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Taxonomic revision of *Chordaria flagelliformis* (Chordariales, Phaeophyceae) including novel use of the intragenic spacer region of rDNA for phylogenetic analysis

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Chordaria flagelliformis is widely distributed in the cold-water areas of the Northern Hemisphere, along the Arctic, Atlantic and Pacific coasts. *Chordaria flagelliformis* f. *chordaeformis,* one of the four new morphological forms first described by Kjellman from Spitsbergen, is characterized by its simple thallus (unbranched or with very few branches) and appears to have a narrower distributional range in colder-water areas compared to the typical form (f. *flagelliformis*). However, the taxonomic character used to distinguish this form is not always reliable, because of the great morphological plasticity of the species. Molecular phylogenetic analyses using the 5.8S, internal transcribed spacer (ITS) and intragenic spacer (IGS, between 26S and 5S) rDNA regions and Rubisco (almost complete *rbc*L gene and its spacer region between *rbc*L and *rbc*S) showed that *C. flagelliformis* is composed of three major genetic groups: 'group 1' (f. *chordaeformis* from Kamchatka, north Pacific), 'group 2' (f. *chordaeformis* from other areas) and 'group 3' (f. *flagelliformis*). 'Group 2' and 'group 3' were composed of two subgroups, corresponding to their Atlantic and Pacific populations. The lengths of the sequences of the ITS1 and ITS2 regions were relatively constant in each group and subgroup, and were considerably different between groups. This is the first report of the use of the 26S–5S IGS region for molecular phylogenetic analysis in any organism; the region was shown to be three to eight times more variable than the 5.8S–ITS region. On the basis of these analyses, *C. flagelliformis* f. *flagelliformis* was concluded to exhibit great morphological plasticity and to contain some specimens that are unbranched, whereas f. *chordaeformis* was more consistent in the number of branches. *Chordaria flagelliformis* f. *chordaeformis* is independent from *C. flagelliformis sensu stricto,* although the taxonomic relationships between the populations of Kamchatka and other areas are still unclear. We propose recognizing f. *chordaeformis* at the species level, as *C. chordaeformis* (Kjellman) Kawai & S.H. Kim *stat. nov.*

INTRODUCTION

Chordaria flagelliformis (Müller) C. Agardh (1817), type of the order Chordariales, was first described from the North Atlantic. This species is widely distributed in the cold-water areas of the Northern Hemisphere along the Arctic, Atlantic (south to France in Europe and south to New Jersey on the American coast) and Pacific coasts (south to northern Japan on the Asian coast and south to Oregon on the American coast) (Rosenvinge 1893; Hamel 1935; Taylor 1937; Rosenvinge & Lund 1943; Inagaki 1958; Kornmann & Sahling 1977; Perestenko 1980; Hansen 1997). Kjellman (1877) described four morphological forms within the species, based on his observations in Spitsbergen (Svalbard): f. *typica* (f. *flagelliformis*), f. *chordaeformis,* f. *ramusculifera,* and f. *subsimplex.* They were characterized principally by their external morphology, and were reported to be indistinguishable in their anatomy (Kjellman 1877). Among these four forms, *C. flagelliformis* f. *chordaeformis,* which is characterized by its simple thallus (unbranched or with very few branches), appears to have a narrower distributional range (being restricted to colder-water areas) compared with the typical form; thus, f. *chordaeformis* has been reported from Greenland (Rosenvinge 1893), the Arctic Sea (Kjellman 1877, 1883; Zinova 1953), and the western north Pacific (Tokida 1934, 1954; Nagai 1940; Yamada & Tanaka 1944; Zinova 1954; Inagaki 1958). However, the taxonomic character used for distinguishing the form is not always reliable, because of the great morphological plasticity of the species.

The life history of *C. flagelliformis* has been studied by three authors (Sauvageau 1929; Caram 1955; Kornmann 1962), who reported direct or heteromorphic life histories; however, none of them obtained the complete life history or observed sexual reproduction. All their specimens were *C. flagelliformis sensu stricto* (= f. *flagelliformis*). Kawai & Kurogi (1982) made a preliminary report of a direct type of life history in *C. flagelliformis* f. *chordaeformis,* in isolates collected from Hokkaido, Japan, and hence no sexual reproduction has been found in this taxon either. Therefore, it appears impossible to clarify the taxonomic relationships among the forms by crossing experiments.

In order to elucidate the taxonomic relationship between the typical form and f. *chordaeformis,* we compared rRNA [5.8S, internal transcribed spacer (ITS) and IGS rDNA regions] and Rubisco gene sequences (almost the complete *rbc*L gene and the spacer region between *rbc*L and *rbc*S), which are coded in the nuclear and plastid genomes, respectively. The IGS (intragenic spacer) region, which is the noncoding sequence region between 26S and 5S rDNA (Kawai *et al.* 1995), is supposed to be more variable than ITS sequences because it is not transcribed, but it has never been used for molecular phy-

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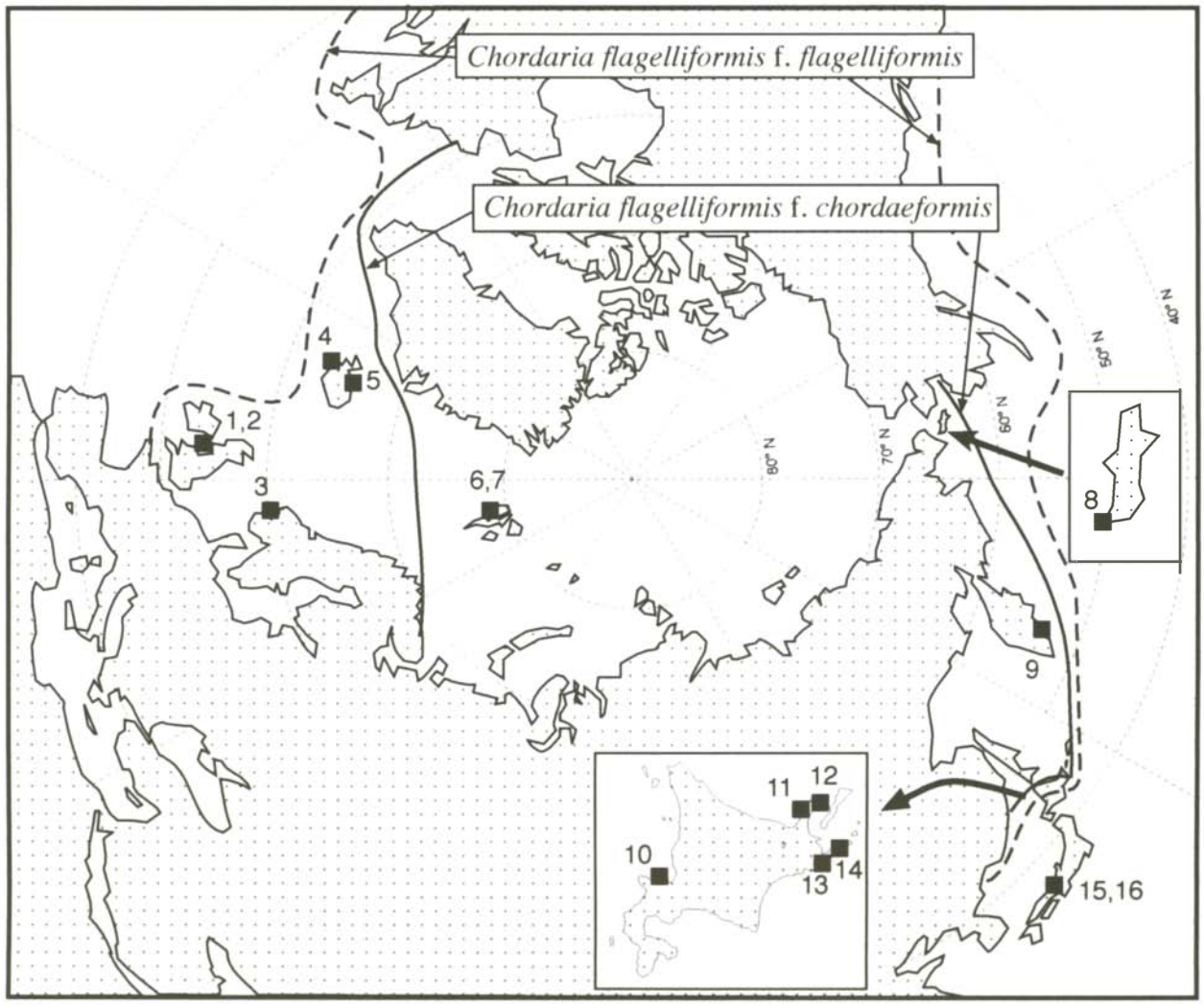


Fig. 1. Map indicating the estimated distributional ranges of *C. flagelliformis* f. *flagelliformis* and *C. flagelliformis* f. *chordaeformis,* based on previous reports (Kjellman 1877; Hamel 1935; Taylor 1937; Rosenvinge & Lund 1943; Inagaki 1958; Kornmann & Sahling 1977; Perestenko 1980; Lüning 1990; Hansen 1997, etc.), and locations of sampling sites of the materials used for the present analyses. (1) Port Erin, Isle of Man; (2) Elby Point, Isle of Man; (3) Bergen, Norway; (4) Reykjavik, Iceland; (5) Orafsfjord, Iceland; (6) Ny Ålesund, Spitsbergen, Norway; (7) Longyearbyen, Spitsbergen, Norway; (8) Gambell, St. Lawrence Island, USA; (9) Abacha Bay, Kamchatka, Russia; (10) Oshoro, Hokkaido, Japan; (11) Osinkosin, Shiretoko Peninsula, Hokkaido, Japan; (12) Sashirui, Shiretoko Peninsula, Hokkaido, Japan; (13) Hanasaki, Hokkaido, Japan; (14) Nemuro, Hokkaido, Japan; (15) Miyazu, Kyoto, Japan; (16) Imagoura, Hyogo, Japan. Broken and solid lines indicate the southern distributional limits of *C. flagelliformis* f. *flagelliformis* and f. *chordaeformis,* respectively.

logenetic analyses of any organism. Therefore, this is the first report using this sequence region for molecular phylogenetic analyses. We also compared the mutation rates between ITS and IGS.

MATERIAL AND METHODS

Morphological observations

Specimens of *C. flagelliformis* (both the typical branched form and the unbranched form assignable to f. *chordaeformis*) were collected at various localities in the northern Atlantic (including Spitsbergen, type locality of f. *chordaeformis*) and northern Pacific (Fig. 1). The number of branches and the presence or absence of secondary branches were recorded for all the specimens collected, including the individuals used for the molecular phylogenetic study (Table 1). Voucher specimens of *C. flagelliformis* collected in Spitsbergen by Kjellman during 1872 and 1873 and housed in the Museum of Evolution, Botany Section (Fytoteket) of Uppsala University (UPS), were also examined.

Molecular phylogenetic analysis

The origins of specimens used for DNA extraction and the sequence data used for the analyses are listed in Table 1. The specimens used for the present study are deposited at the Kobe University Research Center for Inland Seas. Cultures were grown in polystyrene Petri dishes containing 50 ml modified Provasoli's enriched seawater medium (Tatewaki 1966), illuminated by daylight-type white fluorescent lighting of approximately 50 μmol m^{-2} s^{-1} at 10°C or 15°C, in long day (16 : 8 h light–dark) conditions. For DNA extraction, cultures were

Table 1. Sources of *Chordaria flagelliformis* and other specimens used for molecular analyses, their abbreviations, the number of branches in field-collected plants, the length of ITS sequences (bp) and database accession numbers of the sequences. Unless otherwise stated, field-collected plants were rapidly dehydrated using silica gel.[1]

Morphologically assigned taxonomic identity	Collection site	Abbreviation	Origin	No. of branches	ITS1 (bp)	ITS2 (bp)	DDBJ[2] accession no. for 5.8S and ITS rDNA	DDBJ[2] accession no. for IGS (25S-5S) rDNA	DDBJ[2] accession no. for *rbc*L and spacer
f. *flagelliformis*	Orafsfjord, Iceland	Ice1	field plant	10	287	369	AB066039	AB066009	AB066073
f. *flagelliformis*	Keflavik, Iceland	Ice2	field plant	9	287	361	AB066040		
f. *flagelliformis*	Elby Point, Isle of Man	Man1	field plant	4	287	373	AB066041	AB066010	AB066074
f. *flagelliformis*	Port Erin, Isle of Man	Man2	field plant	11	287	370	AB066042	AB066011	AB066075
f. *flagelliformis*	Ny Ålesund, Spitsbergen, Norway	Spi7	field plant	8	286	365	AB066043	AB066007	
f. *flagelliformis*	Ny Ålesund, Spitsbergen, Norway	Spi8	field plant	7	286	365	AB066044		
f. *chordaeformis*[3]	Ny Ålesund, Spitsbergen, Norway	Spi9[4]	field plant	0	283	365	AB066047		AB066077
f. *flagelliformis*	Ny Ålesund, Spitsbergen, Norway	Spi10	field plant	6	283	364	AB066048		AB066076
f. *flagelliformis*	Longyearbyen, Spitsbergen, Norway	Spi11	field plant	13	286	365	AB066045	AB066008	
f. *flagelliformis*	Longyearbyen, Spitsbergen, Norway	Spi12	field plant	9	286	365	AB066046		
f. *flagelliformis*	Bergen, Norway	Ber	field plant	4	286	366	AB066049		AB066078
f. *chordaeformis*[3]	Oshinkoshin, Shiretoko, Hokkaido, Japan	Hok8[4]	field plant	1	264	340	AB066054		
f. *chordaeformis*[3]	Oshinkoshin, Shiretoko, Hokkaido, Japan	Hok9[4]	field plant	0	264	340	AB066055	AB066016	AB066084
f. *chordaeformis*[3]	Oshinkoshin, Shiretoko, Hokkaido, Japan	Hok10[4]	field plant	0	264	340	AB066059		AB066083
f. *flagelliformis*	Sashirui, Shiretoko, Hokkaido, Japan	Hok11	field plant	6	265	340	AB066056	AB066015	AB066086
f. *flagelliformis*	Sashirui, Shiretoko, Hokkaido, Japan	Hok12	field plant	5	264	340	AB066058	AB066014	AB066085
f. *flagelliformis*	Nemuro, Hokkaido, Japan	Hok13	field plant	10	264	340	AB066057		
f. *flagelliformis*	Abacha Bay, Kamchatka, Russia	Kam6	culture (H. Kawai)	4	264	340	AB066050	AB066013	AB066079
f. *flagelliformis*	Abacha Bay, Kamchatka, Russia	Kam7	field plant	6	264	340	AB066051	AB066012	AB066080
f. *flagelliformis*	Gambell, St. Lawrence, Island, USA	StGa3	field plant	6	264	339	AB066052		AB066081
f. *flagelliformis*	Gambell, St. Lawrence Island, USA	StGa4	field plant	7	264	339	AB066053		AB066082
f. *chordaeformis*	Abacha Bay, Kamchatka, Russia	Kam1[4]	field plant	0	434	342	AB066019	AB065997	
f. *chordaeformis*	Abacha Bay, Kamchatka, Russia	Kam2[4]	field plant	1	436	342	AB066020	AB065996	
f. *chordaeformis*	Abacha Bay, Kamchatka, Russia	Kam3[4]	culture (H. Kawai)	0	434	342	AB066023		
f. *chordaeformis*	Abacha Bay, Kamchatka, Russia	Kam4[4]	culture (H. Kawai)	0	434	342	AB066021	AB065999	AB066063
f. *chordaeformis*	Abacha Bay, Kamchatka, Russia	Kam5[4]	culture (H. Kawai)	2	434	342	AB066022	AB065998	AB066064
f. *chordaeformis*	Ny Ålesund, Spitsbergen, Norway	Spi1[4]	field plant	0	608	397	AB066024	AB066001	AB066065
f. *chordaeformis*	Ny Ålesund, Spitsbergen, Norway	Spi2[4]	field plant	0	605	398	AB066027	AB066002	AB066068
f. *chordaeformis*	Ny Ålesund, Spitsbergen, Norway	Spi3[4]	field plant	0	609	398	AB066028		
f. *chordaeformis*	Ny Ålesund, Spitsbergen, Norway	Spi4[4]	field plant	1	607	397	AB066029		
f. *chordaeformis*	Ny Ålesund, Spitsbergen, Norway	Spi5[4]	field plant	0	611	397	AB066025		AB066066
f. *chordaeformis*	Ny Ålesund, Spitsbergen, Norway	Spi6[4]	field plant	0	613	395	AB066026	AB066000	AB066067
f. *chordaeformis*	Hanasaki, Hokkaido, Japan	Hok1[4]	culture (H. Kawai)	1	599	358	AB066030	AB066005	AB066069
f. *chordaeformis*	Nemuro, Hokkaido, Japan	Hok2[4]	field plant	0	600	353	AB066033	AB066004	
f. *chordaeformis*	Oshinkoshin, Shiretoko, Hokkaido, Japan	Hok3[4]	field plant	0	602	358	AB066034	AB066003	AB066071
f. *chordaeformis*	Oshinkoshin, Shiretoko, Hokkaido, Japan	Hok4[4]	field plant	2	602	358	AB066035		
f. *chordaeformis*	Oshinkoshin, Shiretoko, Hokkaido, Japan	Hok5[4]	field plant	1	602	358	AB066036		
f. *chordaeformis*	Oshinkoshin, Shiretoko, Hokkaido, Japan	Hok6[4]	field plant	2	602	358	AB066037		AB066072
f. *chordaeformis*	Oshinkoshin, Shiretoko, Hokkaido, Japan	Hok7[4]	field plant	0	601	358	AB066038		
f. *chordaeformis*	Gambell, St. Lawrence, Island, USA	StGa1[4]	field plant	0	600	358	AB066031	AB066006	AB066070
f. *chordaeformis*	Gambell, St. Lawrence Island, USA	StGa2[4]	field plant	0	600	358	AB066032		
Chordaria linearis	Chile	Cli	database				Z97577 (ITS1), Z98578 (ITS2)		
Sphaerotrichia divaricata	Oshoro, Hokkaido, Japan	Spha1	culture (H. Kawai)				AB066017	AB065994	AB066061
S. divaricata	Miyazu, Kyoto, Japan	Spha2	culture (H. Kawai)				AB066018	AB065995	AB066062
Acrothrix pacifica	Imagoura, Hyogo, Japan	Acro	field plant (frozen)						AB066060

[1] ITS, internal transcribed spacer.
[2] DNA Data Bank of Japan (http://www.ddbj.nig.ac.jp/intro-e.html).
[3] Specimens assigned to f. *chordaeformis* on the basis of morphology.
[4] Classification based on morphology (number of branches) differs from the taxonomy based on the molecular data (see also Figs. 8–11).

frozen in liquid nitrogen and field-collected material was rapidly desiccated in silica gel powder. Air-dried herbarium vouchers were also used. Approximately 40 mg of algal tissue powder ground in liquid nitrogen was used for genomic DNA extractions, using a DNeasy Plant Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions.

We amplified three gene sequences of (1) the 5.8S, ITS1 and ITS2 (internal transcribed spacer regions between 18S, 5.8S and 26S rDNA) rDNA; (2) the IGS (the intragenic spacer region between 26S and 5S); and (3) the Rubisco large subunit gene (*rbc*L) and the spacer region between *rbc*L and *rbc*S. Polymerase chain reactions (PCRs) were carried out using GeneAmp PCR System 2400 and 9700 (Applied Biosystems, Foster City, CA, USA) and a TaKaRa Ex Taq (Takara Shuzo, Shiga, Japan) Reaction Kit (total reaction volume of 25 μl, composed of 2.5 μl 10× Ex Taq buffer, 5.0 μM deoxynucleoside triphosphate mixture, 0.1 μM of each primer, 0.625 units TaKaRa Ex Taq and 2.0 μl DNA solution containing 0.5–1.0 μg DNA). Primers (Table 2) were designed on the basis of known sequences of the corresponding regions reported for related taxa (Assali *et al.* 1990; Valentin & Zetsche 1990; Saunders & Druehl 1992; Tan & Druehl 1993, 1996; Kawai *et al.* 1995; Daugbjerg & Andersen 1997; Stache-Crain *et al.* 1997; Siemer *et al.* 1998; Kogame *et al.* 1999).

The profile of PCR conditions was as follows: initial denaturation at 95°C for 5 min; 30 cycles of denaturation at 95°C for 30 s, annealing at 52°C or 56°C (for 5.8S–ITS rDNA) for 30 s, 48°C or 56°C (for IGS rDNA) for 30 s and 42°C or 50°C (for *rbc*L and spacer) for 30 s, and extension at 72°C for 30 s; and a final extension at 72°C for 7 min. PCR products were directly sequenced using the Cy5 Auto Cycle Sequencing Kit (Pharmacia Biotech, Uppsala, Sweden) and ALF Express DNA Sequencer (Pharmacia Biotech), or the BigDye Terminator Cycle Sequencing Reaction Kit (Applied Biosystems) and an ABI PRISM 310 Genetic Analyzer (Applied Biosystems).

The Clustal W program (Thompson *et al.* 1994) was used for preliminary DNA sequence alignment, followed by a manual final alignment. The aligned sequences were subjected to maximum parsimony (MP) analyses in a general heuristic search using PAUP v. 4.0.2b (Swofford 1999). Five random taxon addition replicates were performed in each heuristic search, using the tree bisection–reconnection (TBR) branch swapping option. Gaps were treated as missing data in every analysis. From the same alignment, two-parameter distances (Kimura 1980) were estimated between taxa, and a phylogenetic tree was constructed with the neighbour-joining (NJ) method, using PAUP. Maximum likelihood (ML) analyses were also performed using PAUP in a general heuristic search with a substitution model of transition–transversion ratio = 2, empirical base frequencies (using the Hasegawa–Kishino–Yano model) and equal among-site rate variation. The robustness of the resulting phylogenies was tested by bootstrap analyses with 1000 (MP and NJ) or 500 (ML) resamplings (Felsenstein 1985). In an additional MP analysis, gaps were recognized as a fifth base. Analyses based on the combined sequence of each aligned sequence data set (5.8S, ITS and IGS rDNA, *rbc*L and spacer) were also done using those specimens in which all these regions had been sequenced.

In the analyses using rDNA, *C. linearis* (J.D. Hooker & Harvey) Cotton (5.8S–ITS) and *Sphaerotrichia divaricata* (C. Agardh) Kylin (Chordariaceae) (5.8S–ITS and IGS) were used as outgroups. For the Rubisco analyses, *Acrothrix pacifica* Okamura & Yamada (Acrotrichaceae) and *S. divaricata* were used as outgroups.

RESULTS

Morphology and anatomy

Specimens assigned to *C. flagelliformis* f. *flagelliformis* (Figs 2, 3) averaged 7.0 (s = 5.1, n = 143) branches on the main axis, and most of the specimens had secondary branches. *Chordaria flagelliformis* f. *chordaeformis* averaged 0.4 (s = 0.6, n = 182) (Figs 4–6) branches and none of them had secondary branches. The significance of the difference between the two forms in these features was confirmed by t test ($P < 0.001$). We measured the branching angles: the branches generally issued at obtuse angles in f. *flagelliformis* and at acute angles in f. *chordaeformis* (Figs 2, 3, 5).

Specimens collected by Kjellman in Spitsbergen during 1872 and 1873, and identified and labelled by him as *C. flagelliformis* f. *chordaeformis,* included seven specimens in the UPS collection. Their external morphology agreed well with the original descriptions by Kjellman (1877); the plants were basically unbranched. The specimen collected on 10 January 1873 at Mosselbay is herein selected as the lectotype of f. *chordaeformis* (Fig. 7).

ITS1, 5.8S and ITS2 rDNA

The aligned ITS1, 5.8S and ITS2 rDNA sequences were 1350 base pairs (bp) in total. There were 221 parsimony-informative nucleotide positions. In the analyses using ITS1, 5.8S and ITS2 sequence data (Fig. 8a, b), three phylogenetic groups were evident in all the three types of analysis (MP, NJ and ML), although the branching order varied a little. We will refer to the groups as 'group 1', 'group 2' and 'group 3'. The members of group 1 (Kam1–5 from Abacha Bay, Kamchatka of the Pacific) and group 2 (Spi1–6 from Spitsbergen, North Atlantic, Hok1–7 from Hokkaido, Japan, and StGa1, 2 from Gambell, St. Lawrence Island, Bering Sea, North Pacific) corresponded to the morphological form f. *chordaeformis.* However, group 3 included both f. *flagelliformis* (Ice1, 2 from Iceland, Man1, 2 from the Isle of Man, Spi7, 8, 10–12 from Spitsbergen and Ber from Bergen, Norway, all in the Atlantic, and Kam6, 7 from Abacha Bay, Kamchatka, Hok11–13 from Hokkaido and StGa3, 4 from St. Lawrence Island, all in the Pacific) and f. *chordaeformis* (Spi9 from Spitsbergen and Hok8–10 from Hokkaido). Groups 2 and 3 were each composed of two subgroups ('group 2a' and 'group 2p', 'group 3a' and 'group 3p'), which clearly corresponded to the Atlantic and Pacific populations within the two main groups.

The lengths of the sequences of the ITS1 and ITS2 regions were relatively constant in each group and subgroup (Table 1), but differed considerably between groups: group 1 had 434 bp of ITS1 and 342 bp of ITS2; group 2a, 605–613 and 395–398 bp, group 2p, 599–602 and 358 bp; group 3a, 287–288 and 361–373 bp, group 3p, 264–265 and 339–340 bp.

In the MP and ML analyses, group 1 [f. *chordaeformis* from Kamchatka (Kam1–5)] branched first, followed by group 2 (f. *chordaeformis* from other regions) and group 3 (all f. *flagel-*

Table 2. Primers used for amplifying and sequencing 5.8S, ITS and IGS rDNA, and *rbc*L and the spacer region between *rbc*L and *rbc*S[1].

DNA regions	Code	Synthesis direction	Sequences (5′–3′)	Annealing position
5.8S–ITS	18F1	forward	AAGGTGAAGTCGTAACAAGG	18S
	5.8F-1	forward	ACGCAGCGAAATGCGATACG	5.8S
	5.8R-1	reverse	CGTATCGCATTTCGCTGCGT	5.8S
	26R-1	reverse	GTTAGTTTCTTTTCCTCCGC	26S
IGS	26F-0	forward	GTAGACGACTTGATA	26S
	26F-1	forward	TGTAAGCATGAGAGT	26S
	26F-2	forward	TTGTCCTACGATCTG	26S
	26F-3	forward	CTCTTGTTCCGAAGA	26S
	25F-4	forward	AGTGCATCTCGCCCC	26S
	5R-0	reverse	AGGAACGGCCATACC	5S
	5R-1	reverse	TCTGTGAAGTTAAGC	5S
*rbc*L–spacer	rbc-F0	forward	ATCGAACTCGAATAAAAAGTGA	*rbc*L
	rbc-F1	forward	CGTTACGAATCWGGTG	*rbc*L
	rbc-F2	forward	AGGTTCWCTWGCTAA	*rbc*L
	rbc-F2.5	forward	TTCCAAGGCCCAGCAACAGGT	*rbc*L
	rbc-F3	forward	CACAACCATTCATGCG	*rbc*L
	rbc-F4	forward	GTAAATGGATGCGTA	*rbc*L
	rbc-F5	forward	ATTTGGTGGTGGTACTATTGG	*rbc*L
	rbc-R1	reverse	TTAGCWAGWGAACCT	*rbc*L
	rbc-R2	reverse	CGCATGAATGGTTGTG	*rbc*L
	rbc-R3	reverse	CCTTTAACCATTAAGGGATC	*rbc*L
	rbc-R4	reverse	GTAATATCTTTCCATAAATCTAA	*rbc*L
	rbc-R5	reverse	AAASHDCCTTGTGTWAGTYTC	*rbc*S
	rbc-R6	reverse	AATAAAGGAAGACCCCATAATTCCCA	*rbc*S

[1] IGS, intragenic spacer; ITS, internal transcribed spacer.

liformis). However, the bootstrap values supporting these branches were generally weak (Fig. 8a). In contrast, in the NJ analysis, group 3 branched first, and groups 1 and 2 formed a clade supported by a 100% bootstrap value (Fig. 8b). Some specimens morphologically referable to f. *chordaeformis,* from the Atlantic (Spi9) and Pacific (Hok8–10), were included in the clade of group 3 (f. *flagelliformis*).

IGS

The aligned 26S–5S IGS rDNA sequences were 1256 bp in total. There were 624 parsimony-informative nucleotide positions. In phylogenetic analyses (Fig. 9a, b), three large clades were recognized, corresponding to groups 1, 2 (including subclades -2a and -2p) and 3 (including subclades -3a and -3p), as in the 5.8S–ITS analysis. However, the tree topologies differed from those evident in the equivalent type of analysis of the 5.8S–ITS data set. Group 1 made a clade with group 2 in the MP and ML analyses (Fig. 9a), as in the NJ analysis of the 5.8S–ITS data, whereas group 1 first formed a clade with group 3 in the NJ analysis (Fig. 9b). The monophyly of each subgroup (Atlantic and Pacific) in groups 2 and 3 was supported by higher bootstrap values (100%) in all analyses (Fig. 9a, b) than in the 5.8S–ITS analyses (Fig. 8a, b). Specimen Hok9, assigned to f. *chordaeformis* on the basis of morphology, was included in the group 3 clade, which was principally composed of specimens referable to f. *flagelliformis* as in the 5.8S–ITS analyses.

*rbc*L and spacer

The aligned *rbc*L–spacer sequences were 1708 bp in total. There were 95 parsimony-informative nucleotide positions. Tree topologies based on data set (Fig. 10a, b) were essentially the same as in the 5.8S–ITS rDNA analyses (Fig. 10a, b). The tree topology connecting the three major clades (groups 1–3) was the same in the MP and ML trees, but different in the NJ tree, although bootstrap values were generally low. Specimens Hok9, 10 and Spi9, whose morphology assigned them to f. *chordaeformis,* were included in group 3 (the f. *flagelliformis* clade), as in the 5.8S–ITS and IGS data.

Combined sequence data

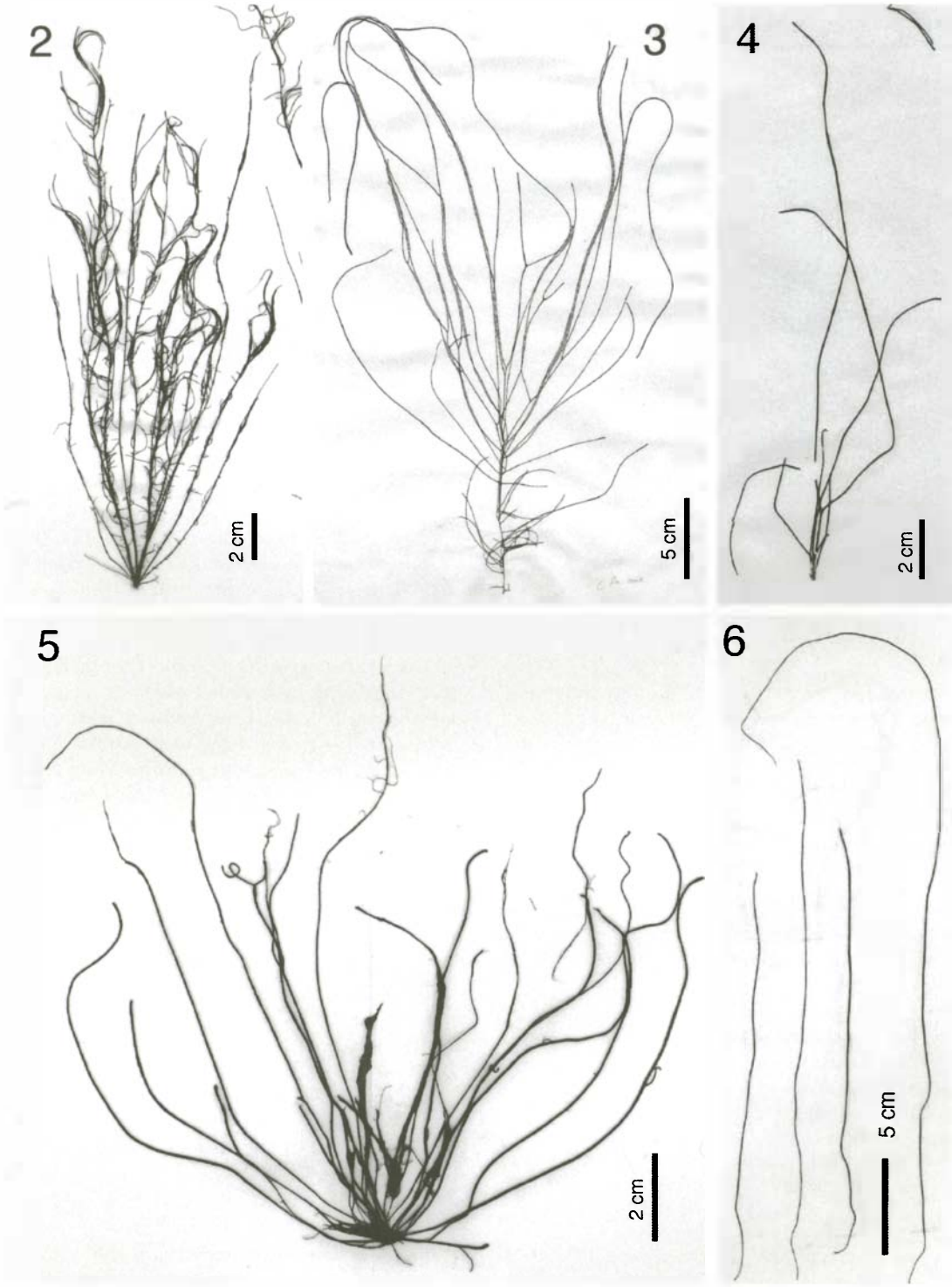
The combined sequence data of aligned 5.8S–ITS, IGS and *rbc*L–spacer sequences were 4231 bp in total, including 1056 parsimony-informative nucleotide positions. Fig. 11 shows the molecular phylogenetic tree based on the combined sequence data set: the tree topology was identical in all analyses (NJ, MP and ML). The bootstrap values supporting the independence of groups 1–3 were generally high (75–100%) and group 1 (f. *chordaeformis* from Kamchatka) formed a clade with group 2 (f. *chordaeformis* from the Atlantic and Pacific). Except for the inclusion of Hok9 (f. *chordaeformis* from the Pacific), f. *flagelliformis* formed a separate clade.

Comparison of pairwise sequence heterogeneity within subgroups

Table 3 compares the percentage of base substitutions (% pairwise sequence heterogeneity) within each subgroup (and group) between 5.8S–ITS, IGS and *rbc*L–spacer sequences. IGS regions showed roughly three to eight times higher substitution rates than ITS regions.

DISCUSSION

Molecular phylogenetic analyses of North Atlantic and North Pacific *C. flagelliformis* revealed that there are three major genetic groups: group 1, representing f. *chordaeformis* from Kamchatka; group 2, representing f. *chordaeformis* from other



Figs 2–6. Morphology of the specimens examined, showing the branching patterns.
Fig. 2. *Chordaria flagelliformis* f. *flagelliformis* (Ny Ålesund, Spitsbergen, Norway; 31 July 2000).
Fig. 3. *Chordaria flagelliformis* f. *flagelliformis* (Nemuro, Hokkaido, Japan; 18 August 2000).
Fig. 4. *Chordaria flagelliformis* f. *chordaeformis* (Abacha Bay, Kamchatka, Russia; 24 July 1998).
Fig. 5. *Chordaria flagelliformis* f. *chordaeformis* (Ny Ålesund, Spitsbergen, Norway; 30 July 2000).
Fig. 6. *Chordaria flagelliformis* f. *chordaeformis* (Oshinkoshin, Shiretoko Peninsula, Hokkaido, Japan; 30 July 2000).

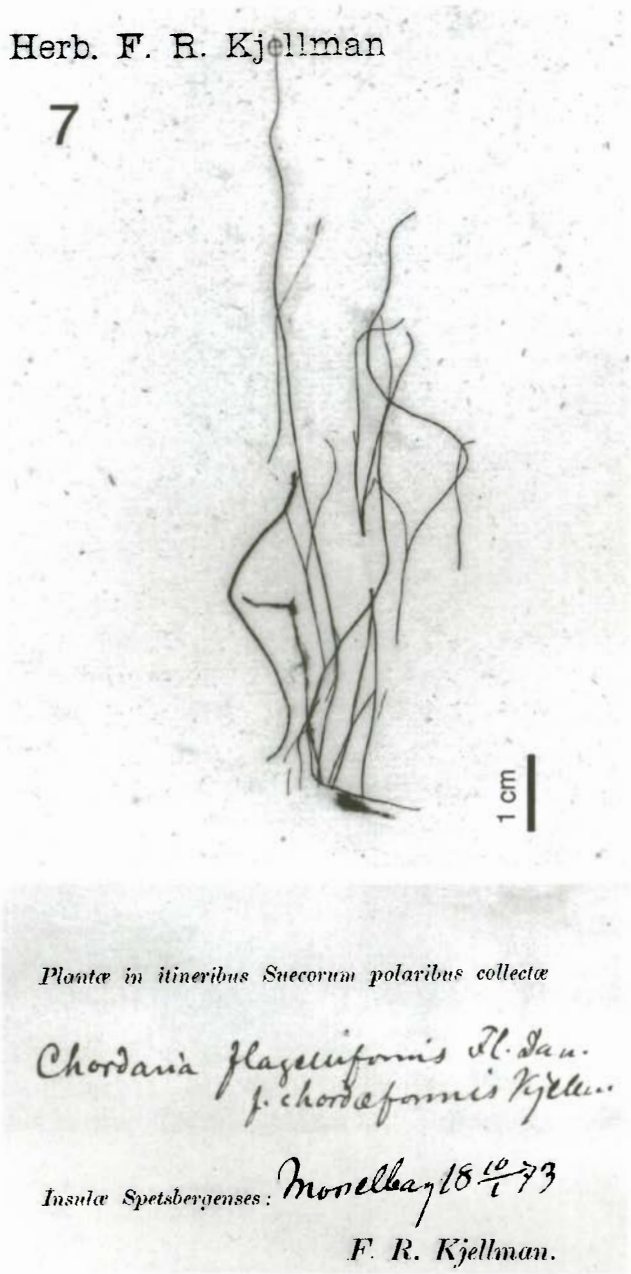


Fig. 7. Morphology of the lectotype specimen of *C. chordaeformis* (Kjellman) Kawai & S.H. Kim *stat. nov.*, collected by F.R. Kjellman at Mosselbay, Spitsbergen, Norway, on 10 January 1873 (UPS).

regions; and group 3, representing all the f. *flagelliformis* as well as some specimens with no or few branches. These groups differed considerably in the length of the ITS rDNA region (Table 1), suggesting their phylogenetic separation. The relationships among the three groups could not be clearly resolved by the use of any one genetic region, because the tree topologies differed depending on the method of analysis (NJ, MP and ML), irrespective of the DNA region used (rDNA or Rubisco gene). The bootstrap values indicating the robustness of the branches connecting the three genetic groups tended to be weak. However, the analysis using the whole-sequence data set obtained in the present study (4231 bp including 5.8S, ITS and IGS rDNA, and *rbc*L and spacer region of Rubisco gene) gave one relatively robust tree, in which group 1 and group 2 formed a clade supported by high bootstrap values (99–100%), except in the ML analysis (51%).

Groups 1 and 2 were composed of specimens morphologically referable to f. *chordaeformis* (basically unbranched thalli), whereas group 3 mostly included specimens with several to many branches (which were therefore referable to f. *flagelliformis*), although it also included a few specimens with no or very few branches. However, despite the inconsistency of these thallus-branching patterns, there is little doubt that these group 3 unbranched specimens belong to the same taxonomic entity as do the other f. *flagelliformis* specimens, judging by the molecular data (length of ITS sequence and the results of phylogenetic analyses using multiple genes). They should therefore be correctly classified as f. *flagelliformis*. These results demonstrate the high morphological plasticity of *C. flagelliformis* f. *flagelliformis*.

The group 1 specimens (from Kamchatka and morphologically referable to f. *chordaeformis*) require further investigation, but the other two groups, 2 and 3, are considered to correspond to two of Kjellman's forms of *C. flagelliformis*, viz. f. *flagelliformis* and f. *chordaeformis*. Each of the groups (clades) 1 and 2 included two subgroups, corresponding to Atlantic and Pacific populations. Regarding the taxonomy of these two taxa, we suggest that it is appropriate to recognize f. *chordaeformis* at the species level as *C. chordaeformis* (Kjellman) Kawai & S.H. Kim *stat. nov.* for the following reasons:

- f. *chordaeformis* (= *C. chordaeformis*) has considerably longer ITS1 and ITS2 sequences of rDNA, compared with *C. flagelliformis* collected from a large part of its worldwide distributional range.
- The independence and monophyletic status of *C. chordaeformis* was clearly supported by all the analyses, using 5.8S–ITS and IGS rDNA as well as *rbc*L–spacer gene sequence data. Furthermore, the molecular phylogenetic trees indicate that *C. chordaeformis* and *C. flagelliformis* have different ancestors, and have diversified independently in the Atlantic and Pacific.
- *Chordaria chordaeformis* has highly stable morphological features; the sporophytes are simple or have very few branches that are borne at acute angles. In contrast, *C. flagelliformis* has a rather variable number of branches and simple unbranched individuals are sometimes encountered.

→

Fig. 8. Molecular phylogenetic trees of *C. flagelliformis* specimens, based on 5.8S–ITS sequences. (a) Maximum parsimony (MP) and maximum likelihood (ML) analyses. The tree shown is based on the MP tree. In the MP analysis, a strict consensus tree of the 631 most parsimonious trees of 631 steps was obtained with a consistency index (CI) of 0.878 and a retention index (RI) of 0.948. In the ML tree, −ln likelihood was 5113.97235. (b) Neighbour-joining (NJ) analysis. Bootstrap values indicate the percentage based on 1000 (MP and NJ) and 500 (ML) replicates (in [a], MP/ML). '*' indicates f. *chordaeformis* morphology.

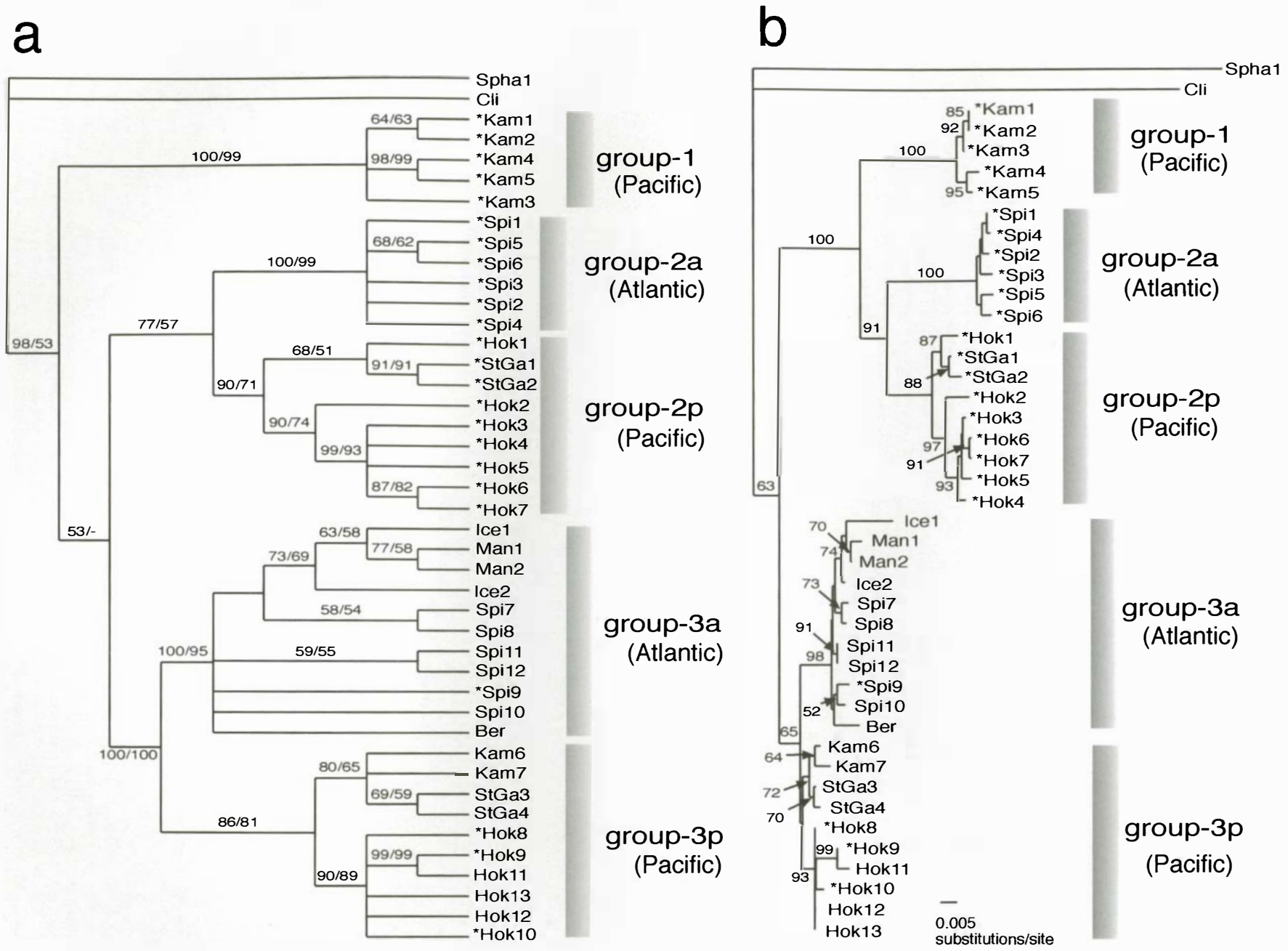

a
Spha1
Cli
64/63
*Kam1
*Kam2
100/99
98/99
*Kam4
*Kam5
*Kam3
group-1
(Pacific)
*Spi1
68/62
*Spi5
100/99
*Spi6
*Spi3
*Spi2
*Spi4
group-2a
(Atlantic)
77/57
98/53
*Hok1
68/51
91/91
*StGa1
90/71
*StGa2
*Hok2
90/74
*Hok3
99/93
*Hok4
*Hok5
87/82
*Hok6
*Hok7
group-2p
(Pacific)
53/-
Ice1
63/58
77/58
Man1
73/69
Man2
Ice2
58/54
Spi7
Spi8
100/95
59/55
Spi11
Spi12
*Spi9
Spi10
Ber
group-3a
(Atlantic)
100/100
Kam6
80/65
Kam7
69/59
StGa3
StGa4
86/81
*Hok8
99/99
*Hok9
90/89
Hok11
Hok13
Hok12
*Hok10
group-3p
(Pacific)
b
Spha1
Cli
85
*Kam1
92
*Kam2
100
*Kam3
*Kam4
95
*Kam5
group-1
(Pacific)
*Spi1
100
*Spi4
*Spi2
100
*Spi3
*Spi5
*Spi6
group-2a
(Atlantic)
91
87
*Hok1
*StGa1
88
*StGa2
*Hok2
*Hok3
97
*Hok6
91
*Hok7
93
*Hok5
63
*Hok4
group-2p
(Pacific)
70
Ice1
Man1
74
Man2
73
Ice2
Spi7
91
Spi8
Spi11
98
Spi12
*Spi9
52
Spi10
Ber
group-3a
(Atlantic)
65
Kam6
64
Kam7
72
StGa3
StGa4
70
*Hok8
99
*Hok9
Hok11
93
*Hok10
Hok12
Hok13
group-3p
(Pacific)
0.005
substitutions/site

Table 3. Pairwise sequence heterogeneity (%, $\bar{x} \pm s$) in each DNA region (5.8S + ITS, IGS and *rbc*L + spacer) within genetic groups and subgroups of *Chordaria flagelliformis*. n = number of sequences in the data set.[1]

	group 1	group 2a	group 2p	group 3a	group 3p
5.8S + ITS	0.6 ± 0.34 (n = 5)	0.7 ± 0.24 (n = 6)	1.4 ± 0.67 (n = 9)	1.0 ± 0.59 (n = 11)	1.0 ± 0.54 (n = 10)
IGS	2.4 ± 0.48 (n = 4)	2.1 ± 0.83 (n = 3)	8.4 ± 0.77 (n = 4)	7.9 ± 2.99 (n = 5)	8.5 ± 2.41 (n = 5)
*rbc*L + spacer	0.3 (n = 2)	0.3 ± 0.14 (n = 4)	0.6 ± 0.14 (n = 4)	0.2 ± 0.13 (n = 6)	0.6 ± 0.25 (n = 8)

[1] IGS, intragenic spacer; ITS, internal transcribed spacer.

The branches of this form are almost perpendicular to the main axes.

- *Chordaria chordaeformis* has a narrower geographical distribution than *C. flagelliformis* and is confined to colder regions, implying a lower tolerance to warm conditions, although there are no experimental data to support this assumption.

One of the additional characteristic features of *C. chordaeformis* is the longer period of fertility of the sporophytes than that occurring in *C. flagelliformis sensu stricto.* This was previously noted by Kjellman (1883): at Spitsbergen, he reported that *C. chordaeformis* bore unilocular sporangia in January, February, May, July, August and December, whereas the typical form, as well as f. *ramusculifera* and f. *simplex,* bore unilocular sporangia only in summer. At Akkeshi, Hokkaido, Japan, *C. flagelliformis* bears unilocular sporangia from August to November, and *C. chordaeformis* from July to January (H. Kawai, unpublished observations). This corresponds to the longer growth period of the latter species at Akkeshi (May to November in *C. flagelliformis* and May to January in *C. chordaeformis*). At Nemuro, near Akkeshi, the basal portion of the fertile thallus of *C. chordaeformis* remained even until the following May (H. Kawai, unpublished observations) and basically agrees with the phenology at Spitsbergen reported by Kjellman (1883).

Although the sampling of the *Chordaria* specimens was not

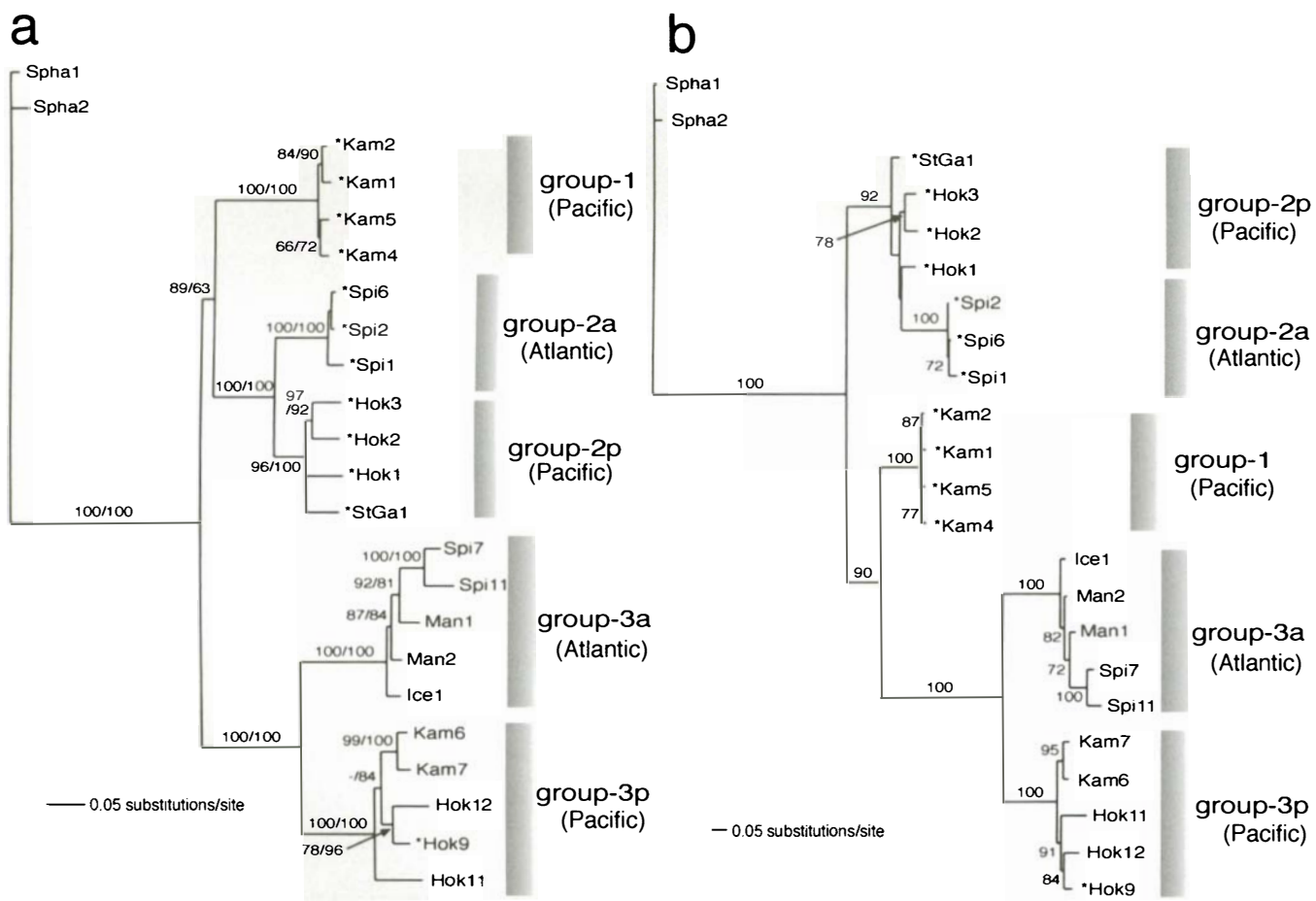


Fig. 9. Molecular phylogenetic trees of *C. flagelliformis* specimens, based on 26S–5S IGS region sequences. (a) Neighbour-joining (NJ) and maximum parsimony (MP) analyses. The tree shown is based on the NJ tree. (b) MP and maximum likelihood (ML) analyses. a. In the MP analysis, the two most parsimonious trees of 1438 steps were obtained with a consistency index (CI) of 0.816 and a retention index (RI) of 0.912. A strict consensus tree of the two most parsimonious trees was obtained with a CI of 0.816 and an RI of 0.912. b. ML analysis. −ln likelihood was 8161.93998. Bootstrap values indicate the percentage based on 1000 (MP and NJ) and 500 (ML) replicates (in 'a', MP/NJ). '*' indicates f. *chordaeformis* morphology.

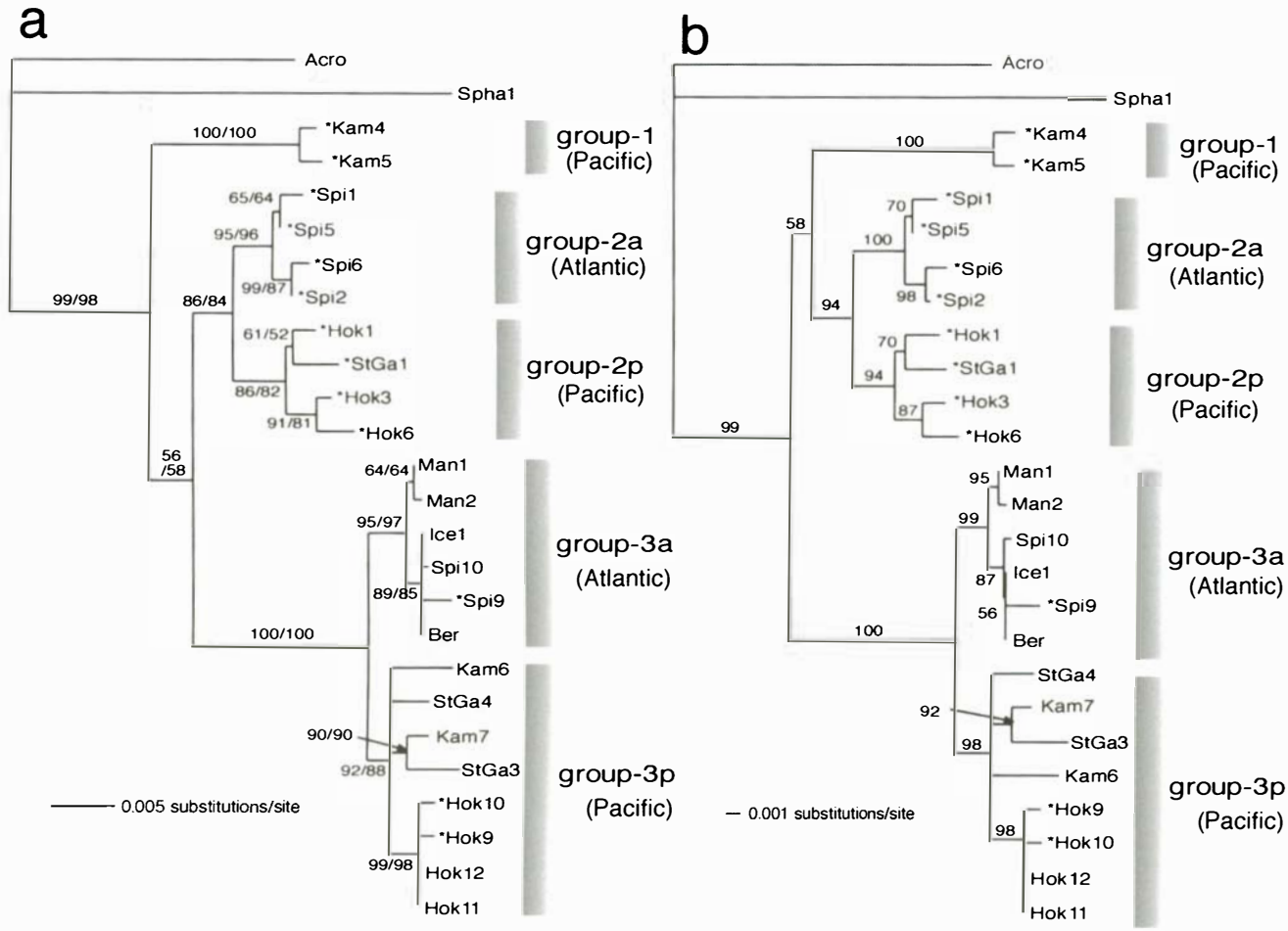


Fig. 10. Molecular phylogenetic trees of *C. flagelliformis* based on *rbc*L and spacer sequences. (a) Maximum parsimony (MP) and maximum likelihood (ML) analyses. The tree shown is based on the MP tree. One most parsimonious tree of 260 steps was obtained with a consistency index (CI) of 0.842 and a retention index (RI) of 0.910. In the ML analysis, −ln likelihood was 4064.70447. (b) Neighbour-joining (NJ) analysis. Bootstrap values indicate the percentage based on 1000 (MP and NJ) and 500 (ML) replicates (in 'a', MP/ML). '*' indicates f. *chordaeformis* morphology.

intended to be restricted to the forms f. *flagelliformis* and f. *chordaeformis,* and relatively diverse morphological forms were found in the branched types of specimens collected, no independent genetic groups representing f. *ramusculifera* and f. *subsimplex* were found in the present analyses. Although some specimens without branches or with only a few branches were collected from Hokkaido, Japan (Hok8–10), they clustered with the typical form with many branches, so they are better regarded as an ecotype of f. *flagelliformis.* This suggests that f. *ramusculifera* and f. *subsimplex* may also be ecotypes of *C. flagelliformis sensu stricto.* Setchell & Gardner (1924) described a new species, *C. gracilis,* from the eastern Pacific, and also reported the occurrence of *C. flagelliformis* f. *ramusculifera* at St. Lawrence Island in the Bering Sea. In the western Pacific, Yamada (1935) and Inagaki (1958) reported the occurrence of *C. gracilis* in the Kurile Islands. However, the diagnostic characters of these taxa are not very clear, so that further investigations are needed to confirm whether they are truly distinct.

Pairwise comparisons of sequence heterogeneity (Table 3) indicate that IGS regions are about three to eight times more variable than 5.8S–ITS regions. This may have contributed the higher bootstrap values in resolving the phylogenetic relationships of the Atlantic and Pacific *C. flagelliformis* and *C. chordaeformis* populations (Fig. 8). Therefore, this DNA region is considered to provide suitable resolution for the study of relatively closely allied taxa, where ITS cannot clearly resolve phylogenetic relationships.

In the *rbc*L–spacer data, the Pacific populations showed greater genetic variation (0.6% in groups 2p and 3p) than did the Atlantic populations (0.3% and 0.2% in groups 2a and 3a, respectively), possibly suggesting their origin in the Pacific. However, this tendency was also found in 5.8S–ITS and IGS data in *C. chordaeformis,* but not in *C. flagelliformis.* It is also noteworthy that group 1 had relatively high genetic diversity, although the collections were made in a rather narrow geographical area within Abacha Bay. In *Chorda filum* (Linnaeus) Stackhouse, the pairwise sequence heterogeneity within the Atlantic populations and Pacific populations [excluding those from Kikonai (Hokkaido), Kamchatka and Puget Sound (northwest America), which formed a distinct clade from other Pacific *C. filum*] has been found to be 0.8% (Atlantic) and 0.7–0.9% (Pacific) for the *rbc*L–spacer, and 0.4–2.6% (Atlantic) and 0.1–3.7% for the 5.8S–ITS sequences (H. Sasaki and

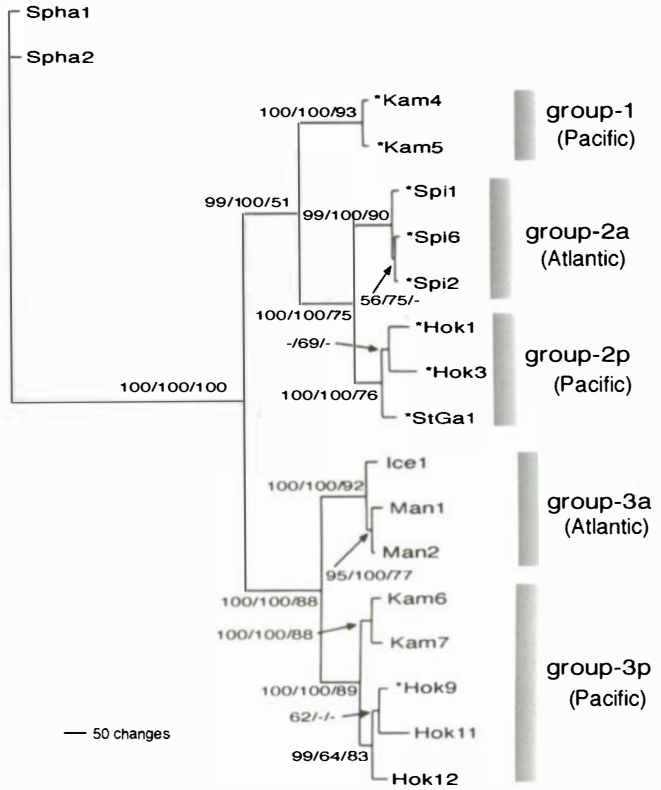


Fig. 11. Molecular phylogenetic tree of *C. flagelliformis* specimens, based on combined sequence data of 5.8S, ITS and IGS regions of rDNA, and the *rbc*L and spacer region of the Rubisco gene. The tree shown is based on the NJ tree. In the MP analysis, one most parsimonious tree of 1961 steps was obtained with a consistency index (CI) of 0.865 and a retention index (RI) of 0.916. In the ML analysis, −ln likelihood was 16361.66369. Bootstrap values indicate the percentage based on 1000 (MP and NJ) and 500 (ML) replicates (MP/NJ/ML). '*' indicates f. *chordaeformis* morphology.

H. Kawai, unpublished observations). Although the two populations are likely to be different species, as judged by the results of phylogenetic analyses (Kawai *et al.* 2001), genetic variation within the Pacific and Atlantic populations of *C. filum* was similar in *rbc*L–spacer, as well as in 5.8S–ITS sequences. The accumulation of such data in various taxa will provide valuable information for discussing the speciation of circumpolar species, as well as for detecting the effects of past geographical changes, specifically the connections between the North Atlantic and North Pacific Oceans.

Chordaria chordaeformis (Kjellman) Kawai & S.H. Kim *stat. nov.*

BASIONYM: *Chordaria flagelliformis* f. *chordaeformis* Kjellman (1877, pp. 28, 29, figs 13–15, table 1).

LECTOTYPE (designated here): UPS, *C. flagelliformis* f. *chordaeformis,* collected at Mosselbay, Spitsbergen, on 10 January 1873, by F.R. Kjellman.

Chordaria chordaeformis is close to *C. flagelliformis* but differs by the consistently simple thallus (unbranched or with a few branches), the longer growth period, the narrower distributional range in colder-water regions, and the considerably longer ITS rDNA sequences.

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REFERENCES

AGARDH C.A. 1817. *Synopsis algarum Scandinaviae.* Lund, Sweden. 135 pp.

ASSALI N.-E., MACHE R. & LOISEAUX-DE GOËR S. 1990. Evidence for a composite phylogenetic origin of the plastid genome of the brown alga *Pylaiella littoralis* (L.) Kjellm. *Plant Molecular Biology* 15: 307–315.

CARAM B. 1955. Sur l'alternance de générations chez *Chordaria flagelliformis. Botanisk Tidsskrift* 52: 18–36.

DAUGBJERG N. & ANDERSEN R.A. 1997. A molecular phylogeny of the heterokont algae based on analyses of chloroplast-encoded *rbc*L sequence data. *Journal of Phycology* 33: 1031–1041.

FELSENSTEIN J. 1985. Confidence limits on phylogenies: an approach using bootstrap. *Evolution* 39: 783–791.

HAMEL G. 1935. *Phéophycées de France,* part 2. Paris. pp. 81–176.

HANSEN G.I. 1997. A revised checklist and preliminary assessment of the macrobenthic marine algae and seagrasses of Oregon. In: *Conservation and management of native flora and fungi* (Ed. by T.N. Kaye, A. Liston, R.M. Love, D.L. Luoma, R.J. Meinke & M.V. Wilson), pp. 175–200. Native Plant Society of Oregon, Corvallis.

INAGAKI K. 1958. A systematic study of the order Chordariales from Japan and its vicinity. *Scientific Papers of the Institute of Algological Research, Faculty of Science, Hokkaido University* 4: 87–197.

KAWAI H. & KUROGI M. 1982. Morphology and ecology of *Chordaria flagelliformis* f. *flagelliformis* and f. *chordaeformis* (Phaeophyceae, Chordariales). In: *Proceedings of the 47th Annual Meeting of the Japanese Botanical Society at Tokyo,* p. 206. The Botanical Society of Japan, Tokyo. (In Japanese)

KAWAI H., MUTO H., FUJII T. & KATO A. 1995. A linked 5S rRNA gene in *Scytosiphon lomentaria* (Scytosiphonales, Phaeophyceae). *Journal of Phycology* 31: 306–311.

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.

KIMURA M. 1980. A simple method for estimating rate of base substitutions through comparative studies of nucleotide sequences. *Journal of Molecular Evolution* 16: 111–120.

KJELLMAN F.R. 1877. Om Spetsbergens marina, klorofyllförande Thallophyter. II. *Bihang til Kongliga Svenska Vetenskaps-Akademiens Handlingar* 4(6): 1–61.

KJELLMAN F.R. 1883. The algae of the Arctic Sea. A survey of the species, together with an exposition of the general characters and the development of the flora. *Bihang til Kongliga Svenska Vetenskaps-Akademiens Handlingar* 20: 1–351.

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.

KORNMANN V.P. 1962. Die Entwicklung von *Chordaria flagelliformis. Helgoländer wissenschaftliche Meeresuntersuchungen.* 8: 276–279.

KORNMANN P. & SAHLING P.-H. 1977. Meeresalgen von Helgoland:

benthische Grün-, Braun- und Rotalgen. *Helgoländer wissenschaftliche Meeresuntersuchungen* 29: 1–289.

LÜNING K. 1990. *Seaweeds. Their environment, biogeography and ecophysiology.* Wiley, New York. 527 pp.

NAGAI M. 1940. Marine algae of the Kurile Islands. 1. *Journal of the Faculty of Agriculture, Hokkaido University* 46: 1–137.

PERESTENKO L.P. 1980. *Vodorosli zaliva Petra Velikogo.* Nauka, Leningrad. 232 pp. (In Russian)

ROSENVINGE L.K. 1893. Grønlands Havalger. *Meddelelser om Grønland* 3: 763–981.

ROSENVINGE L.K. & LUND S. 1943. The marine algae of Denmark. Vol. II. Phaeophyceae. *Det kongelige danske Videnskabernes Selskab, Biologiske Skrifter* 2: 1–59.

SAUNDERS G.W. & DRUEHL L.D. 1992. Nucleotide sequences of the small-subunit ribosomal RNA genes from selected Laminariales (Phaeophyta): implications for kelp evolution. *Journal of Phycology* 28: 544–549.

SAUVAGEAU C. 1929. Sur le development de quelques Phéosporées. *Bulletin de la Station Biologique d'Arcachon.* 26: 253–420.

SETCHELL W.A. & GARDNER N.L. 1924. Phycological contributions, VII. *University of California Publications in Botany* 13: 1–13.

SIEMER B.L., STAM W.T. & OLSEN J.L. 1998. Phylogenetic relationships of the brown algal orders Ectocarpales, Chordariales, Dictyosiphonales, and Tilopteridales (Phaeophyceae) based on RuBisCo large subunit and spacer sequences. *Journal of Phycology* 34: 1036–1048.

STACHE-CRAIN B., MÜLLER D.G. & GOFF L.J. 1997. Molecular systematics of *Ectocarpus* and *Kuckuckia* (Ectocarpales, Phaeophyceae) inferred from phylogenetic analysis of nuclear- and plastid-encoded DNA sequences. *Journal of Phycology* 33: 152–168.

SWOFFORD D.L. 1999. *PAUP*: phylogenetic analysis using parsimony,* version 4.0b2. Sinauer Associates, Sunderland, Massachusetts.

TAN I.H. & DRUEHL L.D. 1993. Phylogeny of the northeast Pacific brown algal (Phaeophycean) orders as inferred from 18S rRNA gene sequences. *Hydrobiologia* 260/261: 699–704.

TAN I.H. & DRUEHL L.D. 1996. A ribosomal DNA phylogeny supports the close evolutionary relationships among the Sporochnales, Desmarestiales, and Laminariales (Phaeophyceae). *Journal of Phycology* 32: 112–128.

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

TAYLOR W.R. 1937. *Marine algae of the northeastern coast of North America.* University of Michigan Press, Ann Arbor. 509 pp.

THOMPSON J.D., HIGGINS D.G. & GIBSON T.J. 1994. CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position specific gap penalties and weight matrix choice. *Nucleic Acids Research* 22: 425–430.

TOKIDA J. 1934. The marine algae from Robben Island, Saghalien. *Bulletin of the Faculty of Fisheries, Hokkaido University* 4: 16–26.

TOKIDA J. 1954. The marine algae of southern Saghalien. *Memoirs of the Faculty of Fisheries, Hokkaido University* 2: 1–164.

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.

YAMADA Y. 1935. Marine algae from Urup, the middle Kuriles, especially from the vicinity of Iema Bay. *Scientific Papers of the Institute of Algological Research, Faculty of Science, Hokkaido University* 1: 1–26.

YAMADA Y. & TANAKA T. 1944. Marine algae in the vicinity of the Akkesi Marine Biological Station. *Scientific Papers of the Institute of Algological Research, Faculty of Science, Hokkaido University* 3: 47–77.

ZINOVA A.D. 1953. *Opredelitel' burnikh vodoroslej severnikh morej SSSR.* Akademii Nauk SSSR, Moscow/Leningrad. 224 pp.

ZINOVA A.D. 1954. Novye semejstvo, rod i vid u burykh vodoroslej. *Trudy Botanicheskogo Instituta im. V.L. Komarova Akademii Nauk SSSR,* ser. 2, 9: 223–244.

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