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Confirmation on Taxonomic Status of *Spatoglossum pacificum* Yendo (Dictyotaceae, Phaeophyceae) Based on Morphology and Plastid Protein Coding *rbcL*, *rbcS*, *psaA*, and *psbA* Gene Sequences

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Phenological, morphological and molecular characteristics of *Spatoglossum pacificum* Yendo are examined. *S. pacificum* has an annual life cycle composed of sporophytes with apparent absence of male and female gametophytes in Korea. The seasonal growth of this species explains that the annual growth is closely related to the monthly variation of water temperature. *S. pacificum* has protruding reproductive structures above the outmost cortical layer. Although this observation is restricted to several species, reproductive structures on the thallus can make *S. pacificum* distinguishable from *S. crassum* and *S. lactum*. The morphogenesis of a midrib at the base of *S. pacificum* in this study is the same as those of *Dictyopteris* but different from those of *S. crassum* and *S. lactum*, suggesting that *S. pacificum* is closely related to *Dictyopteris*. In the comparison of plastid gene sequences among species of *Spatoglossum* and *Dictyopteris*, *S. pacificum* is more similar to *D. divaricata* and *D. undulata* than those of *S. crassum* in *rbcL*, *rbcS*, *psbA* and *psaA*. This result is congruent with the anatomical characteristic of a midrib at the base of the thallus and the protrusion of reproductive organs on the thallus. The phylogenetic relationship based on these plastid genes also shows that *S. pacificum* is included in Dictyotpteris clade and separated from *S. crassum*. We propose the new combination of *Dictyopteris pacifica* (Yendo) I.K. Hwang, H.S. Kim *et* W.J. Lee, comb. nov. based on the differences of anatomical characteristics of the midrib, the existence of reproductive organs on thallus and the molecular analyses.

Key Words: brown algae, *Dictyopteris pacifica* (Yendo) comb. nov., *psaA*, *psbA*, *rbcL*, *rbcS*, *Spatoglossum*

INTRODUCTION

Spatoglossum pacificum Yendo (1920) is found in the West Pacific, especially in Korea and Japan, and is known to share the common morphological characteristics with other *Spatoglossum* species, such as a polystromatic thallus, a small group of meristematic cells in a line at the apex and the absence of a distinct midrib (Tanaka 1991, 1992; Lee and Lee 1996; Lee and Bae 2002). However, this species is distinguished from the closest related species *S. crassum* Tanaka and *S. lactum* Tanaka. They have thin blades, turf phaeophycean hairs and reproductive organs protruding from the cortex (Tanaka 1991, 1992). These reproductive characteristics have also found among the species of genus *Dictyopteris* (Philips 2000) so that there are no reliable characteristics to confirm the taxonomic status of *S. pacificum* except for the absence of a prominent midrib. However, it is very difficult to describe the range of a partial prominent midrib on the old thallus, which makes identification difficult. In their description on morhpology, Lee and Lee (1996) suggested that *S. pacificum* had distinct partial midrib on the lower part of the thallus and their taxonomic status should be reexamined. The partial plastid *rbcL* and 18S *rRNA* gene sequences separated *Spatoglossum* species from *Dictyopteris* but *S. pacificum* is more closely related to the species of *Dictyopteris* than to *S. crassum* (Lee and Bae 2002). There may be need to confirm the taxonomic criteria among species and genera of *Dictyopteris* and *Spatoglossum*.

We confirm the taxonomic status of *Spatoglossum pacificum* based on its morphological and phenological characteristics and the plastid gene sequences of the *rbcL*, *rbcS*, *psaA* and *psbA* gene in this study.

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Table 1. The list of materials and accession number of nucleotide sequences determined and used in these analyses

Species Name	Collection site	GenBank Accession Number		
		rbcL	*psaA*	*psbA*
Dictyopteris divaricata	Korea, Anin, Gangwondo, 19 Jun 2000, I.K. Hwang	AY422676	AY422600	AY422638
D. divaricata	Korea, Manripo, Chungcheongdo, 15 Aug. 1997, I.K. Hwang	AY430320	AY430303	AY430340
D. divaricata	Korea, Huksando, Jeolamando, 14 July 1999, I.K. Hwang	AY430321	AY430304	AY430341
D. divaricata	Korea, Jindo, Jeolanamdo, 20 July 1998. W.J. Lee	AY430322	AY430305	AY430342
D. divaricata	Korea, Anin, Gangwondo, 18 Oct. 1997, W.J. Lee	AY430323	AY430306	AY430343
D. divaricata	Hoshina, R. (2002) unpublished	AB096891	-	-
D. latiuscula	Korea, Haegumgang, Gyoungsangnamdo, 14 July 2000, I.K. Hwang	AY422677	AY422601	AY422639
D. latiuscula	Korea, Haegumgang, Gyoungsangnamdo, 14 July 2000, I.K. Hwang	AY430328	AY430310	AY430347
D. latiuscula	Korea, Sangjokam, Gyoungsangnamdo, 6 Aug. 1998, I.K. Hwang	AY430329	AY430311	AY430348
D. latiuscula	Korea, Sangjokam, Gyoungsangnamdo,15 April 2000, W.J. Lee	AY430330	AY430312	AY430349
D. polypodioides	Hoshina, R. (2002) unpublished	AB096892	-	-
D. prolifera	Korea, Guryoungpo, Gyoungsangbukdo, Korea	AY430324	AY430307	AY430344
D. prolifera	Korea, Gampo, Gyoungsangbukdo, 21 Nov. 2002, W.J. Lee	AY430325	AY430308	AY430345
D. prolifera	Korea, Seongsan, Jejudo, Feb. 2003 W.J. Lee	AY430326	AY430309	AY430346
D. prolifera	Korea, Hachuja, Jejudo, 6 Aug. 2001, I.K. Hwang	AY422678	AY422602	AY422640
D. prolifera	Korea, Hachuja, Jejudo, 6 Aug. 2001, I.K. Hwang	AY430327	AY528444	AY528440
D. prolifera	Hoshina, R. (2002) unpublished	AB096893	-	-
D. undulata	Korea, Seongsan, Jejudo, 20 July, 2001, I.K. Hwang	AY430331	AY528445	AY430350
D. undulata	Korea, Guryoungpo, Gyoungsangbukdo, 19 Aug. 1996, I.K. Hwang	AY430332	AY528446	AY430351
D. undulata	Korea, Huksando, Jeolanamdo, 18 Aug. 2000. W.J. Lee	AY430333	AY528448	AY430352
D. undulata	Hoshina, R. (2002) unpublished	AB096894	-	-
D. undulata	Korea, Seongsan, Jejudo, 15 Aug. 2001, W.J. Lee	AY430334	AY528447	AY430353
Distronium decumbens	Korea, Guryoungpo, Gyoungsangbukdo, 23 Aug. 2001, I.K. Hwang	AY422683	AY422607	AY422645
Padina crassa	Japan, Ishigaki Island, 21 Jan. 1998, W.J. Lee & J.H. OaK	AY422681	AY422605	AY422643
Spatoglossum crassum	Korea, Anin, Gangwondo, 23 Aug. 2001, I.K. Hwang	AY430335	AY430313	AY430354
S. crassum	Korea, Anin, Gangwondo, 23 Dec. 1998, I.K. Hwang	AY430336	AY430314	AY430355
S. crassum	Korea, Dolsando, Jeolamando, 5 July 2001, I.K. Hwang	AY422679	AY422603	AY422641
S. crassum	Hoshina, R. (2002) unpublished	AB096909	-	-
S. pacificum	Korea, Dolsando, Jeolamando, 5 July 2001, I.K.Hwang	AY422679	AY422603	AY422641
S. pacificum	Korea, Anin, Gangwondo, 23 Aug. 2001, I.K. Hwang	AY422680	AY422604	AY422642
S. pacificum	Korea, Gampo, Gyoungsangbukdo, 21 Nov. 2002, W.J. Lee	AY430337	AY430315	AY430356
S. pacificum	Hoshina, R. (2002) unpublished	AB096911	-	-
S. pacificum	Hoshina, R. (2002) unpublished	AB096910	-	-
Zonaria desingiana	Korea, Seongsan, Jejudo, 15 Aug. 2001, W.J. Lee	AY422682	AY422606	AY422644
Z. desingiana	Japan, Ishigaki Island, 21 Jan. 1998, W.J. Lee & J.H. OaK	AY527199	AY528451	AY528442
Pylaiella liittoralis	Assali *et al.* (1990)	X55372	-	-
P. liittoralis	Yoon *et al.* (2002)	-	AY119724	AY9760

MATERIALS AND METHODS

Studies on phenology and morphology

To assess the extent of morphological variation of *Spatogolossum pacificum* from Korea, plants were collected from populations located along the east and south coasts of Korea, namely from Gangnung, Samcheok, Guryoungpo, Sacheon and Dolsando for studies on the phenology and plastid gene sequences analyses. Plants for morphological studies were preserved in 5-10% formaldehyde-seawater immediately after collection. Live plants were brought to the laboratory for DNA extraction. In addition, examination of the morphological characteristics was made using adult tetrasporophytes grown under controlled conditions. Microsections were made by a Leica Cryocut 1800 microtome^{TM} (Nassloch, Germany) and stained with 1% aqueous aniline blue solution, and mounted in 1-5% clear corn sugar syrup prior to microscopic examination. Drawings were made using a camera lucida (Nikon 231412^{TM}, Japan). Voucher specimens are preserved in the herbarium of Kangnung National University.

Plastid gene sequence analyses

Taxa selected for molecular analyses were taken from geographic representatives (Table 1). Genomic DNA were extracted from 0.01g of algal powder ground in liquid nitrogen using a DNeasy Plant Mini Kit (Qiagen), according to the manufacturer's instructions or 2X CTAB (2% hexadecyltrimethylammonium bromide) buffer (Doyle and Doyle 1987).

The partial *rbcL* gene, 3'-terminal region of the *rbcL*, with the RuBisCo spacer and 5'-terminal region of *rbcS*, were amplified as three fragments using the primers set DRL1F-DRL1R, DRL2F-DRL2R and DRL3NF-RU4 and sequenced with these primers (Hwang *et al.* 2004a). The partial *psaA* and *psbA* gene were amplified and sequenced using primers *psaA* 130F-*psaA* 970R, *psaA* 870F- *psaA* 1760, and *psbA* F1- *psbA* R1 (Yoon *et al.* 2002).

The amplified DNA was purified using a PCR product purification kit (High Pure PCR Product Purification Kit^{TM} (Roche), in accordance with the manufacturer's instructions. The sequences of the forward and reverse strands were determined for all samples using an ABI PRISM 377 DNA sequencer. The sequences were aligned using PHYDIT (Chun 1995) with final visual confirmation and submitted to GenBank under the accession number shown in Table 1. The alignment of each gene sequence was based on the alignment of the inferred amino acid sequences and reconfirmed by eye.

We also conducted a partition homogeneity test (PHT, Farris *et al.* 1995; Swofford 2003) to determine the appropriateness of the combining of the four genes. We analyzed the combined four genes dataset with PAUP*4.0b10 (Swofford 2003). We used four outgroup species in the phylogenetic analyses: *Distromium decumbens, Padina crassa, Zonaria diesingiana* and *Pylaiella littoralis*.

Maximum parsimony (MP) analysis was made using by a heuristic search algorithm with the following settings: 1,000 random addition sequence, tree bisection-reconnection (TBR) branch swapping, Mul-Trees, all characters unordered and unweighted, and branches with a maximum length of zero collapsed to yield polytomies.

Minimum evolution (ME, Rzhetsky and Nei 1992) trees were inferred using the general time reversible (GTR) model (Rodriguez *et al.* 1990) + the shape parameter of the gamma distribution (Γ) + the partition of invariable site (I) model and GTR+ Γ model (as determined from Modeltest 3.06 (Posada and Crandall 1998). The optimal ME tree was searched by heuristic searches with stepwise addition sequence starting trees and TBR branch swapping. Best-scoring trees were held at each step. The ME methods were used to infer a tree by the same heuristic research conditions stated above.

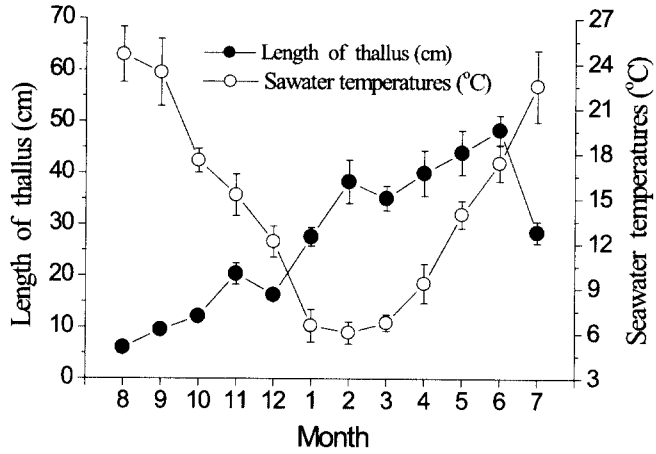

Fig. 1. Variation of seawater temperature and height of *Spatoglossum pacificum* Yendo from Gangnung, Korea.

Maximum likelihood (ML) analysis was conducted using the above GTR+ Γ +I and GTR+ Γ model. Tree likelihoods were estimated using heuristic search with 10 random addition sequence replicates, and TBR branch swapping. The ML bootstrap analyses were conducted with 100 replicates because of high computational demands. Bootstrap values (Felsenstein 1985) were also computed, as implemented in PAUP*, for the MP and ME trees. For bootstrap analysis, 2,000 bootstrap data sets were generated from resampled data (five random sequence additions for parsimony analysis).

RESULTS

Phenology

Plants of *Spatoglossum pacificum* were collected along the east coast of Korea. They grow in cluster on rocks in the lower intertidal and subtidal region. Plants from the east coast are presumed to exhibit asexual reproduction because they have an annual life cycle composed of sporophytes without any male or female gametophytes in the field.

To understand this unusual asexual reproduction plants from Gangnung were monitored monthly from 1994 to 1996 and then every May, July and October until 2003. Throughout the observation period, no sexual plants were encountered. Plants were abundant year-round except for in July and August, when it was difficult to find them (Fig. 1).

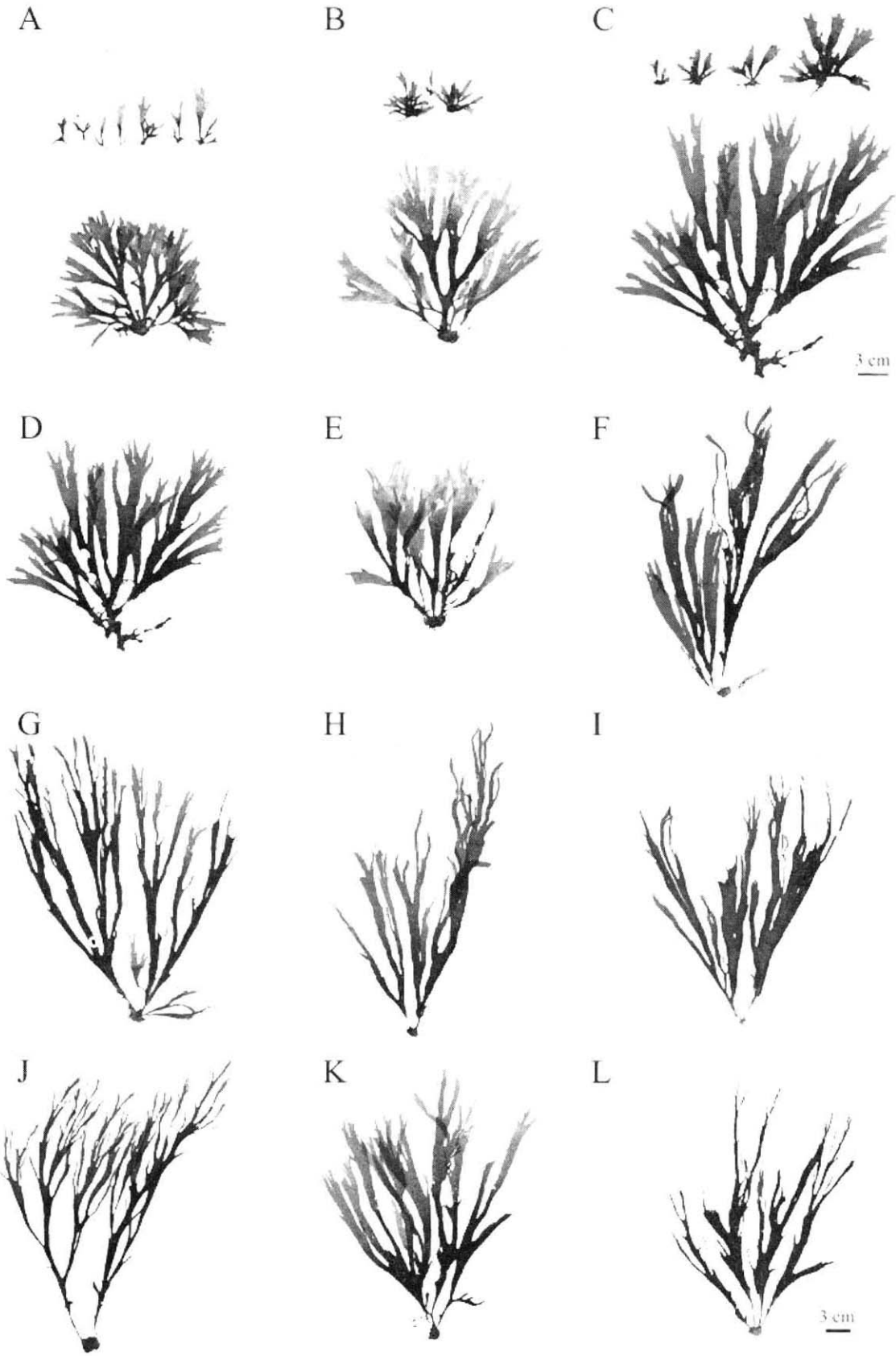

Fig. 2. Habit variation of *Spatoglossum pacificum* Yendo. A-C, plants from August to October; D-F, plants from November to January; G-L, plnats from February to July respectively. Scale bar is the same for all photographs.

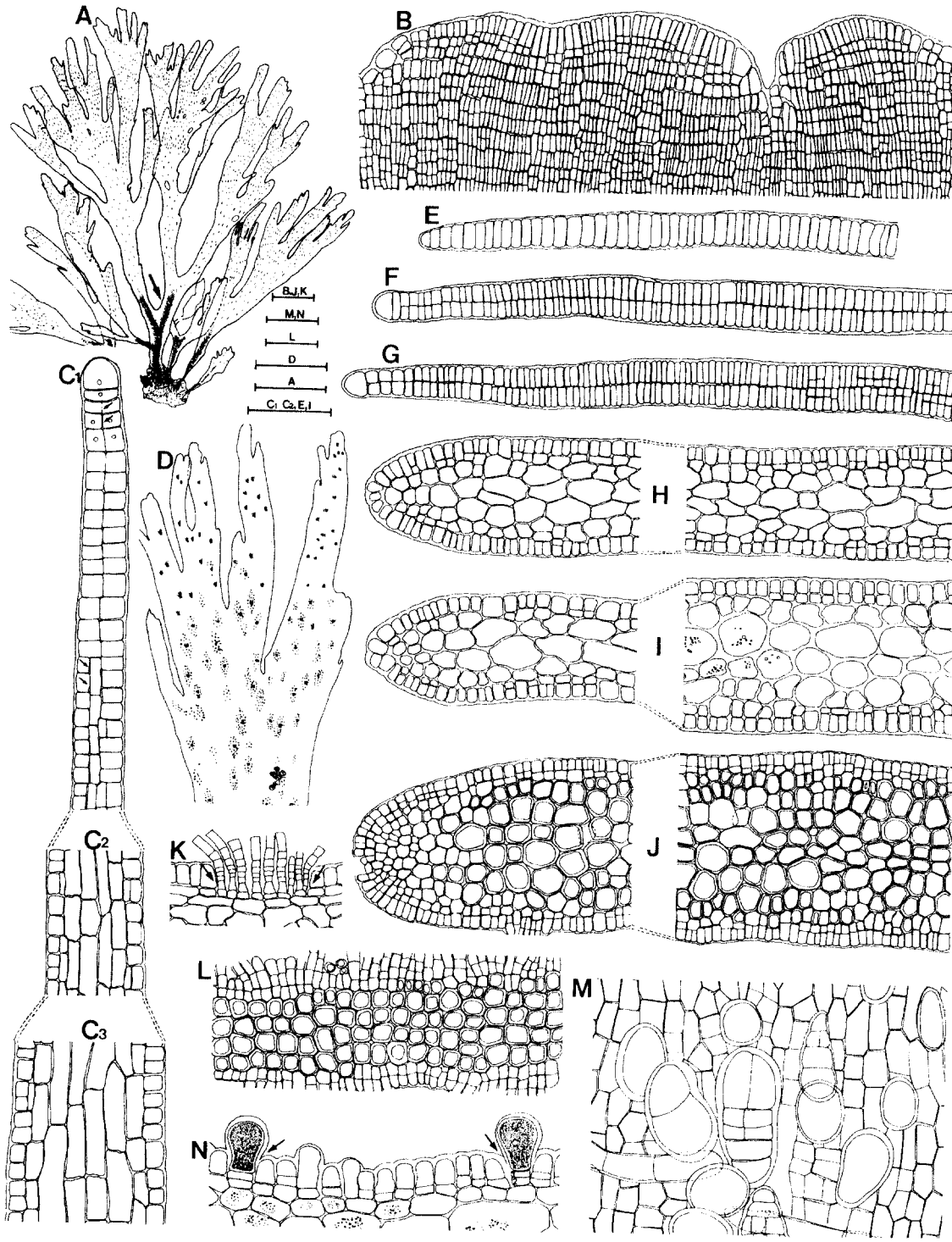

Fig. 3. Characteristics of anatomical structures of *Spatoglossum pacificum*. A, Habit; B, Apical portion; C_1-C_3, Longitudinal section of thallus middle-rib, apical portion (C_1), upper portion (C_2) and middle portion (C_3); E-L, Transverse sections of thallus, apical portion (E-G), 1 cm portion (H) and 4 cm portion (I) from apex, middle portion (H) and basal portion (L); K, Phaeophycean hair pit in transverse section view; M-N, Characteristics of sporangial sturctures, scattered on thallus (M) and transverse section view (N). Scale bars = D, 1 cm; A, 2 cm; B, C1-C3, E-L, 100 μm; M-N, 50 μm.

Juvenile plants can be found in late August and grow to 21 cm in height by early November. The thalli grow to 25-45 cm by the following March and a maximum height of up to 70 cm are observed in June (Fig 2). Most plants grow senile and distorted from early July. Although we can observe monosporangial plants in all seasons, most plants from autumn and winter (from August to December) are non-fertile and develop monosporangia

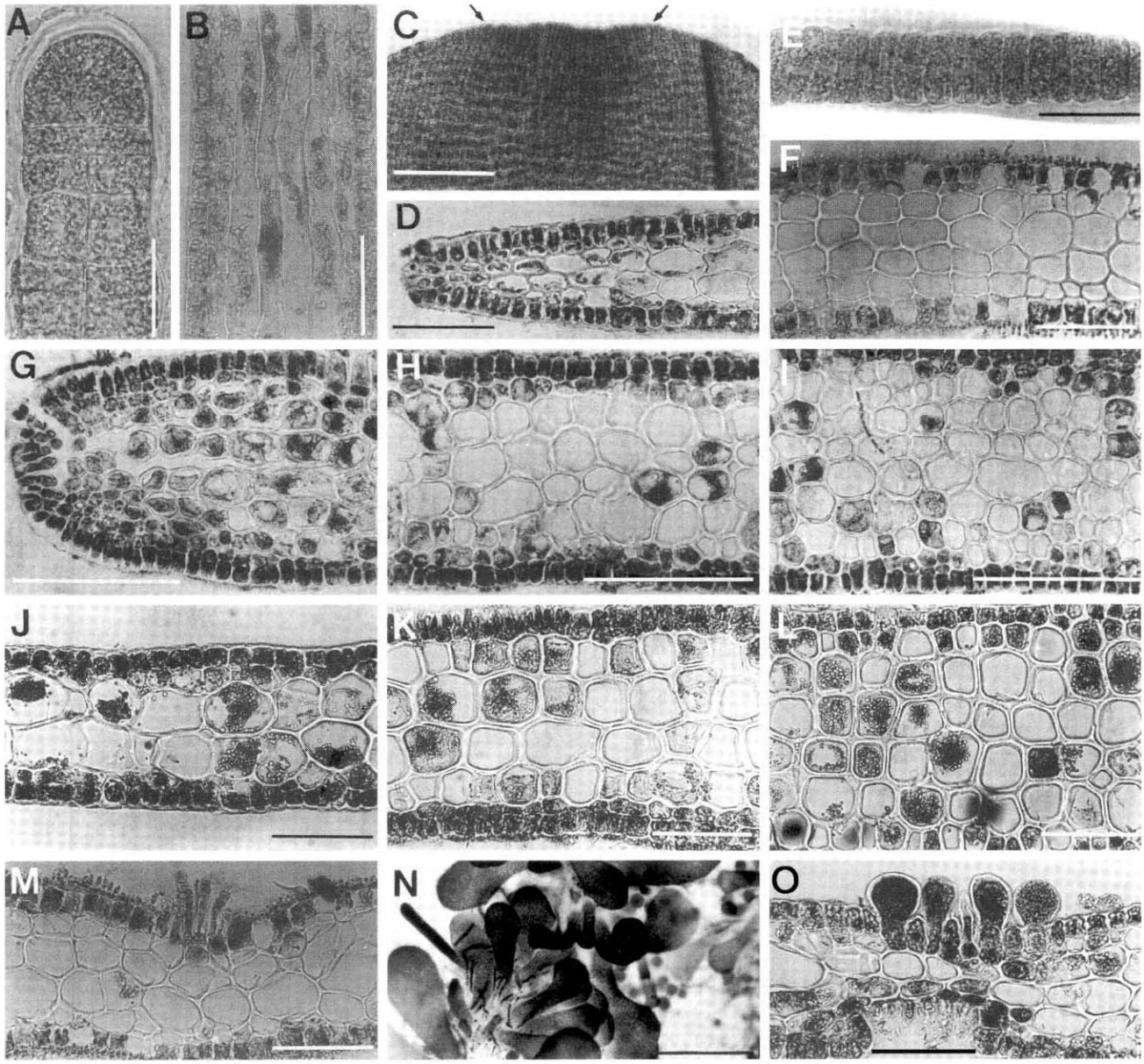

Fig. 4. Characteristics of anatomical structures of *Spatoglossum pacificum*. A-B, Longitudinal section of thallus, apical portion (A), upper portion (B); C, Apical portion of thallus; D-F, Transverse sections, apical portion (E), 1 cm portion (D-F) from apex; G-I, Transverse sections of marginal and middle-rib part on upper portion; J-L, Transverse sections of marginal and middle-rib part (J-L) on basal portion; M, Phaeophycean hair in transverse section view; N, monosporangial germlings *in situ*; O, sporangia on the outmost cortical cells. Scale bars = A-B, 50 μm; C, 450 μm; D-F, J-O, 100 μm; G-H, 200 μm.

in indoor cultures with 15-20°C. Among 27 plants collected in August 1994, most plants are vegetative (70.4%) without any reproductive organs but sporophytes with monosporangia (29.6%). The old sporophytes have numerous monosporangial germlings *in situ* on the surface, which are easily turned off with mild touch and develop into sporophytes in indoor cultures, just like propagules of Sphacelaria.

Gross morphology of thallus

The plants grow in clusters of 5-10 plants and on holdfast of multicellular filamentous rhizoids. The young vegetative plants have yellow brown thalli and thin midribs, but adult plants have dark brown and rudimentary midribs on the lower portion (Fig. 3A). The plants have irregularly bifurcated branches and grow up to 25-70(-80) cm in height and 1.8-2.5 cm in width. No proliferous branches from the midrib are observed. Phaeophycean hairs develop in depressed hair pits

Table 2. Nucleotide composition of four genes and statistics from PAUP analyses of data matrices

	rbcL	*rbcS*	*psaA*	*psbA*	*rbcL*+rbcS+*psaA*+psbA
Number of taxa	26	26	26	26	26
Nucleotides (bp)	1,430	123	1,519	961	4,033
Variable sites (%)	395 (27.62%)	44 (35.7%)	478 (31.4%)	214 (22.2%)	
Informative sites (%)	286 (20.0%)	33 (26.8%)	354 (23.3%)	142 (14.7%)	
Divergence	0.027-0.138	0.023-0.219	0.028-0.177	0.026-0.118	

which are linearly scattered on the thallus.

Anatomical characteristics of thallus

We describe the anatomical characteristics of the thallus based on the longitudinally and transversely sectioned view. The apical meristem is composed of 20-30 rectangular cells 20-62 μm in height and 6-20 μm in width (Figs 3B; 4A). On the longitudinal section of the thallus, apical meristem cells produce subapical cells by unequal cytokinesis and then the subapical cell divides to form a two cell layered thallus around the apical region strains from 7-20 cell rows from the apex (Figs $3C_1$; 5A). Below the two cell layer thallus, these cells are divided into epidermal and internal cells. Epidermal cells become the outmost cortical cell and make periclinal division to form medullae initial cells. Outmost cortical cells repeat transverse and periclinal cell division several times to make medullae cells inward, in increasing the thickness of the thallus (Figs $3C_2$, C_3). After periclinal division of the cortical cell, the inner daughter cell enlarges longitudinally and divides transversely again so that the arrangement of medullae is very irregular (Figs $3C_2$, C_3; 4B). The division pattern of these cells is very similar to meristoderm in Laminariales. So we call this outmost cortical cell layer as meristoderm.

In the transversely sectioned view, the size and the arrangement of medullar cells are variable according to the portion of the thallus. In the upper portion, we can see the meristoderm consisting of one cell layer and medullae 2-4 celled layer 85 μm in thickness (Figs 3H, 4DF). At the middle portion, the meristoderm consists of one cell layer and medullae 5-6 celled layer 238 μm in thickness (Figs 3I, 4G-I). At the lower portion, the meristoderm consistes of 1-2 cell layer and medullae 10-12 celled layer 360 μm in thickness and a very thick cell wall (Fig 3J, 4K, L).

The reproductive structures

We can not find any gametophytes during this study along the coasts of Korea. Sporangia spread across both surfaces of the thallus in clusters around the phaeophycean hair pits. No sporangia are found on the apical, margin or midrib of the thallus. Sporangia develop from the outmost cortical cells (meristoderm), sometimes have one or two stalk cells and protruded out of the cortex (Figs 3N, 4O). Monospores also develop into *in situ* germlings on the thallus surface (Fig. 4N). They fell off easily with a mild touch and developed into sporophytes in indoor cultures.

Molecular characteristics

rbcL sequences determined for 27 taxa are 1430 base pairs (bp) long, this dataset contains 395 variable positions, of which 286 bp (27.6%) are phylogenetically informative. The interspecific "p"-distance values range from 2.71% between *D. divaricata* and *D. undulata* species to 13.84% between *D. divaricata* and *Zonaria diesingiana*, used as a sister group (Table 2). The "p"-distances among *Dictyopteris* from the east and the west coast of Korea range from 2.71% to 9.61%. The "p"-distances between *S. pacificum* and *Dictyopteris* species range from 2.65 to 9.51%. But *S. pacificum* differ from *S. crassum* by 7.41% in the "p"-distances.

Partial *rbcS* sequences are determined for 26 taxa are 123 bp long, this dataset contains 44 variable positions of which 33 bp are phylogenetically informative. Along these *rbcS* sequences, the divergence among the *Dictyopteris* species range from 0.41% between *D. divaricata* and *D. undulata* to 16.26% between *D. divaricata* and *D. latiuscula*. However, the intergeneric "p"-distance values range from 4.88 % between *S. pacificum* and *D. undulata* to 21.95% between *D. undulata* and *Distromium decumbens* used as out-groups.

psaA gene sequences are aligned to 1,519 bp of which 478 nucleotide sites (31.4%) are variable and 354 (23.3%) parsimony informative (Table 2). The intraspecific "p"-distance values range from 2.89% between *D. latiuscula* and *D. prolifera* to 11.32% between *D. undulata* and *D. prolifera*. The intergeneric "p"-distance values range from 5.55% between *S. pacificum* and *D. undulata* to 17.71% between *Distromium decumbens* and *Zonaria desingiana*.

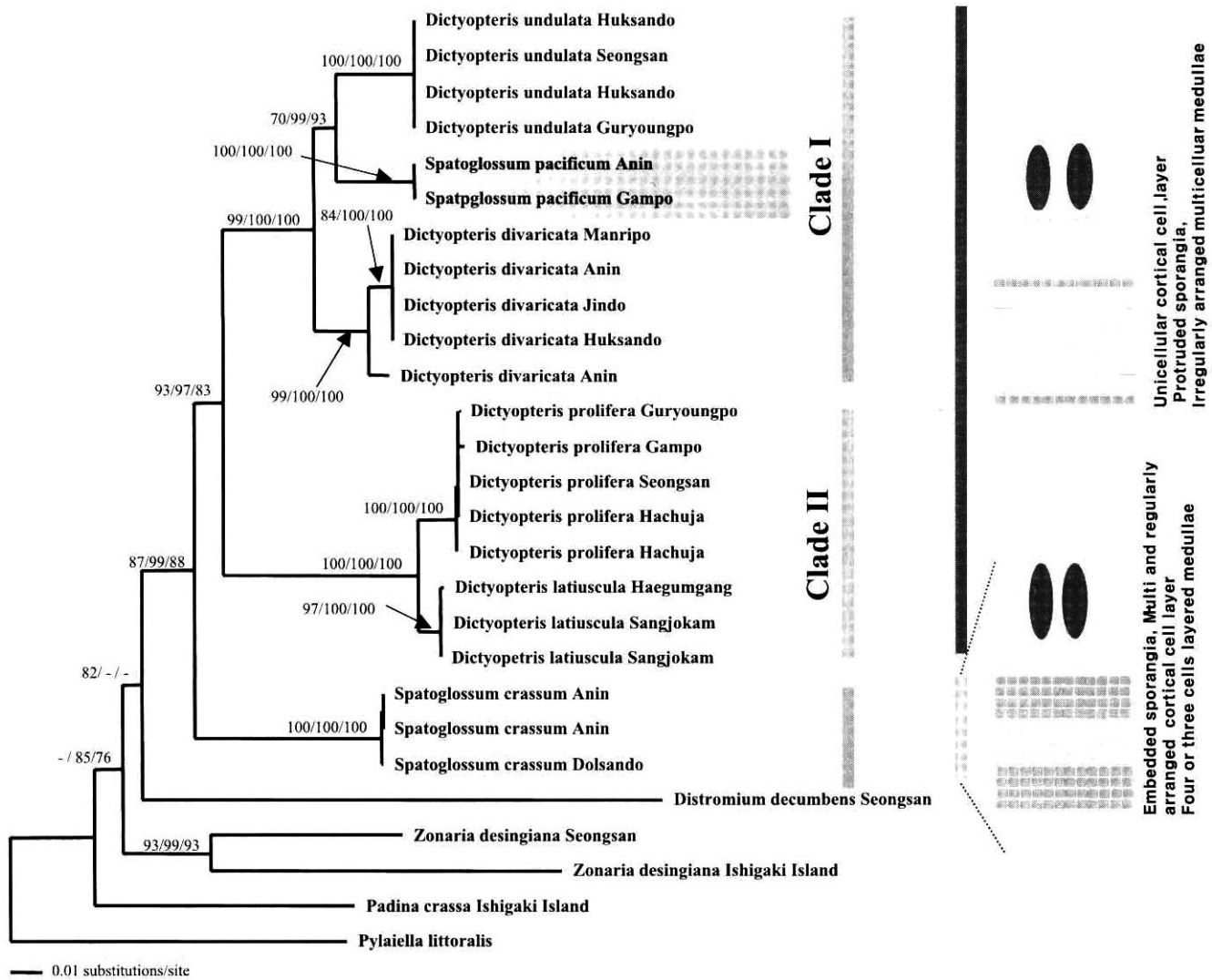

Fig. 5. Maximum likelihood tree for *Spatoglossum* and related taxa estimated from the plastid encoded *rbcL*, *rbcS*, *psaA*, and *psbA* combined sequences data [GTR+Γ+I model, -Log likelihood = 15974.96: A-C, 2.3969; A-G, 6.3162; A-T, 4.2920; C-G, 1.6717; C-T, 14.1794; G-T, 1; Γ, 0.9333; I, 0.5602; different nucleotide frequencies (A = 0.290, C = 0.1563, G = 0.2063, T = 0.3474)]. The bootstrap values shown on the branches (ML/MP/ME) from 2,000 (ME, MP), 100 (ML) resampling.

But *S. pacificum* differed from *S. crassum* by 11.25% in the "p"-distances.

psbA sequences are 961 bp long, this dataset contains 214 variable positions (22.2%), of which 142 (14.7%) were parsimony informative. The interspecific "p"-distance values in *Dictyopteris* range from 2.60% between *D. divaricata* and *D. undulata* to 7.49% between *D. latiuscula* and *D. prolifera*. The intergeneric "p"-distance values range from 3.32% between *S. pacificum* and *D. divaricata* to 11.7% between *Distromium decumbens* and and *D. undulata*. But *S. pacificum* differed from *S. crassum* by 6.67% in the "p"-distance values.

Phylogenetic relationships

The partition homogeneity test revealed congruence between the data sets (p = 0.655) (*rbcL* + *rbcS* + *psaA* + *psbA*). We, therefore, combine the four plastid genes in the phylogenetic analyses. A total of 4030 nucleotides from 27 taxa are used in the phylogenetic analyses. The ML topology of the combined data set is shown in Fig. 5. MP and ME analysis is not significantly different from the ML analysis. *Distromium* is a sister taxa to the monophyletic clade of *Spatoglossum-Dictyopteris* group with low bootstrap support. The *Dictyopteris* species are divided into two clades (Fig. 5): clade I (with bootstrap support over 99%) includes *D. undulata*, *D. divaricata* and *Spatoglossum pacificum*. Clade II includes *D. prolifera* and *D. latiuscula*. *Spatoglossum crassum* is separated from the *Dictyopteris* clade.

The topology of the combined data sets also is

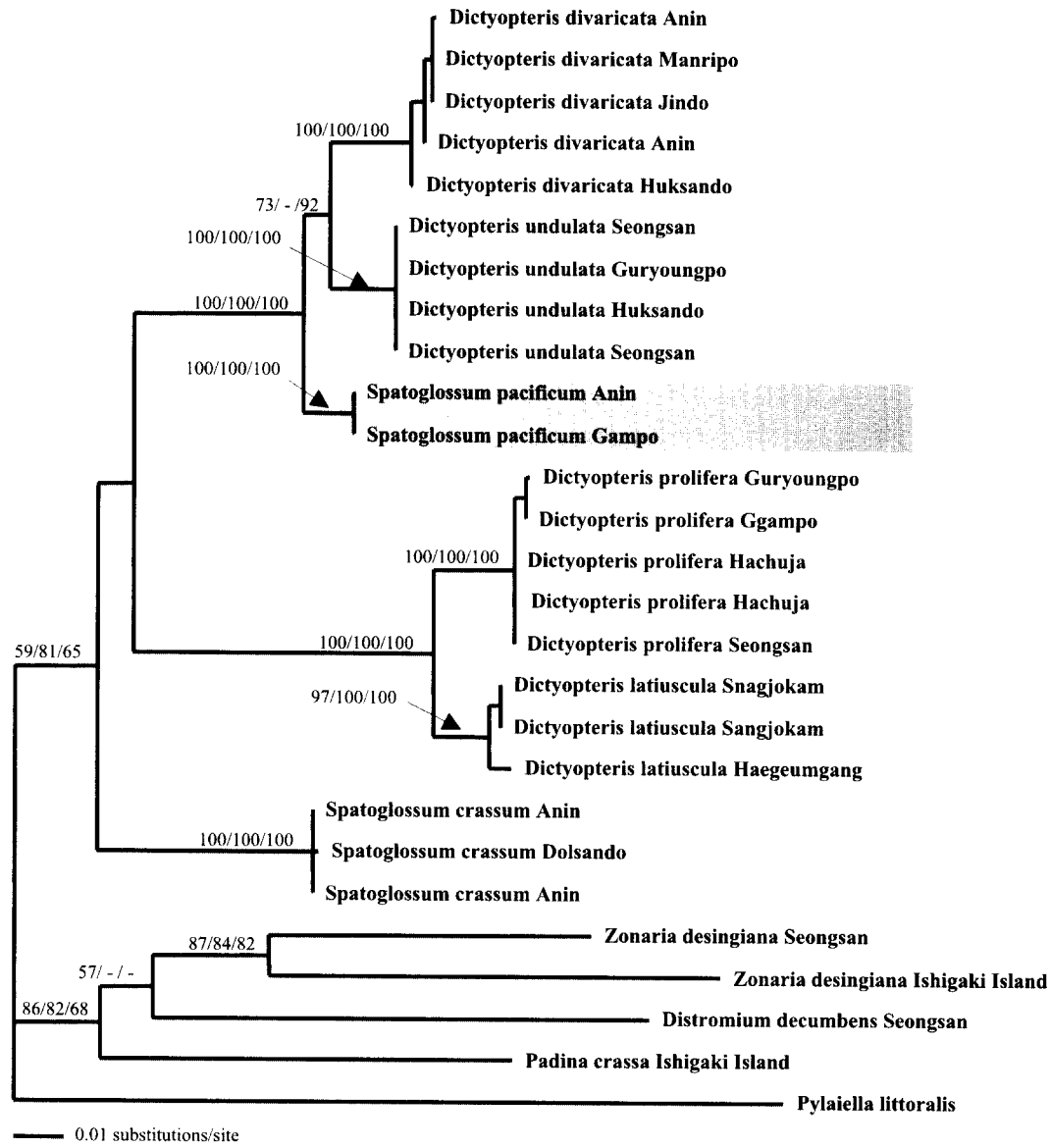

Fig. 6. Maximum likelihood trees for *Spatoglossum* and related taxa estimated from the plastid encoded gene sequences. A: based on *rbcL* (GTR+Γ model, -Log likelihood = 5469.94: A-C, 1.4285; A-G, 4.2255; A-T, 3.9058; C-G, 1.3051; C-T, 9.2059; G-T, 1; Γ, 0.2009), B: based on *psaA* (GTR+Γ+I model, -Log likelihood = 15974.96: A-C, 5.4595; A-G, 9.1355; A-T, 2.2651; C-G, 4.9087; C-T, 22.8868; G-T, 1; Γ, 0.8945; I, 0.5865), C: based on *psbA* sequences (GTR+Γ model, -Log likelihood = 3044.49: A-C, 0.8700; A-G, 3.4280; A-T, 6.00214; C-G, 0.2424; C-T, 13.6216; G-T, 1; Γ, 0.1709). The bootstrap values shown on the branches (ML/MP/ME) from 2,000 (ME, MP), 100 (ML) resampling.

congruent with those based on *psbA*, *bsaA*, and *rbcL* although the *S. crassum* clade makes sister group with *Dictyopteris* by low bootstrap support (Fig. 6A-C). The topologies clearly show that *Spatoglossum pacificum* is included in the *Dictyopteris* clade and separated from *S. crassum*, which agreed with anatomical and reproductive structures.

In the analyses of *rbcL* with other sequences from GeneBank, we distinguish three clades: Clade A; *S. pacificum* makes a clade with *D. divaricata* and *D. undulata* with high bootstrap support. Clade B; only *S. crassum* is involved and grouped as sister clade of clade A with less bootstrap values than others. Clade C; *D. prolifera*, *D. latiuscula*, and *D. polypodioides* is involved with high bootstrap support (more than 99%) (Fig. 8).

DISCUSSION

The plants of *S. pacificum* had been reported from the North Pacific Ocean with little known about their reproduction (Tanaka 1991). During this study, it is observed that plants from the east coast of Korea have an

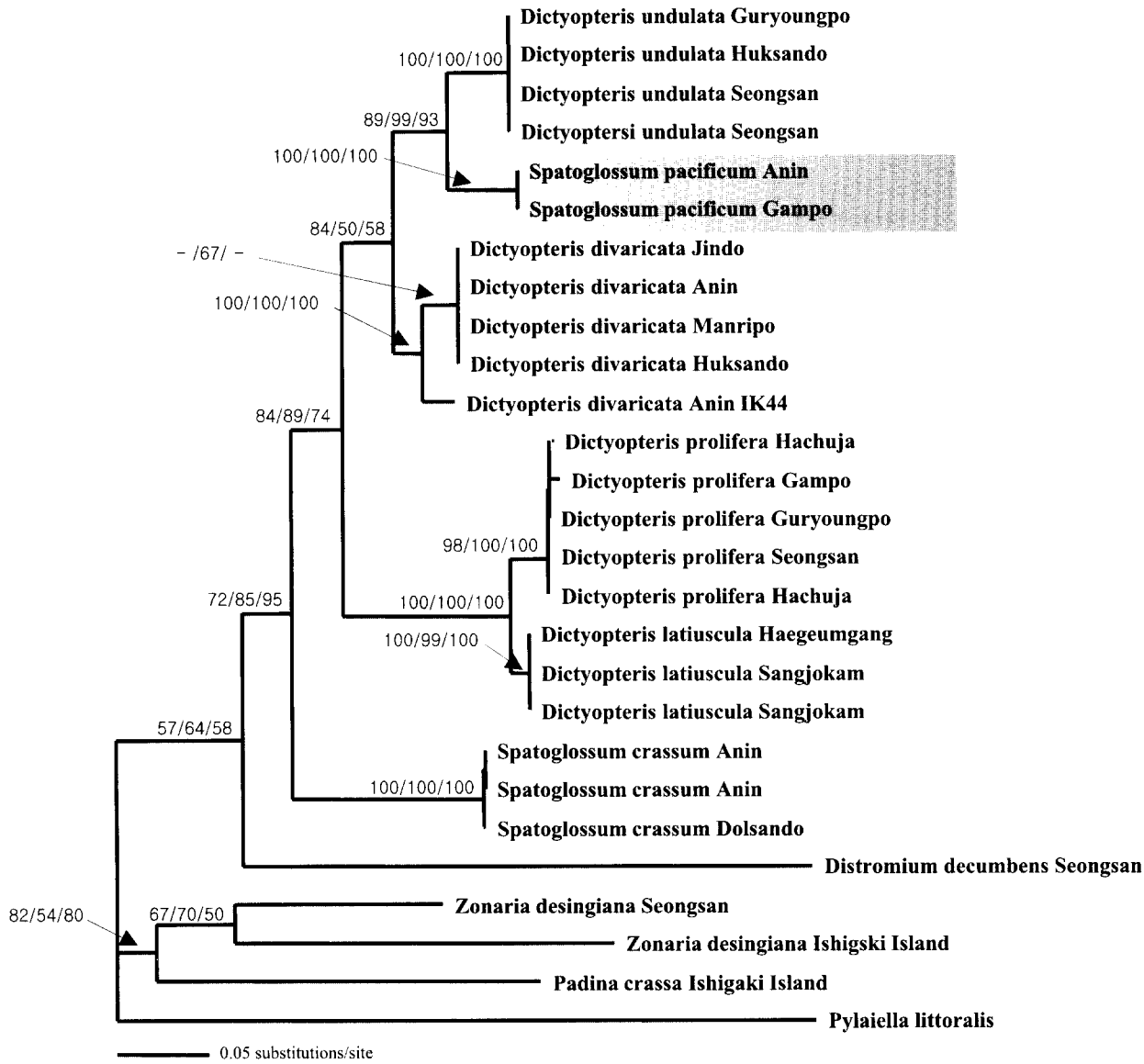

Fig. 6. (continued)

annual life cycle composed of monosporangial sporophytes without any male or female gametophytes in the field. But Tanaka (1991) described the three reproductive organs, sporangia, oogonia, and antheridia, from plants collected from Shirahama, Japan in June. We summarize the phenology of *S. pacificum* in Gangnung as follows. Juvenile plants observed in late August grow up to adult plants with monosporangia by late the following January. Most plants grow senile and distorted from July. They may spend the high water temperature period from July to September as sporophytic germlings, and then may grow up to young thallus in late October or November. The seasonal growth of this species explains that the annual growth is closely related to the monthly variation of water temperature. They start to grow in a period of decreasing water temperature (at 12-15°C in December) and sustain high growth rate below 10°C (in January to April). Their growth rate slows down during the period of increasing water temperature (in May to July). They become senile very fast and the upper part of thallus falls off after the water temperature reach over than 20°C (in late July). The seasonal growth pattern of *S. pacificum* in Korea indicates that it now adapt to more temperate climates. But we cannot elucidate why they have no gametophyte on the coast of Korea.

The embedment of reproductive structures was described as a major characteristic of generictype of *Spatoglossum, S. solierii* (Chauvin *ex* Montagne) Kützing (Hamel 1939) with the absence of a prominent midrib. This characteristic is also found in three species, *S.*

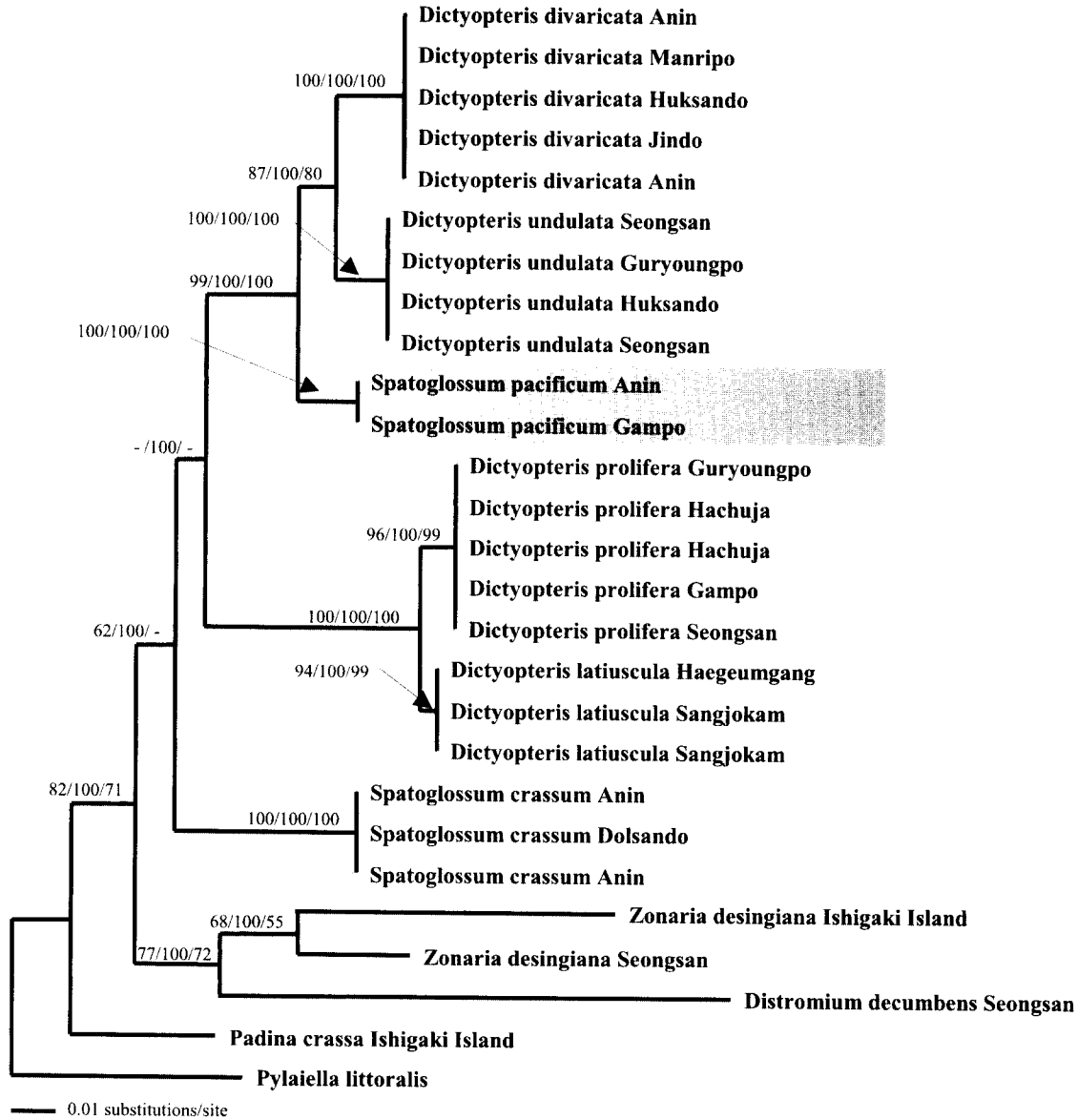

Fig. 6. (continued)

macrodontum (Allender and Kraft 1983; Farrant and King 1989), *S. latum* Tanaka (Tanaka 1992) and *S. crassum* (Tanaka 1991). But *S. pacificum* has protruding reproductive structures above the outmost cortical cells. Moreover, the protrusion of reproductive structures can be found in most species of *Dictyopteris* including generic type, *D. polypodioides* (De Candolle) Lmouroux. However, the protrusion of antheridia, oogonia and sporangia out of the cortex is found in both *Spatoglossum* and *Dictyopteris* so that there is no reliable characteristic to distinguish between the two genera except for the absence of a midrib or the partial prominent midrib on the old plant of *Spatoglossum* (Lee and Bae 2002).

The morphogenesis of midrib at the base of *S. pacificum* in this study is the same as those of *Dictyopteris* but different from those of *S. crassum* and *S. lactum*, suggesting *S. pacificum* is closely related to *Dictyopteris*. *S. crassum* has an outmost cortical cell layer (meristoderm), which is originated from the apical cell, make linear and regular arranged cortical cells inward by several periclinal cell divisions (Hwang *et al.* 2004b). But *S. pacificum* has the inner daughter cell from periclinal division of the cortical cell, which enlarges longitudinally and divides transversely again so that the arrangement of medullae is very irregular as in *Dictyopteris* species, *D. prolifera*, *D. latiuscula*, *D. divaricata*, and *D. undulata*. Although this observation is restricted to several species, this morphogenesis of midrib at the base will be good criterion to distinguish among species of *Dictyopteris* and *Spatoglossum* with the status of reproductive organs on

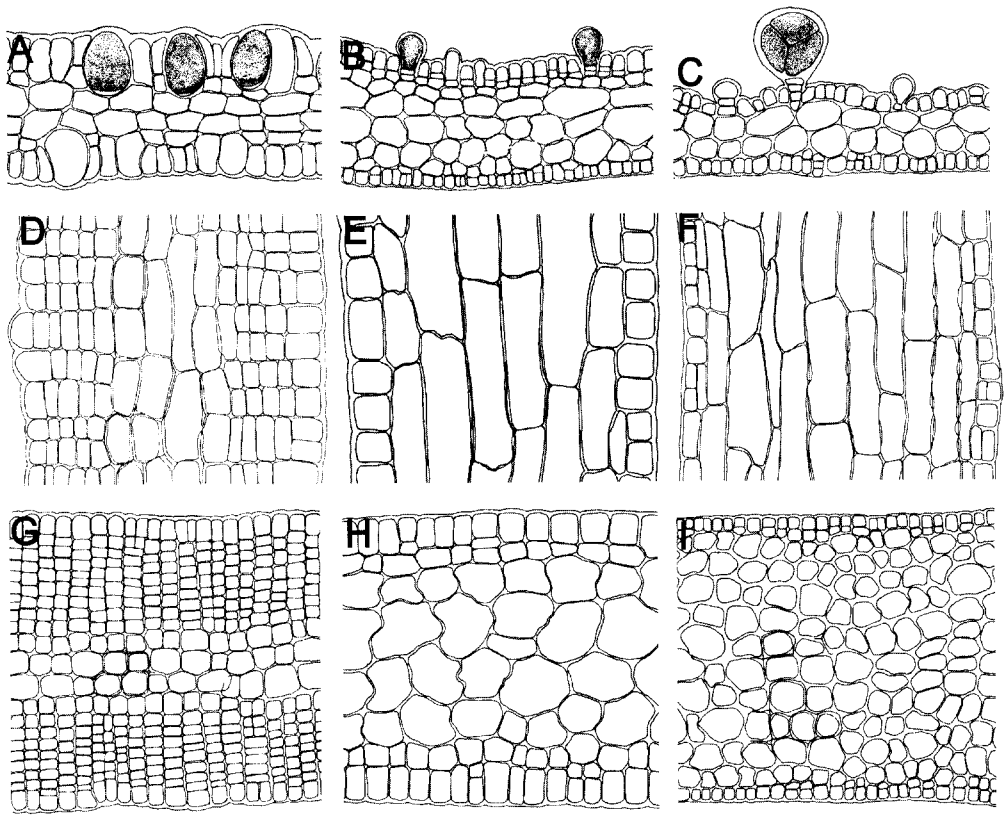

Fig. 7. Comparisons of anatomical characteristics among species of *Spatoglossum* and *Dictyopteris*. A, D, G, *S. crassum*; B, E, H, *S. pacificum*; C, F, I, *D. divaricata*; sporangia (A-C); longitudinal section of thallus (D-F); transverse section of thllaus (G-I).

the thallus (Fig. 7).

In the comparison of plastid gene sequences among *Spatoglossum* and *Dictyopteris*, *S. pacificum* is more similar to *D. divaricata* and *D. undulata* than to *S. crassum* in all compared genes, *rbcL*, *rbcS*, *psbA* and *psaA*. It is congruent with the anatomical characteristic of a midrib at the base and protrusion of reproductive organs on the thallus. The phylogenetic relationship among them based on these plastid genes also say that *S. pacificum* was included in the *Dictyopteris* clade and separated from *S. crassum*. These conclusions were also strongly supported by the analyses of *rbcL* with other sequences of *Dictyopteris* and *Spatoglossum* from GenBank.

In conclusion, the phylogeny based on the plastid gene argues against the taxonomic status of *Spatoglossum pacificum* (Tanaka 1991). Major conclusions are: 1) Species of *Spatoglossum* and *Dictyopteris* are monophyletic but the taxonomic criteria among them should be revised. 2) *S. pacificum* is closely related with *Dictyopteris*. We transfer *Spatoglossum pacificum* Yendo to *Dictyopteris* Lamouroux. This transfer is based upon differences of anatomical characteristics of the midrib, the status of reproductive organs on the thallus and molecular analyses of this species presently included in *Dictyopteris*.

***Dictyopteris pacifica* (Yendo) I.K. Hwnag, H.S. Kim & W.J. Lee, comb. nov.**

Basionym: *Spatoglossum pacificum* Yendo (Bot. Mag. Tokyo 34: 1-12)

The thallus of this species that we transfer to *Dictyopteris* has no prominent but rudimentary midrib on the lower part of the thallus. The midrib has been known as a major characteristic of *Dictyopteris*, but rudimentary midribs are also discerned at the base of *Spatoglossum crassum*. However, there are big differences in the morphogenesis of midribs. It may be better criterion than the absence or presence of midrib to distinguish among two genera. Although there is difference in the status of reproductive organs on the thallus between *Spatoglossum crassum* and *Dictyopteris* observed in this study, these two types have both been found in *Spatoglossum* and *Dictyopteris* (Allder and Kraft 1983; Phillips 2000). This characteristic for definition of genera should be examined as to whether it is suitable or not in more species.

Molecular analyses based on plastid gene sequence demonstrated that two evolutionary clades may exist

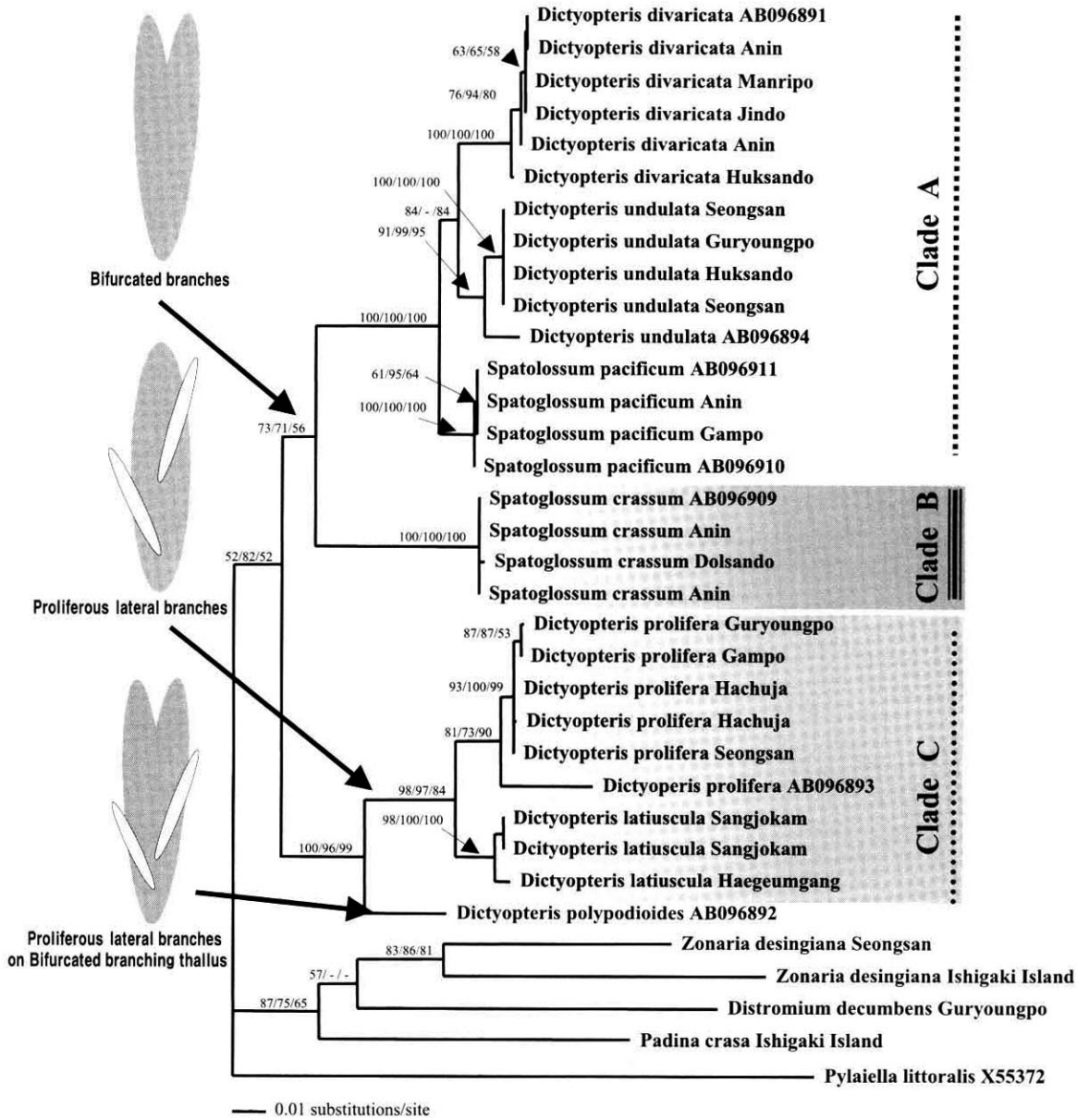

Fig. 8. Maximum likelihood tree for *Spatoglossum* and related taxa estimated from the plastid *rbcL* sequences with others from GenBank (GTR+Γ+I model, -Log likelihood = 5835.48: A-C, 1.3161; A-G, 4.4333; A-T, 3.6272; C-G, 1.4644; C-T, 9.6382; G-T, 1; Γ, 1.0623; I, 0.5549). The bootstrap values shown on the branches (ML/MP/ME) from 2,000 (ME, MP), 100 (ML) resampling.

among species of *Dictyopteris* in this study, one clade composed of species with bifurcated branching which resulted from the splitting of the apical meristem and the other composed of the species with proliferous lateral branching which developed adventitiously on the midrib. The first clade included both species of *Dictyopteris* and *Spatoglossum crassum* in addition to *D. pacifica* comb. nov., especially in the *rbcL* gene sequences with others from GenBank (Fig. 8). These findings show that the presence of a distinctive midrib may be a homoplasy among species of *Spatoglossum* and *Dictyopteris*. Although, the development of proliferous lateral branching is various among species of *Dictyopteris*, they have same ontogenetic characteristics. All branches are proliferous with regular intervals in *D. prolifera* (Okamura) Okamura and *D. latiuscula* (Okamura) Okamura. The bifurcated branching is rare and a pair of proliferous lateral branches arises at regular intervals in the two species. However, the proliferous branches arose sparsely on the low portion of thalli with bifurcated branches in *D. polypodioides* (Newton 1931), *D. acrostichoides* (J. Ag.) Bornet (Phillips 2000), *D. australis* (Sonder) Askenasy (Allen & Kraft 1983), and *D. crassinervia* (Zanardini) Schmidt (Allen and Kraft 1983).

Although *D. polypodioides* formed a clade with *D. prolifera* and *D. latiuscula* based on *rbcL* sequences (Fig. 8), the sequences divergence between them is large. These findings show that the taxonomic and phylogenetic revision among the species of *Dictyopteris* based on type of bifurcated or proliferous lateral branches will make good evolutionary history among species with other molecular characteristics.

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