Phycologia (2007) Volume 46 (6), 657–665 Published 1 November 2007

Genetic diversity of *Scytosiphon lomentaria* (Scytosiphonaceae, Phaeophyceae) from the Pacific and Europe based on RuBisCO large subunit and spacer, and ITS nrDNA sequences

GA YOUN CHO[1], KAZUHIRO KOGAME[2]*, HIROSHI KAWAI[3] AND SUNG MIN BOO[1]

[1]*Department of Biology, Chungnam National University, Daejon 305-764, Korea*
[2]*Department of Natural History Sciences, Faculty of Science, Hokkaido University, Sapporo 060-0810, Japan*
[3]*Kobe University Research Center for Inland Seas, Rokkodai, Kobe 657-8501, Japan*

G.Y. CHO, K. KOGAME, H. KAWAI AND S.M. BOO 2007. Genetic diversity of *Scytosiphon lomentaria* (Scytosiphonaceae, Phaeophyceae) from the Pacific and Europe based on RuBisCO large subunit and spacer, and ITS nrDNA sequences. *Phycologia* 46: 657–665. DOI: 10.2216/06-70.1

Sequence variations of the internal transcribed spacers (ITS) 1 and 2 of the nrDNA and the partial RuBisCO large subunit gene-spacer-partial RuBisCO small subunit gene (*rbc*L-sp-S) region were investigated in samples of *Scytosiphon lomentaria* (Lyngbye) Link from 50 localities in the Pacific (Australia, Japan, Korea, New Zealand, Russia and United States) and the North Atlantic. ITS1 and ITS2 sequences were determined for 83 samples, the *rbc*L-sp-S region for 43 samples, and complete *rbc*L sequences for two European and three Japanese samples. Molecular phylogenetic analyses using *rbc*L sequences were performed including *S. lomentaria* and 15 other scytosiphonacean species. In the *rbc*L analyses the *S. lomentaria* samples made a clade consisting of a Pacific and a European subclade. These two subclades also were supported by the ITS and *rbc*L-sp-S analyses. The nucleotide differences in *rbc*L were 1.8–2.3% (27–33 bp/1,467 bp) between the two subclades. Such differences are so large that they are considered as indicating different, although cryptic, species. In the ITS analyses the Pacific clade was further divided into two well-supported subclades. In the Pacific clade sample localities were not geographically related to the molecular phylogeny: both subclades included samples from Korea, Japan, Oregon and New Zealand. Artificial translocations are suggested to have occurred because identical sequences were found from localities far from each other, for example, Korea and the United States, the United States and New Zealand. The two Pacific groups are possibly two distinct but cryptic species.

KEY WORDS: Cryptic species, Intraspecific genetic variation, ITS, Molecular phylogeny, *rbc*L, RuBisCO spacer, *Scytosiphon lomentaria*

INTRODUCTION

Intraspecific genetic diversity has been studied for some marine benthic algae that have wide geographical distributions both in the Pacific and Atlantic, and those studies have elucidated the presence of genetically isolated populations or cryptic species within a morphological species. For example, the Atlantic and Pacific isolates of the red alga *Dumontia contorta* (Gmelin) Ruprecht clearly showed sufficient genetic divergence in the internal transcribed spacers (ITS) region of the nuclear ribosomal gene and the nuclear small subunit ribosomal gene to warrant recognition as distinct species, and *Dumontia alaskana* Tai, Lindstrom & Saunders was proposed for the Pacific species (Tai *et al*. 2001). In the brown alga *Chordaria flagelliformis* (O.F. Müller) C. Agardh, molecular phylogenetic analyses of the intragenic spacer region (IGS) of rDNA showed three major genetic groups: group 1 (f. *chordaeformis* from Kamchatka, North Pacific), group 2 (f. *chordaeformis* from other areas) and group 3 (f. *flagelliformis*); f. *chordaeformis* was recognized at the species level from morphological data (Kim & Kawai 2002). Furthermore, for *Chorda filum* (Linnaeus) Stackhouse (Kawai *et al*. 2001), *Caulacanthus ustulatus* (Turner) Kützing (Zuccarello *et al*. 2002a), *Sphaerotrichia divaricata* (C. Agardh) Kylin (Kim *et al*. 2003), the *Bostrychia radicans* (Montagne) Montagne/*B. moritziana* (Sonder) J. Agardh complex (Zuccarello & West 2003) and *Colpomenia peregrina* (Sauvageau) Hamel (Cho *et al*. 2005), some intraspecific groups were recognized in widely collected samples by molecular analyses using DNA sequences of ITS, IGS, RuBisCO, as well as the mitochondrial *cox*2–3 spacer, and biogeographic and systematic discussions were provided.

The brown alga *Scytosiphon lomentaria* (Lyngbye) Link has a broad distributional range on cold and temperate coasts (Bold & Wynne 1985), growing in the intertidal and upper subtidal zones. The erect thalli of the alga are gregarious, simple, and tubular, typically with constrictions, but show considerable morphological variations with either complanate or cylindrical thalli and with various degrees of constriction (Wynne 1969; Clayton 1978; Kogame 1998). Furthermore, the morphogenetic responses (formation of erect or crustose thalli) to temperatures and photoperiods are variable depending on culture strains, indicating the existence of ecotypes (Lüning & Dring 1975; Dieck 1987; Kristiansen *et al*. 1991). Based on these features, it could be expected that this species includes several genetically isolated groups or several different species. We analyzed the ITS and the partial *rbc*L-spacer-partial *rbc*S (*rbc*L-sp-S) regions in *S. lomentaria* samples

* Corresponding author (kogame@sci.hokudai.ac.jp).

collected from Pacific and Atlantic coasts to investigate the intraspecific genetic variation in this species. In order to examine the monophyly of *S. lomentaria*, *rbc*L sequences of selected samples also were used for phylogenetic analyses that included additional scytosiphonacean species.

MATERIAL AND METHODS

Thalli of *Scytosiphon lomentaria* were collected from localities worldwide (Fig. 1, Table 1), and were dried in silica gel for morphological observations and molecular analyses. For morphological observations, dried samples were soaked in seawater. Total genomic DNA was extracted and purified from the dried samples or cultured material (Table 1) as described by Cho *et al.* (2005) or Kogame *et al.* (1999). Polymerase Chain Reaction (PCR) was performed in a thermal cycler, either a GeneAmp^{TM} PCR System 9600 or a System 2400 (Applied Biosystems, Foster City, CA, USA), for 35–40 cycles with denaturation at 94°C for 30 s, annealing at 55°C for 30 s and extension at 72°C for 45 s. DNA primers used in this study for PCR and sequencing were previously published by Yoon *et al.* (2001) (LB1, YB1 and LB2 for ITS), Saunders & Druehl (1993) (BC2, reverse primer for sequencing of ITS1), Goff *et al.* (1994) (5.8SBF, forward primer for ITS2), Kogame & Masuda (2001) (25BR2, reverse primer for ITS2), Yoon & Boo (1999) (RS1 and RS2 for *rbc*L-sp-S) and Kogame *et al.* (1999) (for *rbc*L and *rbc*L-sp-S). PCR products were precipitated to remove residual primers and dNTP and were sequenced directly using an ABI PRISM^{TM} BigDye Terminator Cycle Sequencing Kit (Applied Biosystems) and an ABI PRISM^{TM} 310 Genetic Analyzer (Applied Biosystems) DNA sequencer according to the manufacturer's protocols.

Sequences were aligned by eye. In *rbc*L analyses phylogenetic trees were constructed with previously published sequences of *S. lomentaria*, 15 other scytosiphonacean species, *Ectocarpus siliculosus* and *Pylaiella littoralis* (Table 2), setting the last two as outgroup taxa. The alignment of the ITS regions is available in the European Molecular Biology Laboratory (EMBL)-Align database (accession number: ALIGN 001127). The small-subunit (SSU) (135 bp), the 5.8S (162 bp) and the large-subunit (LSU) (35 bp) regions sequenced were almost identical among all samples and were excluded from phylogenetic analyses. Phylogenetic trees were inferred by the neighbor joining (NJ), maximum parsimony (MP), and maximum likelihood (ML) methods, using PAUP*4.0b10 (Swofford 2002). Gaps were treated as missing data. In NJ analyses, Kimura 2-parameter distance (ts/tv = 2.0) was used. MP analyses were performed in a heuristic search with a simple addition sequence and a tree bisection reconnection (TBR) branch-swapping option. ML trees were constructed using a best-fit model selected for likelihood settings by Akaike information criterion (AIC) in Modeltest (v. 3.06; Posada & Crandall 1998). In *rbc*L analyses a TrN + I + G substitution model was used, and the base frequencies were: A = 0.3030; C = 0.1556; G = 0.2107; T = 0.3307 and (A–C) = 1.0000; (A–G) = 4.3545; (A–T) = 1.0000; (C–G) = 1.0000; (C–T) = 8.7918; (G–T) = 1.0000 in the substitution model. In *rbc*L-sp-S analyses the best-fit model was TrN + I, and the base frequencies were: A = 0.3344, C = 0.1391, G = 0.1917, T = 0.3348; (A–C) = 1.0000, (A–G) = 5.6769, (A–T) = 1.0000, (C–G) = 1.0000, (C–T) = 7.2858, (G–T) = 1.0000. Bootstrap analyses (1000 replicates in NJ and MP of *rbc* trees, 500 in MP of ITS trees, 100 in ML trees) were used to estimate the stability of topologies of the inferred trees. ITS sequences determined all were included in NJ analyses, but only 34 sequences were used in MP analyses, excluding identical and similar sequences, because the analyses including all sequences were difficult to complete. Mid-point rooting was used in the ITS analyses. In *rbc*L-sp-S analyses only 17 sequences were used, excluding identical sequences, and a mid-point rooting was used.

RESULTS

Morphology

Thalli were tubular and cylindrical with constrictions except for thalli from Muroran1 and 2, which were complanate and without constrictions. Plurilocular organs were uniseriate and partly biseriate, loosely coherent to each other, lacking a cuticular cover when mature. Ascocysts (paraphyses) accompanied the plurilocular organs in mature thalli.

*Rbc*L analyses

*Rbc*L sequences (1,467 bp) were determined for 5 samples of *S. lomentaria* in this study (Table 1). In all analyses, *S. lomentaria* samples made a well-supported clade with high bootstrap values (99% in NJ, 95% in MP, 96% in ML) although tree topologies were different in other nodes, with low bootstrap values among the three analyses. Two most parsimonious trees (tree length = 537, consistency index (CI) = 0.609, rentention index (RI) = 0.655) were produced in MP analyses. Only the ML tree (−log-likelihood = 4,909.56685) is shown in Fig. 2. The *S. lomentaria* clade consisted of two well-supported subclades (>99% in all analyses) that consisted of the Pacific and European samples, respectively. Nucleotide differences were 27–33 bp (1.8–2.3%) between the Pacific and European clades. Maximum nucleotide differences within each subclade were small, 5 bp (0.3%) in the Pacific and 4 bp (0.3%) in the European clade. Further subclades in these subclades were not well resolved.

ITS analyses

The lengths of ITS1 sequences were variable, ranging from 452 to 523 bp, and ITS2 sequences were from 241 to 255 bp, so there were many gaps in the alignment. The NJ tree based on the ITS1 and ITS2 sequences of all 83 samples is shown in Fig. 3. The MP analysis based on only 34 representative sequences produced 16,065 most parsimonious trees of 318 steps (CI = 0.836, RI = 0.964), and a strict consensus tree of these most parsimonious trees is shown in Fig. 4. The NJ and MP trees showed three clades (A, B, and C), well supported with 100% bootstrap values. Two clades (Clades A and B) consist of only Pacific samples except for one

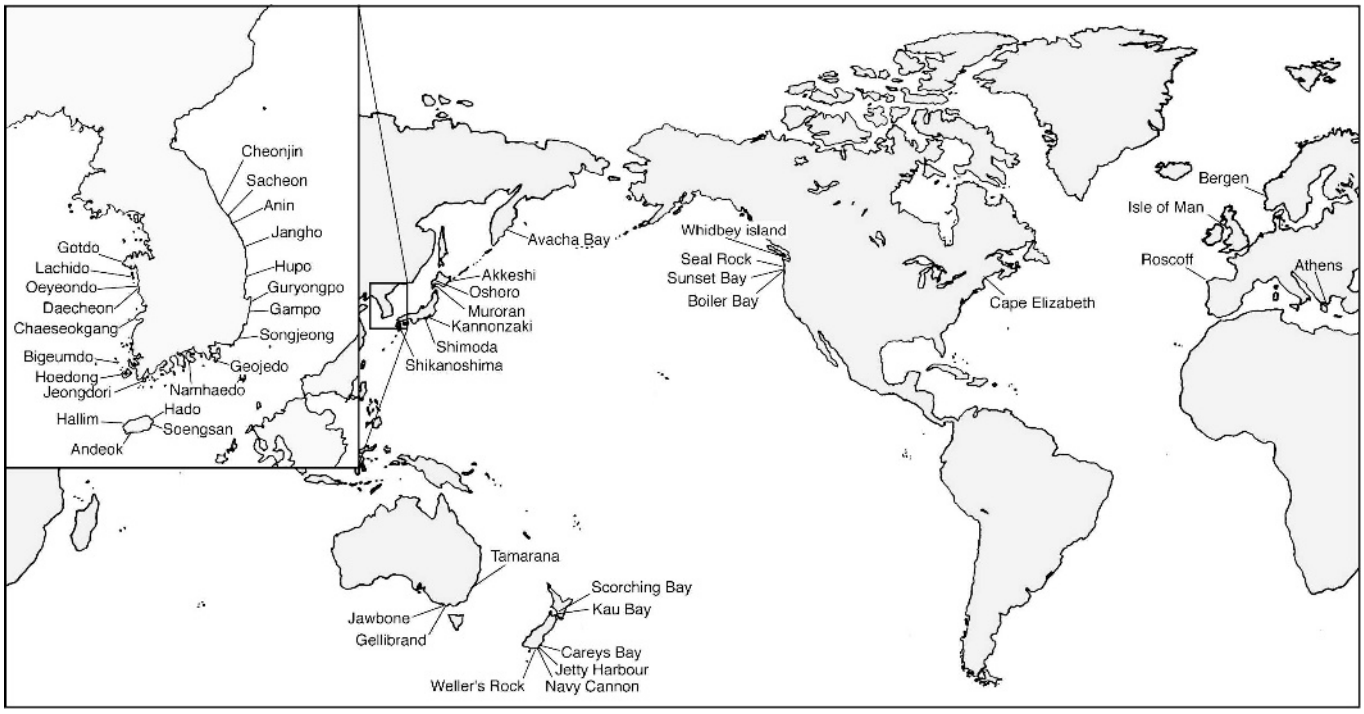

Fig. 1. Map of sample localities.

sample (USA.CapeElizabeth), and another clade (Clade C) consists of European samples. In the Pacific samples, sample localities were not geographically related to the molecular phylogeny. Even in samples from the same locality different sequences were found and were separated into Clades A and B: for example, Kor.Hoedong1 in Clade B, Kor.Hoedong2 in Clade A; USA.BoilerBay1 in A, USA.BoilerBay2–5 in B; USA.SunsetBay1 in A, USA.SunsetBay2 and 3 in B; Jpn.Muroran3 and 4 in A, Jpn.Muroran1 and 2 in B (Fig. 3). Sequence variations in Clade A were very small (1.2% mean character difference), whereas sequence variations in Clade B were far larger (5.4%). Nucleotide differences between Clades A and B ranged from 10.1–11.8%. In Clade C four samples made a subclade with high bootstrap supports (100% in both NJ and MP): UK.PortErinBay, Fr.Roscoff3, Norw.Bergen and UK.ElbyPoint. Clade C (Fig. 3) showed sequence variations of 3.7%.

*Rbc*L-spacer-S analyses

A total of 43 samples was sequenced for the *rbc*L-sp-S region, and 569 bp (261 in *rbc*L, 188 in *rbc* spacer, 120 in *rbc*S) of the data set were aligned. This alignment included 37 variable characters (24 parsimony-informative) and no gaps. The spacer region (188 bp) included 15 variable characters (eight parsimony-informative). The MP analysis produced 1,531 most parsimonious trees of 49 steps (CI = 0.837, RI = 0.855). In this analysis the Pacific and European clades were well supported, but further subclades were not supported except for the clade of Kor.Chaeseokgang3 and Kor.Oeyeondo. NJ and ML analyses showed similar results to that of the MP analysis. Only the ML tree (−log-likelihood = 1054.49089) is shown in Fig. 5.

DISCUSSION

Considerable divergence in the sequences of the *rbc*L, ITS and *rbc*L-sp-S regions was detected in the *Scytosiphon lomentaria* samples collected from localities worldwide. In the phylogenetic trees based on the *rbc*L gene, including 15 other scytosiphonacean species, *S. lomentaria* samples made a well-supported clade, suggesting that they are monophyletic. In the *S. lomentaria* clade, the Pacific and European clades clearly were recognized as independent clades supported by high bootstrap values in all three analyses. Such Pacific and European groups also were clearly recognized in the analyses of the ITS and *rbc*L-sp-*rbc*S regions. Although all Pacific samples used in the *rbc*L analyses were from Japan, they were representatives of two Pacific clades (A and B) that were recognized in the ITS analyses: Jpn.Oshoro1 and Jpn.Muroran1 belong to Clade A, and Jpn.Muroran3 and Jpn.Kannonzaki belong to Clade B in the ITS analyses (Figs 2 and 3). Therefore, the four *rbc*L samples from Japan can be considered as representatives of the two independent phylogenetic groups in the Pacific. In *rbc*L, nucleotide differences within each of the two clades were small and less than 6 bp, and large differences of 27–33 bp were shown between the Pacific and the European samples. Such differences in the *rbc*L gene sequences are greater than those among some scytosiphonacean genera: 15 bp between *Scytosiphon tenellus* and *Petalonia fascia*; 25 bp between *Scytosiphon canaliculatus* and *Petalonia zosterifolia*. The geographically separate distributions and the large differences of *rbc*L between the Pacific and European samples suggest that the Pacific and European *S. lomentaria* are separate species.

Table 1. Sample codes and DNA sequence accession numbers of *Scytosiphon lomentaria* used in this study.

Sample code	Collection site and date	DNA bank accession no.		
		ITS[1]	*rbc*L-sp-*rbc*S	*rbc*L
Australia				
Gellibrand	Gellibrand Reserve, Melbourne; Aug. 2001	AB265596	AB265691	
Jawbone1	Jawbone Reserve1, Melbourne; Aug. 2001	AB265597	AB265692	
Jawbone2	Jawbone Reserve2, Melbourne; Aug. 2001	AB265598		
Tamarana	Tamarana Beach, Sydney; Aug. 2001	AB265599	AB265693	
Japan				
Akkeshi	Akkeshi, Hokkaido; May 1999	AB265600	AB265694	
Kannonzaki	Kannonzaki, Kanagawa Pref.; Apr. 2000	AB265601		AB265734
Muroran1	Muroran, Hokkaido; Mar., 2000	AB265602	AB265695	AB265735
Muroran2	Muroran, Hokkaido; Mar., 2000	AB265603		
Muroran3	Muroran, Hokkaido; Mar., 2000	AB265604	AB265696	AB265736
Muroran4	Muroran, Hokkaido; Mar., 2000	AB265605		
Oshoro1	Oshoro, Otaru, Hokkaido; May 1989	AB195215[2]		AB022238[3]
Oshoro2	Oshoro, Otaru, Hokkaido; Apr. 1998	AB265606		
Shikanoshima	Shikanoshima, Fukuoka Pref.; Mar. 1999	AB265607	AB265697	
Shimoda	Shimoda, Shizuoka Pref.; Jan. 1999	AB265608	AB265698	
Korea				
AndeokM9902	Andeok, Chejudo; Feb. 1999	AB265609		
AndeokY9902	Andeok, Chejudo; Feb. 1999	AB265610	AB265699	
Andeok0003	Andeok, Chejudo; Mar. 2000	AB265611		
Anin	Anin, Gangreung; Feb. 1999	AB265612	AB265700	
Bigeumdo	Bigeumdo, Sinan; Jul. 2000	AB265613	AB265701	
Chaeseokgang2	Gyeokpo, Buan; Feb. 2001	AB265614	AB265702	
Chaeseokgang3	Gyeokpo, Buan; Feb. 2001	AB265615	AB265703	
Cheonjin1	Cheonjin, Sokcho; Apr. 2000	AB265616	AB265704	
Cheonjin2	Cheonjin, Sokcho; Apr. 2000	AB265617		
Daecheon	Daecheon harbour, Daechoen; Jan. 1999	AB265618	AB265705	
Gampo1	Gampo, Geongju; Apr. 2000	AB265619	AB265706	
Gampo2	Gampo, Geongju; Apr. 2000	AB265620		
Geojedo	Geojedo Island, Tongyoun; Mar. 2000	AB265621	AB265707	
Gotdo	Gotdo Island, Dangjin; Jun. 2000	AB265622	AB265708	
Guryongpo9902	Guryongpo, Pohang; Feb. 1999	AB265623	AB265709	
Guryongpo00041	Guryongpo, Pohang; Apr. 2000	AB265624		
Hado	Hado, Chejudo; Mar. 2000	AB265625		
Hallim	Hallim, Chejudo; Mar. 2000	AB265626	AB265710	
Hoedong1	Jindo Island, Jindo; Mar. 2001	AB265627	AB265711	
Hoedong2	Jindo Island, Jindo; Mar. 2001	AB265628	AB265712	
Hupo	Hupo, Uljin; Mar. 2000	AB265629	AB265713	
Jangho	Jangho, Samcheok; Mar. 2000	AB265630	AB265714	
Jeongdori	Wando Island, Wando; Jan. 1999	AB265631	AB265715	
Lachido	Lachido Island, Dangjin; Jun. 2000	AB265632	AB265716	
Namhaedo1	Namhaedo Island, Namhae; Mar. 2001	AB265633		
Namhaedo2	Namhaedo Island, Namhae; Mar. 2001	AB265634		
Oeyeondo	Oeyeondo Island, Daechoen; Mar. 1999	AB265635	AB265717	
Sacheon9902	Sacheon, Gangreung; Feb. 1999	AB265636	AB265718	
Sacheon00031	Sacheon, Gangreung; Mar. 2000	AB265637	AB265719	
Sacheon00038	Sacheon, Gangreung; Mar. 2000	AB265638	AB265720	
Sacheon00039	Sacheon, Gangreung; Mar. 2000	AB265639	AB265721	
Seongsan	Seongsan, Chejudo; Mar. 2000	AB265640	AB265722	
Songjeong	Songjeong Beach, Busan; Apr. 2001	AB265641		
Songtando	Songtando Island, Dangjin; Jul. 2001	AB265642	AB265723	
Woongdo	Woongdo Island, Dangjin; Jun. 2000	AB265643	AB265724	
New Zealand				
CareysBay4	Otago Harbour, Dunedin; Aug. 2001	AB265644		
Jetty1	Jetty Harbour, Dunedin; Aug. 2001	AB265645	AB265725	
Jetty3	Jetty Harbour, Dunedin; Aug. 2001	AB265646		
Jetty4	Jetty Harbour, Dunedin; Aug. 2001	AB265647		
KauBay1	Kau Bay, Wellington; Aug. 2001	AB265648		
KauBay2	Kau Bay, Wellington; Aug. 2001	AB265649		
KauBay3	Kau Bay, Wellington; Aug. 2001	AB265650		
KauBay4	Kau Bay, Wellington; Aug. 2001	AB265651		
NavyCannon	Navy Cannon, Dunedin; Aug. 2001	AB265652		
ScorchingBay1	Scorching Bay, Wellington; Aug. 2001	AB265653		
ScorchingBay2	Scorching Bay, Wellington; Aug. 2001	AB265654		
Weller'sRock2	Otago Harbour, Dunedin; Aug. 2001	AB265655		
Weller'sRock3	Otago Harbour, Dunedin; Aug. 2001	AB265656		

Table 1. Continued.

Sample code	Collection site and date	DNA bank accession no. ITS[1]	*rbc*L-sp-*rbc*S	*rbc*L
United States				
BoilerBay1	Boiler Bay, Oregon; May 2001	AB265657	AB265726	
BoilerBay2	Boiler Bay, Oregon; May 2001	AB265658		
BoilerBay3	Boiler Bay, Oregon; May 2001	AB265659		
BoilerBay4	Boiler Bay, Oregon; May 2001	AB265660		
BoilerBay5	Boiler Bay, Oregon; May 2001	AB265661		
CapeElizabeth	Cape Elizabeth, Maine; May 2001	AB265662	AB265727	
SealRock	Seal Rock, Oregon; May 2001	AB265663		
SunsetBay1	Sunset Bay, Oregon; May 2001	AB265664		
SunsetBay2	Sunset Bay, Oregon; May 2001	AB265665		
SunsetBay3	Sunset Bay, Oregon; May 2001	AB265666		
WhidbeyIsland1	Whidbey Island, Washington; May 2001	AB265667	AB265728	
WhidbeyIsland2	Whidbey Island, Washington; May 2001	AB265668		
WhidbeyIsland3	Whidbey Island, Washington; May 2001	AB265669		
Russia				
AvachaBay	Avacha Bay, Kamchatka; Aug. 2000	AB265670	AB265729	
France				
Roscoff1	Ile de Bartz, Roscoff; Apr. 2000	AB265671	AB265730	
Roscoff2	Ile de Verte, Roscoff; Apr. 2000	AB265672	AB265731	
Roscoff3	Roscoff, Brittany; Jun. 1996	AB265673		
Greece				
Athens	Athens; May 1989	AB195216[2]		
Norway				
Bergen	Bergen; Aug. 1989	AB265674	AB265732	AB265737
United Kingdom				
PortErinBay	Port Erin Bay, Isle of Man; Jul. 2000	AB265675	AB265733	
ElbyPoint	Elby Point, Isle of Man; Aug. 1999	AB265676		AB265738

[1] ITS, internal transcribed spacers.
[2] Camus *et al.* (2005).
[3] Kogame *et al.* (1999).

Table 2. *Rbc*L sequences used in the present study.

Taxa	Accession no.
Pylaiella littoralis (Linnaeus) Kjellman	X55372[1]
Ectocarpus siliculosus (Dillwyn) Lyngbye	X52503[2]
Chnoospora implexa J. Agardh	AB022231[3]
Rosenvingea intricata (J. Agardh) Børgesen	AB022232[3]
Hydroclathrus clathratus (C. Agardh) M.A. Howe	AB022233[3]
Colpomenia sinuosa (Mertens ex Roth) Derbès & Solier	AB022234[3]
Colpomenia peregrina (Sauvageau) Hamel	AB022235[3]
Colpomenia bullosa (Saunders) Yamada	AB022236[3]
Colpomenia phaeodactyla M.J. Wynne & J.N. Norris	AB022237[3]
Scytosiphon lomentaria (Lyngbye) Link	AF207811
Scytosiphon canaliculatus (Setchell & N.L. Gardner) Kogame	AB022239[3]
Scytosiphon gracilis Kogame	AB022240[3]
Scytosiphon tenellus Kogame	AB022241[3]
Petalonia zosterifolia (Reinke) Kuntze	AB022242[3]
Petalonia fascia (O.F. Müller) Kuntze	AB022243[3]
Petalonia binghamiae (J. Agardh) Vinogradova	AB022244[3]
Myelophycus simplex (Harvey) Papenfuss	AY095320[4]
Myelophycus cavus J. Tanaka & Chihara	AY095319[4]

[1] Assali *et al.* 1990.
[2] Valentin & Zetsche 1990.
[3] Kogame *et al.* 1999.
[4] Cho *et al.* 2003.

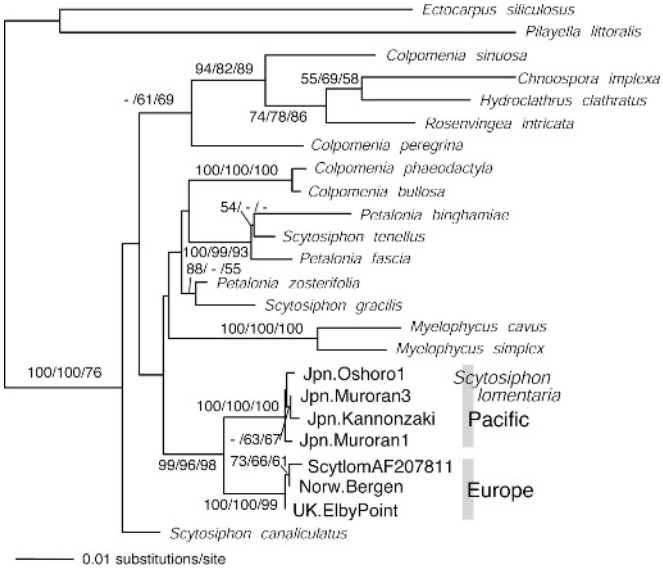

Fig. 2. ML tree inferred from the DNA sequences of *rbc*L in the Scytosiphonaceae. *Pylaiella littoralis* and *Ectocarpus siliculosus* are outgroup taxa. Bootstrap values, NJ (1000 replicates)/MP (500)/ML (100), are indicated near branches.

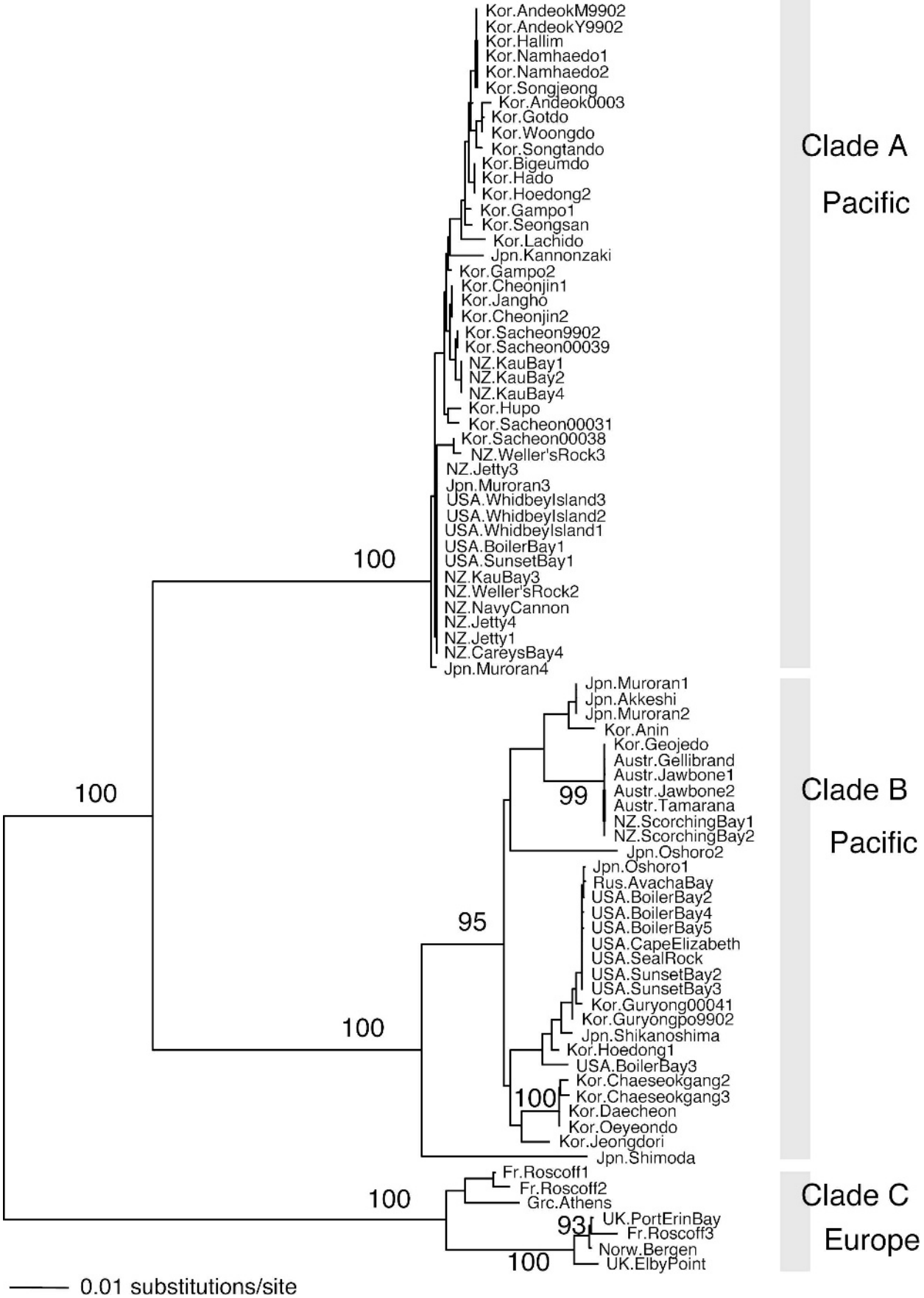

Fig. 3. NJ tree inferred from the sequences of ITS 1 and 2 in *Scytosiphon lomentaria* collected from localities worldwide. Bootstrap values (1000 replicates) are indicated near branches.

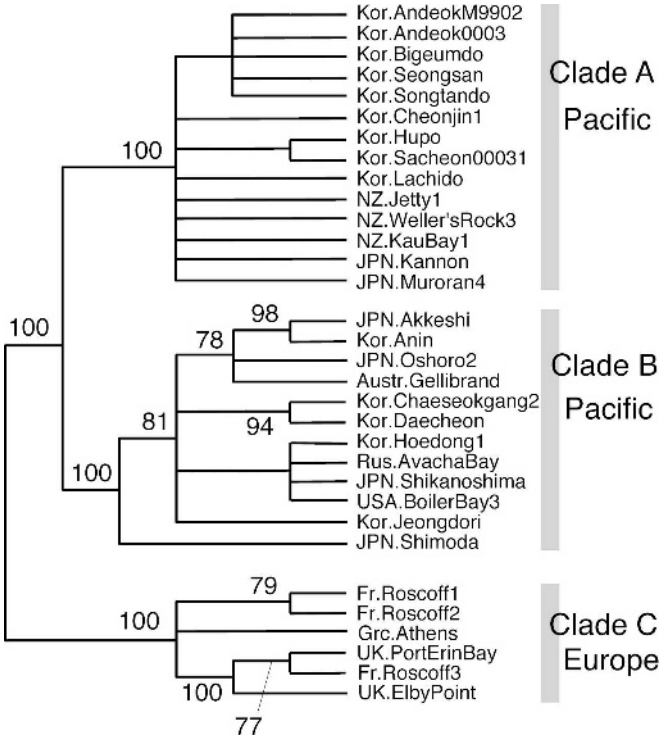

Fig. 4. Strict consensus of 16,065 MP trees inferred from the sequences of ITS 1 and 2 in *Scytosiphon lomentaria* collected from localities worldwide. Bootstrap values (500 replicates) are indicated near branches.

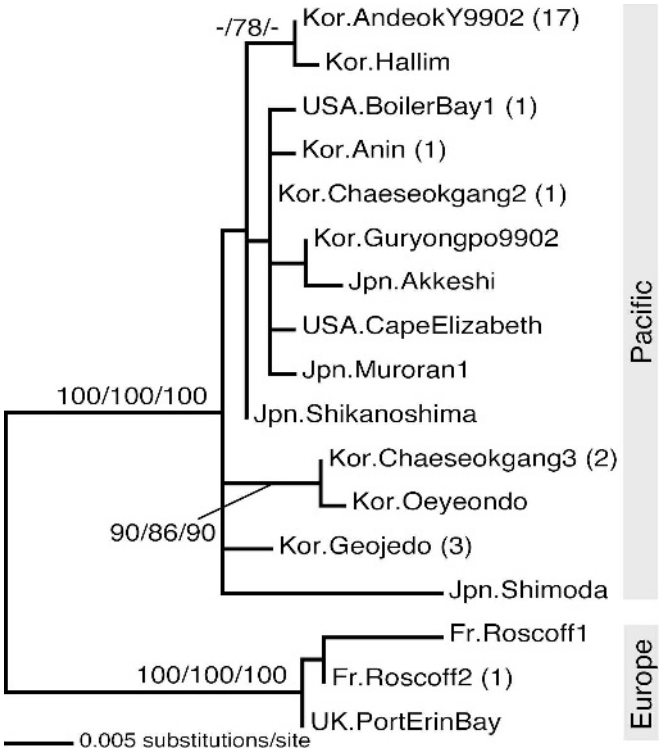

Fig. 5. ML tree inferred from the sequences of the partial *rbc*L-spacer-partial *rbc*S region in *Scytosiphon lomentaria*. Numbers in parentheses after sample codes indicate the number of other samples that had identical sequences: AndeokY9902 = Kor.Bigeumdo, Kor.Cheonjin1, Kor.Gampo1, Kor.Gotdo, Kor.Hoedong2, Kor.Hupo, Kor.Jangho, Kor.Lachido, Kor.Sacheon9902, Kor.Sacheon00031, Kor.Sacheon00038, Kor.Sacheon00039, Kor.Seongsan, Kor.Songtando, Kor.Woongdo, NZ.Jetty1, USA.WhidbeyIsland1; USA.BoilerBay1 = Jpn.Muroran3; Kor.Anin = Kor.Hoedong1; Kor.Chaeseokgang2 = Rus.AvachaBay; Kor.Chaeseokgang3 = Kor.Daecheon, Kor.Jeongdori; Kor.Geojedo = Austr.Gellibrand, Austr.Jawbone1, Austr.Tamarana; Fr.Roscoff2 = Norw.Bergen. Bootstrap values (MP/NJ/ML, > 60) are indicated near branches.

However, morphological differences between the Pacific and European entities of *S. lomentaria* have not been noticed to date. In our observations, both the Pacific and European samples showed tubular, constricted thalli that have ascocysts accompanied by plurilocular organs. These features agree with previous descriptions of the species (Abbott & Hollenberg 1976; Fletcher 1987; Kogame 1998), and we could not find any morphological differences between the two entities. Therefore, the Pacific and European *S. lomentaria* should be referred to as cryptic species at present. *Scytosiphon complanatus* (Rosenvinge) Doty has been reported from Greenland (Rosenvinge 1893; Pedersen 1980) and *S. dotyi* Wynne from California and Europe (Wynne 1969; Fletcher 1987; Furnari *et al.* 1999; Verlaque 2001). These two species were not included in our molecular analyses, but they are clearly distinct from *S. lomentaria* in lacking ascocysts (Rosenvinge 1893; Wynne 1969).

ITS regions showed higher evolutionary rates than *rbc*L, and the ITS analyses included more samples from more localities than the *rbc*L analyses. Because of this, the ITS analyses should elucidate the state of the geographical genetic divergence of *S. lomentaria* in higher resolution than analyses based on *rbc*L and *rbc*L-sp-*rbc*S. Although outgroup taxa were not included in the ITS analyses, we used a mid-point rooting for our ITS trees. We think that the mid-point rooted ITS trees are appropriate because the Pacific and European samples were separated in the rooted ITS trees as well as in the *rbc*L trees.

In the ITS analyses, the presence of two genetically distant groups (Clades A and B) within the Pacific samples was supported by high bootstrap values, although these two groups could not be recognized in the *rbc*L and *rbc*L-sp-S analyses. Kogame *et al.* (2005) also reported the presence of two distinct clades in *S. lomentaria* elucidated by ITS2 and mitochondrial *cox*3 (cytochrome oxidase subunit 3) sequences based on many samples collected from three sites in Hokkaido, Japan. Those two clades correspond to the two Pacific groups indicated by the ITS1 and ITS2 sequences in the present study.

Regarding the two Pacific groups, it is necessary to discuss two questions: (1) Are they different species; and (2) why do they show overlapping distributions? As to the first question, the clear separation of the two Pacific groups in our phylogenetic analyses supports the possibility that they are different species. Although the differences in *rbc*L sequences between the two Pacific groups are small, 3–5 bp, such differences may occur between closely related species of the Scytosiphonaceae, e.g. 5 bp between *Colpomenia bullosa* and *C. phaeodactyla*. Further research, however, is required to elucidate the relationships of the two Pacific groups of *S. lomentaria*. Crossing experiments may be useful for elucidating the problem because sexual reproduction has been reported in plants from Asian Pacific

coasts (Tatewaki 1966; Nakamura & Tatewaki 1975; Kogame 1998).

As to the second question, we consider that the distributions of the two Pacific groups (Clades A and B) came to overlap extensively after they independently differentiated in separate areas. The overlapped distributions are considered to be at least partly due to artificial translocations, because identical ITS sequences were found in localities far from each other; these translocations possibly occurred recently. Artificial introductions of many seaweed species have been reported (Boudouresque *et al.* 1985; Peters *et al.* 1993; McIvor *et al.* 2001; Uwai *et al.* 2006). When there are no pre-existing species that are morphologically similar to the introduced species, the introduction is relatively easy to notice. However, in cases where pre-existing taxa are morphologically similar to or indistinguishable from the introduced taxa in the introduced area, it is very difficult to notice the introduction (McIvor *et al.* 2001; Zuccarello *et al.* 2002a, b). In this study we have demonstrated that such cryptic artificial translocations possibly occurred widely in Pacific *S. lomentaria*.

In contrast to the artificial translocations of *S. lomentaria* within the Pacific, no artificial translocation was detected between the Pacific and European coasts in this study. A scytosiphonacean species, *Colpomenia peregrina*, is known as an introduced seaweed from the Pacific to Europe (Farnham 1980; Cho *et al.* 2005), and this introduction would cause us to expect such introduction of *S. lomentaria*. In fact, Camus *et al.* (2005) reported that Chilean *S. lomentaria* is closely related to European samples based on the ITS1 and RuBisCO spacer regions, possibly suggesting artificial translocation from Europe to Chile. In our study, artificial translocation from the Pacific to the western coast of the Atlantic was suggested by the sample of USA.CapeElizabeth: the sample positioned in the Pacific group in the phylogenetic analyses although it was the only one collected from the northwestern Atlantic.

In the ITS analyses the sequence divergence within Clade A was lower than those of Clades B and C. This result might indicate that in Clade A differentiation has been restricted, or that only a small portion of the differentiated populations have survived. In either case the low divergence of Clade A suggests that the lineage had been distributed in a far smaller area than the modern distributional area, i.e. the temperate and cold coasts of the North and South Pacific, and that expansion of its distribution probably occurred relatively recently. The location of the original area seems to be along the northwestern Pacific coasts because Asian samples show somewhat higher divergence than samples from the northeastern Pacific and the South Pacific in Clade A. In comparison with Clade A, higher divergence within Clade B suggests that this lineage may have had a wider distribution, possibly in the North and South Pacific, for a longer time and may have been affected by geographical separation.

ACKNOWLEDGMENTS

We thank Drs Joanna M. Kain, John A. West, Nathalie Simon, Nina G. Klochkova, Giuseppe C. Zuccarello, Judy Broom, Tae Oh Cho, Han Gu Choi, Michio Masuda, Taizo Motomura, Norishige Yotsukura and Kazuro Shida (Shinta) for their help in collecting specimens. We also thank Dr E.C. Henry for his valuable comments in revising the manuscript. This work was supported by the Korean Science and Engineering Foundation (KOSEF) grant funded by the Korean Government (MOST; R01-2006-000-10207-0) to S.M. Boo and the scientific research grant provided by Japan Society for the Promotion of Science (17570069) to K. Kogame.

REFERENCES

Abbott I.A. & Hollenberg G.J. 1976. *Marine Algae of California* Stanford University Press, Stanford, 827 pp.

Assali N.E., Mache, R. & Loiseaux-de Goër S. 1990. Evidence for a composite phylogenic origin of the plastid genome of the brown alga *Pylaiella littoralis* (L.) Kjellm. *Plant Molecular Biology* 15: 307–315.

Bold H.C. & Wynne M.J. 1985. *Introduction to the Algae: Structure and reproduction,* ed. 2 Prentice-Hall, New Jersey. 720 pp.

Boudouresque C.F., Gerbal M. & Knoepffler-Peguy M. 1985. L'algue japonnaise *Undaria pinnatifida* (Phaeophyceae, Laminariales) en Méditerranée. *Phycologia* 24: 364–366.

Camus C., Meynard A.P., Faugeron S., Kogame K. & Correa J.A. 2005. Differential life history phase expression in two coexisting species of *Scytosiphon* (Phaeophyceae) of northern Chile. *Journal of Phycology* 41: 931–941.

Cho T.O., Cho G.Y., Yoon H.S., Boo S.M. & Lee W.J. 2003. New records of *Myelophycus cavus* (Scytosiphonaceae, Phaeophyceae) in Korea and the taxonomic position of the genus on the basis of a plastid DNA phylogeny. *Nova Hedwigia* 76: 381–397.

Cho G.Y., Boo S.M., Nelson W. & Clayton M.N. 2005. Genealogical partitioning and phylogeography of *Colpomenia peregrina* (Scytosiphonaceae, Phaeophyceae), based on plastid *rbc*L and nuclear ribosomal DNA internal transcribed spacer sequences. *Phycologia* 44: 103–111.

Clayton M.N. 1978. Morphological variation and life history in cylindrical forms of *Scytosiphon lomentaria* (Scytosiphonaceae: Phaeophyta) from southern Australia. *Marine Biology* 47: 349–357.

Dieck I. tom 1987. Temperature tolerance and daylength effects in isolates of *Scytosiphon lomentaria* (Phaeophyceae) of the North Atlantic and Pacific Ocean. *Helgoländer Meeresuntersuchungen* 41: 307–321.

Farnham W.F. 1980. Studies on aliens in the marine flora of southern England. In *The shore environment 2: Ecosystems.* Systematics Association Special Volume 17 (b) (Ed. by J.H. Price, D.E.G. Irvine & W.F. Farnham), pp. 875–914. Academic Press, London and New York.

Fletcher R.L. 1987. *Seaweeds of the British Isles. Volume 3. Fucophyceae (Phaeophyceae) Part 1* British Museum, London. 359 pp.

Furnari G., Cormaci M. & Serio D. 1999. Catalogue of the benthic marine macroalgae of the Italian coast of the Adriatic Sea. *Bocconea* 12: 1–214.

Goff L.J., Moon D.A. & Coleman A.W. 1994. Molecular delineation of species and species relationships in the red algal agarophytes *Gracilariopsis* and *Gracilaria* (Gracilariales). *Journal of Phycology* 30: 521–537.

Kawai H., Sasaki H., Maeda Y. & Arai S. 2001. Morphology, life history, and molecular phylogeny of *Chorda rigida*, sp. nov. (Laminariales, Phaeophyceae) from the Sea of Japan and the genetic diversity of *Chorda filum*. *Journal of Phycology* 37: 130–142.

Kim S.-H. & Kawai H. 2002. Taxonomic revision of *Chordaria flagelliformis* (Chordariales, Phaeophyceae) including novel use of the intragenic spacer region of rDNA for phylogenetic analysis. *Phycologia* 41: 328–339.

KIM S.-H., PETERS A.F. & KAWAI H. 2003. Taxonomic revision of *Sphaerotrichia divaricata* (Ectocarpales, Phaeophyceae), with a reappraisal of *S. firma* from the northwest Pacific. *Phycologia* 42: 183–192.

KOGAME K. 1998. A taxonomic study of Japanese *Scytosiphon* (Scytosiphonales, Phaeophyceae), including two new species. *Phycological Research* 46: 39–56.

KOGAME K. & MASUDA M. 2001. Crustose sporophytes of *Colpomenia bullosa* (Scytosiphonaceae, Phaeophyceae) in nature. *Cryptogamie, Algologie* 22: 201–208.

KOGAME K., HORIGUCHI T. & MASUDA M. 1999. Phylogeny of the order Scytosiphonales (Phaeophyceae) based on DNA sequences of *rbc*L, partial *rbc*S and partial LSU nrDNA. *Phycologia* 38: 496–502.

KOGAME K., UWAI S., SHIMADA S. & MASUDA M. 2005. A study of sexual and asexual populations of *Scytosiphon lomentaria* (Scytosiphonaceae, Phaeophyceae) in Hokkaido, northern Japan, using molecular markers. *European Journal of Phycology* 40: 313–322.

KRISTIANSEN A., PEDERSEN P.M. & MOSEHOLM L. 1991. Growth and reproduction of *Scytosiphon lomentaria* (Fucophyceae) in relation to temperature in 2 populations from Denmark. *Nordic Journal of Botany* 11: 375–383.

LÜNING K. & DRING M.J. 1975. A photoperiodic response mediated by blue light in the brown alga *Scytosiphon lomentaria*. *Planta* 125: 25–32.

MCIVOR L., MAGGS C.A., PROVAN J. & STANHOPE M.J. 2001. *rbc*L sequences reveal multiple cryptic introductions of the Japanese red alga *Polysiphonia harveyi*. *Molecular Ecology* 10: 911–919.

NAKAMURA Y. & TATEWAKI M. 1975. The life history of some species of Scytosiphonales. *Scientific Papers of the Institute of Algological Research, Faculty of Science, Hokkaido University* 6: 57–93.

PEDERSEN P.M. 1980. Culture studies on complanate and cylindrical *Scytosiphon* (Fucophyceae, Scytosiphonales) from Greenland. *British phycological Journal* 15: 391–398.

PETERS A.F., KAWAI H. & NOVACZEK I. 1993. Intraspecific sterility barrier confirms that introduction of *Sphaerotrichia divaricata* (Phaeophyceae, Chordariales) into the Mediterranean was from Japan. *Hydrobiologia* 261: 31–36.

POSADA D. & CRANDALL K.A. 1998. Modeltest: testing the model of DNA substitution. *Bioinformatics* 14: 817–818.

ROSENVINGE L.K. 1893. Grønland Havalger. *Medd. Grønland* 3: 763–981. pls 1–2.

SAUNDERS G.W. & DRUEHL L.D. 1993. Nucleotide sequences of the internal transcribed spacers and 5.8S rRNA genes from *Alaria marginata* and *Postelsia palmaeformis* (Phaeophyta: Laminariales). *Marine Biology* 115: 347–352.

SWOFFORD D.L. 2002. *PAUP*. Phylogenetic Analysis Using Parsimony (*and Other Methods), Version 4*. Sinauer Associates, Sunderland, Massachusetts.

TAI V., LINDSTROM S.C. & SAUNDERS G.W. 2001. Phylogeny of the Dumontiaceae (Gigartinales, Rhodophyta) and associated families based on SSU rDNA and internal transcribed spacer sequence data. *Journal of Phycology* 37: 184–196.

TATEWAKI M. 1966. Formation of a crustaceous sporophyte with unilocular sporangia in *Scytosiphon lomentaria*. *Phycologia* 6: 62–66.

UWAI S., NELSON W., NEILL K., WANG W.D., AGUILAR-ROSAS L.E., BOO S.M., KITAYAMA T. & KAWAI H. 2006. Genetic diversity in *Undaria pinnatifida* (Laminariales, Phaeophyceae) deduced from mitochondria genes — origins and succession of introduced populations. *Phycologia* 45: 687–695.

VALENTIN K. & ZETSCHE K. 1990. Rubisco genes indicate a close phylogenetic relation between the plastids of Chromophyta and Rhodophyta. *Plant Molecular Biology* 15: 575–584.

VERLAQUE M. 2001. Checklist of the macroalgae of Thau Lagoon (Hérault, France), a hot spot of marine species introduction in Europe. *Oceanologica Acta* 24: 29–49.

WYNNE M.J. 1969. Life history and systematic studies of some Pacific North American Phaeophyceae (brown algae). *University of California Publications in Botany* 50: 1–88.

YOON H.S. & BOO S.M. 1999. Phylogeny of Alariaceae (Phaeophyta) with special reference to *Undaria* based on sequences of the RuBisCo spacer region. *Hydrobiologia*. 398/399 47–55.

YOON H.S., LEE J.Y., BOO S.M. & BHATTACHARYA D. 2001. Phylogeny of Alariaceae, Laminariaceae, and Lessoniaceae (Phaeophyceae) based on plastid-encoded Rubisco spacer and nuclear-encoded ITS sequence comparisons. *Molecular Phylogenetics and Evolution* 21: 231–243.

ZUCCARELLO G.C. & WEST J.A. 2003. Multiple cryptic species: Molecular diversity and reproductive isolation in the *Bostrychia radicans*/*B-moritziana* complex (Rhodomelaceae, Rhodophyta) with focus on North American isolates. *Journal of Phycology* 39: 948–959.

ZUCCARELLO G.C., WEST J. & RUENESS J. 2002a. Phylogeography of the cosmopolitan red alga *Caulacanthus ustulatus* (Caulacanthaceae, Gigartinales). *Phycological Research* 50: 163–172.

ZUCCARELLO G.C., SANDERCOCK B. & WEST J.A. 2002b. Diversity within red algal species: variation in world-wide samples of *Spyridia filamentosa* (Ceramiaceae) and *Murrayella periclados* (Rhodomelaceae) using DNA markers and breeding studies. *European Journal of Phycology* 37: 403–417.

Received 6 September 2006; accepted 2 July 2007
Associate editor: Stefano Draisma