Phycologia (2003) Volume 42 (2), 183–192 Published 29 April 2003

Taxonomic revision of *Sphaerotrichia divaricata* (Ectocarpales, Phaeophyceae), with a reappraisal of *S. firma* from the north-west Pacific

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

Specimens of *Sphaerotrichia* in Japan can be classified into two morphotypes, based on their mode of branching and anatomy: (1) specimens that have an obvious (traceable) main axis associated with a relatively high number of first-order branches, and a relatively constant distance between branches; and (2) specimens that branch more or less divaricately, with shorter distances between branches in the distal portion. Molecular analyses of *Sphaerotrichia* from the North Atlantic and North Pacific using ribosomal RNA (ITS1–5.8S–ITS2 and intragenic spacer regions between 26S and 5S rDNA) and Rubisco (almost complete *rbc*L gene and the spacer between *rbc*L and *rbc*S) gene sequences revealed two major genetic groups, Group-1 and Group-2. The sequence length variation in ITS1 suggested subdivisions of Group-2 into two subgroups: Group-2Atlantic and Group-2Pacific. Specimens belonging to Group-1 were generally epilithic, and morphologically corresponded to *S. firma*. Specimens belonging to Group-2 were epiphytic or epilithic and morphologically corresponded to *S. divaricata*.

INTRODUCTION

The taxonomy of *Sphaerotrichia divaricata* (C. Agardh) Kylin (Chordariaceae) has been somewhat complicated and controversial. The species was first described as *Chordaria divaricata* C. Agardh (Agardh 1817) from the Baltic Sea (northeastern Atlantic). Later, Gepp (1904) described a morphologically similar species, *C. firma* E. Gepp, from China (northwestern Pacific). Okamura (1915) described the detailed morphology of *C. firma* based on Japanese and Korean specimens. Kylin (1940) suggested the separation of *C. divaricata* from the genus *Chordaria* C. Agardh, mostly on the basis of differences in the apical structure, and established a new genus *Sphaerotrichia* Kylin. He made a new combination, *S. divaricata* (C. Agardh) Kylin, and a new species, *S. japonica* Kylin, for what had been erroneously identified as *C. cladosiphon* Kützing by Okamura (1915). *Sphaerotrichia* has been widely accepted by later researchers. Yamada & Tanaka (1944) added another species, *S. chordarioides* Yamada, from Japan.

Zinova (1958) re-examined the taxonomy of *Sphaerotrichia* species based on overall morphology and cortical anatomy. He recognized *S. divaricata* and proposed new combinations for two other species: *S. dissessa* (Setchell & Gardner) Zinova and *S. firma* (E. Gepp) Zinova. By contrast, in the same year, Inagaki (1958) proposed the merging of all reported *Sphaerotrichia* taxa into one species, *S. divaricata*, and this treatment was later followed by Peres-tenko (1980). A new species, *S. sadoensis* Noda, was described from Japan (Noda 1975), but Yoshida (1998) considered it to be merely a growth form (ecotype) of *S. divaricata* under mariculture conditions.

Peters *et al.* (1987) demonstrated zygote formation in *S. divaricata* by crossing experiments between gametophytes from Europe and Atlantic and Pacific North America, and the authors regarded this as evidence supporting the merging of *S. firma* and *S. japonica* into *S. divaricata* as proposed by Inagaki (1958). However, Peters *et al.* (1993) subsequently showed that isolates of *Sphaerotrichia* from Japan, the Mediterranean (where the authors infer that the alga was introduced from Japan) and northern Europe could not cross with isolates from Atlantic and Pacific North America. Therefore the authors suggested re-examination of the taxonomic relationships between the species of *Sphaerotrichia*.

In this study, we have re-examined the systematic relationships between Atlantic and Pacific *S. divaricata* by molecular phylogenetic analyses using the nuclear ribosomal RNA [ITS1–5.8S–ITS2 (ITS is the internal transcribed spacer) and the intragenic spacer (IGS) region between 26S and 5S rDNA (Kawai *et al.* 1995)] and the plastidial Rubisco operon (almost the complete *rbc*L gene and the spacer region between *rbc*L and *rbc*S). This study is based on specimens collected in various locations in Japan, as well as on the culture strains used in the crossing experiments of Peters *et al.* (1987, 1993). By using those strains, we have also obtained information for discussing the relationship between genetic divergence and reproductive barriers.

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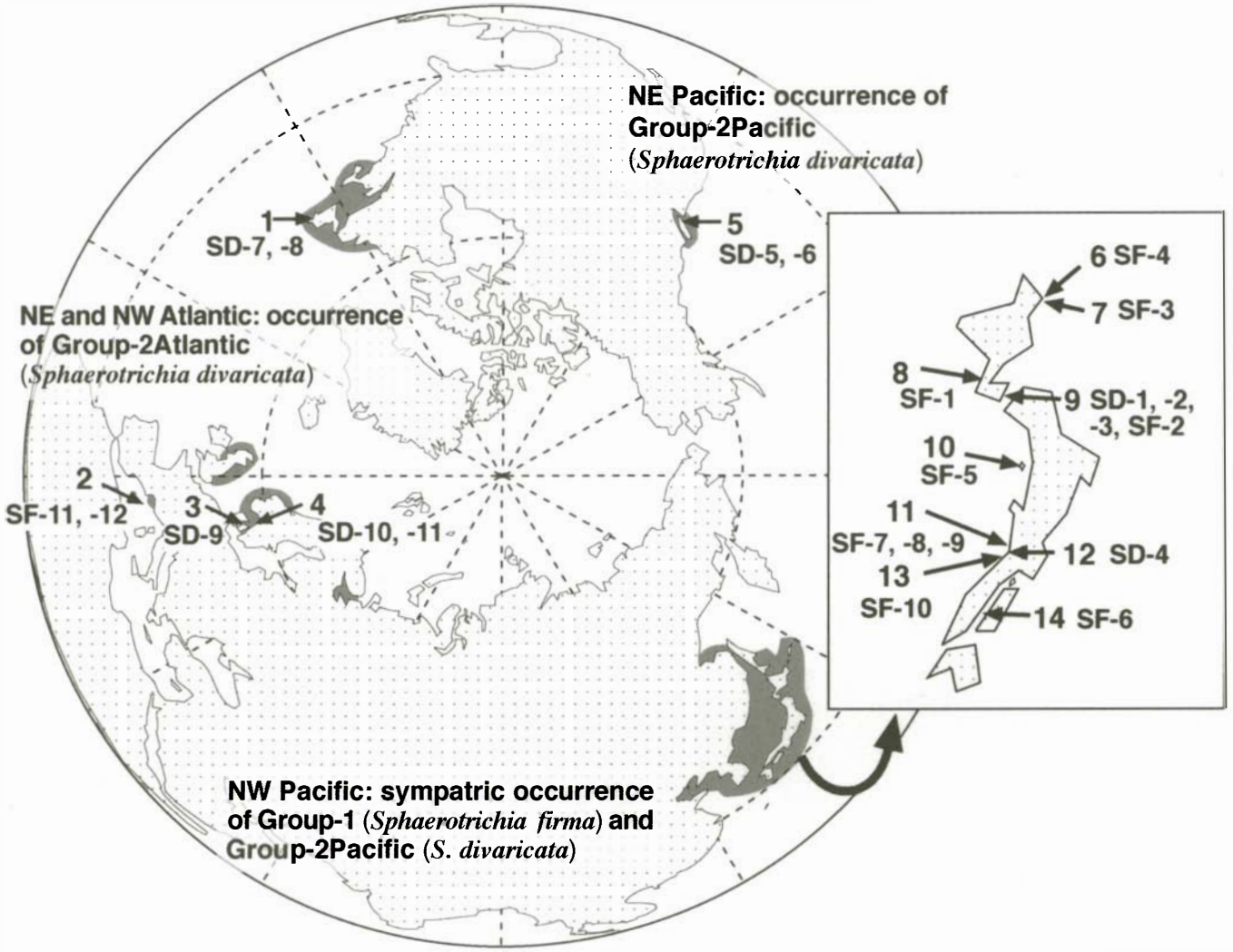


Fig. 1. Distribution of *Sphaerotrichia divaricata* and *S. firma:* collection sites of the materials examined in the present study and specimen codes (see Table 1). 1, Newfoundland, eastern Canada; 2, Étang de Thau, France; 3, Isefjord, Sjaelland, Denmark; 4, Varberg, Kattegat, Sweden; 5, Bamfield, British Columbia, Pacific Canada; 6, Nemuro, Hokkaido, Japan; 7, Hanasaki, Hokkaido, Japan; 8, Oshoro, Hokkaido, Japan; 9, Kikonai, Hokkaido, Japan; 10, Aikawa, Niigata, Japan; 11, Obama, Fukui, Japan; 12, Takahama, Fukui, Japan; 13, Miyazu, Kyoto, Japan; 14, Kurahashi, Hiroshima, Japan.

MATERIAL AND METHODS

Morphological observations and crossing experiments

Specimens used for the molecular phylogenetic study were from Europe, Atlantic and Pacific North America and Japan (Fig. 1, Table 1). Habit, overall morphology (especially the mode of branching) and anatomy were examined in field-collected specimens from Japan. For the anatomical study, thin sections from the upper, middle and lower portions of the thalli were made by hand using razor blades, and an average of 7–10 sections were observed from each portion. Cultures were grown in polystyrene Petri dishes containing 50 ml PESI 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. The interfertility of the newly isolated male strain of *S. divaricata* from Denmark (SD-9) with isolates from other regions was tested as described in Peters *et al.* (1993). In addition, the interfertility of strain SD-6, which Peters *et al.* (1987) had only tested against another strain from the north-east Pacific, was tested with male strains from other regions.

Molecular phylogenetic analysis

For DNA extraction, cultured material was frozen in liquid nitrogen, and field-collected material was blotted and then desiccated rapidly in silica gel powder. Air-dried herbarium vouchers were also used. Approximately 40 mg of algal tissue powder (ground in liquid nitrogen) were used for genomic DNA extractions, using the DNeasy Plant Mini Kit (Qiagen, Hilden, Germany). We amplified three gene sequences of (1) the 5.8S, ITS1 and ITS2 (ITS regions between 18S, 5.8S and 26S rDNA) rDNA; (2) the IGS (between 26S and 5S); and (3) the Rubisco large subunit gene (*rbc*L) and the spacer region between *rbc*L and the small subunit gene (*rbc*S). Polymerase chain reactions (PCR) were carried out using GeneAmp PCR Systems 2400 and 9700 (Applied Biosystems,

Foster City, CA, USA) and a TaKaRa Ex Taq (Takara Shuzo, Shiga, Japan) Reaction Kit. The total reaction volume was 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. Unless otherwise specified, primers (Table 2) were based on 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 for 5 min at 95°C, 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 either a Cy5 Auto Cycle Sequencing Kit (Pharmacia Biotech AB, Uppsala, Sweden) and an ALF express DNA sequencer (Pharmacia Biotech AB) or a 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 the preliminary alignment of DNA sequences, followed by manual final alignment. The alignment data are available from the authors on request. 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 branch-swapping option. Gaps were treated as missing data. From the same alignment, two-parameter distances (Kimura 1980) between taxa were estimated, 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 heuristic search with empirical base frequencies, equal among-site rate variation, and the expected transition–transversion ratio set at 2.0 (using the Hasegawa–Kishino–Yano model). The robustness of the resulting phylogenies was tested by bootstrap analyses with 1000 (MP and NJ) and 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, IGS rDNA, *rbc*L and spacer) were also done using those specimens in which all these regions had been sequenced. *Chordaria chordaeformis* (Kjellman) Kawai & S.H. Kim (Kim & Kawai 2002) and *Chordaria* sp. were used as out-groups for the 5.8S–ITS, IGS and partial *rbc*L–spacer analyses. The percentage of base substitutions (pairwise sequence heterogeneity) within each subgroup (and group) between 5.8S–ITS, IGS and *rbc*L–spacer sequences was calculated by the Kimura two-parameter method using PAUP v. 4.0.2b (Swofford 1999).

RESULTS

Morphology, anatomy and crossing experiments

Specimens of *Sphaerotrichia* collected from Japan could be classified into two groups (morphotypes) based on their habit (epilithic or epiphytic), mode of branching and anatomy. Specimens belonging to the first group (Fig. 2, n = 146) had a more obvious (traceable) main axis and had more first-order branches (Fig. 8, Group-1). The distance between the branches was relatively constant (Fig. 2). The thalli were hollow in their middle to upper portions, but solid near the base (Fig. 3). Specimens belonging to this group were mostly epilithic, but some were epiphytic (Table 1). In contrast, in specimens belonging to the second group (Fig. 4, n = 117), the distance between branches tended to be shorter in the distal portion (Fig. 8), and had more or less divaricate branching (Fig. 4). The number of first-order branches did not exceed 40 even in specimens longer than 30 cm (Fig. 8, Group-2Pacific and Group-2Atlantic). A main axis was not traceable in these specimens (Fig. 4) and the thalli were hollow along their entire length (Fig. 5). Specimens belonging to this group were all epiphytic (Table 1).

Syntypes of *C. divaricata* (LD 46393, LD 46394) had divaricate branches, as in the original description, and the distances between branches were shorter in the distal portions (Fig. 6). The type of *C. firma* (BM 563399) had a more or less traceable main axis, and the distances between first-order branches were relatively constant (Fig. 7).

The female strain SD-6 and the male SD-9 were interfertile, and zygotes were formed in all combinations with sexually compatible strains from the north-east Pacific, north-west Atlantic, or northern Europe. As expected, no gamete fusions occurred in the attempted crosses of the two strains with those from southern France (SF-11, SF-12).

ITS1, 5.8S and ITS2 rDNA

The aligned ITS1, 5.8S and ITS2 rDNA sequences were 1445 base pairs (bp) in total, and there were 246 parsimony-informative nucleotide positions. The length of the ITS2 region was relatively constant (327–337 bp). In contrast, the length of the ITS1 region was more variable, allowing the recognition of three groups: Group-1 (ITS1 502–506 bp) contained SF-1, -2, -3 and -4 (Hokkaido), SF-5 (Niigata), SF-6 (Hiroshima), SF-7, -8 and -9 (Fukui) and SF-10 (Kyoto) from Japan in the north-west Pacific and SF-12 from Mediterranean France; Group-2Pacific (ITS1 602–645 bp) contained SD-1, -2 and -3 (Hokkaido) and SD-4 (Fukui) from Japan and SD-6 and -7 from NW Canada; and Group-2Atlantic (ITS1 818–843 bp) contained SD-7 and -8 from Newfoundland and SD-9, -10 and -11 from northern Atlantic Europe (Table 1, Fig. 9a).

Molecular phylogenetic analyses using ITS1, 5.8S and ITS2 sequence data (Fig. 9a) revealed two major clades, regardless of which analysis was used (MP, NJ or ML), although the branching orders were a little different. Both clades corresponded exactly to the groups defined above on the basis of ITS1 lengths: isolates SF-1 to -10 from Japan in the north-west Pacific and SF-12 from Mediterranean France comprised Group-1; isolates SD-1 to -4 from Japan and SD-5 and -6 from the Canadian north-east Pacific comprised Group-2Pacific; and isolates SD-7 and -8 from the Canadian north-west Atlantic and SD-9 to -11 from the north European north-east Atlantic comprised Group-2Atlantic. The clades comprising the two main groups are supported by high bootstrap values (> 90 except for Group-1 in the ML analysis). However, de-

Table 1. Sources of specimens used for molecular analyses and their sample numbers, habitat, length of ITS1, ITS2 and IGS sequences (base pairs) and DDBJ (DNA Data Bank of Japan) accession codes.

Species	Collection site (source)	Nature of the sample	Sample numbers	Map code (see Fig. 1)	Habitat	ITS1 (bp)	ITS2 (bp)	IGS (26S–5S) (bp)	DDBJ code for 5.8S and ITS rDNA	DDBJ code for IGS (26S–5S) rDNA	DDBJ code for *rbc*L and spacer
Sphaerotrichia divaricata	Kikonai, Hokkaido, Japan	field plant (frozen)	SD-1	9	on *Coccophora langsdorfii*	626	335	863	AB077177	AB077192	AB077157
	Kikonai, Hokkaido, Japan	field plant (silica gel)	SD-2	9	on *Coccophora langsdorfii*	644	335	871	AB077178	AB077193	AB077159
	Kikonai, Hokkaido, Japan	field plant (silica gel)	SD-3	9	on *Coccophora langsdorfii*	621	335	858	AB077180	AB077194	AB077158
	Takahama, Fukui, Japan	field plant (frozen)	SD-4	12	on *Sargassum* sp.	602	335	no data	AB077179	—	AB077156
	Bamfield, BC, Pacific Canada	culture (A.F. Peters)	SD-5	5	on *Zostera marina*	644	333	888	AB077181	AB077195	AB077166
	Bamfield, BC, Pacific Canada	culture (A.F. Peters)	SD-6	5	on *Zostera marina*	645	333	884	AB077182	AB077196	AB077167
	Newfoundland, Atlantic Canada	culture (A.F. Peters)	SD-7	1	no data	843	335	841	AB077183	AB077197	AB077164
	Newfoundland, Atlantic Canada	culture (A.F. Peters)	SD-8	1	no data	818	335	no data	AB077184	—	AB077165
	Isefjord, Sjaelland, Denmark	culture (A.F. Peters)	SD-9	3	drift material	820	335	806	AB077187	AB077200	AB077162
	Varberg, Kattegat, Sweden	field plant (silica gel)	SD-10	4	no data[1]	823	337	817	AB077186	AB077198	—
	Varberg, Kattegat, Sweden	field plant (silica gel)	SD-11	4	no data[1]	820	335	820	AB077185	AB077199	AB077163
Sphaerotrichia firma	Oshoro, Hokkaido, Japan	culture (H. Kawai)	SF-1	8	epilithic	505	331	702	AB066017	AB065994	AB066061
	Kikonai, Hokkaido, Japan	field plant (silica gel)	SF-2	9	epilithic	505	331	no data	AB077175	—	AB077153
	Hanasaki, Hokkaido, Japan	field plant (silica gel)	SF-3	7	epilithic	502	327	no data	AB077170	—	—
	Nemuro, Hokkaido, Japan	field plant (silica gel)	SF-4	6	epilithic	502	328	no data	AB077171	—	AB077154
	Aikawa, Niigata, Japan	culture (H. Kawai)	SF-5	10	epilithic	503	328	704	AB077168	AB077188	—
	Kurahashi Island, Hiroshima, Japan	culture (H. Kawai)	SF-6	14	epilithic	502	331	no data	AB077169	—	—
	Obama, Fukui, Japan	field plant (silica gel)	SF-7	11	epilithic	503	328	651	AB077172	AB077189	AB077155
	Obama, Fukui, Japan	field plant (silica gel)	SF-8	11	epilithic	503	328	no data	AB077173	—	—
	Obama, Fukui, Japan	field plant (silica gel)	SF-9	11	epilithic	503	328	no data	AB077174	—	—
	Miyazu, Kyoto, Japan	field plant (silica gel)	SF-10	13	epilithic	506	328	699	AB066018	AB065995	AB066062
	Étang de Thau, France	culture (A.F. Peters)	SF-11	2	loose lying, not on *Zostera*	no data	no data	743	—	AB077190	AB077160
	Étang de Thau, France	culture (A.F. Peters)	SF-12	2	loose lying, not on *Zostera*	502	330	706	AB077176	AB077191	AB077161

Table 1. Continued.

Species	Collection site (source)	Nature of the sample	Sample numbers	Map code (see Fig. 1)	Habitat	ITS1 (bp)	ITS2 (bp)	IGS (26S–5S) (bp)	DDBJ code for 5.8S and ITS rDNA	DDBJ code for IGS (26S–5S) rDNA	DDBJ code for *rbc*L and spacer
Chordaria sp.	Petropavlovsk, Kamchatka, Russia	Kim & Kawai (2002)	—	—	—	434	342	727	AB066022	AB065998	AB066064
Chordaria chordaeformis	Ny Ålesund, Spitsbergen, Norway	Kim & Kawai (2002)	—	—	—	608	397	597	AB066024	AB066001	AB066065
Chordaria chordaeformis	Hanasaki, Hokkaido, Japan	Kim & Kawai (2002)	—	—	—	599	358	900	AB066030	AB066005	AB066069

[1] In north-west Europe, *Sphaerotrichia* may occur both on hard substratum and as epiphyte, often on *Zostera marina* Linnaeus (Rosenvinge & Lund 1943).

spite the differences in ITS1 length, north-east Pacific (Canadian) specimens of Group-2Pacific (SD-5 and -6) formed a clade with Group-2Atlantic, rather than with north-west Pacific (Japanese) Group-2Pacific. This tree topology was supported by generally high bootstrap values (≥ 83%) in all analyses.

As indicated in Fig. 9a (by the broken lines connecting different strains), interfertility between SF-1 and SF-12 in Group-1 and between SD-5, SD-6 (Group-2Pacific) and SD-7 (Group-2Atlantic) has been demonstrated by Peters *et al.* (1987, 1993). In addition, in the present study, interfertility between SD-6 (north-east Pacific) and SD-9 (north-east Atlantic) was confirmed (see above).

Intragenic spacer

The aligned IGS rDNA sequences were 1137 bp in total, and there were 304 parsimony-informative nucleotide positions. Tree topologies (Fig. 9b) were essentially the same in all analyses (MP/NJ/ML) and were also consistent with the tree based on 5.8S–ITS sequences. The north-west Pacific (Canadian) specimens of Group-2Pacific (SD-5 and -6) first formed a clade with Group-2Atlantic, and then grouped with north-east Pacific (Japanese) Group-2Pacific.

*rbc*L and spacer

The aligned *rbc*L and its spacer sequences between *rbc*L and *rbc*S were 583 bp in total, and there were 35 parsimony-informative nucleotide positions. Tree topologies of the molecular phylogenetic analyses (Fig. 9c) were essentially the same in all of the analyses (MP/NJ/ML), and were also consistent with the trees based on 5.8S–ITS and IGS sequences. Again, the north-west Pacific (Canadian) specimens of Group-2Pacific (SD-5 and -6) first formed a clade with Group-2Atlantic, and then grouped with north-east Pacific (Japanese) Group-2Pacific. However, the bootstrap values supporting the branches connecting the subclades within Group-2 (≤ 85%) were generally lower than those in other analyses (≥ 91% in 5.8S–ITS and IGS data sets).

Comparison of pairwise sequence heterogeneity

Table 3 compares the percentage of base substitutions (pairwise sequence heterogeneity in %) within each subgroup (and group) between 5.8S–ITS, IGS and *rbc*L–spacer sequences. The IGS regions showed roughly 1.6 times (within Group-1) to 2.3 times (within the whole of Group-2) higher substitution rates than the ITS regions. The sequence diversities (differences) within Group-1 were comparable with those within the whole of Group-2 for 5.8S–ITS rDNA (1.8 ± 0.94 vs 1.9 ± 0.76) and partial *rbc*L–spacer (0.4 ± 0.23 vs 0.4 ± 0.27), but somewhat larger within Group-1 for IGS (4.2 ± 1.19 vs 3.0 ± 1.56).

DISCUSSION

Based on the variation in sequence length of the ITS1 region, three groups were resolved (Group-1, Group-2Pacific and Group-2Atlantic), whereas molecular phylogenetic analyses suggested the presence of only two major groups (Group-1

Table 2. List of primers used for amplifying and sequencing 5.8S, ITS and IGS rDNA and *rcb*L and the spacer region between *rbc*L and *rbc*S. Mixtures: S = C + G; W = A + T; Y = C + T.

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-F3	forward	CACAACCATTCATGCG	*rbc*L
	rbc-F4	forward	GTAAATGGATGCGTA	*rbc*L
	rbc-F5	forward	ATTTGGTGGTGGTACTATTGG	*rbc*L
	rbc-R5	reverse	AAASHDCCTTGTGTWAGTYTC	*rbc*S
	rbc-R6	reverse	AATAAAGGAAGACCCCATAATTCCCA	*rbc*S

and Group-2, the latter without distinction between Pacific and Atlantic clades). Specimens collected from the Étang de Thau, Mediterranean France, were included in Group-1, and supported the conclusions by Riouall (1985) and Peters *et al.* (1993) that the *Sphaerotrichia* populations in the Mediterranean originate from Japan and were probably introduced accidentally with oyster spat.

Group-1 and Group-2 differ in anatomy, morphology, ecology and physiology. Specimens belonging to Group-2 were hollow throughout their entire length and had a divaricate branching pattern, and corresponded to *S. divaricata* (type locality in the Baltic Sea). In contrast, specimens belonging to Group-1 were partly solid near the base and had a traceable main axis, and agreed morphologically with *S. firma* (type locality in China). Individuals of Group-1 were generally epilithic, whereas those of Group-2 were either epilithic or epiphytic. The two groups have also been reported to differ in the phototactic behaviour of their gametes and the temperature requirements for the sexual maturation of the gametophytes (Peters *et al.* 1987, 1993). The two groups are sympatric in Japan and we recognize Group-1 as a separate species, *S. firma.*

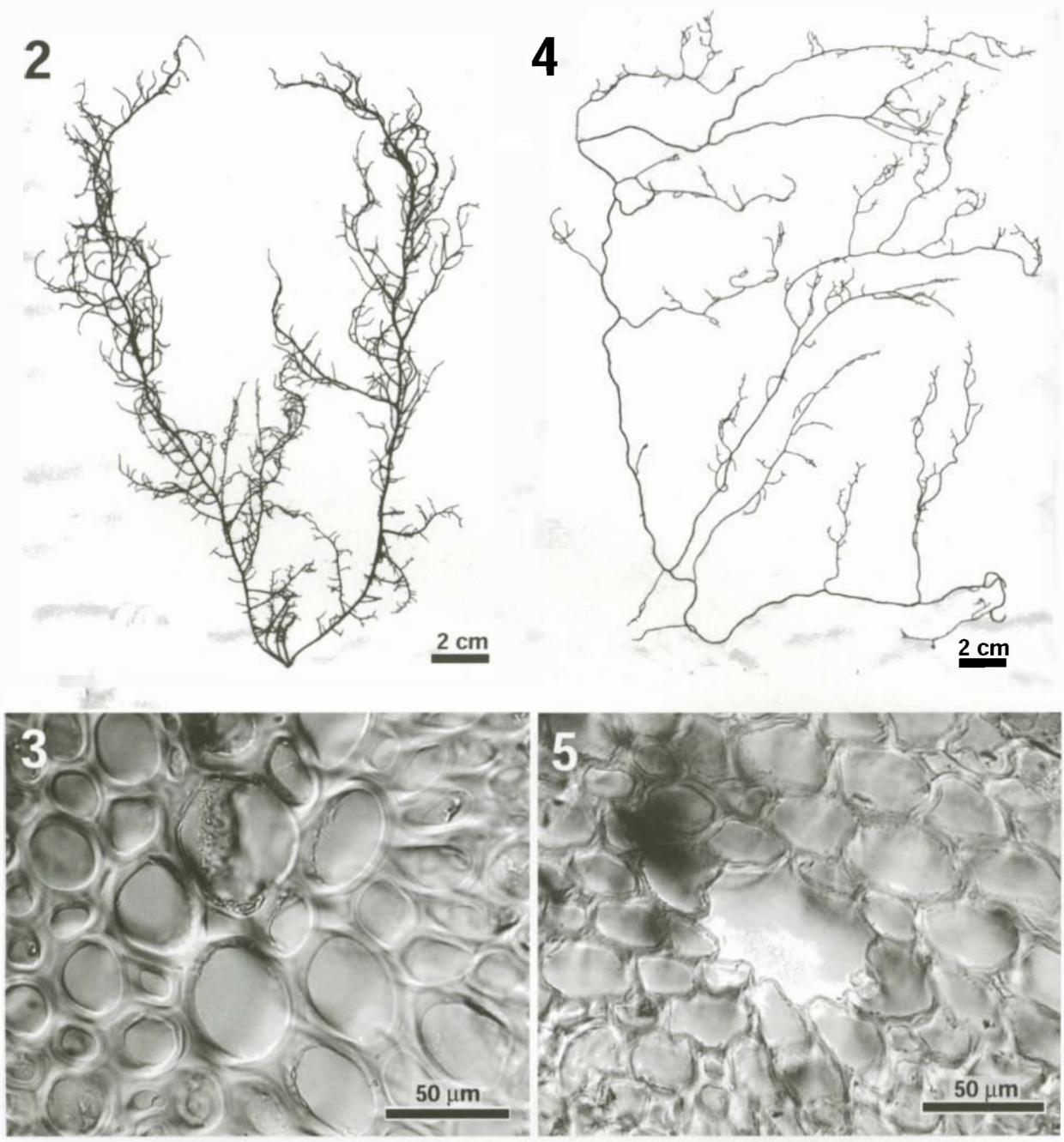
The taxonomic situation in Group-2 is less clear, especially with respect to the distinction between the Pacific and Atlantic populations. Peters *et al.* (1987) demonstrated interfertility at the level of plasmogamy between north-east Pacific plants (SD-5 and -6) and both north-western and north-eastern Atlantic isolates of Group-2 (SD-7); our study has shown in addition that the Canadian SD-6 is interfertile with the Danish SD-9. This is surprising because of the considerable differences in ITS1 length between Atlantic and north-east Pacific isolates. Because the fertility of sporophytes derived from the zygotes was not studied by Peters *et al.* (1987), it is possible that a reproductive barrier between the two populations is present, but manifests itself only after germination of the zygotes. The situation in *Sphaerotrichia* may resemble that in *Ectocarpus* Lyngbye, where two sexually incompatible species [*E. fasciculatus* Harvey and *E. siliculosus* (Lyngbye) Link] occur, with considerable genetic diversity and varying degrees of sexual compatibility (Stache 1989, 1993; Stache-Crain *et al.* 1997). Therefore, *Sphaerotrichia* may also become a suitable model for studying speciation and the evolution of reproductive barriers in brown algae.

The groups based on variations in ITS1 length agree with Zinova's (1958) classification of Pacific specimens into *S. firma* and *S. dissessa,* separate from the north Atlantic *S. divaricata.* However, a morphological re-examination of assimilatory filaments by Peters *et al.* (1993) did not confirm the value of the characters used by Zinova (1958) to distinguish the three taxa.

In conclusion, we propose to recognize both Group-2Pacific and Group-2Atlantic as *S. divaricata* because our present knowledge of morphological variation and the interfertility between these two groups are not sufficient to justify a separation. Further crossing experiments (adding specimens of Group-2Pacific from the north-west Pacific to the analyses) and following the development of the sporophytes derived from zygotes may both provide additional insights into this problem.

The comparisons of pairwise sequence heterogeneity (Table 3) indicate that IGS regions are about 1.3 to 2.3 times more variable than 5.8S–ITS regions in *Sphaerotrichia* spp. This ratio was smaller than that in *Chordaria* spp. (4.0–7.9, Kim & Kawai 2002), but still indicates that the IGS region has a higher mutation ratio than the ITS regions. Therefore, the IGS region is considered to provide suitable resolution for the study of relatively closely related taxa, where ITS cannot clearly resolve the relationship.

Despite the notable difference in the length of ITS1, north-east Pacific populations of *S. divaricata* (Group-2) were genetically closer to north-west Atlantic populations than to north-west Pacific populations (Fig. 9). It can be hypothesized that the species originated in the north Pacific where it first divided into the north-west Pacific and the north-east Pacific subgroups. Subsequently, a subpopulation of the north-east



Figs 2–5. Morphology of *Sphaerotrichia firma* and *S. divaricata.*
Fig. 2. Habit of *Sphaerotrichia firma* (Hakui, Ishikawa, Japan, collected on 25 May 2001) with traceable main axis.
Fig. 3. Cross-section of the thallus near the base indicating solid innermost part of the tissue.
Fig. 4. Habit of *Sphaerotrichia divaricata* (Nozaki, Ishikawa, Japan, collected on 23 June 2001) with divaricate branches. Note that the distances between the branches are shorter near the tip.
Fig. 5. Cross-section of the thallus near the base, demonstrating cavity in the innermost part.

Pacific subgroup spread into the Atlantic through the Arctic region, coincident with an increase in length of the ITS1 sequence. The limited distribution of the closely related *S. firma,* which is genetically as diverse as *S. divaricata* in the Pacific (Table 3), would support the hypothesis that *S. divaricata* is of Pacific origin.

ACKNOWLEDGEMENTS

We are grateful to Dr Eric Henry for reading and correcting the English of the manuscript, Dr Mitsunobu Kamiya for helpful advice, Ms Susanna Riebe of the Botanical Museaum, Lund (LD), Dr R. Huxley and Ms Jenny Bryant of the Natural

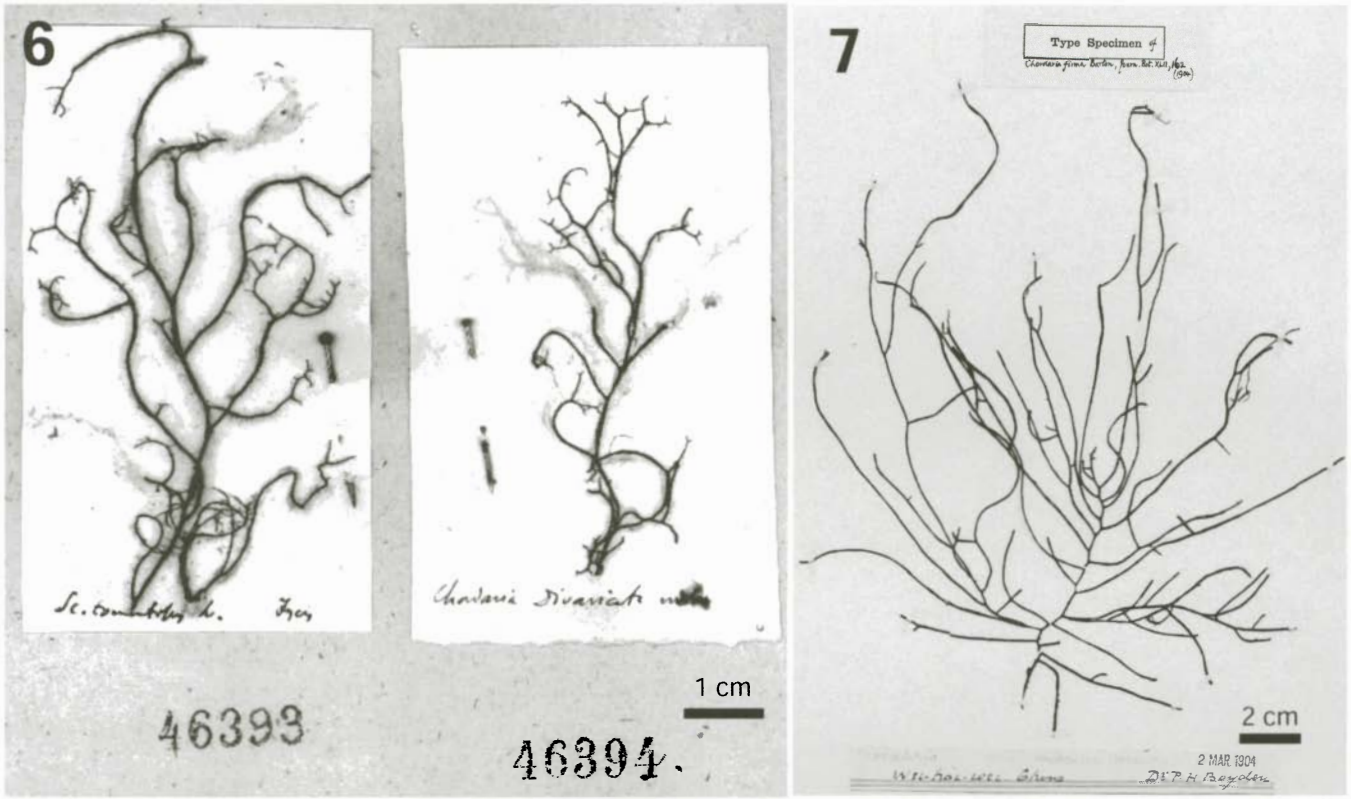


Fig. 6. Syntype specimens of *Chordaria divaricata* C. Agardh [= *Sphaerotrichia divaricata* (C. Agardh) Kylin] (catalogue numbers 46393 and 46394, both from LD).

Fig. 7. Type specimen of *Chordaria firma* E. Gepp [= *Sphaerotrichia firma* (E. Gepp) Zinova] (catalogue number BM 563399).

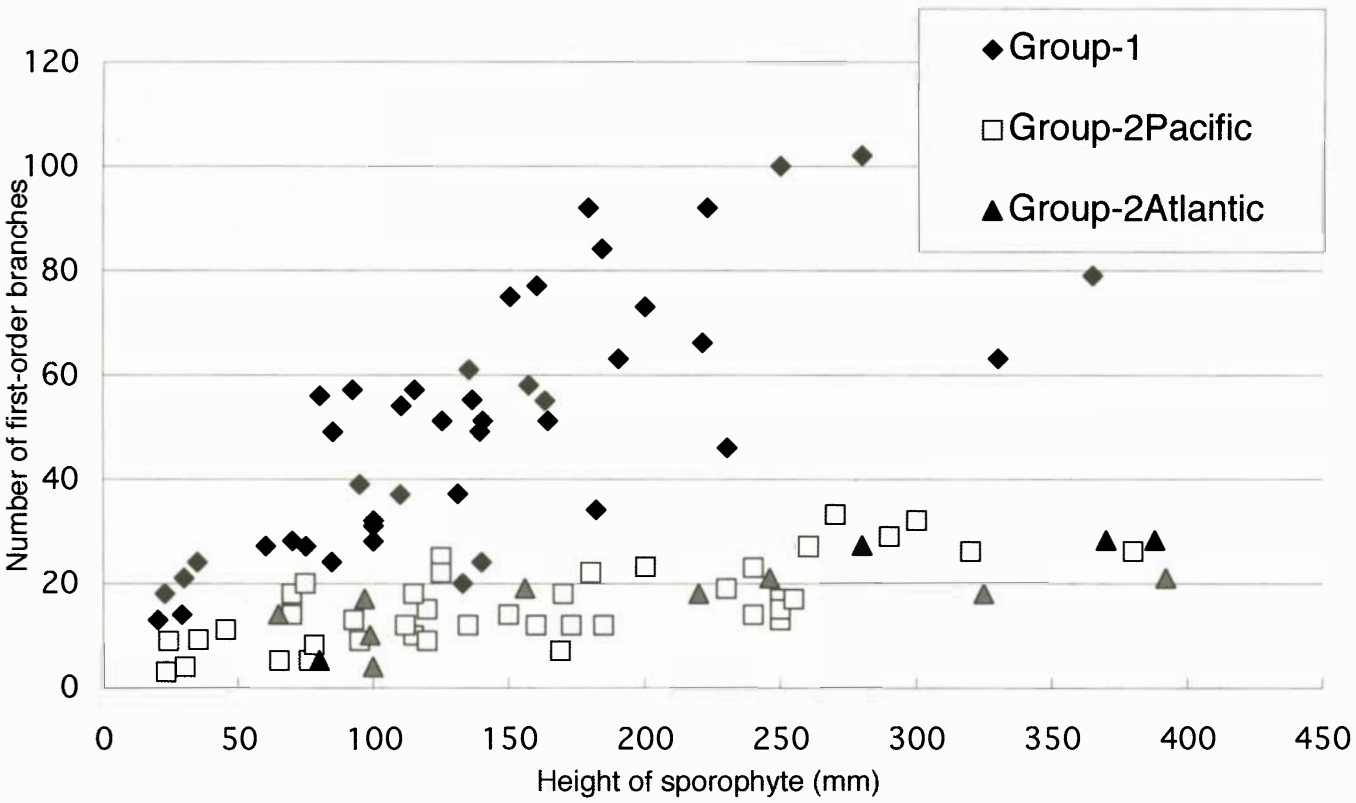


Fig. 8. Relationship between the number of branches and the height of the thallus in the individuals of each group.

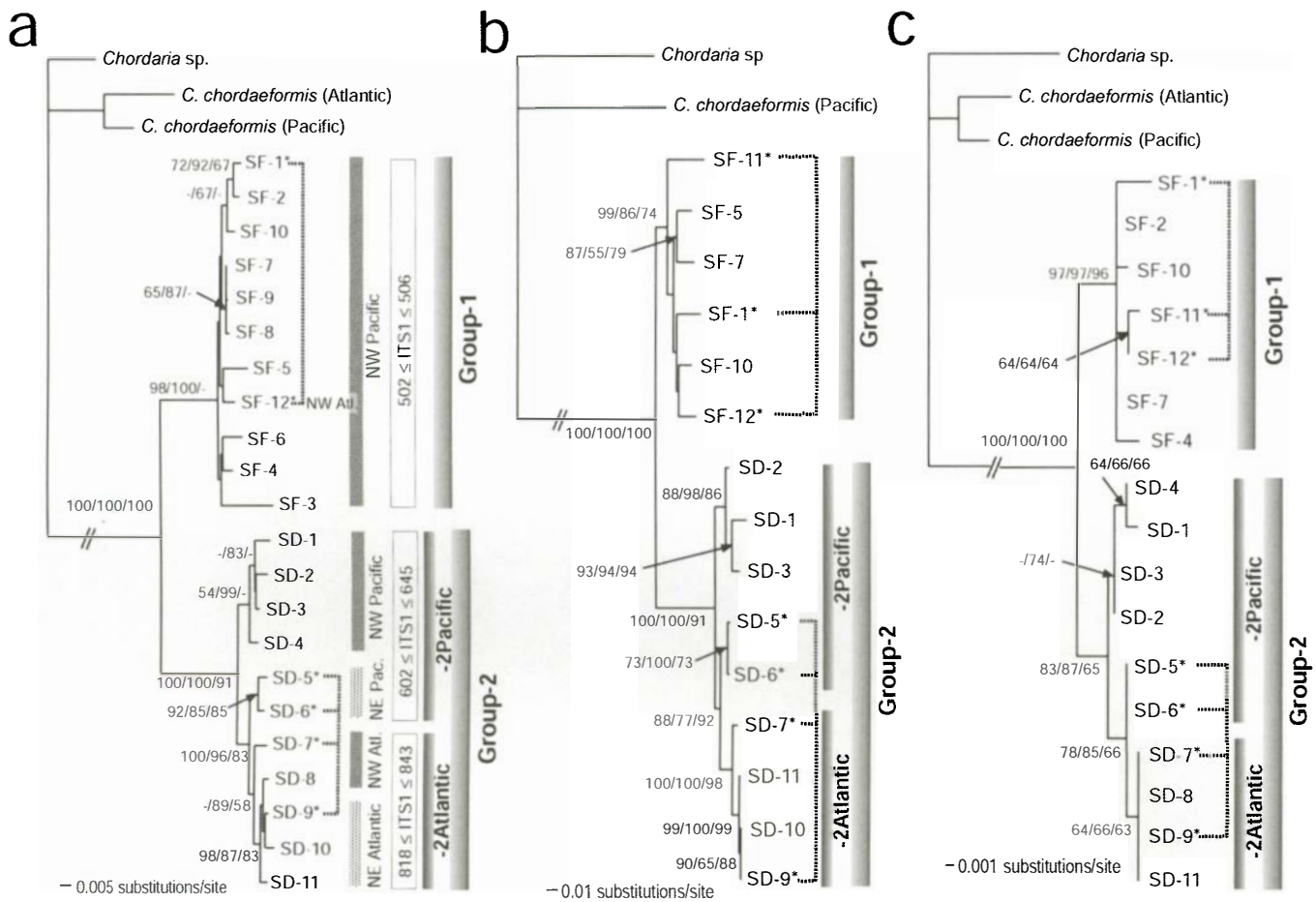


Fig. 9. Molecular phylogenetic trees based on the sequences of three different parts of the genome. The trees are based on the NJ tree and indicate bootstrap values of the MP, NJ and ML analyses in order. '–' Indicates a bootstrap value of < 50%. '*' Indicates the culture strains used for crossing experiments in Peters *et al.* (1993).

a. 5.8S–ITS rDNA. In the MP analysis, 620 most parsimonious trees of 549 steps were obtained with a consistency index (CI) of 0.883 and a retention index (RI) of 0.941. In the ML analysis, −Ln likelihood was 5297.64189. Broken lines at the right of specimen abbreviations (see Table 1) indicate interfertility reported by Peters *et al.* (1987, 1993).

b. IGS. In the MP analysis, two most parsimonious trees of 815 steps were obtained with a CI of 0.926 and a RI of 0.904. In the ML analysis, −Ln likelihood was 4869.36987.

c. *rbc*L and spacer region. In the MP analysis, two most parsimonious trees of 63 steps were obtained with a CI of 0.968 and a RI of 0.981. In the ML analysis, −Ln likelihood was 1169.68088.

History Museum, London (BM), and Dr Eun-Young Lee, Seoul National University, for providing the scanned images of type materials and Ms Tatiana Klotchkova for translating Russian literature. This work was partly supported by the Sasakawa Scientific Research Grant from the Japan Science Society to S.-H.K.

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Table 3. Mean and standard deviation of pairwise sequence heterogeneity (%) in each DNA region within each genetic group and subgroup of *Sphaerotrichia*. *n* = number of sequences in data set.

		S. divaricata		
	Group-1, *S. firma*	Group-2Pacific	Group-2Atlantic	Entire Group-2
5.8S + ITS	1.8 ± 0.94 (*n* = 11)	1.8 ± 0.82 (*n* = 6)	0.9 ± 0.30 (*n* = 5)	1.9 ± 0.76 (*n* = 11)
IGS	4.2 ± 1.19 (*n* = 7)	2.4 ± 1.01 (*n* = 5)	0.9 ± 0.51 (*n* = 4)	3.0 ± 1.56 (*n* = 9)
Partial *rbc*L + spacer	0.4 ± 0.23 (*n* = 7)	0.3 ± 0.20 (*n* = 6)	0 (*n* = 4)	0.4 ± 0.27 (*n* = 10)

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Accepted 6 December 2002