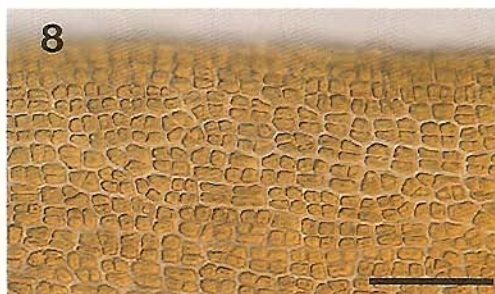
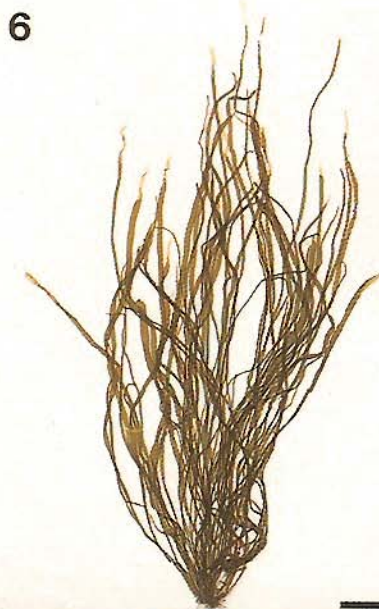


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## Volume 17 Number 3 September 2002

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## First Description of *Petalonia zosterifolia* and *Scytosiphon gracilis* (Scytosiphonaceae, Phaeophyceae) from Korea with Special Reference to nrDNA ITS Sequence Comparisons

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Scytosiphonaceae is an ectocarpalean brown algal family, that is a recent focus of systematics and marine biodiversity. We describe *Petalonia zosterifolia* and *Scytosiphon gracilis* from Korea for the first time. *P. zosterifolia* occurred on the East coast, and had flat, linear and solid thalli. *S. gracilis* was found in Jeju, and had cylindrical to flat and hollow thalli. However, these two species are so similar that it is difficult to identify by morphology alone. In order to determine if the nuclear DNA reveals the distinctness of both species and to know their phylogenies, the ITS region sequences were newly determined in 22 samples of *P. zosterifolia*, *Scytosiphon gracilis*, and other three members of the genera from Korea. We found 0.12% variation among samples of *P. zosterifolia* from different locations, and no variation between *S. gracilis* samples from different years, but extensive interspecific divergences (13.62-22.83%) of each species to other members in *Petalonia* and *Scytosiphon*. The ITS sequence data consistently showed a close relationship between *P. zosterifolia* and *S. gracilis*. This result is congruent with morphology and with the published data of plastid *rbc* and partial nrDNA large subunit gene sequences, and suggests that *P. zosterifolia* and *S. gracilis* might have diverged from the most recent common ancestor.

**Key Words:** ITS region, morphology, *Petalonia zosterifolia*, Phaeophyceae, phylogeny, *Scytosiphon gracilis*, Scytosiphonaceae, taxonomy

### INTRODUCTION

It is generally realized that the biodiversity from brown algae has been underestimated (Peters and Burkhardt 1998). Because of the limited taxonomic characters due to simple morphology, the ectocarpalean families are focus of taxonomy and biodiversity. Scytosiphonaceae, recently altered in rank to a family of the order Ectocarpales from Scytosiphonales (Rousseau and de Reviere 1999; Draisma *et al.* 2001; Peters and Ramirez 2001). It is distinguished by a single plastid bearing a single pyrenoid per each cortical cell, and a heteromorphic life cycle in which a parenchymatous, erect gametophytic thallus alternates with a pseudoparenchymatous, prostrate sporophytic crust (Wynne 1969; Kogame *et al.* 1999). The family in the north Pacific includes five genera: *Colpomenia* (Endlicher) Derbès et Solier in Castagne, *Hydroclathrus* Bory, *Petalonia* Derbès et Solier in Castagne, *Rosenvingea* Borgesen, and *Scytosiphon* C.

Agardh. Of these, some *Petalonia* is similar to *Scytosiphon* in morphology of erect gametophytic thallus (Rosenvinge and Lund 1947). Both genera are related to each other in the plastid *rbcL*, partial *rbcS*, partial nuclear ribosomal DNA 28S (Kogame *et al.* 1999), and the RuBisCo spacer region (Cho *et al.* 2001).

The genus *Petalonia* is characterized by flattened thalli, sori without unicellular paraphyses, and rare to occasional phaeophycean hairs. The genus includes only four species: *P. binghamiae* (J. Agardh) Vinogradova, *P. fascia* (O.F. Muller) Kuntze, *P. filiformis* (Batters) Kuntze, and *P. zosterifolia* (Reinke) Kuntze (Wynne 1969; Fletcher 1987). The genus *Scytosiphon* is distinguished by cylindrical to compressed hollow thalli, sori with unicellular paraphyses, and abundant phaeophycean hairs (Kogame 1998), and consists of seven species; *S. canaliculatus* (Setchell and Gardner) Kogame, *S. complanatus* (Rosenvinge) Doty, *S. crispus* Skottsberg, *S. dotyi* Wynne, *S. gracilis* Kogame, *S. lomentaria* (Lyngbye) Link, and *S. tenellus* Kogame (Wynne 1969; Kogame 1998). Prior to this study, only *P. binghamiae*, *P. fascia*, and *S. lomentaria* have been reported from Korea.

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**Table 1.** Information of taxa included in this study

Species	Collection site and date	GenBank accession number
<i>Petalonia binghamiae</i>	Munseom, Jeju, Korea; 15 Jan. 1997	AY154720
	Sacheon, Gangreung, Korea; 25 Dec. 1996	AY154721
	Shaw's Cove 1, California, USA; 3 Dec. 1999	AY154722
	Shaw's Cove 2, California, USA; 3 Dec. 1999	AY154723
	Shimoda, Shikanoshima, Japan; 8 Jan. 1996	AY154724
<i>P. fascia</i>	Avacha Bay, Kamchatka, Russia; 23 Jul. 1998	AY154725
	Awaji, Hyogo Pref., Japan; 10 Jan. 1998	AY154726
	Guryongpo, Pohang, Korea; 16 Nov. 1997	AY154727
	Onyang, Uljin, Korea; 30 Jan. 2002	AY154728
	Sacheon, Gangreung, Korea; 25 Dec. 1996	AY154729
	Seal Rock, Oregon, USA; 10 Jul. 1998	AY154730
	Sinnam, Samcheok, Korea; 12 Jan. 2002	AY154731
	Anin, Gangreung, Korea; 12 Jan. 2002	AY154732
<i>P. zosterifolia</i>	Ayajin, Sokcho, Korea; 30 Jan. 2002	AY154733
	Hupo, Uljin, Korea; 30 Jan. 2002	AY154734
	Guryongpo, Pohang, Korea; 30 Jan. 2002	AY154735
	Nameae, Gangreung, Korea; 30 Jan. 2002	AY154736
	Onyang, Uljin, Korea; 12 Jan. 2002	AY154737
	Sinnam, Samcheok, Korea; 12 Jan. 2002	AY154738
	Hado, Jeju, Korea; 4 Feb. 2000	AY154739
<i>Scytosiphon gracilis</i>	Hado, Jeju, Korea; 10 Feb. 2002	AY154740
	Hado, Jeju, Korea; 4 Feb. 2000	AY154741

During the continuing survey of the Scytosiphonaceae in Korea, we encountered thalli of *P. zosterifolia* on the East coast and *S. gracilis* in Jeju. The goal of the present paper is to introduce *P. zosterifolia* and *S. gracilis* using our recent collections and to understand phylogeny of both species as inferred from comparisons of nuclear DNA sequences. Since *P. zosterifolia* and *S. gracilis* are very similar to each other, we compared sequences from replicate samples of each species collected from different locations. Because internal transcribed spacer (ITS) region in nuclear ribosomal DNA has been used for species identification and inference of phylogeny of congeneric species in a diverse range of brown algae (Saunders and Druehl 1993; Peters *et al.* 1997; Stache-Crain *et al.* 1997; Serrão *et al.* 1999; Yoon *et al.* 2001), we used ITS region to recognize *P. zosterifolia* and *S. gracilis* from related species and to understand their relationships. In addition to these species, we determined the ITS region sequences of other species from Korea, *P. binghamiae*, *P. fascia*, and *S. lomentaria*. However, because *Colpomenia*, *Hydroclathrus*, and *Rosenvingea* were distantly related to *Petalonia* and *Scytosiphon* in previous molecular phylogenies (Kogame *et al.* 1999; Cho *et al.* 2001), the three genera were excluded from taxon sampling.

## MATERIALS AND METHODS

**Morphology:** Thalli of *Petalonia zosterifolia* and *Scytosiphon gracilis* were collected from the upper to the middle intertidal zone at several places in Korea. Material was preserved in 4% formaldehyde-seawater for further observation. The preserved material was stained with 1% aqueous aniline blue acidified with dilute HCl for light microscopy. Photographs were taken with a camera attached to a Vanox light microscope (Olympus Optical Co., Tokyo). All specimens are deposited in the herbarium of Chungnam National University (CNUK), Daejeon, Korea.

**Analysis of sequences of nrDNA ITS region:** For extraction of DNA, all 22 samples (Table 1) were collected in the field, dried in air, and preserved with silica crystals.

Genomic DNA was extracted from approximately 0.005 g powder ground in liquid nitrogen using the DNeasy Plant Mini Kit (Qiagen GmbH, Hilden, Germany), according to the manufacturer's instructions, and then dissolved in 150  $\mu$ L DW. Polymerase chain reactions (PCR) of the ITS region and sequencing reactions using purified PCR products followed Yoon *et al.*

(2001). The PCR primers were LB1 and LB2 and the sequencing primers were LB1, LB2, JO6, and YB1 (Yoon *et al.* 2001). Sequences of the forward and reverse strands were determined for all taxa using an ABI PRISM™ 377 DNA Sequencer (Foster City, CA). Both electropherogram outputs for each sample were checked using the program Sequence Navigator v. 1.0.1 (Applied Biosystems Inc. CA). All the sequences we determined here were included in the alignment and were aligned visually using SeqPup, a multisequence editing program (Gilbert 1995) by comparing published sequences of *S. lomentaria* from Japan (Kawai *et al.* 1995).

Maximum-parsimony (MP) and neighbor-joining (NJ) methods were used to analyze a data matrix of 1108 aligned positions. All characters were equally weighted. Because of a big size difference between ITS 1 and ITS 2, we analyzed ITS 1 and ITS 2, respectively. Since it is also generally accepted that sequence gaps can sometimes provide significant phylogenetic signals, two different analyses were compared: one treated gaps as missing data and the other as a fifth base. MP analysis (PAUP\* v4.0b8, Swofford 2001) was conducted using heuristic search algorithm with the following options: 10 random additions, tree bisection-reconnection branch-swapping, MulTrees, and branches with a maximum length of zero collapsed to yield polytomies. Other details followed our previous analyses (Cho *et al.* 2001; Yoon *et al.* 2001). A distance tree was constructed with the NJ method (PAUP\*). Bootstrap analyses were undertaken with 2000 replicates using the same parameters for the MP and NJ analysis.

## RESULTS

### *Petalonia zosterifolia* (Reinke) Kuntze (1898): 419

Figs 1-5

Homotypic synonym: *Phyllitis zosterifolia* Reinke (1889): 61

Heterotypic synonym: *Ilea facia* f. *zosterifolia* Setchell and Gardner (1925): 537

Type: KIEL, Botanisches Institut der Universitat Kiel, Kiel

Type locality: Kieler Hafen, Germany

Distribution: Atlantic (France, Spain, and UK) and Pacific (China, Japan, and Korea)

Korean name: Miyeoksil

Representative specimens examined: Ayajin, Sokcho, (Boo & Yang, CNUK 007390 - 007398, 30.i.2002); Namae; Kangreung (Boo & Yang, CNUK 007383 - 007389,

30.i.2002); Anin, Kangreung (Boo & Yang, CNUK 007378 - 007379, 12.i.2002, CNUK 007380 - 007382, 30.i.2002); Sinnam, Samcheok (Boo, Cho & Yang, CNUK 007376 - 007377, 12.i.2002); Onyangri, Uljin (Boo, Cho & Yang, CNUK 007372 - 007375, 12.i.2002); Hupo, Uljin (Boo & Yang, CNUK 007369 - 007371, 31.i.2002); Wolpo, Heunghae (Boo & Yang, CNUK 007365 - 007368, 31.i.2002); Guryongpo, Pohang, East coast (Boo & Yang, CNUK 007360 - 007364, 31.i.2002).

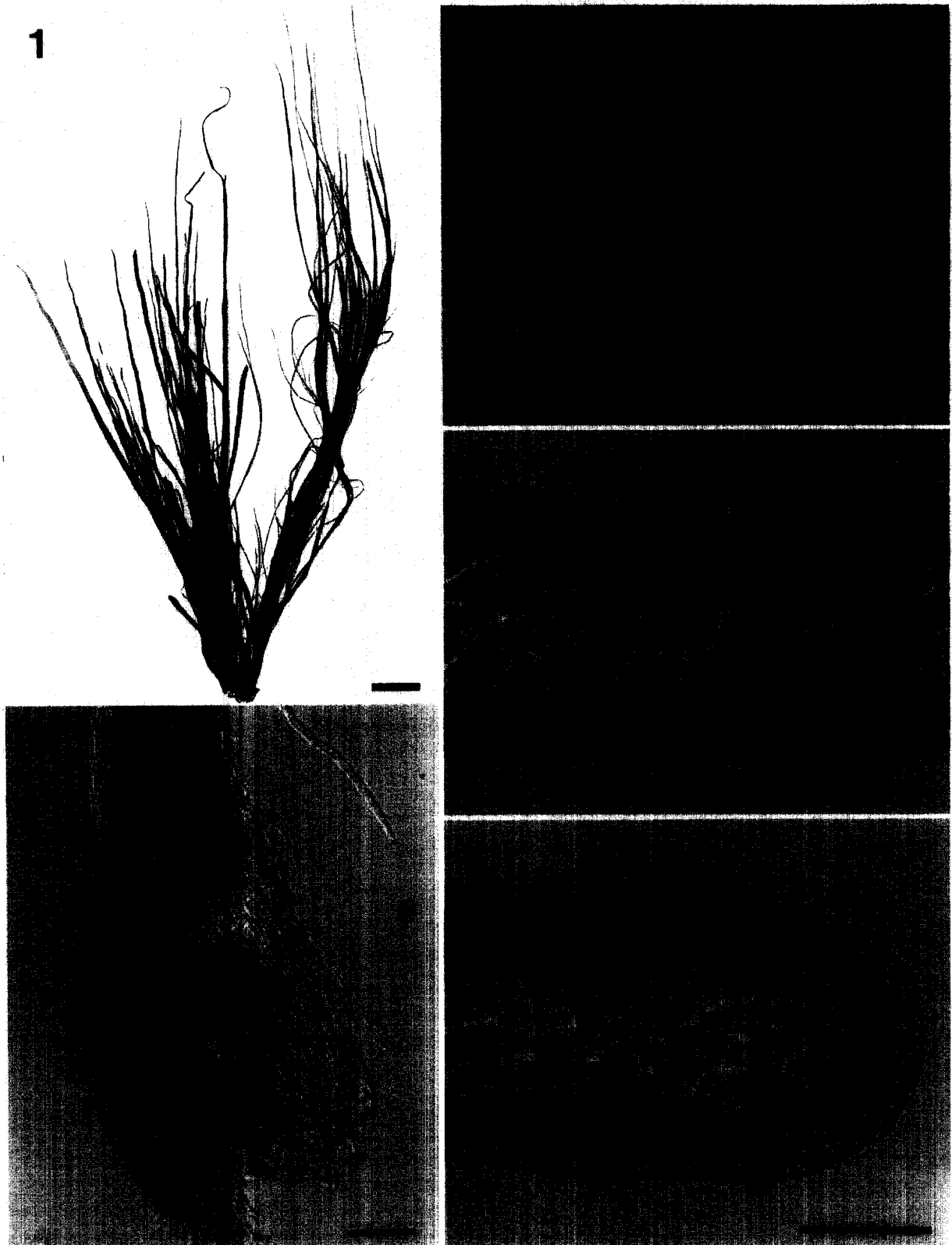
Morphology: Thalli were erect, flattened, unbranched, sometimes twisted in the upper part of narrow thalli, 18-(29) cm long and up to 0.5 cm in width. Rhizoids were developed from the outer cortical cells near the base of the thallus and produced a mat of basally formed, branched filaments.

The thalli were solid, sometimes hollow with small central cavities, and 80-120  $\mu\text{m}$  in thickness. The tissues were parenchymatous and consisted of an outer cortex and medulla. In the middle part, medulla was composed of 2-4 layers of cells without plastids. Medullary cells were round to oval and c. 50 x 24  $\mu\text{m}$  in transverse section. The cortex consisted of 1-3 layers of small, angular to rectangular cells. Each cortical cell had a plastid with a prominent pyrenoid.

Plurilocular sporangia were developed from the surface of the upper parts of thalli and matured basipetally. The sporangia were closely packed and c. 28  $\mu\text{m}$  (4-7 loculi) in heights. Phaeophycean hairs, arising from surface cells, were common in broad thalli and rare in narrow thalli. Unilocular sporangia were not found.

*Petalonia zosterifolia* is epilithic on rock, growing in the upper intertidal zone of exposed locations. Erect thalli occurred often mixed with *S. lomentaria* and were abundant in January to March from Ayajin to Guryongpo on the East coast. Considering that mature thalli were found in the early January, it is probable that they begin to occur from the late fall. Prostrate *Composnema*-type thalli (Kogame and Kawai 1993), which have unilocular sporangia and are considered gametophytes of *P. zosterifolia*, were not found. *P. zosterifolia* was more abundant in the northern part (e.g. Ayajin) than the southern part (e.g. Guryongpo) on the East coast.

Taxonomic remarks: Our collections of *P. zosterifolia* correspond in their habit and anatomy of gametophytic thallus to the descriptions of Reinke (1889) and other previous authors (Rosenvinge and Lund 1947; Dangeard 1962; Fletcher 1987; Kogame and Kawai 1993). *P. zosterifolia* is distinguished by a flattened, linear, twisted, and solid thallus. *P. fascia* is similar to *P. zosterifolia*, but is



**Figs 1-5.** *Petalonia zosterifolia*.

1. Thallus collected on 12 January 2002 from the upper intertidal zone of Onyangri, Jukbyun on the East coast. scale bar = 2 cm.
2. Rhizoidal filaments at the basal part of thallus. scale bar = 100  $\mu\text{m}$ .
3. Small cortical cells on the surface of thallus.
4. Cross section of the middle axis showing medulla and cortex.
5. Cross section of the middle axis showing plurilocular sporangia and medullary cells. scale bars 3- 5 = 50  $\mu\text{m}$ .

characterized by broad thalli (up to 3.5 cm in width) and the presence of rhizoidal cells in medulla (Kogame 1997). *P. binghamiae* is circumscribed by a small holdfast of rhizoid and numerous rhizoidal cells in medulla (Rhew and Boo 1991).

*P. zosterifolia* appears to occur in winter, as does in Japan (Kogame and Kawai 1993). From our survey, Guryongpo appears the southern distribution of that species in Korea. This is the first report on the occurrence of *P. zosterifolia* in Korea, recognized by both morphology and ITS region sequence data as discussed below.

### *Scytosiphon gracilis* Kogame (1998): 39

Figs 6-10

Type: SAP 059720 (collected in 1 February 1990).

Type locality: Ohma, Aomori, Japan

Distribution: Japan and Korea

Korean name: Ganeunmiyeoksil

Representative specimens examined: Hado, Jeju (Boo, CNUK 007399 - 007405, 4.ii.2000; CNUK 007406 - 007498, 10.ii.2002).

Morphology: Thalli were erect, flattened, unbranched, hollow, twisted in the upper part, 14-(25) cm long and up to 0.4 cm in width (Fig. 5). Rhizoids were developed from the outer cortical cells near the base of the thalli and were multiseriate filaments (Fig. 6).

The thalli were hollow in mature and 70-115  $\mu\text{m}$  in thickness. The tissues were parenchymatous and consisted of an outer cortex and medulla. In the middle part, medulla was composed of 1-3 layers of cells without plastids. Medullary cells were round to oval and c. 46 x 36  $\mu\text{m}$  in transverse section. The cortex consisted of 1-3 layers of small, angular to rectangular cells. Each cortical cell had a plastid with a prominent pyrenoid.

Plurilocular sporangia were developed from the surface of the upper parts of thalli and matured basipetally. Plurilocular sporangia were closely packed. Phaeophyceyan hairs were solitary or grouped on the surface. Ascocysts between unilocular sporangia were absent.

*Scytosiphon gracilis* was epilithic on concrete block and rock, growing in the middle to the lower intertidal zone in relatively sheltered areas. Erect thalli occurred in February in Hado on the east side of Jeju. Prostrate sporophytes of *S. gracilis* (Kogame 1998) were not found.

Taxonomic remarks: Our collections of *S. gracilis* from Jeju correspond to the original descriptions of Kogame (1998) and specimens from Aomori and Hyogo Prefecture in Japan collected by Kazuhiro Kogame, the

author of the original paper, and deposited in CNUK. *Scytosiphon lomentaria* is similar to *S. gracilis*, but is distinguished by constricted thalli and *Microspongiium*-like sporophyte (Kogame 1998). *S. tenellus* Kogame, which is reported only in Hokkaido of Japan, differs from our species in having ascocysts between plurilocular sporangia of erect thalli and *Stragularia*-like, crust sporophyte (Kogame 1998). The last species of *Scytosiphon* in Japan is *S. canaliculatus* (Setchell and Gardner) Kogame, which is characterized by *Hapterophycus*-like sporophyte (Kogame 1998).

Although *S. gracilis* was included in our RuBisCo spacer region phylogeny (Cho et al. 2001), this is the first morphological description of the species in Korea. *S. gracilis* may be a winter species in Korea, as in Japan (Kogame 1998). Considering its occurrence from the northern Hokkaido to the southern Fukuoka in Japan (Kogame 1998), detailed collections in the field may probably extend the distributional areas in Korea.

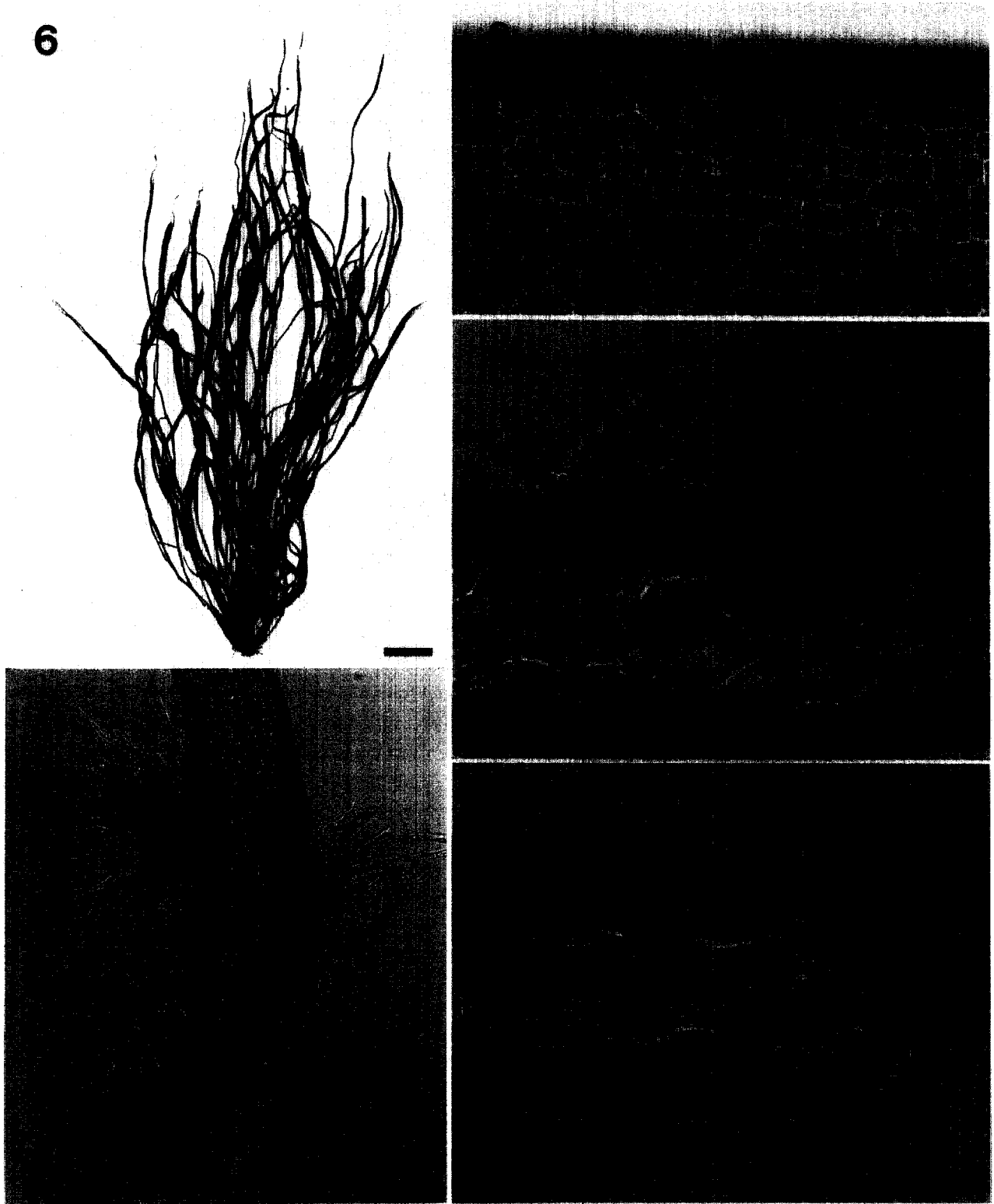
### Analysis of ITS region sequences

The entire ITS region (ITS 1, 5.8S, and ITS 2) in 22 samples of five species ranged from 1069 bp in *Scytosiphon gracilis* to 823 bp in *Petalonia binghamiae*. The ITS 1 region ranged from 638 bp in *S. gracilis* to 385 bp in *P. binghamiae*. The ITS 2 region was much shorter than the ITS 1 region and ranged from 276 bp in *P. binghamiae* to 251 bp in *P. zosterifolia*. The 5.8S subunit sequence was almost conserved and was 162 bp long in all species determined here. A comparison of the ITS region in the Ectocarpales is shown in Table 2.

The pairwise divergences of the ITS region sequences averaged 0.12% among seven samples of *P. zosterifolia*, in Korea, and zero in between two samples of *S. gracilis* from different years. The pairwise divergence averaged 0.33% among five samples of *P. binghamiae* from the north Pacific, and 2.35% among seven samples of *P. fasciata* from the north Pacific and the north Atlantic. The pairwise divergence (22.83-22.43%) between *P. zosterifolia* and other *Petalonia* species was much higher than that (13.62%) between *P. zosterifolia* and *S. gracilis*. In addition, *S. gracilis* differed from *S. lomentaria* by 31.78% in pairwise divergence.

The NJ tree (Fig. 11) showed that seven samples of *Petalonia zosterifolia* from different locations and two samples of *Scytosiphon gracilis* from different years produced a single clade, respectively. These two species were again grouped into a monophyletic clade with a maximum support (Bootstrap value = 100%), which

6



**Figs 6-10.** *Scytosiphon gracilis*.

6. Thallus collected on 4 February 2000 from the intertidal zone of Hado, Jeju. scale bar = 1 cm. 7. Rhizoidal filaments at the basal part of thallus. scale bar = 100  $\mu\text{m}$ . 8. Rectangular cortical cells on the surface of thallus. 9. Cross section of the middle axis showing phaeophycecan hairs, cortical cells, and medullary cells. 10. Cross section of the middle axis showing a large cavity in medulla. scale bars 8-10 = 50  $\mu\text{m}$ .



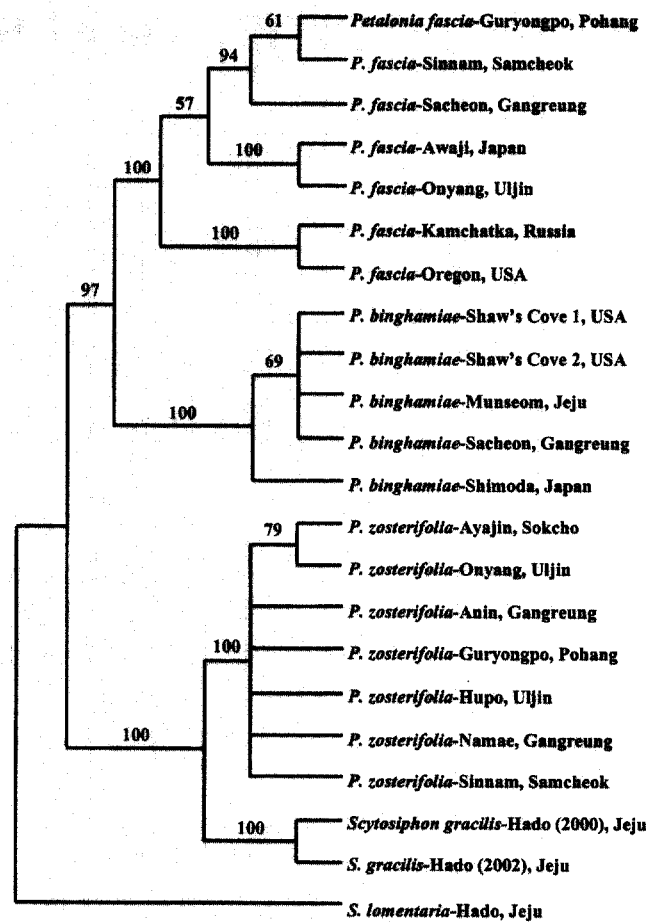
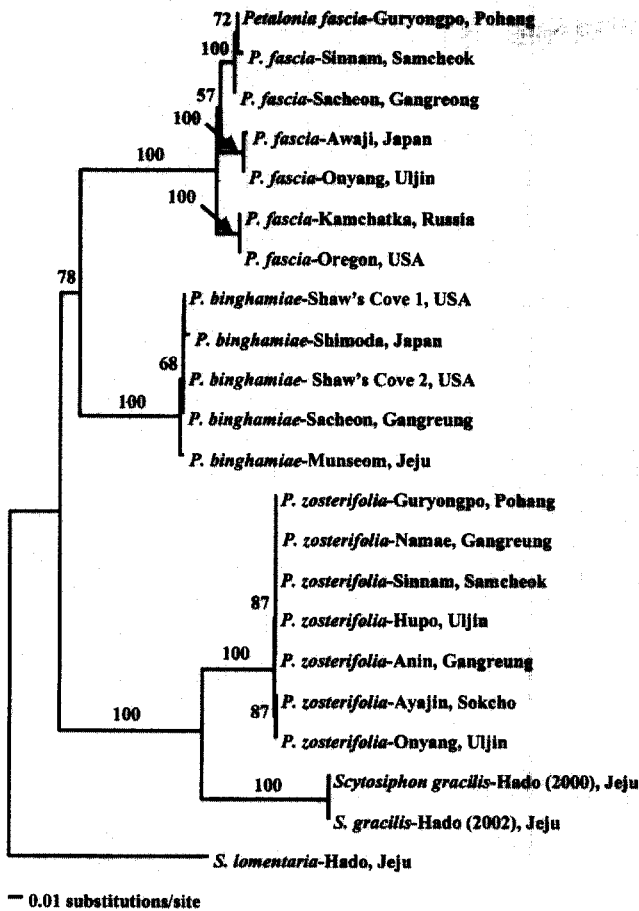


Fig. 11. Neighbor-joining tree for *Petalonia zosterifolia* and *Scytosiphon gracilis* rooted with *S. lomentaria*. Bootstrap values (> 50%) are given above branches.

Fig. 12. Strict consensus of four maximum parsimonious trees for *Petalonia zosterifolia* and *Scytosiphon gracilis* rooted with *S. lomentaria*, with gaps treated as missing data. The tree length was 648 steps, the consistency index was 0.9, and the retention index was 0.966. Bootstrap values (> 50%) are given above branches.

showed sister relationships to the clade of *Petalonia binghamiae* and *P. fascia*. The tree topology of the MP analysis showed a similar pattern to that of the NJ tree. The strict consensus of four most parsimonious trees when gaps were treated as missing data is shown in Figure 12. Despite the big difference in the length (Table 2), the ITS 1 tree was similar to that of the ITS 2 tree in topology and bootstrap support (trees not shown). When gaps were treated as a fifth base, the MP analyses produced four equally parsimonious trees (Fig. 12), having a minimal length of 1054 steps, a CI of 0.891, and a RI of 0.968 (Table 3).

## DISCUSSION

*Petalonia zosterifolia* is very similar to *S. gracilis* in habit and anatomy of the erect gametophytic thallus, and prostrate sporophytic crust (Kogame and Kawai 1993; Kogame 1998). The differences are structure of thallus

and the presence/absence of paraphysis. The thallus of *P. zosterifolia* is solid but, when mature, sometimes has small cavities in medulla. In contrast, the thallus of *S. gracilis* is hollow. However, Kogame et al. (1999) reported that whether erect thalli are hollow or solid does not appear to be useful for distinguishing *Petalonia* and *Scytosiphon*. Paraphyses are absent in *P. zosterifolia* (Rosenvinge and Lund 1947; Kogame and Kawai 1993), while unicellular paraphyses are present between unilocular sporangia in *S. gracilis* (Kogame 1998). Since paraphyses are consistently present in *S. gracilis*, the paraphysis may be an important criterion to recognize *S. gracilis* from *P. zosterifolia* (Kogame 1998). In this study, distribution ranges of *P. zosterifolia*, previously known in China (Tseng 1983), Japan (Kogame and Kawai 1993), USA (Setchell and Gardner 1925), and the Atlantic (Fletcher 1987), and *S. gracilis*, previously reported only

**Table 2.** Sizes of ITS 1, 5.8S and ITS 2 in four families of the Ectocarpales. Classification follows Peters and Ramirez (2001)

Taxa	ITS 1	5.8S	ITS 2	References
Adenocystaceae	326	nd	261	Peters and Burkhardt (1998)
Chordariaceae	425-695	162	253-485	Müller <i>et al.</i> (1998), Peters and Burkhardt (1998), Uwai <i>et al.</i> (2001)
Ectocarpaceae	314-862	159	252-289	Stache-Crain <i>et al.</i> (1997)
Scytosiphonaceae				
<i>Petalonia binghamiae</i>	385-387	162	276	present study
<i>P. fascia</i>	508-511	162	252-257	present study
<i>P. zosterifolia</i>	583	162	251	present study
<i>Scytosiphon gracilis</i>	638	162	269	present study
<i>S. lomentaria</i>	523	162	252	present study
<i>Colpomenia bullosa</i>	479	162	255	unpublished data

**Table 3.** Pairwise divergence matrix of the ITS region within each taxon (bold letters) and between taxa studied

Taxa	1 (n = 5)	2 (n = 7)	3 (n = 7)	4 (n = 2)	5
1. <i>Petalonia binghamiae</i>	<b>0.0033</b>				
2. <i>P. fascia</i>	0.1782	<b>0.0235</b>			
3. <i>P. zosterifolia</i>	0.2283	0.2643	<b>0.0012</b>		
4. <i>Scytosiphon gracilis</i>	0.2837	0.3157	0.1362	<b>0</b>	
5. <i>S. lomentaria</i>	0.2476	0.2931	0.3178	0.3357	-

**Table 4.** Sequence variation in the MP analyses of the ITS region sequences of 22 taxa when gaps were treated as missing data or a fifth base. MPTs: maximum parsimonious trees, CI: consistency index, and RI: retention index.

Characteristic	missing data			fifth base
	ITS 1	ITS 2	ITS region	ITS region
Constant sites (%)	317 (48.2)	191 (66.1)	669 (60.4)	521 (47.0)
Variable sites (%)	340 (51.8)	98 (33.9)	439 (39.6)	587 (53.0)
Informative sites (%)	274 (41.7)	90 (31.1)	365 (32.9)	546 (49.3)
Number of MPTs	14	6	4	4
Tree length	488	149	647	1,054
CI of consensus tree	0.930	0.852	0.9	0.891
RI of consensus tree	0.976	0.953	0.966	0.968

from Japan (Kogame 1998), are extended to Korea.

The ITS region sizes, pairwise divergences, and trees in our study well show the presence of both species in Korea, as do the RuBisCo spacer sequences (unpublished data). The sizes of the ITS 1 in *Petalonia zosterifolia* and *Scytosiphon gracilis* are characteristically longer than those of other scytosiphonian species. The ITS 1 of all the ectocarpalean members is longer than the ITS 2, as seen in Table 2, and the increased length of the ITS 1 in the Ectocarpales may be a synapomorphic character.

The pairwise divergence of the ITS region sequences

within *P. zosterifolia* from Korea is lower (average 0.12%) than 0.33% within *P. binghamiae* from the Pacific and 2.35% within *P. fascia* from the north Pacific to the Atlantic. Two samples of *S. gracilis* from the same location but different years show identical sequences. Therefore, the low infraspecific divergences in the two species included in our study are considered as a response of distance among geographic regions sampled. In contrast, the interspecific pairwise divergence was 13.62% between *P. zosterifolia* and *S. gracilis*, 17.82% between *P. binghamiae* and *P. fascia*, and 22.83-26.42%

between the species of *Petalonia*. The interspecific divergences in the Scytosiphonaceae appear to be lower than those (18-34%) in the genus *Elachista* Duby of the Chordariaceae (Uwai et al. 2001).

Phylogenetic trees from all our data sets, e.g. ITS 1, ITS 2, ITS region with gaps as missing data, and ITS region with gaps as a fifth character, consistently indicate the close relationship (BP = 100) between *P. zosterifolia* and *S. gracilis*, that is congruent with results of analyses of *rbcL*, partial *rbcS* and partial 18S of nuclear ribosomal DNA sequences (Kogame et al. 1999), and the RuBisCo spacer region sequences (unpublished data). The presence of prostrate *Compsoneima*-like thallus in both *P. zosterifolia* and *S. gracilis* is considered as a synapomorphic character (Kogame et al. 1999). Our molecular data together with morphological evidence suggest that *P. zosterifolia* and *S. gracilis* might have shared a common ancestor. Our ITS trees also show that *Petalonia zosterifolia* is clearly separated from other species of *Petalonia*. On the other hand, *P. binghamiae* and *P. fascia* are closely related to each other, sharing *Strangularia*-like sporophytic thalli (Kogame et al. 1999). In addition, *S. gracilis* is distantly related to *S. lomentaria*, having a *Microspongium*-like sporophyte. Kogame et al. (1999) suggested that prostrate sporophytes appear to be phylogenetically important. If this is true in the classification of the Scytosiphonaceae, the family needs a big change at genus level (Kogame et al. 1999; Cho et al. 2001) and the taxonomic revision of *P. zosterifolia* and *S. gracilis* should be followed.

The present study shows that two morphologically similar species of brown algae can be easily classified by the nrDNA ITS region sequences. The ITS region is very powerful for recognizing hidden diversity of simple-morphology brown algae e.g. the Ectocarpales and useful for studying biodiversity of other marine algae.

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