Nat.Hist.Res.,Special lssue No.8 : 69－81. March 2005

Molecular phylogeny of advanced kelps (Laminariales, Phaeophyceae) growing in Japan

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Abstract Phylogenetic relationships of Japanese advanced kelps (Laminariales, Phaeophyceae) were analyzed, based on RuBisCo spacer, ITS-1 and RuBisCo spacer + ITS-1 sequences. In distance and maximum parsimony trees by ITS-1 and by RuBisCo spacer + ITS-1 analyses, which were more informative than those by RuBisCo spacer analysis, five major lineages were found: (1) non-digitate *Laminaria-Cymathaere-Kjellmaniella-Arthrothamnus*, (2) *Eisenia-Ecklonia-Eckloniopsis*-digitate *Laminaria*, (3) *Agarum-Thalassiophyllum-Costaria*, (4) *Alaria* and (5) *Undaria*. Further analysis for non-digitate *Laminaria*, *Cymathaere* and *Kjellmaniella* based on 5S rDNA spacer sequences showed three sublineages: (a) *L. japonica-L. religiosa-L. ochotensis-L. diabolica-L. longipedalis*, (b) *L. angustata-L. longissima*, (c) *L. coriacea-L. cichorioides-L. yendoana*-(*C. japonica*)-*K. gyrata-K. crassifolia*. These sublineages are characterized by (a) bullation which disappears with the progress of their growth, (b) no bullation in their lifetime, and (c) bullation or gyration which remains for life. Compared to the latter two sublineages, genetic distance was so short within the "(a)" sublineage as to suggest that gene flow is occurring between the members of the first sublineage.

Key words: Japanese advanced kelp, Laminariales, molecular phylogeny, DNA sequencing, ITS, RuBisCo spacer, 5S rDNA spacer, taxonomic system.

Introduction

The Phaeophycean order Laminariales includes approximately a hundred species belonging to eight families: Akkesiphycaceae, Pseudochordaceae, Chordaceae, Halosiphonaceae, Phyllariaceae, Alariaceae, Laminariaceae and Lessoniaceae. Of these, the three complex families Alariaceae, Laminariaceae and Lessoniaceae (ALL) have been considered advanced members based on morphological, life cycle and pheromonal features (e. g., Saunders and Druehl, 1993, Kawai and Sasaki, 2000). Many of these advanced kelps have been utilized as foods, manure, feeds, medical supplies and in industry. Molecular phylogeny of laminarialean algae has been pursued since late in the nineteen-eighties. The analyses employed, restriction fragment length polymorphism (RFLP) or nucleotide sequencing for mainly Northeast Pacific materials, showed monophyly of the ALL complex (Fain *et al.*, 1988, Saunders and Druehl, 1993, Druehl *et al.*, 1997, Boo *et al.*, 1999, Yoon *et al.*, 2001), and phylogenetic relationships among the advanced kelps still have remained unresolved.

On the coasts around Japan (Hokkaido, Honshu, Sikoku and Kyushu), we can recognize 34 advanced laminarialean species belonging to Alariaceae and Laminariaceae (Kawashima, 1989a). The present taxonomy of these kelps usually follows the system of either Setchell and Gardner (1925) or Okamura (1936). However, there is disagreement between traditional morphological data and current molecular data for the ALL complex when based on specimens from outside Japan. This has raised uncertainly as to whether the morphological features used for the taxonomy of Japanese kelps reflect their phylogeny. Yotsukura *et al.* (1999) reported little divergence in the internal transcribed spacer -1 and -2 (ITS-1, -2) sequences of the nuclear ribosomal RNA gene (rDNA) among 10 non-digitate laminarian species from Hokkaido, but showed the existence of two phylogenetically distinct groups as (I) the *L. japonica* group encompassing *L. japonica, L. religiosa, L. ochotensis, L. diabolica, L. longipedalis, L. angustata* and

L. longissima, and (II) the *L. saccharina* group including *L. coriacea, L. cichorioides* and *L. yendoana*. Most Japanese laminarian species are valuable edible crops. Although important for future development of kelp breeding, further detailed relationships within the *L. japonica* group and the *L. saccharina* group remain unexplored. Recently Yotsukura *et al.* (2002) found tandemly repeated 5S rDNA, which is located separately from 18S-5.8S-25S rDNA units, in three Japanese laminarian species, and detected genetic differences in this 5S rDNA spacer region among individuals of *L. japonica*, as well as between these three species.

In this paper, I explore phylogenetic relationships of Japanese advanced laminarialean species based on the plastid encoded ribulose-1,5-bisphosphate carboxylase / oxgenase gene spacer between large and small subunits (RuBisCo spacer) and the nuclear encoded ITS-1 sequences. Further analysis of 5S rDNA spacer sequencing was carried out for the two species groups of Japanese *Laminaria*.

Materials and Methods

RuBisCo Spacer and ITS-1 Analyses

Mature or immature sporophytes of laminarialean algae were collected in Hokkaido except for *Laminaria saccharina*, which was from Ireland (Table 1). Total DNA was extracted from zoospores which were released from mature individuals, or from a few small tips of lamina. The processes for DNA extraction-purification from zoospores and lamina were the same as described by Yotsukura *et al.* (1999) and Yotsukura *et al.* (2001), respectively. To amplify RuBisCo spacer, polymerase chain reaction (PCR) was performed using two primers, BLSrbcL1302F: 5'-GAATCTATGG TTCTAGCTCGT-3' and BLSrbcS3R: 5'-A AACATCCTTGTGTAACTCTC-3', which were designed with reference to previous research for several phaeophycean algae (Siemer *et al.*, 1998). For ITS-1, nested-PCR was carried out using three primers (ITS1, ITS2 and ITS4) as in a previous study (Yotsukura *et al.*, 1999). Reaction components and conditions for all PCR, methods for collection of DNA fragments, and sequencing for both RuBisCo spacer and ITS were the same as in Yotsukura *et al.* (1999). Sequences were aligned by using clustal W computer program (Thompson *et al.*, 1994) for RuBisCo spacer and by eye with consideration of the secondary structure derived with mFOLD program (Zuker, 1989) for ITS. In this alignment were included sequences of *Thalassiophyllum clathrum* (Gmelin) Post. et Rupr. (AF31898, AF319028), *Eisenia bicycle* (Kjellman) Setchell (AF318963, AF319012), *Ecklonia cava* Kjellman (AF318967, 319009), *Ecklonia stolonifera* Okamura (AF318968, AF31901 0), *Eckloniopsis radicosa* (Kjellman) Okamura (A F318969, AF319011), *Undaria undarioides* (Yendo) Okamura (AF318962, AF319008), *Undaria peterseniana* (Kjellman) Okamura (AF31896 1, AF319006) (Yoon *et al.*, 2001) and *Ecklonia kurome* from Imako-ura, Hyogo prefecture (Yotsukura, unpub. data). One primitive kelp, *Chorda filum*, was treated as an outgroup. Phylogenetic relationships were derived by comparison of the nucleotide sequences of RuBisCo spacer and ITS-1 independently and conjunctly by distance matrix and maximum parsimony (MP) methods using PAUP version 4.0b10 (Swofford, 2002). The distance matrix was generated by Kimura's two-parameter method and the phylogenetic tree was constructed with the neighbor-joining (NJ) algorithm. MP analysis was executed in a general heuristic search. Gaps were excluded in all analyses. Bootstrap analysis (1000 replicates) was used for all analyses to estimate the stability of tree topologies.

5S rDNA Spacer Analysis

Mature or immature sporophytes of *Laminaria, Kjellmaniella* and *Cymathaere* were collected from throughout coast of Hokkaido, except one individual of *Laminaria japonica* from Omoshige, Iwate Prefecture (Table 2). Methods for obtaining 5S rDNA spacer sequence followed Yotsukura *et al.* (2002). Phylogenetic relationships were derived based on 5S rDNA spacer sequences by the same two methods using PAUP as in the analysis based on RuBisCo spacer and ITS-1 sequences. In parsimony analysis, gaps were treated as fifth bases (gap mode).

Results

RuBisCo Spacer and ITS-1 Analyses

The total length of the RuBisCo spacer and the ITS-1 in these laminarialean species was 214-282 bp and 225-308 bp, respectively. Both RuBisCo spacer and ITS-1 sequences were

Table 1. Collection date and site of Laminariales used for RuBisCo spacer and ITS-1 analyses.

Species	Collection date	Collection site	Species	Collection date	Collection site
Laminaria japonica Areschoug	15 November 1997	Yasuura, Minamikayabe	*Costaria costata* (Turner) Saunders	8 August 1997	Charatsunai, Muroran
L. religiosa Miyabe	13 October 1997	Toyohama, Kumaishi	*Agarum cribrosum* Bory f. *cribrosum*		
L. ochotensis Miyabe	1 October 1997	Hourai, Wakkanai		22 July 1999	Katsuragi, Nemuro
L. diabolica Miyabe	17 November 1998	Funami-cyo, Rausu	*Ag. cribrosum* Bory f. *rugosum* I. Yamada		
L. longipedalis Okamura	13 November 1997	Akkeshi-ko, Akkeshi		5 July 1999	Charatsunai, Muroran
L. angustata Kjellman	30 April 1999	Charatsunai, Muroran	*Arthrothamnus bifidus* (Gmelin) J. Agardh		
L. longissima Miyabe	13 November 1997	Aikappu, Akkeshi		22 July 1999	Katsuragi, Nemuro
L. coriacea Miyabe	13 November 1997	Aikappu, Akkeshi	*Ecklonia kurome* Okamura	9 April 2000	Imako-ura, Kasumi-cyo,
L. saccharina (L.) Lamouroux f. *linearis* J. Agardh					Hyogo Pref.
	1 December 1999	Pier of Spiddal, Ireland	*Alaria praelonga* Kjellman	13 November 1997	Aininkappu, Akkeshi
L. cichorioides Miyabe	19 November 1997	Hourai, Wakkanai	*Al. crassifolia* Kjellman	4 October 1999	Charatsunai, Muroran
L. sachalinensis Miyabe	14 September 1999	Funami-cyo, Rausu	*Undaria pinnatifida* (Harvey) Suringar f. *pinnatifida*		
L. sachalinensis Miyabe (?)	9 September 1999	Heinai-cyo, Nemuro		14 June 1999	Charatsunai, Muroran
L. yendoana Miyabe	6 October 1997	Oinaoshi, Muroran	*Chorda filum* (Linnaeus) Stackhouse		
L. yezoensis Miyabe	18 November 1998	Katsurakoi, Kushiro		15 June 1998	Moheji, Kamiiso
Cymathaere japonica Miyabe et Nagai					
	23 July 1999	Funami-cyo, Rausu			
Kjellmaniella gyrata (Kjellman) Miyabe					
	13 November 1997	Aikappu, Akkeshi			
K. crassifolia Miyabe	31 October 1997	Usujiri, Minamikayabe			

Table 2. Collection date and site of *Laminaria*, *Cymathaere* and *Kjellmaniella* used for 5S rDNA spacer analyses.

Species	Collection date	Collection site	Species	Collection date	Collection site
Laminaria japonica	29 January 2000	Charatsunai, Muroran (Ch)	*L. angustata*	31 January 2000	Charatsunai, Muroran (Ch)
	15 October 2000	Kakkumi, Minamikayabe (Ka)		22 February 2002	Usujiri, Minamikayabe (Uj)
	3 April 2000	Kobui, Esan (Es) [No. 1-5]	*L. longissima*	22 July 1999	Habomae, Nemuro (Ha)
	29 February 2000	Furukawa-cyo, Hakodate (Fr)		27 February 2002	Aikappu, Akkeshi (Ak) [No. 1-5]
	6 March 2000	Shiogama, Fukushima-cyo (Fk)	*L. coriacea*	28 February 2002	Tomoshiri, Nemuro (To)
	21 May 2001	Omoshige, Miyako, Iwate pref. (Mi)		27 February 2002	Aikappu, Akkeshi (Ak) [No. 1-4]
L. religiosa	19 May 2000	Genna, Otobe (Ot) [No. 1-5]	*L. cichorioides*	24 January 2002	Sannai, Wakkanai (Sa) [No. 1-3]
	4 July 2000	Isoya, Suttsu (Is)	*L. sachalinensis*	14 September 1999	Funami-cyo, Rausu (Ra)
	14 February 2000	Osyoro, Otaru (Os)	*L. yendoana*	6 October 1997	Oinaoshi, Muroran (Oi)
	18 January 2000	Minedonari, Atsuta (At) [No. 1-5]	*Cymathaere japonica*	23 July 1999	Funami-cyo, Rausu (Ra)
	21 May 2001	Omoshige, Miyako, Iwate pref. (Mi)	*Kjellmaniella gyrata*	13 November 1997	Aikappu, Akkeshi (Ak)
L. ochotensis	8 February 2000	Hourai, Wakkanai (Wa) [No. 1-5]	*K. crassifolia*	28 November 2001	Charatsunai, Muroran (Ch)
	26 January 2002	Hakunai, Souya (SO)			
L. diabolica	17 November 1998	Hunami-cyo, Rausu (Ra)			
	25 May 2001	Heinai-cyo, Nemuro (He) [No. 1-5]			
	27 February 2002	Aikappu, Akkeshi (Ak)			
L. longipedalis	13 November 1997	Aikappu, Akkeshi (Ak)			

completely identical among the five laminarian species *L. japonica, L. religiosa, L. ochotensis, L. diabolica* and *L. longipedalis* and among the three laminarian species *L. coriacea, L. cichorioides* and *L. yendoana*. The RuBisCo spacer sequence of the latter three species was also identical to that of *L. sachalinensis*. Though identical nucleotide sequences were found in the ITS-1 of *L. angustata* and *L. longissima*, these species were differentiated by one nucleotide substitution in the RuBisCo spacer.

RuBisCo spacer analysis. Aligned sequences with 291 sites in total, were found to contain 117 variable positions and 72 parsimony informative positions. In the distance tree (Fig. 1a), there were six major lineages: non-digitate *Laminaria-Cymathaere-Kjellmaniella-Arthrothamnus, Eisenia-Ecklonia-Eckloniopsis*, digitate *Laminaria, Agarum-Thalassiophyllum, Costaria*, and *Alaria-Undaria*. In a strict consensus MP tree, *L. yezoensis* clustered with *Eisenia-Ecklonia-Eckloniopsis* clade supported with moderate bootstrap value (69%).

ITS-1, RuBisCo spacer + ITS-1 analyses. Of 318 sites aligned, there were 164 variable positions and 134 parsimony informative positions. In both distance trees from ITS-1 (Fig. 1b) and RuBisCo spacer + ITS-1 (Fig. 1c) analyses, *Alaria* formed a sister clade to a large clade including the other genera except for *Undaria*, and this was supported by moderate bootstrap values (77-88%). In both strict consensus MP trees, monophyly of *Alaria*, of *Undaria* and of the other genera were confirmed. All trees showed monophyly for the three major lineages: non-digitate *Laminaria-Cymathaere-Kjellmaniella-Arthrothamnus*, *Eisenia-Ecklonia-Eckloniopsis*-digitate *Laminaria* and *Agarum-Thalassiophyllum-Costaria*, with relatively strong bootstrap support (76-100%), but their branching orders varied partially. In the RuBisCo + ITS-1 analysis, the range of pairwise distances within each clade was 0.000-0.046 within the *Laminaria-Cymathaere-Kjellmaniella-Arthrothamnus* clade, 0.009-0.118 within the *Eisenia-Ecklonia-Eckloniopsis-Laminaria* (*L. yezoensis*) clade, 0.004-0.073 within the *Agarum-Thalassiophyllum-Costaria* clade, 0.039 within *Alaria* and 0.006-0.012 within *Undaria*, and between different clades was 0.116-0.225.

5S rDNA Spacer Analysis

Tandemly repeated arrays of 5S rDNA genes are found in all the Japanese species of non-digitate *Laminaria, Cymathaere* and *Kjellmaniella* that are very closely related by RuBisCo spacer and ITS-1 analyses. The sequence of the 5S rDNA spacer was more variable than those of the RuBisCo spacer and the ITS-1. The nucleotide length of the spacer region between the 5S rDNA repeats in the 14 species examined was 171-254 bp in total. Twenty-one patterns of nucleotide sequences were found for 58 individuals as shown in Table 3.

The alignment included 293 sites, of which 85 were variable and 52 were parsimony informative when gaps were treated as missing. Using gap mode, there were 177 variable and 109 informative sites. An unrooted distance tree (Fig. 2a) showed similar topology with the strict consensus MP tree. In these trees, the non-digitate *Laminaria-Cymathaere-Kjellmaniella* clade was separated into two distinct lineages with 100% bootstrap value: one includes *L. japonica, L. religiosa, L. ochotensis, L. diabolica, L. longipedalis, L. angustata* and *L. longissima*; the other includes *L. coriacea, L. cichorioides, L. sachalinensis, L. yendoana, C. japonica, K. gyrata* and *K. crassifolia*, except for one individual of *L. longissima* from Akkeshi. Moreover in the former lineage, a cluster containing five species (*L. japonica, L. religiosa, L. ochotensis, L. diabolica* and *L. longipedalis*) diverged from the other two species (*L. angustata* and *L. longissima*) supported by relative high (77-90%) bootstrap values. The range of pairwise distances between individuals in the cluster of five species was extremely narrow (0.000-0.012) compared to intraspecific values within *L. coriacea* (0.000-0.099) and *L. cichorioides* (0.023-0.046).

Discussion

For Japanese laminarialean algae, two taxonomic systems, Setchell and Gardner (1925) and Okamura (1936), are currently accepted. These systems were based on traditional morphological investigations, and the most noticeable difference between them was whether Alariaceae was recognized or not. However Kawashima (1989b) pointed out several problems in these earlier taxonomic treatments based on his morphological work. He suggested a new system as follows: Japanese advanced kelps may be divided into two families, the

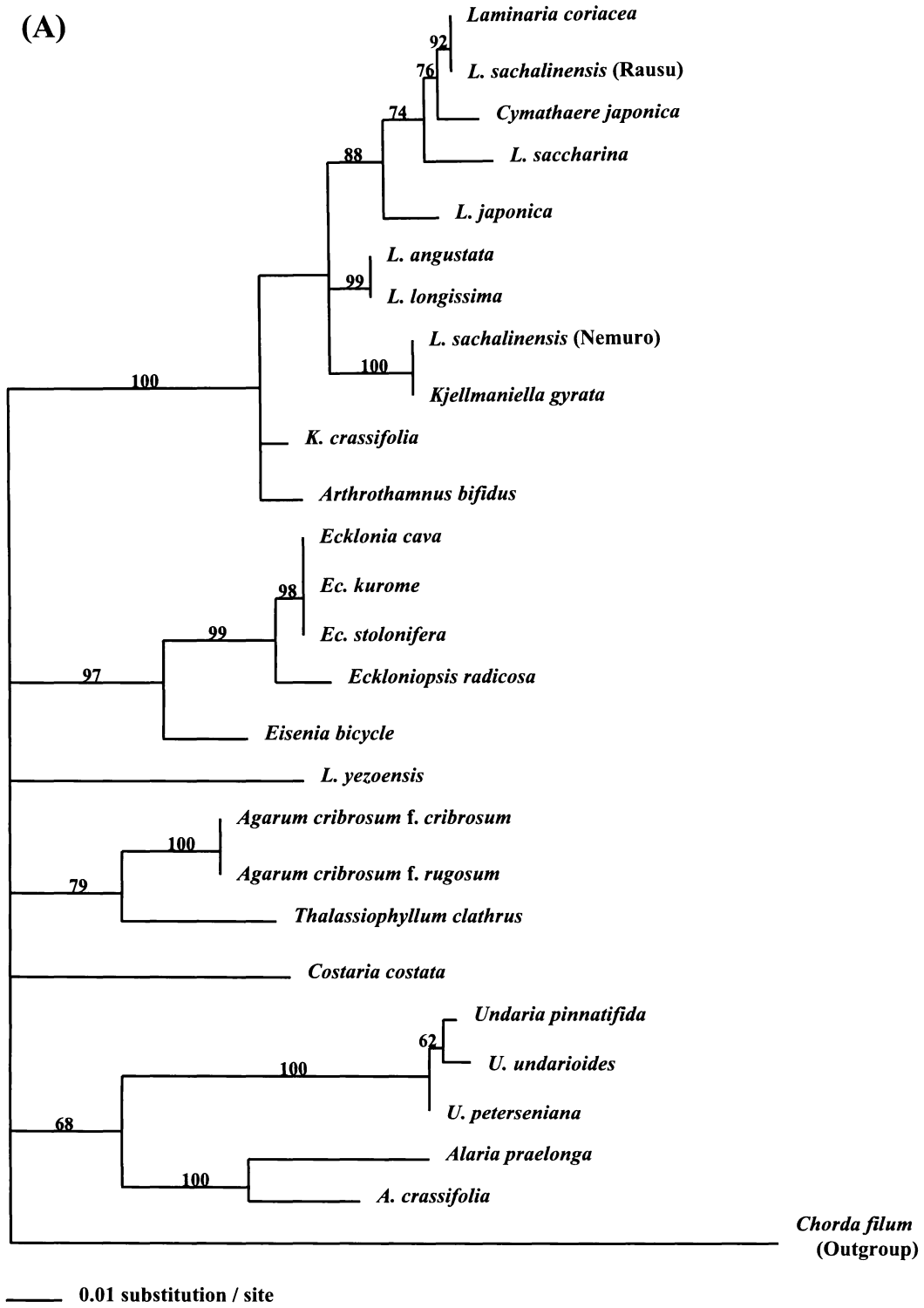

Fig. 1. Distance trees using Neighbor-joining (NJ) method based on RuBisCo spacer (A), internal transcribed spacer (ITS)-1 (B) and combined RuBisCo spacer and ITS-1 (C) sequence data. The number at each node indicates bootstrap values (1000 replicates, given if >50%).

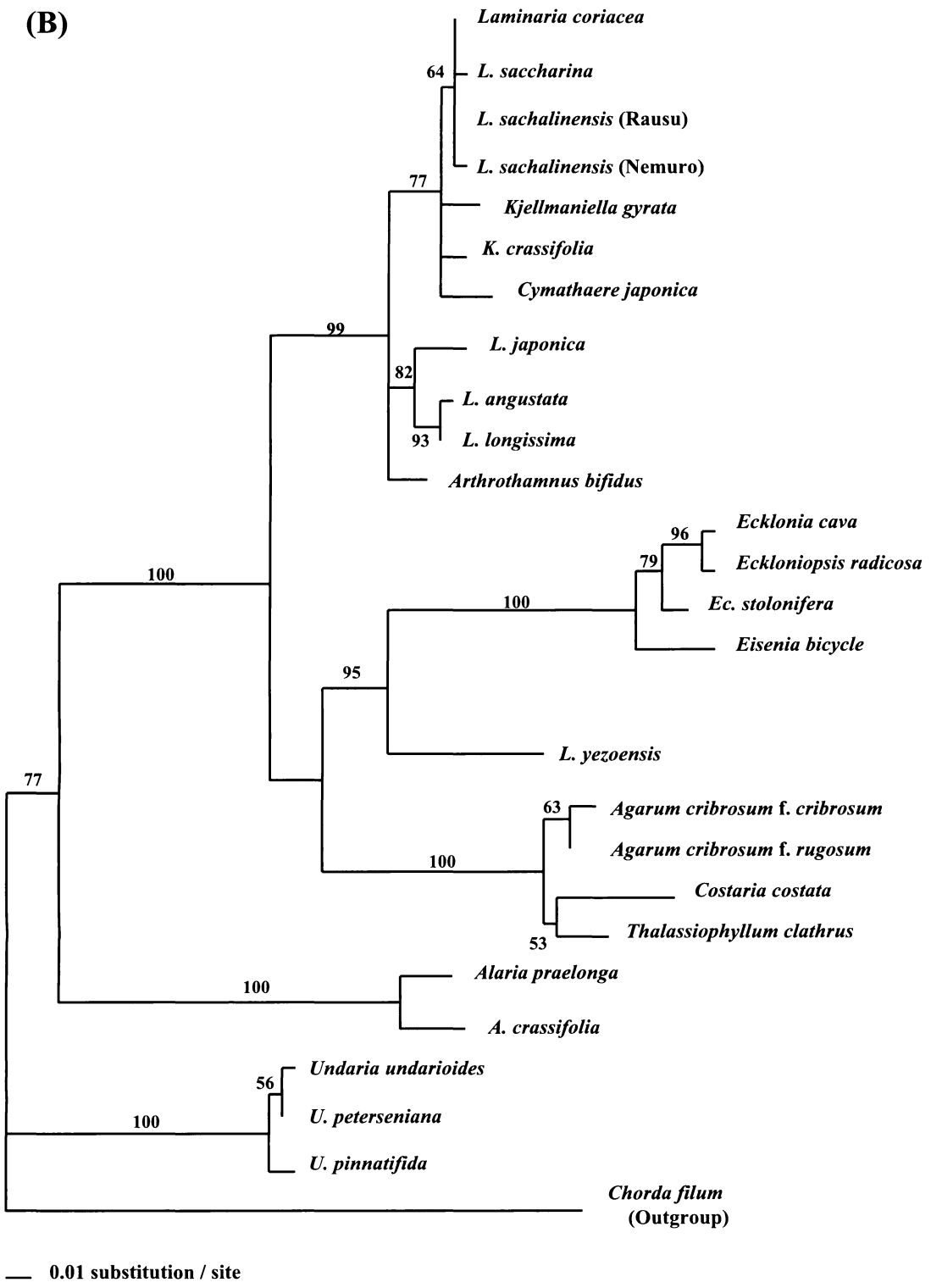

Fig. 1. Continued.

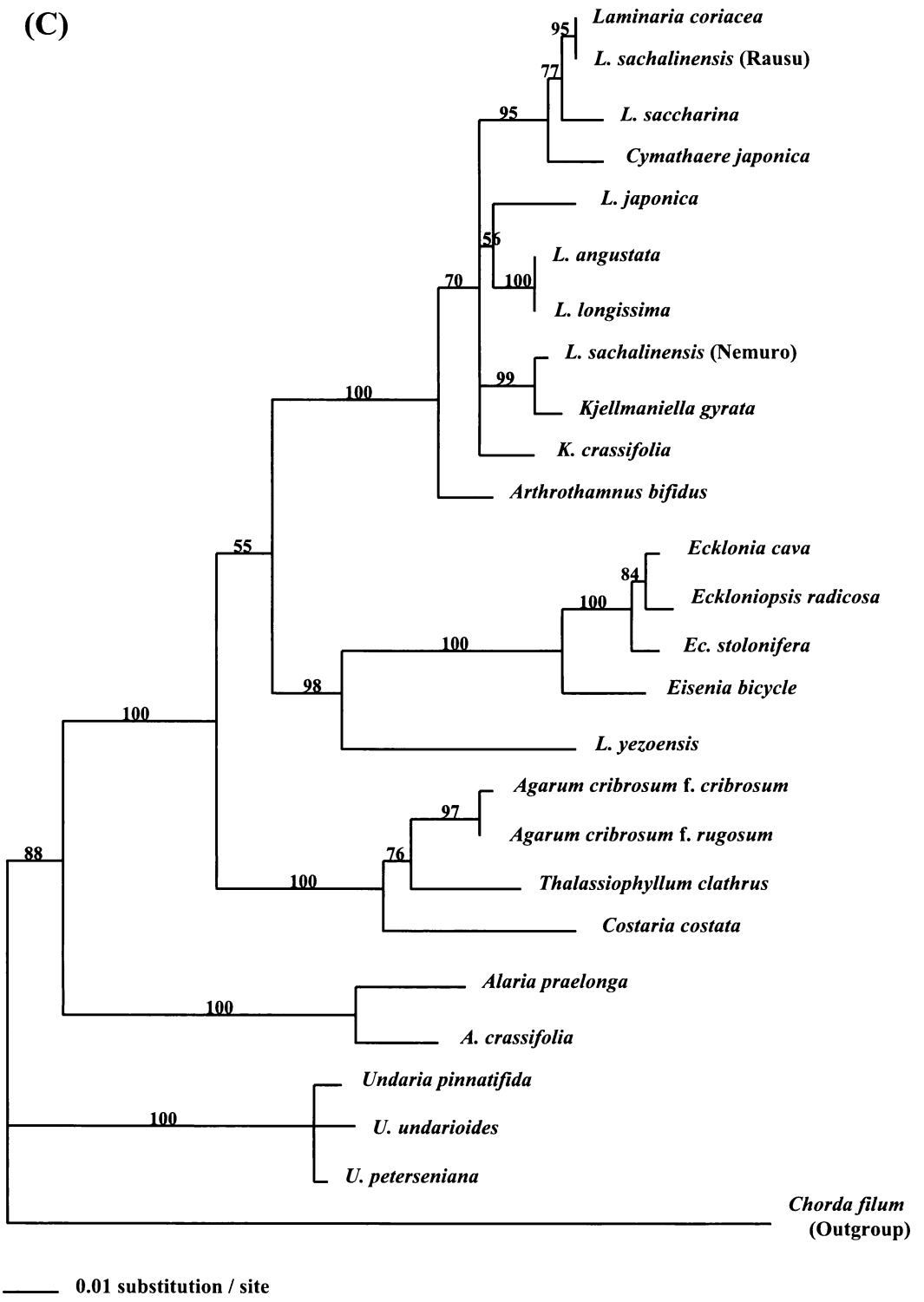

Fig. 1. Continued.

Table 3. Twenty-one patterns of 5S rDNA spacer nucleotide sequence for 59 individuals in *Laminaria*, *Cymathaere* and *Kjellmaniella*.

Laminaria japonica (Ch) 247 bp	
L. japonica (Ka) 252 bp	*L. japonica* (Es-1)
L. japonica (Es-2) 248 bp	*L. japonica* (Es-5, Fr), *L. religiosa* (Ot-4, Os, At-1, 2, 3, 4, 5), *L. ochotensis* (Wa-1, 3, 4), *L. diabolica* (Ra, He-1, 2, 3, 4, 5, Ak), *L. longipedalis* (Ac), *L. longissima* (Ak-3)
L. japonica (Es-3) 252 bp	*L. japonica* (Es-4), *L. religiosa* (Ot-2)
L. japonica (Fk) 252 bp	*L. ochotensis* (Wa-2, 5, So)
L. japonica (Mi) 232 bp	*L. religiosa* (Ot-5, Mi)
L. religiosa (Ot-1) 254 bp	
L. religiosa (Ot-3) 232 bp	
L. religiosa (Is) 252 bp	
L. angustata (Ch) 233 bp	*L. longissima* (Ha, Ak-1, 2, 5)
L. angustata (Uj)	
L. coriacea (To) 206 bp	*L. coriacea* (Ak-1, 2, 3), *L. longissima* (Ak-4)
L. coriacea (Ak-4) 203 bp	
L. cichorioides (Sa-1) 199 bp	
L. cichorioides (Sa-2) 191 bp	
L. cichorioides (Sa-3) 171 bp	*Cymathaere japonica* (Ra) 228 bp
L. sachalinensis (Ra) 213 bp	*Kjellmaniella gyrata* (Ak) 218 bp
L. yendoana (Oi) 222 bp	*K. crassifolia* (Ch) 213 bp

Laminariaceae and Alariaceae. The Laminariaceae may be divided into two subfamilies; Laminariae including *Laminaria, Kjellmaniella, Cymathaere, Agarum* and *Costaria*, and the Thalassiophylleae including *Arthrothamnus, Hedophyllum, Eisenia, Ecklonia* and *Eckloniopsis*. The Alariaceae includes *Alaria* and *Undaria*. Through the present study, I suggest that Japanese advanced kelps belong to the following five major lineages; (1) non-digitate *Laminaria-Cymathaere-Kjellmaniella-Arthrothamnus*, (2) *Eisenia-Ecklonia-Eckloniopsis*-digitate *Laminaria*, (3) *Agarum-Thalassiophyllum-Costaria*, (4) *Alaria*, and (5) *Undaria*. Each lineage is characterized morphologically by (1) simple blade with recognized median fascia, (2) pinnae or digitate blade, (3) holes on the phylloplane, (4) pea pod-like sporophyll on the stipe, and (5) folded sporophyll on the stipe. Accordingly the taxonomic system proposed by Kawashima (1989b) may be emended as follows. A new subfamily Agarumeae including *Agarum, Thalassiophyllum* and *Costaria* should be recognized in the Laminariaceae. In addition, *Arthrothamnus*, where early branch formation is different from that of *Eisenia* in bearing the auriculate outgrowth, should be transferred to the subfamily Laminarieae. Digitate *Laminaria*, where the phylloplane is different from that of non-digitate *Laminaria* in bearing splits and no median fascia, should be transferred to the subfamily Eisenieae [= Thalassiophylleae in Kawashima (1989b)]. The systematic position of *Hedophyllum* remains a subject for future study. Nevertheless, there is an alternative way of classification such that each of the five lineages (1)-(5) may be treated as a tribe of the single family Laminariaceae. In any event, whether the presence or absence of the derivative sporophyll seems to be a useful feature to classify laminarialean families, but the propriety must be discussed taking into consideration the characteristic of the other foreign family, Lessoniaceae, in ALL complex in the future (c. f., Saunders and Druehl, 1993, Yoon *et al.*, 2001).

As for non-digitate laminarian species, al-

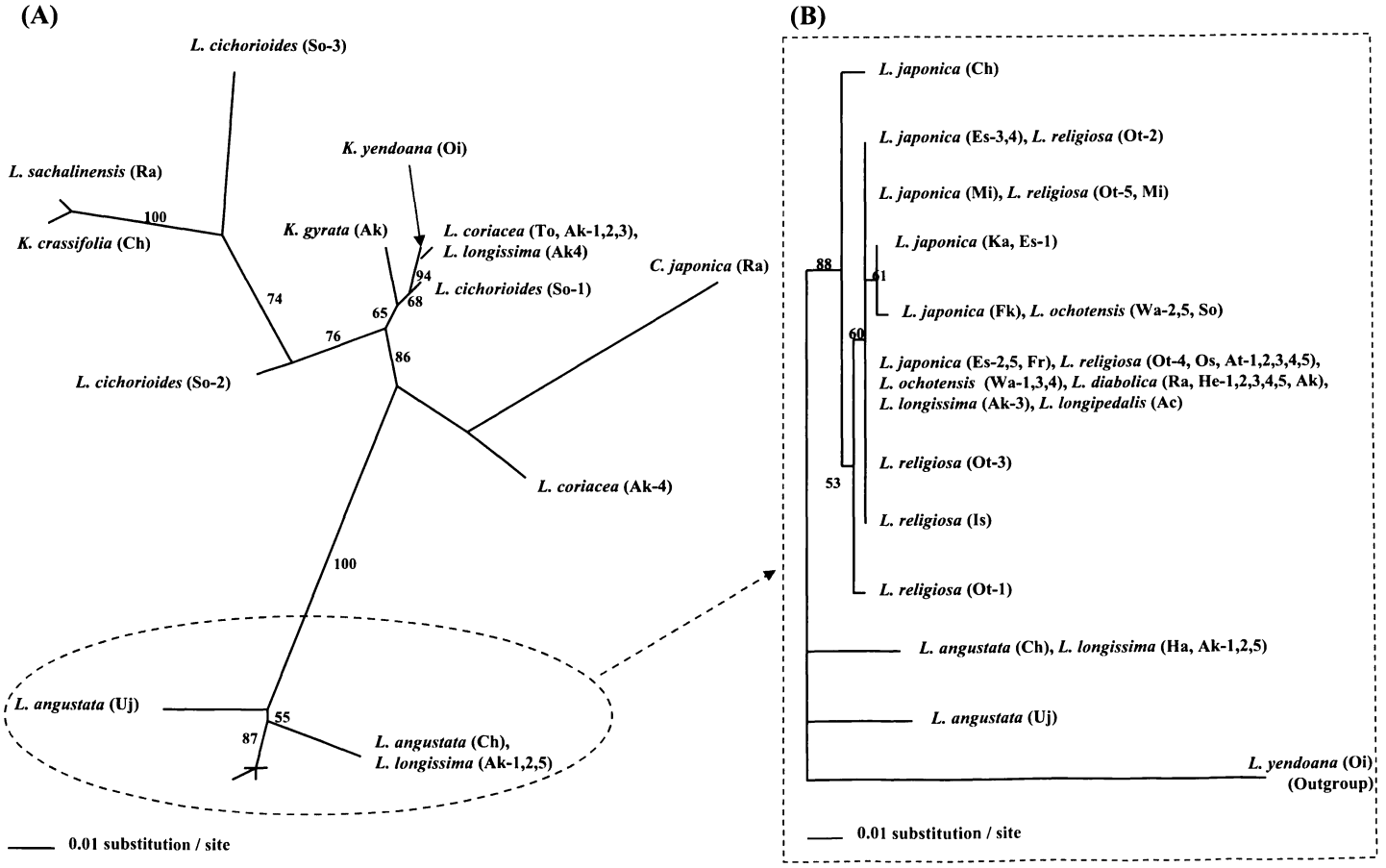

Fig. 2. Distance trees using Neighbor-joining (NJ) method based on 5S rDNA sequence data. (A) Unrooted phylogram for *Laminaria, Cymathaere* and *Kjellmaniella.* (B) Detail of *Laminaria japonica-L. religiosa-L. ochotensis-L. longipedalis-L. angustata-L. longissima* clade. The number at each node indicates bootstrap values (1000 replicates, given if >50%).

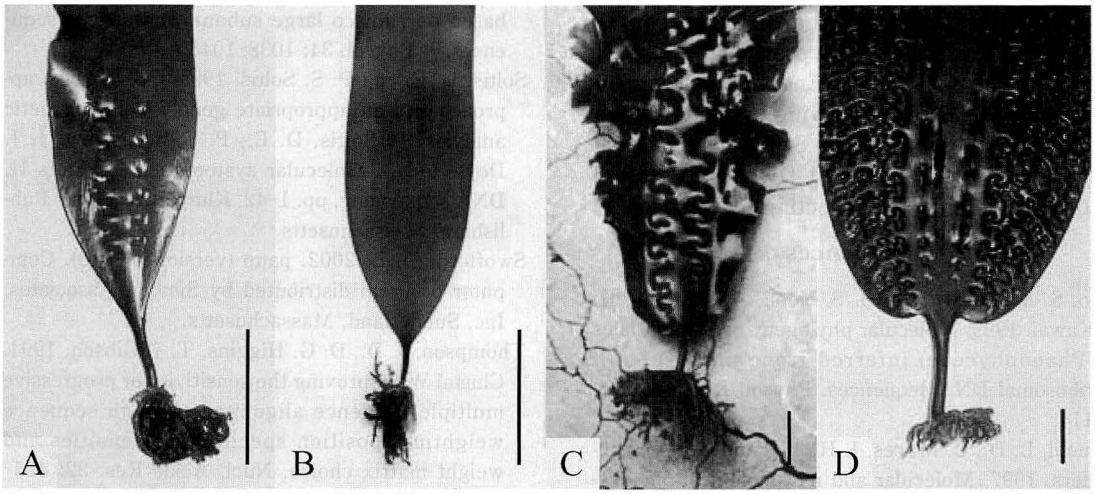

Fig. 3. Bullation or gyration ornamented on the phylloplane. (A) : Bullation of *Laminaria japonica* which disappears with growth. (B) : No bullation or gyration in *L. angustata*. (C) : Bullation of *L. sachalinensis* which remains to maturity. (D) : Gyration of *Kjellmaniella crassifolia* which remains to maturity. Scale=5cm.

though genetic differences among the five species *L. japonica, L. religiosa, L. ochotensis, L. diabolica* and *L. longipedalis* and among the three species *L. coriacea, L. cichorioides* and *L. yendoana* could not be detected in either the RuBisCo spacer or the ITS-1 sequences, differences could be detected in the 5S rDNA spacer sequences which were more variable than those of ITS for land plants (Soltis and Soltis, 1999). The spacer analysis could divide non-digitate *Laminaria* into three sublineages which were contained within two major lineages, although they were divided into two groups by previous study (Yotsukura *et al.*, 1999). Miyabe (1902) classified Japanese laminarian algae into very closely related genera based on several morphological features of their holdfasts, stipes and lamina. However, almost all these features are unstable, depending on their growing environments. Within the three sublineages containing *Kjellmaniella* found in the 5S rDNA spacer analysis, one stable feature seems to be confined to the ornaments on the blade (bullation and gyration). That is (a) bullation which disappears with the progress of their growth (*L. japonica, L. religiosa, L. ochotensis, L. diabolica* and *L. longipedalis*) [Fig. 3 (A)], (b) no bullation in their lifetime (*L. angustata* and *L. longissima*) [Fig. 3 (B)], (c) bullation or gyration which remains to maturity (*L. coriacea, L. cichorioides, L. yendoana, K. gyrata* and *K. crassifolia*) [Fig. 3 (C, D)]. As for *C. japonica*, although a close phylogenetic relationship is presumed to be with the last group, the genetic distance is not particularly close and no bullation (gyration) is seen on the phylloplane. Gyration, not bullation, is thought to be one remarkable feature of *Kjellmaniella* that may divide this genus from *Laminaria*, but it seems that there is no phylogenetic evidence for the origins of bullation and gyration. Thus a reexamination of taxonomic relationships of *Laminaria* and *Kjellmaniella* is required.

In this 5S rDNA spacer analysis, each cluster does not always reflect the taxonomic category and the growing locality within one species. Especially in the *L. japonica-L. religiosa-L. ochotensis-L. diabolica-L. longipedalis* lineage, the situation is puzzling. Any genetic distances within this lineage are extremely short compared to those within each species of *L. coriacea* and *L. cichorioides*. These five species are distributed continuously at the coast of Hokkaido, and thus it is presumed that gene flow is occurring at present. Yabu (1964) asserted that *L. japonica, L. religiosa, L. ochotensis* and *L. diabolica* were identical because cross-fertilization for their reciprocal combinations was possible. The results of the present study strongly support this hypothesis.

Acknowledgments

I would like to thank Professor A. W. Coleman (Brown University) for critically reading

the manuscript. I also thank Professor T. Ichimura (Hokkaido University) and Dr. T. Kawai (Hokkaido Nuclear Energy Environmental Research Center) for giving kind advice and useful comments, and Dr. S. Kraan (National University of Ireland) and Dr. A. Murakami (Kobe University) for collecting materials.

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(Manuscript received 20 January 2003: accepted 28 March 2003.)

日本に生育する高等コンブ類(褐藻綱・コンブ目)の分子系統

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日本で生育が知られている高等コンブ類（チガイソ科・コンブ科）について，ルビスコ遺伝子のスペーサー領域（RuBisCo spacer），およびリボソーム RNA 遺伝子（rDNA）の転写スペーサー領域（ITS-1）の塩基配列比較による系統推定を行った。その結果，解析を行った12属は，（1）単葉状コンブ属－ミスジコンブ属－トロロコンブ属－ネコアシコンブ属，（2）アラメ属－カジメ属－アントクメ属－掌状コンブ属，（3）アナメ属－キクイシコンブ属－スジメ属，（4）アイヌワカメ属，（5）ワカメ属，の5つの系群に分かれることが示された。一方，RuBisCo spacer，ITS-1 でともに変異の乏しい単葉状コンブ属，ミスジコンブ属，トロロコンブ属は，5S rDNA のスペーサー領域（5S rDNA spacer）に基づく解析により，葉面に現れる凹凸にそれぞれ共通した特徴を持つ3つの系群：（a）マコンブ－ホソメコンブ－リシリコンブ－オニコンブ－エナガコンブ，（b）ミツイシコンブ－ナガコンブ，（c）ガッカラコンブ－チヂミコンブ－エンドウコンブ－（アツバスジコンブ）－トロロコンブ－ガゴメ，に分けられた。特に“（a）”内の遺伝的距離は著しく小さく，構成種の分布域が連続していることやこれまでの交雑実験の結果をふまえ，この系列の中で遺伝子流動が起こっている可能性は高い。