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Morphology and Molecular Phylogeny of *Psilothallia dentata* (Ceramiaceae, Rhodophyta)

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Psilothallia is a ceramiaceous red algal genus that includes three species worldwide: *P. dentata, P. siliculosa,* and *P. striata.* The latter two species are limited to Australian waters, and *P. dentata* occurs in Japan. We here report the detailed morphology of *P. dentata,* and also determined plastid protein-coding *psbA* in *P. dentata* and putative relatives. *Psilothallia dentata* is distinguished by compressed thalli with alternate-distichous determinate branchlets, six periaxial cells, rhizoidal filaments in axes, cystocarps with 7-8 involucral filaments, spermatangia on branched filaments, and tetrahedrally divided tetrasporangia on branched filaments. *Psilothallia dentata* is also unusual in that cystocarps, spermatangial clusters, and tetrasporangial tufts are formed on short adventitious indeterminate branches arising on axils of determinate branchlets. The phylogenetic trees of *psbA* sequences show that *P. dentata* was nested in a monophyletic clade comprising *Ptilota, Neoptilota,* and *Plumaria.* This result suggests that the taxonomic position of *P. dentata* may be transferred from the tribe Rhodocallideae to the Ptiloteae.

Key Words: Ceramiaceae, morphology, phylogeny, *Psilothallia dentata, psbA,* Rhodophyta, taxonomy

INTRODUCTION

Psilothallia is a ceramiaceous red algal genus that includes three species; *P. dentata, P. siliculosa* (Harvey) De Toni, and *P. striata* (Harvey) Schmitz. All of the species occurs in Pacific Ocean. The genus *Psilothallia* is characterized by compressed thalli with alternate-distichous determinate branchlets and 6-10 periaxial cells. *Psilothallia* was segregated from *Ptilota* C. Agardh by Schmitz (in Engler and Prantl 1897) for *P. striata,* having alternate-distichous determinate branchlets. *Psilothallia* was classified in the Dasyphileae (Schimitz in Engler and Prantl 1897; Kylin 1956) because of its having alternate-distichous branchlets. However, recently, it is included in the list of the tribe Ptiloteae (Athanasiadis 1996) or classified in the tribe Rhodocallideae (Womersley 1998) on the basis of 6-10 periaxial cells in axes and tetrasporangia derived from surface cortical cells or borne on branched filaments. Thus, the taxonomic position of *Psilothallia,* based on morphology, is controversial.

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The type species of the genus, *Psilothallia striata,* is distinguished by surface view of branches showing transverse striations corresponding to the larger axial cells, axial filaments producing alternate-distichous determinate branchlets every 2 (-3) axial cells, and tetrasporangia on branched filaments on the adaxial sides of branchlets (Harvey 1855; Schimitz in Engler and Prant 1897; Womersley 1998). *Psilothallia siliculosa* has tetrasporangia formed on filaments of densely branched and siliculose branchlets adaxial on determinate branchlets (Harvey 1855; Womersley 1998). These two species are rarely found in the southeastern Australian waters (Womersley 1998). *Psilothallia dentata* is the last member of the genus. It was described as *Ptilota dentata* based on female and tetrasporangial thalli by Okamura (1892), but transferred to the genus *Psilothallia* by Kylin (1956) on the basis of alternate-distichous branchlets. However, despite the systematic value of the sequences of periaxial-cell formation in *Psilothallia* and related groups (Hommersand *et al.* 1998; Womersley 1998), there have been few studies on the anatomy of *P. dentata.*

Psilothallia dentata is reported to occur in Korea and Japan (Okamura 1892; Lee and Kang 1986; Yoshida 1998). Despite many field expeditions, we didn't collect

the species in Korea. However, during a collection trip to Chiba, Japan, where is the type locality of the species (Okamura 1892; Yoshida 1998), we encountered *P. dentata* predominating in intertidal rocky shore in Choshi, Chiba prefecture in summer, 2004. We here observed details of vegetative and reproductive structures of the species, and then investigated phylogenetic relationships of the species and putative relatives from the north Pacific using *psbA* gene, which encodes the photosystem II thylakoid protein D1. To date, the *psbA* has been analyzed for the genus *Campylaephora* J. Agardh (Seo *et al.* 2003) and the tribe Griffithieae (Yang and Boo 2004) and shown to be a suitable marker for a better understanding of the phylogenetic relationships in ceramiaceous red algae.

MATERIAL AND METHODS

Morphology

Morphological observations were made on specimens preserved in 5% formalin-seawater. Whole-mount and sectioned materials were stained with 1% aniline-blue acidified with a drop of 2% hydrochloric acid. Materials observed were collected in Choshi, Japan (P115 and P437). Photographs were taken with a light microscope (Olymphus, Japan). Voucher specimens were deposited in the herbarium of the Department of Biology (CNUK), Chungnam National University, Daejon, Korea.

DNA extraction, sequencing and phylogenetic analyses

For molecular study, 35 specimens (two in *Psilothallia dentata* and 33 in putative relatives) of the tribe Ptiloteae were collected (Table 1). Three species of the Ceramiaceae were chosen as outgroup from our previous study (Yang and Boo 2003). All thalli, after collection, were sorted carefully under a dissecting microscope in laboratory, dried in air, and preserved with silica gel in the field. The sources of algal material used in this study and the GenBank accession numbers of the sequence are listed in Table 1.

Genomic DNA was extracted from approximately 0.005 g of algal tissue using a DNeasy Plant Mini Kit (Qiagen) or Invisorb Spin Plant Mini Kit (Invitek), according to the manufacturers' instructions. Amplifying (*psbA*-F and *psbA*-R2) and sequencing (*psbA*-F, *psbA*-500F, *psbA*-600R, and *psbA*-R2) primers of the *psbA* are listed in Yoon *et al.* (2002). The *psbA* region from all the samples used were easily amplified and sequenced. The PCR products were purified using a High Pure^{TM} PCR Product Purification Kit (Roche Diagnostics GmbH, Mannheim, Germany), in accordance with the users' guide. Nucleotide sequences were determined for all taxa using an ABI PRISM^{TM} 377 DNA Sequencer (Applied Biosystems, Foster City, CA, USA) at Research Center, Chungnam National University, Daejon, Korea. The forward and reverse sequences for each specimen were edited using the program Sequence Navigator v. 1.0.1 (Applied Biosystems). The alignment of each taxon sequence was based on the alignment of the inferred amino acid sequence and was refined by eye. There were no gaps in our alignments of *psbA*.

Maximum parsimony (MP) tree have been reconstructed using PAUP* 4.0b10 (Swofford 2002). Full heuristic search was carried out with 1,000 replicates, random addition sequences of taxa, keeping best trees only, holding one tree at each step, tree bisection-reconection (TBR) branch swapping, collapsed of zero length branches and MULTREES on. Bootstrap values (Bt_{MP}) were calculated performing 1,000 replicates with following options selected: heuristic search, TBR branch swapping, collapse of zero length branches, random sequence addition with one replicate.

Bayesian analysis were conducted with MrBayes v.3.0b4 (Ronquist and Huelsenbeck 2003) using the general time reversible (GTR) + the shape parameter of the gamma distribution (Γ) + proportion of invariable sites (I) model. This model of sequences evolution was chosen based on results from Modeltest v3.6 (Posada and Crandall 1998). The Akaike information criterion (AIC) selected GTR + Γ + I model as the best-fitting model for the *psbA* data. The GTR rates, the shape parameter of the gamma distribution and proportion of invariable site value were not fixed. For the data matrix, 1.3 million generations were performed with four chains and trees sampled every 100 generations. The burn-in period can be identified graphically by tracking the likelihoods at each generation to determine whether the likelihood values reach a plateau. After of preliminary analyses, a burn-in period of 300,000 generations was determined to be appropriate for the data. The 10,000 trees sampled at stationarity were used to infer the Bayesian posterior probability (Bp). Majority-rule consensus trees were calculated using PAUP*.

RESULTS

Morphology

Thalli of *Psilothallia dentata* reach lengths of about 10

Table 1. List of species investigated in this study

Taxa	Locality	Voucher	GenBank number
Neoptilota asplenioides (Esper) Kylin			
	Russia: Kamchatka: Petropavloski	N4	AY865136
N. hypnoides (Harvey) Kylin			
	Canada: Vancouver Island: Bamfield	P430	AY865137
Plumaria plumosa (Hudson) O. Kuntze			
	England: Isle of Man: Port Erin	P2	AY865138
	England: Devon: Wembury Bay 2	P384	AY865139
	Ireland: Clare: Black Head	P373	AY865140
	Ireland: Galway: Kinvarra	P389	AY865141
	Ireland: Galway: Spiddal 1	P369	AY865142
	Ireland: Galway: Spiddal 2	P370	AY865143
	Scotland: Fife: St. Andrews	P435	AY865144
Psilothallia dentata (Okamura) Kylin			
	Japan: Chiba: Choshi	P115	AY865145
	Japan: Chiba: Nagasaki	P437	AY865146
Ptilota filicina J. Agardh			
	Japan: Hokkaido: Denshinhama 1	P123	AY865147
	Japan: Hokkaido: Denshinhama 2	P124	AY865148
	Japan: Hokkaido: Denshinhama 3	P143	AY865149
	Japan: Hokkaido: Muroran 2	P356	AY865150
	Japan: Hokkaido: Sumiyoshi 1	P402	AY865151
	Japan: Hokkaido: Sumiyoshi 2	P403	AY865152
P. gunneri Silva, Maggs et Irvine *in* Maggs & Hommersand			
	Northern Ireland: Moyle: Cushendun 1	P300	AY865153
	Northern Ireland: Moyle: Cushendun 3	P386	AY865154
	Northern Ireland: Larne: Garron 1	P301	AY865155
	Norway: Song og Fjordane: Florø 2	P433	AY865156
	Norway: Møre og Romdal: Ona 2	P424	AY865157
P. phacelocarpoides A. Zinova			
	Japan: Hokkaido: Kitami	P145	AY865158
	Japan: Hokkaido: Okajima	P126	AY865159
	Japan: Hokkaido: Wakkanai	P154	AY865160
	Russia: Maritime Territory: Nakhodka	P148	AY865161
	Russia: Maritime Territory: Vladivostok	P153	AY865162
P. serrata Kützing			
	USA: Oregon: Boiler Bay	P263	AY865163
	USA: Oregon: Seal Rock	P268	AY865164
	USA: Oregon: Yaquina Bay	P155	AY865165
	USA: Oregon: Strawberry Hill 1	P128	AY865166
	USA: Oregon: Strawberry Hill 2	P265	AY865167
	USA: Washington: Libbey Beach 1	P146	AY865168
Ptilota sp.			
	Korea: Gangreung: Anmok	-	AY865169
	Korea: Gangreung: Sageunjin	P405	AY865170
Outgroup			
Antithamnion nipponicum Yamada et Inagaki		A28	AY295157[1]
Ceramium kondoi Yendo		C169	AY178486[1]
Griffithsia corallinoides (Linnaeus) Batters		G96	AY295146[1]

[1]Yang and Boo 2004

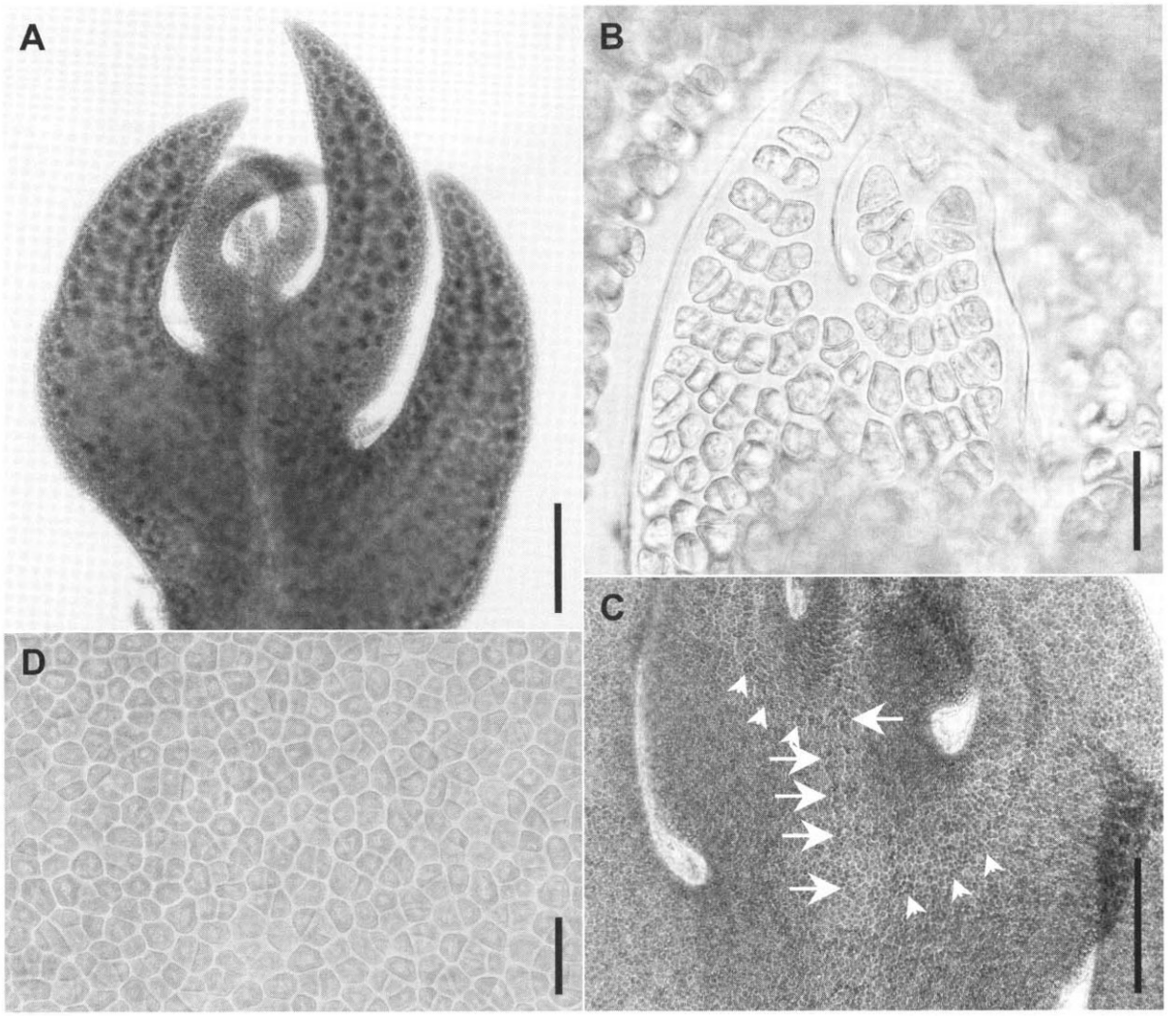

Fig. 1. Morphology of *Psilothallia dentata*. A, Vegetative thallus with alternate-distichous branchlets. Scale bar, 200 μm. B, Apex showing transversely divided axial cell. Scale bar, 20 μm. C, Thallus showing axial cells (arrows). Scale bar, 20 μm. D, Surface cortical cells. Scale bar, 200 μm.

cm, and are compressed with alternately, distichously branched indeterminate and determinate branchlets, and are dark to bright red when fresh. Holdfasts are conical and 2-6 mm across. Thalli are usually epiphytic on *Prionitis* and seaweeds or epilithic. The dried substance is cartilaginous, and the thalli do not adhere to herbarium sheet.

Growth of indeterminate axes takes place by transverse divisions of dome-shaped apical cells (Fig. 1 and Fig. 2A-E). Axial cell becomes cylindrical and increase to 60-100 μm in diameter. The central part of indeterminate branches becomes swollen by extensive rhizoid development around the axial and inner cells, separating these cells from each other. Axial cell cuts off up six periaxial cells; the first and second periaxial cells arise opposite one another, and the third and fourth are initiated as an opposite pair, and the fifth and sixth are formed as an opposite pair. Periaxial cells produce cortical cells. Surface cells of the cortex are small, compact, ovoid, triangle or rectangular to ovioid, and 6-13 μm across.

Indeterminate branches irregularily arise on axes, and bear determinate branchlets. Determinate branchlets arise alternately, distichously per three to four axial cells, tapering, and about 1 mm long.

Male, female, and tetrasporangial thalli are morphologically similar. Spermatangia are formed on branched filaments issued on axil of determinate branchlets. Spermatangial parent cells arise directly from cells of branched filaments. Each parent cell produces one or two spermatangia (Fig. 2G-I).

Carpogonial branches are four-celled and arise on

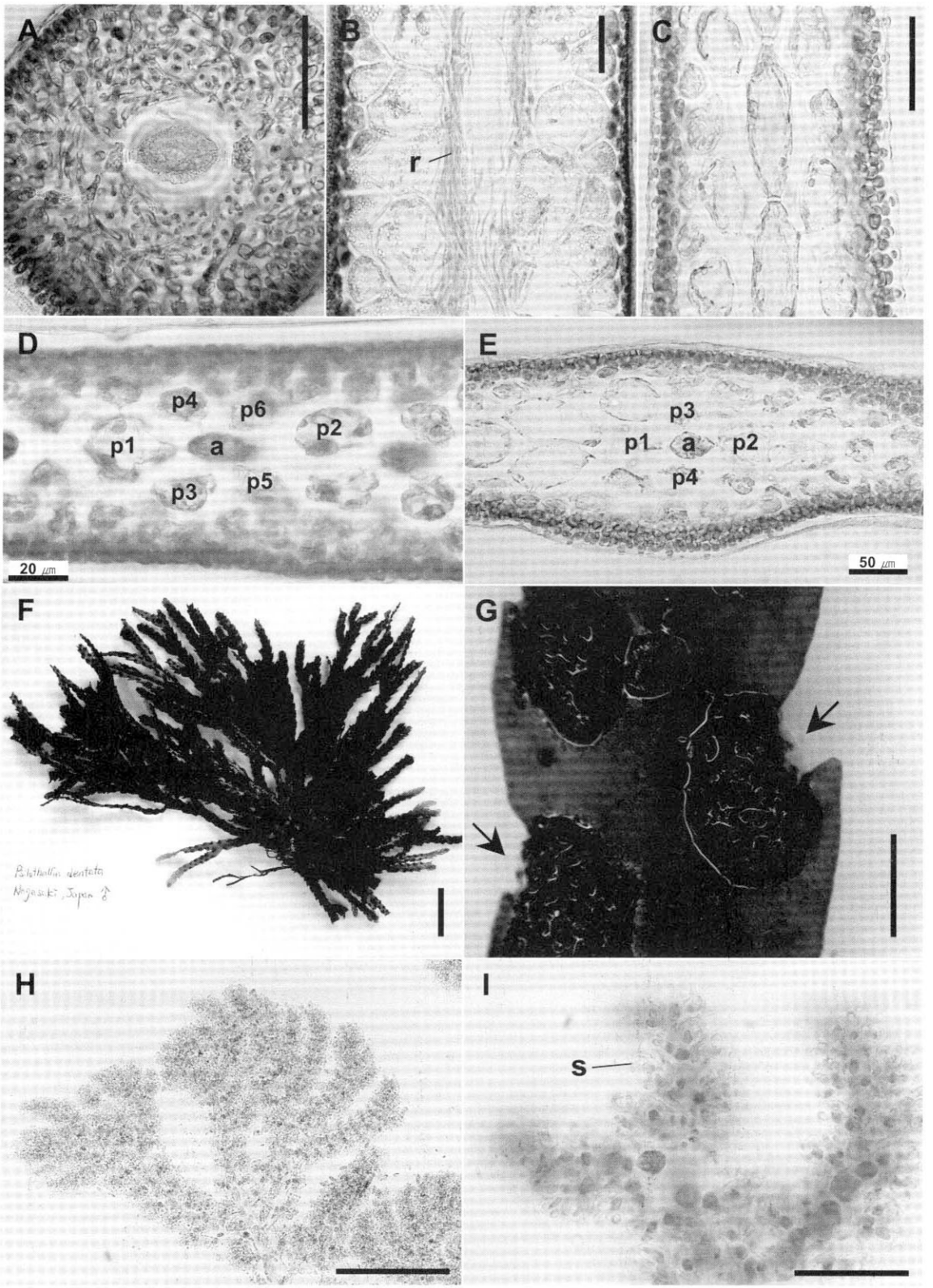

Fig. 2. Anatomy and male thallus of *Psilothallia dentata*. A, Cross-sectioned axis showing axial cell, rhizoidal filaments, and cortical cell. Scale bar, 100 μm. B, Longitudinal section showing rhizoidal filaments (r) and axial cells. Scale bar, 100 μm. C, Longitudinal section showing axial, periaxial and cortical cells. Scale bar, 50 μm. D, Cross-sectioned axis having six periaxial cells (p1-p6) alternately formed from axial cell (a). E, Cross-sectioned axis having four periaxial cells (p1-p4). F, Thallus with spermatangial clusters. Scale bar, 2 cm. G, Enlarged view of Fig. 2F. Arrows indicate spermatangial clusters. Scale bar, 1 mm. H, Spermatangial filaments. Scale bar, 200 μm. I, Spermatangium (s) on spermatangial filaments. Scale bar, 100 μm.

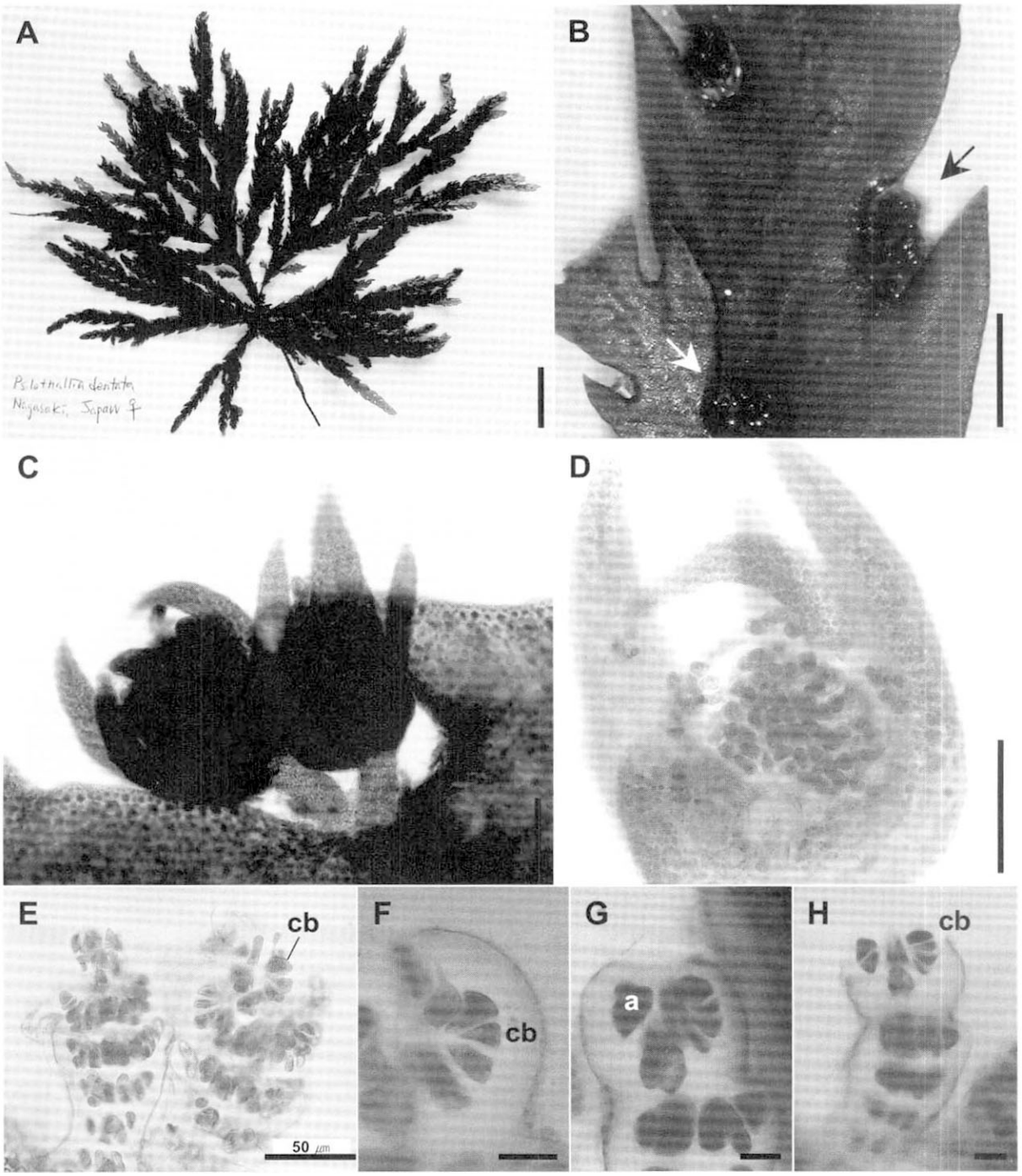

Fig. 3. Female thallus of *Psilothallia dentata*. A, Thallus with cystocarps. Scale bar, 2 cm. B, Enlarged view of Fig. 3A. Arrows indicate cystocarp on short branch. Scale bar, 1 mm. C, Two carposporophytes. Scale bar, 200 μm. D, Cystocarp with involucral brances. Scale bar, 200 μm. E, Branch with carpogonial branch (cb) and hair. F, Four celled carpogonial branch. Scale bar, 10 μm. G, Auxiliary cell (a) and carpogonial branch. Scale bar, 10 μm. H, Division of axiliary cell. Scale bar, 10 μm.

short indeterminate branches issued on axil of determinate branches. Cystocarps are surrounded with 7-8 simple involucral filaments. Involucral filaments are 300-600 μm long and 50-80 μm in diameter, with cells of L/D 0.8-1.4. Gonimolobes are rounded and 70-150 μm across. Carposporangia are ovioid and 10-20 μm in diameter (Fig. 3).

Tetrasporangia are on clustered, branched filaments issued on axils of determinate branchlets. Tetrasporangia are slightly ovoid, 20-40 μm in diameter, and

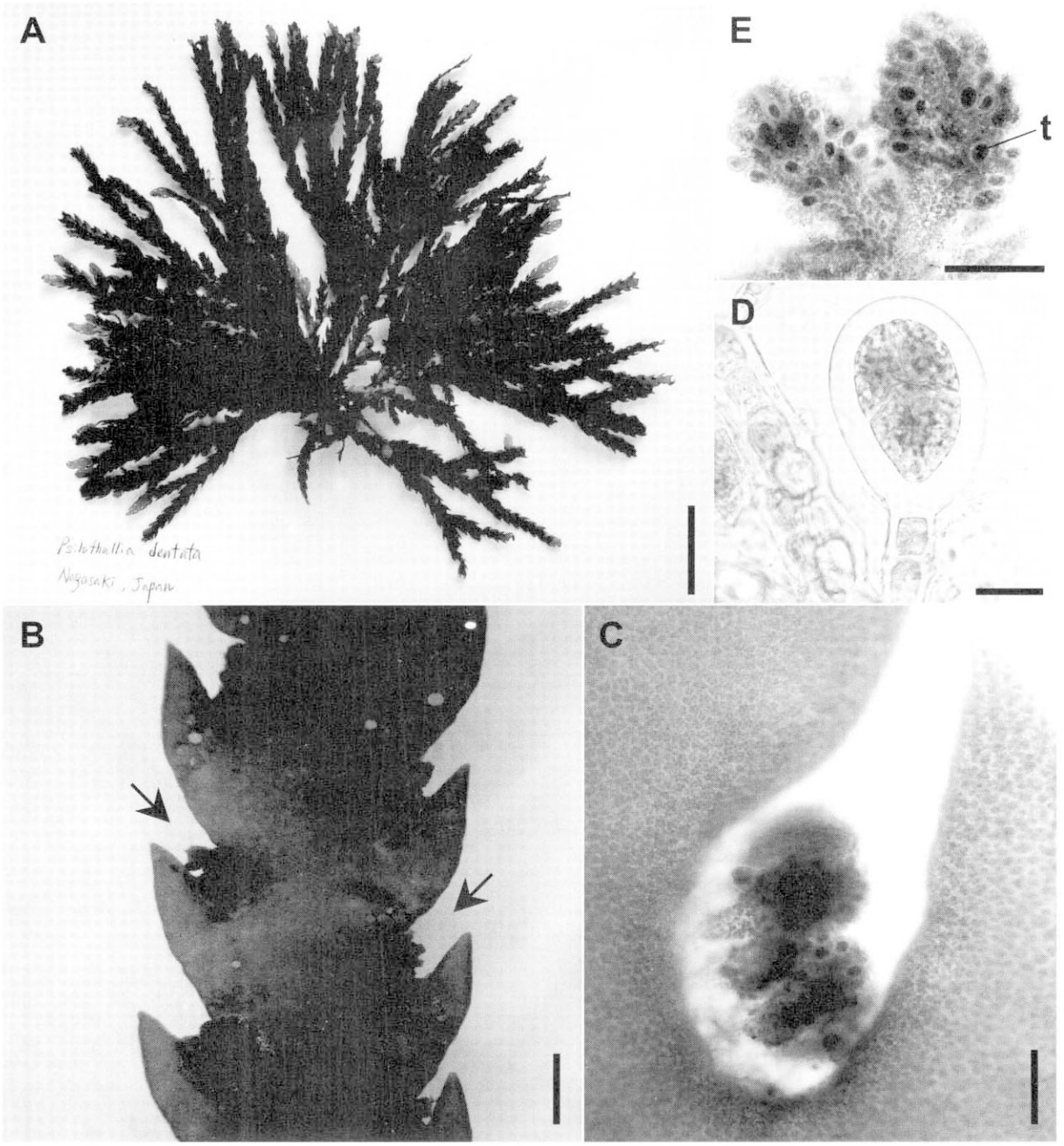

Fig. 4. Tetrasporangial thallus of *Psilothallia dentata*. A, Thallus with tetrasporangial tufts. Scale bar, 2 cm. B, enlarged view of Fig. 4A. Arrows indicate tetraspornagial tufts. Scale bar, 1 mm. C, Enlarged view of Fig. 4B. Scale bar, 200 μm. D, Tetrahedrally divided tetrasporangium on a filament. Scale bar, 20 μm. E, Tetrasporangial filaments. Scale bar, 200 μm.

tetrahedrally divided (Fig. 4).

Protein-coding plastid *psbA* phylogeny

Thirty-five *psbA* sequences of *Psilothallia dentata* and putative relatives were manually aligned due to the lack of length variation between individual sequences. The final alignment of the *psbA* gene yield 894 bp for 38 taxa including three outgroups. Base composition of the gene was slightly AT-biased (61.1%). Transitions abounded [transition/transversion ratio (Ti/Tv) = 3.702] at all codon positions. A total of 166 (18.6%) variable sites were found, of which 103 (11.5%) were parsimoniously informative.

Within the Ptiloteae, average *p*-distance was 2.22%. *Psilothallia dentata* was more related to *Ptilota* (2.2% *p*-distance) than to *Plumaria* (3.13% *p*-distance). However, within the genus *Ptilota*, *P. filicina* differed by 1 bp (99.89% sequence identity) from *P. phacelocarpoides*,

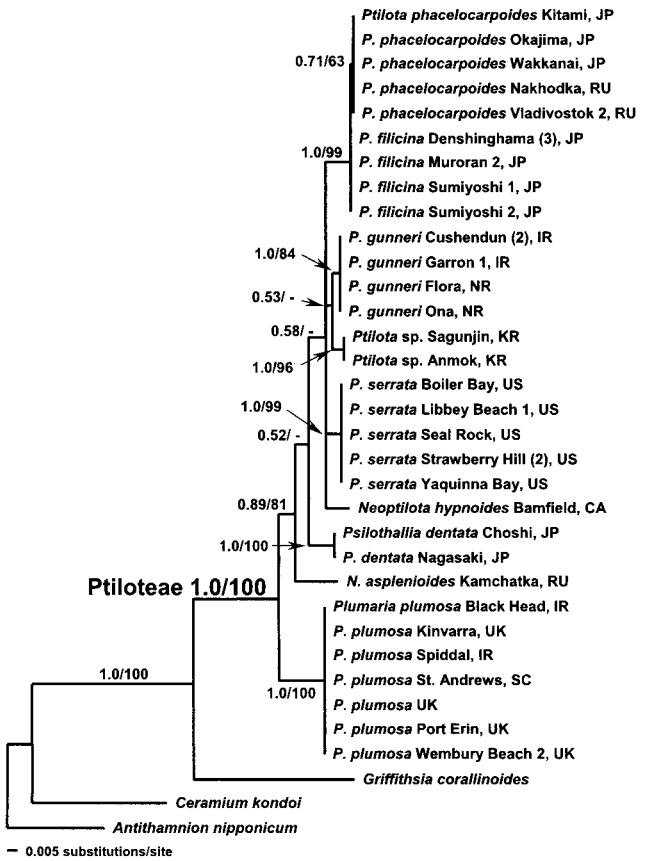

Fig. 5. The 50% majority rule consensus phylogram of the stationary trees of *Psilothallia dentata* and relatives reconstructed in the Bayesian analysis of *psbA* data using the GTR model (R_{AC} = 1.5021, R_{AG} = 2.5101, R_{AT} = 5.4984, R_{CG} = 0.1158, R_{CT} = 32.4762, R_{GT} = 1) with gamma-distributed rate heterogeneity (Γ = 0.6625) and proportion of invariable sites (I = 0.4657) and different base frequencies (π_A = 0.2457, π_C = 0.1918, π_G = 0.1904, π_T = 0.3720). Arithmetic mean of estimated marginal likelihood was -2493.49. The values above or under branches correspond to Bayesian posterior probability (Bp) and maximum parsimony bootstrap (Bt_{MP}) values. The notation "-" indicates support values of nodes with less than 50%. The number in parenthesis indicates the number of specimens analyzed.

which differed by 17 bp (98.1% sequence identity) from an undescribed *Ptilota* species in Korea (Table 2).

Trees have been made using two methods for the *psbA* sequences. In the Bayesian tree marked with Bp and shown in Fig. 5. *Psilothallia dentata* and members of the tribe Ptiloteae produced a strongly supported clade (1.0 Bp and 100% Bt_{MP}). *Psilothallia dentata* was consistently basal to the clade of the *Ptilota* species and *Neoptilota hypnoides*. Two Japanese species of *Ptilota*, *P. filicina* and *P. phacelocarpoides*, made a strong monophyly (1.0 Bp and 99% Bt_{MP}), whereas other species of the genus produced polytomous relationships. *Neoptilota* was not monophyletic, having *N. hypnoides* more related to *Ptilota*, whereas *Neoptilota asplenioides* was sister to the clade comprising *Ptilota*, *Neoptilota hypnoides*, and *Psilothallia*. *Plumaria* was basal to all the Ptiloteae taxa used in the present study.

DISCUSSION

This is the first document to report the morphology and phylogeny of *Psilothallia dentata* since its original description by Okamura (1892). Female, male, and tetrasporangial thalli were collected in one location, and male thalli are described here for the first time. The simultaneous occurrence of the sporophytic and gametophytic phases in Choshi, Chiba indicates the typical *Polysiphonia*-type life history in nature, which is demonstrated in culture in most of ceramiaceous red algae.

Psilothallia dentata has alternate-distichous determinate branchlets and a rhodomelacean sequence of periaxial-cell formation, although it is more similar to *P. siliculosa* than to *P. striata* in the absence of horizontal striation on the determinate branchlets (Womersley 1998). The rhizoidal filaments are well developed in the middle of axes and indeterminate branches in *P. dentata*, as shown in *P. striata* and *P. siliculosa* (Womersley 1998). They originate from periaxial and inner cortical cells. The rhizoidal filaments are present in completely corticated species such as *Ceramium* and *Campylaephora* (Seo *et al.* 2003) as well as members of the tribe Rhodocallideae (Hommersand *et al.* 1998). The rhodomelaceous sequence of the periaxial-cell formation is important enough to make the genus *Psilothallia* in the tribe Dasyphileae to be transfered to the tribe Rhodocallideae (Womersley 1998). *Psilothallia dentata* has six periaxial cells, compared to 6-10 periaxial cells in *P. striata* and *P. siliculosa*. They are formed in a rhodomelacean (alternating) sequence, typical of the tribe Rhodocallideae (Hommersand *et al.* 1998).

Psilothallia dentata is unusual in that reproductive organs such as cystocarps, spermatangial clusters, and tetrasporangial tufts are formed on short indeterminate branches arising on axils of determinate branchlets, as shown in Figs 2-4. In this feature, *P. dentata* is different from *P. striata* and *P. siliculosa*, in which the reproductive organs are formed directly on determinate branchlets (Figs 169 and 170 in Womersley 1998). On the other hand, *P. dentata* is similar to some species of the genus

Table 2. Genetic distances. Above diagonal shows absolute base pair difference between species and below diagonal shows *p*-distance

	1	2	3	4	5	6	7	8	9
1. *Psilothallia dentata*	-	20	19	21	20	18	26	19	28
2. *Ptilota filicina*	2.24	-	11	1	14	16	29	14	30
3. *P. gunneri*	2.13	1.23	-	12	11	7	22	11	27
4. *P. phacelocarpoides*	2.35	0.11	1.34	-	15	17	30	15	29
5. *P. serrata*	2.24	1.57	1.23	1.68	-	10	27	12	28
6. *Ptilota* sp.	2.01	1.79	0.78	1.9	1.12	-	23	14	28
7. *Neoptilota asplenioides*	2.91	3.24	2.46	3.36	3.02	2.57	-	23	33
8. *N. hypnoides*	2.13	1.57	1.23	1.68	1.34	1.57	2.57	-	32
9. *Plumaria plumosa*	3.13	3.36	3.02	3.24	3.13	3.13	3.69	3.58	-

Ptilota in having reproductive organs arising on short indeterminate branches. However, the fertile branches are normal, short branches in *Ptilota* (Masuda and Sasaki 1990; Maggs and Hommersand 1993), whereas the branches are adventitiously formed only in the fertile thalli in *Psilothallia dentata*. The taxonomic value of the fertile branches in *P. dentata* may be reassessed after the type of the genus will be investigated.

The occurrence of spermatangial clusters on branched filaments in *Psilothallia dentata* is similar to *P. siliculosa*, however, the latter has spermatangial clusters on the adaxial side of the short determinate branchlets. In this feature, *Psilothallia* is different from *Rhodocallis* which has spermatangia derived on surface cortical cells (Hommersand *et al.* 1998). The developemental structure of spermatangia is relatively uniform in species or genera and is of limited taxonomic value in the Ceramiaceae.

Carpogonial branches in *Psilothallia dentata* are produced on the subterminal cell of the upper thallus and cystocarps are terminal on the short indeterminate branches. The general condition in which procarps are borne on specialized indeterminate axes that cease growth either following procarp formation or subsequent to the first successful fertilization in *P. dentata* is a common occurrence in the Ceramiaceae (Hommersand *et al.* 1998). The ontogeny of the procarp and cystocarps of *P. dentata* appear similar to that of other ceramiaceous red algae.

Tetraspcrangia are formed terminally on branched filaments issued from axils of determinate branchlets in *Psilothallia dentata*, as does in *P. striata* and *P. siliculosa* (Womersley 1998). Hommersand *et al.* (1998) suggested that the formation of tetrasporangia from surface cortical cells in *Rhodocallis* Kützing is derived, compared to terminal tetrasporangia on either determinate or indeterminate branches, in the Ceramiaceae.

It is expected that the *psbA* sequences were identical between two specimens of *Psilothallia dentata* from different locations in Japan and also among specimens of e.g. *Ptilota gunneri* from UK and Norway. However, the *psbA* sequences are variable enough to identify species and genera within the Ptiloteae, as shown in other ceramiaceous red algae (Seo *et al.* 2003; Yang and Boo 2004). In the *psbA* trees, the monophyly of the Ptiloteae is strongly supported, comprising *Psilothallia dentata*, *Ptilota*, *Neoptilota*, and *Plumaria*. Our *psbA* phylogeny, therefore, contradicts morphology-based classification that *Psilothallia dentata* belongs to the Dasyphileae (Schimitz in Engler and Prantl 1897) or the Rhodocallideae (Womersley 1998). Instead, the molecular phylogeny supports Athanasiadis (1996)'s opinion that *Psilothallia* may be included in the Ptiloteae. In this point, our *psbA* phylogeny is congruent with the *rbc*L and *psa*A phylogenies, using same taxa in the present study (unpublished data, Yang and Boo). However, because *P. striata*, the type of the genus, is not included in the present study, it is early to conclude whether *P. dentata* only will be transferred to the Ptiloteae group or the genus *Psilothallia* will be classified in the Ptiloteae.

Our *psbA* sequence data recognize an undescribed taxon of *Ptilota* in Gangreung, the east coast of Korea. The taxonomy of the taxon will appear elsewhere and is beyond the scope of this paper. The *psbA* phylogeny indicates that the genus *Ptilota* appears paraphyletic. Although *P. filicina* and *P. phacerocarpoides* from Hokkaido, Japan produced a sister relationship, other *Ptilota* species formed a polytomous phylogeny. *Neoptilota* is not monophyletic, *N. hypnoides* being grouped with *Ptilota*, and *N. asplenioides* being basal to the clade of *Neoptilota hypnoides*, *Ptilota*, and *Psilothalia*.

Increased sampling within and outside the tribe Ptiloteae will reconsile uncertainty in branches of *Ptilota* and *Neoptilota*.

In summary, *Psilothallia dentata* has alternate-distichous determinate branchlets and a rhodomelacean sequence of periaxial-cell formation, typical characters of the genus. However, *P. dentata* is distinctly different from *P. siliculosa* and *P. striata* in reproductive organs arising on adventious indeterminate branches issued on axils of determinate branchlets. The protein-coding plastid *psbA* gene phylogeny suggests the broad concept of the tribe Ptiloteae including *P. dentata* (currently classified in the tribe Rhodocallideae) and also non-monophyly of each of the genera *Ptilota* and *Neoptilota*. The preliminary results presented here cast several questions about phylogenetic relationships within the tribe Ptiloteae and should be a guide to future studies in the tribe.

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