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Phylogenetic Relationships of *Soranthera ulvoidea* (Chordariaceae, Phaeophyceae) on the Basis of Morphology and Molecular Data

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The brown algal family Chordariaceae *sensu lato* is a focus of taxonomy because recent studies suggest a broad concept of the family, including genera formerly classified in the Dictyosiphonales. Using morphology, plastid *rbc*L and nrDNA ITS sequences, we evaluated relationships of the monotyic genus *Soranthera* (*S. ulvoidea*), which has been classified in the Punctariaceae. The species occurs in Bering Sea and Aleutian Islands, Alaska to Baja California. Thalli are globose to lobed, hollow, 3-5 cm in diameter, and covered with evenly distributed sori. However, two forms within the species are recognized: f. *ulvoidea* for globose forms and f. *difformis* for lobed forms. Plastid *rbc*L and nuclear ITS region sequences were newly determined in samples of *S. ulvoidea* from the Pacific coast of the North America. We found little variations in the ITS sequences among samples of *S. ulvoidea* from five different locations and in the *rbc*L region from two different locations. These results do not support previous classification of f. *ulvoidea* and f. *difformis* within the species. All analyses of our *rbc*L sequence dataset show that *Soranthera* was placed in the Chordariaceae *s.l.*, but more related to *Botrytella* than *Punctaria* and *Asperococcus*.

Key Words: Chordariaceae, Ectocarpales, ITS region, morphology, Phaeophyceae, phylogeny, *rbc*L, *Soranthera ulvoidea*, taxonomy

INTRODUCTION

Soranthera Postels *et* Ruprecht is a monospecific brown algal genus with *S. ulvoidea* Postels *et* Ruprecht (Postels and Ruprecht 1840; Setchell and Gardner 1925; Bold and Wynne 1985), which was described based on specimens from Sitka, Alaska, USA (Postels and Ruprecht 1840). *S. ulvoidea* is annual and occurs exclusively in Kamchatka, the Bering Sea, Aleutian Islands, Alaska, to Baja California (Wynne 1969; Hansen 1997; Klochkova 1998; Mondragon and Mondragon 2003). Thalli are globose, hollow, and epiphytic on *Neorhodomela* and *Odonthalia* in the mid to low intertidal (Setchell and Gardner 1925; Angst 1926, 1927; Mondragon and Mondragon 2003). The sacs of *S. ulvoidea* are the sporophyte stage that produces swimming spores with flagella. This phase alternates with a microscopic, filamentous gametophyte that produces gametes (Angst 1926; Wynne 1969). *S. ulvoidea* is divided into two forms; f. *ulvoidea* is oval to spherical in shape and has thick membrane and f. *difformis* is deeply lobed and has thin membrane (Setchell and Gardner 1925). *S. ulvoidea* is very similar to other saccate or globose brown algal epiphytes like *Colpomenia*.

On establishing the genus *Soranthera*, the familial position of the genus remained unclear (Postels and Ruprecht 1840). Kjellman (1893 in 1891-1896) placed *Soranthera* and *Asperococcus* in the Asperococcaceae of the Encoeliaceae. Setchell and Gardner (1925) put the genus in the Asperococcaceae of the Ectocarpales, despite the establishment of the Dictyosiphonales. Since Papenfuss (1947) extended the Dictyosiphonales to include the Punctariales and treated the Asperococcaceae as a synonym of the Punctariaceae (Papenfuss 1955), *Soranthera*, together with *Punctaria*, has been placed in the Punctariaceae of the Dictyosiphonales (Wynne 1969; Bold and Wynne 1985). However, Pedersen (1984) doubted the taxonomic position of the genus in the Punctariaceae.

Based on DNA phylogeny, plastid structure, and life histories, the order Ectocarpales consists of five families, viz. Acinetosporaceae, Adenocystaceae, Chordariaceae, Ectocarpaceae, and Scytosiphonaceae (Peters and Ramirez 2001). In this revision, the Chordariaceae

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Table 1. Newly analyzed specimens information of *Soranthera ulvoidea* in this study

Collection site and voucher number	Collection date	GenBank accession number	
		*rbc*L	ITS
Boiler Bay-1, Oregon, USA , PE115	16 May 2001	AY986514	AY986509
Boiler Bay-2, Oregon, USA, PE116	16 May 2001	-	AY986510
Cape Arago, Oregon, USA, PE117	15 May 2001	AY986515	AY986511
Friday Harbor, Washington, USA, PE118	11 June 2003	-	AY986512
Sunset Bay, Oregon, USA, PE120	15 May 2001	-	AY986513

receives all genera, which belonged formerly to the Dictyosiphonales (Peters and Ramirez 2001). This forces *Soranthera* to be putatively classified in the Chordariaceae *sensu lato*. Although morphology of the genus was investigated (Barton 1898; Angst 1926, 1927) and the life history was well studied by Wynne (1969), there are no studies on the phylogeny of *Soranthera* based on molecular data. During the continuing survey of the brown algal diversity in the North Pacific, we encountered thalli of *Soranthera ulvoidea* on the west coast of the North America. The goal of the present paper is to determine both plastid *rbc*L gene and nrDNA ITS region using our recent collections of the species and to reconsider the familial position of the genus. In order to construct phylogeny of the genus, we also downloaded *rbc*L data of *Punctaria*, *Asperococcus*, *Delamarea*, and *Dictyosiphon* of the formerly classified Dictyosiphonales and other relatives of the Ectocarpales *sensu lato* from GenBank.

MATERIALS AND METHODS

Morphology

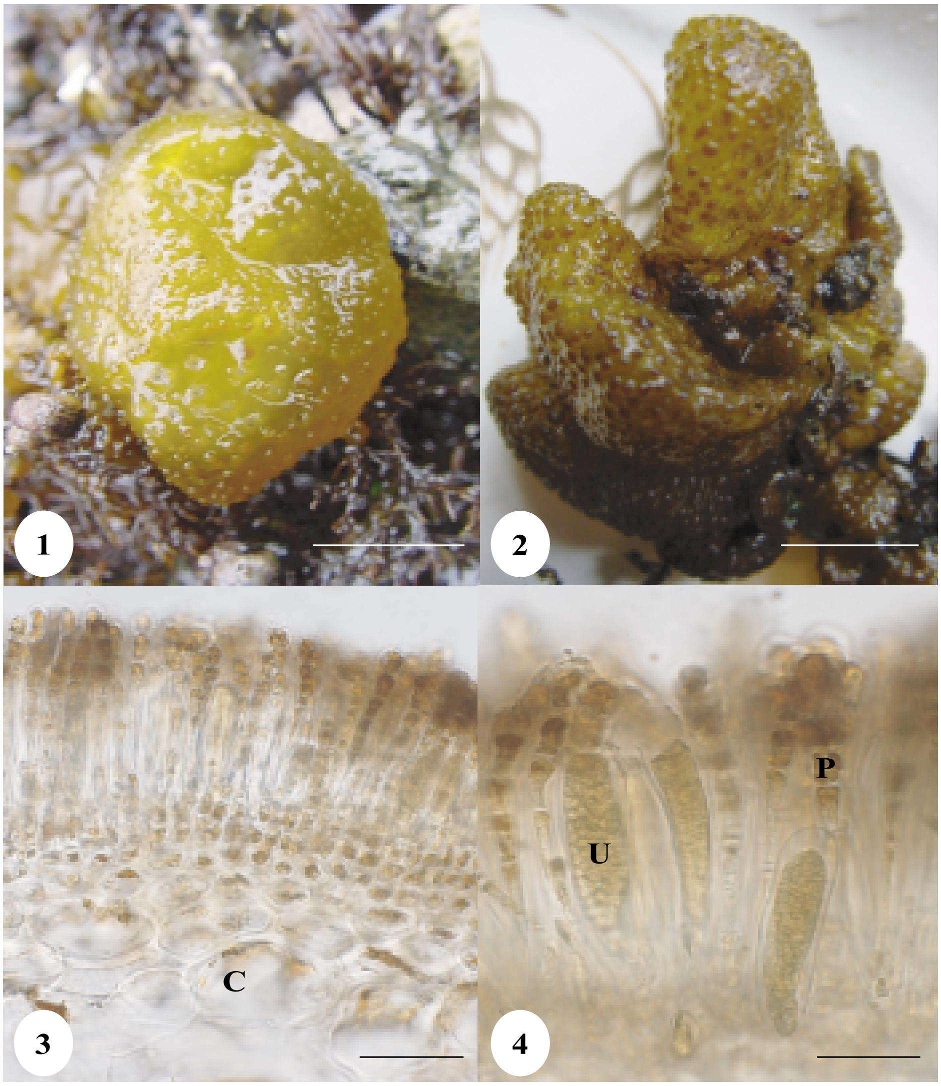
Thalli of *Soranthera ulvoidea* were collected in the middle tidal zone at five different places on the Pacific coast of the North America. Representative specimens were; Boiler Bay, Oregon, USA (Boo & TO Cho, PE115, PE116, 16 v 2001), Cape Arago, Oregon, USA (Boo & TO Cho, PE117, 15 v 2001), Friday Harbor Lab., San Juan Island, Washington, USA (Boo, MS Kim & GY Cho, PE118, 11 vi 2003), Seal Rock, Oregon, USA (Boo, MS Kim & GY Cho, PE119, 16 vi 2003), Sunset Bay, Oregon, USA (Boo & TO Cho, PE120, 15 v 2001). Material for observations was preserved in 4% formaldehyde-seawater. Microscopic observations were made from material stained with 1% aqueous aniline blue acidified with dilute HCl. Photos were made with a camera lucida attached to an Olympus microscope (VANOX AHBT3). All specimens are deposited in the herbarium of Chungnam National University (CNUK), Daejon, Korea.

Analyses of sequences of nrDNA ITS region and plastid *rbc*L gene

For extraction of DNA, five samples (Table 1) were collected in the field, dried in air, and preserved with silica crystals. Genomic DNA was extracted from approximately 0.05 g powder using the DNeasy Plant Mini Kit (Qiagen Gmbh, Hilden, Germany), according to the users' guide, and then dissolved in 150 μL DW. Polymerase chain reactions (PCR) of the ITS region and *rbc*L and sequencing reactions followed Cho *et al.* (2002, 2003). The two *rbc*L sequences we analyzed here were included in the previous alignment by Draisma *et al.* (2001) and Cho *et al.* (2003, 2004) and were aligned visually. However, because the five ITS sequences newly determined here were too variable to align with previously published sequences of putative relatives, we compared the sequences within *S. ulvoidea* only.

For maximum likelihood and Bayesian analyses of the *rbc*L sequences, we performed a likelihood ratio test using Modeltest 3.5 (Posada and Crandall 1998) to determine the best available model for our sequence data. The best model was a general time reversible (GTR) model with a gamma correction for among-site variation (Γ) and proportion of invariable sites (I). Maximum likelihood tree was constructed by heuristic search option with 100 random sequence additions. GTR + I + Γ model was set as follow: $A \leftrightarrow C = 1.38638$, $A \leftrightarrow G = 4.16994$, $A \leftrightarrow T = 1.38266$, $C \leftrightarrow G = 1.27445$, $C \leftrightarrow T = 10.33266$, $I = 0.565401$ and $\Gamma = 0.850432$.

The sequence data were performed using MrBayes v. 3.0b4 (Huelsenbeck and Ronquist 2001). The analysis was initiated from a random starting tree and the program was set to run four chains of Markov chain Monte Carlo iterations simultaneously for 2,000,000 generations with trees sampled every 100th generation. Parameter values and trees of first 5001 trees were burned to get the stabilized likelihood scores. Bayesian



Figs 1-4. Habit of *Soranthera ulvoidea*. **Fig. 1.** Morphology of f. *ulvoidea*. **Fig. 2.** Morphology of f. *difformis*. Both were collected in 11 June 2003 at the front of Friday Harbor Marine Lab, San Juan Island, Washington, USA. Scale bar = 1 cm. **Fig. 3.** Anatomy of thallus showing cortical layers (C) and paraphyses. Scale bar = 50 μm. **Fig. 4.** Unilocular sporangium (U) and paraphysis (P). Scale bar = 25 μm.

posterior probabilities were obtained from the majority-rule consensus tree of 15,000 saved trees.

Maximum parsimony (MP) analyses for *rbc*L data was done using a heuristic search algorithm of PAUP * 4.0b10 (Swofford 2002) with the following settings: 1,000 random sequence-addition replicates, tree bisection-reconnection (TBR) branch swapping, MulTrees, all characters unordered and unweighted, and branches with a maximum length of zero collapsed to yield polytomies. Nonparametric bootstrap values for nodes were calculated based on 500 and 2,000 resamplings (5 and 10 random sequence-addition replicates) for the ML and MP respectively.

RESULTS

Morphology

Thalli of *Soranthera ulvoidea* were epiphytic on *Neorhodomela* and *Odonthalia*, in tide pools, in the intertidal region. The thalli were globose to lobed, hollow, 2-3 cm in diameter, and olive-brown in color (Figs 1, 2). The saccate thalli were composed of two kinds of tissue. The surface layer included small, angular cells having 1-2 plastids with pyrenoid. The cortex consisted of 4-5 layers of cells, being large and relatively colorless. The cortical tissue was parenchymatous (Figs 3-4).

Sori of unilocular sporangia were abundant and scattered fairly evenly over the surface of the saccate thalli. Unilocular sporangia were interspersed with

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CCGAAGTGGC TCGTAGCCAA GTTAAAAACT GCGAAATTAA GTGCCGCGCC   [ 50]
GAGTTCGCCT CCCCATCCGT AGGCTTTTCG GTGTCTCTCT CTCTAGTATT   [100]
GTAAAGAAAA GGAGAAGGAA CGAGAACACT ATGATGGATC AGACGCGAAC   [150]
GAGAGAGGCA GGCCGTAAAA CCCCGCGAAA GAAAACTGTT ATGCGATGTC   [200]
GGGCGAGGGG CGTCTCGCCG AGCTTTGCTA AGCTCGAAAC AAAGCGTACC   [250]
CCACAATTCA ACCCCAATAA ACTCTGAATC TGAACTTAAA AGGAGGCGGC   [300]
GCCTCCGCTC TTTTGTTAGC GCGCGGCGCG CCTTCTCAAC CTTTAACGTT   [350]
GTAAAACTTT CAGCGACGGA TGTCTTGGCT CCCACAACGA TGAAGAACGC   [400]
AGCGAAATGC GATACGTCTT GCGACTTGCA GAATCCAGTG AATCATCAAA   [450]
ACTTTGAACG CATCTTGCGC TTCCGGGATA TGCCTGGGAG CATGCTTGTC   [500]
GGAGTGTCTG TTGACACCAC TCGCCCCACG CCTTGCTTTC CTTTCGGGGT   [550]
CTGTTTGTTA TGGTGGCGGA CTCTGCGTGC TCCGGAGCTC GCGCTCCGAG   [600]
TGCACCGAAC CTCGTGAACG AGACCCCCCT GCCGGTTGGA AGATGCGGAA   [650]
CCAGGACTTC AAAACTCCCG GGGATGCGCG GCTTCACCTC CGTGAGCAGG   [700]
ATTGTTGACG GCATGGCGGT TTGTTATAGT CACGCGTTCG TTGACTTCGT   [750]
CTGGACATCT TCGCCTCGGC GTTGACCACA TTCGTGGTTG ACCTCCGCTG   [800]
TCTAGGCAGT TCGTTACGAC TTCTACAATC CGTAATACGC TCTCATGCCG   [850]
TAAATTCCAG CAGGTATGGT CGCCAAGTTC CGGTCGCCTT GTGTCCGCTG   [900]
CTTCTTCCCT ATCATCTCTC TGTTTCCTTC TTCGGGAAGG AGGAGGAGGA   [950]
AGGAAGGAAG GAAGGAACGG CGCACAAACT ACCCCAACTT TCG          [993]
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Fig. 5. ITS sequence of *Soranthera ulvoidea* from San Juan Island, Washington. Only sequence was changed at 255 position of ITS 1 in sample from Sunset bay, Oregon (A ↔ C, bold). 5.8S rRNA gene was located between ITS 1 and ITS 2 (underlined).

numerous clavate assimilatory filaments, which were approximately 16 celled. Phaeophycean hairs, arising from the surface cells, were common with unilocular sporangia.

Gametophytes, being microscopic, filamentous and bearing plurilocular sporangia, were not found in our collections.

Of the specimens collected in the present study, most specimens were f. *ulvoidea* forms, and those from San Juan Island, Washington and Seal Rock, Oregon were f. *difformis* type (Figs 1-2).

Analyses of ITS and *rbc*L sequences

All the ITS sequences except that from Sunset Bay in Oregon, which differed by only base, were identical (Fig. 5). The ITS1 was 350 bp length, and ITS2 481 bp length. The 5.8S region was 162 bp length.

The *rbc*L region was 1349 bp in specimen from Boiler Bay (PE115) and 1299 nt in specimen from Cape Arago (PE117), because of the use of different primers. However, both *rbc*L sequences were identical. For the *rbc*L sequences, 459 characters (31.3%) were variable and 311 characters (21.2%) were parsimony-informative sites. A total three equally most parsimonious trees were constructed. Tree length was 1382 steps, consistency index 0.457, and retention index 0.532. Bayesian tree of the *rbc*L sequences showed that *Soranthera ulvoidea* clustered with members of the Chordariaceae (98% Bayesian support) and was a sister to *Botrytella micromorus* (more than 90% Bayesian probability, 71% MP, and 64% ML bootstrap values, Fig. 6). The Chordariaceae clade was supported with Bayesian probability, although less supported by MP (58%) and ML (below 50%) bootstrap values. However, the Scytosiphonaceae and Acinetosporaceae were strongly supported. Only *Ectocarpus* in the Ectocarpaceae was included in the present study.

DISCUSSION

This is the first document to include the genus *Soranthera* in the molecular phylogeny, and the results recognize only *S. ulvoidea*, denying infraspecific taxa within the species, and place it firmly within the Chordariaceae *s.l.* *Soranthera ulvoidea* has been classified into two forms (Setchell and Gardner 1925). *S. ulvoidea* f. *ulvoidea* Setchell and Gardner has oval to spherical thalli with thick membrane, and *S. ulvoidea* f. *difformis* Setchell and Gardner possesses variously and deeply lobed fronds with thin membrane. Although Setchell and Gardner (1925) reported that f. *ulvoidea* was more common in the southern part of the range than f. *difformis*, both taxa occur in Oregon (Markham and Celestino 1976). In our study, despite a distance of 750 km from Cape Arago, where globose thalli were collected (Fig. 1), to Friday Harbor, where lobed fronds

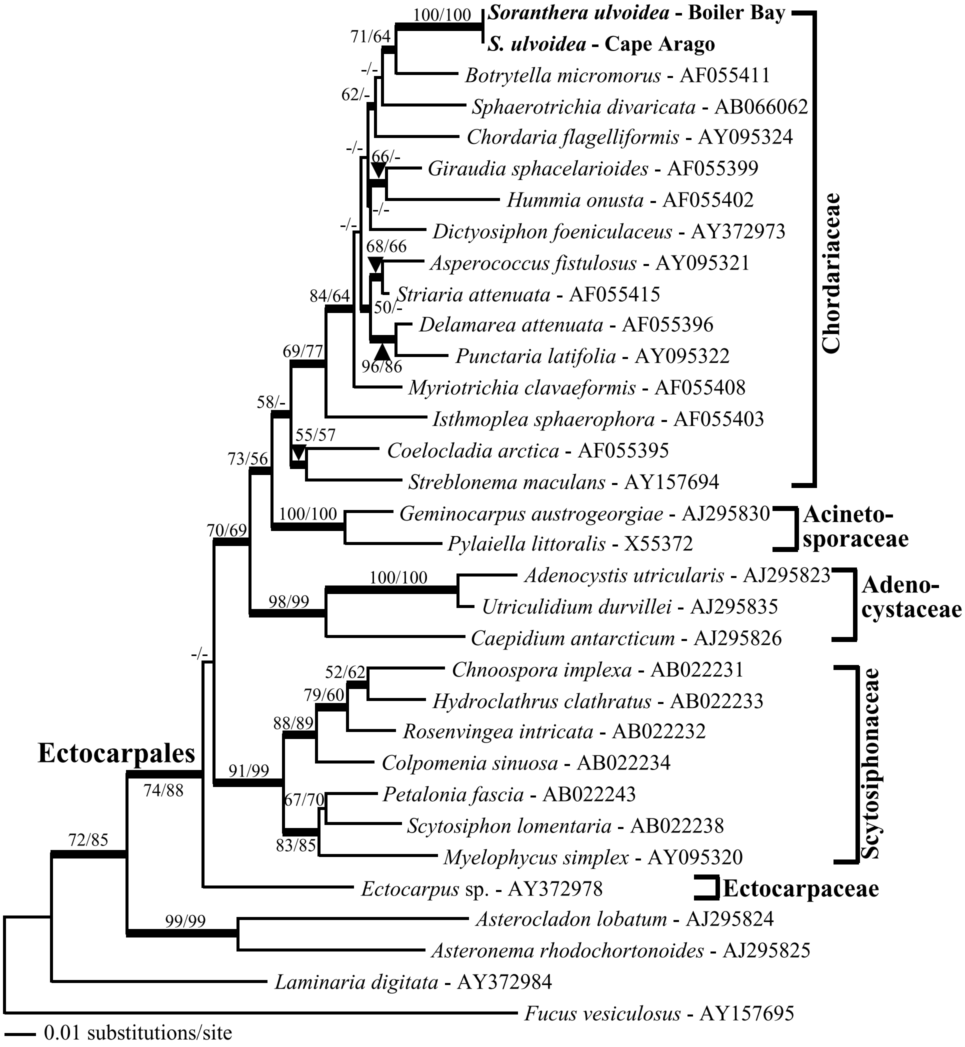

Fig. 6. Maximum likelihood tree of *rbc*L sequences for *Soranthera ulvoidea* (bold). Bootstrap values (≥ 50%) of maximum likelihood (left) and maximum parsimony (right) are above branches. Bayesian probabilities (≥ 0.9) represented as hard bar on the branches.

were sampled (Fig. 2), the ITS sequences were identical except specimen from Sunset Bay, which differed by only base (0.04% pairwise divergence). The *rbc*L sequences were identical between specimens from Boiler Bay and Cape Arago. We also found identical sequences of *rbc*L and ITS regions from typical forms of f. *ulvoidea* and f. *difformis* from Friday Harbor, Washington, as seen in Fig. 1. Our *rbc*L and ITS sequence data therefore do not support the presence of two infraspecific taxa such as f. *ulvoidea* and f. *difformis* within the species. Our results are in accordance with the views of Bold and Wynne (1985) and Abbott and Hollenberg (1976) that *Soranthera* includes a single species, *S. ulvoidea*, without infraspecific taxa. Although it is difficult to identify *S. ulvoidea* thallus when young from *Colpomenia* and *Leathesia* in the field, dark bumps of sori on the surface of the thalli makes it easy to distinguish the species in the field from the latter species (Mondragon and Mondragon 2003). The *rbc*L tree revealed that the former is clearly separated from the latter species (Cho and Boo, unpublished data).

It is unexpected that *Soranthera ulvoidea* was consistently a sister taxon to *Botrytella micromorus* (= *Sorocarpus micromorus*), although both being placed in the Chordariaceae. Although sporophytes of *Soranthera* are polystichous, while those of *Bortytella* haplostichous, both have phaeophycean hairs. Gametophytes of both taxa are filamentous, having discoid plastids with pyrenoid in each cell (Angst 1926; Wynne 1969 for *Soranthera*; Kim1996 for *Botrytella*). Further taxon sampling and alternate molecular markers such as *psa*A will improve resolution on the sister relationship between *Soranthera* and *Botrytella*. Instead, *Soranthera* did not link with *Asperococcus* and *Punctaria* of the Punctariaceae, or *Delamarea* of the Delamareaceae, *Striaria* of the Striariaceae, or *Dictyosiphon* of the Dictyosiphonaceae, although the latters formed a clade. Thus the Pun-ctariaceae or the other families in the Dictyosiphonales may no more accommodate *Soranthera*. Our results agree with previous molecular phylogenies that the Dictyosiphonales have close relationships with some chordarialean or ectocarpalean algae (Siemer *et al.* 1998; Rousseau and de Reviers 1999; Draisma *et al.* 2001; Peters and Ramirez 2001; Cho *et al.* 2003, 2004).

Our phylogenetic analyses of the *rbc*L sequences support the classification of the Ectocarpales into five families, as proposed by Peters and Ramirez (2001). However, the *rbc*L sequences in the present study reveal that phylogenetic relationships of *Soranthera* to putative relatives need more resolution. If the core groups of the Chordariaceae such as *Coilodesme*, *Coelocladia*, and *Phaeostroma* will be included, the phylogenetic position of *Soranthera* will be more resolved. Together with this, the phylogenetic relationships of the Chordariaceae *s.l.* should be urgently reinvestigated.

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REFERENCES

Abbott I.A. and Hollenberg G.J. 1976. *Marine Algae of California*. Stanford University Press, Stanford, CA.

Angst L. 1926. The gametophyte of *Soranthera ulvoidea. Publ. Puget Sound Biol. Stat.* **5:** 159-163.

Angst L. 1927. The holdfast of *Soranthera ulvoidea. Publ. Puget Sound Biol. Stat.* **5:** 267-275.

Barton E.S. 1898. On the structure and development of *Soranthera*, Post. & Rupr. *Linn. Journ. Bot.* **33:** 479-486.

Bold H.C. and Wynne M.J. 1985. *Introduction to the Algae: Structure and Reproduction*. 2nd ed. Prentice Hall Inc, New Jersey.

Cho G.Y., Lee S.H. and Boo S.M. 2004. A new brown algal order, Ishigeales (Phaeophyceae), established on the basis of plastid protein-coding *rbc*L, *psa*A, and *psb*A region comparisons. *J. Phycol.* **40:** 921-936.

Cho T.O., Cho G.Y., Yoon H.S., Boo S.M. and Lee W.J. 2003. New records of *Myelophycus cavus* (Scytosiphonaceae, Phaeophyceae) in Korea and the taxonomic position of the genus on the basis of a plastid DNA phylogeny. *Nova Hedw*. **76:** 381-397.

Cho G.Y., Yang E.C., Lee S.H. and Boo S.M. 2002. First description of *Petalonia zosterifolia* and *Scytosiphon lomentaria* (Scytosiphonaceae, Phaeophyceae) from Korea with special reference to nrDNA ITS sequence comparison. *Algae* **17:** 135-144.

Draisma S.G.A., Prud'homme van Reine W.F., Stam W.T. and Olsen J.L. 2001. A reassessment of phylogenetic relationships within the Phaeophyceae based on RUBISCO large subunit and ribosomal DNA sequences. *J. Phycol.* **37:** 586-603.

Hansen G.I. 1997. A revised checklist and preliminary assessment of the macrobenthic marine algae and seagrasses of Oregon. In: Kaye T.N., Liston A., Love R.M., Luoma D.L., Meinke R.J., and Wilson M.V. (eds), *Conservation and Management of Native Flora and Fungi. Native Plant Society of Oregon, Covaliis, Or*. pp. 175-200.

Huelsenbeck J.P. and Ronquist F. 2001. MrBayes: Bayesian inference of phylogenetic trees. *Bioinformatics* **17:** 754-755.

Kim H.S. 1996. Morphotaxonomic studies on the Korean Ectocarpaceae (Phaeophyta). IV. *Botrytella parvus* (Takamatsu) comb. nov., life history and morphogenesis based on light-temperate gradient culture. *Algae* **11:** 45-57.

Kjellman F.R. 1891- 1896. Phaeophyceae (Fucoideae). In: Engler A. and Prantl K. (eds), *Die naturlichen Planzenfamilien. I. Teil, 2.* Abteilung, Leipzig, pp. 176-192 (1891), 193-288 (1893), 287-297 (1896).

Klochkova N.G. 1998. An annotated bibliography of marine macroalgae on northwest coast of the Bring Sea and the southeast Kamchatka: the first revision of flora. *Algae* **13:** 375-418.

Markham J.W. and Celestino J.L. 1976. Intertidal marine plants of Clatsop County, Oregon. *Syesis* **9:** 253-266.

Mondragon J. and Mondragon J. 2003. *Seaweeds of the Pacific Coast: Common Marine Algae from Alaska to Baja California*. Sea Challengers, California.

Papenfuss G.F. 1947. Extension of the brown algal order Dictyosiphonales to include the Punctariales. *Bull. Torrey Bot. Club* **74:** 398-402.

Papenfuss G.F. 1955. Classification of the algae. In: A Century of Progress in the Natural Sciences 1853-1953. California Academy of Science, San Francisco, pp. 115-224.

Pedersen P.M. 1984. Studies on primitive brown algae (Fucophyceae). *Opera Bot*. 74: 1-76.

Peters A.F. and Ramirez M.E. 2001. Molecular phylogeny of small brown algae, with special reference to the systematic position of *Caepidium antarcticum* (Adenocystaceae, Ectocarpales). *Cryptogamie, Algol.* **22:** 187-200.

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

Postels A. and Ruprecht F. 1840. *Illustrationes Algarum*. St. Petersburg.

Rousseau F. and de Reviers B. 1999. Circumscription of the order Ectocarpales (Phaeophyceae): bibliographical synthesis and molecular evidence. *Cryptogamie, Algol.* **20:** 5-18.

Rousseau F. and de Reviers B. 1999. Circumscription of the order Ectocarpales (Phaeophyceae): bibliographical synthesis and molecular evidence. *Crytogamie, Algol.* **20:** 5-18.

Setchell W.A. and Gardner N.L. 1925. *The Marine algae of the Pacific coast of North America: Part III. Melanophyceae. Univ. Calif. Publ. Bot*. **8:** 383-898.

Siemer B.L., Stam W.T., Olsen J.L. and Pedersen P.M. 1998. Phylogenetic relationships of the brown algal orders Ectocarpales, Chordariales, Dictyosiphonales, and Tilopteridales (Phaeophyceae) based on RUBISCO large subunit and Spacer sequences. *J. Phycol.* **34:** 1038-1048.

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

Wynne M.J. 1969. Life history and systematic studies of some Pacific North American Phaeophyceae (brown algae). *Univ. Calif. Publ. Bot*. **50:** 1-88.

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