

RuBisCO cistron sequence variation and phylogeography of *Ceramium kondoi* (Ceramiaceae, Rhodophyta)

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Abstract

Ceramium kondoi is a morphologically variable ceramiaceous red alga that occurs commonly in the northwest Pacific Ocean region and has recently been reported in the United States. Forty-five specimens of *C. kondoi* from 29 locations in Korea, Japan, Russia, and the USA were examined for DNA sequence variation along the whole plastid-encoded RuBisCO cistron: 1406 bp for *rbcL*, 103 bp for the spacer, and 387 bp for *rbcS*. *C. kondoi* specimens were variable, having up to nine haplotypes, with six found in Korea, southern Japan, far-eastern Russia, and the USA, and three found in northern Japan and far-eastern Russia. The occurrence of the same haplotype on both sides of the North Pacific Ocean provides evidence of recent introduction of the species from the west to the east. Phylogenetic reconstructions revealed the monophyly of *C. kondoi* and two well-supported lineages: a 'southern lineage' that contained specimens from Korea, southern Japan, far-eastern Russia and the USA, and a 'northern lineage' that included specimens from northern Japan and far-eastern Russia. The two genetic lineages are morphologically indistinguishable and referred to as cryptic species. The boundary between the two cryptic species is at the Tsugaru Strait, Japan.

Keywords: Ceramiaceae; *Ceramium kondoi*; phylogeography; RuBisCO cistron.

Introduction

The genus *Ceramium* Roth is one of the largest red algal genera, consisting of some 190 species (Boo and Lee 1994). The genus is cosmopolitan and is distributed from tropical to boreal waters around the world. It is common as an epiphyte on other algae in marine and estuarine habitats and is frequently part of the fouling community on floating docks (Cho et al. 2001).

Ceramium is circumscribed by ovoid to angular cortical cells growing in both acropetal and basipetal directions, three to five corticating filaments produced from each

periaxial cell, spermatangia produced from cortical cells, cystocarps with involucre branches, and tetrasporangia immersed in axes and branches (e.g., Dixon 1960, Hommersand 1963, Boo and Lee 1994). The genus is lectotypified by *C. virgatum* Roth, which commonly occurs in the North Atlantic Ocean region (Maggs et al. 2002). *C. virgatum* is fully corticated on the axis, and is very similar in habit to *C. kondoi* Yendo from the northwestern Pacific Ocean (Yendo 1920, Nakamura 1965, Boo and Yoon 1993).

Ceramium kondoi is an annual alga commonly found in Japan, its type locality (Yendo 1920, Nakamura 1965), and also occurs frequently in Korea (Boo and Yoon 1993), China (Tseng 1983), and far-eastern Russia (Adrianov and Kussakin 1998). It is distinguished by fully corticated axes, alternate and trichotomous branches, and tetrasporangia formed from periaxial cells or elongated cortical cells (Yendo 1920, Nakamura 1950, 1965, Boo and Yoon 1993). The type locality of *C. kondoi* is Harius, Hokkaido, Japan (Yendo 1920, Boo and Yoon 1993). The species has a *Polysiphonia*-type life history in culture (Notoya and Yabu 1979), and its morphology and life history are identical in the field and in culture (Boo and Yoon 1993). The chromosome number of haploid tetraspores of *C. kondoi* from Japan is $n=12-15$ (Notoya and Yabu 1979).

Ceramium kondoi is abundant from spring to summer on rocks associated with sand, or as an epiphyte on other algae in Korea (Boo and Yoon 1993) and Japan (Nakamura 1965). The species is so variable in its mode of ramification and degree of cortication that four forms have been described: forma *abbreviatum* Nakamura, f. *ambiguum* Nakamura, f. *kondoi* Nakamura, and f. *trichotomum* Nakamura (Nakamura 1950, 1954, 1965). However, these forms are not currently recognized owing to the occurrence of intermediates and our limited knowledge of environmental variation (Boo and Yoon 1993). Although *C. kondoi* also occurs from Alaska (Wynne 1990) to Oregon (Cho et al. 2001), its occurrence in the northeastern Pacific Ocean is thought to result from a recent introduction from the northwestern Pacific because of its single collection site in Alaska (Wynne 1990) and its recent and rare occurrence on the coast of North America (Cho et al. 2001, this study).

DNA sequence data are now recognized as an integral tool in addressing questions on species identification and phylogenetic relationships (Bickford et al. 2006). It is crucial to find 'natural' species that are supported by both morphological and phylogenetic data to contribute to proper evaluation of biodiversity. In red algae, several examples of multiple cryptic species have been described: five cryptic species are found in the *Bostrychia radicans* E. Post /*B. moritziana* E. Post complex (Zuccarello et al. 1999), three species that are described from *Grateloupia filicina* (C. Agardh) Lamouroux, (De

Clerck et al. 2005), and at least eight cryptic species found in *Plocamium cartilagineum* (Linnaeus) P.S. Dixon (Saunders and Lehmkuhl 2005). However, although it is very likely that single red algal taxa harbor cryptic species, there are still relatively few reports of this phenomenon in the phylum.

The RuBisCO cistron is plastid-encoded and consists of a large subunit (*rbcL*), a small subunit (*rbcS*), and its intervening spacer (Valentin and Zetsche 1989). The *rbcL* and spacer sequences are commonly used to address relationships within and between species of red algae (Zuccarello et al. 2000, Yang and Boo 2004). Recently, *rbcS* has been used to identify species of *Antithamnion* Nägeli (Lee et al. 2001).

In the present study, we examined sequences of the RuBisCO cistron from 45 specimens of *Ceramium kondoi* collected over its distributional range to uncover genetic variation in the species and to use plastid haplotypes in explaining the current distribution. We also analyzed the *rbcL* region from *C. boydenii* A. Gepp et E.S. Gepp and *C. japonicum* Okamura and, together with previously published *rbcL* sequences for *Ceramium*, constructed trees for a better understanding of the phylogeny of this group.

Materials and methods

Sampling

Forty-five specimens of *Ceramium kondoi* were used in this study: 14 from Korea, 21 from Japan, 4 from Russia, and 6 from the USA. All samples, including material from Harius, Hokkaido, Japan, the type locality of the species (Nakamura 1950, Boo and Yoon 1993), were taken from the field (Table 1, Figure 1). We also included two fully corticated species, *C. boydenii* and *C. japonicum*, from Korea and Japan. All thalli were air-dried or preserved with silica gel in the field. Epiphytes were removed under a dissecting microscope in the laboratory. The sources of the algal material and the GenBank accession numbers are listed in Table 1. Vouchers of all taxa used in the analyses are deposited in the herbarium of Chungnam National University, Daejeon, Korea (CNUK) and the herbarium of the University of Washington, Seattle, WA, USA (WTU).

DNA extraction, amplification, and sequencing

Genomic DNA was extracted from approximately 5 mg of dry thalli ground in liquid nitrogen using the Qiagen DNeasy Plant Mini Kit (Qiagen, Hilden, Germany) or the Invisorb Spin Plant Mini Kit (Invitek, Berlin-Buch, Germany) according to the manufacturers' instructions. The *rbcL*-spacer region was amplified in two sections using primers F7–R753 and F645–RrbcS start (Freshwater and Rueness 1994, Lin et al. 2001) and sequenced using the same primers. The *rbcS* region was amplified and sequenced using primers *rbcS5* and *rbcS2-2* (Lee et al. 2001). The PCR products were purified using a High Pure PCR Product Purification Kit (Roche Diagnostics, Mannheim, Germany) in accordance with the user's guide.

The sequences of the forward and reverse strands were determined for all taxa using an ABI PRISM™ 377 DNA Sequencer with the BigDye terminator cycle sequencing kit (Applied Biosystems, Foster City, CA, USA) at the Research Center, Chungnam National University, Daejeon, Korea. The electropherogram outputs for each specimen were edited using the program Sequence Navigator v. 1.0.1 (Applied Biosystems). Alignment of each gene sequence was based on alignment of the inferred amino acid sequence and was refined by eye. There were no gaps in the aligned RuBisCO sequences from *Ceramium kondoi*.

Phylogenetic analyses

Haplotype genealogies for the RuBisCO cistron sequences from 45 *Ceramium kondoi* specimens were computed using the Java computer program TCS Version 1.21 (Clement et al. 2000). The TCS program calculates the minimal number of mutational steps by which the sequences can be joined with >95% confidence.

Analysis of phylogenetic relationships among *rbcL* haplotypes of *Ceramium kondoi* was conducted using PAUP* 4.0b10 (Swofford 2002). The *rbcL* data set consisted of nine haplotypes of *C. kondoi*, *C. boydenii*, *C. japonicum*, and published sequences of 17 species. *Antithamnion nipponicum* Yamada et Inagaki and *Centroceras clavulatum* (C. Ag.) Montagne were used as outgroups, since they belong to the subfamily Ceramioideae, which includes *Ceramium* (Hommersand 1963). Maximum parsimony (MP) analysis was performed using a heuristic search algorithm with the following settings: 1000 random addition sequences, tree bisection reconnection (TBR) branch swapping, MulTrees, all characters unordered and unweighted, and branches with a maximum length of zero collapsed to yield polytomies (Felsenstein 1985). The robustness for individual nodes was determined by bootstrapping (MPB) the data set 1000 times (Felsenstein 1985).

Before maximum likelihood (ML) and Bayesian analyses (BA), we applied the Akaike Weights criterion implemented in Modeltest v.3.7 (Posada and Crandall 1998) to determine the best model for the *rbcL* data set. The recommended model was a general time reversible (GTR) model with the proportion of invariable sites (I) and gamma distribution (γ). ML analyses were performed using PAUP* with a GTR+I+ γ model, and the parameters followed were: $R_{AC}=1.0505$, $R_{AG}=4.1244$, $R_{AT}=2.2825$, $R_{CG}=1.0461$, $R_{CT}=18.9146$, $R_{GT}=1$, $I=0.6056$, $\gamma=1.5856$ and empirical base frequencies ($\pi_A=0.3225$, $\pi_C=0.1611$, $\pi_G=0.2074$, and $\pi_T=0.309$). Tree likelihood was estimated using a heuristic search with 100 random addition sequence replicates and TBR branch swapping. The robustness of individual nodes was determined by bootstrapping (MLB) the data set with 100 replicates.

Bayesian analyses were performed using MrBayes 3.1.2 software (Ronquist and Huelsenbeck 2003) with the GTR+I+ γ model. Two independent analyses were initiated from a random starting tree and the program was set to run four chains of Markov chain Monte Carlo iterations simultaneously for 2 million generations, with trees sampled every 100th generation. The likelihood scores

Table 1 Information on samples used in the present study.

Taxon and locality	Voucher	Haplotype	GenBank accession number		
			<i>rbcL</i>	Spacer	<i>rbcS</i>
<i>Ceramium kondoi</i>					
Korea: Boryong: Daechun	C149	A	AY770629	AY178522	DQ350390
Korea: Boryong: Oeyondo	C158	B	AY770630	AY770658	DQ350391
Korea: Sinan: Sudori	C157	B	AY770631	AY770659	DQ350392
Korea: Buan: Gyukpo	C145	B	AY770632	AY770660	DQ350393
Korea: Jindo: Hoidong	C173	B	AY770633	AY770661	DQ350394
Korea: Namhaedo: Seosang	C148	E	AY770634	AY178523	DQ350395
Korea: Sachun: Daebang	C378	E	AY770635	AY770662	DQ350396
Korea: Tongyong: Songbang 1	C155	C	AY770636	AY770663	DQ350397
Korea: Tongyong: Songbang 2	C156	C	AY770637	AY770664	DQ350398
Korea: Tongyong: Songbang 3	–	C	–	AY770665	–
Korea: Taean: Bangpo	C554	A	AY770638	AY770666	DQ350399
Korea: Taean: Hakampo	C169	B	AY295176	AY770667	DQ350400
Korea: Taean: Manripo	C152	B	AY770639	AY770668	DQ350401
Korea: Taean: Padori	C159	B	AY770640	AY770669	DQ350402
Japan: Aomori: Asamushi 1	C1307	D	DQ350377	DQ350364	DQ350403
Japan: Aomori: Asamushi 2	C1308	D	DQ350378	DQ350365	DQ350404
Japan: Aomori: Asamushi 3	C1309	D	DQ350379	DQ350366	DQ350405
Japan: Aomori: Asamushi 4	C1310	D	DQ350380	DQ350367	DQ350406
Japan: Aomori: Asamushi 8	C1314	D	DQ350381	DQ350368	DQ350407
Japan: Aomori: Senjoujiki 1	C1316	E	DQ350382	DQ350369	DQ350408
Japan: Aomori: Senjoujiki 2	C1317	E	DQ350383	DQ350370	DQ350409
Japan: Aomori: Senjoujiki 3	C1318	F	DQ350384	DQ350371	DQ350410
Japan: Aomori: Senjoujiki 7	C1322	E	DQ350385	DQ350372	DQ350411
Japan: Chiba: Nagasaki 1	C866	E	AY770647	AY770676	DQ350421
Japan: Chiba: Nagasaki 3	C868	E	AY770648	AY770677	DQ350422
Japan: Fukuoka: Shikanoshima	C178	E	AY770649	AY770678	DQ350423
Japan: Hokkaido: Akkeshi	C163	H	AY770641	AY770670	DQ350412
Japan: Hokkaido: Asari	C165	I	AY770642	AY770671	DQ350413
Japan: Hokkaido: Otamoi 1	C1305	I	DQ350386	DQ350373	DQ350414
Japan: Hokkaido: Otamoi 2	C1306	I	DQ350387	DQ350374	DQ350415
Japan: Hokkaido: Harius	C170	I	AY770643	AY770672	DQ350416
Japan: Hokkaido: Rishiri	C176	G	AY770644	AY770673	DQ350417
Japan: Hokkaido: Kitami Esashi	C174	G	AY770645	AY770674	DQ350418
Japan: Hokkaido: Wakkanai	C183	I	AY770646	AY770675	DQ350420
Japan: Niigata: Itoigawa	C1315	E	DQ350388	DQ350375	DQ350419
Russia: Nakhodka	C175	G	AY770650	AY770679	DQ350424
Russia: Vladivostok 1	C180	E	AY770651	AY770680	DQ350425
Russia: Vladivostok 2	C181	E	AY770652	AY770681	DQ350426
Russia: Vladivostok Harbor	C182	G	AY770653	AY770682	DQ350427
USA: Alaska: Sitka: Halibut Point 1	C1595	E	EF186997	EF186993	EF187001
USA: Alaska: Sitka: Halibut Point 3	C1597	E	EF186998	EF186994	EF187002
USA: Alaska: Sitka: Ferry terminal	AC182	E	EF186999	EF186995	EF187003
USA: Washington: San Juan Island: Argyle	AC240	E	EF187000	EF186996	EF187004
USA: Oregon: Coos Bay 1	C167	E	AY770654	AY770683	DQ350428
USA: Oregon: Coos Bay 2	C168	E	AY770655	AY770684	DQ350429
<i>Ceramium boydenii</i>					
Korea: Jindo: Hoidong	C116		AY770656	AY178519	DQ350430
Japan: Hokkaido: Hakodate	C1304		DQ350389	DQ350376	–
<i>Ceramium japonicum</i>					
Korea: Namhaedo: Gasun	C135		AY770657	–	–

stabilized at approximately 500 000 generations; thus, the first 5000 trees were removed as a burn-in point. Remaining trees were used to infer the Bayesian posterior probabilities (BPP).

Results

A total of 1896 bp of the RuBisCO cistron (1406 bp for *rbcL*, 103 bp for the spacer, and 387 bp for *rbcS*) was aligned for 45 *Ceramium kondoi* samples. *C. kondoi* had 26 (1.4%) variable positions over the RuBisCO cistron

(Table 2). The *rbcL* region contained 15 variable sites (1.1%); 12 substitutions were at the third codon position and were silent, and three substitutions (positions 255, 792, and 1014) were at the first codon position and were non-synonymous (Table 2). The spacer region included only two variable sites (1.9%). The *rbcS* region had nine variable sites (2.3%): seven synonymous changes at the third codon position and two non-synonymous changes (positions 1696 and 1714) at the first codon position (Table 2).

The pairwise divergence among *Ceramium kondoi* haplotypes ranged from 1 bp change (0.05%) to 19

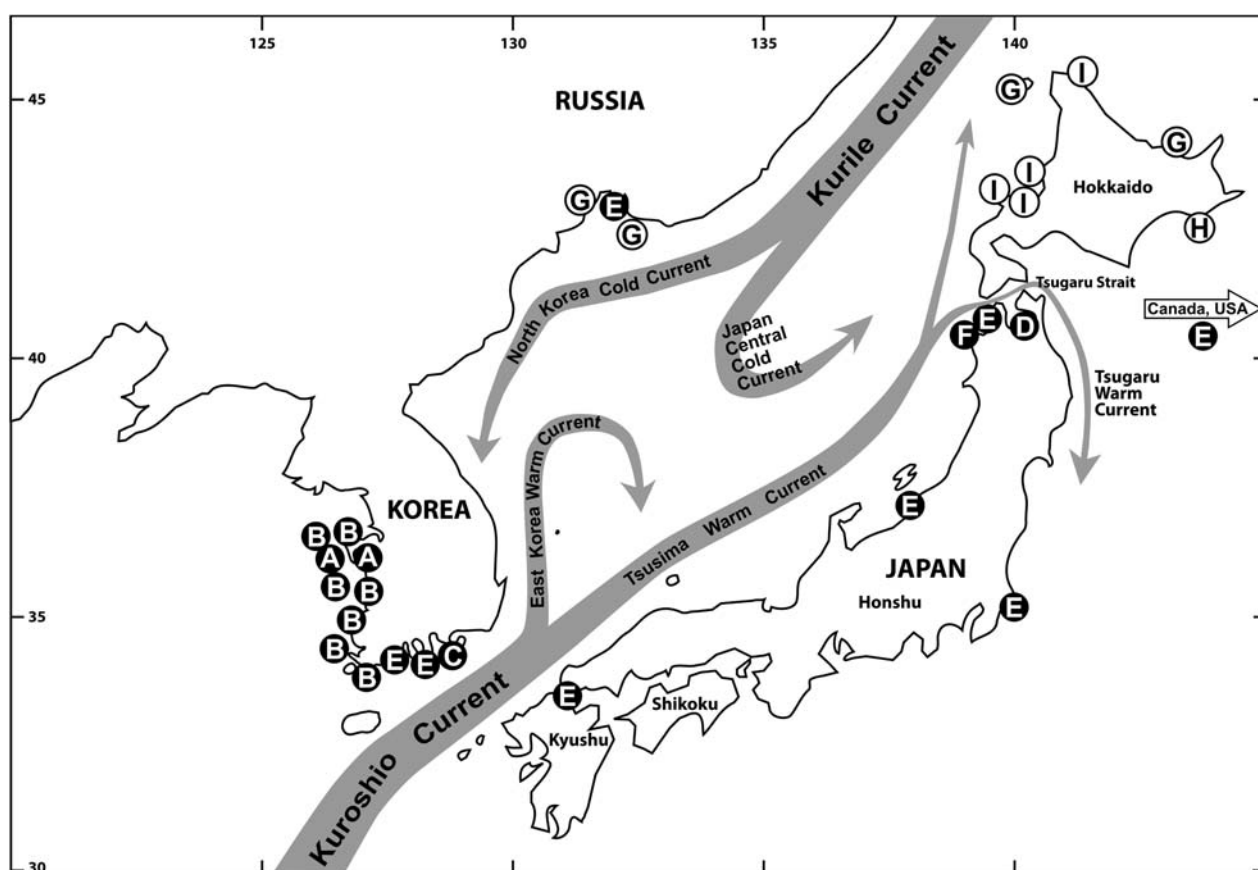


Figure 1 *Ceramium kondoi*: distribution of haplotypes in the northwestern Pacific Ocean.

Currents are marked by bold lines with arrows. Dark circled haplotypes (A–F) indicate the southern lineage and open circled haplotypes (G–I) indicate the northern lineage. Other details are in Table 1.

changes (1%). In total, nine haplotypes of *C. kondoi* were found (Tables 1 and 2, and Figure 1). Haplotypes A and B, sharing two non-synonymous mutations, occurred on the west coast of Korea. Haplotype D occurred on the south coast of Korea. Haplotypes C and F were found in northern Honshu, Japan. Haplotype E occurred in Korea, Japan, Russia, and the USA. Haplotype H occurred in northeastern Hokkaido, and haplotype G was found in Vladivostok, Russia and northwestern and northern Hokkaido. Haplotype I occurred in western and northern Hokkaido.

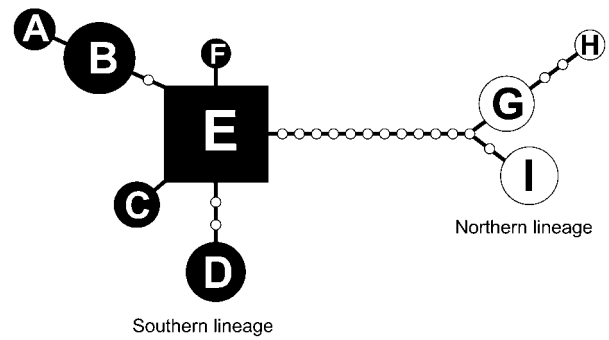
A parsimony network of the RuBisCO cistron haplotypes clearly identified two clades, which were divided into two major assemblages designated as the ‘southern lineage’ and the ‘northern lineage’. The southern lineage included six haplotypes (A–F) from Korea, southern Japan, far-eastern Russia, and the USA, whereas the northern lineage contained three haplotypes (G–I) from Hokkaido, Japan, and far-eastern Russia (Figure 2). Within the southern group, six haplotypes differed from each other by 1–6 bp. Haplotype A was connected to haplotype B, having two mutational steps to haplotype E. Haplotype E was basal, connecting with haplotypes B, C, D, and F. Haplotypes C and F had more sequence similarity than haplotype D, which had two mutational steps to haplotype E. In the northern lineage, three haplotypes differed from each other by 3–6 bp. Haplotype G had two mutational steps with haplotypes H and I.

A total of 1406 bp of the *rbcL* region was available for analysis within the genus *Ceramium*. Of these, 419 positions were variable (29.8%) and 302 (21.5%) were parsimoniously informative. The ML tree based on the *rbcL* sequences is illustrated in Figure 3, and is congruent with the Bayesian and MP trees (tree length, 1094; consistency index, 0.482; and retention index, 0.623). *C. kondoi* was grouped into two lineages corresponding to the previously defined ‘southern’ and ‘northern’ lineages (Figures 2 and 3). The monophyly of each lineage was well supported (MLB/MPB/BPP=65:86:0.98 and 89:95:0.99, respectively). The two divergent lineages of *C. kondoi* were strongly monophyletic.

The sister relationship of *Ceramium kondoi* was not resolved, but it produced a clade with *C. boydenii*, *C. californicum* J. Agardh, *C. inkyuii* T.O. Cho, S. Fredericq et S.M. Boo, *C. tenerrimum* (G. Martens) Okamura, *C. brevizonatum* H.E. Petersen, *C. paniculatum* Okamura, and *C. affine* Setchell et N.L. Gardner (84% for MLB, 95% for MPB, and 1.0 for BPP). However, the basal position of *C. japonicum* was not supported. Northeastern Pacific species such as *C. sinicola* Setchell et Gardner, *C. codicola* J. Agardh, *C. interruptum* Setchell et Gardner, and *C. horridum* Setchell et Gardner produced a strong clade (100% for MLB and MPB and 1.0 for BPP). The Atlantic group, consisting of *C. secundatum* Lyngbye, *C. botryocarpum* A.W. Griffiths ex Harvey, *C. virgatum* Roth, *C. pallidum* (Nägeli ex Kützinger) Maggs et Hommersand, *C. diaphanum* (Lightfoot) Roth, *C. deslongchampsii* Chau-

Table 2 *Ceramium kondoi*: compressed alignment of variable sites.

Haplotype	rbcL										Spacer										rbcS									
	113	134	255	407	497	578	698	734	792	806	878	1014	1136	1187	1337	1453	1491	1617	1635	1668	1696	1714	1716	1734	1788	1806				
A	T	A	T	A	G	G	T	C	A	G	T	A	C	T	A	G	C	G	C	A	G	C	G	T	T	T				
B	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	A	-	-	-	-	-				
C	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	G	A	A	-	-	-	-				
D	-	-	-	-	-	-	C	-	-	-	-	-	-	-	-	A	-	-	-	-	A	A	T	-	-	-				
E	-	-	-	-	-	-	-	-	-	-	-	G	-	-	-	-	-	-	-	-	A	A	-	-	-	-				
F	-	-	-	-	-	A	-	-	-	-	-	G	-	-	-	-	-	-	-	-	A	A	-	-	-	-				
G	C	-	G	G	T	-	-	-	G	A	C	G	T	C	G	-	-	A	T	-	-	A	-	-	-	C				
H	C	G	G	G	T	-	-	G	G	A	C	G	T	C	G	-	T	A	T	-	A	A	-	-	-	C				
I	C	-	G	G	T	-	-	-	G	A	-	G	T	C	G	-	-	A	T	-	A	A	C	-	-	C				

**Figure 2** *Ceramium kondoi*: the most parsimonious network based on RuBisCO (1896 bp) estimated by the TCS algorithm. Filled circles and box indicate the southern lineage, and open circles indicate the northern lineage. Haplotype E (filled box) was inferred as the hypothetical ancestral haplotype of the species. Small unlabelled circles represent missing haplotypes. Each line represents a single mutational step connecting two haplotypes. Circle sizes are proportional to haplotype frequencies.

vin ex Duby, and *C. echionotum* J. Agardh, was moderately supported (71% for MLB, 59% for MPB, and 1.0 for BPP).

Discussion

This study involved analyses of RuBisCO cistron sequences for a better understanding of the genetic variation and distribution of *Ceramium kondoi* collected from the North Pacific Ocean over a wide geographic range. The main finding of the study is that *C. kondoi* is strongly monophyletic and falls into two divergent groups, 'southern' and 'northern' lineages that are both characterized by a high number of substitutions and support values. The southern lineage contains samples from Korea, Honshu (Japan), Russia, and the USA, and the northern lineage contains samples from Hokkaido (Japan) and Russia. The boundary between the southern and northern lineages in Japan is at the Tsugaru Strait between Honshu and Hokkaido, which is a channel approximately 160 km long and 24–40 km wide. The differentiation between lineages is most likely due to a vicariant event, i.e., disruption of a formerly continuous distribution area, and survival in disjunctive glacial refugia.

Our results are consistent with those of a mitochondrial ND5 sequence study on ground beetles (*Damaster blaptoides rugipennis* Kollar and *D. b. viridipennis* Lewis) between Hokkaido and northern Honshu, which are tightly linked to geography, with a sharp distribution boundary, the Tsugaru Strait, between them (Su et al. 1998). In this case, the ancestor in northern Honshu moved to Hokkaido and the two groups were then geographically isolated approximately 13 million years ago (MYA). The Tsugaru Strait is also considered to be a distribution boundary between two groups of *Tomocarabus opaculus* Putzeys (another species of ground beetle), which possibly separated approximately 20 MYA, after which the Hokkaido group diversified approximately 13 MYA (Su et al. 2000).

The water surrounding Hokkaido is colder, being influenced mostly by the cold Kurile and East Sakhalin

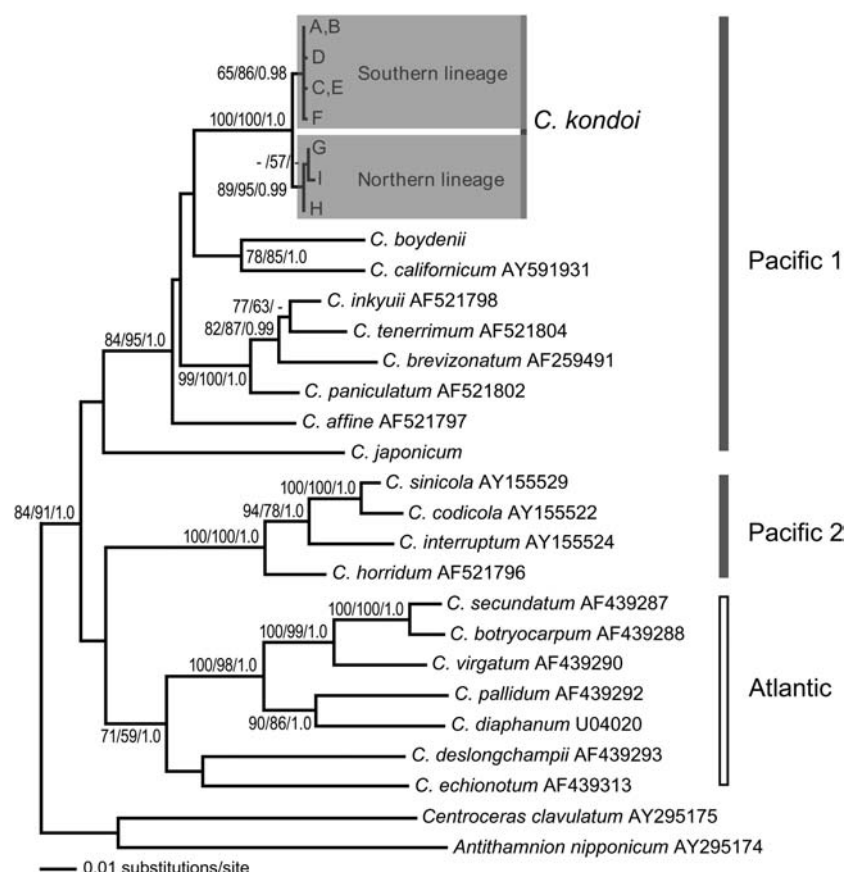


Figure 3 Maximum likelihood tree for *Ceramium kondoi* and other species of the genus inferred from the *rbcL* sequences. Numbers above or below the branches indicate bootstrap support values for maximum likelihood/maximum parsimony/Bayesian posterior probabilities. Dashes represent nodes with values <50% and 0.95.

Currents, than Honshu, which is influenced mostly by two branches of the warm Kuroshio Current (Yamada 1980). Therefore, these two lineages of *Ceramium kondoi* appear to reflect the oceanographic difference between the areas they inhabit. In the southern lineage, haplotypes A and B are adapted to the west coast of Korea, where considerable amounts of nutrients and freshwater flow from surrounding rivers. *C. kondoi* grows well in areas with freshwater influence, such as estuarine areas and intertidal regions on the west coast of Korea, which sometimes receive an influx of freshwater and nutrients from rivers in Korea and China (Boo and Yoon 1993); it is occasionally the most abundant intertidal alga in Korea and Japan during the summer months (Boo and Lee 1994). Its distribution appears punctuated, thereby creating limited gene flow between neighboring populations and resulting in the geographic structure of the species. Haplotypes C, D, and F are each limited to their own areas. Haplotype E is the most abundant and is discussed below.

The northern lineage is also geographically structured, with haplotype G in northern Hokkaido and Vladivostok, haplotype H in eastern Hokkaido, and haplotype I in western and northwestern Hokkaido. The occurrence of haplotype G in Japan and far-eastern Russia may be due to a natural dispersal event because both areas are under the influence of the cold Kurile Current.

Haplotype E is the most common and probably ancestral haplotype owing to its central position in the network (Figure 2). It is distributed most widely in the Northwest Pacific Ocean (Korea, Japan, and Russia), and is also found in the Northeast Pacific Ocean. In North America, it was first reported in Alaska (Wynne 1990), and we also reported the occurrence of the species in Bamfield, British Columbia, Canada, and Coos Bay and Netarts Bay, Oregon, USA (Cho et al. 2001). The occurrence of an ancestral haplotype on both sides of the Pacific Ocean may indicate a widely distributed species that was partitioned, with populations on either side of the Pacific Ocean being isolated with minimal gene flow. Subsequent haplotypes would arise that are unique to the region, as happened in Korea and Japan. However, although we collected samples of the species from Alaska to Oregon, locations much more distantly located than the distance from Korea to Japan, only one haplotype was found there. This is one reason why we do not believe that this 'wide ancestral distribution' scenario is correct. Another relevant fact is that *Ceramium kondoi* is not common in North America and has been recorded only recently. The earliest report in Alaska was by Wynne (1990); however, he mentioned that "it is likely that material has been questionably identified as *Ceramium rubrum*." In the herbarium of the University of Washington and Exsiccatae of North American Marine algae

(Collins et al. 1895–1919), there are no specimens of *C. kondoi*. Despite three intensive collection trips during the present study, we did not find the species in California. *C. kondoi* was not found in any harbor known for international transport; however, it has been encountered adjacent to a ferry terminal in Sitka, Alaska, where the ferries from Washington State land. This is also in close proximity to the site of a former pulp mill that was owned and operated by a Japanese company from 1959 to 1993; Japan's reliance on Alaskan timber ensured consistent boat traffic between these areas throughout this time period (http://www.commerce.state.ak.us/oed/forest_products/forest_products1.htm). We confirmed its occurrence near oyster farms in Washington and Oregon through our molecular data. It is also found in floating docks at Willapa Bay, Washington, which is filled with oyster farms, further supporting our hypothesis that *Ceramium kondoi* was recently introduced (Gayle Hansen, unpublished data). There are other marine species that have been transported with oysters used to seed farms (Ruiz et al. 1997). The above results lead to the conclusion that *C. kondoi* was recently introduced from the Northwest Pacific (occurring abundantly there and having high genetic diversity) to the Northeast Pacific Ocean. We believe that haplotype E had more opportunity for introduction to the Northeast Pacific coast than other haplotypes because of its common occurrence in the Northwest Pacific Ocean.

Should the two genealogical groups be recognized as distinct species? The disjunctive distributions of the two *Ceramium kondoi* groups and 12 substitutions in the RuBisCO cistron between them suggest that these are different species. However, morphological differences between the species (related to the distribution areas) have not been observed to date (Nakamura 1965, Boo and Yoon 1993). In the present study, we could not find any morphological variation between the two genetic groups collected in the two areas, and most of the features are closely congruent with previous descriptions of the species (Yendo 1920, Nakamura 1965, Boo and Yoon 1993). Therefore, the two phylogenetically divergent lineages of *C. kondoi* should be referred to as cryptic species at present.

The present RuBisCO data indicate that *Ceramium kondoi* is distantly related to *C. virgatum* and its putative relatives from the Atlantic Ocean. *C. kondoi* formed a monophyletic group with other Northwest Pacific Ocean species and is distantly related to a clade containing species from the Northeast Pacific Ocean. *Ceramium* from the North Atlantic Ocean is monophyletic and has no resolution with any of the Pacific groups. It is also interesting to note that *C. brevizonatum* Shperk from the Caribbean Sea clustered with some Pacific elements, indicating a route of dispersal for the genus across the present Isthmus of Panama, which was submerged until the late Pliocene, as proposed for other red algae by Zuccarello and West (2003). Additional sampling is necessary to address interesting phylogeographic questions as to the center of origin and current distribution patterns of the genus.

In summary, *Ceramium kondoi*, commonly occurring in the Northwest Pacific Ocean, is genetically variable in its RuBisCO cistron sequences, having nine haplotypes.

The recent report of the species in the USA is likely a result of anthropogenic transport from the west to the east of the North Pacific Ocean. The two cryptic species are separated by the Tsugaru Strait, which is a narrow channel between Hokkaido and Honshu, Japan. It is hypothesized that geographic fragmentation might have played an important role in the distribution of both lineages. This hypothesis may be further tested by similar studies of other marine algal species that occur on both sides of the Tsugaru Strait, Japan.

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