ePublishing

ΕΘΝΙΚΟ ΚΕΝΤΡΟ ΤΕΚΜΗΡΙΩΣΗΣ
EKT NATIONAL DOCUMENTATION CENTRE

Mediterranean Marine Science

Vol 17, No 3 (2016)

Phenological and molecular studies on the introduced seaweed Dictyota cyanoloma (Dictyotales, Phaeophyceae) along the Mediterranean coast of the Iberian Peninsula

J. ARAGAY, D. VITALES, A. GÓMEZ GARRETA, M. A. RIBERA SIGUAN, F. STEEN, O. DE CLERCK, T. GARNATJE, J. RULL LLUCH

doi: 10.12681/mms.1872

To cite this article:

ARAGAY, J., VITALES, D., GÓMEZ GARRETA, A., RIBERA SIGUAN, M. A., STEEN, F., DE CLERCK, O., GARNATJE, T., & RULL LLUCH, J. (2016). Phenological and molecular studies on the introduced seaweed Dictyota cyanoloma (Dictyotales, Phaeophyceae) along the Mediterranean coast of the Iberian Peninsula. *Mediterranean Marine Science*, *17*(3), 766-776. https://doi.org/10.12681/mms.1872

https://epublishing.ekt.gr | **e-Publisher:** EKT | **Downloaded at:** 11/09/2026 13:28:01

Research Article

Mediterranean Marine Science
Indexed in WoS (Web of Science, ISI Thomson) and SCOPUS
The journal is available on line at http://www.medit-mar-sc.net
DOI: http://dx.doi.org/10.12681/mms.1872

Phenological and molecular studies on the introduced seaweed *Dictyota cyanoloma* (Dictyotales, Phaeophyceae) along the Mediterranean coast of the Iberian Peninsula

J. ARAGAY[1,2], D. VITALES[1,2], A. GÓMEZ GARRETA[1], M.A. RIBERA SIGUAN[1], F. STEEN[3], O. DE CLERCK[3], T. GARNATJE[2] and J. RULL LLUCH[1]

[1] Laboratori de Botànica, Facultat de Farmàcia i Ciències de l'Alimentació & Institut de Recerca de la Biodiversitat (IRBio), Universitat de Barcelona, Av. Joan XXIII s.n, 08028 Barcelona, Catalonia, Spain
[2] Institut Botànic de Barcelona (IBB-CSIC-ICUB), Passeig del Migdia s.n. 08038 Barcelona, Catalonia, Spain
[3] Phycology Research Group and Center for Molecular Phylogenetics and Evolution, Ghent University, Ghent, Belgium

Corresponding author: daniel.vitales@ibb.csic.es

Handling Editor: Athanasios Athanasiadis

Received: 8 August 2016; Accepted: 6 October 2016; Published on line: 16 November 2016

Abstract

Dictyota cyanoloma, a distinctive brown algal species characterized by a blue-iridescent margin, was recently reported as an introduced species in the Mediterranean Sea but little is known about its distribution dynamics, morphological plasticity and genetic structure. In this integrative study, we evaluate its past and present occurrence along the Mediterranean Iberian coast, assess the species' phenology in Palamós (Girona, Spain) and analyze the haplotype diversity by sequencing 49 individuals from nine sampling sites for different chloroplast and mitochondrial DNA regions. Although *D. cyanoloma* currently occurs along all the Mediterranean Iberian coasts (in 19 of 36 localities sampled, mostly in marinas and harbour environments), we were not able to find any herbarium material of this species (at BCN-Phyc and MA) predating the year 1987. In Palamós, *D. cyanoloma* is present all through the year, with a maximum development in winter and a minimum in summer. Fertile specimens are absent during summer (July and August). Sporophytes are dominant from January to June and gametophytes were found only in February, March and June. Information about the antheridia, which has never been described before, is provided. Two chloroplast and three mitochondrial haplotypes were observed, indicating that multiple introductions of *D. cyanoloma* occurred in the study area. Additionally, the genetic structure suggests that spread did not occur through simple advancing wave fronts but by several long-distance dispersal events. Further studies employing microsatellite markers could potentially offer a better resolution to unravel expansion and colonisation dynamics of *D. cyanoloma* in the Mediterranean Sea.

Keywords: *Dictyota*, Iberian coast, introduced species, phenology, phylogeography.

Introduction

The Mediterranean Sea is considered to be one of the most anthropogenically affected water masses in the world (Coll *et al.,* 2012). As an example, nearly 1000 alien marine species have been reported to date (Zenetos *et al.*, 2010; Hoffman, 2014). Non-indigenous marine species are introduced into new areas primarily as a result of accidental transport via culture of exotic shellfish or shipping (by fouling or ballast tank emptying) (Boudouresque & Verlaque, 2002; Ribera Siguan, 2003; Streftaris *et al.*, 2005; Zenetos *et al.,* 2006; Galil, 2009). In this context, more than 115 introduced benthic algae and seagrass taxa have been found in the Mediterranean Sea (Zenetos *et al.*, 2010; Mineur *et al*., 2015; Verlaque *et al*., 2015).

The genus *Dictyota* J.V.Lamour (Dictyotales, Phaeophyceae) was established by Lamouroux (1809) and 91 specific and infraspecific taxa are currently accepted (Guiry & Guiry, 2016). Six species were recognized as indigenous in the Mediterranean Sea (Tronholm *et al*., 2010; Cormaci *et al.,* 2012), including the recently described *Dictyota cyanoloma* Tronholm, De Clerck, Gómez Garreta & Rull Lluch in Tronholm *et al.* (2010). This species was first reported on the Mediterranean Iberian coast by Rull Lluch *et al*. (2007) as *Dictyota ciliolata* Sonder ex Kützing, on the basis of several specimens collected at Barcelona, Sitges, Palamós, and Sancti Petri. Later, Tronholm *et al.* (2010) demonstrated that despite morphological similarity, the Iberian specimens belonged to a new species which they described as *D. cyanoloma.*

The type of *D. cyanoloma* was a specimen from Palamós harbour (Girona, Spain), and new records of *D. cyanoloma* from the Canary Islands, Morocco, Madeira, Açores, Southern Portugal, Aegean coast, Adriatic Sea, French Mediterranean, Great Brittany and Spanish North Atlantic coasts were soon reported (Taskin, 2013; Bárbara *et al.,* 2014; Santolaria, 2014; Steen *et al.,* 2016). Steen *et al*. (2016), based on phylogenetic affinities, haplotype networks and global distributions of *Dictyota* species, concluded that the species is non-native in the Mediterranean and North Atlantic regions and indicated Australia as its native region. Steen *et al*. (2016) also found herbarium records of *D. cyanoloma* from the Adriatic Sea

dating as early as 1935, questioning whether the species remained unreported despite being widespread in the Mediterranean or whether it only spread recently from the Adriatic Sea.

In this study we aim to provide a deeper insight into the dynamics and genetic diversity showed by local populations of *D. cyanoloma* on the Mediterranean coast of the Iberian Peninsula. We therefore conducted: (i) a screening of the principal Spanish herbaria looking for historical records of this species; (ii) a large scale survey of the most important harbours from the Mediterranean Iberian coast to describe the current distribution of *D. cyanoloma* in the region where the species was first reported; (iii) a one year detailed phenology study of one population from Palamós harbour (Girona, Spain) to characterise the sporophytic and gametophytic reproduction; and (iv) a phylogeographic study in order to assess the variability of *D. cyanoloma* across the Mediterranean Iberian coast.

Materials and Methods

Herbaria screening and distribution survey

Looking for evidence of historical records of *D. cyanoloma* in Spain, we screened the BCN-Phyc herbarium (Centre de Documentació de Biodiversitat Vegetal, Universitat de Barcelona) and the MA herbarium (Real Jardín Botánico de Madrid - CSIC Herbarium), which hold the most important collection data of seaweeds from the Mediterranean coast of the Iberian Peninsula. Specifically, we surveyed all the material of *Dictyota* species (mainly *D. dichotoma* (Hudson) Lamouroux) that could have been misidentified as *D. cyanoloma*.

To characterise the current distribution of *D. cyanoloma* along the Mediterranean coasts of the Iberian Peninsula, and taking into account that virtually all its citations were from harbours, the most important fishing, leisure and commercial harbours throughout the area were visited. In total, 36 harbours were explored from Llançà (Girona) to Algeciras (Cádiz) mainly during winter and spring of 2013 and 2014 (Table 1). The presence or absence of *D. cyanoloma* in each harbour was assessed, collecting some specimens from those harbours in which the species was present. All samples were preserved in 4% formalin in seawater and / or pressed on herbarium sheets and deposited in the BCN-Phyc herbarium (Centre de Documentació de Biodiversitat Vegetal, Universitat de Barcelona, Spain).

Phenology and morphological-anatomical study

The type locality of *D. cyanoloma* (Palamós harbour) was selected to carry out the study. In this harbour, the seaweed grows on rocks in a shallow area (0.5 m depth) with low hydrodynamism. Monthly sampling was carried out from December 2012 to December 2013. Each month two samples were collected by scraping off all vegetation from two squares of 10 x 10 cm (100 cm^2) using a chisel and a hammer. For each specimen of *D. cyanoloma,* the thallus length, the number of dichotomies from the base to the apex and the width of the third dichotomy were measured as comparative morphological features. The reproductive stage was also assessed by recording the presence/absence of reproductive structures. For each quadrat, the total abundance of *D. cyanoloma* was quantified. The abundance of specimens of each reproductive type (sterile, fertile sporophytes and fertile male and female gametophytes) was also quantified measuring the cover of the horizontal surface in cm^2 after spreading the algal thalli in a laboratory tray (Ballesteros, 1986). For the anatomical study of the male specimens, transverse sections were made by hand with a razor blade. Photographs were taken with an AxioCam ERc 5s (Carl Zeiss) digital camera coupled with either a Nikon Eclipse 50i microscope or a Zeiss Stemi 2000-C stereomicroscope. Field photographs were taken with a Nikon Coolpix 4500.

Sampling for DNA extraction and marker selection

Specimens from nine localities (harbour environments) were sampled along the Mediterranean coast of the Iberian Peninsula (Table 1). From each sampling site, five to seven individuals were collected and preserved in silica gel.

Total genomic DNA was extracted following the CTAB method (Doyle & Doyle, 1987) with modifications (Soltis *et al.,* 1991; Cullins, 1992). Following preliminary polymorphism test of several nuclear, mitochondrial and chloroplast markers, the regions *cox*1, *nad*6-*nad*11 and *atp*9-*orf*11 of the mtDNA and the region *rbc*L-*rbc*S of the cpDNA were chosen for further sequencing of multiple individuals per sampling site (Table S1). Nucleotide sequencing was carried out at the Centres Científics i Tecnològics of the Universitat de Barcelona on an ABI PRISM 3700 DNA analyser (PE Biosystems, Foster City, CA, USA). The sequences were edited with Chromas LITE v2.01 (Technelysium Pty, Tewantin, Australia) and manually aligned in Bioedit (Hall, 1999). See Table S2 of the supporting information for Genbank accession numbers.

Analysis of haplotype variation and geographic inference

Molecular diversity indices, such as number of haplotypes (n), number of polymorphic sites (s), haplotype diversity (Hd) and nucleotide diversity (π) for each sampling site were calculated in DnaSP 4.1 (Librado & Rozas, 2009). Additionally, we constructed two haplotype networks - one for the mtDNA dataset and the other for cpDNA dataset - using the software TCS v. 1.21 (Clement *et al.,* 2000). The partitioning of the genetic diversity among and within *D. cyanoloma* localities was evaluated by molecular variance analysis (AMOVA) using ARLEQUIN v. 3.5 (Excoffier & Lischer, 2010). ARLEQUIN

Table 1. Province, localities, coordinates and date of visit of each sampled site of *Dictyota cyanoloma*. Harbours where *Dictyota cyanoloma* was found are indicated by (*). Populations studied from the point of view of genetic diversity are indicated by (§).

Code	Province / Harbour	Coordinates		Date of visit / sampling
	Girona			
LLA	Llançà (*)(§)	42°22’25.74”N,	3° 9’47.12”E	27/09/2013
POR	Port de la Selva (*)	42°20’29.34”N,	3°12’10.85”E	24/03/2013
ESC	L’Escala (*)	42° 7’8.67”N,	3° 8’46.72”E	25/03/2013
ROS	Roses (*)	42°15’15.04”N,	3°10’51.58”E	12/06/2013
EST	L’Estartit (*)(§)	42° 3’10.21”N,	3°12’18.63”E	25/03/2013
PAL	Palamós (*)(§)	41°50’43.62”N,	3° 7’39.39”E	19/12/2012, 16/01/2013, 15/02/2013, 25/03/2012,18/04/2013, 06/05/2013, 16/06/2013, 24/07/2013, 22/08/2013, 20/09/2013, 15/10/2013, 28/11/2013, 28/12/2013
STF	Sant Feliu de Guíxols (*)	41°46’48.60”N,	3° 1’57.46”E	16/01/2013
BLA	Blanes (*)	41°40’31.96”N,	2°47’57.55”E	15/02/2013
	Barcelona			
ADM	Arenys de Mar	41°34’42.48”N,	2°33’33.68”E	08/02/2013
MAT	Mataró (*)	41°31’43.47”N,	2°26’29.05”E	08/04/2013
PRE	Premià de Mar	41°29’21.82”N,	2°21’56.96”E	15/01/2013
ELM	El Masnou	41°28’35.66”N,	2°18’44.85”E	16/01/2013
BAD	Badalona	41°26’5.19”N,	2°14’28.73”E	16/01/2013
BAR	Barcelona (*)	41°21’47.14”N,	2°10’58.76”E	08/11/2008, 4/12/2008, 25/02/2009, 29/04/2009, 18/05/2009, 26/05/2009, 03/05/2014
GAR	Garraf	41°15’3.53”N,	1°53’58.14”E	25/02/2013
SIT	Sitges	41°14’2.90”N,	1°49’25.06”E	25/02/2013
	Tarragona			
TAR	Tarragona (*)	41° 6’13.09”N,	1°13’44.74”E	13/02/2013
LAM	L’Ametlla de Mar	40°52’49.13”N,	0°48’7.46”E	16/04/2014
LMP	L’Ampolla	40°48’38.65”N,	0°42’35.80”E	16/04/2014
SCR	Sant Carles de la Ràpita	40°37’1.69”N,	0°35’48.54”E	16/04/2014
	Castelló			
VIN	Vinaròs	40°27’50.20”N,	0°28’20.98”E	17/04/2014
PEÑ	Peñíscola	40°21’25.08”N,	0°24’20.63”E	17/04/2014
CAS	Castelló de la Plana (*)(§)	39°58’3.66”N,	0° 1’19.02”E	17/04/2014
	València			
SAG	Sagunt	39°38’50.22”N,	0°12’33.30”O	22/03/2013
VAL	València (*)	39°26’1.40”N,	0°18’47.17”O	21/03/2013
GAN	Gandia	38°59’50.43”N,	0° 9’23.62”O	23/03/2013
	Alicante			
DEN	Dénia	38°50’25.70”N,	0° 6’55.24”E	23/03/2013
ALI	Alicante	38°19’43.63”N,	0°29’58.47”O	24/03/2013
SPO	Santa Pola	38°11’23.78”N,	0°33’48.54”O	25/03/2013
TOR	Torrevieja	37°58’15.99”N,	0°40’56.04”O	25/03/2013
	Murcia			
CAR	Cartagena (*)(§)	37°35’4.91”N,	0°59’5.68”O	26/03/2013, 25/11/2013, 17/05/2014
	Almería			
ALM	Almería (*)(§)	36°49’50.35”N,	2°28’1.68”O	16/04/2013, 24/11/2013, 17/05/2014
MOT	Motril (*)(§)	36°43’26.00”N,	3°31’33.60”O	17/01/2013
	Málaga			
MAL	Málaga (*)	36°43’3.15”N,	4°24’47.92”O	17/04/2013, 23/11/2014
ETP	Estepona (*)(§)	36°24’53.71”N,	5° 9’34.83”O	16/05/2014
	Cádiz			
ALG	Algeciras (*)(§)	36° 7’2.34”N,	5°26’7.62”O	18/04/2013, 23/11/2013

was also used to perform a Mantel test in order to determine whether genetic distance was correlated with geographical distance between sampling sites. Mantel tests were performed between the pairwise fixation index (F_{ST}) matrix and the geographical distance matrix of *D. cyanoloma* locations, estimating the significance level by 100,000 permutations.

Results

Historical data and distribution survey

After surveying all the Spanish *Dictyota* specimens of the herbaria BCN-Phyc (336 sheets, from 1950 to date) and MA (30 sheets, since the early nineteenth century to date), we did not find any voucher which could correspond to a misidentified collection of *D. cyanoloma*. In the field surveys, the species was found in 19 of the 36 harbours visited along the entire Mediterranean coast of the Iberian Peninsula from the South (Algeciras, Cádiz) to the North (Llançà, Girona) and occurred in all important commercial harbours except in Alicante (Fig. 1 and Table 1). Individuals of *D. cyanoloma* were observed growing in infralittoral habitats with low hydrodynamism, on breakwaters or artificial structures, at 0.3-1 m deep, forming well-developed populations in some of the harbours (Algeciras, Almería, Motril, Tarragona, Palamós, L'Estartit) or only as few scattered individuals in the other harbours.

Vegetative and reproductive phenology

Specimens of *D. cyanoloma* were observed throughout the year. In summer (July, August and September) the individuals had the lowest mean value in thallus length, the minimum observed in August (0.9 ± 0.33 cm). The maximum thallus length was found in winter and early spring (January, February and March), February being the month with the longest thallus length (7.64 ± 2.03 cm). The width of the third dichotomy, as well as the number of dichotomies from the base to the top, displayed the same tendency: maximum values in January, February and March, February being the month with the widest values (6.7 ± 0.9 mm) and maximum number of dichotomies (6.11 ± 1.34). The minimum values were found in summer, August being the month with the lowest values for width of dichotomies (1.32 ± 0.48 mm) and September the month with the lowest values in number of dichotomies (1.89 ± 1.02) (Fig. 2). The total cover of the 10x10 cm quadrats follows the same tendency, maximum cover values in winter (February 256 cm^2) and minimum values in summer (August 10 cm^2) (Fig. 3).

Sterile individuals were found throughout the year except in February, March and April. Sporophytes were found almost all year through excluding July and August. Male gametophytes were observed only in December and February, and female individuals in February and June. Fertile thalli were more abundant than non-fertile thalli from January to June (64-100%) whereas the non-fertile thalli were dominant from July to December (65-100%). In July and August all specimens were sterile.

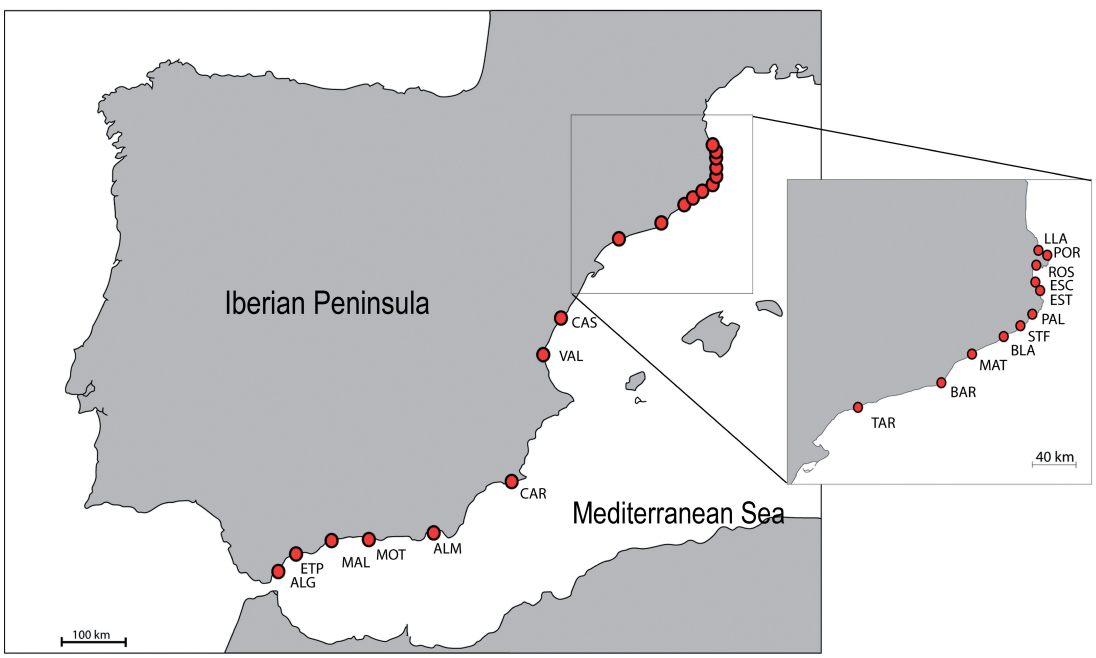

Fig. 1: Distribution map of *Dictyota cyanoloma* on the Mediterranean coast of the Iberian Peninsula. (ALG) Algeciras, (ETP) Estepona, (MAL) Málaga, (MOT) Motril, (ALM) Almería, (CAR) Cartagena, (VAL) València, (CAS) Castelló de la Plana, (TAR) Tarragona, (BAR) Barcelona, (MAT) Mataró, (BLA) Blanes, (STF) Sant Feliu de Guíxols, (PAL) Palamós, (EST) L' Estartit, (ESC) L'Escala, (ROS) Roses, (POR) Port de la Selva, (LLA) Llançà.

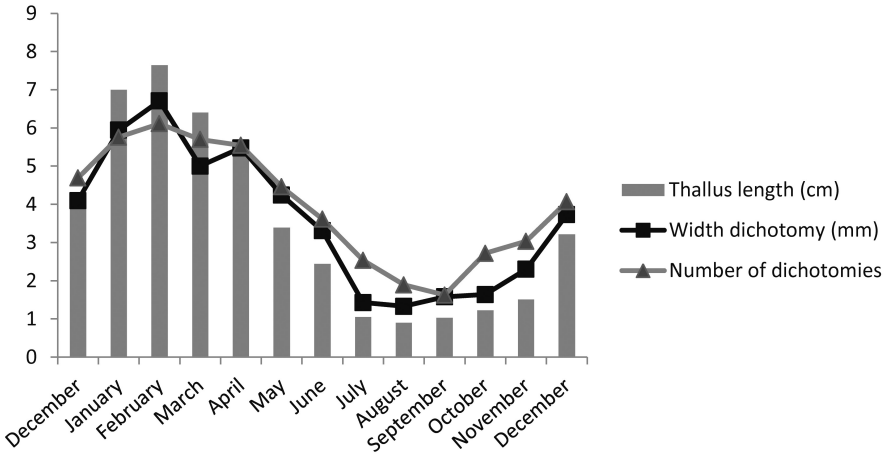

Fig. 2: Average of thallus length (grey colour), width of the third dichotomy (black line) and number of dichotomies (grey line) of *Dictyota cyanoloma* per month.

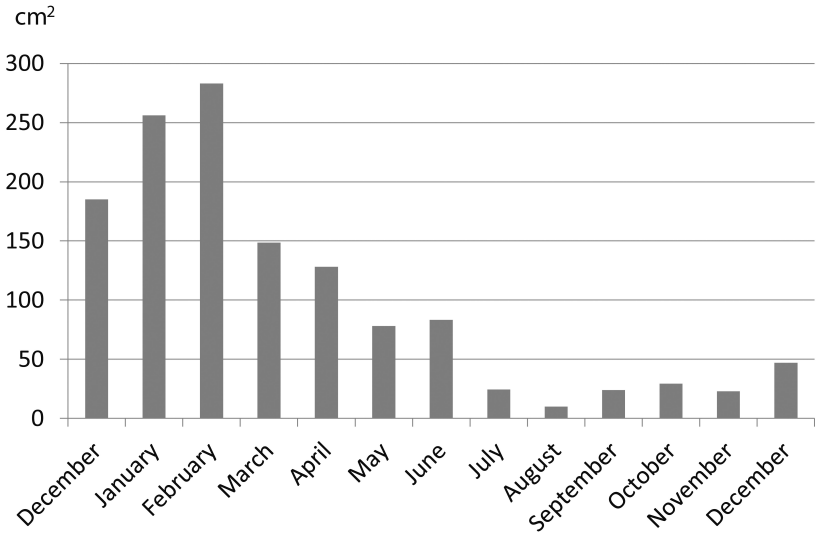

Fig. 3: Total cover of *Dictyota cyanoloma* per month.

Sporophytes were found in 10 of the 12 months, being dominant from January until June (45-100%). Dominance of sporophytes over gametophytes and sterile individuals reached a maximum in March and April, all individuals being sporophytes. The percentage of gametophytes (Fig. 4) never reached more than 27%, February being the month with the maximum percentage of male individuals (12.3%) and female individuals (14.8%).

Antheridia were grouped in ellipsoidal sori on both sides of the frond, leaving a sterile zone near the margin (Fig. 5). The length of the sori was (220) 300-640 (901) µm and (100) 140-320 (360) µm wide, with (36) 60-180 (247) antheridia per sorus. Sori were surrounded by 3-4 (5) layers of unicellular paraphyses and covered by an indusium. The antheridia were (31) 37-70 (78) µm high and (10) 14-25 (29) µm wide. Each antheridium had one stalk cell.

Haplotype diversity

The mitochondrial *cox*1, *nad*6-*nad*11, *atp*9-*orf*11 and the chloroplast *rbc*L-*rbc*S DNA regions were sequenced for 49 individuals of *D. cyanoloma* from nine localities along the Mediterranean coast of the Iberian Peninsula, obtaining two alignments of 1848 bp (mtDNA) and 495 bp (cpDNA). Sequence comparisons revealed nine polymorphic sites for mtDNA regions and one for the cpDNA region, defining three and two haplotypes respectively. The mitochondrial *cox*1 region revealed one nucleotide polymorphism, *nad*6-*nad*11 region revealed two nucleotide polymorphisms and two insertions/deletions, *atp*9-*orf*11 showed four nucleotide polymorphisms and finally, the plastid *rbc*L-*rbc*S region revealed a single nucleotide polymorphism. Within the mitochondrial dataset, haplotype mt1 occurred in five localities; haplotype mt2 occurred in five others; and haplotype mt3 was found only in two sampling sites (Fig. 6A; Table 2). The frequency of the three mitochondrial haplotypes is not very different among them: mt1 is the most frequent (41%), followed by mt2 (39%) and mt3 (20%). The plastid dataset revealed only two haplotypes (Fig. 6B): cp1 being more abundant (61%) and occurring

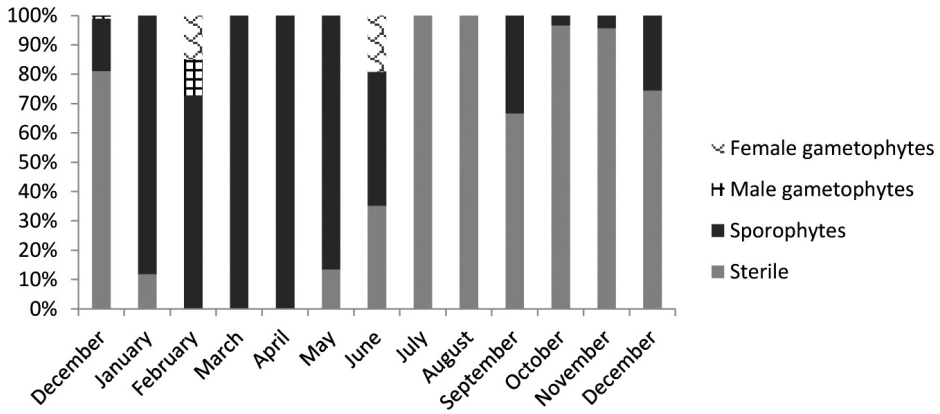

Fig. 4: Percentage of female gametophytes (patron), male gametophytes (squares), sporophytes (black colour) and sterile individuals (grey colour) of *Dictyota cyanoloma* per month.

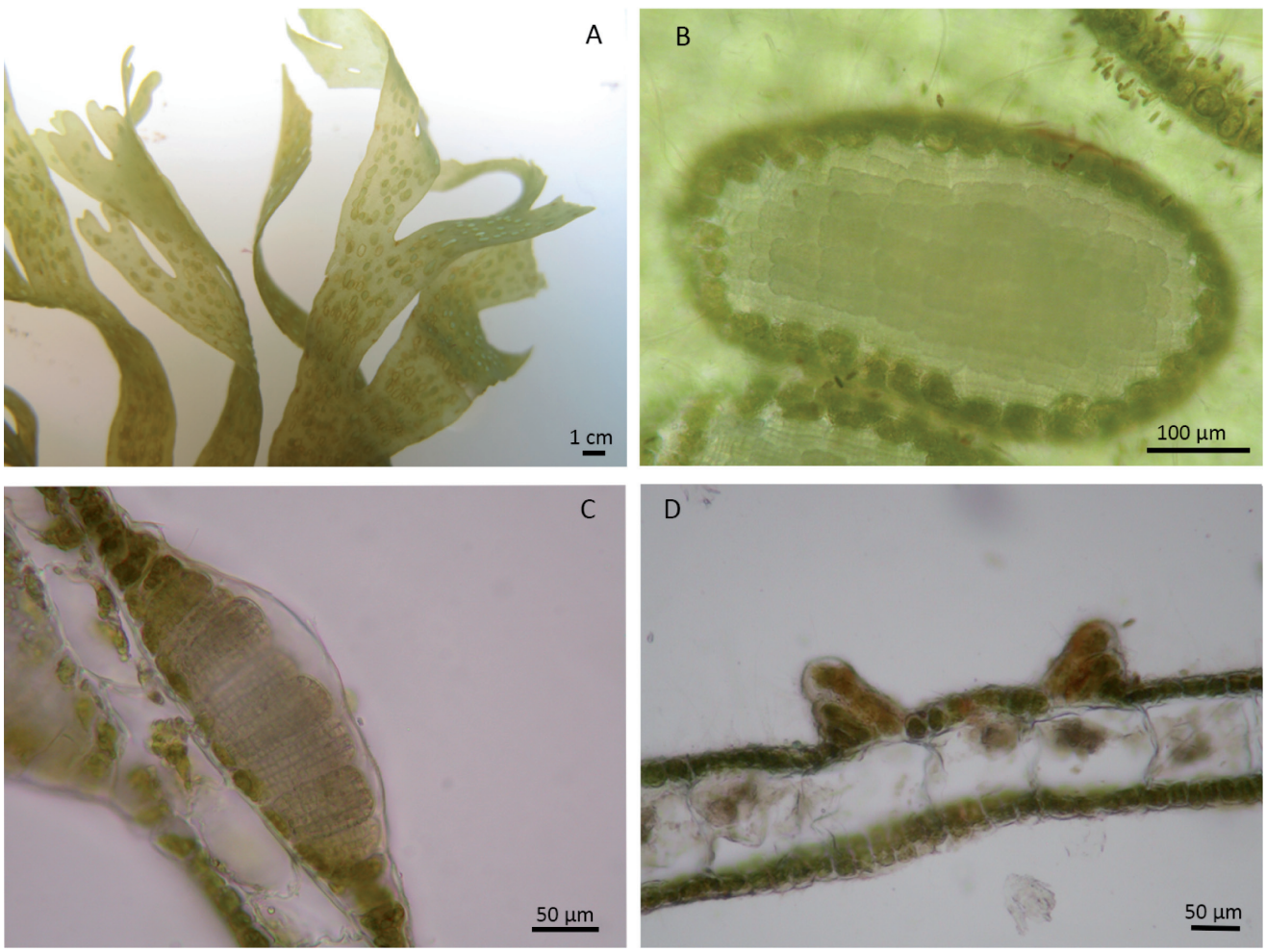

Fig. 5: *Dictyota cyanoloma*. A) Antheridia distribution; B) Surface view of a male sorus; C) Section of a male sorus with paraphyses; D) An empty male sorus showing only the paraphyses.

in seven localities; cp2 revealed as less abundant (39%), being found in five sampling sites.

Genbank accession numbers of the haplotype and nucleotide diversities of the nine *D. cyanoloma* locations for both plastid and mitochondrial datasets are shown in Table 2. Six sampling sites (Llançà, L'Estartit, Palamós, Castelló de la Plana, Cartagena and Almería) failed to reveal any haplotype diversity, having one fixed haplotype for both regions (mtDNA and cpDNA). In contrast, two haplotypes were found in both regions (mtDNA and cpDNA) for the other three sampling sites (Motril, Estepona & Algeciras). Haplotype diversity within localities varied from Hs=0

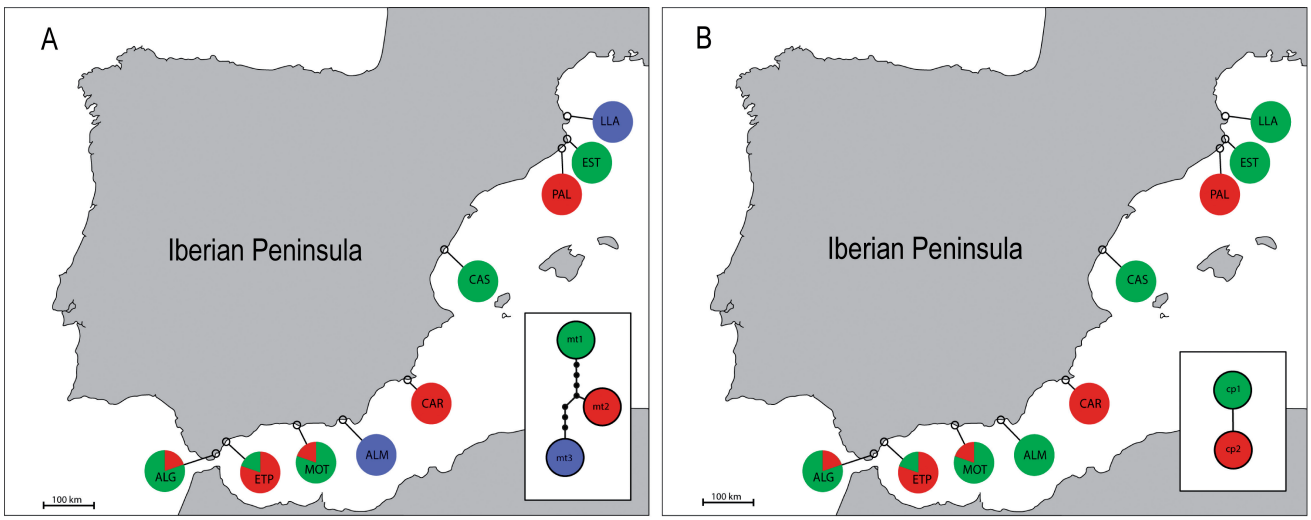

Fig. 6: Distribution of the haplotypes of *D. cyanoloma* sampled from the Mediterranean coast of the Iberian Peninsula, and the respective haplotype composition (pie charts) per sampling site. The different colours in the pie charts represent different haplotypes. LLA, Llançà; EST, L'Estartit; PAL, Palamós; CAS, Castelló de la Plana; CAR, Cartagena, ALM, Almería, MOT, Motril; ETP, Estepona; ALG, Algeciras. A) Mitochondrial haplotypes. B) Chloroplast haplotypes.

to Hs=0.4 (mean Hs=0.1259) for both mitochondrial and chloroplast regions. Overall haplotype diversity for mtDNA is Hd= 0.655 and Hd=0.485 for cpDNA. The AMOVA analyses grouping sequences by sampling sites are shown in Table 3. On average, 76.81% of the total variation was attributable to differentiation among sampling sites for cpDNA and 85.23% for mtDNA region, while the rest corresponds to differences within sampling sites (23.19% for cpDNA and 14.77% for mtDNA). The Mantel test showed that geographical and genetic distances between localities were not correlated (r=0.1104, *P*=0.733 for cpDNA and r=0.168, *P*=0.878 for mtDNA).

Table 2. Diversity measures for populations of *Dictyota cyanoloma*. Number of individuals (n) and haplotypes (h), values of haplotype diversity (Hd) and nucleotide diversity (π) for each location sampled.

	Mitochondrial				Chloroplast			
	n	h	Hd	π	n	h	Hd	π
Llançà	6	mt3	0	0	6	cp1	0	0
L'Estartit	5	mt1	0	0	5	cp1	0	0
Palamós	7	mt2	0	0	7	cp2	0	0
Castelló de la Plana	5	mt1	0	0	5	cp1	0	0
Cartagena	6	mt2	0	0	6	cp2	0	0
Almería	4	mt3	0	0	4	cp1	0	0
Motril	5	mt1/mt2	0.3333	0.00072	5	cp1/cp2	0.3333	0.00067
Estepona	5	mt1/mt2	0.4	0.00086	5	cp1/cp2	0.4	0.00081
Algeciras	5	mt1/mt2	0.4	0.00086	5	cp1/cp2	0.4	0.00081
Total	49	3	0.655	0.0016	49	2	0.485	0.00098

Table 3. AMOVA results grouped by sampling sites according to the two regions (mitochondrial and chloroplast).

	Source of variation	d.f.	Sum of squares	Variance components	Percentage of variation	F_{ST}	P
Mitochondrial	Among populations	8	78.650	1.755	85.23		<0.0001
	Within populations	40	12.167	0.304	14.77		<0.0001
	Total	48	90.816	2.059		0.852	
Chloroplast	Among populations	8	9.313	0.199	76.81		<0.0001
	Within populations	41	2.467	0.060	23.19		<0.0001
	Total	49	11.780	0.259		0.768	

Discussion

Distribution

After surveying all material assigned to different *Dictyota* species from BCN-Phyc and MA herbaria, we were not able to observe any misidentified historical collection of *Dictyota cyanoloma* prior to the first specimens collected at Palamós in 1987 (Rull Lluch *et al*., 2007) and preserved at BCN-Phyc herbarium. Moreover, even though it is not usual to sample in harbours, some previous studies on benthic communities along the Mediterranean coast of the Iberian Peninsula had included harbour habitats (Ballesteros *et al*., 1984; Ballesteros, 1989) but in no case had the authors referred to an unidentified *Dictyota* species. In 1988, an extensive study on the algal communities from the harbour of Blanes – where *D. cyanoloma* has currently been found (Table 1) – did not report the occurrence of any *Dictyota* species (Rodríguez-Prieto *et al.,* 1997). These results contrast with the data from the Croatian coast, where some herbarium records (since 1935) of *D. cyanoloma* have been found (Steen *et al.,* 2016) suggesting that the colonization of the Spanish coast could have occurred later. Alternatively, the presence of this species along the Mediterranean coast of the Iberian Peninsula could have been overlooked since older times, not expanding its range or frequency until recently.

Dictyota cyanoloma currently shows a wide distribution along the Iberian Mediterranean coast, being present in more than half of the surveyed harbours from the French border to Gibraltar. Certainly, some environmental characteristics of harbours such as water and sediment pollution, structures providing artificial substrates, altered temperature regime and marine traffic have been associated with increased introduction incidence (Carlton, 1996; Glasby *et al.,* 2007; Schaffelke & Hewitt, 2007). However, despite that the first records of *D. cyanoloma* in the Mediterranean Sea corresponded to anthropogenic environments, the species has also been recently reported in more natural habitats (Steen *et al.,* 2016). The occurrence of this seaweed outside harbour environments on the Iberian Mediterranean coast needs to be studied more thoroughly.

Phenology

It is assumed that sexual reproduction may enhance the success of exotic species, since recombination of genetic material allows more rapid adaptive evolution (Sakai *et al*., 2001; Lambrinos, 2004). The presence of all life stages of *D. cyanoloma* in the Mediterranean region implies that the species can reproduce sexually outside its native range. The Palamós population persists throughout the year, and shows a clear annual vegetative and reproductive cycle. In summer the population's cover is sparse; the individuals are small and sterile, with a reduced number of dichotomies. In autumn the thallus length starts to increase, the individuals are taller and mainly sterile, but a few fertile sporophytes also occur in this period of the year. In winter the population shows its greatest development; the specimens reach their maximum size and their greatest number of dichotomies; the population's cover also reaches its higher values. In this period almost all specimens are fertile sporophytes, although some fertile male and female gametophytes also occur in February. Through spring, the average size of the specimens, mainly fertile sporophytes, progressively decreases until disappearing at the beginning of summer, when new young individuals start to appear. Therefore, in Palamós, *D. cyanoloma* occurs all year round, but it is particularly abundant and fertile during the winter-spring period.

No previous data exist about the phenology of *D. cyanoloma*. Concerning other *Dictyota* species, most phenological studies refer to the seasonal variation. In this respect, the phenology of Iberian *D. cyanoloma* is similar to other *Dictyota* species occurring in the same geographical area, such as *D. dichotoma, Dictyota spiralis* Montagne and *Dictyota fasciola* J.Agardh. All these species occur throughout the year and they are always fertile, except *D. fasciola* which in autumn is exclusively sterile (Polo, 1978; Ribera Siguan, 1978; Rodríguez-Prieto & Polo, 1986). The seasonal phenology of *D. cyanoloma* – with a maximum development in winter and a minimum in summer – also matches other *Dictyota* species occurring in Valencian and Basque Countries (Barceló, 1987; Casares, 1987), even though no gametophytes were found in these areas. In Málaga (Conde, 1981) and Argentina (Gauna *et al*., 2013), *D. dichotoma* occurs throughout the year, while it is lacking in October in the Canary Islands (Tronholm *et al.,* 2008) and from April to August in Mallorca (Gómez Garreta, 1982). Regarding the period where *Dictyota* species reach their greatest size, in Mallorca the tallest specimens of both *D. fasciola* and *D. spiralis* occur in spring, and in the Canary Islands the tallest *D. dichotoma* occurs in early summer, unlike *D. cyanoloma,* which has its maximum development in winter. In the Palamós population of *D. cyanoloma* both maximum abundance and tallest specimens occur during the same period of the year (winter). This also happens in *D. dichotoma* from Argentina (Gauna *et al*., 2013), although in this area the higher values occur in summer. In contrast, in the *D. dichotoma* population from the Canary Islands, maximum abundance and thallus length do not coincide at the same time (Tronholm *et al*., 2008).

In our study, the proportion between fertile and sterile specimens of *D. cyanoloma* follows the same pattern of temporal variation observed in cover and size of specimens. In summer and autumn sterile specimens dominate over fertile specimens and in winter and spring fertile individuals dominate over sterile individuals. Sporophytes always dominate over gametophytes when the latter exist. Very few studies take into account the temporal variation of the different life-cycle generations proportion, as

well as the fertile and sterile specimen proportion in the genus *Dictyota*. In *D. dichotoma* from the Canary Islands and Argentina, unlike *D. cyanoloma*, the fertile specimens dominate over sterile ones throughout the year. However, while in the Canary Islands the sporophytes always dominate over the gametophytes, in Argentina gametophytes are the dominant life stage in all months of the year (Tronholm *et al*., 2008; Gauna *et al*., 2013).

Our phenological observations suggest that *D. cyanoloma* on the Mediterranean coast of the Iberian Peninsula may benefit from increased growth rates during winter, when native species of *Dictyota* sharing similar niches [e.g. *D. dichotoma* var. *intricata* (C.Agardh) Greville] show lower abundance and a smaller size (Benedetti-Cecchi & Cinelli, 1993). Similarly, the phenology of *D. fasciola* and *D. spiralis* in Mallorca (Gómez Garreta, 1982) and that of *D. dichotoma* in the Canary Islands (Tronholm *et al.,* 2008) could support this idea. This hypothesis should be further tested by studying the community ecology of the habitat (harbour platforms) over the whole year in this geographical area.

Haplotype diversity

Our indicative results revealed noticeable molecular polymorphism within and among sampling sites of *D. cyanoloma* from the Mediterranean Iberian Peninsula coast. Interestingly, mtDNA regions *nad*6-*nad*11 and *atp*9-*orf*11 as adapted from Steen *et al.* (2016) proved four times more variable than *cox*1, a typical barcoding marker between seaweed species (e.g. Leliaert *et al*., 2014 and references therein). The plastid RubisCo spacer (*rbc*L-*rbc*S) has been already used in several phylogenetic studies (e.g. Siemer *et al*., 1998; Yoon *et al.,* 2001; Lee *et al*., 2011), showing significant variability at the interspecific level. However, this marker is significantly less variable than *cox*1 at intra-specific level among different red seaweed species (Robba *et al*., 2006). In *D. cyanoloma*, we have observed the same amount of variability in *rbc*L-*rbc*S spacer as in *cox*1. The results confirm the potential usefulness of these mitochondrial and plastid regions at the intra-specific level in brown algae as previously suggested by Engel *et al.,* (2008).

The five haplotypes observed for both mitochondrial and chloroplast markers suggest that several thalli or propagules (either in multiple colonization events or in a single one carrying several different genotypes) have been responsible for the introduction of this species along the Mediterranean coast of the Iberian Peninsula. The distributional pattern of the haplotypes show that six of the nine studied sampling sites of *D. cyanoloma* present only one haplotype per population and only three localities (Algeciras, Estepona and Motril) show two haplotypes. In accordance with this low haplotype diversity within the sampling sites (mean Hs=0.1259), AMOVA results show that *D. cyanoloma* from the Mediterranean Iberian Peninsula coast presents significantly more genetic differentiation among sampling sites than within sampling sites. Additionally, spatial autocorrelation analysis indicates that the haplotypic structure of *D. cyanoloma* in this region does not correlate to geography. Being particularly evident in the northern distribution area, nearby localities do not share the same haplotype but a different one according to both mitochondrial and plastid DNA markers (Fig. 6). This pattern of genetic structure also suggests that geographic spread did not occur through a smoothly expanding range with simple advancing wave fronts [the "wave of advance" model (Fisher, 1937)], but by hopscotch jumps with long-distance dispersal typical of human transport (Lawson Handley *et al*., 2011). Moreover, the three sampling sites that show a greater number of haplotypes are the southern ones, located near to the Strait of Gibraltar, a place of intensive maritime traffic, whereas the northern ones are more uniform.

Considering our limited sampling data, these evidences could reflect the role of marine navigation as a dispersal vector of this species in the area. In this context, extending this fine-scale sampling to include more samples per locality, over longer time periods and also expanding the study to include the entire reported range of *D. cyanoloma,* would provide more information to clarify the colonization process of the species.

Acknowledgements

Financial support was provided by Fundación Biodiversidad and the Catalan government (project 2014SGR514). We thank all the harbour authorities for sampling permissions. We also acknowledge Samuel Pyke for considerably improving the English of the text.

References

Ballesteros, E., 1986. Métodos de análisis estructural en comunidades naturales, en particular del fitobentos. *Oecologia Aquatica*, 8, 117-131.

Ballesteros, E., 1989. Els fons marins de la costa. Barcelona. *Metròpolis Mediterrània*, 12, 87-92.

Ballesteros, E., Pérez, M., Zabala, M., 1984. Aproximación al conocimiento de las comunidades algales de la zona infralitoral superior en la costa catalana. *Collectanea Botanica*, 15, 69-100.

Bárbara, I., Peteiro, C., Peña, V., Altamirano, M., Piñeiro-Corbeira, C. *et al.,* 2014. Adiciones florísticas y aportaciones corológicas para la flora bentónica marina del Atlántico ibérico. *Acta Botanica Malacitana*, 39, 207-237.

Barceló, M.C., 1987. *Estudi de les algues marines del País Valencià*. PhD Thesis. University of Barcelona.

Benedetti-Cecchi, L., Cinelli, F., 1993. Seasonality and reproductive phenology of algae inhabiting littoral pools in the western Mediterranean. *Marine Ecology*, 14, 147-157.

Boudouresque, C., Verlaque, M., 2002. Biological pollution in the Mediterranean Sea: invasive versus introduced macrophytes. *Marine Pollution Bulletin*, 44, 32-38.

Carlton, J.T., 1996. Biological invasions and cryptogenic species. *Ecology*, 77, 1653-1655.

Casares, C., 1987. *Estudio de la Flora Bentónica Marina de la Costa de Guipúzcoa*. PhD Thesis. University of Barcelona.

Clement, M., Posada, D., Crandall, K., 2000. TCS: a computer program to estimate gene genealogies. *Molecular Ecology*, 9, 1657-1659.

Coll, M., Piroddi, C., Albouy, C., Ben Rais Lasram, F., Cheung, W.W.L. *et al.,* 2012. The Mediterranean Sea under siege: spatial overlap between marine biodiversity, cumulative threats and marine reserves. *Global Ecology and Biogeography*, 21, 465-480.

Conde, F., 1981. *Estudio sobre las algas bentónicas del litoral de la provincia de Málaga*. PhD Thesis. University of Málaga.

Cormaci, M., Furnari, F., Catra, M., Alongi, G., Giaccone, G., 2012. Flora marina bentonica del Mediterraneo: Phaeophyceae. *Bollettino dell'Accademia Gioenia di Scienze Naturali di Catania*, 45, 1-508.

Cullins, K.W., 1992. Design and testing of a plant-specific primer for ecological and evolutionary studies. *Molecular Ecology*, 1, 233-240.

Doyle, J.L., Doyle, J.J., 1987. A rapid DNA isolation procedure for small quantities of fresh leaf tissue. *Phytochemical Bulletin*, 19, 11-15.

Engel, C.R., Billard, E., Voisin, M., Viard, F., 2008. Conservation and polymorphism of mitochondrial intergenic sequences in brown algae (Phaeophyceae). *European Journal of Phycology*, 43, 195-205.

Excoffier, L., Lischer. H., 2010. Arlequin suite ver 3.5: a new series of programs to perform population genetics analyses under Linux and Windows. *Molecular Ecology Resources*, 10, 564-567.

Fisher, R.A., 1937. The wave of advance of advantageous genes. *Annals of Human Genetics*, 7, 355-369.

Galil, B.S., 2009. Taking stock: inventory of alien species in the Mediterranean Sea. *Biological Invasions,* 11(2), 359-372.

Gauna, M.C., Caceres, E.J., Parodi, E.R., 2013. Temporal variations of vegetative features, sex ratios and reproductive phenology in a *Dictyota dichotoma* (Dictyotales, Phaeophyceae) population of Argentina. *Helgoland Marine Research*, 67, 721-732.

Glasby, T.M., Connell, S.D., Holloway, M.G., Hewitt, C.L., 2007. Nonindigenous biota on artificial structures: could habitat creation facilitate biological invasions? *Marine Biology*, 151(3), 887-895.

Guiry, M.D., Guiry, G.M., 2016. AlgaeBase. World-wide electronic publication, National University of Ireland, Galway. http://www.algaebase.org; searched on 11 May 2016.

Gómez Garreta, A., 1982. *Estudio fenológico de la vegetación marina de la isla de Mallorca*. PhD Thesis. University Complutense of Madrid.

Hall, T.A., 1999. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symposium Series*, 41, 95-98.

Hoffman, R., 2014. Alien benthic algae and seagrasses in the Mediterranean Sea and their connection to global warming. p. 159-181. In: *The Mediterranean Sea*. Goffredo S., Dubinski Z. (Eds.). Springer, Dordrecht.

Lambrinos, J.G., 2004. How interactions between ecology and evolution influence contemporary invasion dynamics. *Ecology*, 85, 2061-2070.

Lamouroux, J.V.F., 1809. Exposition des caractères du genre *Dictyota*, et tableau des espèces qu'il renferme. *Journal de Botanique (Desvaux),* 2, 38-44.

Lawson Handley, L.-J., Estoup, A., Evans, D. M., Thomas, C. E., Lombaert, E. *et al.,* 2011, Ecological genetics of invasive alien species. *BioControl*, 56, 409-428.

Lee, S.R., Oak, J.H., Keum, Y.S., Lee, J., Chung, I.K., 2011. Utility of rbcS gene as a novel target DNA region for brown algal molecular systematics. *Phycological research*, 59 (1), 34-41.

Leliaert, F., Verbruggen, H., Vanormelingen, P., Steen, F., Lopez-Bautista, J. M., Zuccarello, G. C. & De Clerck, O., 2014. DNA- based species delimitation in algae. *European Journal of Phycology*, 49, 179-96.

Librado, P., Rozas, J., 2009. DnaSP v5: a software for comprehensive analysis of DNA polymorphism data. *Bioinformatics,* 11, 1451-1452.

Mineur, F., Arenas, F., Assis, J., Davies, A. J., Engelen, A. H. *et al.,* 2015. European seaweeds under pressure: Consequences for communities and ecosystem functioning. *Journal of Sea Research*, 98, 91-108.

Polo, L., 1978. *Estudio sobre las algas bentónicas de la Costa Catalana*. PhD Thesis. Autonomous University of Barcelona.

Ribera Siguan, M.A., 1978. *Aportación al estudio sistemático y fenológico de las algas bentónicas de la Costa Brava Catalana*. Master Thesis. University of Barcelona.

Ribera Siguan, M.A., 2003. Pathways of biological invasions of marine plants. P. 183–226. In: *Invasive species vectors and management strategies.* Ruiz G.M., Carlton J.T. (Eds.). Island Press, Washington, DC.

Robba, L., Russell, S.J., Barker, G.L., Brodie, J., 2006. Assessing the use of the mitochondrial cox1 marker for use in DNA barcoding of red algae (Rhodophyta). *American Journal of Botany*, 93, 1101-1108.

Rodríguez-Prieto, C., Sala, E., Clavell, A., Polo, L., 1997. Composición y estructura de las comunidades de algas bentónicas de ambientes portuarios: El puerto de Blanes. *Collectanea Botanica*, 23, 29-40.

Rodríguez-Prieto, C., Polo, L. 1986. Fenologia i distribució de les algues del litoral Català. *Scientia*, 12, 67-86.

Roman, J., Darling, J.A., 2007. Paradox lost: genetic diversity and the success of aquatic invasions. *Trends in Ecology & Evolution*, 9, 454-464.

Rull Lluch, J., Ballesteros, E., Barceló, M.C., Gómez Garreta, A., Ribera Siguan, M.A., 2007. *Dictyota ciliolata* Sonder ex Kützing (Phaeophyceae, Dictyotales) in the Mediterranean Sea. *Cryptogamie, Algologie,* 28, 89-97.

Sakai, A.K., Allendorf, F.W., Holt, J.S., Lodge, D.M., Molofsky, J., *et al*., 2001. The population biology of invasive species. *Annual Review of Ecology, Evolution, and Systematics*, 32, 305-332.

Santolaria De Castro, A., 2014. *Dinámica del macrofitobentos marino de sustrato duro en un área portuaria en proceso de recuperación ambiental*. PhD Thesis. University of the Basque Country.

Schaffelke, B., Hewitt, C.L., 2007. Impacts of introduced seaweeds. *Botanica Marina*, 50(5/6), 397-417.

Siemer, B.L., Stam, W.T., Olsen, J.L., Pedersen, P.M., 1998. Phylogenetic relationships of the brown algal orders Ectocarpales, Chordariales, Dictyosiphonales, and Tilopteridales (Phaeophyceae) based on RUBISCO large subunit and spacer sequences. *Journal of Phycology*, 34(6), 1038-1048.

Soltis, D.E., Soltis, P.S., Collier, T.G., Edgerton, M.L., 1991. Chloroplast DNA variation within and among genera of the *Heuchera* group (Saxifragaceae): evidence for chloroplast transfer and paraphyly. *American Journal of Botany*, 78, 1091-1112.

Steen, F., Aragay, J., Zuljevic, A., Verbruggen, H., Bunker, F. *et al.,* 2016. Tracing the introduction history of the brown seaweed *Dictyota cyanoloma* (Phaeophyta, Dictyotales) in Europe. *European Journal of Phycology* (in press).

Streftaris N., Zenetos A., Papathanassiou, E., 2005. Globalisation in marine ecosystems: the story of non-indigenous marine species across European seas. *Oceanography and Marine Biology- an Annual Review*, 43, 419-453.

Taskin, E., 2013. New records of three dictyotalean brown algae for Turkey. *Botanica Marina*, 56, 299-302.

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

Tronholm, A., Steen, F., Tyberghein, L., Leliaert, F., Verbruggen, H. *et al.,* 2010. Species delimitation, taxonomy, and biogeography of *Dictyota* in Europe (Dictyotales, Phaeophyceae). *Journal of Phycology*, 46, 1301-1321.

Verlaque, M., Ruitton, S., Mineur, F., Boudouresque, C.-F., 2015. *CIESM atlas of exotic species of the Mediterranean. Macrophytes.* CIESM Publishers, Monaco, 362 pp.

Yoon, H.S., Lee, J.Y., Boo, S.M., Bhattacharya, D., 2001. Phylogeny of Alariaceae, Laminariaceae, and Lessoniaceae (Phaeophyceae) based on plastid-encoded RuBisCo spacer and nuclear-encoded ITS sequence comparisons. *Molecular Phylogenetics and Evolution*, 21(2), 231-243.

Zenetos, A., Cinar, M., Pancuci-Papadopoulou, M.A., Harmelin, J.G., Furnari, G. *et al.,* 2006. Annotated list of marine alien species in the Mediterranean with records of the worst invasive species. *Mediterranean Marine Science,* 6, 63-118

Zenetos, A., Gofas, S., Verlaque, M., Cinar, M., Garcia, R. *et al.,* 2010. Alien species in the Mediterranean Sea. A contribution to the application of European Union's Marine Strategy Framework Directive (MSFD). Part I. Spatial distribution. *Mediterranean Marine Science*, 11, 381-493.