)82–89(–136)		(79–)88–92(–107)		(74–)90–94(–107)
Oogonia stalk cell height (µm)	(10–)16–17(–21)	(11–)16–18(–43)		(12–)15–17(–21)		(14–)18–20(–26)
Distribution	Mediterranean Sea, southern Portugal, Azores, Madeira, Canary Islands	Northern Europe, Mediterranean Sea, southern Portugal, Azores, Madeira, Canary Islands, South Africa	Mediterranean Sea, southern Portugal, Madeira, Canary Islands	Mediterranean Sea, southern Portugal, Azores, Madeira, Canary Islands, Bermuda	Mediterranean Sea	Northern Europe, Mediterranean Sea, southern Portugal, Madeira, Canary Islands
References	This study	Tronholm et al. (2008)	This study	This study	This study	This study

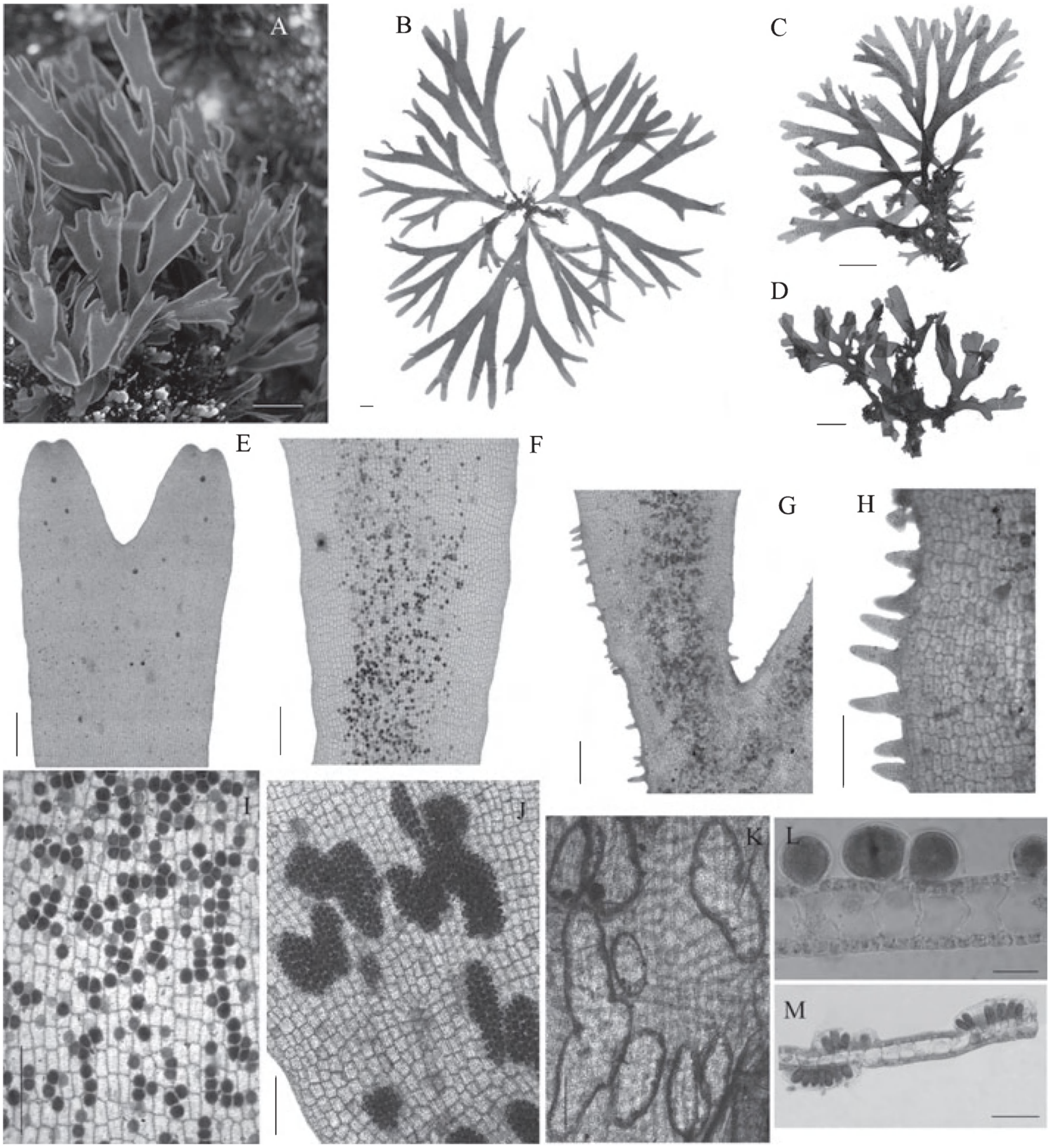

FIG. 5. *Dictyota cyanoloma.* (A) In situ habit of a specimen showing brightly iridescent margins; (B) holotype, sporophyte, Port de Palamós, Girona, Spain (BCN-Phyc 5892); (C) a female gametophyte, from Madeira Island (GENT HEC 15777); (D) a male gametophyte, from Azores (AZB SMG-06-58); (E) detail of rounded to obtuse apices (FAO006); (F) detail of a sporophyte segment showing smooth margins (FAO006); (G) detail of a sporophyte segment showing dentate margins (FAO006); (H) detail of teeth becoming proliferations (FAO006); (I) surface view of solitary sporangia (FAO006); (J) surface view of female sori (TFC Phyc 14431); (K) surface view of empty male sori of a pressed specimen, note persistent rings of rows of paraphyses (AZB SMG-06-58); (L) transverse section of the thallus showing sporangia borne on a single stalk cell, note unilayered medulla (FAO006); (M) transverse section showing female sori in both surfaces of the thallus (TFC Phyc 14431). Scale bars: (A–D), 1 cm; (E–G), 1 mm; (H–I), 500 μm; (J–K), 200 μm; L, 50 μm; M, 100 μm.

entity is more difficult. Several species have been described to accommodate Mediterranean *Dictyota* specimens with narrow axes. The oldest available name is probably *Fucus implexus* Desfontaines (1799: 423), described from the Mediterranean Sea. Other names that are important with respect to the priority rule are *D. pusilla* Lamouroux (1809) and *D. divaricata* Lamouroux (1809). Early in the nineteenth century, all three taxa were already considered to represent growth forms of *D. dichotoma* (Gray 1821, Agardh 1848). The complicated taxonomic history of these intraspecific taxa was clarified by Papenfuss (1944), who pointed out that the correct name for the taxon is *D. dichotoma* var. *intricata* (C. Agardh) Greville. However, by demonstrating that the *D. dichotoma* complex consists of two lineages,

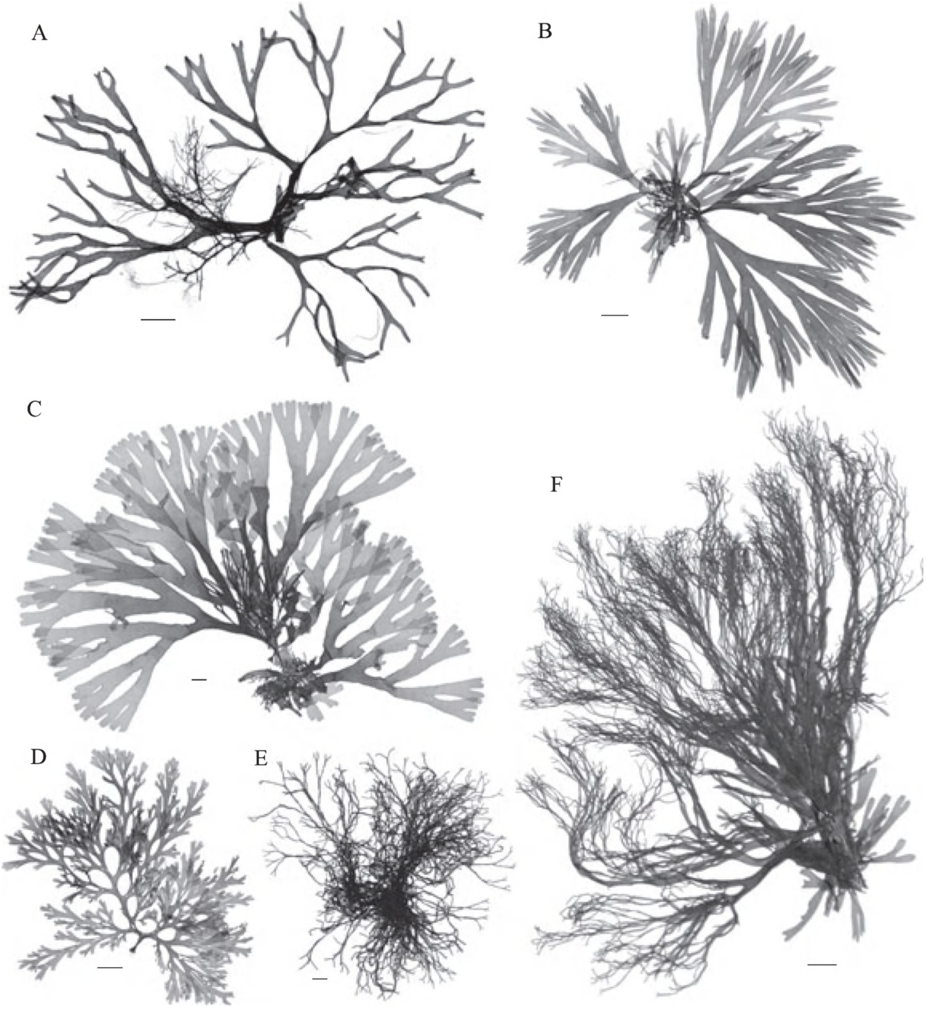

FIG. 6. Morphological plasticity of *Dictyota dichotoma.* (A) Epitype of *D. dichotoma* (Hudson) Lamouroux from the type locality, Walney Island, England (GENT ODC 1689); (B) specimen from the Netherlands (GENT IC 17); (C) specimen from Brittany, France (GENT HEC 9572); (D) female gametophyte from the Canary Islands (TFC Phyc 13085); (E) *D. dichotoma* var. *intricata* from Brittany, France (GENT HEC 15606); (F) *D. dichotoma* var. *intricata* from Wimereux, N. France (GENT HEC 3309). Scale bars, 1 cm.

the nomenclature needs to be reinvestigated. The type specimens of *D. divaricata, D. pusilla, Zonaria dichotoma* var. *intricata* C. Agardh (1820), and *Zonaria linearis* C. Agardh (1820), were examined in CN, P, and LD, respectively (Fig. 8A) (Fig. S1 in the supplementary material). Earlier types, of which there are plenty (see De Clerck 2003), can most probably be attributed to any of the above mentioned names and therefore are not crucial for the nomenclature. Establishing the relationships of type specimens with the extant diversity is troublesome because the types cannot be sequenced, and we acknowledge that any decision to adopt a name will be arbitrary to some extent. In accordance with Art. 11 of the International Code of Botanical Nomenclature (ICBN, Vienna Code), the name *D. implexa* has priority over all other epithets, and the type (PC0146187, Fig. 8A) corresponds very well with our morphological concept of this lineage. In addition, we select a recently collected specimen, GENT FS338 as epitype (Fig. 8B).

Dictyota mediterranea (Schiffner) G. Furnari in Cormaci et al. 1997: 214 (Fig. 9, A–E; Table 3).

The species was originally described as *Dilophus mediterraneus* Schiffner (1931) from various localities in the Adriatic Sea. It was reduced to a synonym of *D. fasciola* by Feldmann (1937). Our analyses, however, show a clear molecular divergence between both taxa. Although both species exhibit similarities in color, width of the axes, and shape of the apices, *D. mediterranea* can be differentiated by its terete thallus and multilayered medulla (Fig. 9, C–E). Axes of *D. fasciola* are complanate, and a multilayered medulla is restricted to the basal parts of the thallus. In addition, *D. mediterranea* is usually much more branched than *D. fasciola* and grows mainly on *Cystoseira* spp.

Dictyota spiralis Montagne, 1846: 29–30 (Fig. 10, A–J; Table 3).

The species was originally described from El Kala (Annaba) and a locality near Alger in Algeria. A lectotype, PC MA8030 from among Montagne's materials, is designated here (Fig. 10A). Because of its multilayered medulla in the lower portions of the thallus, Hamel (1939) transferred the species to the genus

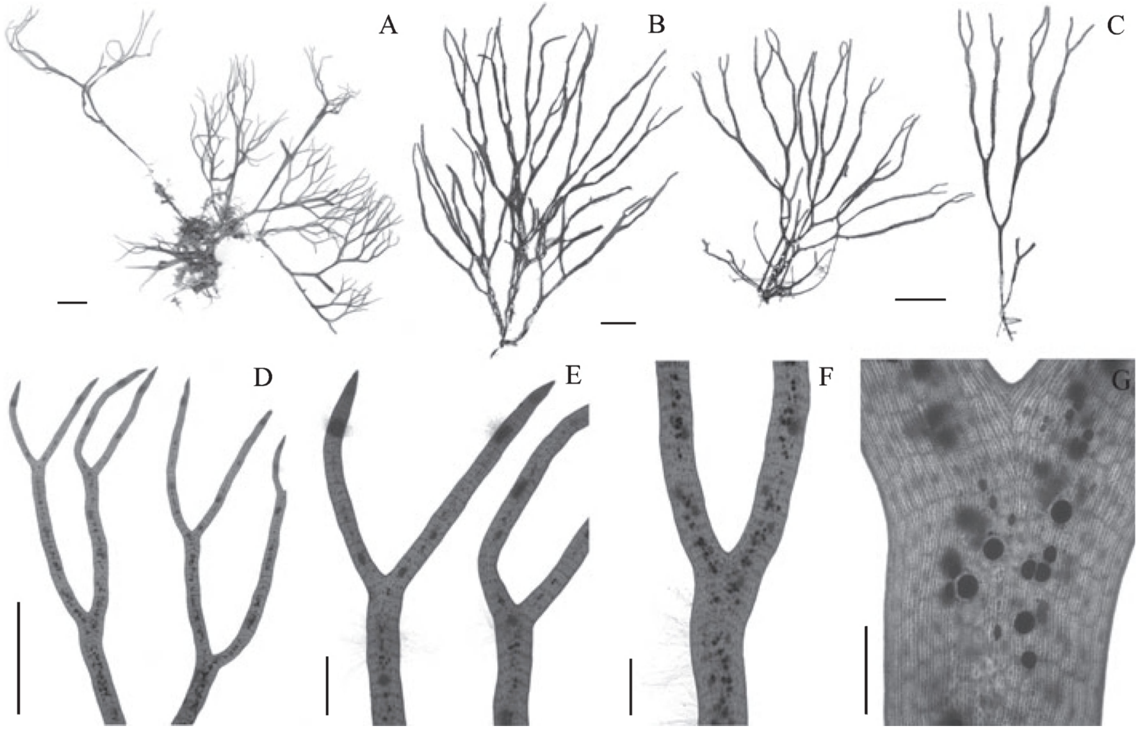

FIG. 7. *Dictyota fasciola.* (A) Epitype of *D. fasciola* (Roth) Lamouroux (GENT ODC 1057); (B–C) habit of pressed specimens (GENT HEC 3040a); (D) detail of terminal portion showing dichotomous branching and acute apices (GENT ODC 1054); (E) detail of acute apices (GENT ODC 1054); (F) sporangia arrangement over the whole thallus (GENT ODC 1054); (G) detail of solitary sporangia (GENT ODC 1054). Scale bars: (A–C), 1 cm; (D), 5 mm; (E–F), 1 mm; (G), 500 μm.

Dilophus. Even though the status of the latter genus is not completely clarified yet, *Dilophus spiralis* (Montagne) Hamel is clearly more related to species of *Dictyota* than to the generitype of *Dilophus*, *Dilophus fastigiatus* (see De Clerck et al. 2006). Diagnostic characters of *D. spiralis* include a multilayered medulla near the base and the margins of the thallus, spathulate or at least broadly rounded apical branches, and sporangia occurring in blocklike patches. The branching pattern is dichotomous, and the thallus is only sparsely branched, as is the case for *D. fasciola.* Specimens with only a single dichotomy are commonly observed. *D. spiralis* shows an olive-greenish color in situ with green-yellow light iridescent margins, turning into a yellowish brown once dried. Medullary cells exhibit lomentaceus thickenings in the inner walls, which are observed in transverse section (Fig. 10J). This species is usually found growing on sandy substrates of rocky platforms, attached to the substrate by stoloniferous holdfasts.

DISCUSSION

Genetic diversity and species delineation. The majority of *Dictyota* species are notoriously difficult to identify based on morphological, anatomical, or reproductive characters. Phylogenetic analysis based on a densely sampled *psb*A data set resulted in the delimitation of six clearly defined ESUs which we equate to species. This finding is considerably more than the three species proposed by Hörnig et al. (1992a,b): *D. dichotoma* (incl. var. *intricata*), *D. fasciola*, and *D. spiralis.* DNA sequence data demonstrate that *D. dichotoma* as defined by Hörnig et al. (1992a) is clearly polyphyletic, consisting of two unrelated clades. Neotype material from the type locality (Walney Island) clusters with specimens from the NE Atlantic, Mediterranean Sea, and Macaronesian Islands. The other clade, for which we adopt the name *D. implexa*, is composed of Mediterranean (incl. southern Portugal) and Macaronesian specimens. The latter taxon has generally been considered a synonym of *D. dichotoma* var. *intricata.* It is likely that most reports of *D. linearis* from the Mediterranean region refer to the same taxon. A specimen (CL030101) from Bermuda that was reported as *D. pulchella* in a previous study (De Clerck et al. 2006) is also contained in this ESU.

All ESUs are clearly delineated (i.e., they are preceded by a long and highly supported branch. The divergence of *psb*A sequences within the ESUs was very low (<1%) for *D. dichotoma, D. fasciola, D. mediterranea*, and *D. cyanoloma*, but somewhat higher for *D. implexa* and *D. spiralis* (1%–2%). Two subclades, receiving low bootstrap support, can be discerned within *D. implexa.* The haplotype networks of *cox*1 and *psb*A sequences confirm the split in the

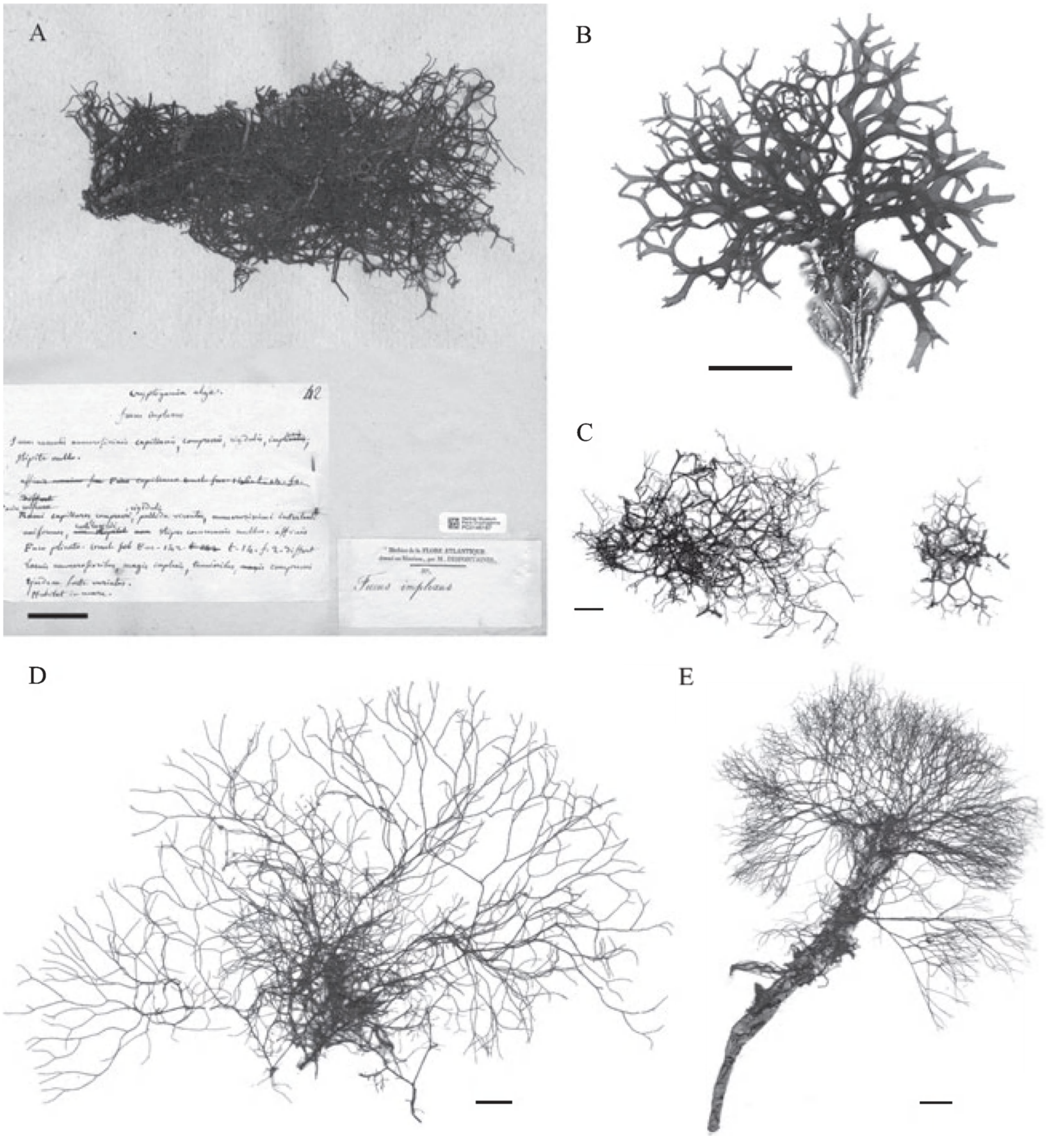

FIG. 8. *Dictyota implexa.* (A) Holotype of *D. implexa* (Desfontaines) Lamouroux; (B) epitype (GENT FS 338); (C) habit of a pressed specimen (GENT HEC 15758); (D) habit of a slender specimen (GENT HEC 3914); (E) habit of an epiphyte slender specimen (GENT HEC 41). Scale bars, 1 cm.

D. implexa clade. The divergence of cytoplasmic markers between both subclades suggests that these genetic entities have evolved separately. However, since this split is not observed in the LSU rDNA sequences, we adopted a conservative approach at present and do not recognize the subclades as different species. Similarly, *D. spiralis* also displays considerable genetic variation compared to other densely sampled ESUs. This divergence does not correlate with any morphological differentiation. Increased intraspecific variation displayed by *D. spiralis* may reflect lower dispersal abilities, compared to, for example, *D. dichotoma,* which occupies a similar distribution range but is characterized by significantly lower nucleotide diversity. Using simulation studies and empirical data, Papadopoulou et al. (2008) demonstrate that clustering of mtDNA sequences in discrete groups and genetic divergence are greatly affected by different levels of dispersal.

Morphological comparison of European species. Although it may prove difficult to identify every single specimen based on morphological characters, several characters or combinations of characters aid in the morphological characterization of the species. Table 3 summarizes the morphological, anatomical, and reproductive characters of the European species. It is clear from this table that all characters show considerable overlap for at least a subset of the species. Especially, the extensive morphological

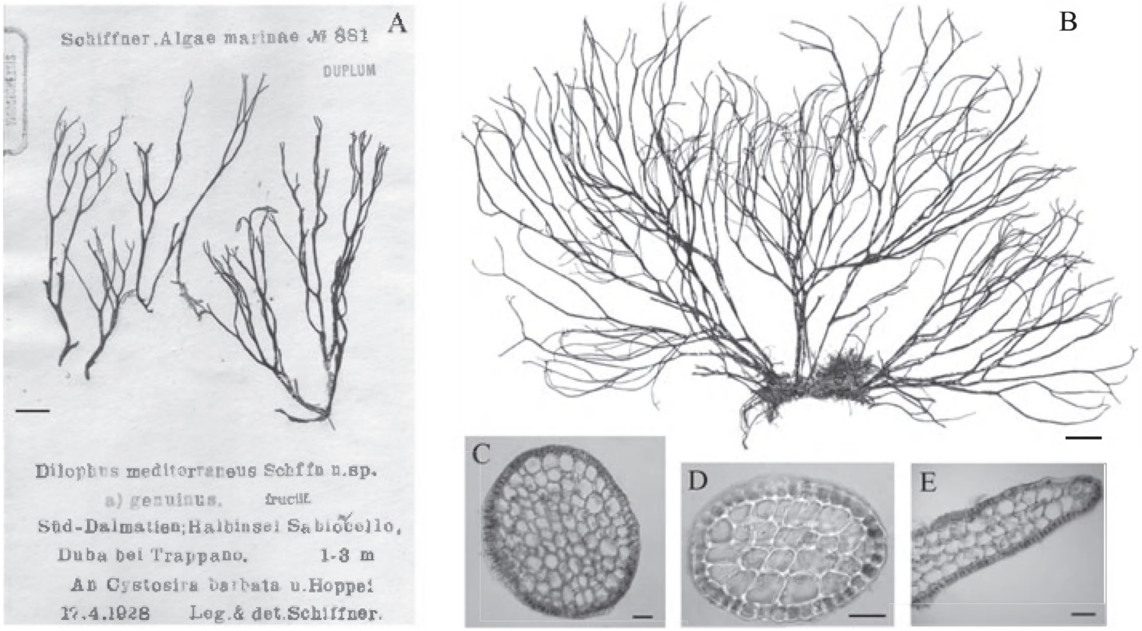

FIG. 9. *Dictyota mediterranea.* (A) Holotype of *Dilophus mediterraneus* Schiffner (Schiffner. Algae marinae 881); (B) habit of a pressed specimen (GENT HEC 3040b); transverse sections of the thallus showing multilayered medulla in basal (C) and apical (D) parts, and in middle flattened parts (E) (ABH Algae 199). Scale bars: (A–B), 1 cm; (C–E), 50 μm.

variation in *D. dichotoma* and *D. implexa* makes it impossible to assign less typical growth forms of these species to the right species without the aid of sequence data.

The structure of the medulla remains a principal characteristic to differentiate two groups of species. *D. fasciola, D. mediterranea,* and *D. spiralis* are all characterized by a multilayered medullary layer and the presence of stoloniferous holdfasts. In *D. fasciola,* duplications of medullary cells are restricted to the basal parts of the upright axes and the stoloniferous holdfasts, while in *D. spiralis,* duplicated medullary cells are also present near the margins. *D. mediterranea* exhibits a multilayered medulla over the entire thallus, with varying numbers of layers along the axes. *D. dichotoma* and *D. implexa* are characterized by a unilayered medulla throughout the entire thallus. The character may be misleading at times because rare and local duplications of medullary cells can occur in the basal parts of many species, as in *D. dichotoma.* Our analyses also demonstrate the distinctiveness of *D. mediterranea* and *D. fasciola.* The taxa are sister species, but molecular data as well as morphological and anatomical characters allow for a clear-cut differentiation. *D. mediterranea* is characterized by a terete and more branched thallus and an entirely multilayered medulla. The new species, *Dictyota cyanoloma,* most closely resembles *D. dichotoma* but can be distinguished by its blue iridescent margins with small teeth and proliferations.

Species concepts and reproductive isolation. Schnetter et al. (1987) and Hörnig et al. (1992a,b) applied a biological species concept in their studies of European *Dictyota.* Several strains of Mediterranean and NE Atlantic *Dictyota dichotoma-linearis-pusilla* were grown in culture and crossed in no-choice interbreeding experiments. All crosses yielded viable, diploid sporophytes that were capable of undergoing meiosis, and their tetraspores developed into fertile gametophytes. From these observations, Schnetter et al. (1987) concluded that the *D. dichotoma* complex consisted of a single biological species. These results are only superficially contradictory to our interpretation of European *Dictyota* diversity and come down to the application of different species concepts. We follow Mayden (1997) and De Queiroz (1998, 2007) in their attempts to reconcile different species concepts by distinguishing between the primary defining property of the species category and secondary defining properties. Most species concepts agree that species are separately evolving lineages (the primary species criterion). During the process of speciation, most incipient species gradually accumulate differences in secondary defining properties (e.g., reproductive isolation, morphological differences, ecological differentiation, etc.). Because secondary properties arise at different times during the process of speciation, species concepts based on secondary species criteria are often at least partially incompatible. In this particular case, the observation that *Dictyota* specimens belonging to different genealogical lineages, some of them being quite distantly related, may still be capable of producing viable offspring is very intriguing. Reproductive isolation is

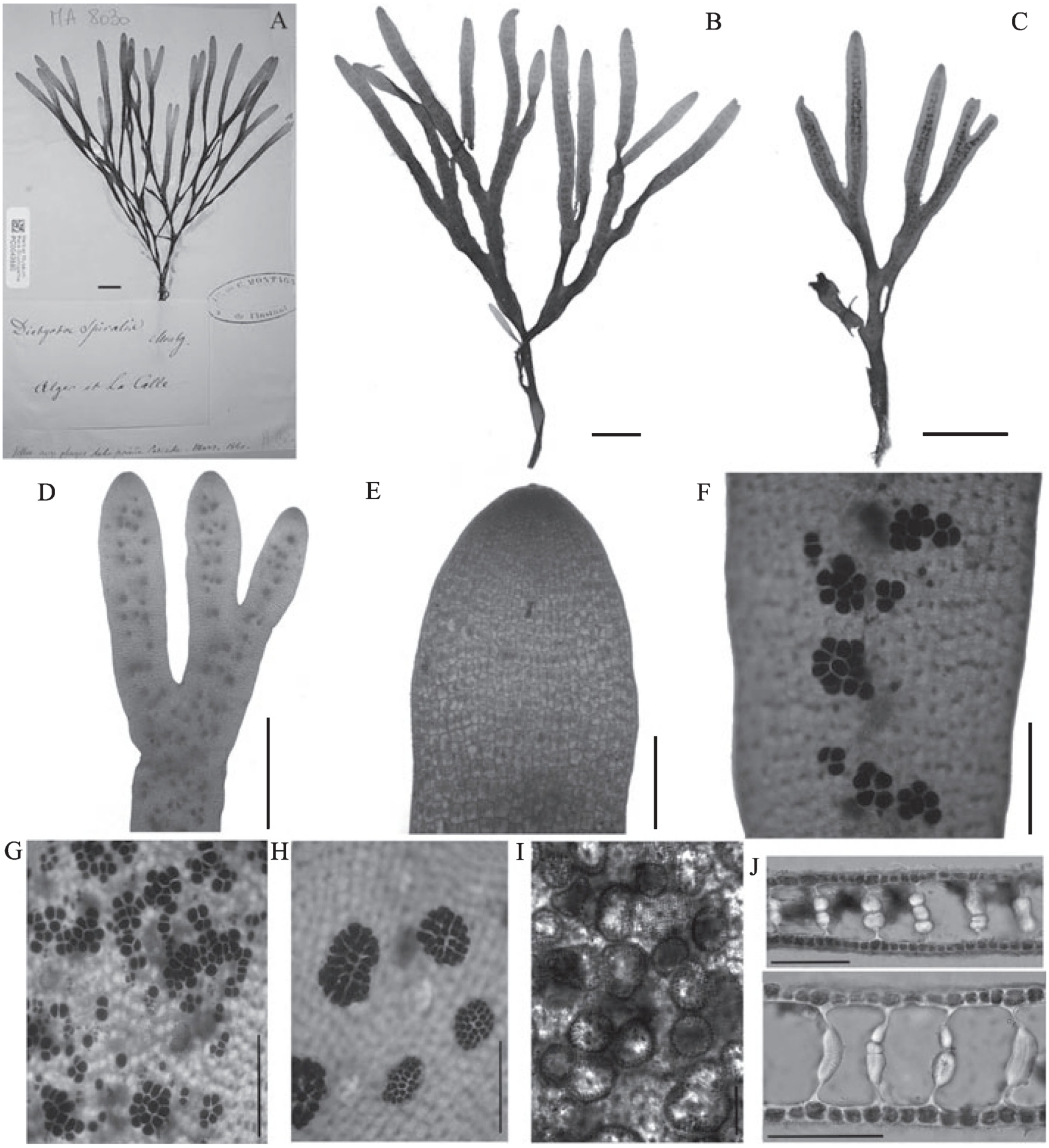

FIG. 10. *Dictyota spiralis.* (A) Lectotype of *D. spiralis* Montagne (MA 8030); (B) habit of a sporophyte (GENT HEC 4814); (C) habit of a sporophyte (GENT HEC 2686); (D) detail of terminal portion showing dichotomous branching and spathulate apices (GENT ODC 1056); (E) detail of rounded to spathulate apex showing a protruding apical cell (GENT ODC 1056); (F) sporangia arrangement in block-like patches (TFC Phyc 14444, D308); (G) detail of sporangia (TFC Phyc 14444, D308); (H) detail of female sori (TFC Phyc 14445, D752); (I) detail of male sori (TFC Phyc 14538); (J) detail of transverse sections of the thallus showing lomentaceus thickenings in the inner walls of medullary cells (TFC Phyc 14444, D308). Scale bars: (A–C), 1 cm; (D), 5 mm; (E–H), 500 μm; (I), 200 μm; (J), 100 μm.

often regarded as an incidental by-product of adaptation to alternative selection regimes (Mayr 1942, Schluter 2001, Coyne and Orr 2004). Although hardly investigated in macroalgae, scattered evidence from a limited number of well-studied taxa seems to corroborate the by-product mechanism. The formation of viable hybrids as a result of crosses between divergent lineages is not uncommon. In red algae, reports of naturally formed hybrids are scarce (Niwa et al. 2009, Destombe et al. 2010), and the resulting offspring is often sterile or reproduces exclusively apomictically (Zuccarello et al. 2005, Kamiya and West 2008). Hybridization seems more widespread in brown algae. Ecologically and morphologically differentiated sister species in *Fucus* are known to form hybrids under natural conditions, and backcrosses of the reproductive offspring result in introgressed parent species (Coyer et al. 2002a, 2007, Engel et al. 2005).

Many studies have dealt with the hybridization of kelp species (see Bartsch et al. 2008). Even though the reported results of interfertility between genera and families of the Laminariales have to be judged with care as demonstrated by Liptack and Druehl (2000) and Druehl et al. (2005), it is believed that reproductive isolation is a slowly evolving character in kelps. Interestingly, the scarcity of naturally occurring hybrids of Laminariales indicates the presence of effective pre- or postzygotic reproductive barriers. Similar reproductive boundaries may be at play in the case of *Dictyota*. Using molecular evidence from differentially inherited markers, we could not detect natural hybrids between *D. dichotoma* and *D. implexa.* Diagnostic signatures of partial LSU rDNA sequences segregate congruently with cytoplasmic markers (*psb*A and *cox*1). Nuclear-encoded ribosomal DNA may not present the ideal marker to detect introgression due to intergenic sequence homogenization during meiosis (Alvarez and Wendel 2003); the diploid offspring resulting from interspecific hybrids should be readily detected as having divergent rDNA copies. Despite the screening of 57 individuals, we did not detect any recombinant incongruence between cytoplasmic and nuclear-encoded markers. Classical crossing experiments are typical no-choice experiments. Such experiments give a rather limited picture of hybridization potential. Especially in cases where reproductive isolation is not complete, no-choice experiments have the tendency to inflate the rate of heterospecific fertilization considerably (Lessios 2007). Experiments in which mating choices are available are therefore believed to provide more insightful information of the evolution of reproductive barriers and the various pre- or postzygotic isolation mechanisms at play. Geyer and Palumbi (2005) demonstrated that sea urchins, which in no-choice experiments appear to be almost perfectly compatible, can actually discriminate in a mixture between homospecific and heterospecific sperm, so that the eggs are fertilized by their own species. At present, we have no clear idea about the reproductive barriers that might prevent or limit the formation of natural hybrids between *D. dichotoma* and *D. implexa.* However, preliminary results on periodicity of gamete release (oogonia) indicate that *D. implexa* shows a weekly pattern (A. Tronholm unpublished results) in contrast to the fortnightly behavior reported for *D. dichotoma* by several authors (Williams 1905, Müller 1962, Tronholm et al. 2010).

Species distributions and biogeography. Our data set of DNA sequences delivers reliable species occurrence records that can serve to infer species distributions. Based on literature reports, many species appear widespread, not being confined to a single biogeographic region. *D. dichotoma*, for example, has historically been reported from tropical to warm-temperate regions worldwide. Even though there were indications that many of these non-European reports referred to different species, the claim that *D. dichotoma* is confined to the NE Atlantic, Mediterranean Sea, and Macaronesia was never substantiated. Hörnig and Schnetter (1988) segregated *D. pulchella* and *D. menstrualis* to accommodate tropical and temperate western Atlantic specimens of the *D. dichotoma* complex, respectively. De Clerck (2003) was of the opinion that *D. dichotoma* was not present in the tropical Indian Ocean. Specimens identified as *D. dichotoma* most probably represented misidentifications belonging to a whole suite of species. De Clerck (2003) applied the name *D. ceylanica* to Indo-Pacific *Dictyota* specimens characterized with a *D. divaricata*–like morphology that had previously been referred to as *D. dichotoma* var. *intricata*, *D. divaricata*, or *D. linearis.* Genuine *D. dichotoma* var. *intricata* was thought to occur in the Red Sea, Persian Gulf, and the warm-temperate coast of South Africa.

In this study, we have confirmed the distribution of *D. dichotoma* along the NE Atlantic coast, Mediterranean Sea, and Macaronesia. Confirming the ideas of Hörnig et al. (1992a), the species is probably absent in the western Atlantic Ocean. In the Indo-Pacific Ocean, *D. ceylanica* is clearly distinct from *D. dichotoma.* Interestingly, our *psb*A data set shows that *D. dichotoma* occurs along the temperate south coast of South Africa. Whether the presence of this species in South Africa can be attributed to human-mediated dispersal, natural dispersal, or historical vicariance cannot be inferred from the present data set. Limited taxon sampling does not allow us to make a firm statement about the presence of *D. dichotoma* in the Red Sea or Persian Gulf. *D. dichotoma* is widespread in the Canary Islands (see Tronholm et al. 2008), but the species was not collected in the Cape Verde Islands during a recent expedition. The presence of *D. dichotoma* along the West African coast needs to be confirmed. We included sequences deposited in GenBank identified as *D. dichotoma* from the NW Pacific Ocean (Japan) and temperate Australia in our *psb*A data set, but these specimens are clearly unrelated and represent different species awaiting formal taxonomic treatment.

Other European *Dictyota* species appear equally geographically confined to the Mediterranean Sea and NE Atlantic coasts. All species are also widespread in the Macaronesian archipelagos, with the exception of *D. mediterranea,* which was not observed outside of the Mediterranean Sea. The *D. implexa* ESU also contains a sequence of *D. pulchella* from Bermuda. *D. pulchella* was described by Hörnig and Schnetter (1988) from the Caribbean coast of Colombia to accommodate tropical western Atlantic specimens of the *D. dichotoma* complex. Crossing experiments revealed at least a partial interbreeding barrier between European *D. dichotoma* and Caribbean *D. pulchella* specimens. The status of *D. pulchella* becomes uncertain now that our analyses reveal an amphi-Atlantic distribution for *D. implexa.* *D. pulchella* and *D. implexa* could be synonyms, in which case, the name *D. implexa* has priority, but because we were unable to include specimens of *D. pulchella* from the type locality, we cannot make a definite statement at present.

The European *Dictyota* diversity represents a subset of the species richness encountered in the Macaronesian region. We speculate that the Mediterranean Sea was reinvaded by species occurring in Macaronesian Islands and the NW African coast following the Messinian salinity crisis (5.9–5.3 million years ago; Krijgsman et al. 1999). However, unlike the situation in several groups of marine animals (Huyse et al. 2004, Sotelo et al. 2009), subsequent Pliocene flooding did not trigger substantial endemic speciation in *Dictyota*. The only evidence for endemic speciation in *Dictyota* comes from *D. mediterranea*, which may have speciated from *D. fasciola* in the Mediterranean basin. Alternatively, *D. fasciola* and *D. mediterranea* speciated in allopatry and formed a secondary contact zone following reinvasion of the Mediterranean basin from their respective allopatric sources after the Messinian crisis. These two species also represent the only example of species that share the same distribution and have evolved from a common ancestor. In all other cases, the closest relatives of European *Dictyota* are non-European species. This observation is unlikely to change with a more exhaustive taxon sampling.

CONCLUSIONS

Using DNA sequence data, we have demonstrated the presence of six ESUs in European waters and have attributed names to these lineages by relating them to their respective types, where extant. Future research should concentrate on the mechanisms that shape diversification in *Dictyota* on microecological as well as macroevolutionary scales. The incongruence between sexual compatibility and the presumed absence of hybrids in natural populations opens perspectives to identify pre- and postzygotic barriers. On a macroevolutionary scale, a more comprehensive sampling should result in a better understanding of global diversity patterns and identify the mechanisms that shaped the current European diversity.

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Supplementary Material

The following supplementary material is available for this article:

Figure S1. Type specimens of: (A) *Dictyota divaricata* J. V. Lamoroux (CN X7F67); (B) *Dictyota pusilla* J. V. Lamoroux (CN C7F75); drawing of *Dictyota pusilla* J. V. Lamoroux (CN C7F75); (D) *Zonaria dichotoma* var. *intricata* C. Agardh (LD 48910); and (E) *Zonaria linearis* C. Agardh (LD 487921 Scale bars, 1 cm).

Table S1. Specimens used in the *psbA* analysis with indication of collecting data and accession numbers. Non-European taxa are indicated below the grey bar. Asterisks indicate the subset of 57 sequences used for the haplotype network.

Table S2. Specimens used in the multigene alignment constructed using six (*rbc*L, *psb*A, *nad*1, *cox*1, *cox*3, and LSU rDNA) and a single representative of each species, with indication of collecting data.

Table S3. GenBank accession numbers of the sequences used in the concatenated alignment, including strain numbers and sequence length.

Table S4. 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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