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Genetic diversity and biogeography in *Chaetomorpha melagonium* (Ulvophyceae, Cladophorales) based on internal transcribed spacer (ITS rDNA) sequences

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Abstract: *Chaetomorpha melagonium* is a morphologically distinct species of green algae that occurs throughout the North Atlantic, the North Pacific and the Arctic Ocean. In this study, we analyzed the intraspecific genetic diversity among 14 samples of *C. melagonium* from across the distribution range based on nuclear large subunit ribosomal DNA (LSU rDNA) and rDNA internal transcribed spacer (ITS) DNA sequences. All samples had identical LSU sequences. The ITS sequences had very few mutations that nevertheless divided the specimens into two groups: one included samples from Iceland, Svalbard, Massachusetts and Alaska with identical ITS sequences; members of this group differed in samples from Europe (France, Germany, Scotland, Sweden, and Wales) by three mutations (two point mutations and one five base pair indel). The European specimens had identical ITS sequences with the exception of a single sample from Brittany that differed by one base pair. The maximum ITS sequence divergence within the samples of *C. melagonium* was less than 0.5%. This low intraspecific variation in the frequently used highly variable ITS region is discussed in the context of past geological and climatic scenarios.

Keywords: Arctic; *Chaetomorpha*; distribution; internal transcribed spacer (ITS); intraspecific variation; large subunit (LSU); North Atlantic; North Pacific; phylogeography; ribosomal DNA (rDNA) sequences.

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Introduction

Chaetomorpha melagonium (Weber and Mohr) Kützing is a marine green macro-alga that consists of rigid, straight, unbranched filaments that are attached by a discoid holdfast to sublittoral rocks down to 15 m depth and in low littoral pools, often where slight sedimentation occurs (Kornmann 1972, Leliaert and Boedeker 2007). Filaments are up to 1 mm in diameter and are typically less than 30 cm long (maximum 60 cm), are attenuated near the base and have marked constrictions between cells producing the distinctive moniliform appearance (Kornmann 1972, Leliaert and Boedeker 2007). The conspicuous (up to 3 mm long) basal cell (Figure 1A) and the large drum-shaped or barrel-shaped cells of the filaments with a distinct dark blue-green color and thick cell walls (Figure 1B) are characteristic for this species. *Chaetomorpha melagonium* is distributed in the cold-temperate Northern hemisphere, from Greenland, Spitsbergen, Iceland, the White Sea and Norway to the Atlantic coasts of France and Spain; also along the northwestern Atlantic coasts from the Canadian Arctic, south to Cape Cod; and along the Pacific coasts of North America from Alaska to Oregon and in the western Pacific from Kamchatka to Japan and Korea (Guiry and Guiry 2016).

Chaetomorpha melagonium is the type species of the genus *Chaetomorpha* (see Silva 1950) and was described originally as *Conferva melagonium* (Weber and Mohr) based on material from the North Sea (Varberg, Sweden). The type specimen is lost and the original illustration represents the lectotype (Silva et al. 1996, Leliaert and Boedeker 2007). A recent molecular study showed that *C. melagonium* represents a separate lineage from the clade containing all other species of *Chaetomorpha* and it was recommended to conserve *Chaetomorpha* with a new type and to create a new genus to accommodate *C. melagonium* in the future (Boedeker et al. 2016). While *C. melagonium* is a morphologically well-defined species, descriptions of some unattached growth forms have led to taxonomic confusion. An unattached form from the Pacific coast of

Figure 1: *Chaetomorpha melagonium*: morphological characteristics.
(A) Conspicuous long basal cell with discoid holdfast. Scale bar = 1 mm. (B) Large, barrel-shaped cells with dark blue-green color and a sheen. Scale bar = 500 µm.

North America had been named *C. atrovirens* W.R. Taylor and is currently regarded as a synonym of *C. picquotiana* Montagne ex Kützing (Blair 1983). Electrophoretic enzyme banding patterns imply a close relationship between *C. picquotiana* and *C. melagonium* (Blair et al. 1982), but a later study argued convincingly against the conspecificity of *C. melagonium* and *C. picquotiana* (Blair 1983).

A number of macroalgal species, including *Acrocladus pygmaeus* (Reinke) Boedeker, *Cladophora rupestris* (L.) Kützing, *Acrosiphonia arcta* (Dillwyn) Gain, *Coccotylus truncatus* (Pallas) M.J. Wynne & J.N. Heine, *Phycodrys rubens* (L.) Batters and *Desmarestia aculeata* (L.) J.V. Lamouroux (van Oppen et al. 1993, 1995, Peters et al. 1997, Lindstrom 2001) occurs in both the North Pacific and the North Atlantic and one possible explanation for this distribution pattern is dispersal between the two oceans via the Arctic Ocean since the opening of the Bering Strait in the Miocene or Pliocene, approximately 5 million years ago (mya) (Lüning 1985, Lindstrom 1987, 2001). Since then, repeated glaciation periods have likely led to extinctions on one or both sides of the Atlantic and the Pacific (van den Hoek 1984, Lüning 1985), have effectively isolated eastern and western Atlantic populations of many taxa by ice fields (Novaczek et al. 1990), and have shaped the current distribution patterns of many algae. Therefore marine species with an amphioceanic distribution represent interesting study systems to investigate population structure and phylogeography. In this study, we analyzed the intraspecific genetic diversity in *C. melagonium* collections from both the Atlantic and Pacific using large subunit ribosomal DNA (LSU rDNA) and rDNA internal transcribed spacer (ITS) sequences.

Materials and methods

Fourteen samples of *C. melagonium* were used in the molecular analyses (listed with collection information, as well as voucher and GenBank accession numbers in Table 1), spanning most of the distribution range, including the northeast and northwest Atlantic, Arctic and Pacific Oceans (Figure 2). Genomic DNA was isolated from samples preserved in silica gel using the Chelex method (Goff and Moon 1993). Polymerase chain reaction (PCR) amplifications were carried out with an initial denaturation step of 94°C for 5 min followed by 30–34 cycles of 1 min at 94°C, 1 min at 57°C for the LSU or at 62°C for the whole ITS fragment or at 54°C for the ITS1 and ITS2 regions and 1 min at 72°C and a final extension step of 5 min at 72°C. The reaction volume consisted of approximately 0.1–0.4 µg genomic DNA, 1.25 nmol of each deoxynucleotide triphosphate (dNTP), 6 pmol of each primer, 1× reaction buffer, 1 mM $MgCl_2$, 5% bovine serum albumin (BSA) and one unit of Taq polymerase. Approximately 590 nucleotides of the LSU rDNA were amplified using the universal primers C′1 forward and D2 reverse (Hassouna et al. 1984, Leliaert et al. 2003). The whole ITS region was amplified with the primers 9F and 7R (Hayakawa et al. 2012), resulting in an approximately 1000 base pairs (bp) long fragment. For some samples (see Table 1), the ITS1 and ITS2 regions were amplified separately. ITS1 was amplified using the primers ITS5–ITS2, and ITS2 was amplified using the primers ITS3–ITS4 (White et al. 1990). Amplifications were checked for correct size by electrophoresis on 1% agarose gels. PCR products were purified with Montage PCR filter units (Millipore, Billerica, MA, USA) or with ExoSAP-IT® (Affymetrix, Santa Clara, CA, USA) following the manufacturer's protocol. Cleaned PCR products were commercially sequenced (Macrogen Inc.,

Table 1: *Chaetomorpha melagonium*: list of sequenced specimens with collection data and GenBank accession numbers.

Taxon	Sample no.	Origin	Date	Collector	Voucher[a]	ITS	LSU
Chaetomorpha melagonium	A62/Cm1/CMASS	Wood's Hole, Mass., USA	1971	n.i.	n.i.[b]	KY569003	KY568989
C. melagonium	A63/Cm3/CBRIT	Roscoff, Brittany, France	1986	I. Novaczek	n.i.[b]	KY569004	KY568990
C. melagonium	A64/Cm4/CHELG	Helgoland, Germany	1987	I. Novaczek	n.i.[b]	KY569005	KY568991
C. melagonium	A65/Cm6/CICE1	Iceland	1987	I. Novaczek, H. Rietema	n.i.[b]	KY569006	KY568992
C. melagonium	A66/Cm7/CICE2	Iceland	1987	I. Novaczek, H. Rietema	n.i.[b]	KY569007	KY568993
C. melagonium	A88	Kongsfjord, Svalbard	14.07.2004	Kai Bischof	WELT: A033502	KY569008	KY568994
C. melagonium	B33	Sandgerdi, Iceland	06.09.2004	Christian Boedeker	WELT: A033503	KY569009	KY568995
C. melagonium	B63	Sandgerdi, Iceland	15.09.2004	Christian Boedeker	WELT: A033504	KY569010	KY568996
C. melagonium	B64	Helgoland, Germany	29.08.2004	Andreas Wagner	WELT: A033505	KY569011	KY568997
C. melagonium	C64	Gulmarnsfjord, Sweden	July 2001	Christian Boedeker	WELT: A033506	KY569012	KY568998
C. melagonium	E47	Newburgh, Scotland, UK	24.09.2005	Christian Boedeker	n.i.	KY569013	KY568999
C. melagonium	E74	Kongsfjord, Svalbard	Sept. 2005	Jan Rueness	WELT: A033507	KY569014	KY569000
C. melagonium	H22	Kenai Peninsula, Alaska, USA	16.05.1991	Gayle Hansen	WELT: A033508, OSC-2801	KY569015	KY569001
C. melagonium	J31/FL1018	Broad Haven, Wales, UK	17.04.2006	Christine Maggs	WELT: A033509, GENT: FL1018	KY569016	KY569002

[a]WELT, National Museum of New Zealand Te Papa Tongarewa, Wellington, New Zealand; GENT, Ghent University, Ghent, Belgium; OSC, Oregon State University, Corvallis, OR, USA.
[b]The DNA was extracted from the original cultures used in Novaczek et al. (1990), however, the cultures do not exist anymore and no voucher specimens have been preserved.
n.i., no information.

Seoul, South Korea). Sequences were edited and assembled with Geneious 5.1.5. (Biomatters Limited, Auckland, New Zealand). The sequences obtained in this study have been deposited in GenBank (Table 1). The sequences were aligned by eye in Se-Al v2.0a11 (Rambaut 2007).

Results

All 14 samples had identical LSU sequences, while the ITS sequences had few mutations that nevertheless divided the specimens into two groups (Figure 2). The samples from Iceland, Svalbard, Massachusetts and Alaska had identical ITS sequences and differed from the European samples by three mutations (two point mutations and one five bp indel) in the ITS1 region. The European specimens had identical ITS sequences with only a single sample from Brittany differing by one bp in the ITS2 region. The maximum ITS sequence divergence within the samples of *C. melagonium* is less than 0.5%.

Discussion

The ITS frequently shows considerable intraspecific variation. In the marine genera *Phyllodictyon* and *Boodlea* (Cladophorales), ITS sequences showed intraspecific variation of up to 4%, in contrast to between-species divergence of 7%–29% (Leliaert et al. 2008, 2009). Even higher ITS sequence variation of 18%–33% was found within the marine species *Cladophora albida* and *Cladophora vagabunda* (Bakker et al. 1992, 1995, Marks and Cummings 1996), but it is likely that these numbers reflect divergence between cryptic species rather than intraspecific variation. In contrast, some globally distributed genotypes of the easily dispersed freshwater morphospecies *Cladophora glomerata* have identical ITS sequences (Marks and Cummings 1996, Ross 2006, Boedeker et al. unpublished data). Low or no ITS variation was also surprisingly found in species with an assumed low dispersal capacity, with only up to 0.5% divergence throughout the holarctic range for the freshwater lacustrine species *Aegagropila linnaei* Kützing (Boedeker et al. 2010a) and no variation found in populations of *Wittrockiella lyallii* (Harvey) C. Hoek et al. from as far apart as New Zealand and Chile (Boedeker et al. 2010b). Observed differences in intraspecific ITS sequences are not necessarily simply a measure of the time since populations were separated, but are linked to factors such as secondary structure of ITS, generation time, population size and dynamics and mode of

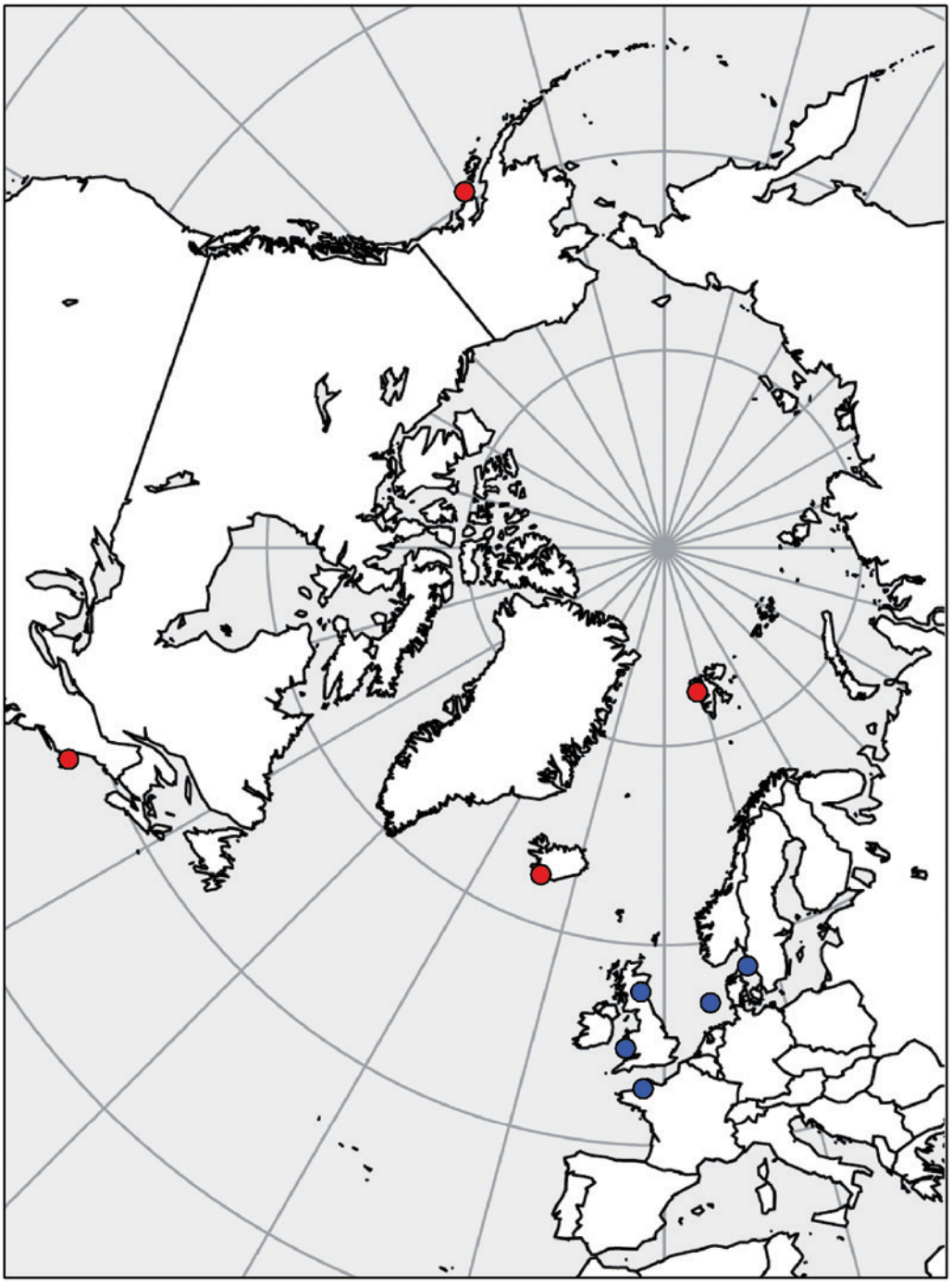

Figure 2: *Chaetomorpha melagonium*: map of the distribution range with the locations of sequenced samples indicated by dots. The blue dots represent the European ITS genotypes, the red dots represent the Pacific-Arctic ITS genotype.

reproduction and must therefore be interpreted differently for every species (Baer et al. 2007, Bromham 2009).

Similar to *C. melagonium*, no or low genetic divergence between Atlantic and Pacific populations has been found in several macroalgal species. No variation in three markers (*tuf*A, rbcL and *psa*B) was found between the Atlantic *Prasiola stipitata* Suhr ex Jessen and the Pacific *Prasiola meridionalis* Setchell and N.L. Gardener (Moniz et al. 2014). Low divergence in ITS sequences between Atlantic and Pacific populations has been discovered in *Acrosiphonia arcta* (van Oppen et al. 1993), *Desmarestia aculeata* (Peters et al. 1997) and *Fucus evanescens* C. Agardh/*Fucus distichus* L./*Fucus gardneri* P.C. Silva (Serrão et al. 1999). These patterns

of low divergence between Atlantic and Pacific populations have been hypothesized to be due to dispersal after the opening of the Bering Strait about 5 mya and subsequent colonization of the Atlantic from a Pacific source population or vice versa (Lindstrom 2001). The grouping of sequences of *C. melagonium* from Svalbard, Iceland and Massachusetts with the specimen from Alaska could indicate that the northwest Atlantic and the Arctic were recently recolonized from the Pacific; alternatively, recent dispersal from the Atlantic to the Pacific is also a possibility. More sequence data and an estimate of a rate of nucleotide evolution based on dated phylogenies would be useful to investigate these questions. The unique ITS genotype from Brittany (France) hints at additional intraspecific genetic diversity and additional samples from France and Spain would be useful to investigate the possibility of several refugia in the Atlantic during the last ice age.

Additional samples from Pacific regions, such as the Russian Far East, Japan, Korea, Canada and Oregon, are needed to fully understand the intraspecific patterns and colonization history. Several geographical regions for which *C. melagonium* has been reported are likely based on misidentifications (Turkey, India, Australia and Chile/temperate Pacific South America; Guiry and Guiry 2016). *Chaetomorpha melagonium* has a lethal temperature of 25°C (Novaczek et al. 1990) and the Indian records most likely represent *Chaetomorpha antennina* (Bory) Kützing, a morphologically somewhat similar tropical species. The records from Turkey seem doubtful as *C. melagonium* has not been reported from other areas of the Mediterranean and summer surface temperatures are likely too high. The illustration of Australian *C. melagonium* in van den Hoek and Womersley (1984) does not show the typical straight and elongated basal cell, but instead depicts a curved basal cell more representative of *Chaetomorpha clavata* (C. Agardh) Kützing. Chilean records possibly represent the morphologically similar, large-celled species *Chaetomorpha firma* Levring.

A supposedly closely related species, *Chaetomorpha picquotiana* (see Blair et al. 1982, Blair 1983), has a distribution on both the Pacific (Alaska to Oregon) and Atlantic (Labrador to Connecticut) coasts of North America (Guiry and Guiry 2016) and molecular sequence data of this species is required to establish whether it is in fact a synonym of *C. melagonium* (as in Burrows 1991, Leliaert and Boedeker 2007) or a separate species (as indicated by Blair et al. 1982, Blair 1983). Recent molecular studies have shown that *C. melagonium* forms a monotypic lineage separate from the rest of *Chaetomorpha* (Boedeker et al. 2016). If *C. picquotiana* is a distinct species, it will be very interesting to see if DNA sequences will place this species with *C. melagonium*, or with all the other *Chaetomorpha* species.

In conclusion, *C. melagonium* has a distribution in the temperate North Atlantic and North Pacific and displays an intraspecific genetic structure correlated with location. The distribution of genotypes appears to be structured by past geological and/or climate events and represents a suitable system to study colonization history and possible refugia by using more variable molecular markers and population genetics.

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Bionotes

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