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# Phylogenetic Relationships of *Pelvetia* and *Pelvetiopsis* (Phaeophyceae) Based on Small Subunit Ribosomal DNA Sequences

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**Nucleotide sequences of the small-subunit (SSU) ribosomal DNA were determined for *Pelvetia babingtonii*, *P. canaliculata*, *Pelvetiopsis limitata*, and *Ascophyllum nodosum* in the family Fucaceae. A total of 1755 positions were aligned for the whole sequence. The positional differences in the primary structure among the taxa ranged from 16 to 30 nucleotide changes in pairwise comparisons. There was a minimum divergence between *Ps. limitata* and *P. babingtonii* while a maximum between *Ps. limitata* and *P. canaliculata*. The SSU rDNA trees showed that the genus *Pelvetia* was not monophyletic and the genus *Pelvetiopsis* was not closely related to *Pelvetia*. Our results suggest that the taxonomic revision of the genus *Pelvetia* as well as the family Fucaceae is needed based on detailed morphological observations.**

*Keywords*: *Pelvetia*, *Pelvetiopsis*, Phaeophyceae, phylogeny, SSU rDNA

The genus *Pelvetia* Decaisne et Thuret is typified by *P. canaliculata* (L.) Decaisne et Thuret (1845) from Europe and is distinguished by having stem-like blades and two functional eggs in a single oogonium. The genus includes three other species, which occur in the Pacific coast: *P. babingtonii* Yoshida et Silva (1992) from Japan, *P. compressa* (J. Agardh) De Toni (1895) from the west coast of North America, and *P. siliquosa* Tseng et Chang from China and Korea (Tseng, 1953). *P. canaliculata* differs from the Pacific species in divisional pattern of functional egg cells: the transversal division in the former and the longitudinal division in the latter (Gardner, 1913; Song *et al.*, 1996).

The genus *Pelvetiopsis* Gardner is monotypic based on *Ps. limitata*. The genus is characterized by having a single functional egg in an ogonium, but show few differences of external morphologies from *Pelvetia* (Gardner, 1913). This led Powell (1963) to suggest that *Pelvetiopsis* originated from the Pacific *Pelvetia* species on the accounts of the number of functional egg, while *Pelvetia* from *Fucus*. Clayton (1984), however, gave a phylogenetic inference that *Pelvetia*, *Pelvetiopsis*, and even *Fucus* evolved from a common hypothetical ancestor. Song *et al.* (1996) observed that the number of functional eggs and the divisional pattern were variable in *P. siliquosa* from Korea.

The small subunit (SSU) rDNA sequence data have contributed to address phylogenetic relationships in the brown algae (Saunders and Druehl, 1992; Tan and Druehl, 1993, 1996). The molecular data supported the traditional taxonomic system and also gave a new insight on phylogenetic relationships of the taxa studied. Saunders and Kraft (1995) showed that SSU rDNA sequence data were suitable for inferring phylogenetic relationship among the interfamilies of the fucalean algae. On the other hand, Rousseau *et al.* (1997) have studied molecular phylogeny of European Fucales based on large-subunit ribosomal DNA sequences.

This study is aimed to address the phylogenetic relationship and taxonomic position of *Pelvetia* and *Pelvetiopsis* using the molecular data. We compared here the whole sequences of SSU rDNA from *P. canaliculata* as the type species of the genus, *P. babingtonii* as the representative of Pacific species, and *Pelvetiopsis limitata*. *Ascophyllum nodosum* (L.) Le Jolis and *Fucus gardneri* Silva, which are related members in the family Fucaceae, were also treated in this study.

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## MATERIALS AND METHODS

Collection data of the fucacean algae used in this study are shown in Table 1. All the voucher specimens in this study have been kept in the herbarium of Chungnam National University.

Thalli dried in silica gel were washed by filtered seawater and distilled water before grinding. Total genomic DNA was extracted using CTAB buffer (Lee and King, 1996). Quality and quantity of genomic DNA were estimated on 0.8% agarose gels including EtBr. To amplify the SSU rDNA, polymerase chain reaction (PCR) was performed in an automatic thermocycler (MJ research, Watertown). Two primer pairs of SR1+SR7 and SR4+SR12 were used for each reaction (Nakayama *et al.*, 1996). PCR profile was as follows: an initial denaturation at 95°C for 2 min, repeated by 28 cycles of denaturation at 95°C for 1 min, annealing at 45°C for 30 sec, and extension at 72°C for 2 min, with the final extension for 10 min at 72°C. Reaction cocktail followed the recipe of Nakayama *et al.* (1996). A negative control without target DNA template was included in each set of reaction. To check the yield and size of amplified products, PCR products were separated on 0.8% agarose gels including EtBr through electrophoresis. Amplified DNAs in gel slices were purified by GeneClean Kit II (Bio 101, La Jolla, CA).

Direct sequencing of SSU rDNA was done following Tan and Druehl (1996). The nested primers newly designed in this study are in Table 2 and other sequencing primers are in Nakayama *et al.* (1996). Denatured DNAs at 95°C were labeled with $^{35}$S-dATP using Sequenase 2.0 DNA Sequencing Kit (United States Biochemicals, Cleveland). The DNA fragments were separated on 0.8% gels following the manufacturer's protocol. The gels were fixed for 15 min with the mixed solution (5% of glacial acetic acid and 15% of methanol), and autoradiographs were made after the exposure of 96 h.

**Table 1.** Sources of materials used for SSU rDNA sequences analyses

| Species | Locality of collection | Sources |
|---|---|---|
| *Ascophyllum nodosum* | Neeltjejans, Netherlands | This study |
| *Fucus gardneri* | Vancouver Island, Canada | Bhattacharya *et al.*, 1992 |
| *Pelvetia babingtonii* | Murroran, Hokkaido, Japan | This study |
| *Pelvetia canaliculata* | Isle of Man, Great Britain | 〃 |
| *Pelvetiopsis limitata* | Bamfield, Vancouver Island, Canada | 〃 |
| *Scytosiphon lomentaria* | Murroran, Hokkaido, Japan | Kawai *et al.*, 1995 |

**Table 2.** Primers designed in this study. Annealing positions was determined from Kawai *et al.* (1995)

| Code | Sequence (5'-3') | Position |
|---|---|---|
| Forward | | |
| S34 | CAAGTCTGGCAATTGGAATGAGA | 894-916 |
| S56 | AGCATGGAATAATGAGATAGGG | 1,312-1,333 |
| S78 | CAAGTCTGGCAATTGGAATGAGA | 1,573-1,595 |
| S11 | CGAGGAATTCCTAGTAAACG | 1,988-2,009 |

The SSU sequences were assembled and aligned with CLUSTAL W computer program (version 1.5; Thompson *et al.*, 1994). Final alignment was manually redefined. The published SSU rDNA sequence of *F. gardneri* was also used for alignment and included in phylogenetic analysis. The end region of 5' upstream and 3' downstream of each species were excluded because of the ambiguity of reading nucleotides as primer regions. *Scytosiphon lomentaria* (Lyngbye) J. Agardh was used as an outgroup based on its morphological primitiveness of vegetative and reproductive organs (Wynne and Loiseaux, 1978).

To compare phylogenetic relationships inferred from SSU rDNA sequences, pairwise distances were calculated by DNADIST of PHYLIP package (version 3.57; Felsenstein, 1995) with the Kimura two-parameter model of nucleotide change (Kimura, 1980). The distances were converted to neighbor-joining (NJ) trees with PHYLIP program. Maximum parsimony (MP) analysis was done with PAUP program (version 3.1.1; Swofford, 1993). All nucleotides were equally weighted and gaps were treated as missing data. The heuristic search option with MULTIPARS option in effect was adopted to find the shortest tree. Although bootstrap support has been criticized (Hillis and Bull, 1993), we use it here as a measure of robustness of the clades obtained in both NJ and MP trees (Felsenstein, 1985).

## RESULTS

Nucleotide sequences of SSU rDNA were determinded for *Pelvetia* babingtonii, *P. canaliculata*, and *Pelvetiopsis limitata* as well as *Ascophyllum nodosum*. A total of 1,755 positions were aligned for the whole sequence, excluding about 85 bases at the 5' and 3' end near primer region of PCR (Fig. 1).

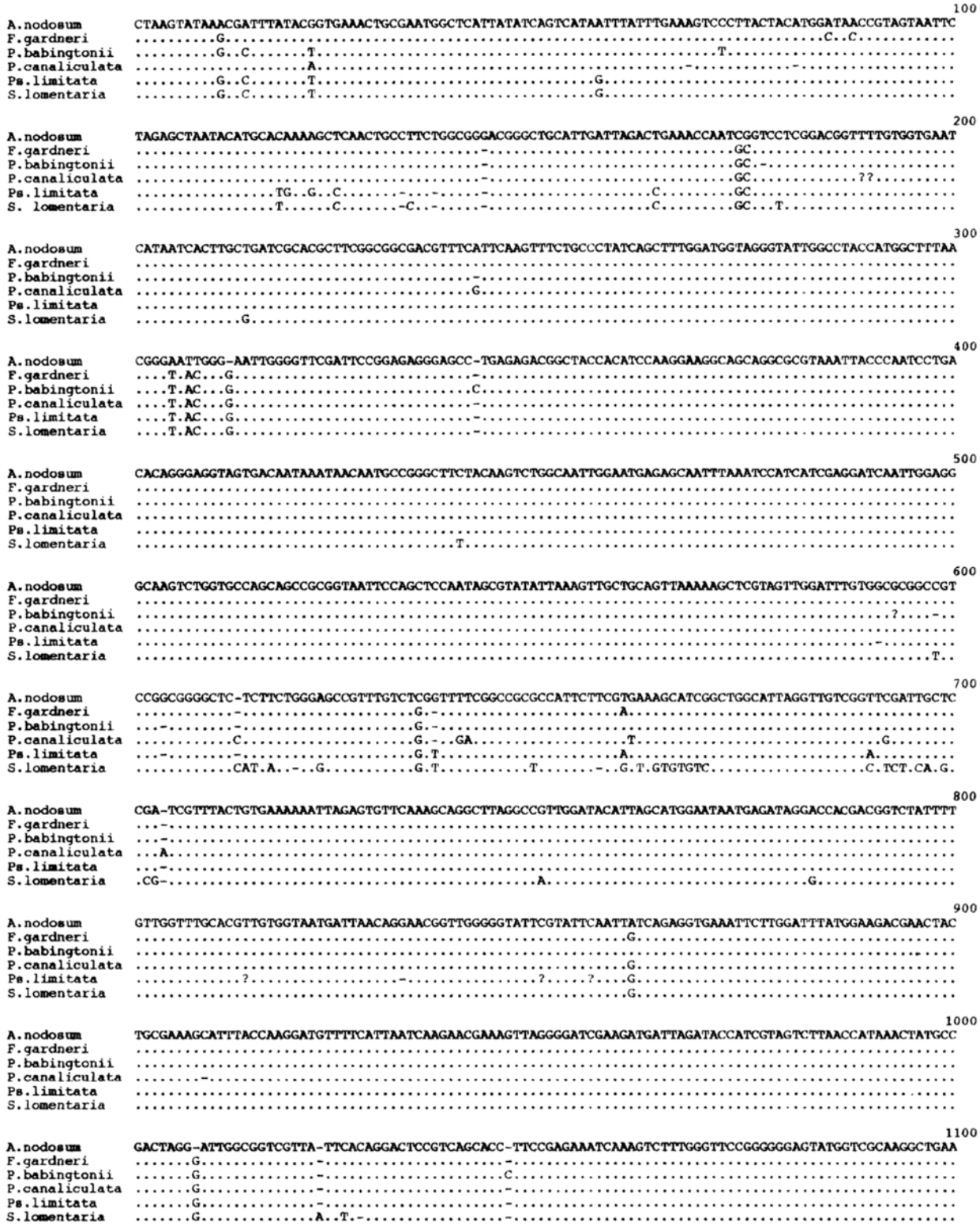


**Fig. 1.** Aligned sequences of SSU rDNA of *Pelvetia babingtonii, P. canaliculata, Pelvetiopsis limitata*, and *Ascophyllum nodosum*. Dots represent bases identical with the sequence of the first line, dashes gaps alignment, and question marks ambiguous bases. Sequences of *Fucus gardneri* and *Scytosiphon lomentaria* were cited from the published data (Bhattacharya *et al.*, 1992; Kawai *et al.*, 1995).

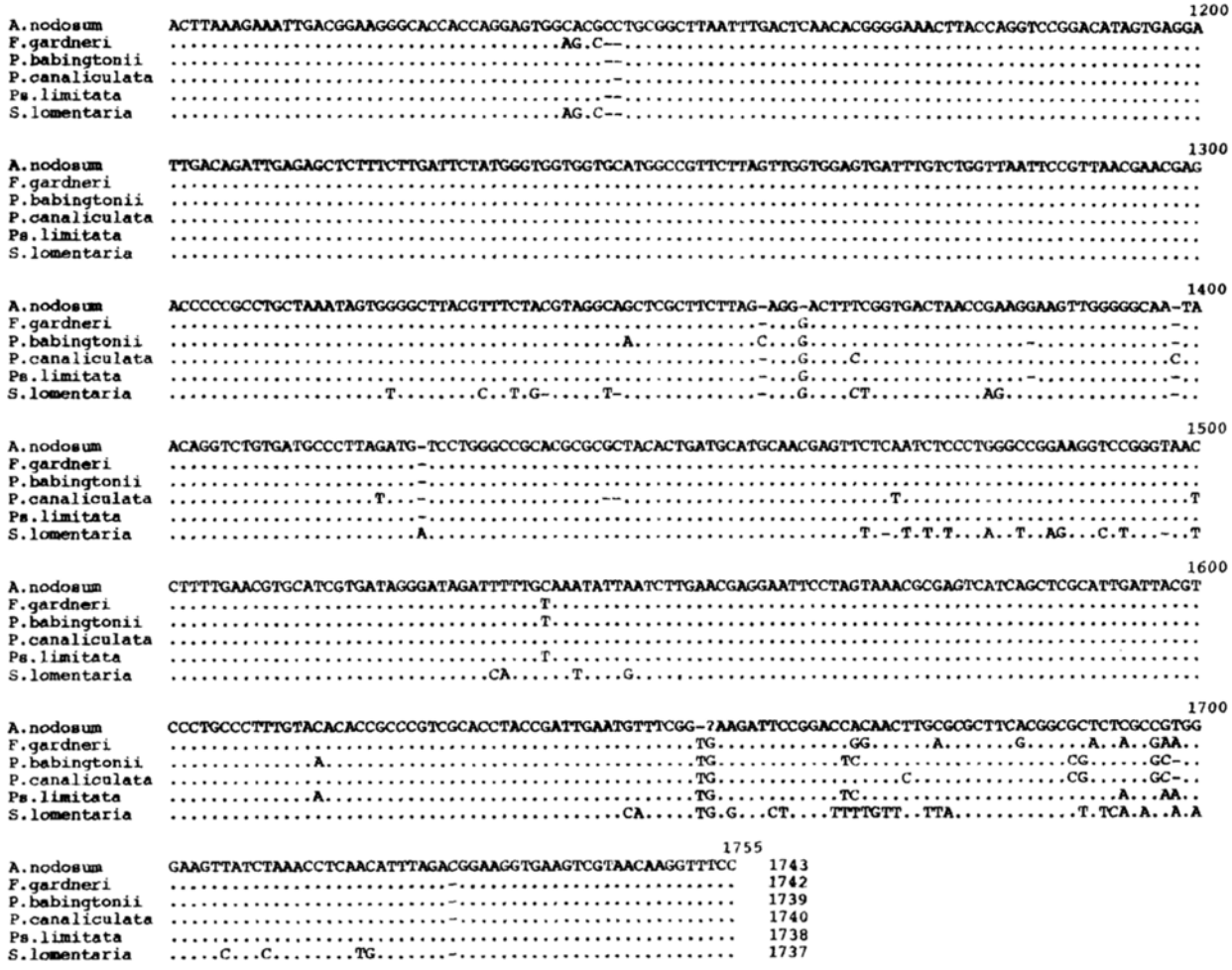


**Fig. 1.** Continued

Most of positions were unambiguously aligned. One position in *A. nodosum* and *P. babingtonii*, two positions in *P. canaliculata*, and three positions in *Ps. limitata* were ambiguous and excluded for data analysis.

The positional differences in the primary structure of the SSU rDNA ranged between 16 and 30 nucleotide changes in pairwise comparisons. Pairwise divergences of the sequences were lowest between *P. babingtonii* and *Ps. limitata* (0.009), while highest between *Ps. limitata* and *P. canaliculata* (0.017). The divergence between two species of *Pelvetia* was 0.011, which was the same value between *A. nodosum* and *P. babingtonii* (Table 3). The divergence of sequences among genera was highest between *Ascophyllum* and *Pelvetiopsis* (0.015) and lowest between *Ascophyllum* and *Pelvetia* (0.012).

MP analyses showed that two *Pelvetia* species

**Table 3.** Pairwise distances of the SSU rDNA sequences. Relative mean distances are above the diagonal and absolute distances below the diagonal

| Taxa | *A. nodosum* | *F. gardneri* | *P. babingtonii* | *P. canaliculata* | *Ps. limitata* | *S. lomentaria* |
|---|---|---|---|---|---|---|
| *Ascophyllum nodosum* | - | 0.014 | 0.011 | 0.013 | 0.015 | 0.057 |
| *Fucus gardneri* | 24 | - | 0.013 | 0.016 | 0.013 | 0.053 |
| *Pelvetia babingtonii* | 19 | 22 | - | 0.011 | 0.009 | 0.054 |
| *Pelvetia canaliculata* | 22 | 28 | 19 | - | 0.017 | 0.057 |
| *Pelvetiopsis limitata* | 26 | 22 | 16 | 30 | - | 0.050 |
| *Scytosiphon lomentaria* | 99 | 92 | 93 | 98 | 86 | - |

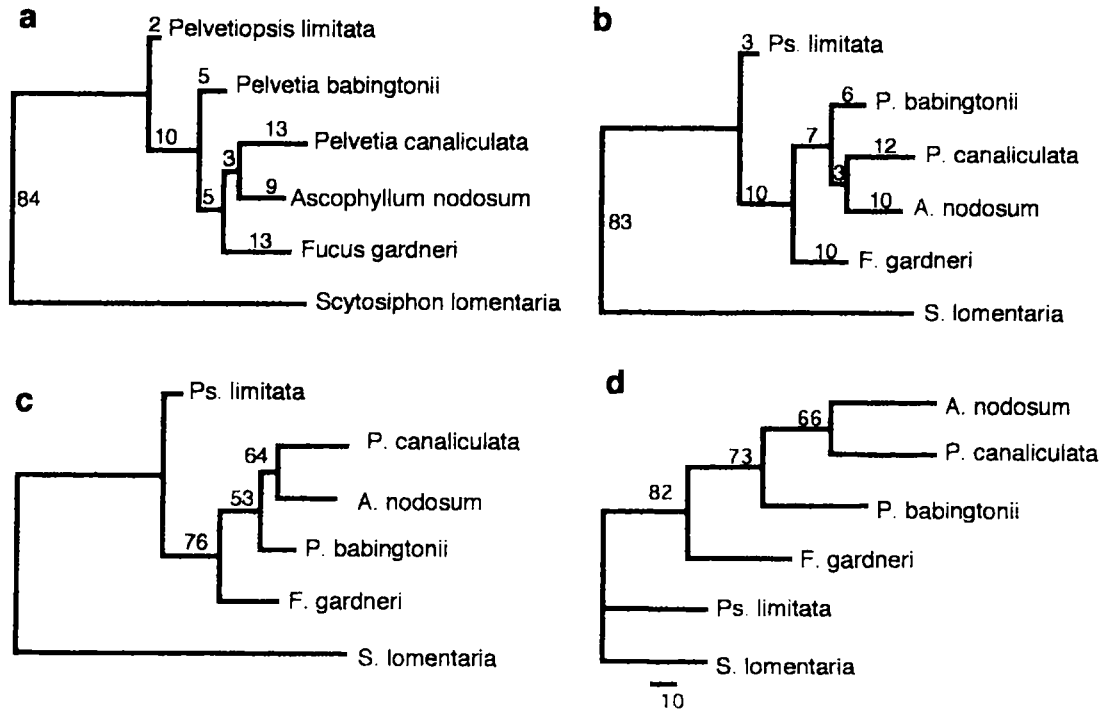


**Fig. 2.** Phylogenetic trees of *Pelvetis* and *Pelvetiopsis* using SSU rDNA sequences. a-b; Two equally most parsimonious trees. The number of steps of evolutionary change are shown at the internal node. c; A 50% majority-rule consensus tree from two most parsimonious trees. d; Neighbor-joining tree. Bootstrap percentage values are shown at the internal nodes of both consensus and NJ tree.

appeared paraphyletic, and *P. canaliculata* and *A. nodosum* were rather recovered as a monophyletic clade (Fig. 2a, b). The analyses yielded two equally parsimonious trees (tree length=144, CI=0.896, RI= 0.483). The number of evolutionary changes for branches ranged from 2 to 13. Although the bootstrap values were low in the 50% majority rule consensus tree (Fig. 2c), all branches were supported by 53-76% bootstrap replicates.

NJ analysis yielded a single tree which is similar to MP trees in topology (Fig. 2d). Futhermore *Ps. limitata* was found to be far related to a clade of other fucacean genera. The bootstrap supports of each clade, however, were relatively low with 66-82%. Both NJ and MP analyses showed that the genus *Pelvetia* is not monophyletic and the genus *Pelvetiopsis* is a sister taxon to other fucacean algae.

## DISCUSSION

Our SSU rDNA sequence data strongly suggest that the genus *Pelvetia* is not monophyletic. In addition, the data show that the genus *Pelvetiopsis* is not closely related to *Pelvetia*. These results contrast with the previous morphological and biogeographical studies (Shchapova, 1945; Tseng and Chang, 1953; Song *et al.*, 1996).

The range of 16-30 nucleotide changes within the family Fucaceae obtained in this study is similar to the level of nucleotide changes occurred among the families. For example, the partial SSU rDNA sequences differed in a range of 6-47 nucleotide changes in the fucalian families. The low differences between taxa are often reported in SSU rDNA sequences of other brown algae (Tan and Druehl, 1996) and mosses (Capesius and Stech, 1997). This may be due to slow accumulation of nucleotide changes or recent dispersal only over the last several million years (Wray *et al.*, 1995). The hypothesis of young history coupled with morphological evolution in the brown algae has been supported by molecular data (van den Hoek *et al.*, 1995). Lim *et al.* (1986) proposed that the brown algae were diversified quite recently based on 5S ribosomal RNA sequence data. Saunders and Druehl (1992) reported that SSU molecular clock suggested the diversification of kelp just 16-30 million years ago. The low levels of our SSU rDNA sequence divergence may suggest the

recent divergence of the fucacean algae from a hypothetical ancestor. This inference agrees with a biogeographical suggestion that the migration of the Pacific *Pelvetia* species might occur during the postglacial period of the Quaternary (Shchapova, 1946).

*P. canaliculata* has channelled blade, bulge conceptacle and transversal division of egg cell, while three Pacific *Pelvetia* species share the morphological features of flat blade and invasive conceptacles (Gardner, 1913; Tseng and Chang, 1953; Song *et al.*, 1996). As Shchapova (1946) noted, *P. canaliculata* is morphologically more remote from *P. compressa* than the latter from *P. babingtonii*. Our SSU rDNA sequence data reflect the morphological differences between *P. canaliculata* and *P. babingtonii*. As is seen in Fig. 2, *P. canaliculata* are not linked to *P. babingtonii*, but rather with *A. nodosum*. The SSU rDNA sequence data also agree with the ITS sequence data of Serrao and Brawley (1997), in which the gene trees showed a nonmonophyletic grouping of the *Pelvetia* species. It is probable that *P. babingtonii* from Japan and *P. canaliculata* from Europe might have different ancestors.

*Pelvetiopsis* appears to be not closely related to the other members of the family Fucacae. In the phylogentic trees of SSU rDNA (Figs. 2), *Pelvetiopsis* represented by *Ps. limitata* forms a sister taxon to the clade of *Pelvetia*, *Ascophyllum* and *Fucus*. This result contradicts the morphological inference of Powell (1963) that *Pelvetiopsis* might originate from the Pacific *Pelvetia* species. Although *Hesperophycus* is characterized by having fronds with distinct percurrent midrib (Setchell and Gardner, 1925), the diagnostic feature of a single functional egg in *Pelvetiopsis* is also shown in the former genus (Gardner, 1913).

All members of *Pelvetia* and *Pelvetiopsis* occur to be epilithic on rock in the upper-mid tidal zone. The similar morphologies observed in these two genera might be due to the same selection pressure of the habitats. *Pelvetia*, *Pevetiopsis*, *Ascophyllum* and *Fucus* have been suggested to originate separately from a common hypothetical ancestor with eight eggs in a single oogonium (Clayton, 1984; Lee, 1989).

The SSU rDNA trees show that *Pelvetiopsis* was basal to the clade of other fucacean genera treated here. Rousseau *et al.* (1997) also reported that *Pelvetia* shared less than three synapomorphies with *Ascophyllum* and *Fucus*. Therefore, the molecular data of SSU and LSU rDNA, and ITS sequences don't reflect the evolutionary hypothesis that most of the fucacean genera might originate from *Fucus* based on morphological features (Setchell and Gardner, 1925; Powell, 1963). The molecular data rather appear to postulate an evolutionary mode of adaptive radiation in the family Fucaceae. The detailed reinvesitgation of morphologies would give a new insight on the systematic treatment of *Pelvetia* and *Pelvetiopsis* members as well as the family Fucaceae.

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