Phylogenetic relationships between Pelvetia and Pelvetiopsis (Fucaceae, Phaeophyta) inferred from sequences of the RuBisCo spacer region

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Phylogenetic relationships between *Pelvetia* and *Pelvetiopsis* (Fucaceae, Phaeophyta) inferred from sequences of the RuBisCo spacer region

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As a basis for inferring phylogenetic relationships between *Pelvetia* and *Pelvetiopsis* in the family Fucaceae, the complete sequences of the plastid-encoded RuBisCo spacer region were determined for all four species of *Pelvetia*, for the type species of *Pelvetiopsis*, *P. limitata*, and for three other fucacean species (*Ascophyllum nodosum*, *Fucus gardneri* and *F. vesiculosus*). All trees based on the sequences of the RuBisCo spacer region showed that *Pelvetia* is not monophyletic and consists of two clades, one including *P. babingtonii*, *P. compressa*, and *P. siliquosa*, the other comprising only the type species, *P. canaliculata*. *P. canaliculata* is not closely related to any of the other Fucaceae studied, and it may represent a monotypic genus. RuBisCo spacer sequences support the establishment of a new genus for the three Pacific *Pelvetia* species: *P. babingtonii*, *P. compressa* and *P. siliquosa*. The congruence of the RuBisCo spacer data with reported nuclear rDNA 18S and ITS sequences, and with biogeographical and morphological data, indicates that the RuBisCo spacer region, including its flanking areas, is useful for inferring phylogenetic relationships within the Fucaceae.

Key words: Fucaceae, molecular systematics, *Pelvetia*, *Pelvetiopsis*, Phaeophyta, phylogeny, RuBisCo spacer

Introduction

After studying reproductive structures in various European members of the Fucaceae, Decaisne & Thuret (1845) concluded that the number of 'spores' or 'sporules' (i.e. eggs) produced during maturation of 'perispores' (i.e. oogonia) was taxonomically important. Combining this character with vegetative features, they recognized four genera: *Cymaduse* Decaisne & Thuret (now known as *Bifurcaria* Stackhouse in the Cystoseiraceae) with a single egg; *Pelvetia* Decaisne & Thuret with two eggs; *Ozothallia* Decaisne & Thuret (now known as *Ascophyllum* Stackhouse) with four eggs; and *Fucus* Linnaeus with eight eggs. Four species of *Pelvetia* are currently recognized. The type species, *P. canaliculata*, is characterized by flattened and channelled axes and a transverse partitioning of the oogonium (Oltmanns, 1889; Ardré, 1970). It is distributed along the eastern coasts of the North Atlantic from Portugal to England and Norway. The other three species occur on Pacific coasts: *P. siliquosa* in China and Korea (Tseng & Chang, 1953; Song *et al.*, 1996), *P. babingtonii* in Japan (= *P. wrightii* Harvey; see Yoshida & Silva, 1992) and *P. compressa* (= *P. fastigiata* (J. Agardh) De Toni; see Silva, 1996) on the west coast of North America (Setchell & Gardner, 1925). The Pacific members all have an unchannelled blade and a longitudinal partitioning of the oogonium (Yendo, 1907; Gardner, 1910; Song *et al.*, 1996). Therefore, the species of *Pelvetia* can be separated into two groups based on morphology and biogeography,

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but there have been few systematic studies to support this separation.

Pelvetiopsis was established by Gardner (1910) for *P. limitata* on the basis of its having a single egg per oogonium. Later, Gardner (1940) described *Pelvetiopsis arborescens*. While the type species is common along the Pacific coast from Vancouver Island, Canada, through northern California, USA, to northern Baja California, Mexico, *P. arborescens* is known only from a few populations in the vicinity of Monterey, California. Gardner (1913) concluded that *Pelvetiopsis* was more closely related to *Pelvetia* than to other genera of the Fucaceae based on thallus colour, texture and absence of a midrib. Powell (1963) and Clayton (1984) agreed that *Pelvetia* and *Pelvetiopsis* were closely related.

Recent molecular analyses of the ribosomal DNA (rDNA) gene in the Fucaceae have not been able to resolve phylogenetic relationships among *Ascophyllum*, *Fucus* and *Pelvetia*, because there was insufficient variation among the 18S sequences (Lee *et al.*, 1998) and too few informative sites in the 26S sequence data (Rousseau *et al.*, 1997). However, the above 18S rDNA sequence data conflicted with the traditional view of taxonomy (i.e. Song *et al.*, 1996) and evolutionary history (Powell, 1963) within the Fucaceae.

Sequences of the spacer region of the plastid-encoded ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBisCo) gene have been successfully used to investigate phylogenetic problems at the genus and species level in brown (Stache-Crain *et al.*, 1997; Siemer *et al.*, 1998; Yoon & Boo, 1999) and red algae (Destombe & Douglas, 1991;

Maggs *et al.*, 1992; Goff *et al.*, 1994; van Oppen *et al.*, 1995; Brodie *et al.*, 1996). However, there are few studies on the molecular systematics of fucacean genera using RuBisCo spacer sequences.

As a basis for inferring phylogenetic relationships between *Pelvetia* and *Pelvetiopsis*, we investigated the RuBisCo spacer sequences of all species of both genera except for *Pelvetiopsis arborescens*. *Ascophyllum nodosum*, *Fucus gardneri* and *F. vesiculosus* were also included in this study.

Materials and methods

Information on the samples used is summarized in Table 1. Fresh material was cleaned by removing epiphytes and rinsing repeatedly with seawater, and rinsed again with sterile seawater just before extracting DNA. All vouchers are deposited in the herbarium of Chungnam National University, Daejon, Korea.

Apical tips from a thallus for each sample were ground in liquid nitrogen for 2–3 min and 10–20 mg of powder was added to 200 ml Chelex 100 resin (BioRad, Richmond, CA) (6% Chelex 100 w/v in a sterile solution of 90 mM Tris-HCl (pH 8·0) and 50 μM ethylenediaminetetra-acetate) (Goff & Moon, 1993).

The RuBisCo spacer region was amplified as a single fragment using the primers RS 1 and RS 2 (RS 1: 5′-GCC AAA TGC ACC AAC TTC TT-3′, RS 2: 5′-AGA CCC CAT AAT TCC C-3′), which were designed by Ian Tan and have been used for the Alariaceae (Yoon & Boo, in press). A negative control without the template was included in every set of polymerase chain reactions (PCR). The PCR cocktail followed Tan & Druehl (1996) and the reactions were performed in an automated thermal cycler (MJ Research, Watertown, MA). The initial cycle was carried out at 95 °C for 5 min, followed by 28 cycles of 30 s at 94 °C, 30 s at 45 °C, and 1 min at 72 °C. The final temperature was 72 °C for 10 min for complete primer extension. The quality of the PCR products was checked by gel electrophoresis and visualized with ethidium bromide. The amplified DNA was further purified using a Gene clean kit II (Bio 101, La Jolla, CA), according to the manufacturer's protocol.

The purified DNA was sequenced directly using the Sequenase ver. 2·0 DNA sequencing kit (US Biochemical, OH) with a modification of the dideoxynucleotide chain terminating protocol (Tan & Druehl, 1996). The sequencing protocol was amended to include dimethyl sulphoxide (DMSO) in the initial denaturing step and to include ^{35}S-dATP in the labelling step of the double-stranded template. The RuBisCo spacer region of amplified DNA was sequenced with RS1, RS2 and RS3 (5′-AAA GCG GCT TTA GAT TTA TG-3′). The sequencing cocktail included 150 ng of purified DNA, 5 ng primers, 10% DMSO, and other chemicals recommended by the manufacturer (US Biochemical, OH). The sequencing reactions followed the manufacturer's protocol. Electro-phoresis was run on 8% acrylamide-bisacrylamide gels with 1 × TBE buffer. The gel was fixed in a 5% glacial acetic acid/15% ethanol mixture for 15 min. The dried gel was then exposed to autoradiograph film for a week in a − 70 °C freezer. Multiple sequences were read manually from the autoradiographs and edited using Seq-App, a multiple-sequence editing program.

The sequence of each RuBisCo spacer and its flanking regions was confirmed using BigDyeTerminator Cycle Sequencing Kit (Applied Biosystems (ABI), Perkin-Elmer Cetus) following the manufacturer's recommendations. The sequence data were collected with the ABI model PRISM 377 DNA Sequencer (Foster City, CA). The

Table 1. Collection data for *Pelvetia*, *Pelvetiopsis*, *Ascophyllum* and *Fucus* samples used for sequence analysis of the RuBisCo spacer region, and GenBank accession number of the sequences

Species	Collection sites	Legit et det.	GenBank accession no.
Pelvetia babingtonii (Harvey) De Toni	Muroran, Hokkaido, Japan	T. Motomura	AF106508
	Akkeshi, Hokkaido, Japan	S.M. Boo	AF132473
P. canaliculata Decaisne *et* Thuret	Isle of Man, Great Britain	A. Star	AF106509
	Cap Blanc Nez, France	J.H. Kim	AF132472
P. compressa (J. Agardh) De Toni	Sheltered, Pacific Grove, California, USA	J. Watanabe	AF106510
	Exposed, Pacific Grove, California, USA	J. Watanabe	AF106511
P. siliquosa Tseng *et* Chang	Padori, Seosan, west coast, Korea	S.M. Boo	AF106512
	Namhaedo, south coast, Korea	S.M. Boo	AF106517
	Rushan, Shandong, China	S. Lu	AF106513
Pelvetiopsis limitata (Setchell) Gardner	Bamfield, Vancouver Island, Canada	S.M. Boo	AF106514
Ascophyllum nodosum (L.) Le Jolis	Neeltjejans, The Netherlands	M.D. Guiry	AF106515
Fucus gardneri Silva	Bamfield, Vancouver Island, Canada	S.M. Boo	AF106516
Fucus vesiculosus Linnaeus	Neeltjejans, The Netherlands	M. D. Guiry	AF132474

```
                              3' rbcL                                                                       100
                                           .         .         .         .         .         .         .
P. caniculata-British     TCACCCTGATGGTATACAAGCAGGTGCTACAGCGAATCGTGTAGCGCTAGAAGCTATGGTTTTAGCTCGTAATGAAGGGCGTGATTATGTGGGTGAAGGC
P. caniculata-France      ....................................................................................................
P. babingtonii-Japan (M)  .......................................T...T.......................G..T..........T........T
P. babingtonii-Japan (A)  .......................................T...T.......................G..T..........T........T
P. compressa-USA (S)      .......................................T...T.......................G..T..........T........T
P. compressa-USA (E)      .......................................T...T.......................G..T..........T........T
P. siliquosa-Korea (P)    .......................................T...T.......................G..T..........T........T
P. siliquosa-Korea (N)    .......................................T...T.......................G..T..........T........T
P. siliquosa-China        .......................................T...T.......................G..T..........T........T
Ps. limitata-Canada       .......................................T...T.........................T..........T........T
F. gardneri-Canada        .......................................T...T.........................T....T.....T........T
F. vesiculosus-Netherlands ......................................T...T.........................T..........T........T
A. nodosum-Netherlands    ...........G...........................T...T........C.............G..T..........T........T

                                                                                                           200
P. caniculata-British     CCTGAGATTTTACGTACAGCTGGTGGTACTTGTGGACCATTAAAAGCAGCTTTAGATTTATGGAAAGATATTACTTTTGAATATACTTCAACAGATACAC
P. caniculata-France      ....................................................................................................
P. babingtonii-Japan (M)  .T...................AA.............................................................................
P. babingtonii-Japan (A)  .T...................AA.............................................................................
P. compressa-USA (S)      .T...................AA...............................................A.............................
P. compressa-USA (E)      .T...................AA...............................................A.............................
P. siliquosa-Korea (P)    .T...................AA.............................................................................
P. siliquosa-Korea (N)    .T...................AA.............................................................................
P. siliquosa-China        .T...................AA.............................................................................
Ps. limitata-Canada       .....................A.......C.....................................................................
F. gardneri-Canada        .....................A.......G.....................................................................
F. vesiculosus-Netherlands .....A...............C.A...........................................................................
A. nodosum-Netherlands    .....................A.............................................................................

                                              RuBisCo spacer                                               300
P. caniculata-British     CTGATTTCGTTGAAATGGCAACTGAAAGTAACTAAATAATATATTAAAAGTAGTATAATACTTACTATAAAGAGGATCAAAGTTTAAGCTTAAATTAGGT
P. caniculata-France      ....................................................................................................
P. babingtonii-Japan (M)  ..............G.A..................AGT....T......T.............A...-----------------------
P. babingtonii-Japan (A)  ..............G.A..................AGT....T......T.............A...-----------------------
P. compressa-USA (S)      ..............G...................AGT..A..TC.....T.............A.......A.....T........AA.
P. compressa-USA (E)      ..............G...................AGT..A..TC.....T.............A.......A.....T........AA.
P. siliquosa-Korea (P)    ..............G...................AGT....T......T.............A.......A.....T........C..
P. siliquosa-Korea (N)    ..............G...................AGT....T......T.............A.......A.....T........C..
P. siliquosa-China        ..............G...................AGT....T......T.............A.......A.....T........C..
Ps. limitata-Canada       ..............G...........-----------------------------.T.......-------------
F. gardneri-Canada        ..............G.....................................GT........A......------..A..A.
F. vesiculosus-Netherlands ........AC....G.....................................GT........A...........T.....A..A.
A. nodosum-Netherlands    ........AC....G...................AGT....T......T...A.....G..A...........C...T.......A..

                                                                                                           400
P. caniculata-British     TTTCTTTATAGTAAAAAGTTATTGTTATTTTAAAAATTTTTAATACTTTACATTAAAGTTAAATAAATAAAAAATTTAGTAGTTAACTAAAAACAAAAAT
P. caniculata-France      ....................................................................................................
P. babingtonii-Japan (M)  ------------------------------.....................................G.........G.......
P. babingtonii-Japan (A)  ------------------------------.....................................G.........G.......
P. compressa-USA (S)      ...T...-..T...G..T.....AG......G.................................G.........G.......
P. compressa-USA (E)      ...T...-..T...G..T.....AG......G.................................G.........G.......
P. siliquosa-Korea (P)    ..GT.........T.T.....AG......C...................................G.........G.......
P. siliquosa-Korea (N)    ..GT.........T.T.....AG......C...................................G.........G.......
P. siliquosa-China        ..GT.........T.T.....AG......C...................................G.........G.......
Ps. limitata-Canada       ------------------------------.............................G.........C.G.......
F. gardneri-Canada        ..................TTC....AA.T...A.....................................C.G.......
F. vesiculosus-Netherlands .................TTC....AA.G.........................................TGG.......
A. nodosum-Netherlands    ..................A....AG.......T...................................G.........G.......

                                              5' rbcS                                                      500
P. caniculata-British     TTCAGATTTATATTAAATAAAATATTTGAAGAGTGATGAGACTTACACAAGGATGTTTTTCATTTTTACCAGATTTAAGTGATGAGCAAATTAAAAGCCA
P. caniculata-France      ....................................................................................................
P. babingtonii-Japan (M)  ....T....................................................................A.........AT..
P. babingtonii-Japan (A)  ....T....................................................................A.........AT..
P. compressa-USA (S)      ....T....................................................................A.........AT..
P. compressa-USA (E)      ....T....................................................................A.........AT..
P. siliquosa-Korea (P)    ....T....................................................................A.........AT..
P. siliquosa-Korea (N)    ....T....................................................................A.........AT..
P. siliquosa-China        ....T....................................................................A.........AT..
Ps. limitata-Canada       ....T.............................................................................AT..
F. gardneri-Canada        .................................................................................AT..
F. vesiculosus-Netherlands ................................................................................AT..
A. nodosum-Netherlands    ....T............................................................................AT..

                                           550
P. caniculata-British     AATTAATTATGCTATTTCAAAAAGTTGGGCTGTTAGTGTTGAATGGACAGACGAT
P. caniculata-France      ......................................................
P. babingtonii-Japan (M)  .............................................T...
P. babingtonii-Japan (A)  .............................................T...
P. compressa-USA (S)      .............................................T...
P. compressa-USA (E)      .............................................T...
P. siliquosa-Korea (P)    .............................................T...
P. siliquosa-Korea (N)    .............................................T...
P. siliquosa-China        .............................................T...
Ps. limitata-Canada       .............................................T...
F. gardneri-Canada        ...................A.........................T...
F. vesiculosus-Netherlands ..................A.........................T...
A. nodosum-Netherlands    .................G.........A..........T...
```

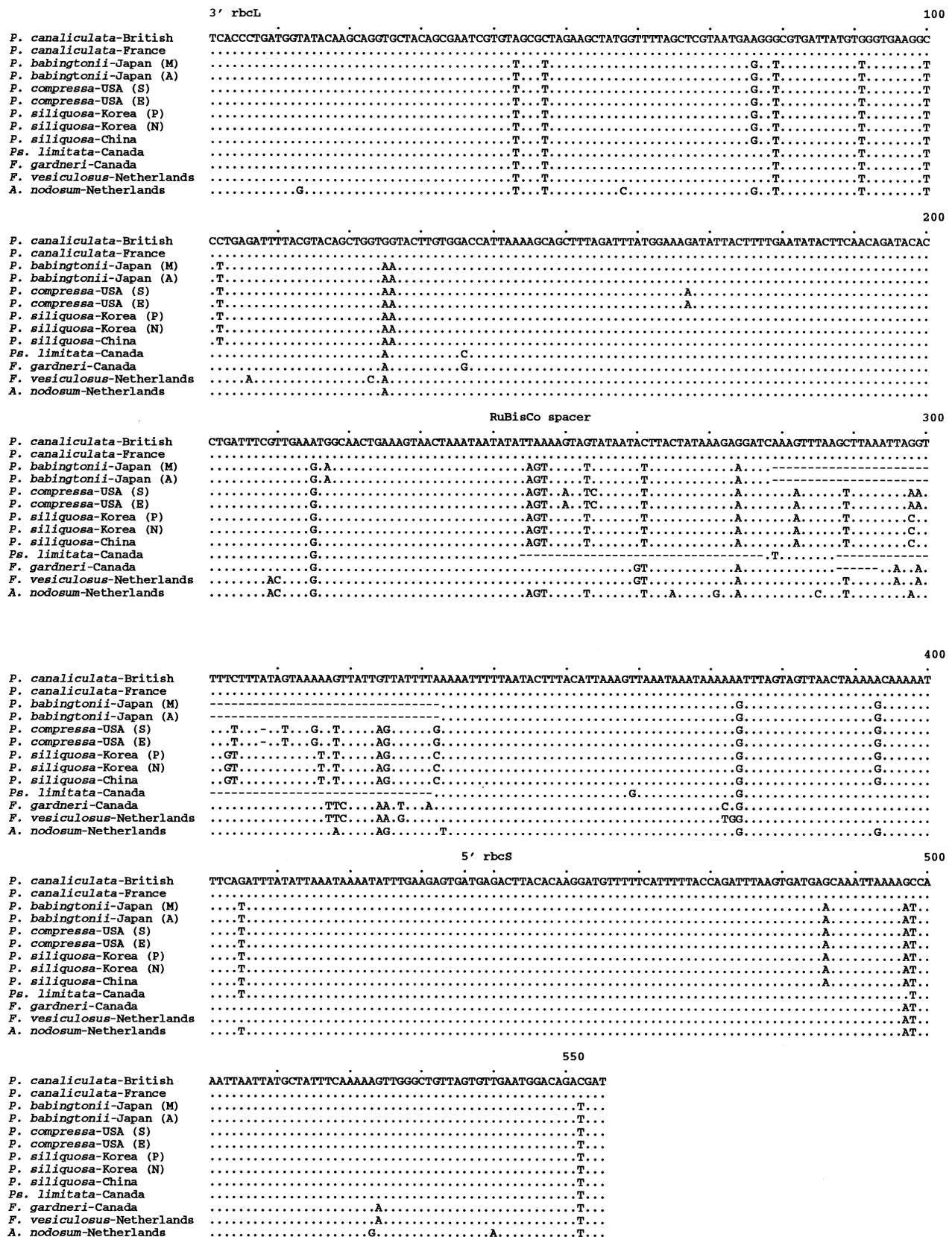**Fig. 1.** Sequence alignment of the RuBisCo spacer region of 13 samples of *Pelvetia, Pelvetiopsis, Ascophyllum* and *Fucus*. Positions of nucleotides are numbered consecutively above from 1 to 555 (5′ to 3′) on the *rbc*L/RuBisCo spacer/*rbc*S region. Dots represent nucleotides identical to those in the first sequence, and dashes indicate indels (insertions/deletions).

sequences were aligned using the CLUSTAL W (ver. 1·4) program (Thompson *et al.*, 1994). Final alignments were done visually. The RuBisCo spacer regions were compared with published data on the primitive brown alga *Pylaiella littoralis* (L.) Kjellman (GenBank accession no. X55371; Assali *et al.*, 1990).

Parsimony analysis was carried out using Phylogenetic Analysis Using Parsimony (PAUP version 3.1.1; Swofford, 1993) on a Power Mac. A branch and bound search option was used to find the most-parsimonious trees (MPTs). All nucleotides were equally weighted and gaps were treated as missing characters. To assess the robustness of each node in the phylogenetic trees, both bootstrap and decay analyses were done. Bootstrap analysis was performed using 1000 resamplings (Felsenstein, 1985). Decay analysis was performed until all branches collapsed (Morgan, 1997). The g-statistic (Hillis, 1991) was calculated based on 100 000 randomly generated trees. To root the trees, two representatives of *Fucus* (*F. gardneri* and *F. vesiculosus*) were used as outgroups since they were the most basal in unrooted trees.

A pairwise sequence divergence was obtained using the Phylogeny Inference Package (PHYLIP, version 3.5c; Felsenstein, 1995) by invoking the DNADIST algorithm. Two-parameter distance values of Kimura (1980) were generated from the aligned sequence. The distance matrix was subjected to the neighbor-joining (NJ) algorithm to reconstruct a phylogenetic tree using the NEIGHBOR program. The maximum likelihood (ML) tree was constructed using the DNAML program of PHYLIP, in which the global rearrangement option was given. A bootstrap analysis of 1000 resamplings was performed for both the NJ and ML analyses.

Results

Complete sequences of the RuBisCo spacer and 359 base pairs (bp) of the flanking regions were determined for eight species: four species of *Pelvetia*, *Pelvetiopsis limitata*, *Ascophyllum nodosum*, *Fucus gardneri* and *F. vesiculosus*. For each *Pelvetia* species, thalli from two or three different locations were included. Most nucleotide positions were unambiguously aligned with a total of 555 positions including insertions/deletions (indels) (Fig. 1). Length of the flanking region sequenced in this study was 359 bp: 239 bp of the 3′ end of *rbc*L and 120 bp of the 5′ end of *rbc*S. The size of the RuBisCo spacer ranged from 142 bp in *P. babingtonii* to 196 bp in *Pelvetia siliquosa*. It was

118 bp in *Pelvetiopsis limitata*, 190 bp in *Fucus gardneri*, and 196 bp in both *Ascophyllum nodosum* and *Fucus vesiculosus* (Table 2). Within the RuBisCo spacer region there was one large indel of 54 bp in *Pelvetia babingtonii* and two large indels of 34 bp and 44 bp in *Pelvetiopsis limitata*. All samples had a similar G + C nucleotide content in the RuBisCo spacer regions. The G + C content in the spacer was 12·3–15·3% in the *Pelvetia* species, 13·6% in *Pelvetiopsis limitata*, and 13·8–14·8% in *Ascophyllum nodosum* and the two *Fucus* species. The G + C of *rbc*L of all taxa treated here was 37·6–40·2% and of the *rbc*S 28·3–31·7% (Table 2).

RuBisCo spacer and flanking region sequences were the same for conspecific samples of all *Pelvetia* species from different locations, but they clearly differed between the species as well as the genera treated here. The nucleotide divergence averaged 0·9% within the Pacific members of *Pelvetia*, 3·5% within the genus *Pelvetia*, and 6·1% between *P. canaliculata* and the Pacific *Pelvetia* species. The intergeneric divergence averaged 2·5% between *Pelvetia* and *Pelvetiopsis limitata*, 4·3% between *Pelvetia* and *Ascophyllum* and 5·0% between *Pelvetia* and *Fucus* (Table 3).

Of 555 nucleotide positions, 64 positions (11·5%) were variable, 41 positions (7·4%) uninformative (i.e. substitutions were not shared between two or more samples) and 23 positions (4·1%) were informative. Parsimony analysis using the exhaustive search option found six equally MPTs. The trees were 75 steps in length, with a consistency index of 0·947 and a retention index of 0·889. The frequency distribution of tree length, for all trees, showed a skew to the left ($g1 = -0.889$). Therefore, the MPTs showed strong phylogenetic signal.

All six MPTs showed the genus *Pelvetia* to be non-monophyletic, as indicated in the strict consensus tree. *Pelvetia* was separated into two groups (Fig. 2). One clade, which consisted of *P. siliquosa*, *P. babingtonii* and *P. compressa*, had 98% bootstrap support and a decay index of 3. Within this clade, these three species formed a polytomy. *Ascophyllum nodosum* was basal to the clade with 96% bootstrap support and a decay index of 2. All members of *Pelvetia*, *Pelvetiopsis* and *Ascophyllum* treated

Table 2. Size and G + C composition of the RuBisCo spacer, and the lengths and G + C composition of the flanking regions of *rbc*L and *rbc*S that were sequenced for *Pelvetia*, *Pelvetiopsis*, *Ascophyllum* and *Fucus*

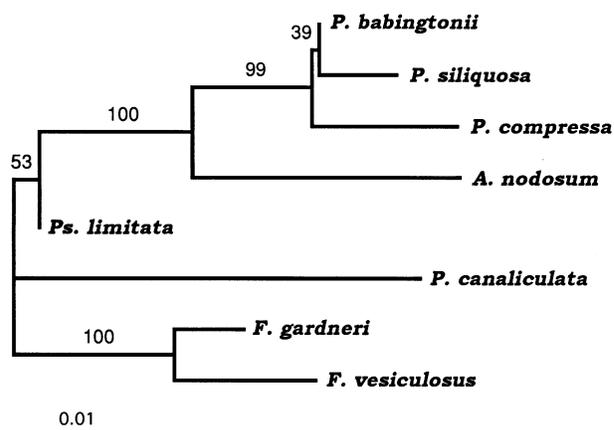| Taxon	*rbc*L 3′-end		RuBisCo spacer		*rbc*S 5′-end	
	Size (bp)	G + C (%)	Size (bp)	G + C (%)	Size (bp)	G + C (%)
Pelvetia canaliculata	239	40·2	196	15·3	120	31·7
Pelvetia babingtonii	239	37·6	142	14·1	120	28·3
Pelvetia compressa	239	37·6	195	12·3	120	28·3
Pelvetia siliquosa	239	38·0	196	13·8	120	28·3
Pelvetiopsis limitata	239	38·9	118	13·6	120	30·0
Ascophyllum nodosum	239	39·3	196	13·8	120	28·3
Fucus gardneri	239	38·9	190	14·2	120	28·3
Fucus vesiculosus	239	38·1	196	14·8	120	28·3

Table 3. Pairwise sequence divergences of the RuBisCo spacer region in *Pelvetia, Pelvetiopsis, Ascophyllum* and *Fucus*. Nucleotide divergences are shown on the diagonal and actual number of nucleotide differences below

	1	2	3	4	5	6	7	8
1. *Pelvetia canaliculata*	–	0·0496	0·0701	0·0624	0·0301	0·0663	0·0492	0·0563
2. *Pelvetia babingtonii*	24	–	0·0080	0·0020	0·0195	0·0265	0·0306	0·0390
3. *Pelvetia compressa*	37	4	–	0·0183	0·0256	0·0428	0·0551	0·0603
4. *Pelvetia siliquosa*	33	1	10	–	0·0235	0·0372	0·0513	0·0565
5. *Pelvetiopsis limitata*	14	9	12	11	–	0·0301	0·0149	0·0257
6. *Ascophyllum nodosum*	35	13	23	20	14	–	0·0573	0·0547
7. *Fucus gardneri*	26	15	29	27	7	30	–	0·0166
8. *Fucus vesiculosus*	30	19	32	30	12	29	9	–
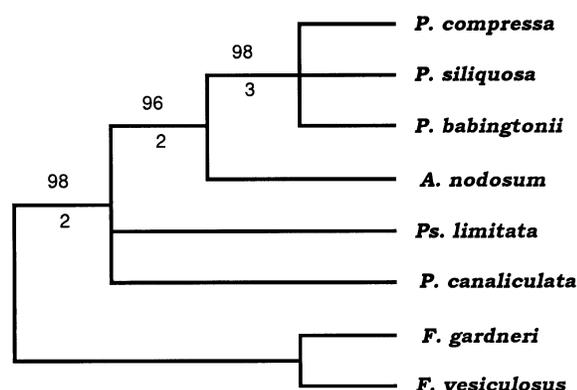
Fig. 2. Strict consensus of six most parsimonious trees showing the phylogenetic relationships of *Pelvetia, Pelvetiopsis, Ascophyllum* and *Fucus*. Numbers above the nodes are bootstrap values (1000 resamplings) and numbers below the nodes are decay indices.
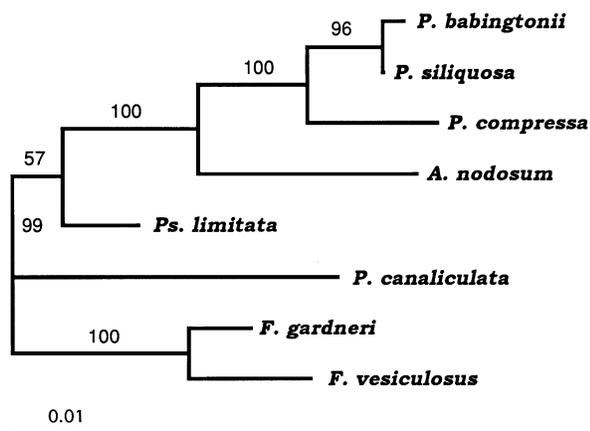
Fig. 3. Maximum likelihood tree showing showing the phylogenetic relationships of *Pelvetia, Pelvetiopsis, Ascophyllum* and *Fucus*. Numbers above the branches are bootstrap values (1000 resamplings).

Fig. 4. Unrooted neighbor-joining tree showing phylogenetic relationships of *Pelvetia, Pelvetiopsis, Ascophyllum* and *Fucus*. Numbers above the branches are bootstrap values (1000 resamplings).

Ascophyllum nodosum was basal to the clade with 100% bootstrap support and *Pelvetiopsis limitata* followed this with very weak bootstrap support (57%). *P. canaliculata* had a long branch and was not grouped with any other members. The NJ tree (Fig. 4) was identical to the ML tree, but without resolution (39% bootstrap support) for the clade of *P. babingtonii* and *P. siliquosa*. *Pelvetia* was again separated into two groups, as in both the MP and ML trees.

Discussion

All the analyses based on our RuBisCo spacer sequences show that *Pelvetia* is not monophyletic. *P. canaliculata* is separated by a long branch from all fucacean algae treated here, suggesting that *P. canaliculata* may represent a monotypic genus. On the other hand, three Pacific species – *P. babingtonii, P. compressa* and *P. siliquosa* – form a well-resolved clade (98–100% bootstrap support in different analyses).

Analysis of 18S rDNA sequences (Lee *et al.*, 1998) and ITS sequences (Serrão *et al.*, 1999) has already shown that the genus *Pelvetia* is not monophyletic. The results of the most comprehensive molecular evolutionary study of the Fucaceae to date using rDNA ITS sequences (Serrão *et al.*, 1999) were basically congruent with those of RuBisCo spacer sequences (particularly with respect to the relative

here formed a single clade with 98% bootstrap support and a decay index of 2. Within this, the positions of *P. canaliculata* and *Pelvetiopsis limitata* were unresolved.

The topology of the ML tree (Fig. 3) was similar to that of the MP consensus. Although *Pelvetia compressa* was basal to the subclade of *P. babingtonii* and *P. siliquosa* with 96% bootstrap support, the first clade consisting of the Pacific members was again strongly (100%) supported.

position of Pacific members of *Pelvetia*, *P. canaliculata*, *Pelvetiopsis limitata* and *Ascophyllum nodosum*). Therefore, both the plastid-encoded RuBisCo spacer and nuclear rDNA (18S and ITS) sequence data are in conflict with the circumscription of a single genus, *Pelvetia*, characterized by production of two eggs in a single oogonium (Decaisne & Thuret, 1845; Yendo, 1907; Gardner, 1910; Tseng & Chang, 1965). However, although all species of *Pelvetia* produce two eggs, egg cells in the oogonium are produced by a transverse division in *P. canaliculata* (Decaisne & Thuret, 1845; Oltmanns, 1889) and by a longitudinal division in *P. babingtonii*, *P. compressa* and *P. siliquosa* (Gardner, 1910; Song *et al.*, 1996). Yendo (1907) mentioned that the division pattern of the oogonium was less important than the number of eggs. We hypothesize that the longitudinal division of the oogonium is a synapomorphy for these three Pacific *Pelvetia* species and we concur with Serrão *et al.* (1999) that it is important enough to warrant establishing a new genus – *Silvetia* Serrão, Cho, Boo *et* Brawley – for the Pacific species. This interpretation correlates with the biogeographical distribution of the taxa: *P. canaliculata* is an Atlantic species, while *P. babingtonii*, *P. compressa* and *P. siliquosa* occur in the North Pacific. As was speculated by Shchapova (1946), the Pacific species, but not *P. canaliculata*, might have evolved recently from a common ancestor.

Pelvetiopsis limitata is linked very weakly with the group (((*Pelvetia siliquosa* + *P. babingtonii*) + *P. compressa*) + *Ascophyllum nodosum*) in both the ML and NJ trees (Figs 3, 4) and its branch collapses in the MP tree (Fig. 2). This result contradicts the phylogenetic views of Powell (1963), based on number of eggs, gross morphology of thallus and arrangement of conceptacles, that *Pelvetiopsis* evolved from *Pelvetia compressa* (as *P. fastigiata*) and of Clayton (1984, fig. 40), based on the formation of either one or two eggs per oogonium, that *Pelvetia* and *Pelvetiopsis* were more related to each other than to other fucacean genera. Instead, our RuBisCo spacer sequence data suggest that *Pelvetiopsis* might have evolved independently from *Pelvetia canaliculata* and the Pacific members of *Pelvetia*. It would be very interesting to examine *Hesperophycus* (Gardner, 1910), which resembles *Pelvetiopsis* in having one egg in the oogonium but differs in gross morphology of the thallus.

Ascophyllum nodosum is strongly linked with the Pacific members of *Pelvetia* in the MP (96% bootstrap support and a decay index of 2), ML (100% bootstrap support) and NJ trees (100% bootstrap support). This result indicates that species with two eggs (*P. babingtonii*, *P. compressa* and *P. siliquosa*) and four eggs (*A. nodosum*) may share a more recent common ancestor than do the Pacific *Pelvetia* species and *P. canaliculata*. This inference supports the hypothesis of Clayton (1984, p. 34) that the reduction of the number of the eggs in the oogonium might have occurred independently more than once during the evolution of the various families of the Fucales.

The general characteristics of the RuBisCo spacer of fucacean algae treated here are in accordance with those of

other browns (Stache-Crain *et al.*, 1997; Siemer *et al.*, 1998). The spacer length is shorter than that (268–287 bp) of the laminarialian family Alariaceae (Yoon & Boo, 1999), but is comparable to that (151–204 bp) of other brown algae (Stache-Crain *et al.*, 1997; Siemer *et al.*, 1998). There is an extreme bias towards AT (85–87%) in the sequences of the Fucaceae. The AT-rich spacer is very similar in other brown (Valentin & Zetsche, 1990; Yoon and Boo, 1999) and red algae (Valentin & Zetsche, 1990). Length and sequence variation of the RuBisCo spacer of some fucacean algae confirms its suitability for phylogenetic studies, as demonstrated in previous works (Stache-Crain *et al.*, 1997; Siemer *et al.*, 1998)

The present study shows congruence between the RuBisCo spacer region sequences, rDNA sequences, biogeographical and morphological groupings for *Pelvetia*. It would be desirable to extend this approach to include all members of the family Fucaceae and information from the other fucacean members would also help to complete a plastid-genome phylogeny of the Fucaceae.

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