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ORIGINAL ARTICLE

Delineation of *Chondrus* (Gigartinales, Florideophyceae) in China and the origin of *C. crispus* inferred from molecular data

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Abstract

Complete nuclear ribosomal DNA (nrDNA) internal transcribed spacer (ITS) sequences were obtained for 18 *Chondrus* populations collected at 15 sites from eight countries worldwide. Pairwise comparisons with the multiple alignment revealed that intraspecific divergences of ITS sequences ranged from 0.3 to 1.8% in *C. crispus* Stackhouse (except for the entity SVLH from France) and from 0.0 to 0.6% in *C. ocellatus* Holmes, whereas interspecific divergences in *Chondrus* varied from 1.4 to 5.0%. Three phylogenetic methods (neighbour joining, maximum parsimony and maximum likelihood) confirmed three main lineages: the North Atlantic lineage containing entities of *C. crispus* from Canada, France, Germany, England, Portugal, Ireland and Wales; a second lineage comprising three species: *C*. sp. 1, *C. armatus* (Harvey) Yamada et Mikami, and *C. pinnulatus* (Harvey) Okamura from the Northern Pacific; and a third lineage containing just one species: *C. ocellatus* from the Northern Pacific. *Chondrus yendoi* Yamada et Mikami separated from other *Chondrus* species singly. nrDNA ITS data indicate that a previous assignment of *C*. sp. 2 to *Mazzaella japonica* (Mikami) Hommersand may be incorrect, and additional evidence is needed to resolve the generic placement of this entity. It is inferred from the nrDNA ITS data that three *Chondrus* species are presently known in China with two, *C. ocellatus* and *C. nipponicus*, in Qingdao and two, *C. armatus* and *C. nipponicus*, in Dalian. We hypothesize that the ancestor of North Atlantic *C. crispus* had a Pacific origin, and that the present distribution of *C. crispus* in the Atlantic Ocean correlates with a trans-Arctic dispersal and vicariance events associated with Pleistocene glaciation maxima.

Key words: *Biogeography,* Chondrus *Stackhouse, internal transcribed spacer, phylogeny, taxonomy*

Introduction

Chondrus Stackhouse 1797 is a perennial red seaweed with alternate phases of gametophyte and tetrasporophyte. It is a major source of the commercially important phycocolloid carrageenan (Cheney & Mathieson 1979). *Chondrus* exhibits great variation in thallus morphology. Thomas (1938) recognized 21 forms of *C. crispus* Stackhouse based on morphological differences and reproductive characteristics. These differences of form have been ascribed to different environmental conditions, such as emersion period, salinity, depth and light intensity (Mathieson & Burns 1975), exposure to wave action (Gutierrez & Fernandez 1992), and life history phases (Chen & Taylor 1980). The large part played by environmental factors therefore limits the usefulness of morphological features in determining species.

To date there have been few investigations regarding the phylogenetic relationships among species of *Chondrus*. Cheney & Mathieson (1978) examined the isozyme patterns of eight populations of *C. crispus* from New Hampshire and the Maritime Provinces, Canada, and found substantial genetic differentiation over short distances. Hommersand et al. (1993, 1999) proposed a revised classification of the family Gigartinaceae based on *rbc*L sequences and morphological evidence, and they suggested that *Chondrus* was one of seven genera in the family in

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which *Chondrus* and *Mazzaella* form a complex of related species. Chopin et al. (1996) determined that several *C. crispus* isolates examined were all the same species inferred from 2.18% sequence divergence of the internal transcribed spacer (ITS) regions. Fewer reports about *Chondrus* have been published in China. Tseng (1962) first reported the presence of *C. ocellatus* Holmes in Weihai, China. Luan & Zhang (1998) reported *C. armatus* (Harvey) Yamada et Mikami and *C. yendoi* Yamada et Mikami for the first time from Dalian. Subsequently, Su et al. (2002) conducted morphological observations and partial *rbc*L sequence analyses for *Chondrus*, and suggested that three species were present in the Liaodong peninsula: *C. ocellatus*, *C. nipponicus* and *C. armatus*. They also concluded that the species previously referred to *C. yendoi*-should be called *Mazzaella japonica* (Mikami) Hommersand. The nomenclature and taxonomy of *Chondrus* in China remains confused.

A variety of nuclear- and chloroplast-specific sequences have been utilized in an effort to define useful molecular characters for delineating species and populations of red algae. One of these, the ITS region separating 18S and 26S nuclear ribosomal DNA (nrDNA), which includes two spacers (ITS1 and ITS2) and the intervening 5.8S coding sequence, has become well characterized across interspecific–intergeneric-level divergences (van Oppen et al. 1995; Hughey et al. 2002). It can be used to diagnose the origin and phylogenetic relationships of organisms at different taxonomic levels and provides a useful paradigm for molecular evolutionary studies (Hershkovitz & Lewis 1996; Coleman & Mai 1997). It has also been widely used to investigate the intrageneric relationships in Chlorophyta (Meusnier et al. 2004), Phaeophyta (Yoon et al. 2001) and in members of the Rhodophyta (Hughey et al. 2002; Bellorin et al. 2002).

In this study, we sampled 18 populations of *Chondrus* from 15 sites in eight countries worldwide and sequenced and compared the nrDNA ITS regions. The primary aims were to delineate the genus *Chondrus* in China and to propose a hypothesis regarding the origin and distribution of *C. crispus* in the North Atlantic.

Materials and methods

Algal materials and DNA extraction

The materials used in this study are listed in Table I, along with collection information and GenBank accession numbers. Collecting sites are given in Figure 1, along with the number of samples sequenced from each site. Voucher specimens were prepared and lodged in the Key Laboratory of Experimental Marine Biology, Institute of Oceanology, Chinese Academy of Sciences (KEMB). Su et al. (2002) conducted morphological observations and *rbc*L sequence analyses for *Chondrus* samples in Xiaoping Dao, Dalian, China from materials collected by R. X. Luan, who worked in the Natural Museum of Dalian. In this study, the three *Chondrus* entities (*C. armatus*, *C.* sp. 1 and *C.* sp. 2) from Xiaoping Dao were also sampled by R. X. Luan from the same material that Su et al. (2002) had analysed. Based on morphological and molecular evidence, they suggested that only two species of *Chondrus* are present in Xiaoping Dao: *C. nipponicus* (*C.* sp. 1 in this study) and *C. armatus*, and that the species previously called *C. yendoi* should be regarded as *Mazzaella japonica* (*C.* sp. 2 in this study). We found that *C.* sp. 1 and *C.* sp. 2 differed greatly morphologically from other *Chondrus* species (Figure 2), especially *C.* sp. 2, whose generic placement remains undefined. Accordingly, we list these entities as *C.* sp. 1 and *C.* sp. 2, rather than giving them species names. Figure 2 shows their gross morphology and that of three other *Chondrus* species: *C. ocellatus*, *C. nipponicus* and *C. armatus*. Algal samples were processed and genomic DNA extracted according to the protocol of Hu et al. (2004). There were two to five replicates for each population.

Polymerase chain reaction (PCR) amplification and sequencing

The nrDNA ITS regions were amplified using the synthetic primers TW18:5 ′GGGATCCGTTTCCGTAGGTGAACCTGC3′ (annealing to the 3′ end of 18S rDNA) and AB28: 5 ′GGGATCCATATGCTTAAGTTCAGCGGGT3′ (annealing to the 5′ end of 28S rDNA), as described by Goff & Moon (1993). Amplification was performed in an Eppendorf Master thermocycler personal (Germany). The reaction mixture (20 µl) contained 10 mM Tris.HCl (pH 8.3), 50 mM KCl, 1.5 mM $MgCl_2$, 2.0 mM dATP, dCTP, dGTP, dTTP, respectively, 50 ng nuclear DNA, 1.0 mM of each primer and 1.0 U *Taq* DNA polymerase (Takara Co. Ltd., Dalian). The PCR parameters were 5 min at 95°C; 1 min at 90°C and 2 min at 50°C for five cycles; followed by 30 cycles of 1 min at 90°C, 1 min at 60°C, 1 min at 72°C; finally left at 72°C for 10 min. A negative control was run with each PCR reaction to check for contamination. The PCR products were checked on a 1.5% agarose gel for 1 h at 80 V.

Table I. Sample information for the species investigated in this study.

Species	Collector	Collection location and date	Specimen voucher	GenBank no.	Sample size
C. ocellatus Holmes (S1)	B. M. Xia	Ba Da Guan, Qingdao, China, 26 March 2002	EMBLC H307002	AY699051	2
C. nipponicus Yendo (S1)	B. M. Xia	No. 3 Swimming Beach, Qingdao, China, 27 September 2003	EMBLC H307001	AY699050	5
C. nipponicus Yendo (S2)	B. M. Xia	No. 3 Swimming Beach, Qingdao, China, 27 September 2003	EMBLC H307018		5
C. yendoi Yamada et Mikami	T. Motomura	Muroran, Charatsunai, Japan, 22 May 2003	EMBLC H307003	AY699052	2
C. pinnulatus (Harvey) Okamura	T. Motomura	Muroran, Charatsunai, Japan, 22 May 2003	EMBLC H307004	AY699053	3
C. armatus (Harvey) Yamada et Mikami	R. X. Luan	Xiao Ping Dao, Dalian, China, 17 July 2000	EMBLC H307005	AY699054	3
C. sp. 1	R. X. Luan	Xiao Ping Dao, Dalian, China, 19 July 2000	EMBLC H307006	AY699055	3
C. sp. 2	R. X. Luan	Xiao Ping Dao, Dalian, China, 19 July 2000	EMBLC H307007	AY699056	2
C. crispus Stackhouse	L. A. Franklin	Helgoland North Sea, Germany, 8 July 2003	EMBLC H307008	AY699057	2
C. crispus Stackhouse	A. T. Critchley	Ireland, 9 February 2004	EMBLC H307009	AY699058	2
C. crispus Stackhouse	A. T. Critchley	Sune, Porto, Portugal, June 2003	EMBLC H307010	AY699059	2
C. crispus Stackhouse	G. W. Saunders	Green Point, Lepreau, New Brunswick (NB), Canada, 19 November 2003	EMBLC H307011	AY699060	3
C. crispus Stackhouse	M. Brown	Wembury Beach, Devon, England, 5 December 2003	EMBLC H307012	AY699061	3
C. crispus Stackhouse	S. L. Morrell	Black Rock, Dale Pembrokeshire, Wales, UK, 5 December 2003	EMBLC H307013	AY699062	2
C. crispus Stackhouse	A. T. Critchley	Prince Edward Island (PEI), Canada, September 2003	EMBLC H307014	AY699063	3
C. crispus Stackhouse	A. T. Critchley	St. Vaast La Hougue (SVLH), France, 24 November 2003	EMBLC H307015	AY699064	3
C. crispus Stackhouse	A. T. Critchley	France, 20 January 2004	EMBLC H307016	AY699065	2
C. ocellatus Holmes (S2)	Z. M. Hu	Ba Da Guan, Qingdao, China, 26 March 2002	EMBLC H307000		5

The nrDNA ITS region was completely sequenced in both directions, using the Sanger dideoxy chain termination method with an ABI PRISM™ 370 genetic analyser. Sequencing primers were the amplification primer AB28 and revised TW18-1: 5′CGTTTCCGTAGGTGAACC3′, the revised TW 18-1 subtracted 9 bp at both laterals from TW 18 for convenient sequencing.

Alignment and phylogenetic analyses

The boundaries of the ITS regions were determined by comparison with sequences published in Goff et al. (1994), Van Oppen et al. (1995) and Hughey et al. (2002). Sequence alignments were made with Clustal X (Thompson et al. 1997) and refined manually. DNAsis v2.5 and MEGA 2.1 (Kumar et al. 2001) were applied to estimate GC content and pairwise distance for the ITS regions separately. Four *Mazzaella* species (GenBank accession numbers AY225290, AY225230, AY225231 and AY225232) were selected as outgroups and included in the alignments.

As indels are important characters for phylogenetic analysis, especially among closely related species, gaps were treated as both fifth bases and missing values in separate analyses to compare with the topology of resulting phylograms. Trees were constructed by the maximum parsimony (MP) and neighbour joining (NJ) methods available in MEGA 2.1. The reliability of the trees was tested by non-parametric bootstrap analysis (Felsenstein 1985) with 1000 replications. The maximum likelihood (ML) algorithm with the modified Hasegawa–Kishino–Yano (HKY) model (Hasegawa et al. 1985) for multiple substitutions was used as implemented by the online PHYML (Guindon et al. 2005).

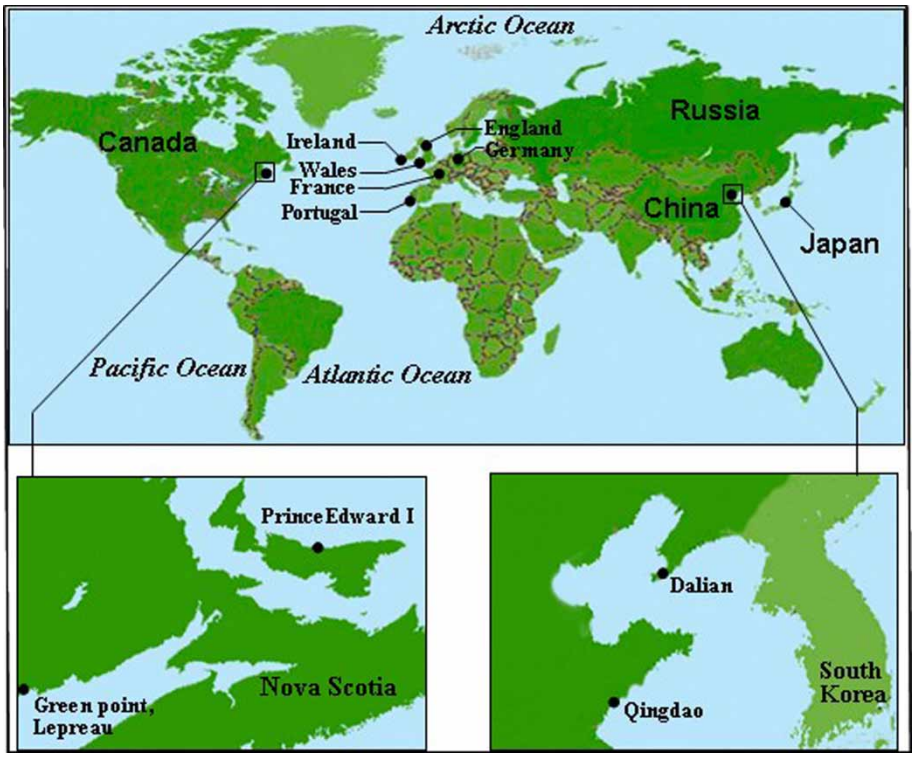

Figure 1. A map showing the sampling sites of *Chondrus* Stackhouse.

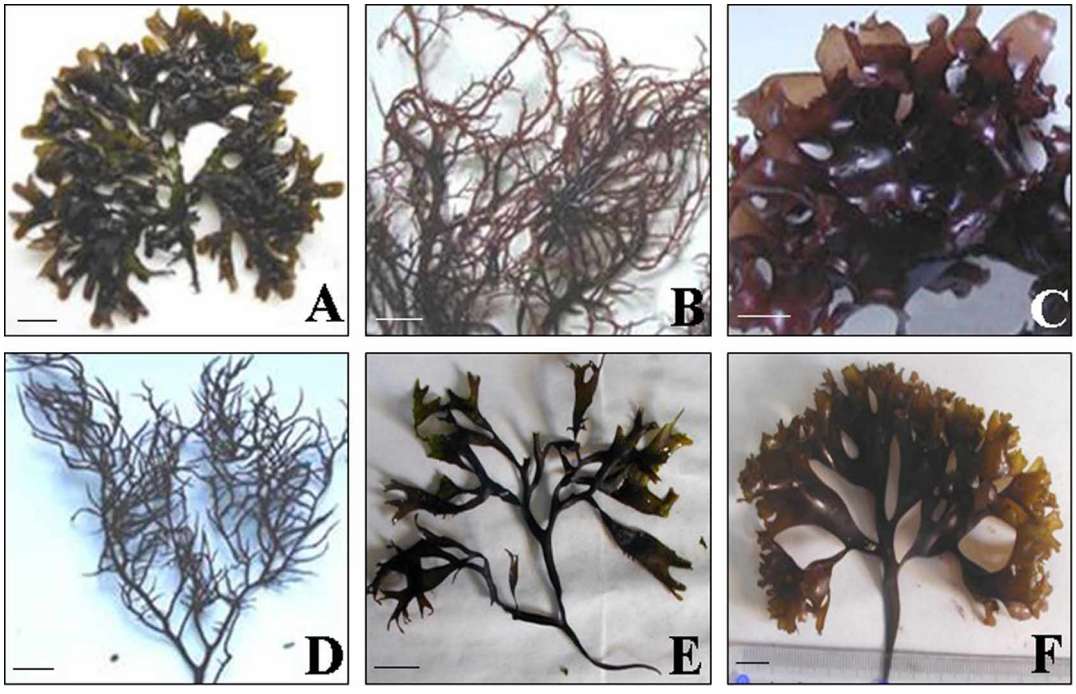

Figure 2. Photographs of four *Chondrus* entities collected from China: (A) *C. ocellatus*, Qingdao; (B) *C. armatus*, Dalian; (C) *C.* sp. 2, Dalian; (D) *C.* sp. 1, Dalian; (E) *C. nipponicus* (S1), Qingdao; (F) *C. nipponicus* (S2), Qingdao. The scale bar shows 1 cm.

Results

Length, GC content and sequence divergence of the ITS region

Eighteen complete nrDNA ITS sequences were obtained from our *Chondrus* samples. The size and G+C% variation among ITS sequences are listed in Table II. The length of ITS1 varied from 143 to 151 bp, and that of ITS2 from 398 to 404 bp. The coding 5.8S region was 158 bp in length. The range in GC content of ITS1, 5.8S rDNA and ITS2 was 33.0–36.0, 48.5–50.3 and 44.3–46.9%, respectively. In general, the GC content in ITS1 was lower than that in ITS2, whereas that in the 5.8S region was significantly higher than in the two spacer regions. *Chondrus yendoi* from Charatsunai, Japan, had a relatively longer sequence (714 bp) for the entire ITS region, whereas sequence lengths in *C. nipponicus* (S2) from Qingdao, China, *C. pinnulatus* from Charatsunai, Japan and *C. armatus* from Dalian, China were all 704 bp.

To determine the extent of inter-individual differences in the ITS region, the entire ITS region was sequenced for two to five individuals of each population from 15 collecting sites. In most cases, the sequences obtained were identical. In four cases 1–3 bp differences were seen. Pairwise comparisons (including insertions and deletions) in the multiple alignment revealed that intraspecific divergences in ITS sequences ranged from 0.3 to 1.8% within *C. crispus*, except for the entity SVLH from France, which had higher values (2.4–3.5%). Within *C. ocellatus*, divergence varied from 0.0 to 0.6%. In contrast, interspecific divergences ranged from 1.4 to 5.0% within the genus *Chondrus*.

Molecular phylogenetic analyses

The three phylogenetic reconstruction methods (NJ, MP and ML) produced consistent tree topologies (Figures 3–5). MP yielded 48 equally most-parsimonious trees of 456 steps. For the ML analysis, the empirical base frequencies were unequal (A = 0.27099, C = 0.19973, G = 0.25063, T = 0.27865), –Ln likelihood = 3069.29834. With gaps treated as missing values and fifth bases, we obtained nearly the same phylogenetic topologies. Three main lineages were consistently identified in all analyses: a North Atlantic lineage and two lineages from the Pacific. *Chondrus crispus* from Canada, France, Germany, England, Portugal, Ireland and Wales formed the first condensed clade (NJ = 99%, MP = 93%, ML = 99%). The second clade contained *C.* sp. 1 and *C. armatus* from Dalian, China, *C. nipponicus* (S1) from Qingdao, China and *C. pinnulatus* from Japan. The third clade just included three *C. ocellatus* entities, including *C. "nipponicus"* (S2), which was identified as *C. ocellatus*) from Qingdao, China (NJ= 100%, MP=99%, ML=100%), and *C. yendoi* from Japan singly separated from other *Chondrus* species. In NJ, MP and ML trees, it is clear that the two morphological forms of *C. ocellatus* (S1 and S2) from Badaguan in Qingdao, China clustered closely (NJ= 92%, MP=96%, ML=100%) and are actually the same species. In contrast, the two morphological forms of *C. nipponicu* (S1 and S2) from the no. 3 swimming beach in Qingdao were widely separated

Table II. Internal transcribed spacer (ITS) size and base composition of *Chondrus* Stackhouse and the outgroup.

	ITS1		5.8S rDNA		ITS2		Total ITS	
Species (no.)	Length	%G+C	Length	%G+C	Length	%G+C	Length	%G+C
C. ocellatus (S1)	148	35.5	158	49.8	404	46.9	710	45.7
C. nipponicus (S1)	148	34.8	158	49.8	399	46.2	705	45.1
C. nipponicus (S2)	143	36.0	158	49.8	403	46.5	704	45.6
C. yendoi	148	34.8	158	49.8	408	45.4	714	44.7
C. pinnulatus	148	35.5	158	49.8	398	45.6	704	44.9
C. armatus	147	34.4	158	49.8	399	46.2	704	45.1
C. sp. 1	147	34.4	158	49.8	400	46.3	705	45.1
C. sp. 2	150	33.7	158	49.8	402	44.6	710	44.0
C. crispus (Germany)	149	33.0	158	49.8	400	44.6	707	44.3
C. crispus (Ireland)	149	33.9	158	48.5	401	45.7	708	44.7
C. crispus (Portugal)	148	34.1	158	49.8	401	45.2	707	44.4
C. crispus (New Brunswick, Canada)	151	34.8	158	50.3	400	45.1	709	44.6
C. crispus (England)	149	33.9	158	48.5	400	45.6	707	44.6
C. crispus (Wales)	148	34.1	158	49.8	400	45.1	706	44.4
C. crispus (PEI, Canada)	149	35.3	158	49.8	400	45.1	707	44.6
C. crispus (SVLH, France)	151	33.5	158	48.5	399	44.3	708	43.4
C. crispus (France)	150	34.4	158	49.8	400	45.1	708	44.4
C. ocellatus (S2)	148	35.5	158	49.8	404	46.9	710	45.7

SVLH, St Vaast La Hougue; PEI, Prince Edward Island.

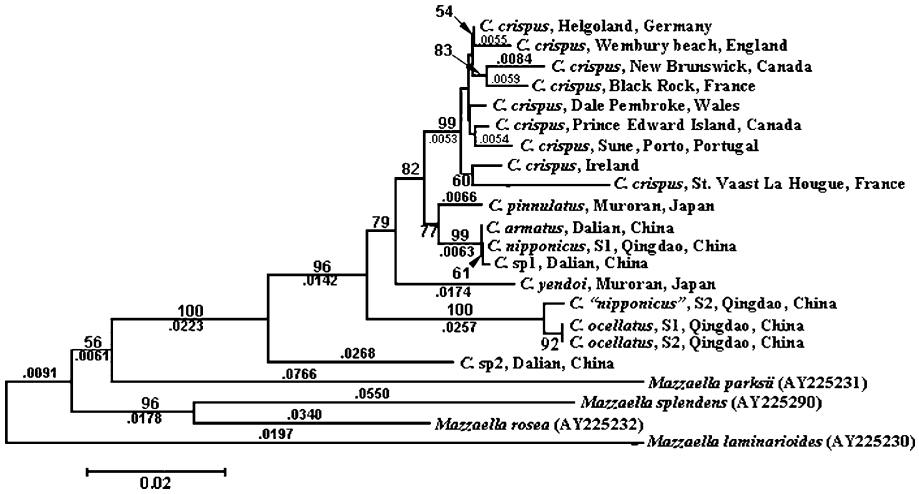

Figure 3. The neighbour joining phylogenetic tree inferred from the analysis of nuclear ribosomal DNA internal transcribed spacer (ITS) sequences. The numbers above the branches indicate bootstrap values (1000 replicates).

genetically, indicating that the two (S1 and S2) cannot both be referred to *C. nipponicus*. The S2 form is evidently *C. ocellatus* and S1 is referable to *C. nipponicus*. We conclude that the *Chondrus* samples from the no. 3 swimming beach in Qingdao consist of only two species: *C. ocellatus* and *C. nipponicus*. Three phylogenetic trees (Figures 3–5) showed that *C.* sp. 1 had close relationships with *C. nipponicus*, but there was only moderate bootstrap support (NJ=61%, ML=68%). *Chondrus* sp. 2 from Dalian exhibited the highest genetic distance (Table III) compared with all other *Chondrus* species. Its basal position, remote from the main *Chondrus* clade, received strong bootstrap support (NJ=96%, MP=89%, ML=95%). Because its position at the base of the *Chondrus* clade lies between *Chondrus* and *Mazzaella*, its correct generic placement is uncertain. The nine entities of *C. crispus* from the Atlantic region clustered strongly despite their collection from disparate sites.

Discussion

Molecular delineation and taxonomy of Chondrus *in China*

Similarities in the morphology of *C. ocellatus* and *C. nipponicus* make it difficult to separate the two species in the field. Surveys of *Chondrus* in Liaodong Peninsula from 1987 to 1992 had suggested that only *C. ocellatus* was widely distributed. However, samples varied considerably in size, and species could readily be divided into four morphological forms (Li et al. 1994). The two forms of *C. ocellatus* (S1 and S2) from Badaguan, Qingdao, China yielded identical ITS sequences, indicating that they are the same species. The explanation for the apparent discrepancy is that they represent two separate reproductive phases of the life cycle, as reported by Chen & Taylor (1980) for *C. crispus*. In contrast, the two forms of *C. nipponicus* (S1 and S2) that were morphologically distinct were also widely

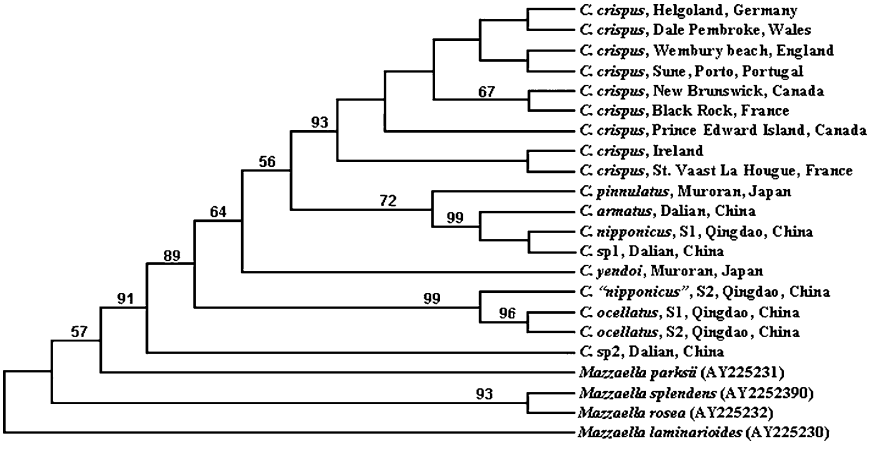

Figure 4. The maximum parsimony phylogenetic tree inferred from nuclear ribosomal DNA internal transcribed spacer (ITS) data. The numbers above the branches indicate bootstrap values (1000 replicates).

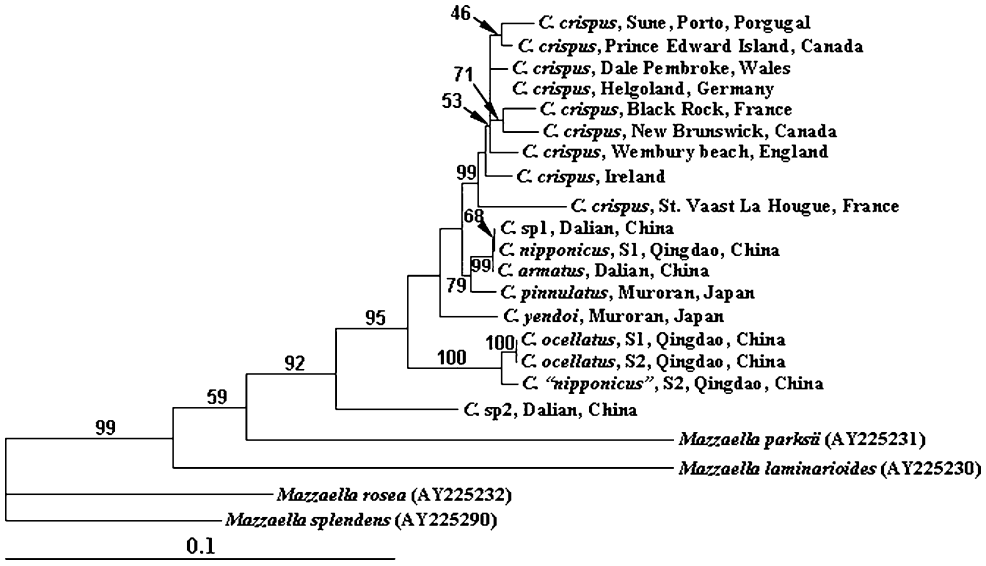

Figure 5. The maximum likelihood phylogram generated from internal transcribed spacer (ITS) sequences with PHYML online. The numbers above the branches indicate bootstrap values (100 replicates).

separated genetically. Our results show that form S2 from Qingdao (formerly referred to *C. nipponicus*) should be called *C. ocellatus* from which it is separated by only 4 bp or 0.6% (Table III). The form S2, which has traditionally been referred to *C. nipponicus*, and the one designated S1 here had relatively high pairwise sequence divergence (1.4–3.4%) compared with other *Chondrus* species (Table III). The nrDNA ITS data indicated that S1 from Qingdao is the true *C. nipponicus*. Accordingly, we concluded that two species of *Chondrus*, *C. ocellatus* and *C. nipponicus*, occur in Qingdao, China, not just one, as previously recorded.

Chondrus sp. 1 from Dalian, China (Figure 2D) was morphologically distinct from other *Chondrus* species. However, its nrDNA ITS sequence was identical to that of *C. nipponicus* (S1) from Qingdao (Table III). Our molecular data support that the entity *C.* sp. 1 corresponds genetically to *C. nipponicus*, even though it is very different morphologically (Figure 2E), but this conclusion should be consolidated on more morphological data. With regard to the entity *C.* sp. 2 (Figure 2C), formerly reported as *C. yendoi* (Luan & Zhang 1998) and later renamed as *Mazzaella japonica* (Su et al. 2002), our nrDNA ITS data showed that it possessed the highest pairwise divergence (5.3–7.3%; 37–51 bp difference) among the *Chondrus* species investigated. In fact, the genetic distance exceeded the average interspecific pairwise divergence found in the genus *Chondrus* (2.18%). A BLAST search for homologous sequences based on similarity of the entire length of the nrDNA ITS regions conducted through GenBank showed a 98% sequence homology of *C.* sp. 2 to *C. ocellatus* and *C. armatus*. This suggests that *C.* sp. 2 is related at some level to the genus *Chondrus*. The taxonomy *C.* sp. 2 remains unknown and further studies are needed in order to determine its precise generic placement.

Hommersand et al. (1999) examined relationships among species in the red algal family Gigartinaceae and placed *C. nipponicus* in the *C. ocellatus* clade, whereas in this study *C. nipponicus* was shown to belong in the clade containing *C. armatus* and *C. pinnulatus*. This relationship is further supported by morphological comparisons (Su et al. 2002). The branching of *C. nipponicus* and *C. armatus* was dichotomous or subdichotomous, in contrast to that of *C. ocellatus*, which was strictly dichotomous. In addition, the height of the frond (10–15 cm), the size of cells in cross-section (width 7–12 μm, length 7–15 μm) and the size of the carposporangia (length 18–35 μm) in *C. nipponicus* and *C. armatus* were greater than in *C. ocellatus*, whose measurements were height 4–10 cm, cell length 5–6 μm, cell width 4–5.5 μm, cell and carposporangial diameter 10–20 μm. We find the placement of *C. nipponicus* in the clade with *C. armtaus* and *C. pinnulatus* to be more appropriate than in the clade with *C. ocellatus*.

The origin and vicariance of the North Atlantic C. crispus

Previous studies strongly support a red algal invasion of the North Atlantic from the North Pacific. Van Oppen et al. (1995) interpreted nrDNA ITS sequences of *Phycodrys* isolates to indicate two trans-Arctic passages. The authors suggested that the North Atlantic was invaded first by a North Pacific progenitor shortly after the opening of the Bering

Table III. Pairwise distance comparison of internal transcribed spacer (ITS) sequence variation in *Chondrus* Stackhouse and outgroups. The lower half of the matrix shows the mean distances between taxon pairs; the upper half of the matrix shows the actual numbers of base pair changes between taxon pairs.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22
1 *C. ocellatus* (S1)	***	0	4	20	30	28	28	24	25	29	26	26	35	24	23	24	25	43	84	65	82	96
2 *C. ocellatus* (S2)	0.000	***	4	20	30	28	28	24	25	29	26	26	35	24	23	24	25	43	84	65	82	96
3 *C. nipponicus* (S2)	0.006	0.006	***	20	30	27	27	23	24	28	25	25	34	23	22	23	25	42	83	64	80	93
4 *C. yendoi*	0.028	0.028	0.029	***	25	22	22	18	20	23	18	21	31	18	17	18	19	43	84	64	80	97
5 *C. crispus* (NB, Canada)	0.043	0.043	0.043	0.036	***	10	12	8	10	12	10	13	25	19	19	20	22	46	92	71	88	98
6 *C. crispus* (France)	0.040	0.040	0.039	0.031	0.014	***	9	5	7	9	6	9	22	15	15	16	17	43	89	70	85	99
7 *C. crispus* (England)	0.040	0.040	0.039	0.031	0.017	0.013	***	4	6	8	6	9	21	15	15	16	18	40	88	68	83	97
8 *C. crispus* (Germany)	0.034	0.034	0.033	0.026	0.011	0.007	0.006	***	2	4	2	5	17	11	11	12	14	38	84	65	80	93
9 *C. crispus* (PEI, Canada)	0.036	0.036	0.035	0.028	0.014	0.010	0.008	0.003	***	6	4	5	19	13	13	14	16	40	85	67	81	95
10 *C. crispus* (Ireland)	0.041	0.041	0.040	0.033	0.017	0.013	0.011	0.006	0.008	***	6	9	18	15	15	16	18	42	89	69	84	94
11 *C. crispus* (Wales)	0.037	0.037	0.036	0.026	0.014	0.008	0.008	0.003	0.006	0.009	***	5	17	13	13	14	16	40	84	64	81	93
12 *C. crispus* (Portugal)	0.037	0.037	0.036	0.030	0.018	0.013	0.013	0.007	0.007	0.013	0.007	***	18	14	14	15	17	42	86	69	84	98
13 *C. crispus* (SVLH, France)	0.050	0.050	0.049	0.044	0.035	0.031	0.030	0.024	0.027	0.026	0.024	0.026	***	24	24	25	27	51	96	76	93	106
14 *C. nipponicus* (S1)	0.034	0.034	0.033	0.026	0.027	0.021	0.021	0.016	0.018	0.021	0.018	0.020	0.034	***	0	10	11	37	86	63	83	93
15 *C.* sp. 1	0.033	0.033	0.032	0.024	0.027	0.021	0.021	0.016	0.018	0.021	0.018	0.020	0.034	0.000	***	10	10	37	86	63	83	93
16 *C. armatus*	0.034	0.034	0.033	0.026	0.028	0.023	0.023	0.017	0.020	0.023	0.020	0.021	0.036	0.014	0.014	***	11	38	86	63	83	93
17 *C. pinnulatus*	0.036	0.036	0.036	0.027	0.031	0.024	0.026	0.020	0.023	0.026	0.023	0.024	0.038	0.016	0.014	0.016	***	41	88	69	85	97
18 *C.* sp. 2	0.062	0.062	0.061	0.062	0.066	0.062	0.057	0.054	0.057	0.060	0.057	0.060	0.073	0.053	0.053	0.055	0.059	***	87	59	82	97
19 *M. splendens*	0.125	0.125	0.124	0.125	0.137	0.132	0.131	0.125	0.126	0.132	0.125	0.128	0.142	0.128	0.128	0.128	0.131	0.129	***	53	79	109
20 *M. rosea*	0.107	0.107	0.106	0.105	0.117	0.115	0.111	0.107	0.110	0.113	0.105	0.113	0.124	0.104	0.103	0.104	0.113	0.096	0.083	***	78	89
21 *M. parksii*	0.125	0.125	0.123	0.121	0.134	0.129	0.126	0.121	0.123	0.127	0.123	0.127	0.140	0.126	0.126	0.126	0.129	0.124	0.117	0.123	***	107
22 *M. laminarioides*	0.145	0.145	0.141	0.145	0.147	0.148	0.145	0.139	0.142	0.141	0.140	0.147	0.159	0.140	0.140	0.140	0.146	0.146	0.161	0.144	0.162	***

NB, New Brunswick; PEI, Prince Edward Island; SVLH, St Vaast La Hougue.

Strait, but that a second invader from the North Pacific colonized the Western Atlantic more recently. Lindstrom (2001) published a long list of vicariant pairs of species reported to occur in the North Pacific and North Atlantic and suggested that water flow across the Arctic Ocean would have been from the North Pacific to the North Atlantic after the closure of the Panamanian Isthmus at approximately 3.5 Ma.

In this study, we hypothesize that the ancestor of the North Atlantic *C. crispus* had a North Pacific origin. Although no red algal molecular clock has been inferred, an 18S rRNA gene clock has been calibrated for green algae (Olsen et al. 1994), which fits with the more general angiosperm 18S rRNA gene clock of 1% divergence per 25 Ma (Ochman & Wilson 1987). Bakker et al. (1995) estimated that for the cold-water green alga *Cladophora*, the ITS regions were evolving at about 0.8–2% Ma^{-1}. If this evolutionary rate applies to the Northern Pacific *Chondrus* species pairs and *C. crispus* from the North Atlantic, the average ITS distance of 3.1% would represent 1.55–3.875 Ma, a range that suggests that the separation between Atlantic *C. crispus* and other Pacific *Chondrus* species correlates with the opening of the Bering Strait ca. 3.5–6.4 Ma ago (Vermeij 1991; Marincovich & Gladenkov 1999) and the closure of the Panamanian Isthmus (Lindstrom 2001).

We interpret the current distribution of the Northern Atlantic *C. crispus* as resulting from subsequent vicariant events. Dunton (1992) dubbed the "paradox of the trans-Arctic exchange" and attributed the lower intrusion rate between the Atlantic and the Pacific to poor dispersal capacities, unfavourable current regimes, lack of sufficiently hard substrata and ice scouring, which would have affected seaweeds more than other benthic faunal assemblages. It has also been assumed that the presence of a richer endemic marine flora in the North Pacific empirically demonstrates the putatively restricted interchange (Lüning 1990) between the Pacific and the Atlantic. However, in a recent review of the Arctic marine flora, Lindstrom (2001) concluded that 85–100% of the Arctic flora is shared with the North Atlantic and 55–80% with the North Pacific, and argued that marine floral exchanges have occurred with greater frequency from both directions than previously supposed. We therefore believe that the trans-Arctic exchange has played an important role in the speciation of *C. crispus* within the North Atlantic. Late Miocene and early Pliocene temperatures were around 5–6°C warmer in the North Atlantic and Arctic Oceans than now, permitting the initial trans-Arctic passage of cold temperate species (Vermeij 1991). However, two dramatic changes took place beginning around 3.0 Ma. At that time, the cold-water Labrador Current displaced warm North Atlantic currents. This event created a significant thermal gradient in the North Atlantic, and warm temperature floras were abruptly replaced with polar, subpolar and cool temperate floras along the continental shelf of Nova Scotia and New England (Cronin 1988). Subsequently, northern hemisphere glaciations displaced the flora latitudinally, giving rise to the present day floral provincialization (Berggren & Hollister 1974). The dramatic cooling of the northwestern North Atlantic probably initiated the separation of North Atlantic *Chondrus* into European and North American populations. Subsequent Pleistocene glaciation would have prevented the long-term establishment of new populations in New England, as most of the North American coast was covered by a kilometre of ice during glaciation maxima (Kelley et al. 1995). There are a greater number of *C. crispus* populations along the European coast and fewer along the North American coast.

In this study, nrDNA ITS data suggest that the ancestor of the North Atlantic *C. crispus* clade originated in the North Pacific was dispersed through the Arctic Ocean after the opening of the Bering Strait during the Pliocene or Pleistocene and radiated the forms that comprise the North Atlantic species: *C. crispus*. The present distribution of North Atlantic *C. crispus* may be attributed to trans-Arctic dispersal and climate change followed by vicariance, and may serve as a useful example for understanding the historical biogeography of other marine algae.

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