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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*[1]

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***Chorda rigida* Kawai et Arai, sp. nov. (Chordaceae, Laminariales) is described from the Sea of Japan, NW Pacific. This species resembles *Chorda filum* (Linnaeus) Stackhouse but is distinguished by the following characteristics: 1) the sporophytes grow on more or less exposed rocks at 2–7 m depth and do not form dense tufts; 2) compared with *C. filum*, sporophytes of *C. rigida* are much more rigid and are composed of denser cortical layers (6–18 cells thick); 3) *C. filum* becomes fertile and disappears in late spring to summer, whereas *C. rigida* appears in early summer, oversummers, and becomes fertile only in late autumn at the same localities; 4) in culture, *C. rigida* sporophytes tolerate higher temperature conditions (20 and 25° C) than *C. filum*; and 5) *C. rigida* has considerably longer sequences of the rDNA ITS region than does *C. filum*. The independence of the species is further supported by molecular phylogenetic analyses using sequence of the ITS + 5.8S ribosomal DNA. Interestingly, *C. filum* is shown to be genetically diverse and possibly paraphyletic, and it may require subdivision into several species or subspecies. The *rbc*L and associated spacer sequence data established monophyly of the genus *Chorda* among Laminariales, but the resolution was limited for discussing the phylogenetic relationships within the genus.**

Key index words: **5.8S rDNA; Chordaceae; *Chorda filum*; *Chorda rigida* sp. nov.; ITS; Laminariales; molecular phylogeny; Phaeophyceae; *rbc*L; Rubisco spacer**

Abbreviations: **ITS, internal transcribed spacer; ML, maximum likelihood; MP, maximum parsimony; NJ, neighbor joining**

The order Laminariales (so-called kelps) is a well-defined systematic group and is regarded as one of the most derived in the Phaeophyceae (Bold and Wynne 1985, van den Hoek et al. 1995). Six families are included in the order at present: Alariaceae, Chordaceae, Laminariaceae, Lessoniaceae, Phyllariaceae, and Pseudochordaceae (Setchell and Gardner 1925, Tilden 1935, Kawai and Kurogi 1985, Henry and South 1987). Although phylogenetic relationships relative to other phaeophycean orders are unclear (Clayton 1984, Müller et al. 1985, Kawai 1992, Tan and Druehl 1996, Boo et al. 1999), within the Laminariales the families with terete sporophytes (Chordaceae and Pseudochordaceae) have been considered to be primitive based on the following characteristics (Kawai and Kurogi 1985, Henry and South 1987, Kawai and Nabata 1990): 1) relatively simple organization of sporophytes without differentiation between blade and stipe and lack of a meristematic rhizoidal holdfast, 2) annual nature of sporophytes and lack of distinct intercalary meristem (except for *Chorda filum*), 3) lack of mucilage organs (e.g., mucilage gland cells or mucilage ducts) and mucilage caps on paraphyses, 4) presence of eyespots in zoospores, 5) occurrence of monoecious (*Chorda tomentosa* Lyngbye = *Halosiphon tomentosus* [Lyngbye] Jaasund) or monomorphic dioecious (*Pseudochorda* nagaii [Tokida] Inagaki) gametophytes, and 6) lack of inner hyphae (*C. tomentosa, Pseudochorda* spp.).

The placement of *H. tomentosus* in the family Chordaceae has been controversial because the species differs from the generitype *C. filum* in various basic features: 1) occurrence of long assimilatory filaments instead of unicellular paraphyses, 2) absence of an intercalary meristem, 3) absence of trumpet-shaped hyphae or an obvious differentiation between cortical layer and peripheral (meristodermal) layer, 4) occurrence of monoecious gametophytes, and 5) presence of different sexual pheromones (Maier 1995). More recently, Peters (1998) established a relatively substantial genetic distance between *C. filum* and *H. tomentosus* based on molecular phylogenetic data. (He reinstated the generic name *Halosiphon* at that time, which was established by Jaasund [1957] based on a misunderstanding of an epiphyte as the gametophyte of the species.) Peters (1998) discussed the possibility that *Halosiphon* was incorrectly placed in the Chordaceae but did

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not present a formal taxonomic treatment. Therefore, the genus *Chorda* (and probably the family Chordaceae) only includes the type species *C. filum*.

Here we describe a new species in the genus *Chorda*, found in a relatively limited area in the Sea of Japan, NW Pacific. We describe its morphology, phenology, life history in culture, and phylogenetic affinities based on sequences of the nuclear internal transcribed spacer (ITS) regions of the rDNA (ITS1-5.8S-ITS2) and the Rubisco large subunit gene (*rbc*L) and the spacer region between *rbc*L and *rbc*S.

MATERIALS AND METHODS

Morphological observations and anatomical comparison. The specimens of *Chorda rigida* sp. nov. and *C. filum* preserved in 5% formaldehyde seawater as listed in Table 1 were used for morphological observations by Nomarski light microscopy and anatomical investigations of the sporophyte generation. For comparisons of the number of cortical cell layers for sporophytes, cross-sections were made by hand using razor blades, and an average of 8–10 sections were observed for the following regions on each plant: intercalary meristem (except for old plants that had lost their meristem), the middle portion between the meristem and base, and the lower part of the thallus near the base. The herbarium voucher specimens of *C. filum* as listed in Table 2 housed in SAP (Herbarium of the Department of Botany, Graduate School of Science, Hokkaido University) were also examined for morphological comparisons of the number of cell layers constituting the cortex. The Statview v. 4 program (Abacus Concepts, Berkeley, CA) was used for the statistical analysis (*t*-test) of the data.

Culture experiments. Cultures of *C. rigida* sp. nov. were started from zoospores released from unilocular sporangia (unispores) on erect thalli collected on November 1, 1991 at Kashiwazaki. The zoospores were pipetted onto glass slides and cultured in glass vessels containing 200 mL of PESI medium, or they were pipetted directly into plastic petri dishes containing 50 mL PESI medium (Tatewaki 1966). The sets of culture conditions used were as follows: 5° C short day (SD; 8:16h light:dark), 5° C long day (LD; 16:8h light:dark), 10° C SD, 10° C LD, 15° C SD, 15° C LD, 20° C SD, 20° C LD, and 25° C LD. All culture conditions were illuminated under daylight-type white fluorescent tubes of approximately 50 μmol photons·m^{-2}·s^{-1} using a temperature gradient culture chamber (TG-100AD, TG-200AD, Nippon Medical and Chemical Instruments, Osaka, Japan). To compare growth differences under the culture conditions and to make anatomical comparisons, unialgal culture strains of *C. filum* isolated from Oshoro, Hokkaido and Hakui, Ishikawa Pref., Japan and Bergen, Norway were used. For anatomical comparisons, well-developed sporophytes of 50–100 mm in length grown under 10° C (*C. filum*) and 20° C (*C. rigida* sp. nov.) LD conditions were used.

Molecular phylogenetic analysis. The origins of specimens used for molecular phylogenetic studies are listed in Table 3. Genomic DNA was extracted using the DNeasy Plant Mini Kit (Qiagen, Hilden, Germany), following the manufacturer's instructions. Algal material was ground in liquid nitrogen, and approximately 40 mg of algal tissue was used. Extracted total DNA was monitored on ethidium bromide-stained 1.0% agarose gels.

PCR amplification of the ITS regions and the Rubisco large subunit gene (*rbc*L) and the spacer region between *rbc*L and *rbc*S were carried out with a GeneAmp PCR Cycler 2400 and 9700 (Perkin Elmer, Foster City, CA) using a TaKaRa Ex Taq (Takara Shuzo, Shiga, Japan) reaction kit (total volume of 25 μL composed of 2.5 μL 10× Ex Taq Buffer, 5.0 μM dNTP mixture, 0.1 μM of each primer, 0.625 units TaKaRa Ex Taq, and 2.0 μL DNA solution including 0.5–1.0 μg DNA). Primers (Table 4) were designed based on published sequences of the corresponding regions reported for related taxa (Tan and Druehl 1993, 1996, Kawai et al. 1995, Stache-Crain et al. 1997 for ITS; Assali et al. 1990, Valentin and Zetsche 1990, Daugbjerg and Andersen 1997 for *rbc*L and spacer). The profile of PCR conditions was as follows: an initial denaturation at 95° C for 5 min, followed by 30 cycles of denaturation at 95° C for 30 s, annealing at 42 or 58° C (for *rbc*L and spacer) for 30 s and 54 or 58° C (for ITS) for 30 s, extension at 72° C for 30 s, and a final extension at 72° C for 7 min. PCR products were monitored on ethidium bromide-stained 1.0% agarose gels. PCR products were directly sequenced using the Cy5 Auto Cycle Sequencing Kit (Pharmacia Biotech AB, Uppsala, Sweden), following the

TABLE 1. List of liquid-preserved specimens of *Chorda rigida* and *Chorda filum* used for anatomical comparisons.

Species	Locality	Collection date	n[a]	Collector
Chorda rigida, sp. nov.	Kashiwazaki, Niigata Pref.	4 July 1988	1	S. Arai
	Kashiwazaki, Niigata Pref.	22 September 1990	7	S. Arai
	Kashiwazaki, Niigata Pref.	26 October 1990	6	S. Arai
	Kashiwazaki, Niigata Pref.	3 June 1991	2	S. Arai
	Kashiwazaki, Niigata Pref.	1 November 1991	5	A. Arai
	Awashima Isl., Niigata Pref.	4 August 1991	2	S. Arai
	Futami, Sado Isl., Niigata Pref.	16 October 1991	3	H. Kawai
	Nanao, Ishikawa Pref.	7 August 1992	2	S. Arai
Chorda filum	Rishiri Isl., Hokkaido	19 June 1991	5	H. Kawai
	Oshoro, Hokkaido	27 July 1982	2	T. Kudo
	Oshoro, Hokkaido	25 April 1988	2	H. Kawai
	Oshoro, Hokkaido	3 June 1988	7	Y. Hattori
	Oshoro, Hokkaido	11 July 1988	6	Y. Hattori
	Kikonai, Hokkaido	21 April 1988	8	H. Kawai
	Kikonai, Hokkaido	16 May 1988	5	K. Kogame
	Kikonai, Hokkaido	24 July 1988	3	Y. Hattori
	Shichigahama, Miyagi Pref.	20 May 1984	2	H. Kawai
	Shiga, Ishikawa Pref.	14 June 1993	2	S. Arai
	Fukuura, Ishikawa Pref.	31 May 1993	2	S. Arai
	Takahama, Fukui Pref.	29 April 1993	1	Y. Maeda
	Takahama, Fukui Pref.	7 June 1993	2	Y. Maeda
	Aidani, Hyogo Pref.	15 July 1993	1	H. Kawai
	Shodoshima Isl., Kagawa Pref.	28 May 1987	1	H. Kawai
	Takashima, Ehime Pref.	14 June 1991	2	H. Kawai
	Futashima, Nagasaki Pref.	14 May 1991	5	S. Arai

[a]Values are numbers of erect thalli (individual sporophytes) used for anatomical comparisons.

TABLE 2. List of voucher specimens of *Chorda filum* used for anatomical comparisons.

Locality	Collection date	Collector
Outside Japan		
Dairen, China	27 July 1937	Y. Yamada
Bohuslän, Råttholmen, Fiskebäkskil, Sweden	16 August 1894	H. G. Simmons
Sverige, Bohuslän, Bonden, Sweden	18 July 1946	T. Levring
Frederikshaven, Denmark	2 August 1985	T. Yoshida
Roscoff, France	21 November 1972	T. Yoshida
Roscoff, France	30 June 1973	T. Yoshida
Inside Japan		
Soyamisaki, Hokkaido	27 July 1980	M. Kurogi
Shiretoko Peninsula, Hokkaido	16 September 1943	Y. Yamada
Tomari, Kunashiri Isl.	30 July 1936	M. Nagai
Notsuke, Hokkaido	28 May 1987	T. Yoshida
Muroran, Hokkaido	23 June 1936	T. Muraoka
Muroran, Hokkaido	1 July 1966	T. Funano
Usu, Hokkaido	13 June 1957	Y. Enomoto
Kikonai, Hokkaido	7 April 1988	I. Mine
Asamushi, Aomori Pref.	June 1927	Y. Yamada
Takonoura, Iwate Pref.	19 May 1951	S. Kawashima
Ozuchi, Iwate Pref.	28 May 1979	S. Kawaguchi
Hayata, Yamagata Pref.	2 July 1984	K. Ikehara
Aikawa, Niigata Pref.	12 June 1987	K. Ikehara
Senami, Niigata Pref.	1 June 1968	S. Kikuchi
Miyako, Miyagi Pref.	14 May 1952	M. Kurogi
Matsushima, Miyagi Pref.	24 May 1952	M. Kurogi
Misaki, Kanagawa Pref.	April 1923	Y. Yamada
Misaki, Kanagawa Pref.	May 1927	S. Akiyama
Shinmaiko, Aichi Pref.	11 April 1944	N. Segi
Takashima, Ehime Pref.	12 June 1987	S. Enomoto
Ikata, Ehime Pref.	1 June 1957	Y. Nomura
Hirado, Nagasaki Pref.	25 May 1983	T. Yoshida
Nomozaki, Nagasaki Pref.	25 April 1933	Y. Yamada
Amakusa, Kumamoto Pref.	5 May 1958	M. Ichinoki

manufacturer's instructions. Reactions were electrophoresed, and the sequence data were collected with the ALF express DNA sequencer (Pharmacia). Sequences were aligned for phylogenetic analysis using the Clustal W computer program (Thompson et al. 1994) or manually.

The aligned sequences were subjected to maximum parsimony (MP) analyses in a general heuristic search using PAUP v. 4.0.2b (Swofford 1999). Fifty random taxon addition replicates were performed in each heuristic search, using the option TBR branch swapping. Gaps were not taken into account in every analysis. From the same alignment, two-parameter distances (Kimura 1980) between taxa were estimated, and a phylogenetic tree was constructed with the neighbor-joining (NJ) method, using PAUP. Maximum likelihood (ML) analyses were also performed using PAUP in a general heuristic search (10 random taxon additions) with a substitution model of transition/transversion ratio = 2 and empirical base frequencies (using 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) and 200 (ML) resamplings (Felsenstein 1985). In an additional MP analysis, gaps were recognized as a fifth base. *Pseudochorda nagaii* and *P. gracilis* Kawai et Nabata (Pseudochordaceae, Laminariales), *Laminaria diabolica* Miyabe, and *L. yendoana* Miyabe (Laminariaceae, Laminariales) were used as outgroups in ITS-5.8S trees, whereas *Ectocarpus siliculosus* (Dillwyn) Lyngbye and *Pilayella littoralis* (Linnaeus) Kjellman (Ectocarpales) were used for *rbc*L + spacer analyses.

RESULTS

Chorda rigida Kawai et Arai, sp. nov.

Sporophyton macroscopicum epilithicum sublittorale, solitarium vel sparsim, simplex filiforme, 0.3–0.5 (−1.2) m longum et circa 3.5 mm diametro, fulvescens vel fuligineum, parenchymatosum cavum, cum hypha, cortice, epiderme, pilis, paraphysibus unicellularibus sine pileolibus, et hapteris discoideum rhizoideis. Cortex densum, ad 9–13 (−17) cellula crassum. Sporangium uniloculare sessile, anguste ovatum. Gametophyton minutum, oogamum, dioecium, dimorphum. Antheridium solitarium. Cellulae sporophyticae et gametophyticae cum chloroplastis numerosis discoideis sine pyrenoidibus.

Quoad fabricam ad Chordae filum accedit, sed ab ea differt essentialiter robore maiore thalli, etiam in maturitate cum meristema intercalare.

Holotype: No. 21416. 1990.10.26. Collected from Kashiwazaki, Niigata, Japan. Deposited in the Herbarium of the Science Faculty of Kobe University.

Etymology: The specific epithet originates from the rigid sporophytic thallus of the species.

Sporophyte macroscopic, epilithic, sublittoral, solitary or sparse, simple, cord-shaped, 0.3–0.5 (–1.2) m in length and about 3.5 mm in diameter, medium brown to dark brown, parenchymatous, hollow, composed of hypha, cortex, epidermis, and hairs, unicellular paraphyses without mucilagenous cap, and disk-shaped rhizoidal holdfast. Cortex dense composed of 9–13 (−17) cells. Unilocular sporangia sessile and narrowly ovate. Microscopic gametophytes dioecious, sexually dimorphic, oogamous. Antheridium solitary. Sporophytic and gametophytic cells containing many disk-shaped chloroplasts without pyrenoid.

TABLE 3. Source of specimens used for molecular analyses, their abbreviations, length of ITS1 and ITS2 sequences (base pairs), and database accession numbers of the sequences (DDBJ: DNA Data Bank of Japan).

Species (taxonomic position)	Collection site (source)	Origin	Abbreviations	ITS1 (bp)	ITS2 (bp)	DDBJ accession no. for ITS-5.8S rDNA	DDBJ accession no. for *rbc*L and spacer respectively
Chordaceae (Laminariales)							
Chorda rigida sp. nov	Futami, Sado Island, Niigata, Japan	Field plant (Frozen)	CR-Sad	358	303	AB035776	—
C. rigida	Kashiwazaki, Niigata, Japan	Culture (H. Kawai)	CR-Kas	360	302	AB035777	AB035788, AB041874
C. rigida	Nanao, Ishikawa, Japan	Field plant (Frozen)	CR-Nan	359	302	AB035778	—
Chorda filum (L.) Stackhouse	Roscoff, Brittany, France	Culture (H. Kawai)	CF-Ros	268	275	AB035748	AB035781, AB041867
C. filum	Santec, Brittany, France	[Peters 1998]	CF-San	270	266	Z98585, Z98586	—
C. filum	Portsmouth, U.K.	Field plant (Silica gel)	CF-Por	271	272	AB035749	—
C. filum	Elby Point, Isle of Man	Field plant (Silica gel)	CF-IME	270	275	AB035750	—
C. filum	Port Erin, Isle of Man	Field plant (Silica gel)	CF-IMP	270	274	AB035751	—
C. filum	Bergen, Norway	Culture (H. Kawai)	CF-Ber	270	273	AB035752	—
C. filum	Orafsfjord, Iceland	Field plant (Silica gel)	CF-Ora	270	275	AB035753	—
C. filum	Reykjavik, Iceland	Field plant (Silica gel)	CF-Rey	270	274	AB035754	—
C. filum	Newfoundland, Canada	Culture (E. Henry)	CF-NFL	271	261	AB035755	AB035782, AB041868
C. filum	St. Lawrence Island, Bering Sea, USA	Field plant (Silica gel)	CF-StL	259	259	AB035756	AB035783, AB041869
C. filum	Puget Sound, WA, USA	Field plant (Voucher)	CF-Pug	289	269	AB035757	AB035784, AB041870
C. filum	Petropavlovsk, Kamchatka, Russia (1)	Field plant (Voucher)	CF-Pet1	285	264	AB035758	—
C. filum	Petropavlovsk, Kamchatka, Russia (2)	Field plant (Voucher)	CF-Pet2	262	255	AB035759	—
C. filum	Kikonai, Hokkaido, Japan (1)	Culture (H. Kawai)	CF-Kik1	290	266	AB035760	AB035785, AB041871
C. filum	Kikonai, Hokkaido, Japan (2)	Field plant (Frozen)	CF-Kik2	288	265	AB035761	—
C. filum	Kikonai, Hokkaido, Japan (3)	Field plant (Frozen)	CF-Kik3	289	266	AB035762	—
C. filum	Oshoro, Hokkaido, Japan (1)	Culture (H. Kawai)	CF-Osh1	277	259	AB035763	AB035786, AB041872
C. filum	Oshoro, Hokkaido, Japan (2)	Culture (H. Kawai)	CF-Osh2	280	262	AB035764	—
C. filum	Oshoro, Hokkaido, Japan (3)	Culture (H. Kawai)	CF-Osh3	276	261	AB035765	—
C. filum	Kamiiso, Hokkaido, Japan (1)	[Yotsukura et al. 1999]	CF-Kam1	279	259	AB22815, AB22816	—
C. filum	Kamiiso, Hokkaido, Japan (2)	Culture (H. Kawai)	CF-Kam2	276	260	AB035766	—
C. filum	Ohzuchi, Miyagi, Japan	Field plant (Frozen)	CF-Ohz	280	260	AB035767	—
C. filum	Aikawa, Niigata, Japan	Field plant (Frozen)	CF-Aik	277	260	AB035768	—
C. filum	Fukuura, Ishikawa, Japan	Field plant (Frozen)	CF-Fuk	277	260	AB035769	—
C. filum	Hakui, Ishikawa, Japan	Culture (H. Kawai)	CF-Hak	279	260	AB035770	—
C. filum	Takahama, Fukui, Japan	Field plant (Frozen)	CF-Tak	276	261	AB035771	—
C. filum	Imagoura, Hyogo, Japan	Field plant (Frozen)	CF-Ima	278	261	AB035772	AB035787, AB041873
C. filum	Sasajima, Hiroshima, Japan	Culture (H. Kawai)	CF-Sas	276	267	AB035773	—
C. filum	Mukaishima, Hiroshima, Japan	Field plant (Frozen)	CF-Muk	275	264	AB035774	—
C. filum	Futashima, Nagasaki, Japan	Culture (H. Kawai)	CF-Fut	278	266	AB035775	—

continued

TABLE 3. Continued.

Species (taxonomic position)	Collection site (source)	Origin	Abbreviations	ITS1 (bp)	ITS2 (bp)	DDBJ accession no. for ITS-5.8S rDNA	DDBJ accession no. for *rbc*L and spacer respectively
Pseudochordaceae (Laminariales)							
Pseudochorda gracilis Kawai et Nabata	Isoya, Hokkaido, Japan	Culture (H. Kawai)	—	331	280	AB035780	AB035790, AB041876
Pseudochorda nagaii (Tokida) Inagaki	Hanasaki, Hokkaido, Japan	Culture (H. Kawai)	—	343	293	AB035779	AB035789, AB041875
Laminariaceae (Laminariales)							
Agarum clathratum Dumortier	Muroran, Hokkaido, Japan	Field plant (Silica gel)	—	—	—	—	AB035791, AB041877
Laminaria diabolica Miyabe	Hokkaido, Japan	(Yotsukura et al. 1999)	—	236	256	AB022795, AB022796	
Laminaria yendoana Miyabe	Hokkaido, Japan	(Yotsukura et al. 1999)	—	241	262	AB022807, AB022808	
Kjemaniella crassifolia Miyabe	Muroran, Hokkaido, Japan	Field plant (Silica gel)	—	—	—	—	AB035792, AB041878
Thalassiophyllum clathrus (Gmel.) Postels et Ruprecht	Abacha Bay, Kamchatka, Russia	Field plant (Silica gel)	—	—	—	—	AB035793, AB041879
Undaria peterseniana (Kjellman) Okamura	Tokushima, Japan	Culture (Y. Kogame)	—	—	—	—	AB035794, AB041880
Ectocarpaceae (Ectocarpales)							
Ectocarpus siliculosus (Dillw.) Lyngbye	—	(Valentin and Zetsche 1990)	—	—	—	—	X52503
Pilayella littoralis (L.) Kjellman	—	(Assali *et al.* 1990)	—	—	—	—	X55372

TABLE 4. List of primer sequences and their annealing positions.

Code	Direction	Sequences (5′–3′)	Annealing position
18F1	Forward	AAGGTGAAGTCGTAACAAGG	18S (1768–1787)
5.8F-1	Forward	ACGCAGCGAAATGCGATACG	5.8S (47–66)
5.8R-1	Reverse	CGTATCGCATTTCGCTGCGT	5.8S (66–47)
26R-1	Reverse	GTTAGTTTCTTTTCCTCCGC	26S (70–51)
rbc-F0	Forward	ATCGAACTCGAATAAAAAGTGA	*rbc*L (20–41)
rbc-F1	Forward	CGTTACGAATCWGGTG	*rbc*L (43–58)
rbc-F2	Forward	AGGTTCWCTWGCTAA	*rbc*L (342–356)
rbc-F2.5	Forward	TTCCAAGGCCCAGCAACAGGT	*rbc*L (454–474)
rbc-F3	Forward	CACAACCATTCATGCG	*rbc*L (635–650)
rbc-F4	Forward	GTAAATGGATGCGTA	*rbc*L (953–967)
rbc-F5	Forward	ATTTGGTGGTGGTACTATTGG	*rbc*L (1212–1232)
rbc-R1	Reverse	TTAGCWAGWGAACCT	*rbc*L (356–342)
rbc-R2	Reverse	CGCATGAATGGTTGTG	*rbc*L (650–635)
rbc-R3	Reverse	CCTTTAACCATTAAGGGATC	*rbc*L (1040–1021)
rbc-R4	Reverse	GTAATATCTTTCCATAAATCTAA	*rbc*L (1406–1384)
rbc-R5	Reverse	AAASHDCCTTGTGTWAGTYTC	*rbc*S (23–3)
rbc-R6	Reverse	AATAAAGGAAGACCCCATAATTCCCA	*rbc*S (167–142)

This species is similar to *C. filum* in habit but differs essentially by the more robust thallus with an intercalary meristem even at maturity.

Geographical distribution, phenology, and morphology. Specimens referable to the new species were collected at several localities in Ishikawa and Niigata Prefectures, central Honshu on the Sea of Japan (Fig. 1). This species grows subtidally on rocks in slightly muddy areas at a depth of 2–7 m below mean low water level forming relatively dense populations associated with *Sargassum pallidum* (Turner) C. Agardh. Few other macroscopic algae were found at the same sites. Thalli grew in sparse tufts or solitary from a small discoid holdfast up to 4 mm in diameter (Fig. 2, a–c). The holdfast was well developed and composed of densely packed rhizoidal filaments issuing from the lower part of the erect thalli (Fig. 2h). The erect thalli were simple, cord-shaped, medium to dark brown, and resembled *C. filum* but were distinguished by their solitary or sparse habit and by short and rigid erect thalli with a persistent meristematic zone (Fig. 2d). Young specimens with an intact distal portion above the intercalary meristem were collected in early June (Fig. 2a). Plants grew throughout the summer and attained a maximum length of 30–50 cm (rarely up to 120 cm) and a diameter of 3.5 mm by August–September. Specimens collected in August still retained the distal portions beyond the meristem; however, this portion was absent in specimens collected in September (Fig. 2b). Most of the specimens, including fertile individuals, retained a rigid solid meristematic zone (Fig. 2, d and e).

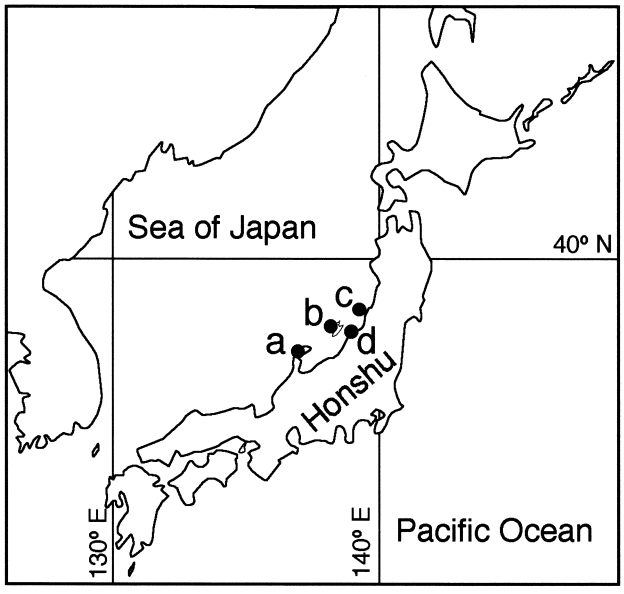

FIG. 1. Geographical distribution of *Chorda rigida* sp. nov. (a) Kannonzaki, Nanao, Ishikawa. (b) Futami, Sado Isl., Niigata. (c) Awashima Isl., Niigata. (d) Kashiwazaki, Niigata.

The new species largely had the same anatomical features as *C. filum*. Thalli had a solid meristem composed of small almost equal-sized meristematic cells (Fig. 2, e and f). Epidermal cells of the meristem were not tightly coherent in cross-section, and they contained many disk-shaped chloroplasts without pyrenoids (Fig. 2g). Pyrenoids were not detected in the field or in culture materials throughout the life history investigations. Phaeophycean hairs of 20–25 μm in diameter were abundant in the meristematic region (Fig. 2f). The central part of the thallus near the meristem was composed of two types of inner hyphae (i.e. trumpet-shaped hyphae and secondary hyphae), cortical cells, and epidermal cells (Fig. 3, a and b). The inner trumpet-shaped hyphae were formed by the elongation of inner meristematic cells (Fig. 3, c and d). The thallus was hollow in the thicker portion further below, and the hyphae were attached on the inner surface of the cortex (photograph not shown). The cortical layer was dense, up to 18 cells thick (see below for detailed descriptions). Cells of the cortical layer were almost hyaline, cylindrical, ca. 40–80 μm in diameter. In the epidermal layer, unicellular paraphyses without mucilaginous appendages developed from epidermal cells in August, but the thalli remained vegetative during

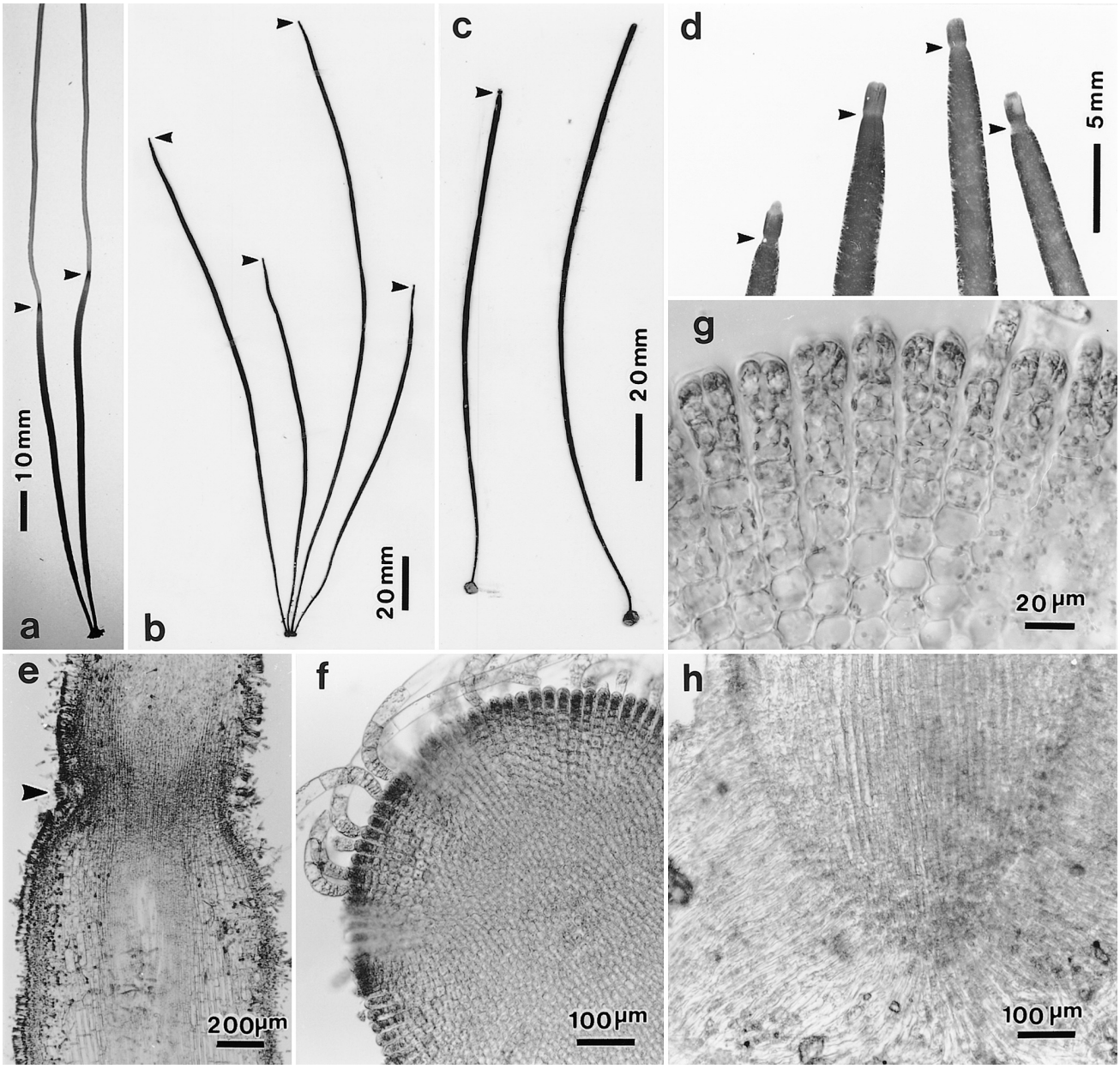

FIG. 2. Field-collected plants of *Chorda rigida*, sp. nov. Arrowheads indicate intercalary meristem. (a) Young sporophytes with long portion distal to meristem (3 June 1991). (b) Vegetative sporophyte without portion distal to meristem but retaining meristem (26 October, 1990). (c) Fertile specimen (1 November, 1991). (d) Rigid intercalary meristem. (e) Longitudinal section of sporophyte through meristematic zone. (f) Cross-section through meristem. (g) Outermost part of meristematic zone in cross-section. (h) Longitudinal section of lowermost part of sporophyte and rhizoidal filaments composing basal holdfast.

summer and autumn. Cylindrical-oblong unilocular sporangia appeared on the entire surface of the thallus in October and November (Fig. 3, e and f). They contained about 16 zoospores and had conspicuous thickenings of the upper sporangial walls. Collections could not be made during midwinter because of the rough wave conditions. No sporophytes were seen in early spring, and regeneration from old erect thalli or the holdfast did not occur; hence, this species is an annual.

Life history. Zoospores were teardrop shaped, flagellated with the usual longer anterior and a shorter posterior flagellum, and contained one chloroplast with an eyespot and showed negative phototaxis. Zoospores germinated by forming a germ tube and developed into sexually dimorphic male and female gametophytes (Fig. 4, a–c, f). The gametophytes matured under LD and SD conditions at 5 and 10° C. Male gametophytic filaments were 5–6.5 μm in diameter (Fig. 4a), forming terminal antheridia (Fig. 4, b

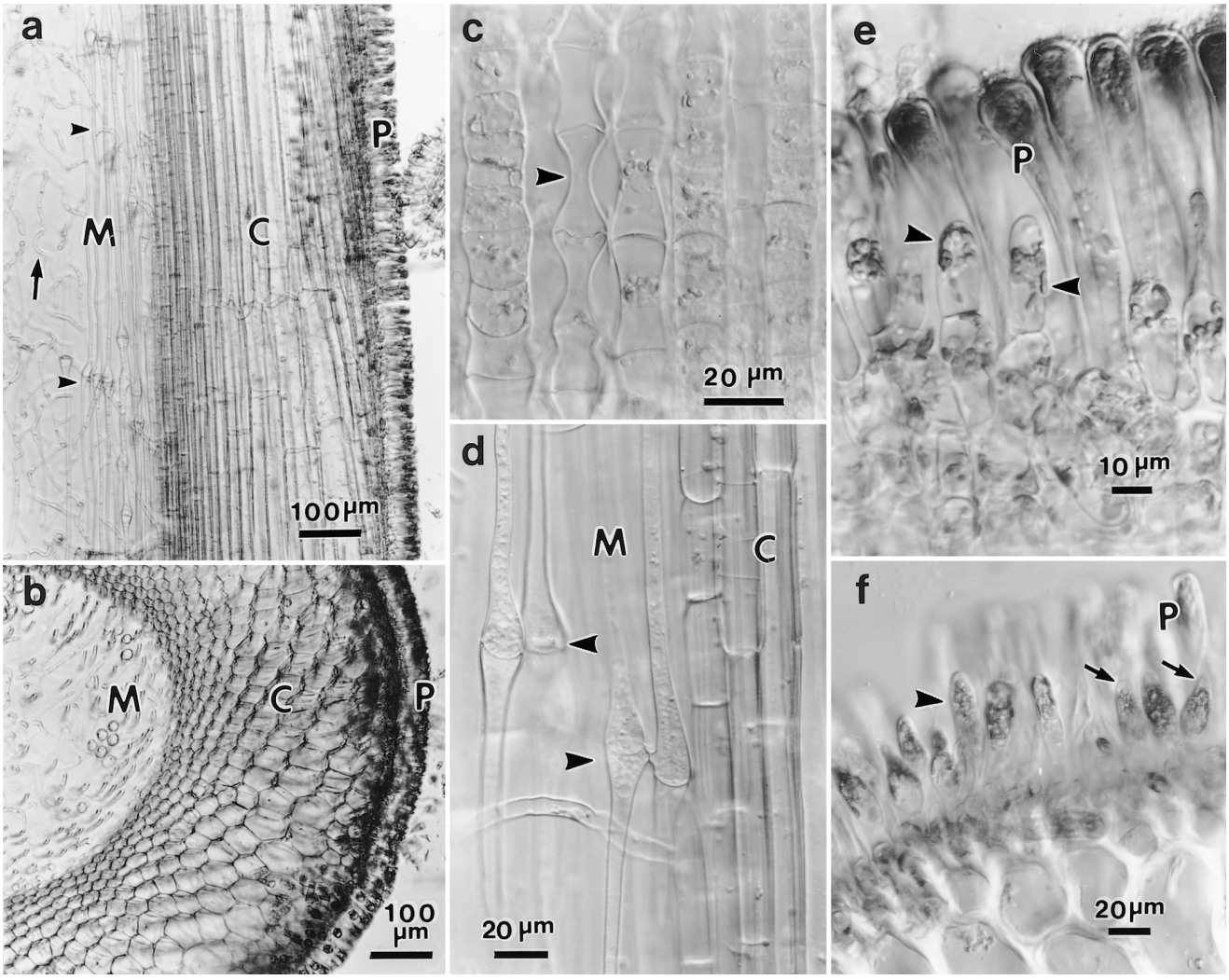

FIG. 3. Field-collected plants of *Chorda rigida*, sp. nov. (a) Longitudinal section just below meristem indicating secondary hyphae (arrow), trumpet-shaped hyphae (arrowheads), cortical layer, and paraphyses. (b) Cross-section just below meristem. (c) Initials of trumpet-shaped hyphae (arrowhead) in the central portion of meristem. (d) Developed trumpet-shaped hyphae (arrowheads). (e) Unicellular paraphyses without mucilage caps and initials of unilocular sporangia (arrowheads). (f) Mature (arrowheads) and premature (arrows) unilocular sporangia among paraphyses. C, cortical layer; M, medullary layer; P, paraphyses.

and c). Sperm were 7–13 µm in length and 4–5 µm in width, with two laterally inserted flagella, a shorter anterior (ca. 16 µm long) and a longer posterior one (42–46 µm long including ca. 25 µm of a distal thinner portion) (Fig. 4, d and e). The shape of sperm was rather variable depending on the time that had elapsed since release. Newly released sperm were relatively elongated but tended to become rounder with time (5–10 min or longer after release). They contained several small chloroplasts ca. 1.5 µm in diameter in the posterior part of the cell and had no eyespot (Fig. 4, b–e). The chloroplasts were not spatially associated with the base of the posterior flagellum (Fig. 4, d and e). Female gametophytic filaments were rich in chloroplasts and measured 13–15 µm in diameter (Fig. 4f).

Although actual fertilization was not observed, presumptive zygotes (in mixed-sex cultures) and unfertilized eggs (in female cultures) developed into sporophytes attached to the mouth of the oogonia (Fig. 4f). They developed into 12- to 16-celled uniseriate embryos forming cross-walls by diffuse growth and then became polystichous by forming longitudinal walls in the distal portions (Fig. 4, g–j). Terminal and lateral hairs (often in whorls in later stages; photographs not shown) occurred in young sporophytes under LD conditions (Fig. 4i) but were rare or absent under SD conditions (Fig. 4, g and h). The thalli were occasionally flattened in cross-section in earlier developmental stages (Fig. 4, g and k) but later became terete. Secondary rhizoids issued from the lower part of the erect thalli (Fig. 4l). The intercalary meristem appeared near the base (Fig. 4l) of the erect thalli, and then the elongation of the portion below the meristem became more active and new growth displaced the meristem toward the distal portion of the thallus.

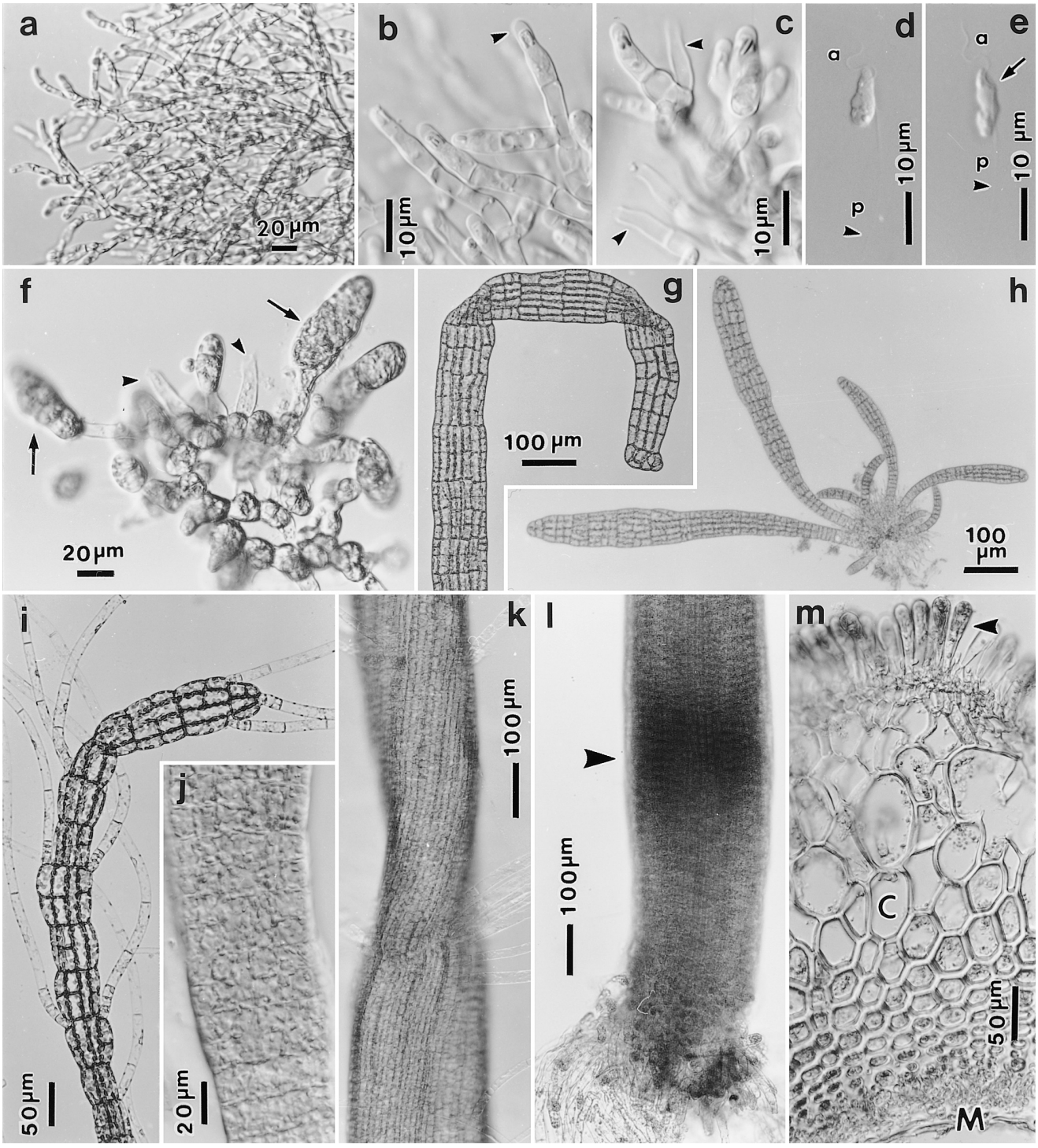

FIG. 4. Gametophytes and sporophytes in culture of *Chorda rigida*, sp. nov. (a) Male gametophyte. (b) Antheridia with terminal thickening of the wall (arrowhead). (c) Antheridia and walls of emptied antheridia (arrowheads). (d and e) Sperm. a, anterior flagellum; arrow, basal part of anterior and posterior flagella; p and arrowheads, posterior flagellum. (f) Female gametophyte and embryos of young sporophytes (arrows). Arrowheads show walls of emptied oogonia. (g) Young flat sporophyte without phaeophycean hairs. (h) Young sporophytes without phaeophycean hair on female gametophyte. (i) Young cylindrical sporophyte with phaeophycean hairs. (j) Basal part of young sporophyte with basal uniseriate portion. (k) Slightly flat young sporophyte. (l) Lower part of young sporophyte with initial of intercalary meristem (arrowhead). (m) Cross-section of fertile sporophyte in culture. Arrowhead indicates unicellular paraphysis. C, cortical layer; M, medullary layer.

The sporophytes elongated up to ca. 10 cm within 2–3 months at 10, 15, 20, and 25° C but did not grow well at 5° C. They became fertile at 15° C, forming unilocular sporangia among unicellular paraphyses (Fig. 4m), and completed the life history. The cultured sporophytes had relatively dense cortical layers composed of as many cell layers (up to 16 cells thick) as field-collected thalli.

In contrast, the strains of *C. filum* (Ishikawa Pref. and Hokkaido, Japan and Bergen, Norway) showed relatively good growth at 5, 10, and 15° C culture conditions (5 and 10° C in Bergen strain) but did not grow well at 20 and 25° C.

Anatomical comparison with Chorda filum. *Chorda rigida* has a considerably denser cortical layer than *C. filum*, and hence the sporophyte is conspicuously more rigid. To clarify the intraspecific morphological variations within these two taxa and to compare their differences more clearly, the anatomies of sporophytes of *C. filum* and *C. rigida* were compared with the number of cell layers comprising the intercalary meristem and cortical layers of the middle and lower portions of sporophytes in field-collected, as well as cultured, material.

Chorda rigida had more cortical cell layers than *C. filum* in all parts of the thallus. The average number of cells forming the meristem (from center to surface) was 19.0 ± 2.6 (SD) in *C. filum* and 25.2 ± 2.9 in *C. rigida*. The average number of cortical cell layers in non-meristematic portions was 5.0 ± 1.1 (middle part of sporophyte) and 5.5 ± 1.0 (near the base) in *C. filum* versus 8.7 ± 1.5 (middle part) and 12.8 ± 1.7 (near the base) in *C. rigida*.

This tendency was maintained even in sporophytes grown in culture. The average number of cells forming the meristem (from center to surface) was 16.5 ± 2.3 in *C. filum* (strains from Oshoro, Hokkaido and Hakui, Niigata Pref., Japan) and 22.3 ± 1.9 in *C. rigida*. The average number of cortical cell layers in the non-meristematic portions was 7.1 ± 1.0 (middle part of sporophyte) and 7.1 ± 1.0 (near the base) in *C. filum* and 11.2 ± 1.7 (middle part) and 10.8 ± 1.2 (near the base) in *C. rigida*. Significance of the difference in these measurements between the two species in all these features was confirmed by *t*-test ($P < 0.001$).

Molecular phylogenetic analysis. ITS +5.8S rDNA region: As seen in Table 3, the sequences of ITS1 and ITS2 regions were considerably longer in *C. rigida* (358–360 base pair [bp] for ITS1 and 302–303 bp for ITS2) than in *C. filum* (259–290 bp for ITS1 and 255–275 bp for ITS2).

The aligned ITS + 5.8S rDNA sequence data included 1054 sites in total. There were 260 parsimony-informative nucleotide positions in our alignment. All *C. rigida* specimens formed a close monophyletic cluster supported in all analyses by 100% bootstrap values (Fig. 5, a–c). In contrast, *C. filum* was shown to be relatively genetically diverse. In the NJ tree (Fig. 5b) *C. filum* was monophyletic, but in the MP (Fig. 5a) and ML (Fig. 5c) trees it was paraphyletic, although the bootstrap support was weak in all cases. The species was resolved as 4–5 groups in all analyses. North Atlantic specimens, including those from France (CF-Ros, CF-San), Norway (Ber), Iceland (Ora, Rey), Britain (Por, IME, IMP), and NE Canada (NFL), formed a cluster supported by high bootstrap values in all analyses. There appear to be two groups in the northernmost Pacific Ocean, including specimens from NW America (Pug), the Bering Sea (StL), and Kamchatka (Pet1 and Pet2), but their phylogenetic relationships with other populations appear to be relatively unclear. Most Japanese specimens from various localities formed a single cluster supported by bootstrap values as high as for the Atlantic cluster. Interestingly, however, the specimens from Kikonai (southern part of Hokkaido) did not cluster with other Japanese *C. filum* but rather joined specimens from Kamchatcka (Pet1) and NW America (Pug). In a second parsimony analysis with gaps recognized as a fifth base, the tree topology was basically the same (data not shown).

rbc*L* + *spacer region:* The aligned sequence data of the *rbc*L gene and spacer region consisted of 1834 bp, of which 311 were informative. Among the Laminariales clades, including Chordaceae, Pseudochordaceae, and Laminariaceae, *Pseudochorda* was basal in distance (Fig. 6b) and ML (Fig. 6c) analyses, although they formed a trichotomy with *Chorda* and the advanced Laminariales in the MP tree (Fig. 6a). Within the *Chorda* clade, a North Atlantic group was basal in distance (Fig. 6b) and ML (Fig. 6c) trees. The genetic diversity of *C. filum*, especially the independence of the North Atlantic (Ros, NFL) and North Pacific (Osh1, Ima, Kik1, Pug) groups, was supported by *rbc*L trees. *Chorda rigida* (CR-Kas) clustered with Pacific *C. filum* (CF-Osh1, Ima, Kik1, Pug) in all analyses, and *C. filum* was again paraphyletic. Phylogenetic analyses using amino acid sequence for the *rbc*L gave essentially the same tree topology as those based on DNA sequences. In addition, combined analyses of *rbc*L gene and spacer regions with ITS + 5.8S rDNA sequence data yielded tree topologies essentially the same as those based on *rbc*L gene and spacer region sequence data (trees not shown).

DISCUSSION

Chorda rigida, sp. nov. resembles *C. filum*, generitype and until now was the only species of the genus in basic morphological features of the sporophyte and occurrence of sexually dimorphic dioecious gametophytes (Reinke 1892, Kylin 1918, South and Burrows 1967, Kogame and Kawai 1996). However, the new species is distinguished from *C. filum* by the following characteristics of the sporophytes: 1) relatively short, more rigid and solitary, or sparsely clustered thalli; 2) different phenology; 3) greater tolerance to considerably higher temperature conditions; and 4) thicker and firmer meristem and cortical layers. Furthermore, *C. rigida* has considerably longer ITS1 and ITS2 regions when compared with *C. filum* collected from a

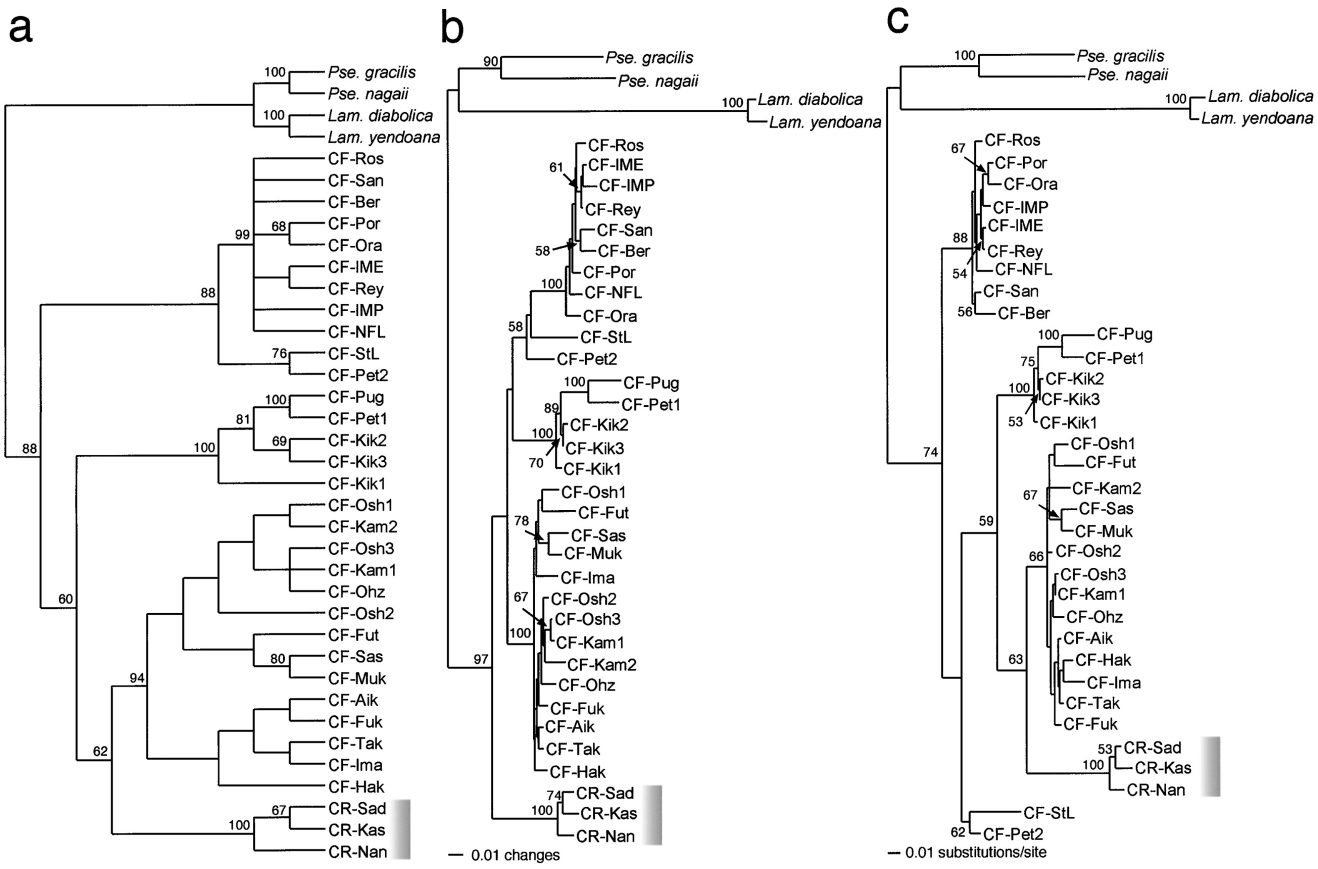

FIG. 5. Molecular trees based on ITS1, 5.8S, and ITS2 rDNA sequences. Bootstrap values indicate percents based on 1000 (a and b) and 200 (c) replications. See Table 3 for collection abbreviations. *Chorda rigida* highlighted by gray bar. (a) MP analysis. (Strict consensus tree of 87 MP trees. Tree length, 712; consistency index, 0.7219; retention index, 0.8210.) (b) NJ analysis. (c) ML analysis (one of the two ML trees, –Ln likelihood = 5356.77325).

large proportion of its distributional range. The independence and monophyletic status of *C. rigida* was also supported by ITS sequence analysis. A detailed discussion of these features follows.

Character 1. Chorda rigida grows in more exposed habitat than *C. filum*. Perhaps due to the shorter and more rigid thalli that are firmly attached to stable rocks, the sporophytes of *C. rigida* can resist relatively strong wave action. The two species do not mix in the areas where they both occur.

Characters 2 and 3. On the Japanese coast, new sporophytes of *C. filum* usually appear in early spring and become fertile soon after attaining their maximum length, disappearing before mid-summer. In contrast, sporophytes of *C. rigida* appear in late spring to early summer, attain their maximum length in summer, but remain vegetative during summer (oversummer) and become fertile in late autumn to winter. In colder habitats (e.g. northern Europe where the monthly average surface water temperature in August is 11–13° C) (South and Burrows 1967) where summer water temperatures are considerably lower, growth of *C. filum* may be slower and the sporophytes often oversummer (Lund 1959, South and Burrows 1967, our own observations). However, even in such localities, the sporophytes do not remain vegetative after attaining their maximum length in summer (South and Burrows 1967). As shown in the present culture experiments, Japanese *C. filum* grew well at 10 and 15° C but poorly or not at all at 20° C and could not tolerate temperatures over 25° C. In contrast, *C. rigida* showed tolerance to higher temperature ranges and grew even at 25° C. This result agrees with the field water temperature conditions of the locality where the monthly average surface water temperature in August is ca. 24–25° C (Funahashi 1974).

Character 4. In *C. filum*, the distal portion of sporophytes above the meristem is gradually lost as they grow, and a meristem is rarely found in sporophytes that have reached their maximum length (South and Burrows 1967, our own observations). However, in *C. rigida* the decay stops at the meristem, and the meristem is often retained even in fertile sporophytes, probably because of the rigid meristem and cortex below the meristem.

Although the number of cortical cell layers is more or less predetermined by the number of cells composing the meristem (Kogame and Kawai 1996), it is

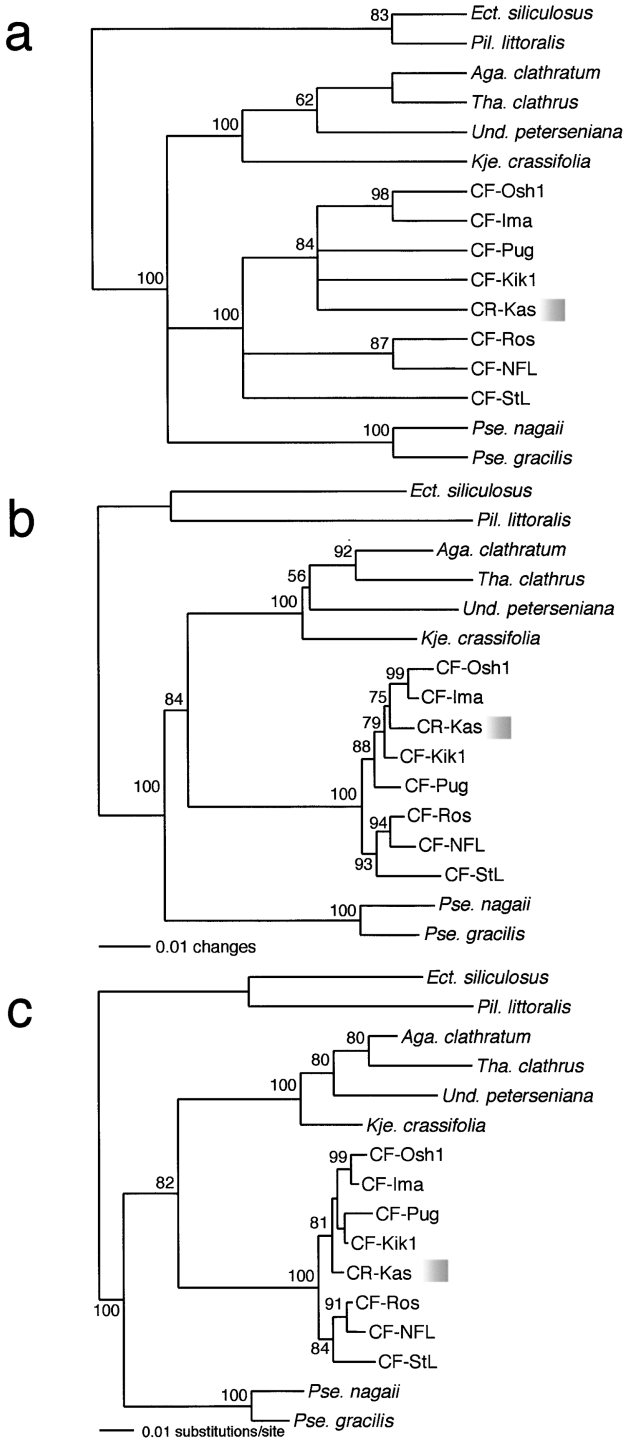

FIG. 6. Molecular phylogenetic trees based on *rbc*L and spacer sequences. Bootstrap values indicate percents based on 1000 (a and b) and 200 (c) replications. See Table 3 for collection abbreviations. *Chorda rigida* highlighted by gray bar. (a) MP analysis. (Strict consensus tree of 6 maximum parsimonious trees. Tree length, 808; consistency index, 0.7500; retention index, 0.7699). (b) NJ analysis. (c) ML analysis (–Ln likelihood = 6930.18599).

somewhat variable depending on the portion of the sporophyte. *Chorda filum* is reported to have 4–10 cortical layers (Kylin 1918, South and Burrows 1967, Russell 1985, Kogame and Kawai 1996), whereas *C. rigida* has 6–18. The cell numbers composing the meristem and cortical cell layers might possibly change depending on the environmental conditions; however, they were found to be rather stable in culture experiments.

The morphology of juvenile sporophytes was rather variable depending on the culture conditions; phaeophycean hairs were abundant under LD conditions but sparse or totally absent under SD conditions. In brown algae, hair formation has been reported to be controlled by light quality in *Scytosiphon lomentaria* (Lyngbye) Link (Dring and Lüning 1975); however, in the present species it is apparently controlled by day length. Juvenile sporophytes of *C. rigida* became relatively flat in culture, but it is still unclear whether *C. filum* has the same tendency.

Chorda filum sensu stricto (excluding *C. rigida*) has a broad geographical distribution along Arctic, Atlantic (south to the Spanish coast in Europe and south to New Jersey on the American coast), and Pacific coasts (south to southern Japan on the Asian coast and south to Washington on the American coast). The ITS + 5.8S rDNA and *rbc*L + spacer sequence data showed great genetic diversity and possibly paraphyly for *C. filum*, and this species may require taxonomic revision. However, at present there are insufficient morphological and physiological data to support any useful taxonomic recognition of genetically distinct populations of *C. filum*. Variations in the number and size of cells comprising the cortical layer have been noted in European populations (South and Burrows 1967, Russell 1985). Specimens from Kikonai, Japan, which were shown to be genetically distant from other *C. filum* in the current study, have been noted to have cortical layers composed of fewer cells compared with other Japanese specimens (H. Kawai and Y. Kogame, unpublished data). Therefore, detailed morphological comparisons, especially those of cortical layers, and responses to different temperature and day-length conditions may provide useful information to clarify the taxonomic relationship between the Kikonai population and other *C. filum*.

Kanda (1938) reported that sperm of *C. filum* had a shorter posterior flagellum and a distinct eyespot unlike all other laminarialean species (Henry and Cole 1982, Kawai 1992). However, in our observations, *C. filum* (data not shown) and *C. rigida* had a longer posterior flagellum and lacked an eyespot. This latter sperm morphology basically agrees with *Halosiphon tomentosus* (Maier 1984), Pseudochordaceae (Kawai and Kurogi 1985, Kawai and Nabata 1990), Phyllariaceae (Henry 1987), and advanced Laminariales (Henry and Cole 1982). Therefore, all known members of the order Laminariales are considered to lack eyespots in sperm and have a longer posterior flagellum.

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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. *Pl. Mol. Biol.* 15:307–15.

Bold, H. C. & Wynne, M. J. 1985. *Introduction to the Algae: Structure and Reproduction*, 2nd ed. Prentice-Hall, Englewood Cliffs, NJ, 720 pp.

Boo, S. M., Lee, W. J., Yoon, H. S., Kato, A. & Kawai, H. 1999. Molecular phylogeny of Laminariales (Phaeophyceae) inferred from small subunit ribosomal DNA sequences. *Phycol. Res.* 47: 109–14.

Clayton, M. 1984. Evolution of the Phaeophyta with particular reference to the Fucales. *In* Round, F. E. & Chapman, D. J. [Eds.] *Progress in Phycological Research.* Vol. 3. Biopress, Bristol, pp. 11–46.

Daugbjerg, N. & Andersen, R. A. 1997. A molecular phylogeny of the heterokont algae based on analyses of chloroplast-encoded rbcL sequence data *J. Phycol.* 33:1031–41.

Dring, M. J. & Lüning, K. 1975. Induction of two-dimensional growth and hair formation by blue light in the brown alga *Scytosiphon lomentaria. Z. Pflanzenphysiol.* 75:107–17.

Felsenstein, J. 1985. Confidence limits on phylogenies: an approach using bootstrap. *Evolution* 39:783–91.

Funahashi, S. 1974. Distribution of marine algae in the Japan Sea, with reference to the phylogeographical positions of Vladivostok and Noto Peninsula districts. *J. Fac. Sci. Hokkaido Univ., Ser. V (Botany)* 10:1–31.

Henry, E. C. 1987. Primitive reproductive characters and a photoperiodic response in *Saccorhiza dermatodea* (Laminariales, Phaeophyceae). *Br. Phycol. J.* 22:23–31.

Henry, C. E. & Cole, K. M. 1982. Ultrastructure of swarmers in the Laminariales (Phaeophyceae). II. Sperm. *J. Phycol.* 18:570–9.

Henry, E. C. & South, G. R. 1987. *Phyllariopsis* gen. nov. and a reappraisal of the Phyllariaceae Tilden 1935 (Laminariales, Phaeophyceae). *Phycologia* 26:9–16.

Jaasund, E. 1957. Marine algae from Northern Norway. *Bot. Not.* 110:205–31.

Kanda, T. 1938. On the gametophytes of some Japanese species of Laminariales. II. *Sci. Pap. Inst. Algol. Res. Fac. Sci., Hokkaido Imp. Univ.* 2:87–111, plates 17 and 18.

Kawai, H. 1992. A summary of the morphology of chloroplasts and flagellated cells in the Phaeophyceae. *Kor. J. Phycol.* 7:33–43.

Kawai, H. & Kurogi, M. 1985. On the life history of *Pseudochorda nagaii* (Pseudochordaceae fam. nov.) and its transfer from the Chordariales to the Laminariales (Phaeophyta). *Phycologia* 24:289–96.

Kawai, H., Muto, H., Fujii, T. & Kato, A. 1995. A linked 5S rRNA gene in *Scytosiphon lomentaria* (Scytosiphonales, Phaeophyceae). *J. Phycol.* 31:306–11.

Kawai, H. & Nabata, S. 1990. Life history and systematic position of *Pseudochorda gracilis* sp. nov. (Laminariales, Phaeophyceae). *J. Phycol.* 26:721–7.

Kimura, M. 1980. A simple method for estimating rate of base substitutions through comparative studies of nucleotide sequences. *J. Mol. Evol.* 16:111–20.

Kogame, Y. & Kawai, H. 1996. Development of the intercalary meristem in *Chorda filum* (Laminariales, Phaeophyceae) and other primitive Laminariales. *Phycol. Res.* 44:247–60.

Kylin, H. 1918. Studien über die Entwicklungsgeschichte der Pheophyceen. *Svensk. Bot. Tidskr.* 12:1–64.

Lund, S. 1959. The marine algae of East Greenland 1. Taxonomical part. *Medd. Grønland.* 156:1–247.

Maier, I. 1984. Culture studies of *Chorda tomentosa* (Phaeophyta, Laminariales). *Br. Phycol. J.* 19:95–106.

Maier, I. 1995. Brown algal pheromones. *In* Round, F. E. & Chapman, D. J. [Eds.] *Progress in Phycological Research.* Vol. 11. Biopress, Bristol, pp. 51–102.

Müller, D. G., Maier, I. & Gassmann, G. 1985. Survey on sexual pheromone specificity in Laminariales (Phaeophyceae). *Phycologia* 24:475–7.

Peters, A. 1998. Ribosomal DNA sequences support taxonomic separation of the two species of *Chorda*: reinstatement of *Halosiphon tomentosus* (Lyngbye) Jaasund (Phaeophyceae, Laminariales). *Eur. J. Phycol.* 33:65–71

Reinke, J. 1892. *Atlas Deutscher Meeresalgen. Heft. II*, Berlin, pp. 35–70.

Russell, G. 1985. Some anatomical and physiological differences in *Chorda filum* from coastal waters of Finland and Great Britain. *J. Mar. Biol. Assoc. UK* 65:343–9.

Setchell, W. A. & Gardner, N. L. 1925. The marine algae of the Pacific Coast of North America. Part III. Melanophyceae. *Univ. Calif. Pub. Bot.* 8:383–898.

South, G. R. & Burrows, E. M. 1967. Studies on marine algae of the British Isles. 5. *Chorda filum* (L.) Stackh. *Br. Phycol. Bull.* 3:379–402.

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. *J. Phycol.* 33:152–68.

Swofford, D. L. 1999. *PAUP: Phylogenetic Analysis Using Parsimony.* (version 4.0b2). Computer program distributed by Sinauer Associates, Inc., 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). *J. Phycol.* 32:112–8.

Tatewaki, M. 1966. Formation of crustaceous sporophyte with unilocular sporangia in *Scytosiphon lomentaria. Phycologia* 6:62–6.

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. *Nucl. Acids Res.* 22:425–30.

Tilden, J. E. 1935. *The Algae and Their Life Relations.* University of Minnesota Press, Minneapolis, 550 pp.

Valentin, K. & Zetsche, K. 1990. Rubisco genes indicate a close phylogenetic relation between the plastids of Chromophyta and Rhodophyta. *Pl. Mol. Biol.* 15:575–84.

van den Hoek, C., Mann, D. G. & Jahns, H. M. 1995. *Algae: An Introduction to Phycology.* Cambridge University Press, Cambridge, 623 pp.

Yotsukura, N., Denboh, T., Motomura, T., Horiguchi, T., Coleman, A. W. & Ichimura, T. 1999. Little divergence in ribosomal DNA internal transcribed spacer-1 and -2 sequences among non-digitate species of Laminaria (Phaeophyceae) from Hokkaido, Japan. *Phycol. Res.* 47:71–80.