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Molecular phylogeny of the Family Scytosiphonaceae (Phaeophyceae)

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Recent phylogenetic studies of scytosiphonacean brown algae show many conflicts with current classification. In order to clearly define the phylogenetic relationships of the family, we newly sequenced the photosystem I coding *psa*A gene (1488 base pairs) from 13 taxa (15 samples), of the family, and, for comparison, *rbc*L from four taxa. The *psa*A region has more informative sites (17.9%) than the *rbc*L (13.1%) and the number of nodes supported by over 50% bootstrap values is more in the *psa*A phylogeny (53 /57 nodes; 93%) than in the *rbc*L (47/63 nodes; 74.6%). The *psa*A phylogenies are basically congruent with the *rbc*L trees, recognizing two major groups in the monophyletic Scytosiphonaceae. The first group included *Myelophycus*, *Petalonia*, *Scytosiphon*, and elongate sack-shaped species of *Colpomenia*, primarily cold temperate elements with unilocular zoidangia on sporophytes. The second group, although not resolved, consisted of *Hydroclathrus*, *Chnoospora*, *Rosenvingea*, and ball-shaped *Colpomenia*, primarily warm-temperate taxa with both unilocular and plurilocular zoidangia on sporophytes. *Chnoospora* is not monophyletic, as was previously shown the paraphyly of *Colpomenia*, *Petalonia*, and *Scytosiphon*. *Hydroclathrus clathratus* from Korea and Japan was not monophyletic. Our studies show that gametophytic characters are the main source of conflict for the present taxonomy of the family. The *psa*A region is a useful tool for resolution of phylogenetic relationships within the Scytosiphonaceae.

Key Words: brown algae, phylogeny, *psa*A, Phaeophyceae, *rbc*L, Scytosiphonaceae

INTRODUCTION

The scytosiphonacean brown algae are an ecologically important group in the intertidal zones of the temperate regions where they predominate in spring. Some species such as *Scytosiphon lomentaria* is a model species for life history and molecular studies in brown algae (Wynne 1969; Kawai *et al.* 1995; Nagasato *et al.* 2004).

Traditionally, two families, the Chnoosporaceae and Scytosiphonaceae, are classified in the order Scytosiphonales that have a single cup-shaped plastid bearing a single pyrenoid and a heteromorphic life cycle in which a parenchymatous, erect gametophytic thallus alternates with a pseudoparenchymatous sporophytic prostrate thallus (Feldmann 1949; Wynne 1969; Nakamura and Tatewaki 1975; Clayton 1980; Kogame 2001). The Chnoosporaceae is characterized by having subapical growth and includes the genus *Chnoospora* only. The Scytosiphonaceae is distinguished from the Chnoosporaceae by having thalli with diffuse growth (Farlow 1881; Setchell and Gardner 1925). The Scytosiphonaceae traditionally includes *Colpomenia* (Endlicher) Derbès et Solier in Castagne, *Hydroclathrus* Bory, *Iyengaria* Børgesen, *Jolyna* Guimarês, *Petalonia* Derbès et Solier, *Rosenvingea* Børgesen, and *Scytosiphon* C. Agardh (Wynne 1969; Kogame *et al.* 1999). *Endarachne* J. Agardh was treated as a synonym of *Petalonia* (Vinogradova 1973). However, Cho *et al.* (2003) proposed inclusion of *Myelophycus* Kjellman in Engler and Prantl, which has an isomorphic life history, in the Scytosiphonaceae based on *rbc*L sequence data.

Recent works on nuclear ribosomal DNA (Reviers and Rousseau 1999; Rousseau and Reviers 1999) and plastid *rbc*L sequences (Siemer *et al.* 1998; Draisma *et al.* 2001; Peters and Ramírez 2001) show that the order Ectocarpales could include the Scytosiphonales as a family, together with the Chordariales, Dictyosiphonales, and Punctariales and excluding the Ralfsiales and the taxa with stellate plastids. Peters and Ramírez (2001) proposed a new system in which the Ectocarpales consists of five families: Ectocarpaceae, Scytosiphonaceae, Acinetosporaceae, Chordariaceae and Adenocystaceae. In the new system, the Chnoosporaceae was included in

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the Scytosiphonaceae.

The objective of our study was to compare the sequences of the *psa*A with the previously published molecular data of the Scytosiphonaceae. The *psa*A gene encodes photosystem I P700 apoprotein A1 in algae to vascular plants. We observed that the *psa*A is as variable and informative as the *rbc*L in brown algae (Cho *et al.* 2004). Despite its variability, the *psa*A sequences are easily aligned because of no gaps even among the whole brown algal groups. In the present study, we introduce 15 novel *psa*A sequences from 13 species of the Scytosiphonaceae, representing seven genera, and added five previously published sequences by us to improve resolution within the family (Cho *et al.* 2004). For comparison, we also obtained *rbc*L data from four taxa and added 17 published data from Kogame *et al.* (1999) and Cho *et al.* (2003) for constructing *psa*A, *rbc*L, and combined *psa*A + *rbc*L phylogenies.

MATERIALS AND METHODS

Taxon sampling

Thirteen species of Scytosiphonaceae were collected in the Korean coast (Fig. 1). Twenty-one samples of seven genera of the family Scytosiphonaceae were available for the present study. Of 20 taxa available for the *psa*A phylogeny, we analyzed 13 taxa (15 samples) of the family and downloaded 5 taxa from GenBank. We also analyzed the *rbc*L from four taxa and took 17 sequences from the GenBank. Each representative of the remaining families of the Ectocarpales was chosen as outgroups; *Adenocystis utricularis* of the Adenocystaceae, *Chordaria flagelliformis* of the Chordariaceae, *Ectocarpus* sp. of the Ectocarpaceae, and *Pylaiella littoralis* of the Acinetosporaceae. Both *psa*A and *rbc*L sequences from these four taxa were taken from Genbank (Cho *et al.* 2004). Voucher specimens of the present study are deposited in the herbaria of Chungnam National University (CNUK), Daejon, Korea and Graduate School of Science, Hokkaido University. The collection sites and GenBank accession numbers of the ingroup and outgroup taxa included here are listed in Table 1.

Analyses of the *psa*A and *rbc*L regions

Total DNA was extracted from approximately 0.01 g of dried thalli ground in liquid nitrogen using a DNeasy Plant Mini Kit (Qiagen Gmbh, Hilden, Germany), according to the manufacturers' instructions, and then dissolved in 150 μL of distilled water. Extracted DNA was stored at -20°C and used to amplify the *psa*A and *rbc*L regions.

The *psa*A region was amplified and sequenced using primers *psa*A130F, *psa*A870F, *psa*A970R and *psa*A1760R (Yoon *et al.* 2002). The amplification and sequencing reactions were the same as those used by Yoon *et al.* (2002). The same DNA aliquot was used to amplify the *rbc*L region, and the amplification and sequencing reactions for this region were the same as those used by Kogame *et al.* (1999) and Yoon and Boo (1999). Primers PRB-F0, F2, F3, R1A, R2, R3A, RS1, and RS2 have been used for all taxa. The polymerase chain reaction (PCR) products were purified using a High Pure^{TM} PCR Product Purification Kit (Roche Diagnostics GmbH, Mannheim, Germany), according to the manufacturer's instructions. The sequences of the forward and reverse strands were determined for all taxa using an ABI PRISM^{TM} 377 DNA Sequencer (Applied Biosystems, Foster City, CA, USA) at the Center for Research, Chungnam National University, Daejon, Korea. The electropherogram output for each sample was edited using the program Sequence Navigator v. 1.0.1 (Applied Biosystems).

All the sequences of the *psa*A gene from 17 taxa (20 samples) of the Scytosiphonaceae and four outgroups were collated using the multisequence editing program, SeqPup (Gilbert 1995), and aligned by eye to compare our sequences with those published previously (Cho *et al.* 2004). The *rbc*L sequences from 25 taxa, including the same four outgroups, were also aligned by eye. Both the *psa*A and *rbc*L sequence data sets were combined for phylogenetic analyses.

Phylogenetic analyses

Three data sets were used for the phylogenetic analyses: 24 sequences for *psa*A, 25 sequences for *rbc*L, and 22 sequences for the combined *rbc*L + *psa*A data sets. Since we previously performed the saturation and incongruence length difference tests for both genes (Cho *et al.* 2004), we did not repeat the tests.

Maximum parsimony (MP) trees were constructed for each dataset using PAUP* (Swofford 2002) using a heuristic search algorithm with the following settings: 100 random sequence-addition replicates, TBR branch swapping, MulTrees, all characters unordered and unweighted, and branches with a maximum length of zero collapsed to yield polytomies. The bootstrap values (BS) for the resulting nodes were assessed using bootstrapping with 1,000 replicates.

For maximum likelihood (ML) and Bayesian analyses

Fig. 1. Korean scytosiphonacean algae. A. *Myelophycus cavus* from Chujado, Jeju. B. *M. simplex* from Chujado, Jeju. C. *Petalonia zosterifolia* from Onyangri, Uljin. D. *P. binghamiae* from Shinnam, Samcheok. E. *P. fascia* (arrow) and *Scytosiphon lomentaria* (arrow head) from Gangjeong, Jeju. F. *S. gracilis* from Gangjeong, Jeju. G. *Hydroclathrus clathratus* from Chagwido, Jeju. H. *Colpomenia bullosa* from Wuelpo, Pohang. I. *C. phaeodactyla* from Sangjokam, Goseong. J. *C. sinuosa* from Wuelpo, Pohang. K. *C. peregrina* from Seosang, Namhae. L. *Colpomenia* sp. from Sangjokam, Goseong.

(BA) performed the likelihood ratio test using the Modeltest 3.06 version (Posada and Crandall 1998) to determine the best available model for the *psa*A data set. For each of both analyses, the best model was a TrN model with shape parameter of the gamma distribution (Γ) and portion of invariable sites (I). Maximum likelihood tree for the *psa*A data was constructed by heuristic search methods (100 random sequence-additions) - substitution rate matrix. Bootstrap values for ML tree was evaluated with 100 bootstrap replicates by heuristic search (5 random sequence-additions).

Bayesian phylogenetic analyses were performed using

Table 1. Species information and GenBank accession numbers used in this study.

Species	Collection sites or references and voucher numbers of *psa*A/*rbc*L	GenBank accession numbers	
		*psa*A	*rbc*L
Chnoospora implexa J. Agardh	Sesoko, Okinawa, Japan; Ko1 /Kogame *et al*. 1999	**DQ239772**	AB022231
C. minima (K. Hering) Papenfuss	Yonaguni-jima, Okinawa, Japan; Ko2	**DQ239773**	**DQ239768**
Colpomenia bullosa (Saunders) Yamada *in* Yamada *et* Kinoshita	Bamfield, Vancouver Isl., Canada; PE681/Kogame *et al*. 1999	**DQ239774**	AB022236
	Weller's Rock, Dunedin, New Zealand; PE049	**DQ239775**	AY398466
C. peregrina (Sauvageau) Hamel	Anin, Gangreung, Korea; PE018/Cho *et al*. 2005	**DQ239776**	AY398435
C. phaeodactyla Wynne *et* Norris	Hoedong, Jindo, Korea; PE231/Kogame *et al*. 1999	**DQ239777**	AB022237
C. sinuosa (Mertens *ex* Roth) Derbès *et* Solier *in* Castagne	Cho *et al*. 2004/Kogame *et al*. 1999	AY372950	AB022234
Hydroclathrus clathratus (C. Agardh) Howe	Cho *et al*. 2004/Kogame *et al*. 1999	AY372951	AB022233
	Chagwido, Jeju, Korea; PE142	**DQ239778**	**DQ239769**
H. tenuis C. K. Tseng *et* Lu	Batac, Ilos Cortes, Philippine; PE609	**DQ239779**	**DQ239770**
	Ohama, Ishigaki Island, Japan; PE381	**DQ239780**	-
Myelophycus cavus Tanaka *et* Chihara	Woongdo, Taean, Korea; PE606/ Cho *et al*. 2003	**DQ239781**	AY095319
M. simplex (Harvey) Papenfuss	Cho *et al*. 2004/Cho *et al*. 2003	AY372952	AY095320
Petalonia binghamiae (J. Agardh) Vinogradova	Munseom, Jeju, Korea; PE627/Kogame *et al*. 1999	**DQ239782**	AB022244
P. fascia (O. F. Muller) Kuntze	Cho *et al*. 2004/Kogame *et al*. 1999	AY372953	AB022243
P. zosterifolia (Reinke) Kuntze	Onyangri, Uljin, Korea; PE635/Kogame *et al*. 1999	**DQ239783**	AB022242
Rosenvingea intricata (J. Agardh) Børgesen	Gushikawa, Okinawa, Japan; Ko3/Kogame *et al*. 1999	**DQ239784**	AB022232
Scytosiphon canaliculatus (Setchell *et* Gardner) Kogame	Kogame *et al*. 1999	-	AB022239
Scytosiphon dotyi Wynne	Monterey, CA, USA; PE628	**DQ239785**	**DQ239771**
S. gracilis Kogame	Hado, Jeju, Korea; PE610/Kogame *et al*. 1999	**DQ239786**	AB022240
S. lomentaria (Lyngbye) Link	Cho *et al*. 2004/Kogame *et al*. 1999	AY372954	AB022238
S. tenellus Kogame	Kogame *et al*. 1999	-	AB022241
Adenocystis utricularis (Bory) Skottsberg	Cho *et al*. 2004/Peters and Ramírez 2001	AY372939	AJ295823
Chordaria flagelliformis (O. F. Muller) J. Agardh	Cho *et al*. 2003/Cho *et al*. 2004	AY372941	AY095324
Ectocarpus sp.	Cho *et al*. 2004/Hoedong, Jindo, Korea; PE011	AY372949	AY372978
Pylaiella littoralis (Linnaeus) Kjellman	Yoon *et al*. 2002/Assali *et al*. 1990	AY119724	X55372

MrBayes 3.0 (Huelsenbeck and Ronquist 2001). Each analysis was initiated from a random starting tree and the program was set to run four Markov chain Monte Carlo iterations simultaneously for 2,000,000 generations with trees sampled every 100th generation. The likelihood scores stabilized at approximately 300,000 generations, so the first 3,000 trees were burned. For comparison with bootstrapping, we considered nodes with Bayesian probabilities (BP) greater than 0.9 (i.e., the node appears in more than 90% of the sampled trees) as being well supported.

RESULTS

General characteristics of *psa*A and *rbc*L sequences and phylogenetic trees

General characteristics of the *psa*A and *rbc*L sequences investigated in the present study are summarized in Table 2. The *psa*A sequences determined in the present study totaled 1488 base pairs (Table 2). For the 20 aligned sequences from scytosiphonacean taxa, 424 (28.5%) sites were variable and 266 (17.9%) were parsimoniously informative. There were excesses of adenine and thymine at all codon positions (29.69% and 35.37%, respectively). Transitions occurred higher than transversions for all codon positions (Ti/Tv = 1.58).

The *rbc*L sequences were trimmed with 1467 base pairs (Table 2). For the 21 aligned sequences from scytosiphonacean taxa, 325 (22.2%) bases were variable and 192 (13.1%) were parsimoniously informative. There were excesses of adenine and thymine at all codon positions (29.5% and 32.04%, respectively). Transitions occurred two times higher than transversions for all codon positions (Ti/Tv = 2.23).

In MP analysis, the *psa*A and *psa*A + *rbc*L data sets produced a single tree, while the *rbc*L data produced six

Table 2. Information of analyses of individual and combined data sets.

	*psa*A	*rbc*L	*psa*A + *rbc*L
Number of ingroup taxa	20	21	18
Number of outgroup taxa	4	4	4
Analyzed size	1488	1467	2955
Base frequency (A/C/G/T)	0.2969/0.1567/0.1927/0.3537	0.2950/0.1626/0.2220/0.3204	-
Number of transitions/transversions (Ti/Tv ratio)	18425/11697 (1.58)	15464/6922 (2.23)	-
Variable sites	424 (28.5%)	325 (22.2%)	745 (25.2%)
Parsimoniously informative sites	266 (17.9%)	192 (13.1%)	447 (15.1%)
Number of MP tree	1	6	1
Tree length of MP	1002	726	1716
Consistency index	0.565	0.558	0.561
Retention index	0.618	0.591	0.552
Selected model for likelihood analysis	TrN + I + Γ	TrN + I + Γ	TrN + I + Γ
A ↔ G substitution rate	4.4707911	4.0197483	4.1743831
C ↔ T substitution rate	6.9286303	8.2525349	7.3057072
Proportion of invariable sites	0.601325	0.639244	0.615285
Gamma shape parameter	1.049559	0.822098	0.882258
-*ln* L	6928.21866	5779.58776	12661.16524
Number of nodes supported by over 50% bootstrap values per total no. of nodes	53/57 (93%)	47/63 (74.6%)	53/57 (93%)

trees. The consistency and retention indices were higher in the *psa*A tree than the *rbc*L and *psa*A + *rbc*L trees. Proportion of invariable sites was lower in the *psa*A tree than the *rbc*L and *psa*A + *rbc*L trees, while the value of -*ln* L was higher in the *psa*A + *rbc*L tree than in the *psa*A tree. Nodes supported by over 50% ML and MP bootstrap values were 34 in both trees constructed by the *psa*A and *psa*A+ *rbc*L data sets, and 27 in the *rbc*L tree. In Bayesian analysis, nodes supported by over 90% were 17 in the *psa*A + *rbc*L trees, and 14 in the *psa*A and *rbc*L trees.

Phylogenetic relationships

In the *psa*A phylogenies (Fig. 2), all the scytosiphonacean algae investigated here produced a monophyletic clade (99% BS for ML, 100% BS for MP, and BP=100%), which were grouped into two major clades. Group I consisted of *Myelophycus*, *Petalonia*, *Scytosiphon*, and the elongate sack-shaped species of *Colpomenia*, being strongly supported (91% BS for ML, 88% BS for MP, and BP=97%). In this clade, although the genus *Myelophycus* only was monophyletic, the genera *Scytosiphon* and *Petalonia* were paraphyletic, respectively. *Scytosiphon gracilis* clustered with *Petalonia zosterifolia* with maximum support. *Colpomenia bullosa*, and *C. phaeodactyla* were very closely related to each other.

Group II included *Chnoospora*, *Rosenvingea*, *Hydroclathrus* and two saccate species of *Colpomenia*. This clade was not supported by bootstrap analyses of ML and MP, but the subclade except *Chnoospora minima* and *Colpomenia peregrina* was well retrieved (100% BS for ML, 95% BS for MP, and BP=100%). The genus *Hydroclathrus* was monophyletic with maximum support, but *H. clathratus* from Korea and Japan did not form a clade. *Chnoospora implexa* was basal to *Hydroclathrus*, while *Chnoospora minima* clustered with *Colpomenia peregrina*.

The *psa*A + *rbc*L phylogenies (Fig. 3) are almost identical with those of the *psa*A data except bootstrap values in some nodes, but the *rbc*L phylogenies are not shown because they are congruent with those of the *psa*A + *rbc*L trees. However, *Myelophycus* did not cluster with the *P. zosterifolia*/*S. gracilis* group in the *rbc*L trees.

DISCUSSION

This is the first report to document photosystem I coding *psa*A phylogeny of the Scytosiphonaceae. Although the *psa*A and *rbc*L analyzed in the present study are similar for length (1488 bp in *psa*A and 1467 bp in *rbc*L), the former has more informative sites (17.9%) than the latter (13.1%). The number of nodes supported by over 50% bootstrap values is more in the *psa*A trees (53 /57 nodes; 93%) than in the *rbc*L (47/63 nodes; 74.6%), as in Table 2. There is no sign of saturation of the *psa*A region even in a big tree of diverse brown algae (Cho *et al.* 2004). At the levels of the family and genus, the *psa*A phylogenies are generally consistent with those of *rbc*L in the present study and previous *rbc*L and/or LSU rDNA phylogenies

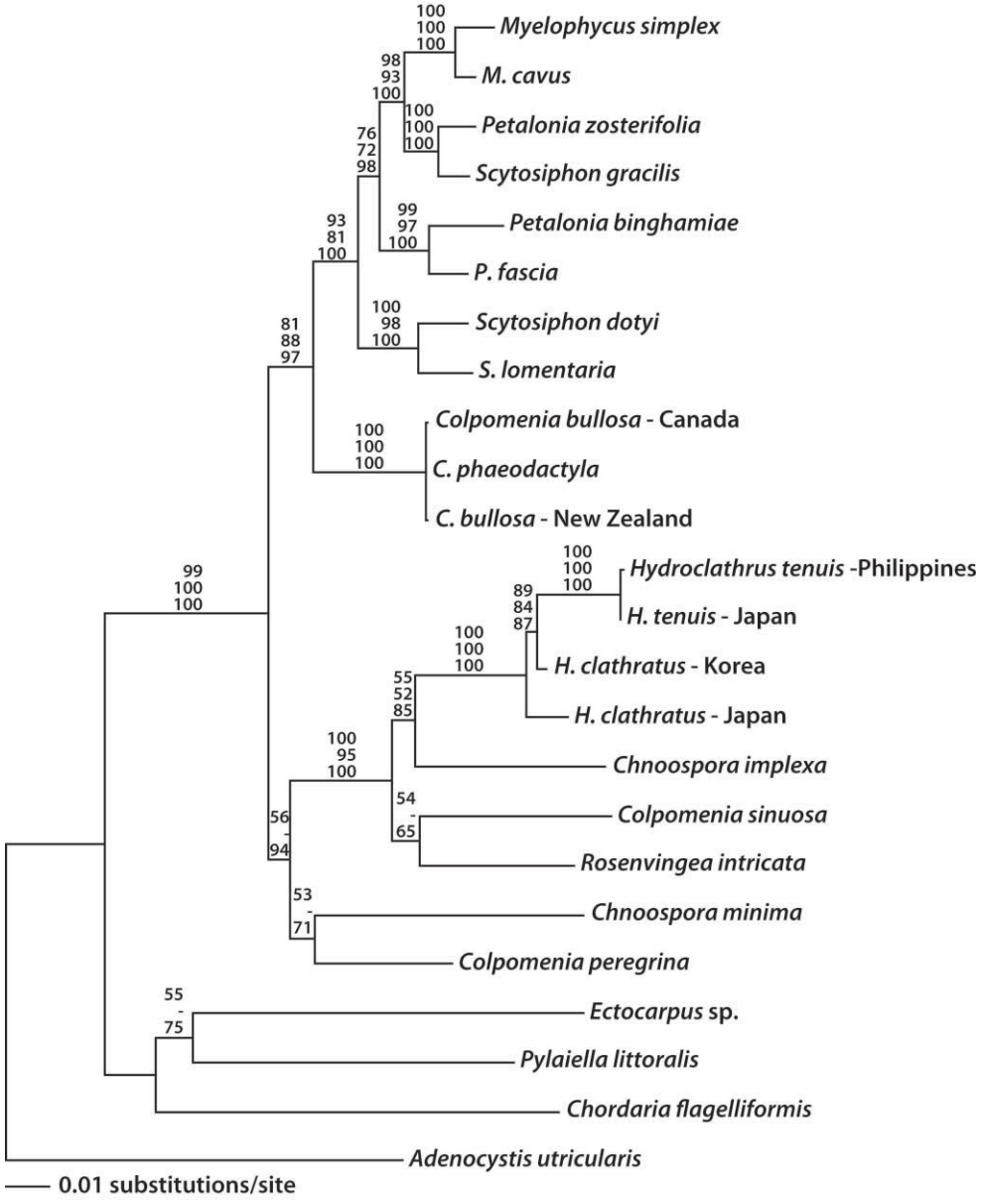

Fig. 2. Maximum likelihood tree for the Scytosiphonaceae estimated from the *psa*A sequence data (TrN + I + G model, -ln L = 6928.21866; AG = 4.4707911, CT = 6.9286303, I = 0.601325, G = 1.049559). The numbers above the branches are bootstrap values from the maximum likelihood, maximum parsimony, and Bayesian analyses (ML/MP/BP).

(Kogame *et al.* 1999; Cho *et al.* 2003), and are also not different with RuBisCO spacer trees (Cho *et al.* 2001). All of these results indicate that the *psa*A region is a useful tool for resolution of phylogenetic relationships within the Scytosiphonaceae.

Both *psa*A and *rbc*L sequence data highlight a single evolutionary origin for the family Scytosiphonaceae, as shown in previous published *rbc*L and rDNA trees (Kogame *et al.* 1999; Cho *et al.* 2001, 2003). Like *rbc*L trees, the *psa*A phylogenies place two species of *Myelophycus* in the Scytosiphonaceae. The present study is a good confirmation that the life cycle of *Myelophycus* is derived with the Scytosiphonaceae and that a reduced sporophyte is a synapomorphy of the family (Cho *et al.* 2003).

The *psa*A trees are consistent with the *rbc*L in recognizing two major groups within the family and paraphyly of each of the genera *Colpomenia*, *Petalonia*, and *Scytosiphon*, the relationships of these genera being well reviewed by Kogame *et al.* (1999). One of the two major groups consists of *Myelophycus*, *Petalonia*, *Scytosiphon*, and elongate sack-shaped *Colpomenia*. All of these have tubular or flat, linear thalli and produce only unilocular zoidangia as reproductive organs on sporophytes (Nakamura and Tatewaki 1975; Clayton 1980; Hori 1993; Kogame 1998;

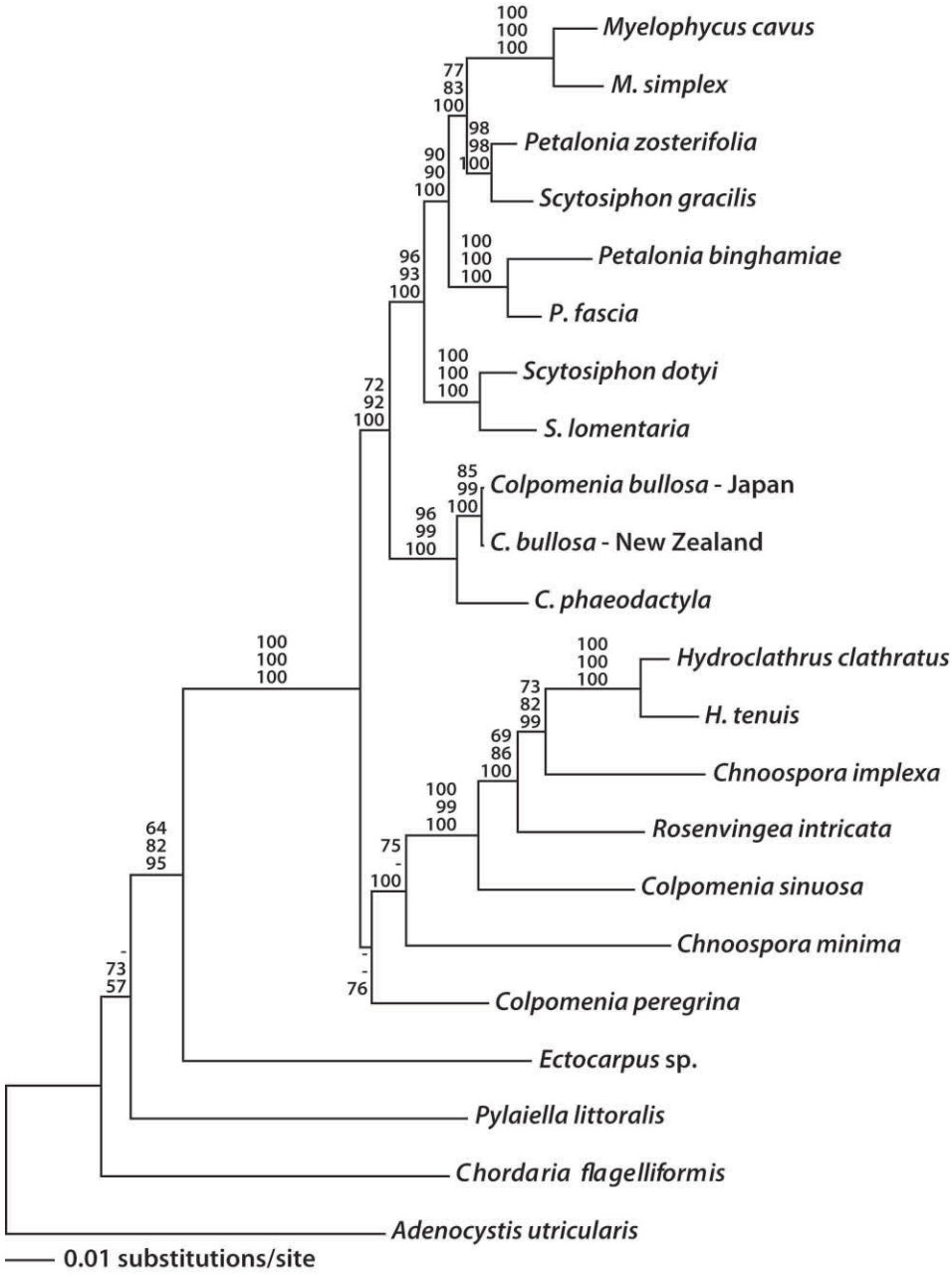

Fig. 3. Maximum likelihood tree for the Scytosiphonaceae estimated from the *psa*A + *rbc*L sequence data (TrN + I + G model, -ln L = 12661.16524; AG = 4.1743831, CT = 7.3057072, I = 0.615285, G = 0.882258). The numbers above the branches are bootstrap values from the maximum likelihood, maximum parsimony, and Bayesian analyses (ML/MP/BP).

Kogame *et al.* 1999). They usually occur in cold-temperate water (Kogame *et al.* 1999). However, it is very interesting that *Myelophycus* with an isomorphic life history is the sister-taxon to the *Petalonia zosterifolia*/ *Scytosiphon gracilis* clade. The second group, although not resolved, consisted of *Hydroclathrus*, *Chnoospora*, *Rosenvingea*, and ball-shaped *Colpomenia*. They produce plurilocular and unilocular zoidangia on sporophytes, and are mostly distributed in warm-temperate waters (Kogame 1997; Kogame and Yamagishi 1997; Kogame *et al.* 1999; Kogame 2001; Toste *et al.* 2003). It is therefore concluded that the Scytosiphonaceae consists of two phyletic groups recognized by reproductive organs, distributional patters, and *psa*A and *rbc*L data. These results propose two possibilities in taxonomy. The first idea is that the family may be classified into two tribes such as the Scytosiphonieae for elongate sack-shaped *Colpomenia*, *Myelophycus*, *Petalonia*, *Scytosiphon* and the Chnoosporieae for *Chnoospora*, ball-shaped *Colpomenia*, *Hydroclathrus*, and *Rosenvingea*. This proposal, however, does not

resolve the paraphyly of the scytosiphonacean genera mentioned above. Alternately, the second idea is that the first group is classified in the genus *Scytosiphon* and the second group is placed in the genus *Hydroclathrus*, according to the priority of generic names. In this case, however, the first group includes the isomorphic genus *Myelophycus* and heteromorphic genera. It may be better that such life-history features are treated as a generic feature. Further, the monophyly of the second group has not been demonstrated by molecular analyses: it is possible that the second group is paraphyletic.

Chnoospora implexa and *C. minima* possess a subapical meristem which characterizes this genus. The presence of plurilocular zoidangia on erect thalli and the plastid features in *C. minima* were studied by Fotos (1981), and Kogame (2001) confirmed a heteromorphic and diphasic life history of *C. implexa* in culture, and also observed a direct-type life history. These morphological and life-history observations confirmed that these *Chnoospora* species belong to the Scytosiphonaceae (as Scytosiphonales). Both *psa*A and *rbc*L data analyzed here show that the two species are never recovered as a monophyletic clade, *C. implexa* being placed between the genus *Hydroclathrus* and *R. intricata*, whereas *C. minima* between *Colpomenia peregrina* and *C. sinuosa*. These results indicate that subapical meristem is not synapomorphic for the genus. Together with designation of the type of the genus, a formal taxonomic revision will be followed.

The genus *Hydroclathrus* is a widespread tropical to temperate genus, the thalli consisting of hollow sacks or torn, sheet-like expanses that are irregularly but abundantly punctuated by various holes (Kraft and Abbott 2003). *Hydroclathrus tenuis* is different from *H. clathratus* in having spider-webbed skeins of narrow membranes and particularly large hole-to-tissue ratio (Tseng and Lu 1983). However, Kraft and Abbott (2003) reported that, since both species appear closely related in cortical cell, hairs, and soral features, two species could be settled more decisively by DNA fingerprinting than by the establishment of firm anatomical/morphological discontinuities. Our trees using *psa*A and *rbc*L data clearly show that two species form a monophyletic clade, but are different species. However, *H. clathratus* from Korea and Japan is not monophyletic. The relationships of European *H. clathratus* with specimens from Korea and Japan should also be taken into account, because the type of the species is from Belle Ile, France. Unfortunately, we could not include any specimen from the type locality in our studies. Because the species is widely distributed in tropical to temperate waters and difficult to generalize morphological limit of the species, a comparative study on different populations of putative *H. clathratus* is necessary (Kraft and Abbott 2003).

The *psa*A trees studied here are a demonstration of previously published *rbc*L and LSU rDNA trees (Kogame *et al.* 1999; Cho *et al.* 2003). The criterion of monophyly to two groups of scytosiphonacean algae that are emerging from molecular studies is strongly supported by their sporophyte morphology and distribution patterns, as well discussed by Kogame *et al.* (1999). Certainly, the current classification of the Scytosiphonaceae appears to show an extremity of confusion in view of paraphyly of four (*Chnoospora*, *Colpomenia*, *Petalonia*, and *Scytosiphon*) of six genera, in the family, consisting of more than two species. In this point, inclusion of two other *Hydroclathrus* species, recently described by Kraft and Abbott (2003) and of more species of *Rosenvingea*, in which only species was included in the present study, will also be of great interests, especially in view of the monophyly of each of both genera. Taxonomic problems also become visible in species such as *S. lomentaria* (Cho *et al.* 2001), *C. peregrina* (Cho *et al.* 2005), and *H. clathratus* in the present study. All of our results together with previous studies require an urgent revision of the family at species and genus level, much different from the current classification system. In order to reduce taxonomic confusion, however, the revision should be done after broad sampling of *Rosenvingea* and *Hydroclathrus*, and/or inclusion of *Iyengaria* and *Jolyna*, which were not addressed here.

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