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## Tracing the introduction history of the brown seaweed *Dictyota cyanoloma* (Phaeophyceae, Dictyotales) in Europe

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### ABSTRACT

*Dictyota cyanoloma* has recently been described from the Mediterranean Sea and Macaronesia but doubt had arisen as to whether this species was truly native in Europe. The species is mainly found on non-natural substrata (harbour walls, marinas, boat hulls, etc.), strongly suggesting that it is an introduction. Molecular sequence information from historical herbarium samples proves the presence of *D. cyanoloma* in the Adriatic Sea as early as 1935. Since approximately the year 2000, however, the number of records as well as the geographic range of the species has expanded significantly. The present-day distribution of *D. cyanoloma* occupies most of the Mediterranean Sea, Macaronesia, NW Africa and southern Portugal, but recent records from Galicia and SW England (Falmouth, Cornwall) indicate that the species is rapidly expanding northward. Collections from Australia demonstrated that the species is also present from Perth in Western Australia, over much of the southern Australian coastline up to Minnie Water in New South Wales. Phylogenetic analyses resolve *D. cyanoloma* in a sister clade to a previously unreported Australian *Dictyota* species. Analysis of genetic diversity of the mitochondrial markers (*nad6-nad11* and *atp9-orf11*) reveals that even though Australian populations contain a much higher haplotype richness, European populations are also fairly diverse. Furthermore, only two out of 25 haplotypes are shared between both regions. These somewhat counterintuitive results could be indicative of a more complicated introduction history.

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**KEYWORDS** Alien species; cryptogenic species; *Dictyota cyanoloma*; herbarium species; mitochondrial DNA

### Introduction

Invasive species are widely recognized as a major threat to marine biodiversity. By changing the relative abundance of native species, invasive species may profoundly affect the structure and functioning of native communities, thereby often negatively impacting local economies (Molnar *et al.*, 2008; Simberloff, 2011). Even though only a minority of introduced species can be considered invasive, their impact can be devastating. Hence there is the necessity to document introductions of non-native species and to assess the possible risk they impose on the invaded ecosystem (Molnar *et al.*, 2008). The Mediterranean Sea and the Atlantic coasts of Europe are considered hotspots of marine species introductions (Galil *et al.*, 2014; Mineur *et al.*, 2015). A total of 986 alien species have been reported for the Mediterranean Sea, of which 128 are macroalgae (Zenetos *et al.*, 2012). This number, however, is likely to be an underestimation of the total number of introduced species owing to the difficulties surrounding morphology-based algal identification (Leliaert *et al.*, 2014; Verbruggen, 2014). A combination of a limited set

of morphological, and potentially diagnostic characters (the ‘low morphology problem’; Van Oppen *et al.*, 1996) and considerable morphological plasticity that characterizes many algal species, hampers species identification based on morphological characteristics in algae. As a result, distinguishing native from non-native species becomes difficult (Grosholz, 2002). This taxonomic uncertainty also influences an understanding of the biogeography of the species, which is often insufficiently characterized. In a context of introduced species, uncertainty regarding native and introduced distributions led Carlton (1996) to coin the term ‘cryptogenic species’, i.e. species that are not demonstrably native or introduced.

Here we aim to ascertain the origins of *D. cyanoloma*, which was recently described from the Mediterranean Sea in Tronholm *et al.* (2010). The species was first reported from the Iberian Peninsula as *D. ciliolata* by Rull Lluç *et al.* (2007). Sequence data, however, demonstrated unequivocally that the Iberian specimens were clearly distinct from *D. ciliolata* (Tronholm *et al.*, 2010). Because the species did not correspond to the type material of any *Dictyota* of

Mediterranean or Macaronesian origin and in the absence of evidence for it to be present outside of Europe, *D. cyanoloma* was described as a new species native to Europe. This study also significantly expanded the known distribution range of the species to include the Adriatic Sea as well as Macaronesia (Tronholm *et al.*, 2010). Nevertheless, some aspects relating to the ecology and morphology of the species cast doubt on its status as a native species. First, historical interest in the diversity of *Dictyota* in Europe (e.g. Hamel, 1931–1939; Weberpeukert, 1986; Schnetter *et al.*, 1987; Hornig & Schnetter, 1988; Hornig *et al.*, 1992a, 1992b), and the fact that the species is easily discernible by its blue iridescent margins, raises the question why *D. cyanoloma* had gone unnoticed for so long. Second, the specimens of *D. cyanoloma* are often collected from non-natural habitats such as marinas (Rull Lluh *et al.*, 2007; Aragay Soler *et al.*, 2014). Last, a recent sampling campaign recovered what appears to be the same species from the south of Australia (De Clerck and Verbruggen, unpublished). The combination of these factors challenges the status of *D. cyanoloma* as an indigenous European species. However, to date it remains unknown whether *D. cyanoloma* is native to Europe and introduced to Australia, or vice versa.

The main objective of this study was to provide molecular support for the origins of this cryptogenic species. Therefore, we first applied a phylogenetic approach to determine the affinities of *D. cyanoloma* with Australian species of the genus. We then compared the diversity and genetic structure of Australian and European populations based on two variable mitochondrial loci (*nad6–nad11* and *atp9–orf11*). Last, the current and historical geographic range of the species within Europe was assessed employing both historical and recent records.

## Materials and methods

### DNA extraction, amplification and sequencing

*Dictyota cyanoloma* Tronholm, De Clerck, Gómez Garreta & Rull Lluh was sampled from Australia, the North-east Atlantic, the Mediterranean and Macaronesia (Table 1). Specimens were taken fresh from the field and apical tips were dried in silica gel for rapid dehydration and storage. In addition, the Ghent and the Zagreb herbaria were screened for *D. cyanoloma* vouchers and subsampled for DNA extraction. DNA was extracted employing a cetyltrimethyl ammonium bromide extraction method as outlined in Steen *et al.* (2015). We amplified and sequenced two variable intergenic spacer regions of the mitochondrial genome, the *nad6–nad11* and *atp9–orf11* regions (Table 2). Primers were designed in the flanking coding region of both markers, using

PRIMER3 software (Untergasser *et al.*, 2012). Primer design was aided by the availability of the mitochondrial genome of *D. dichotoma* (Secq *et al.*, 2006) and a draft mitochondrial genome of *D. cyanoloma* (De Clerck & Steen, unpublished). Primer sequences are displayed in Table 2. Plastid-encoded PSII reaction centre protein D1 (*psbA*) and RUBISCO large subunit (*rbcL*), mitochondrial-encoded cytochrome oxidase subunit 1 and 3 (*cox1*, *cox3*) and NADH dehydrogenase subunit 1 (*nad1*), and partial nuclear ribosomal LSU rDNA genes were amplified and sequenced following De Clerck *et al.* (2006), Hwang *et al.* (2009) and Silberfeld *et al.* (2010).

### Phylogenetic position of *Dictyota cyanoloma* within the genus *Dictyota*

A phylogenetic tree was constructed from a multigene alignment, using one representative per species, sequenced for six markers (*psbA*, *rbcL*, *cox1*, *cox3*, *nad1*, LSU rDNA) (Supplementary Table 1S). All *Dictyota* species native to the North-east Atlantic, the Mediterranean and Macaronesia were included (Tronholm *et al.*, 2010) as well as species closely related to *D. cyanoloma*, selected based on a preliminary *psbA* NJ phylogeny (data not shown). Partitioning strategies were selected with Partitionfinder (Lanfear *et al.*, 2012). A partitioning scheme with seven partitions organized by codon position and organelle and a GTR+G model of sequence evolution was adapted in a subsequent Bayesian analysis with MrBayes 3.2 (Ronquist *et al.*, 2012). Two parallel runs, consisting of three heated chains and one cold chain each, were run for 9 million generations, using default priors and chain temperature settings. The cold chain was sampled every 1000 generations. Tracer 1.4 was used to assess stationarity of log-likelihoods and parameter values (Drummond & Rambaut, 2007). A burn-in sample of 1000 trees was removed before construction of a majority rule consensus tree.

A maximum likelihood ML analysis, using the same partitioning strategy, was conducted employing RAXML v.8.1.11 under a GTRCAT substitution model. The robustness of the resulting phylogenies was tested using 1000 replicates of a rapid bootstrap heuristic (Stamatakis *et al.*, 2008).

### Geographic distribution of *Dictyota cyanoloma*

Geographic data from a global Dictyotales dataset (<http://www.phycology.ugent.be/Dictyotales/distribution.pdf>), summarizing taxonomic and geographic data of more than 4400 specimens, were plotted on a global map, highlighting the occurrences of *D. cyanoloma*. Additional maps of the presumed introduced and native range were constructed indicating the localities

**Table 1.** Specimens of *D. cyanoloma* used for the network analysis, with indication of haplotype number (h), habitat, locality with coordinates, year of sampling, and accession number per phylogenetic marker.

h	ID	Locality	Habitat	Country	Latitude	Longitude	Sampling year	Nad6	Atp9
1	LT0030	New South Wales: Minnie Waters	natural	Australia	-29.7761	153.3021	2009	LN871845	LN871881
2	LT0384	Western Australia: Albany: Vancouver Peninsula: Whaling Cove	natural	Australia	-35.0552	117.9314	2009	LN871846	LN871882
3	HV2123	New South Wales: Minnie Waters	natural	Australia	-29.7761	153.3021	2009	LN871847	LN871883
4	HV2301	Victoria: Bellarine Peninsula: Point Lonsdale: Glaneuse Reef	natural	Australia	-38.2914	144.6078	2009	LN871848	LN871884
5	HV2335	South Australia: Port McDonnell	natural	Australia	-38.0602	140.6629	2009	LN871849	LN871885
6	HV2155	New South Wales: Newcastle	natural	Australia	-32.9277	151.7919	2009	LN871850	LN871886
7	ED138	Doukkala-Abda: Oualidia	natural	Morocco	32.7258	-9.0560	2010	LN871851	LN871887
	ED141	Doukkala-Abda: Oualidia	natural	Morocco	32.7258	-9.0560	2010		
	ED197	Doukkala-Abda: Safi	natural	Morocco	32.3209	-9.2596	2010		
	ED151	Marrakech-Tensift-El Haouz: Essaouira	natural	Morocco	31.5089	-9.7757	2010		
	ED283	Tangier-Tétouan: Asilah	natural	Morocco	35.4647	-6.0423	2010		
	ED284	Tangier-Tétouan: Asilah	natural	Morocco	35.4647	-6.0423	2010		
	ED477	Tangier-Tétouan: Tangier: Cap Spartel	natural	Morocco	35.7607	-5.9395	2010		
	FS0480	Algarve: Albufeira: Olhos de Agua	natural	Portugal	37.0886	-8.1935	2009		
	SMG-09-01	Azores: São Miguel: Lagoa	natural	Portugal	37.7411	-25.5738	2009		
	HEC15777	Madeira Island: Rais Magos	natural	Portugal	32.6024	-16.8036	2006		
	Algecires2	Andalusia: Cádiz: Algeciras	marina	Spain	36.1173	5.4355	2014		
	Algecires3	Andalusia: Cádiz: Algeciras	marina	Spain	36.1173	5.4355	2014		
	Motril1	Andalusia: Granada: Motril	marina	Spain	36.7239	3.5260	2014		
	Motril2	Andalusia: Granada: Motril	marina	Spain	36.7239	3.5260	2014		
	D1567	Canary Islands: El Hierro	natural	Spain	27.6383	-17.9849	2009		
	D1568	Canary Islands: El Hierro	natural	Spain	27.6383	-17.9849	2009		
	Castell5	Castelló: Castelló de la Plana	marina	Spain	39.9677	0.0220	2014		
	Castell6	Castelló: Castelló de la Plana	marina	Spain	39.9677	0.0220	2014		
	D621	Catalonia: Barcelona	marina	Spain	41.3314	2.1736	2008		
	Estartit2	Girona: Costa Brava: Estartit	marina	Spain	42.0528	3.2052	2014		
	Estartit3	Girona: Costa Brava: Estartit	marina	Spain	42.0528	3.2052	2014		
8	KD879	Victoria: Williamstown: Gloucester reserve	natural	Australia	-37.8690	144.8818	2010	LN871852	LN871888
	KD880	Victoria: Williamstown: Gloucester reserve	natural	Australia	-37.8690	144.8818	2010		
9	LT0287	South Australia: Port Noarlunga	natural	Australia	-35.1486	138.4642	2009	LN871853	LN871889
	HV2384	South Australia: Port Noarlunga	natural	Australia	-35.1491	138.4656	2009		
	HV2389	South Australia: Port Noarlunga	natural	Australia	-35.1491	138.4656	2009		
10	LT0057	New South Wales: Newcastle	natural	Australia	-32.9277	151.7919	2009	LN871854	LN871890
	FS0478	Algarve:Albufeira: Olhos de Agua	natural	Portugal	37.0886	-8.1935	2009		
	Almeria1	Andalusia: Almería	marina	Spain	36.8307	2.4671	2014		
	Almeria2	Andalusia: Almería	marina	Spain	36.8307	2.4671	2014		
	D502	Canary Islands: Gran Canaria: Arinaga: Zoco del Negro	natural	Spain	27.8661	-15.3847	2008		
	LLan2	Girona: Llançà	marina	Spain	42.3738	3.1631	2014		
	LLan3	Girona: Llançà	marina	Spain	42.3738	3.1631	2014		
	FS0715	Huelva: Chipiona	marina	Spain	36.7240	-6.4391	2009		
	FS0734	Huelva: Zahara de los Atunes: Playa de los Alemanes	natural	Spain	36.1028	-5.8247	2009		
11	FS0405	Dalmatia: Split: Bacvice	natural	Croatia	43.5003	16.4461	2009	LN871855	LN87189A
	ED199	Doukkala-Abda: Doukkala-Abda: Safi	natural	Morocco	32.3209	-9.2596	2010		
	ED079	Doukkala-Abda: El Jadida: Sidi Bouزيد	natural	Morocco	33.2314	-8.5541	2010		
	ED389	Tangier-Tétouan: Ben Younech	natural	Morocco	35.9116	-5.3846	2010		
	ED408	Tangier-Tétouan: Ben Younech	natural	Morocco	35.9116	-5.3846	2010		
	ED014	Tangier-Tétouan: Larache: Port Lixus	natural	Morocco	35.2042	-6.1465	2010		
	ED018	Tangier-Tétouan: Larache: Port Lixus	natural	Morocco	35.2042	-6.1465	2010		
	FS0547	Algarve: Lagos: Praia da Dona Ana	natural	Portugal	37.0908	-8.6693	2009		
	Estepona1	Andalusia: Málaga: Estepona	marina	Spain	36.4149	5.1597	2014		
	Estepona2	Andalusia: Málaga: Estepona	marina	Spain	3.4149	5.1597	2014		
	Palamos3	Girona: Palamós	marina	Spain	41.8455	3.1276	2014		
	Palamos4	Girona: Palamós	marina	Spain	41.8455	3.1276	2014		
	FS0733	Huelva: Zahara de los Atunes: Playa de los Alemanes	natural	Spain	36.1028	-5.8247	2009		
	Cartagena1	Murcia: Cartagena	marina	Spain	37.5847	0.9849	2014		
	Cartagena2	Murcia: Cartagena	marina	Spain	37.5847	0.9849	2014		
12	FS0429	Alentejo: Sines: Porto Covo	natural	Portugal	37.8272	-8.7922	2009	LN871856	LN871892
	FS0716	Huelva: Chipiona: marina	marina	Spain	36.7240	-6.4391	2009		
13	LT0095	New South Wales: Jervis Bay: Bowen Island	natural	Australia	-35.1118	150.7688	2009	LN871857	LN871893
14	HV2182	New South Wales: Jervis Bay: Bowen Island	natural	Australia	-35.1118	150.7688	2009	LN871858	LN871894

(Continued)

**Table 1.** (Continued).

h	ID	Locality	Habitat	Country	Latitude	Longitude	Sampling year	Nad6	Atp9			
15	D3814	Dalmatia: Split	harbour	Croatia	43.5037	16.4298	2013	LN871859	LN871895			
	D4009	Istria: Rovinj	natural	Croatia	45.0769	13.6336	2014					
	D4010	Istria: Rovinj	natural	Croatia	45.0769	13.6336	2014					
	D4011	Istria: Rovinj	natural	Croatia	45.0769	13.6336	2014					
	D3813	Catalonia: Barcelona	marina	Spain	41.3538	2.1780	2005					
	D4006	Galicia: Oleiros: Ría de A Coruña	natural	Spain	43.3472	-8.3794	2014					
16	HV2387	South Australia: Port Noarlunga	natural	Australia	-35.1491	138.4656	2009	LN871860	LN871896			
17	FS1752	West Australia: Perth: Port of Fremantle	harbour	Australia	-32.0558	115.7333	2014	LN871861	LN871897			
	FS1739	Victoria: Portarlington	natural	Australia	-38.1109	144.6529	2014					
	FS1740	Victoria: Portarlington	natural	Australia	-38.1109	144.6529	2014					
	FS1746	Victoria: Portarlington	natural	Australia	-38.1109	144.6529	2014					
	FS1728	Victoria: Williamstown: South	natural	Australia	-37.8690	144.8818	2014					
	FS1729	Victoria: Williamstown: South	natural	Australia	-37.8690	144.8818	2014					
	FS1732	Victoria: Williamstown: South	natural	Australia	-37.8690	144.8818	2014					
	18	FS1733	Victoria: Williamstown: South	natural	Australia	-37.8690	144.8818			2009	LN871862	LN871898
		FS1748	West Australia: Perth: Port of Fremantle	harbour	Australia	-38.1453	115.7333			2014		
	19	HV5285	Victoria: Frankston	natural	Australia	-38.1453	145.1141			2014	LN871863	LN871999
		FS1738	Victoria: Portarlington	natural	Australia	-38.1109	144.6529			2014		
		FS1742	Victoria: Portarlington	natural	Australia	-38.1109	144.6529			2014		
		FS1744	Victoria: Portarlington	natural	Australia	-38.1109	144.6529			2014		
		FS1747	Victoria: Portarlington	natural	Australia	-38.1109	144.6529			2014		
		FS1734	Victoria: Williamstown: South	natural	Australia	-37.8690	144.8818			2014		
		FS1731	Victoria: Williamstown: South	natural	Australia	-37.8690	144.8818			2014		
		D3819	Dalmatia: Split (Zagreb herbarium)	harbour	Croatia	43.5037	16.4298			1958		
20		FS1735	Victoria: Williamstown: South	natural	Australia	-37.8690	144.8818	2014	LN871864	LN871900		
		D3936A	Cornwall: Falmouth	marina	UK	50.1653	-5.0845	2014				
21		D3936B	Cornwall: Falmouth	marina	UK	50.1653	-5.0845	2014	LN871865	LN871901		
	D3936C	Cornwall: Falmouth	marina	UK	50.1653	-5.0845	2014					
	D3936D	Cornwall: Falmouth	marina	UK	50.1653	-5.0845	2014					
	D3817	Côte d'Azur: Cannes	marina	France	43.5494	7.0149	1999					
22	D3817	Côte d'Azur: Cannes	marina	France	43.5494	7.0149	1999	LN871866	LN871902			
23	D3818	Dalmatia: Split (Zagreb herbarium)	harbour	Croatia	43.5037	16.4298	1935	LN871867	LN871903			
24	D3815b	Dalmatia: Split (Zagreb herbarium)	harbour	Croatia	43.5037	16.4298	1935	LN871868	LN871904			
25	D3815	Dalmatia: Split (Zagreb herbarium)	harbour	Croatia	43.5037	16.4298	1935	LN871869	LN871905			

**Table 2.** Primer sequences for the mitochondrial markers *nad6-nad11* and *atp9-orf11*.

Marker	Forward primer	Reverse primer
<i>nad6-nad11</i>	TTT TAT TGT GTG	CAC AAG AAA CTT
	CGG TGG TG	GAG GCT TCG
<i>atp9-orf11</i>	ATA CGC GAT TTT	TGC GGG TAA AAC
	AGG TTT TGC	TGA TAC GG

of all samples. A distinction was made between those records published earlier and those either confirmed within this study, or obtained from historical herbarium specimens, or unconfirmed based on photographic evidence. Annual mean minimum and maximum sea surface temperature isotherms obtained from Bio-ORACLE (Tyberghein *et al.*, 2012), flanking the inferred species range in both hemispheres were indicated.

### Genetic diversity and haplotype network construction

Concatenated *nad6-nad11* and *atp9-orf11* sequences (1235bp), corrected for indels following Simmons & Ochoterana (2000), were collapsed into haplotypes. Haplotype networks were constructed based on traditional phylogenetic algorithms, an approach which has been demonstrated to outperform widespread network constructing methods (Salzburger *et al.*, 2011). Maximum parsimony- and maximum

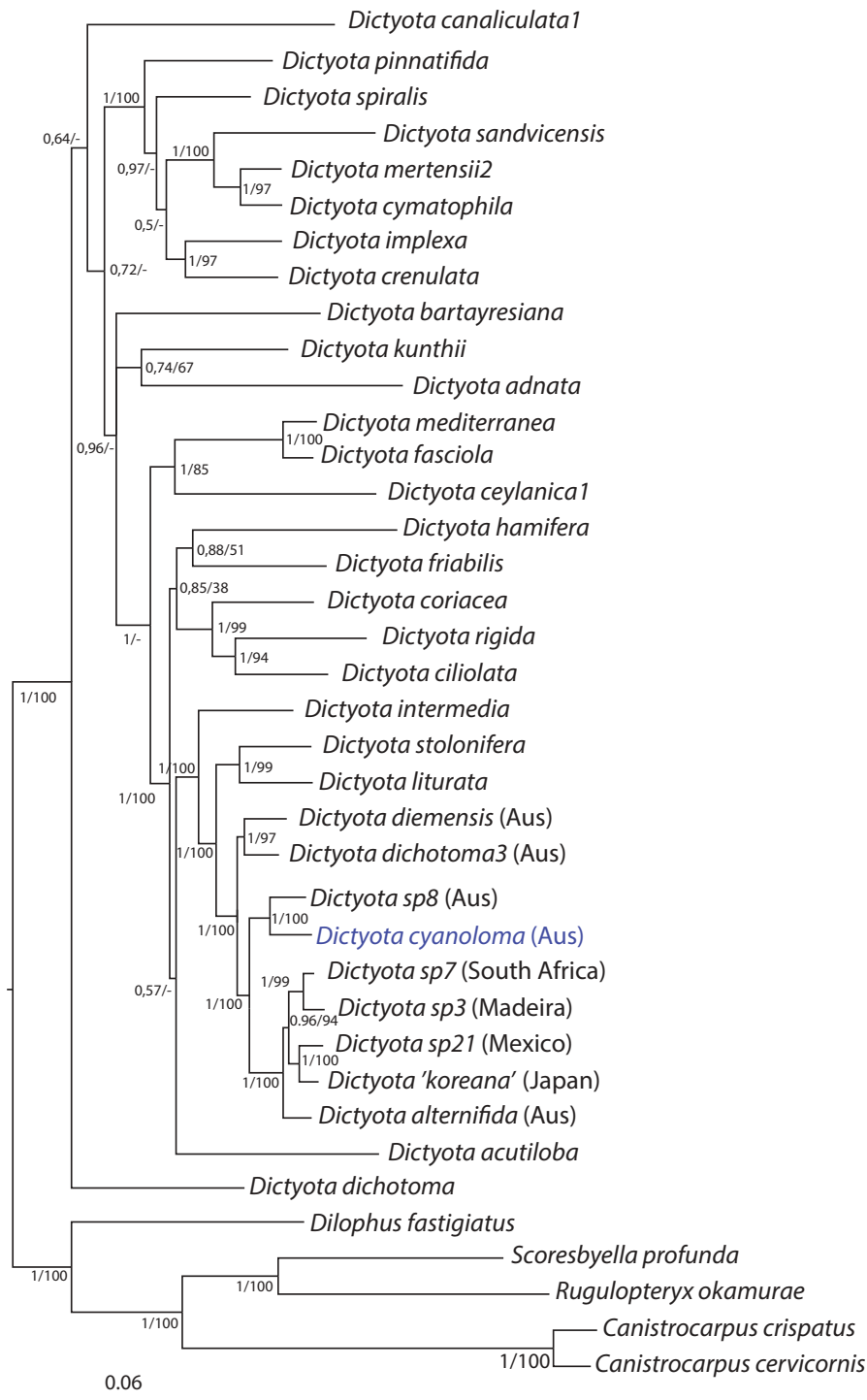
likelihood phylogenies were estimated in MEGA6 (Tamura *et al.*, 2013) and RaxML v.8.1.11 (Stamatakis *et al.*, 2008), respectively, and converted into haplotype networks using Haploviewer (available at <http://www.cibiv.at/~greg/haploviewer>).

Levels of genetic variation (haplotype diversity, nucleotide diversity and haplotype richness) in European and Australian populations were estimated in Arlequin 3.5 (Excoffier & Lischer, 2010). Estimates of haplotype richness after rarefaction were obtained with a rarefaction calculator based on the program HP-RARE (Kalinowski, 2005). Haplotype richness was standardized to the smallest population size (32) to obtain an unbiased estimate of the haplotype richness for inter-population comparisons (Leberg, 2002).

## Results

### Phylogenetic position of *Dictyota cyanoloma* within the genus *Dictyota*

Bayesian and maximum likelihood analyses of a multilocus dataset based on six markers (*psbA*, *rbcL*, *cox1*, *cox3*, *nad1*, LSU rDNA) resulted in highly congruent and reasonably well-supported phylogenetic trees (Fig. 1). *Dictyota cyanoloma* was resolved (BS-values = 100; BPP = 1.0) as the sister taxon to an unidentified *Dictyota* species from Western Australia (*Dictyota* sp8, HV2563). The



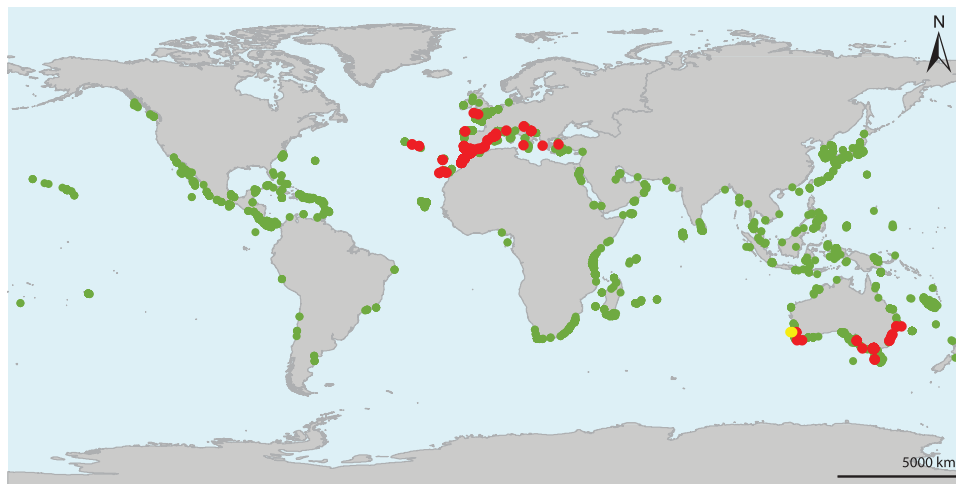
**Fig. 1.** Phylogenetic tree obtained by ML-inference of a dataset containing six genes (partial LSU rDNA, *rbcL*, *psbA*, *cox1*, *cox3* and *nad1*). Numbers at the nodes indicate posterior probabilities followed by ML-bootstrap values.

larger clade to which *D. cyanoloma* belongs, contained several other Australian species (*D. alternifida*, *D. dichotoma* 3, *D. diemensis*, *D. intermedia*) next to species of which the actual distribution is hitherto unclear (*D. sp7*, *D. sp21*, *D. liturata*, *D. stolonifera*) due to restricted taxon sampling.

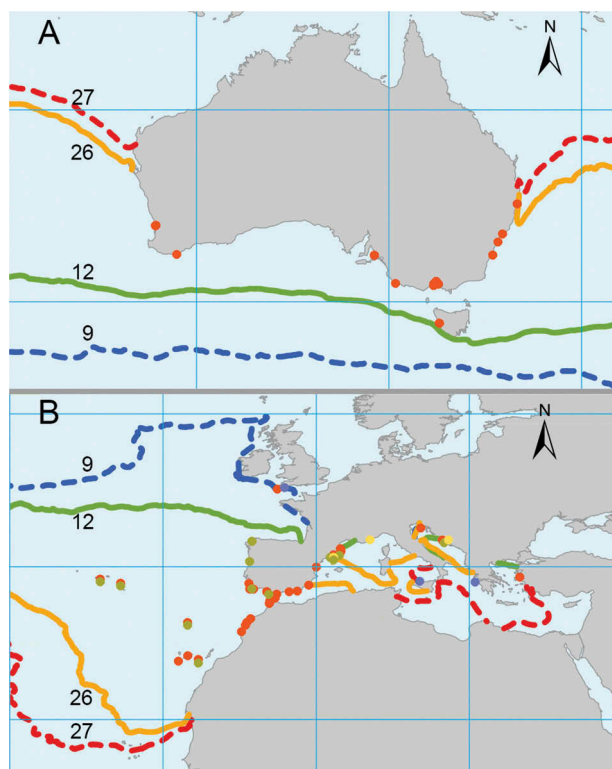
#### Geographic distribution of *Dictyota cyanoloma*

A map displaying the distributions of more than 4400 Dictyotales specimens indicated that *D. cyanoloma* is

present both in Europe and South Australia, but presumably absent elsewhere (Fig. 2). European records stretch from Cornwall, England to the Canary Islands and North-west Africa in the South and the Gulf of Izmir in the eastern Mediterranean Sea. This range is bordered by a 9°C minimum sea surface temperature (SSTmin) and a 27°C maximum sea surface temperature (SSTmax) (Fig. 3A). No samples from the southern hemisphere were found outside a thermal range bordered by the 12°C SSTmin and 26°C SSTmax (Fig. 3B).



**Fig. 2.** Distribution map of a global *Dictyota* data set (green) highlighting the occurrences of *D. cyanoloma* (red) and *D. sp8* (yellow).



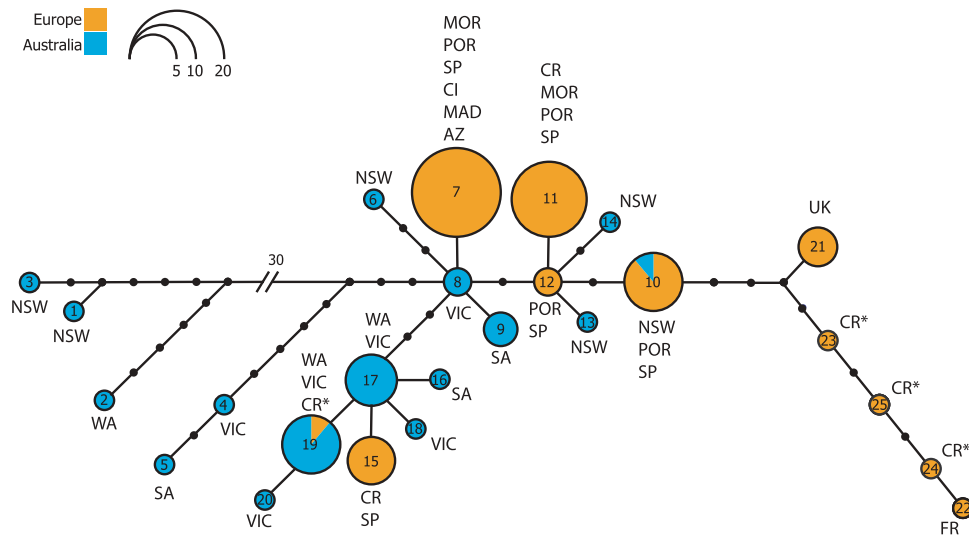
**Fig. 3.** The sample locations (dots) within the native range (A) and the region of introduction (B) are reported. The reported isotherms on both maps are the annual mean maximum and minimum sea surface temperatures bordering the distribution range of *Dictyota cyanoloma* within the native region (full lines) contrasted with the annual mean maximum and minimum sea surface temperatures bordering the distribution within the introduced range (dashed lines). A distinction is made between samples from this study (red), earlier published records (green), photographic evidence (blue) and herbarium samples (yellow).

The earliest *D. cyanoloma* records in Europe were from the Adriatic Sea (Split), collected in 1935. Additional records from 1947, 1958 and 1976 (herbarium), 2009 (Tronholm *et al.*, 2010) and 2014 (this study) form circumstantial evidence of its intermittent presence

in the Adriatic Sea. The first published record of *D. cyanoloma* appeared in 2007 by Rull Lluich *et al.* (2007) and was incorrectly identified as *D. ciliolata*. Herbarium species sequenced in this study from Cannes (1999) and Barcelona (2005) precede this record. Records within the NE-Atlantic were noted for Aveiro (Barbara *et al.*, 2014) and A Coruña (Barbara *et al.*, 2014; Peña *et al.*, 2014) and SW Britain (2014) (Table 1). More southern records were sampled in the Canary Islands (2009) and Morocco (2010) (Table 1). A single record was reported for the Aegean Sea, in the Gulf of Izmir in Turkey (Taskin, 2013). Next to these published records, evidence based on photographs searchable on the internet indicated the presence of *D. cyanoloma* in Greece and Sicily (Supplementary Table 2S). All records were confirmed genetically except the specimens from Turkey, Galicia, Greece and Sicily.

#### **Genetic diversity and haplotype networks**

A total of 25 haplotypes were found among 93 *D. cyanoloma* samples. In the haplotype network a 37 bp gap separated three Australian specimens (LT0030, LT0384, HV2123) from the remainder of the specimens. The *cox1* gene sequences of the two clusters, however, differed by no more than 1.6%. Compared with typical interspecific distances in *Dictyota* (~20–30%) this is a low value that persuaded us to treat both clusters as the same species. Australian samples contained 16 haplotypes (32 samples), including 12 singletons, while in Europe 11 haplotypes are recorded for 61 samples, including four singletons. Only two haplotypes, however, were shared between Australia and Europe. One of the shared haplotypes was from a Croatian herbarium sample from 1958. Haplotype networks based on ML- (Fig. 4) or MP-trees (Supplementary Fig. 1S) were generally consistent in the patterns they reveal, except in the reciprocal positioning of the singleton haplotypes from three out of



**Fig. 4.** Haplotype network based on a ML phylogeny indicating Australian (blue), European (orange) and sequenced Split herbarium samples (asterisks). The size of the circles is proportional to the amount of individuals of that haplotype. The number specifies the haplotype. (WA: West Australia, NSW: New South Wales, VIC: Victoria, SA: South Australia, MOR: Morocco, POR: Portugal, AZ: Azores, MAD: Madeira, SP: Spain, CI: Canary Islands, CR: Croatia, UK: United Kingdom, FR: France.)

four of the herbarium specimens and in the position of haplotype 15. These minor differences do not, however, alter the general phylogeographic pattern.

For the calculation of the genetic diversity indices, haplotypes of four European specimens collected in 1935 and 1958 in Split (D3815, D3815b, D3818, D3819) were removed as these were not representative of the current genetic diversity within the introduced range. The corresponding haplotype diversity for Australia and Europe were  $0.8931 \pm 0.0370$  and  $0.7713 \pm 0.0327$  respectively. The nucleotide diversities were  $0.008967 \pm 0.004653$  for Australia compared with  $0.002904 \pm 0.001660$  for Europe. Haplotype richness after rarefaction brought the total number of Australian haplotypes to 16 compared with 7 for Europe.

## Discussion

Using a combination of phylogenetic and phylogeographic analyses, we have demonstrated that *Dictyota cyanoloma*, recently described from Europe (Tronholm *et al.*, 2010) is also present in large parts of Australia. Below we discuss the plausibility of a number of alternative hypotheses that could potentially account for this pattern.

### Alternative hypotheses for the disjunct distribution of *Dictyota cyanoloma*

An initial hypothesis, that Australian and European specimens belong to different species, is not supported by the genetic data. Genetic divergence between Australian and European populations of

typical barcoding markers such as the *cox1* gene is very close to 0%. As a comparison, interspecific p-distances among *Dictyota* species vary from 7–8% to about 25%. In addition, a haplotype network based on mitochondrial *nad6–nad11* and *atp9–orf11* regions fails to illustrate two clusters corresponding to European and Australian samples. Haplotypes between both regions are interspersed.

It is also unlikely that *D. cyanoloma* is characterized by a continuous biogeographic range extending from Europe to Australia. First, seaweeds are predominantly characterized by relatively narrow thermal tolerances (Santelices, 1980; Van den Hoek, 1982; Eggert, 2012), making exceptionally wide latitudinal ranges spanning tropical as well as temperate regions uncommon. Second, no specimens were sampled in tropical latitudes or in other temperate areas (e.g. southern Africa) as shown by our global *Dictyotales* dataset (Fig. 2). It is therefore most likely that *D. cyanoloma* has a truly disjunct distribution, with established populations in Europe and Australia. In the light of our present knowledge of natural seaweed distributions and cases of anthropogenic introductions of non-native species through maritime traffic and aquaculture, a discontinuous distribution pattern as demonstrated by *Dictyota cyanoloma* is highly indicative for an introduction history.

### Evidence supporting an Australian native range of *Dictyota cyanoloma*

Under the hypothesis that the current biogeographic range of *Dictyota cyanoloma* involves an introduction, the question remains as to which area should



be considered the native range. Several lines of evidence support the hypothesis that *D. cyanoloma* has been introduced in Europe from Australia. First, a phylogenetic analysis shows that *D. cyanoloma* belongs to a clade consisting of species with a mainly South Pacific distribution, many of them restricted to Australia. Furthermore, the fact that *D. sp8*, the sister species of *D. cyanoloma*, is recorded only from Australia is additional evidence for an Australian native range. Australasia is known as one of the main three regions from which species are introduced into the Mediterranean, next to the Red Sea and the North-western Pacific (Mineur *et al.*, 2015). While Lessepsian migration and the transport of live shellfish, especially oysters, are the main introduction pathways and vectors from the latter two regions respectively, it has not been demonstrated what caused the introductions of Australasian species into the Mediterranean (Mineur *et al.*, 2015), except for aquarium trade in the case of *Caulerpa taxifolia* (Jousson *et al.*, 1998).

Even though reduced genetic diversity of invasive populations may not be as commonplace as once hypothesized (Roman & Darling, 2007; Dlugosch & Parker, 2008), for the marine environment the opposite pattern seems to be the rule rather than the exception. At meta-analysis by Rius *et al.* (2015) revealed that no less than 74% of the marine introduced species for which genetic data are available, display similar genetic diversity values in both native and introduced ranges. These results strongly favour the notion that genetic diversity is not necessarily impaired when species are introduced. A reduction of genetic diversity, as documented for the invasive seaweed species *Codium fragile* ssp. *fragile* (Provan *et al.*, 2005) and for *Sargassum muticum* (Bae *et al.*, 2013; Cheang *et al.*, 2010), could therefore represent an exception rather than the rule.

For *Dictyota cyanoloma*, allelic richness is higher in Australia than in Europe, but haplotype and nucleotide diversities indicate that Australian as well as European populations harbour a high level of genetic diversity. Australian haplotype diversities are only slightly higher than in Europe, but the nucleotide diversities differ significantly. This discrepancy can be indicative of a bias in calculated diversity measures (Goodall-Copestake *et al.*, 2012). Diverging haplotypes within a sample suggest undersampling and inflate estimates of nucleotide diversity, as is the case for the Australian population. The high proportion of singleton haplotypes (12 out of 16) together with the large genetic gap for Australian specimens suggest that this region is undersampled, and thus might inflate nucleotide diversities. Allelic richness is higher for Australia (16 versus seven haplotypes).

Recent insights, however, claim that genetic diversity is not necessarily depauperate after an aquatic invasion. In many cases, large inocula and multiple introductions

boost the genetic diversity in the introduced range (Roman & Darling, 2007). Introduced populations of *Undaria pinnatifida* clearly illustrate the importance of the above factors. While Australasian introduced populations show a high genetic diversity, European introduced populations have a much lower diversity in comparison to the Asian source populations. This disparity stems from the fact that the European introduction was the consequence of an intentional introduction for aquaculture, while the Australasian populations were the consequence of recurrent introductions by accidental translocations associated with maritime traffic (Voisin *et al.*, 2005). Multiple introductions or great genetic diversity within the inocula together with beneficial life history characteristics as clonal propagation could have enabled *D. cyanoloma* to by-pass successfully a bottleneck associated with the founder event and to establish a genetically diverse introduced population.

Within Europe, *D. cyanoloma* is thriving in non-natural environments such as ports, marinas and yachting clubs (Table 1, Fig. 5), and artificial substrata such as pontoons, jetties and neglected ship hulls are often fouled by the species (Fig. 6). Along the Iberian



**Figs 5 and 6.** *Dictyota cyanoloma*. **Fig. 5.** *Dictyota cyanoloma* clearly showing its characteristic blue iridescent margin, photograph taken by Joana Aragay, port of Almeria, 2013. **Fig. 6.** Boat hull fouled by *Dictyota cyanoloma*, photograph taken by Olivier De Clerck at Garachico Bay, Tenerife, 2014.

Peninsula and the NW Moroccan coast, some natural habitats harbour populations of the species. In comparison sampling localities within Australia are almost strictly natural environments. This observation is concurrent with the idea of Europe being the introduced range, as marine invaders are often growing on artificial substrata.

The combination of these findings offers support for the hypothesis of an introduction of *D. cyanoloma* from the southern hemisphere into the Mediterranean and Europe.

### **Introduction and potential spread within Europe**

Molecular data demonstrated that the species was at least present in the Adriatic Sea as early as 1935. Its presence in the Split area is confirmed by molecular data obtained from herbarium samples collected in 1947, 1958 and 1976. Other herbarium records from Cannes (1999) and Barcelona (2005) were confirmed to be genuine *D. cyanoloma* and all pre-date the finding of Rull Lluçh *et al.* (2007). Since the publication of Tronholm *et al.* (2010), species observations (UK, Greece, Sicily) and published records (Taskin, 2013; Barbara *et al.*, 2014; Peña *et al.*, 2014) increased steadily, with reports for the North-east Atlantic until the eastern Mediterranean and Macaronesia. The question remains as to whether *D. cyanoloma* was unreported until about a decade ago despite being widespread in southern Europe or whether the species only spread recently despite being locally present in the Mediterranean Sea for several decades. Accepting the historical records in the Adriatic as evidence for a species introduction within this area would be premature, as no other collections than the Ghent and Zagreb herbarium were screened for this species. In this respect and based on our data, we cannot exclude the hypothesis that *D. cyanoloma* was present along most European coastlines throughout most of the 20th century. The same was the case for *Codium fragile*, which had been colonizing habitats across the world in some cases nearly 100 years before it was noticed and remaining cryptically present (Provan *et al.*, 2005).

The occurrence of populations of *D. cyanoloma* in marinas and yachting clubs suggest an important role of recreational boating as a secondary dispersal vector. This could explain the presence of *D. cyanoloma* in the Falmouth marina in Cornwall. This marina is one of the most famous and well-visited yachting marinas in the south of the UK, often used as a stop-over point for yachts returning from southern Europe.

Comparison of the thermal boundaries of the native and introduced ranges shows that these are similar, except that *D. cyanoloma* has established sustainable populations in significantly lower temperatures in its

introduced range than in its native range. The most southern sample from Tasmania does not go below a mean minimum temperature of 12°C, while in Europe, populations are established in the south-west of Britain (UK), where mean minimum temperatures are as low as 9°C. This could indicate that the climatic tolerance for the species is wider than the conditions to which it is subjected in its native range. The same has been noted for several invasive tropical algae in the Mediterranean, which thrive in temperatures lower than the limit of tropical temperatures (Boudouresque & Verlaque, 2002). Extreme mean maximum temperatures isotherms for the species are comparable in both the native and introduced range (26 and 27°C, respectively). Due to this northward extension of the minimum thermal boundary in Europe, *D. cyanoloma* has been able to establish itself in a geographic range exceeding the size of its presumed potential range.

### **Conclusions and future prospects**

Using gene sequence data we provide ample evidence for the introduced nature of *D. cyanoloma* in Europe. However, several questions remain unanswered to date. The main question is whether *D. cyanoloma* was ubiquitous but unrecognized within the Mediterranean throughout the 20th century, or whether it was an introduction that remained contained during a lag phase of several decades before expanding its range (Crooks *et al.*, 1999; Mack *et al.*, 2000; Sakai *et al.*, 2001). An initial step to resolve this matter would be to screen other European herbaria for historical records. Additionally, a population genetic study including thorough sampling of both the introduced and native range, together with the use of more variable multilocus molecular markers such as microsatellites, could shed a light on different ecological processes as colonization and dispersal. Contrasting the multilocus genetic diversity of both native and introduced populations could pinpoint source populations and allow us to infer whether there was a single or multiple successive introductions within Europe. This approach might answer important questions regarding pathways and dynamics of secondary spread within the introduced range. There are indications that recreational boating acts as a secondary vector since *D. cyanoloma* is growing abundantly in marinas and harbours, where polluted conditions hamper the growth of native species. Recent reports of populations in natural environments raise suspicions that they might spread from these marina populations. These patterns should be monitored, as well as the ecological impact of the species on the native communities. Last, it seems evident that *D. cyanoloma* is still spreading north. Predicting the eventual range in Europe therefore is important. Regarding the extent to which the species has spread, it is important to assess what exactly the potential range within Europe could be.

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## Author contributions

F. Steen, O. De Clerck: original concept, generating data and drafting manuscript; J. Aragay, D. Vitales, A. Gomez Garreta sampling and generating molecular data; F. Bunker, H. Verbruggen and F.P. Mancuso sampling invaded and native range; A. Zuljevic screening herbarium collections and sampling. All authors contributed to the writing of the manuscript.

## Supplementary information

The following supplementary material is accessible via the Supplementary Content tab on the article's online page at <http://dx.doi.org/10.1080/09670262.2016.1212998>

**Supplementary fig. 1S.** Haplotype networks based on both best scoring MP phylogenies

**Supplementary table 1S.** Specimen table employed for phylogenetic inference

**Supplementary table 2S.** Links to photographic evidence of *D. cyanoloma* occurrences

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