Algae
Volume 21(1): 91-101, 2006

The Occurrence of *Griffithsia okiensis* (Ceramiaceae, Rhodophyta) from Korea on the Basis of Morphology and Molecular Data

Hyung-Seop Kim[1], Eun Chan Yang[2] and Sung Min Boo[2]*

[1]*Department of Biology, Kangnung National University, Gangreung 210-702, Korea*
[2]*Department of Biology, Chungnam National University, Daejon 305-764, Korea*

Despite continued studies on red algal flora in Korea, the taxonomy of the tiny ceramiaceous algae has received little attention. We report for the first time *Griffithsia okiensis* from Korea on the basis of morphology and molecular data. The species is small in thalli height (0.3-1.5 cm), and in diameter of vegetative cells (50-500 μm), and the ratio of cell length/breadth is 2-3 times. It has two carpogonial branches from the supporting cell of procarp. We generated *psb*A and *rbc*L sequences from ten specimens of *G. okiensis* isolated from Korea and Japan and from one *G. japonica* species isolated Japan. Eight specimens of *G. okiensis* from Korea were almost identical in both *psb*A and *rbc*L regions, nevertheless they differed from Japanese specimens by 4 nucleotides in *psb*A and 7 in *rbc*L. In all analyses of *psb*A, *rbc*L, and *psb*A + *rbc*L data sets, *G. okiensis* was determined to be a different species from *G. japonica* isolated from Japan, although both species showed a sister relationship. For all that extensive collection trips, we found no evidence for the occurrence of *G. japonica* in Korea.

Key Words: Ceramiaceae, *Griffithsia okiensis*, morphology, *psb*A, phylogeny, *rbc*L, Rhodophyceae, taxonomy

INTRODUCTION

Griffithsia C. Agardh is an annul, ceramiaceous red algal genus that has characteristic large cells visible to naked eye and thousands of nuclei in a single cell at maturity (Waaland and Cleland 1974). The genus is distinguished by filamentous thalli with dichotomous to irregular branches, tetrasporangial fascicles in the constrictions between axial cells near apex, spermatangial fascicles in the constrictions between axial cells or on apical cells, and cystocarps surrounded by two-celled involucral branches (Baldock 1976; Kim and Lee 1987; Womersley 1998; Boo and Cho 2001). Although about 27 species have been reported to occur intertidally along temperate to tropical coasts (Womersley 1998), diversity of the genus is still underestimated.

In the northwest Pacific Ocean, eight *Griffithsia* species are currently recognized (Itono 1977; Yoshida 1998). Of these species, *G. japonica* and *G. okiensis* are so similar in their morphology that it is very difficult to identify the two species in the field. Okamura (1930) first described *G. japonica* based on material from Chiba, Pacific coast of Japan, which is distinguished by filamentous flabellate thalli with dichotomous branches, tetrasporangial and spermatangial fascicles lying in the constrictions between the cells of distal axes near the apex and surrounded by involucrals arising separately from the cells bearing the fascicles, and cystocarps terminating on laterals and surrounded by simple or branched involucres. *G. japonica* occurs in Korea, Japan, China, and Australia (Tseng 1941; Kang 1966; Itono 1977; Miller 1998). *G. okiensis* Kajimura was described based on specimens from Oki Island on the west side of Japan (Kajimura 1982) and is distinguished by tiny thalli and two carpogonial branches on a single supporting cell on female thalli. Prior to the present study, *G. okiensis* has been considered to occur in Oki Island, Japan (Yoshida 1998).

During our study on the marine biodiversity from Korea, we found an alga in Jeju and Wando, which is morphologically similar to *G. japonica* and genetically different from the species (Yang and Boo 2004). We extended collection of the alga along the whole coast of Korea and compared it with *G. japonica* and *G. okiensis*. Here we identify the alga as *G. okiensis* on the basis of its vegetative and reproductive morphology and provide further evidence of its taxonomic validity using comparative analyses of protein-coding plastid *psb*A and

*Corresponding author (smboo@cnu.ac.kr)

Table 1. Species studied in the present study. Locations underlined are type locality of the species. F. female gametophyte; M. male gametophyte; T. tetrasporophyte; V. vegetative thalli.

Taxa	Locality	Collection date	Voucher	GenBank accession number	
				*psb*A	*rbc*L
G. okiensis	Korea: Jeju: Chaguido	16 Apr. 2002 (V)	G164	AY604851	AY604860
	Korea: Jeju: Gangjeong	18 Apr. 2002 (F, M, T, V)	G165	AY604852	AY604861
	Korea: Jeju: Moonsum	22 Aug. 2002 (T, V)	G98	AY2951481	AY2951661
	Korea: Gangreung: Anin	20 Mar. 2004 (F, V)	G283	AY604850	AY604859
	Korea: Geojedo: Haegumgang	29 Jun. 2003 (V)	G170	AY604854	AY604862
		21 Mar. 2004 (M)	G284	AY604855	AY604863
	Korea: Namhaedo: Songbang	18 Apr. 2001 (V)	G99	-	-
	Korea: Pohang: Gampo	18 Jul. 2001 (V)	G97	AY604853	-
	Korea: Ulreungdo: Tonggumi	27 Aug. 2003 (F, V)	G185	AY604856	AY604864
	Korea: Wando: Jeongdori	18 Feb. 2003 (V)	G149	AY2951491	AY2951671
	Japan: Oki Island: Kamo Bay	6 May 2003 (F)	G166	AY604857	AY604865
	Japan: Oki Island: Kamo Bay Cave	7 May 2003 (V)	G167	AY604858	AY604866
G. japonica Okamura	Japan: Chiba: Choshi	27 Jul. 2002 (V)	G100	AY2951471	AY2951651

*rbc*L genes. The *rbc*L gene has most commonly been used for red algae at a variety of taxonomic levels (e.g. Yang and Boo 2004). Recently we found that *psb*A gene, which encode photosystem II proteins in plastid, is variable enough for identifying ceramiaceous red algal species and have more resolution when combining with other molecular data such as the *rbc*L sequences (Seo *et al*. 2003; Yang and Boo 2004).

MATERIALS AND METHODS

Sampling, Microscopy and Culture

Twenty samples of *G. okiensis* were collected in the field and each was separated into three portions: preserved in 4% formaldehyde-seawater, air-dried and kept preserved in silica gel, and unialgal culture. For microscopic observation, thalli were stained with aqueous 1% aniline blue solution, and mounted in 10% Karo-syrup mixed with phenol. Drawings were made with a camera lucida attached to an Olympus microscope (BX50). Cell-size ranges and means were based on 30 measurements. Natural seawater from Gangreung (salinity 33‰) enriched with PES medium was used for laboratory culture. Cultures were kept at 20°C under white fluorescence light at 30-50 $\mu \cdot mol \cdot m^{-2}$ sec^{-1}. Herbarium specimens and voucher slides are deposited in the herbaria of Kangnung National University and Chungnam National University.

Specimens examined

Korea; KNU243 (Jumoonjin, Gangreung, 5 May 1984), KNU286 (Impo, Dolsando, 5 Apr. 1985), KNU 295 (Jeongdori, Wando, 31 Jul. 1985), KNU315 (Sungsan, Jeju, 6 Apr. 1986), KNU324 (Pyosun, Jeju, 9 Apr. 1986), KNU326 (Eoyoung, Jeju, 9 Apr. 1986), KNU357 (Huksando, Jeonam, 18 Jul. 1986), KNU1156 (Daebo, Guryungpo, 14 Jun. 1997), KNU1360 (Impo, Dolsando, 12 Jul. 2001), KNU1412 (Chaguido, Jeju,16 Apr. 2002) KNU1482 (Gangjeong, Jeju, 22 Aug. 2002), KNU1812 (Jeongdori, Wando, 18 Feb. 2003), KNU1892 (Tonggumi, Ulreungdo, 13 May 2003), KNU2197 (Summok, Ulreungdo, 13 May 2003), KNU 2343 (Anin, Gangreung, 20 Mar. 2004, KNU3012 (Gacheon, Namhaedo, 13 May 2004). KNU3024 (Sachoen, Gangreung, 17 Jul. 2004), KNU3029 (Jangho, Samcheok, 18 Jul. 2004). Japan; CNUG166 (Gamo Bay, Oki Island, 6 May 2003), CNUG17 (Gamo Bay, Oki Island, 7 May 2003).

Analyses of the *psb*A and *rbc*L sequences

The sources of used in the present study and the GenBank accession numbers of *psb*A and *rbc*L sequences are listed in Table 1. Published *psb*A and *rbc*L sequences were downloaded from GenBank (Yang and Boo 2004).

Genomic DNA was extracted from approximately 5 mg algal powder ground in liquid nitrogen using a DNeasy Plant Mini Kit (Qiagen, GmbH, Hilden, Germany) or Invisorb Spin Plant Mini Kit (Invitek, GmbH, Berlin-Buch, Germany), according to the manufacturers' instructions. PCR was done with specific primers for each of the plastid genes; *psb*A was amplified using primers *psb*A-F and *psb*A-R1 and sequenced using additional primers: *psb*A-500F, *psb*A-600R, and *psb*A-R2 (Yoon *et al*. 2002). The primers used to amplify (F7 - R753 and F645 - RrbcS start) and sequence (F7, F645, R753, and

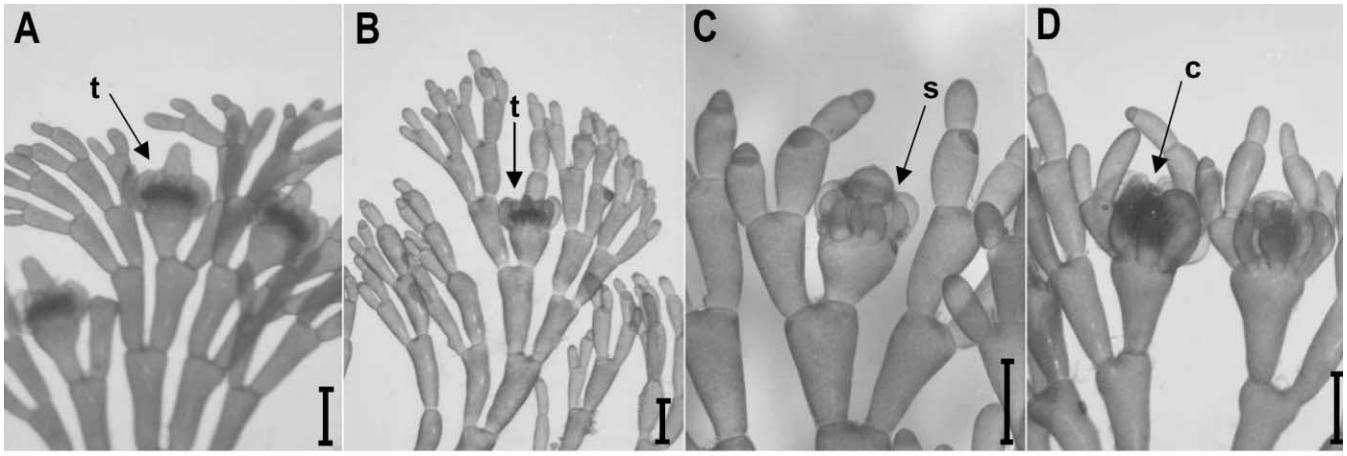

Fig. 1. Morphology of *Griffithsia okiensis* isolated from Korea. A. Thallus having tetrasporangial fascicles (t) between inflated cells near the apex of laterals. B. Development of normal vegetative branch from the cell bearing tetrasporangial fascicles. C. Male thallus bearing spermatangial fascicles (s). D. Female thallus bearing cystocarps (c). Scale bars = 500 μm.

RrbcS start) *rbc*L are listed in Freshwater and Rueness (1994), Lin *et al*. (2001), and Gavio and Fredericq (2002). The *psb*A and *rbc*L regions from all the samples used were easily amplified and sequenced. The PCR products were purified using a High Pure PCR Product Purification Kit (Roche, Mannheim, Germany) in accordance with the user's guide. The sequences of the forward and reverse strands were determined for all taxa using an ABI PRISM^{TM} 377 DNA Sequencer (Applied Biosystems, Foster City, CA, USA).

The alignment of each gene 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 *psb*A and *rbc*L. The final alignment is available from the corresponding author upon request.

To find the best model of sequence evolution for the maximum likelihood (ML) analysis using PAUP* (Swofford, 2002), we used MODELTEST (v3.6, Posada and Crandall, 1998). The MODELTEST chose the GTR + G + I model for *psb*A, *rbc*L and combined sequence data. Tree likelihoods were estimated using a heuristic search with 500 random addition sequence replicates, and tree bisection-reconection (TBR) branch swapping. To test the stability of monophyletic groups, bootstrap (BS) analyses were undertaken with 500 replicates.

Maximum parsimony (MP) analysis was done using a heuristic search algorithm with the following settings: 1,000 random addition sequence, TBR branch swapping, MulTrees, all characters unordered and unweighted, and branches with a maximum length of zero collapsed to yield polytomies. Bootstrap analyses were undertaken with 1,000 replicates.

RESULTS

Habitat and Seasonality

Thalli commonly occur in the intertidal zone to the subtidal zone on the south and east coast of Korea. The species grows as epiphytes on *Cladophora* and other algae or epilithic tufts on rock in sheltered and shared rocky side, especially in clefts of rock or in small caves. They occur throughout the year in Gangreung, east coast of Korea. Tetrasporangial and sexual thalli occurred in spring to summer (March to August).

Habit and Vegetative Structure

Thalli are fan-shaped, 0.3-1.5 cm high, rose-pink to bright red in color, erect and filiform, and stiff in living state. Attachment to the substratum is by rhizoidal filaments from the basal portion. The lower thallus is regularly dichotomous, complanate, and the upper thallus is compact and rarely trichotomous. The holdfasts are digitate, consisting of branched rhizoids. Axial cells at the mid-frond also produce rhizoids that terminate in unattached tips.

Cells of erect axes are cylindrical to slightly cuneate. The axes are ecorticate throughout and invariably dichotomously branched. Lateral initials are cut off with slightly oblique divisions of subapical cells and adaxially on axial cells, and laterals then divide into two cells (Fig. 1). The laterals are slender at first, but rapidly reach the same diameters to the main axes (Fig. 1A-B). Branch initiation in a given axis is second and occurs at regular intervals per every segment, the branches always ending

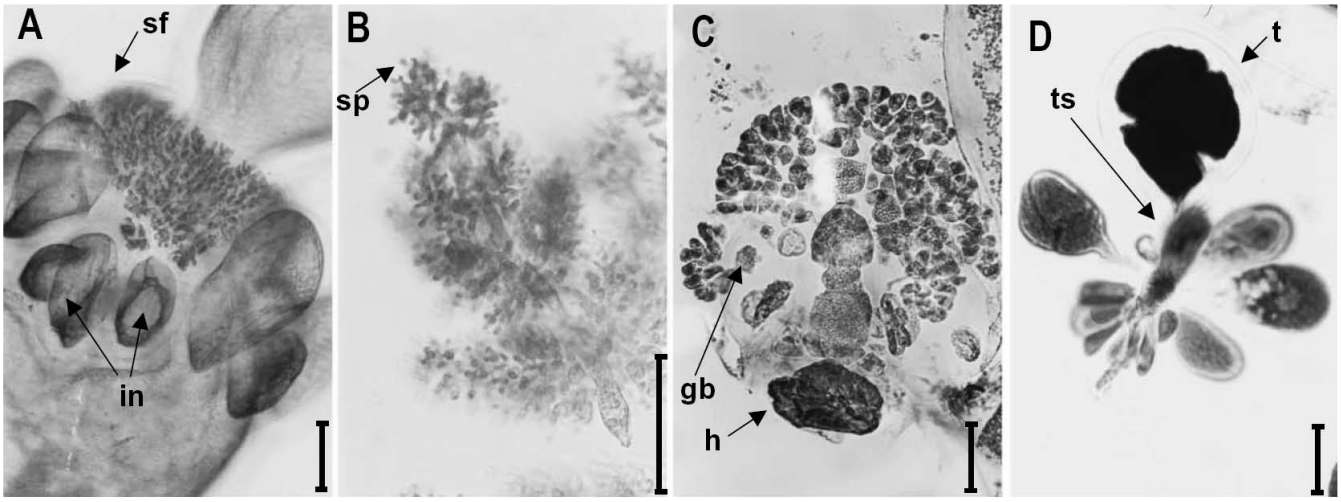

Fig. 2. Reproductive structures of *Griffithsia okiensis* isolated from Korea. A. Young spermatangial fascicles (sf) on shoulder of the inflated subapical cell with involucres (in). B. Spermatangial fascicles consisted of 5-6 polychotomous branched axis with spermatia (sp). C. Cystocarp having 8-10 distal gonimolobes (gb) and hypogenous cell (h). D. Tetrasporangial fascicle bearing a mature tetrasporangium (t) and immature tetrasporangia on a single stack cell (ts). Scale bars = 50 μm.

blunt tips that are slightly incurved (Fig. 1A-B). Adventitious branches occur frequently on basal portion of thallus but rarely in low to upper potion. Trichoblast are not produced.

The apical cells are small, 50-90 μm long (mean = 76.2 μm) at first and rapidly grow to be 220-420 μm long (mean = 361.9 μm) and 120-220 μm broad (mean = 160.4 μm). The second segments from apex, which have two new apical cells upwardly, are more or less cuneate having broader distal ends than at the proximal ends, and are 84-110 μm broad (mean = 102.6 μm) in the proximal end (BP), 145-210 μm broad (mean = 182.8 μm) in distal end (BD), and 330-580 μm long (L) (mean = 455.0 μm). The 4^{th} to 5^{th} segments from apex are clearly cuneate and 97-157 μm in BP (mean = 125.2 μm), 218-280 μm in BD (mean = 243.7 μm), and 480-660 μm in L (mean = 554.2 μm). The axial cells at the basal part are 170 - 220 μm in BP (mean = 193.3 μm), 390-530 μm in BD (mean = 440.4 μm), and 790-1330 μm in L (mean= 958.8 μm). Thus the ratio of cell length (L)/breadth (BD) are ca. 2.5 times in the first intermediate cells, ca. 2.3 times in the 4^{th} to 5^{th} segments from apex, and ca. 2.2 times in the basal cells. The cell dimensions are very similar among populations from Korea.

Reproductive Structure

Whorls of tetrasporangial fasicles are clustered in the constriction between a distally globular cell and an inflated pyriform axial cell near the apex. The fertile pyriform subapical axial cells, being 650-900 μm long and 540-610 μm broad, produce the whorls of numerous unicellular stalked tetrasporangial mother cells (Figs 1A, B, 2D). The each of mother cell produces 7-12 non-synchronously maturing tetrahedrally divided tetrasporangia on both (sub-) terminally and radially (Fig. 2D). Mature tetrasporangia are elliptical to spherical, and are 57-76 μm long and 57-70 μm broad. The involucral cells are non-stalked and one-celled, surrounding tetrasporangial fasicles. They are produced separately and peripherally from the pyriform subapical cells. The involucres are slightly incurved inwardly and 330-400 μm long. 150-180 μm broad.

Whorls of spermatangial fasicles are clustered in the constriction between a distally globular bearing cell and an inflated pyriform axial cell near the apex. However, the globular apical cells of spermatangia are usually caduceus, the spermatangial masses then appearing terminal on pyriform cells surrounded by involucres (Fig. 1C). The spermatangial fertile initial from the upper shoulders of pyriform subapical cell divide 2 times transversely to form 3-celled fertile axis, then successively divide di-/polychotomously, and ultimately bears large numbers of spermatangial parent cells that each produce one or two spermatangia (Fig. 2A, B). The spermatangial fascicles are surrounded by 14-18 non-stacked, and one-celled synchrononic involucres that are produced separately and peripherally from the pyriform subapical cells (Fig. 1C). The involucres, after formation of 3-celled spermatangial fertile initials, are slightly incurved inwardly.

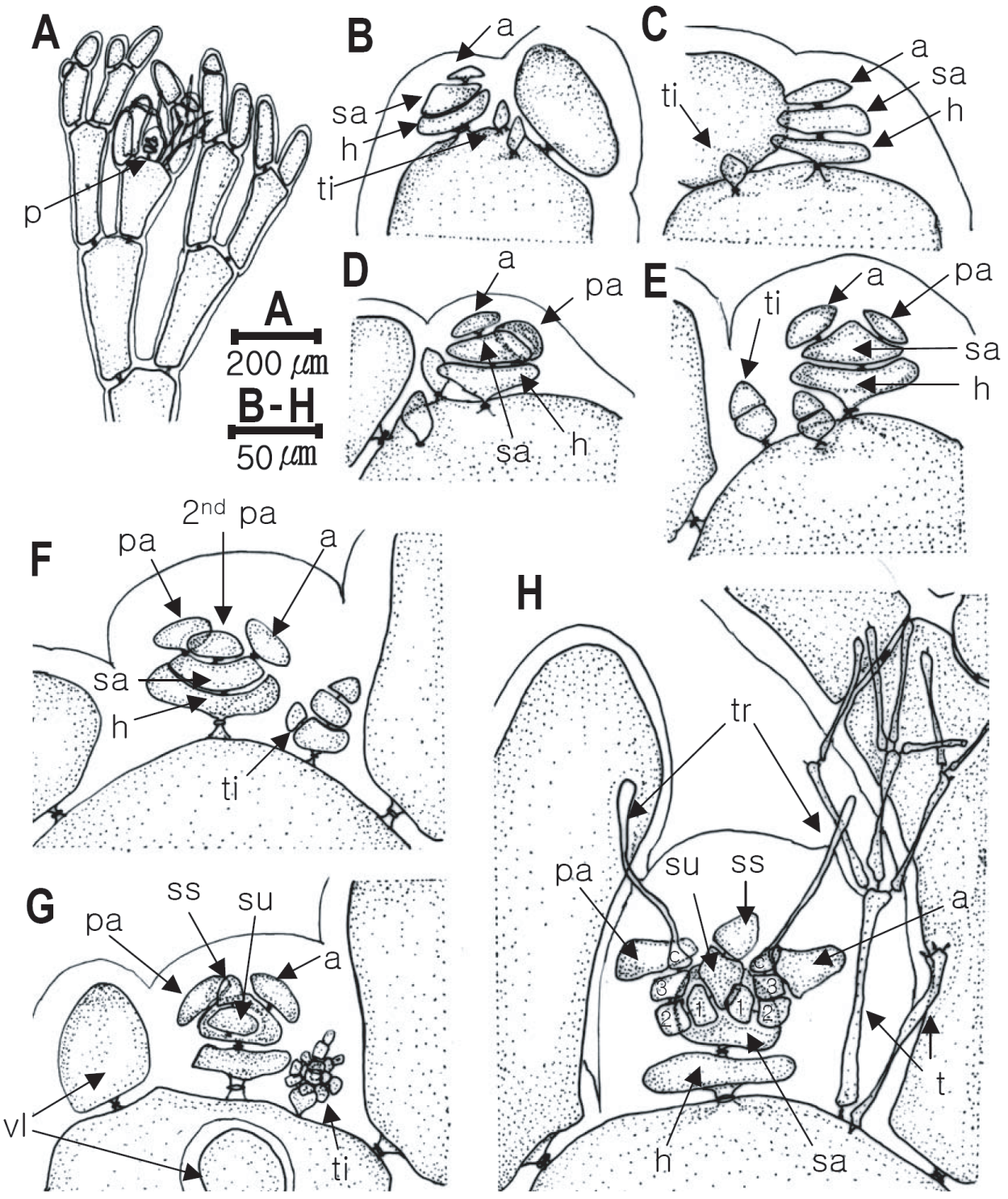

Fig. 3. Development of procarp of *Griffithsia okiensis* in Korea. A. Female thallus having a procarp on apex of lateral enclosed by 2-4 vegetative laterals. B-H. Synchronous development of procarp and trichoblasts (B-G) and a mature procarp consisting of two carpogonial branches on a supporting cell (H). a, apical cell of fertile axis. h, hypogenous cell of fertile axis. p, procarp. pa, primary periaxial cell. 2nd pa, secondary periaxial cell. sa, subapical cell of fertile axis. su, supporting cell. ss, supporting sterile cell. t, trichoblast. ti, trichoblast initial. tr, trichogyne.

The female thalli are easily distinguished from male or tetrasporangial thalli by the unusual tri-/tetrachotomous branches and a pair of trichoblasts (Fig. 1D, 3A). The initial of procarp divides two times to form a dwarf and colorless three-celled fertile axis (hypogenous, subapical, and apical cell) on the adaxial side of subapical cell (Fig.

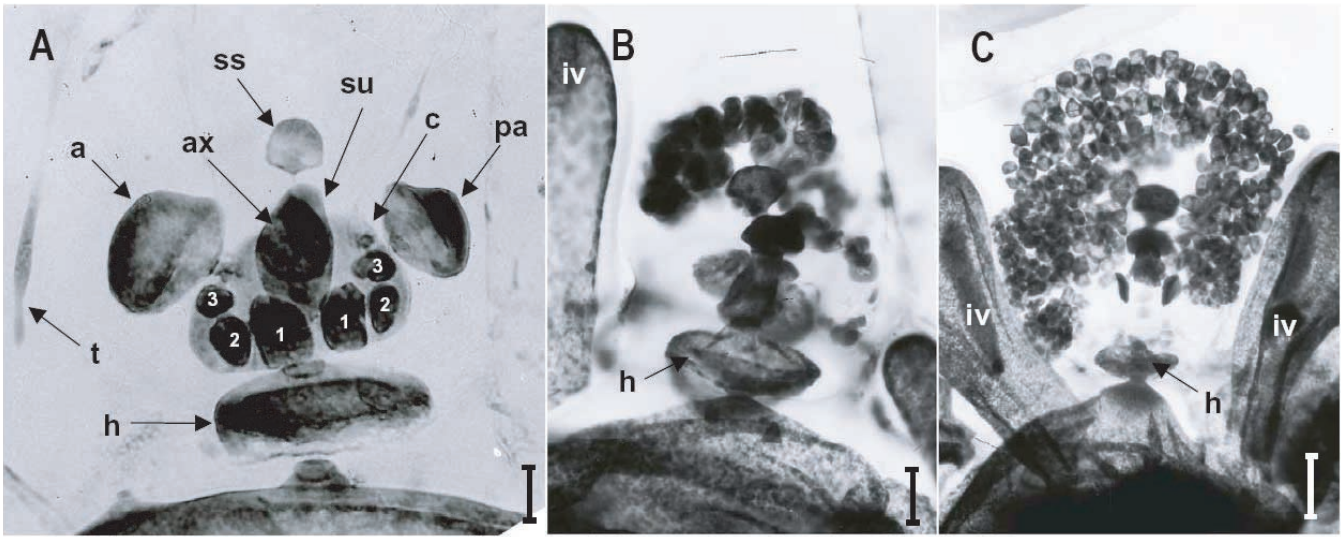

Fig. 4. Female structure of *Griffithsia okiensis* in Korea. A. Two carpogonial branches and auxiliary cell on procarp. B. Young gonimolobe. C. Mature gonimolobe. Abbreviations are same as in Fig. 4. Scale bars, A-B = 20 μm; C = 50 μm.

3B-C). The first division is transverse and the second is adaxially oblique, thus the apical cell being located on the adaxial side. The subapical cell successively cuts off obliquely two periaxial cells; the former is located abaxially and remains sterile, and the later is located perpendicularly to the first periaxial cell and becomes a supporting cell. The supporting cell cuts off terminally a single sterile cell, the supporting sterile cell, and abaxially two 4-celled carpogonial branches in the both sides (Fig. 3D-H).

The developmental processes of procarp are synchronized with the development of vegetative laterals and fertile trichoblasts. A pair of trichoblast initials arises on the abaxial side to the fertile axis, thus being positioned between vegetative lateral and fertile axial cells. The initials soon become two-celled trichoblasts after cutting off the sterile periaxial cell (Fig. 3C-E).

The second periaxial cell is produced after the trichoblasts were three-celled and the second vegetative lateral arises. At that time the 3-celled dwarf trichoblasts start the division of branch from basal cell, and the procarpic fertile axis is located at center between two laterals (Fig. 3D-E). At this stage the apical cell and first periaxial cell of fertile axis are almost same sized and are located oppositely each other on the same plane with lateral. The apical cell of fertile axis is always located toward trichoblasts and larger lateral cell. When the second periaxial cell cuts off terminally a supporting sterile cell, the trichoblasts are multi-celled and the third vegetative lateral arise perpendicularly to the two laterals. The mutli-celled trichoblasts are dwarf at first and then fully elongated. Most of procarps have the third vegetative lateral arises at the perpendicular position (Fig. 3G). The perpendicular lateral in procarp and in female plant is extraordinary because the third lateral in tri-chotomous branch always arise from the same plane with the other branch.

After presumed fertilization, an auxiliary cell produced perpendicularly from supporting cell, displaced apically by the continued growth itself (Fig. 4A). During this stage, a pair of connecting cells is usually cut off from either side of the base of the carpogoium, and 5-8 one-celled involucral cells produced newly from the subhypogenous cell. The auxiliary cell probably is fused with one of the connecting cell, divides apically and successively 3-4 gonimoblast initials. Successively 3-4 additional gonimoblast initials are developed from the fusion cell, which is a mushroom to columnar shaped cell by fusion of the supporting cell and subapical cell of fertile axis (Figs. 3C, 4B-C).

Each gonimoblast initial divides obliquely to irregularly to produce a mass of gonimolobe cells, which are roughly equal-sized and enclosed by common gelatinous sheath. About 8-10 distal gonimolobes consist of a cystocarp, being 450-550 μm broad and 600-750 μm long at maturity. Mature carpospores are spherical to ovoid and 57-82 μm broad and 64-96 μm long. The cystocarps are surrounded by two kinds of involucres; one is 2-4 vegetative laterals produced before fertilization, and the other is 5-8 one-celled involucres

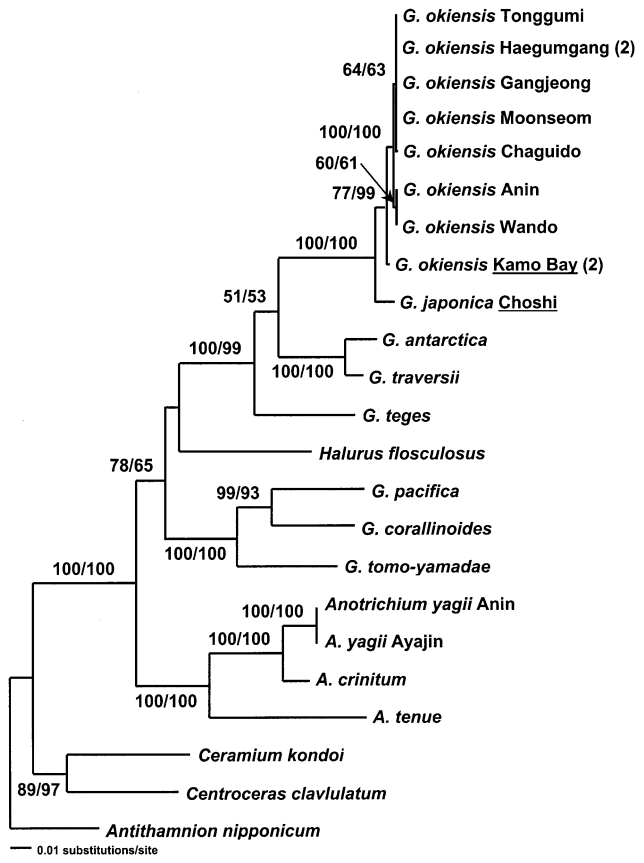

Fig. 5. Maximum likelihood tree for *Griffithsia okiensis* and relatives estimated from the plastid-encoded *psb*A + *rbc*L sequence data [GTR + Γ + I model, - Log likelihood = 10805.261; A ↔ C = 1.0276, A ↔ G = 5.0705, A ↔ T = 5.6461, C ↔ G = 1.3363, C ↔ T = 18.387, G ↔ T = 1; Γ = 2.0476; I = 0.6053; and different nucleotide frequencies (A = 0.2945, C = 0.1735, G = 0.2042, T = 0.3278)]. The bootstrap values are calculated from 500 bootstrap resamplings with the ML method using the GTR + Γ + I model and 1,000 bootstrap resamplings with the MP method (ML/MP).

produced after fertilization. All involucres arise from the subhypogenous cell (vegetative pyriform cell), not from the hypogenous cell.

Phylogenetic analyses

Plastid *psb*A and *rbc*L region from eleven populations of *G. okiensis* from Korea and Japan and one population of *G. japonica* from its type locality of Japan were determined in present study. In *psb*A gene (941 bp aligned), 235 positions (25%) were variable and 197 (19%) were parsimoniously informative (Table 2). There was a slight AT bias for the *psb*A gene fragment (60.7%). The infraspecific divergence in nine populations of Korean *G. okiensis* ranged from 0% to 0.21% (Chaguido and Wando). The sequences of *G. okiensis* from Korea and Japan differ by 3-4 nucleotides (0.32-0.43% sequence divergence) and from *G. japonica* by 10-11 nucleotides (1.06-1.17% sequence divergence).

In the *rbc*L gene (1379 bp aligned), 458 (33.2%) were variable, 359 (26%) were informative and with a G + C content of 36.8%. The *rbc*L sequences of *G. okiensis* from Korea were identical except Anin and Wando, which differed from others by 2 and 1 nucleotides, respectively. The *rbc*L sequences of *G. okiensis* from Korea and Japan differed by 6-7 nucleotides (0.44-0.56% sequence divergence) and from *G. japonica* by 28-29 nucleotides (2.03-2.1% sequence divergence). There was a difference of 24 nucleotides (1.74% sequence divergence) between *G. japonica* and *G. okiensis* from Japan (Table 2).

For the *psb*A and *rbc*L regions, the partition homogeneity test revealed significant congruence (p = 0.125). Based on the partition homogeneity test, sequence data from the two plastid gene regions were combined for the phylogenetic analyses.

All analyses of the *psb*A + *rbc*L, *psb*A, and *rbc*L sequences showed the monophyly of *G. okiensis*, which was clearly separated from *G. japonica* (Figs 5 and 6). In the combined *psb*A + *rbc*L ML tree, monophyly of *G. okiensis* was strongly supported by bootstrap analyses. The ML tree for the concatenated dataset showed close relationships among *G. okiensis* and *G. japonica*. The genus *Griffithsia* was clearly separated into two clades (Fig. 6). The monophyly of each clade was supported by high bootstrap values (100% for ML and 99% for MP).

The topology of the ML tree for *psb*A (Fig. 6A) was similar to that for the concatenated data. However, the *psb*A tree was different from those of *rbc*L and combined data in having *G. okiensis* from Korea as a sister to *G. japonica*. The *G. okiensis* and *G. japonica* clade was well supported (100% for ML and MP).

The ML tree for *rbc*L (Fig. 6B) showed that *G. okiensis* was monophyletic with high bootstrap values (99% for ML and 97% for MP). The *rbc*L ML tree had a similar topology to the concatenated tree in having close relationships between *G. okiensis* from Korean and Japan.

DISCUSSION

Our recent collections of *Griffithsia okiensis* from Korea correspond in their habit and vegetative and reproductive structures to the description of Kajimura (1982, 1989). *Griffithsia okiensis* is smaller in thalli height (0.3-1 cm), diameter of vegetative cells (50-500 μm), and the ratio of cell length/breadth (2-3 times) than those of

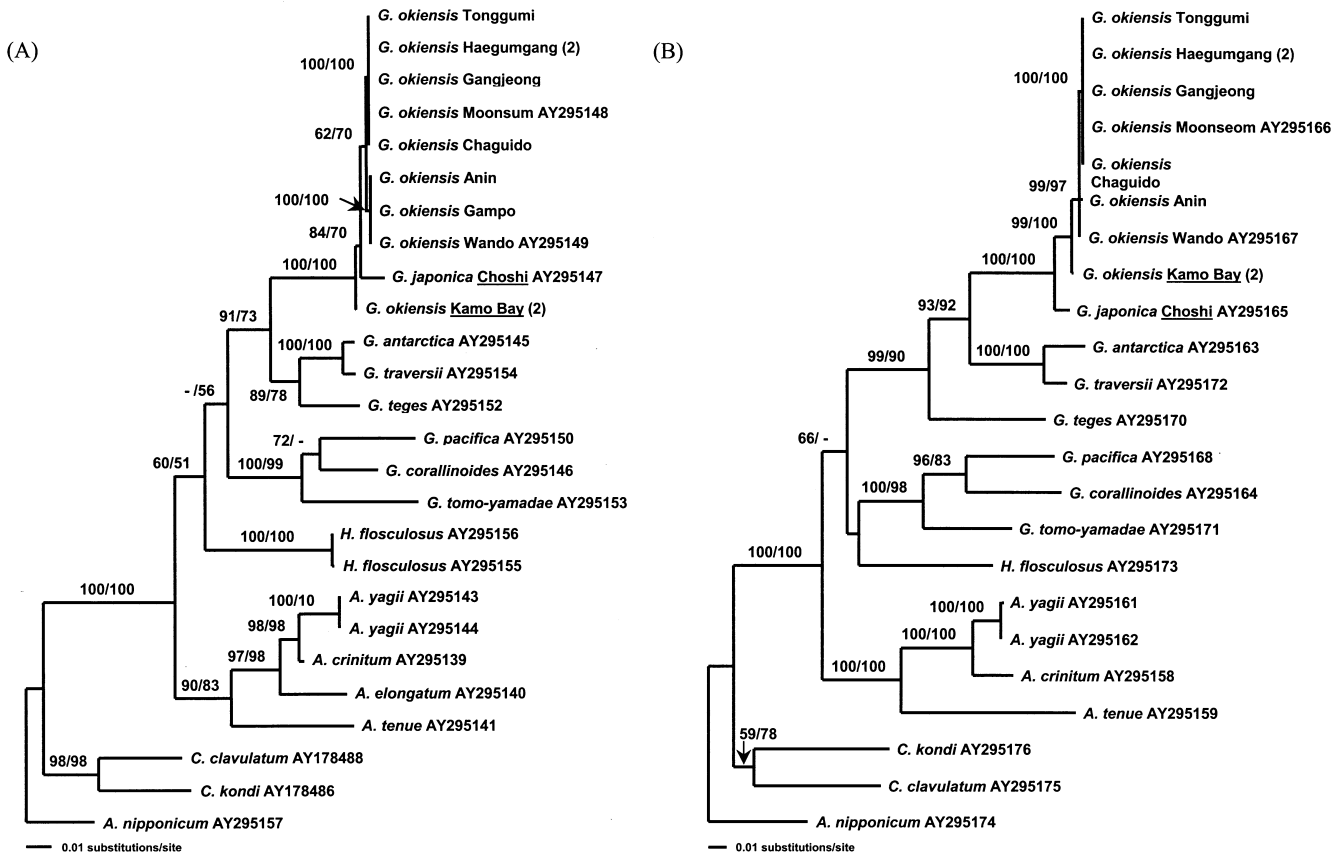

Fig. 6. Maximum likelihood trees for *Griffithsia okiensis* and relatives using GTR + Γ model for (A) *psb*A sequences [-Log likelihood = 3734.296; A ↔ C = 0.5626, A ↔ G = 5.8843, A ↔ T = 9.804, C ↔ G = 0.6269, C ↔ T = 32.5359, G ↔ T = 1; Γ = 0.175; A = 0.2547, C = 0.1925, G = 0.2003, T = 0.3524]. (B) *rbc*L sequences [-Log likelihood = 7106.292; A ↔ C = 1.3513, A ↔ G = 4.7034, A ↔ T = 4.5312, C ↔ G = 1.727, C ↔ T = 14.9034, G ↔ T = 1; Γ = 0.2023; A = 0.3216, C = 0.161, G = 0.2067, T = 0.3107]. The bootstrap values for each gene are calculated from 500 bootstrap resamplings with either the ML method using the GTR + Γ + I model and 1,000 with MP method (ML/MP).

Table 2. Absolute sequence divergence of *psb*A (the lower-left matrix) and *rbc*L (the upper-right matrix) sequences between *Griffithsia okiensis* (Korean population), *G. japonica*, and *G. okiensis* (Japan Population). Numeral in parentheses is the number of samples. Type locality of the species is underlined.

Taxa			1	2	3	4	5	6	7	8	9	10	11
1.	*G. okiensis*	Moonsum	-	0	0	0	0	2	-	1	7	7	29
2.		Tonggumi	0	-	0	0	0	2	-	1	7	7	29
3.		Haegumgamg (2)	0	0	-	0	0	2	-	1	7	7	29
4.		Gangjeong	0	0	0	-	0	2	-	1	7	7	29
5.		Chaguido	1	1	1	1	-	2	-	1	7	7	29
6.		Anin	1	1	1	1	2	-	-	1	7	7	29
7.		Gampo	1	1	1	1	2	0	-	-	-	-	-
8.		Wando	1	1	1	1	2	0	0	-	6	6	28
9.		Kamo Bay	3	3	3	3	4	4	4	4	-	-	24
10.		Kamo Bay, cave	3	3	3	3	4	4	4	4	0	-	24
11.	*G. japonica*	Choshi	10	10	10	10	11	11	11	11	11	11	-

G. japonica (3-6 cm, 575-920 μm, and 2-6 times, respectively). In addition, *G. okiensis* has two carpogonial branches from the supporting cell, while *G. japonica* has one carpogonial branch (Itono 1977; Kajimura 1982, 1989). The procarpic structures are consistent ontogenetically in most species of the tribe Griffithseae (Baldock 1976; Kim and Lee 1987; Boo and Cho 2001). There have been reported three types of procarp: the first type is reported

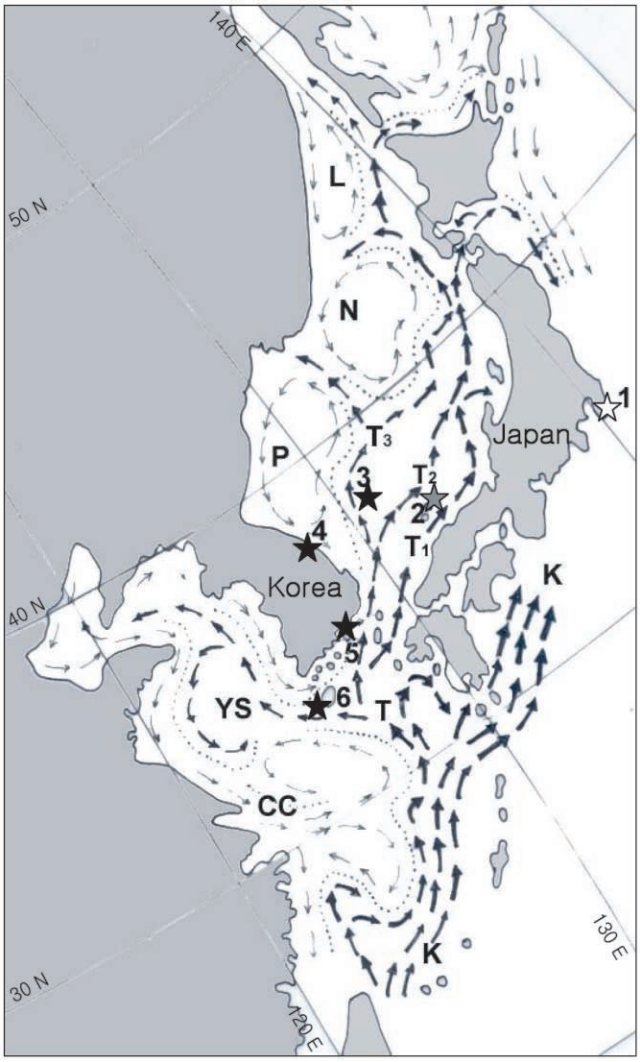

Fig. 7. Distribution of *Griffithsia okiensis* (Korean types = dark stars; Japanese type = gray star) and *G. japonica* (white star) and current systems in Korea and Japan (modification from Nishimura 1983). CC = Chinese coastal currents, K = Kuroshio currents, N = Liman current, P = Primorskoi currents, T = Tsushima (T1=coastal branch, T2 and T3= offshore branches), YS = Yellow Sea Currents.

in *G. corallinoides*, which produces three periaxial cells from subapical cell of fertile axis and two carpogonial branches arise from each other of two supporting cells (Kylin 1916, 1956). The second type is reported in *G. monilis* var. cincta and *G. okinesis*, which produces two periaxial cells from subapical cell of fertile axis and two carpogonial branches arise from one supporting cell, as seen previous studies (Baldock 1976, Kajimura, 1982, 1989). The third type is reported in *Ossiella pacifica*, which produces two periaxial cells from subapical cell of fertile axis and two carpogonial branches arose from one supporting cell without supporting-sterile cell (Miller and Abbott 1997). The supporting-sterile cell developed into another carpogonial branch in *Ossiella pacifica*. Additionally, Itono (1977) reported three periaxial cells in procarps of *G. japonica* and *G. rhizophora* (= *G. heteromorpha* Kützing, see Abbott 1999). However, phylogentic importance of these procarpic types was not supported in previous molecular study by Yang and Boo (2004).

It is also interesting to note that *G. monilis* var. *cincta* Baldock has two carpogonial branches on a supporting cell and spermatangial fascicles enclosed by involucres, whereas *G. monilis* var. *monilis* has one carpogonial branch and naked spermatangial fascicles without involucres (Baldock 1976). Molecular analyses are expected to resolve a taxonomic query between both species that we issue here.

The morphological difference between *G. okiensis* and *G. japonica* is reflected in the *rbc*L and *psb*A sequences: both species differed by 10-11 nucleotides (1.06-1.17% divergence) in *psb*A sequences and by 28-29 nucleotides (2.03-2.1% divergence) in *rbc*L sequences. Such levels in *rbc*L are mostly higher than or equal to the interspecific variation in other ceramiaceous algae: 0.7-1.8% between *Ceramium secundatum* and *C. botrycarpum* Griffiths ex Harvey (Maggs *et al.* 2002), 1.2-3.8% among *C. codicola* J. Agardh, *C. interruptum* Setchell et Gardner, and *C. sinicola* Setchell et Gardner (Cho *et al.* 2003). This is the first report on the occurrence of *G. okiensis* outside Japan, corroborated by both morphological and molecular data. Since *G. okiensis* is very similar to *G. japonica* in habit, branching pattern and cystocarps, the thalli have probably been misidentified under the name of *G. japonica*. Detailed observations and molecular analyses of field-collected material will probably enable a more realistic evaluation of distribution of *G. okiensis* to be made in the northwest Pacific.

It is very interesting to note that *G. okiensis* from Korea and Japan differed by 3-4 nucleotides (0.32-0.43% sequence divergence) in *psb*A and by 6-7 nucleotides (0.44-0.56% sequence divergence) in *rbc*L, despite low sequence difference (0% to 0.21% in *psb*A and 1-2 nucleotides in *rbc*L) within samples from Korea. However, samples from Korea and Japan were morphologically undistinguishable. Molecular variation but morphological similarity between *G. okiensis* from Korea and Japan may imply that *G. okiensis* is in the early stages of speciation. Both *rbc*L and psaA data suggest that the current distribution of *G. okiensis* may follow Tsushima currents, which flows up from Korea to Japan (Fig. 7).

Despite many previous reports for the occurrence of *G. japonica* in Korea (see review by Lee & Kang 2001), we found no evidence for the occurrence of the species in Korea in the present study. The results cast a question on the occurrence of *G. japonica* in China, Vietnam (Pham-Hoang 1969), and Solitary Island in Australia (Miller 1998), as well as Japan (Yoshida 1998). For example, the Chinese alga under the name of *G. japonica* (Tseng 1941) is questioned and looks *G. okiensis* because of its small size and its distribution on the South China Sea, which is near Jeju, Korea.

Both *psb*A and *rbc*L trees reveal the sister relationship between *G. okinesis* and *G. japonica*. This relationship may be reflected in the Kuroshio currents. The main Kuroshio current flows from the South China Sea to Pacific Ocean side of Japan, where *G. japonica* is distributed. Meanwhile, the Tsushima current flows from southern Korea to the western parts of Japan, where *G. okiensis* occur. However, the species from Korea and Japan is under different branches of Tsushima currents (Fig. 7, Nishimura 1983).

ACKNOWLEDGEMENTS

We thank Drs Muneo Kajimura for help with the collection and valuable comments. Drs. Ga Youn Cho and Il Ki Hwang helped us with collection. This study was supported by KRF grant 2002-070-C00083 to SMB and by academic research grant of Kangnung Nat. Univ. to HSK (2005).

REFERENCES

Abbott I.A. 1999. *Marine Red Algae of the Hawaiian Islands*. Bishop Museum Press, Honolulu.

Baldock R.N. 1976. The Griffithsieae group of the Ceramiaceae (Rhodophyta) and its southern Australian representatives. *Aust. J. Bot*. **24:** 509-593.

Boo S.M. and Cho T.O. 2001. The morphology of *Griffithsia tomo-yamadae* Okamura (Ceramiaceae, Rhodophyta): a little-known species from the northeast Pacific. *Bot. Mar*. **44:** 109-118.

Cho T.O., Fredericq S. and Boo S.M. 2003. *Ceramium inkyuii* sp. nov. (Ceramiaceae, Rhodophyta) from Korea: a new species based on morphological and molecular evidence. *J. Phycol*. **39:** 236-247.

Freshwater D.W. and Rueness J. 1994. Phylogenetic relationships of some European *Gelidium* (Gelidiales, Rhodophyta) species based on *rbc*L nucleotide sequence analysis. *Phycologia* **33:** 187-194.

Gavio B. and Fredericq S. 2002. *Grateloupia turuturu* (Halymeniaceae, Rhodophyta) is the correct name of the non-native species in the Atlantic known as *Grateloupia doryphora*. *Eur. J. Phycol*. **37:** 349-360.

Itono H. 1977. Studies on the ceramiaceous algae (Rhodophyta) from southern parts of Japan. *Bibl. Phycol*. **35:** 1-498.

Kajimura M. 1982. On a new species of *Griffithsia* (Rhodophyta, Ceramiaceae), *G. okiensis*, from Japan. *Mem. Fac. Sci. Shimane Univ*. **16:** 77-90.

Kajimura M. 1989. Morphological note on *Griffithsia okiensis* Kajimura (Ceramiaceae, Rhodophyta). *Mem. Fac. Sci. Shimane Univ*. **23:** 107-111.

Kang J.W. 1966. On the geographic distribution of marine algae in Korea. *Bull. Pusan Fish. Coll*. **7:** 1-125.

Kim H.S. and Lee I.K. 1987. Morphology and reproduction of *Griffithsia venusta* Yamada (Ceramiaceae, Rhodophyta). *Korean J. Phycol*. **2:** 51-60.

Kylin H. 1916. Die Entwicklungsgeschichte von *Griffithsia corallina* (Light.) Ag. *Zeitchr. Bot*. **8:** 97-123.

Kylin H . 1956. *Die Gattungen der Rhodophyceen*. CWK Gleerups Forlag, Lund.

Lee Y.P. and Kang S. 2001. *A Catalogue of the Seaweeds in Korea*. Jeju University Press, Jeju.

Lin S.M., Fredericq S. and Hommersand M.H. 2001. Systematics of the Delesseriaceae (Ceramiales, Rhodophyta) based on large subunit rDNA and *rbc*L sequences, including the Phycoryoideae, subfam. nov. *J. Phycol*. **37:** 881-899.

Maggs C.A., Ward B. A., McIvor L.M., Evans C.M., Rueness J. and Stanhope M. J. 2002. Molecular analyses elucidate the taxonomy of fully corticated, non-spiny species of *Ceramium* (Ceramiaceae, Rhodophyta) in the British Isles. *Phycologia* **41:** 409-420.

Miller A.J.K. 1998. *Marine algae of the northern section of the Solitary Islands Marine Park*. Report No. 13. Sydney, NSW.

Miller A.J.K. and Abbott, I.A. 1997. The new genus and species *Ossiella pacifica* (Griffithsieae, Rhodophyta) from Hawaii and Norfolk Island, Pacific Ocean. *J. Phycol*. **33:** 88-96.

Nishimura S. 1983. Okhotsk Sea, Japan Sea, East China Sea, In: Ketchum, B.H. (ed.), *Ecosystems of the world 26. Estuaries and enclosed seas*. Elsevier Sci. Pub. Co. New York. pp. 375-401.

Okamura K. 1930. Icones of Japanese algae. 6: 28-30. Tokyo.

Pham-Hoang H. 1969. *Marine algae of south Vietnam*. Trung-Tàm Hoc-Liêu Xuât-Bán.

Posada D. and Crandall K.A. 1998. MODELTEST: testing the model of DNA substitution. *Bioinformatics* **14:** 817-818.

Seo K.S., Cho T.O., Park J.S., Yang E.C., Yoon H.S. and Boo S.M. 2003. Morphology, basiphyte range, and plastid DNA phylogeny of *Campylaephora borealis* stat. nov. (Ceramiaceae, Rhodophyta). *Taxon* **52:** 9-19.

Swofford D.L. 2002. *PAUP*: Phylogenetic Analysis Using Parsimony (* and other methods). Version 4.0b8*. Sinauer Associates, Sunderland, MA.

Tseng C.K. 1941. Studies on Chinese species of *Griffithsia*. *Pap. Michigan Acad. Sci. Arts Letters* **27:** 105-116.

Waaland S.D. and Cleland R.E. 1974. Cell repair through cell fusion in the red alga *Griffithsia pacifica*. *Protoplasma* **79:** 185-196.

Womersley H.B.S. 1998. *The Marine Benthic Flora of Southern Australia, Rhodophyta. Part III*C. State Herbarium of South Australia, Richmond, South Australia.

Yang E.C. and Boo S.M. 2004. Evidence for two independent lineages of *Griffithsia* (Ceramiaceae, Rhodophyta) based on plastid protein-coding psaA, *psb*A, and *rbc*L gene sequences. *Mol. Phylogen. Evol*. **31:** 680-688.

Yoon H.S., Hackett J.D. and Bhattacharya D. 2002. A single origin of the peridinin- and fucoxanthin-containing plastids in dinoflagellates through tertiary endosymbiosis. *Proc. Nat. Acad. Sci. U. S. A*. **99:** 11724-11729.

Yoshida T. 1998. *Marine algae of Japan*. Uchida Rokakuho Publishing, Tokyo.

Submitted 27 January 2006
Accepted 17 February 2006