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New records of *Myelophycus cavus* (Scytosiphonaceae, Phaeophyceae) in Korea and the taxonomic position of the genus on the basis of a plastid DNA phylogeny

by

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With 12 figures and 3 tables

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Abstract: *Myelophycus* is a brown algal genus that includes only two species worldwide: *M. cavus* and *M. simplex*. *M. simplex* occurs commonly in the Northwest Pacific and *M. cavus* was reported only in Japan. Here we report the occurrence of *M. cavus* in Korea for the first time. The species occurred in the upper intertidal on the west and south coast. Thalli were twisted and hollow, having small plurilocular zoidangia (c. 90 µm long) and unilocular sporangia (c. 50 µm long). Plastid DNA (*rbc*L and Rubisco spacer) sequences were determined in *Myelophycus* and compared with homologous positions of newly sequenced putative relatives (*Analipus japonicus*, *Asperococcus fistulosus*, *Chordaria flagelliformis*, and *Punctaria latifolia*) and with published sequences of other brown algae. Extensive interspecific divergences of the *rbc*L and Rubisco spacer were found in *Myelophycus*. Supporting the recent classification of five families in the Ectocarpales, our data of plastid DNA sequences also show that *Myelophycus* consistently formed a monophyletic clade with a maximum support together with the Scytosiphonaceae. Within this lineage, *Myelophycus* clustered with taxa having only unilocular sporangia on their sporophytes, while internal branches were less resolved. Based on the plastid DNA sequences and synapomorphic characters such as parenchymatous tissue, a single plastid with a pyrenoid per cell, and hormosirene, we propose to place *Myelophycus* (formerly classified in the Asperococcaceae or in the Punctariaceae or in the Chordariaceae sensu lato) in the Scytosiphonaceae and emend the circumscription of that family to include also isomorphic life histories in addition to those with heteromorphic patterns.

Key words: *Myelophycus*, Phaeophyceae, Phylogeny, *rbc*L, Rubisco spacer, Scytosiphonaceae, Taxonomy.

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Introduction

Myelophycus Kjellman in Engler et Prantl is a genus of brown algae containing two species. They are annuals and occur exclusively in the northwest Pacific Ocean. The genus is characterized by cylindrical, unbranched thalli, having one plate-like plastid with one pyrenoid in each cell, and by an isomorphic life history (Wynne 1969; Tanaka & Chihara 1984; Kawai et al. 1994). The genus was based on *M. caespitosum* Kjellman, which was described from material collected from Japan (Kjellman 1893). However, Papenfuss (1967) showed that *M. caespitosum* is a later homonym of *Chordaria simplex* Harvey, making the name of the type species *Myelophycus simplex* (Harvey) Papenfuss. *Myelophycus simplex* occurs in the upper intertidal zone along the coasts of China (Tseng 1983), Japan (Tanaka & Chihara 1984), and Korea (Cho & Boo 1998). Tanaka & Chihara (1984) described *M. cavus* (= *M. cavum*), the second member of the genus, based on material from Shimoda on the Pacific coast of Japan. Prior to the present study, all *Myelophycus* specimens from outside Japan have been considered to be *M. simplex*.

The taxonomy of the genus *Myelophycus*, which is based on morphology, is controversial. Kjellman (1893) placed the genus under the Encoeliaceae (= Punctariaceae) because of its polystichous growth. Wynne (1969), however, assigned the genus to the Chordariales and suggested its possible relationships to *Analipus* Kjellman and *Heterochordaria* Setchell et Gardner, interpreting *M. simplex* as having pseudoparenchymatous tissues. Tanaka & Chihara (1984) reconfirmed the presence of parenchymatous tissues in the *Myelophycus* species and classified the genus in the Punctariaceae within the Dictyosiphonales. On the other hand, Yoshida (1998) classified *Myelophycus*, together with *Asperococcus* Lamouroux and *Melanosiphon* Wynne, in the Asperococcaceae, as did Setchell & Gardner (1925). According to Yoshida (1998), the family Asperococcaceae, having unilocular sporangia protruding from the surface of the thallus, is separated from the Punctariaceae, which consists of *Pogotrichum* Reinke, *Punctaria* Greville, and *Trachynema* Pedersen.

Recently, Rousseau & de Reviers (1999) circumscribed the Ectocarpales *sensu lato*, including the Chordariales, Dictyosiphonales, Ectocarpales sensu stricto, and Scytosiphonales. This is corroborated by other DNA phylogenies (de Reviers & Rousseau 1999; Draisma et al. 2001; Peters & Ramírez 2001; Rousseau et al. 2000, 2001). Based on plastid structure, life histories, and DNA sequences, Peters & Ramírez (2001) proposed a new classification scheme, stating that the Ectocarpales can be classified into five families, viz. Acinetosporaceae, Adenocystaceae, Chordariaceae, Ectocarpaceae, and Scytosiphonaceae. In the new scheme, previous dictyosiphonalean families such as the Punctariaceae and Dictyosiphonaceae are synonymized with the Chordariaceae (Peters & Ramírez 2001). Then, *Myelophycus*, together with *Punctaria* Greville and *Dictyosiphon* Greville, which were assigned to the Punctariaceae, will be forced to be included in the Chordariaceae *sensu lato*.

The Chordariaceae, having macroscopic sporophyte and microscopic gametophyte with pariatal discoid plastids (Peters & Ramírez 2001), however, does not include *Myelophycus*. The family Ectocarpaceae may contain *Myelophycus* in having an isomorphic life history, but is distinct in having ribbon-shaped plastids (Peters &

Ramírez 2001). Although genera of the Scytosiphonaceae show a different type of life history from *Myelophycus*, all these members have the same type of plastid and sexual pheromone (Kawai 1992; Kawai et al. 1994).

The goal of the present paper is to describe the developmental morphology of *Myelophycus cavus* using our recent collections and to determine the taxonomic position of the genus based on plastid DNA. The *rbc*L gene has already often been used for assessing deep branches of brown algal phylogenies (Siemer et al. 1998; Kogame et al. 1999; Draisma et al. 2001; Peters & Ramírez 2001) and, in an extensive sampling of phaeophycean algae, the *rbc*L is found to have more resolving power than nuclear-encoded ribosomal DNA (Draisma et al. 2001). In addition to *M. cavus* and *M. simplex*, we determined the *rbc*L sequences of *Analipus japonicus, Asperococcus fistulosus, Chordaria flagelliformis,* and *Punctaria latifolia,* which have been mentioned to be morphologically related to *Myelophycus* (Kjellman 1893; Setchell & Gardner 1925; Wynne 1969; Tanaka & Chihara 1984; Kawai et al. 1994). In order to give a better understanding of the phylogeny of *Myelophycus,* we compiled a total of 42 *rbc*L sequences including published data of other brown algae. We also determined the Rubisco spacer sequences from replicate samples of *M. cavus* (four samples) and *M. simplex* (two samples), collected from different locations, and from the four species mentioned above. The Rubisco spacer is used for interspecific variation and phylogenetic relationships of closely related genera or families (Stache-Crain et al. 1997; Siemer et al. 1998; Yoon et al. 2001).

Materials and methods

MORPHOLOGY: Thalli of *Myelophycus cavus* were collected in June and July 1999 and 2000 in the upper intertidal zone at several places in Boryong, Sinan, and Taean Provinces on the west coast, and in the Wando Province on the south coast, of Korea (Fig. 1). Material for observations was preserved in 4% formaldehyde-seawater. Microscopic observations were made on material stained with 1% aqueous aniline blue acidified with 2% HCl. Drawings 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.

ANALYSIS OF SEQUENCES OF RBCL AND RUBISCO SPACER: *Myelophycus cavus* specimens were collected in four islands on the West coast in order to determine intraspecific variation using the Rubisco spacer. Material of *M. simplex* was collected in two different sites. Four putative relatives, *Analipus japonicus, Asperococcus fistulosus, Chordaria flagelliformis,* and *Punctaria latifolia,* were sampled in Korea, Far East Russia, Pacific North America, and Great Britain (Table 2). All samples were air-dried and preserved with silica crystals.

Genomic DNA was extracted from approximately 0.005 g powder of thalli ground in liquid nitrogen using the DNeasy Plant Mini Kit (Qiagen Gmbh, Hilden, Germany), according to the manufacturer's instructions, and then dissolved in 150 mL distilled water. Polymerase chain reactions (PCR) of the *rbc*L and Rubisco spacer using genomic DNA and their sequencing reactions using purified PCR products followed Kogame et al. (1999) and Cho et al. (2001), respectively. Primers PRB-F0, F2, F3, R1A, R2, and R3A developed by Kogame et al. (1999) were used for amplification of the *rbc*L gene and the primer RbcL68F of Draisma et al. (2001) was also available for *Analipus* only because priming problem. Primers of Cho et al. (2001) were used for amplification of the Rubisco spacer. Sequences of the forward and reverse strands were determined for all taxa using an ABI PRISM™ 377 DNA Sequencer (Foster City, CA). Both electropherogram outputs for each sample were checked using the program Sequence Navigator v. 1.0.1 (Applied Biosystems Inc. CA).

Fig. 1. Distribution of *Myelophycus cavus* (arrows) in Korea, and collection sites of *M. cavus* (dark circles) and *M. simplex* (arrow heads) specimens for a molecular comparison.

Three data sets were used for the phylogenetic analyses: *rbc*L, Rubisco spacer, and the concatenated *rbc*L + Rubisco spacer. In the *rbc*L data, a total of 42 taxa were included (Table 1); 36 ectocarpalean members and, as six outgroup taxa, *Analipus japonicus*, *Asteronema rhodochortonoides*, *Asterocladon lobatum*, *Desmarestia aculeata*, *Scytothamnus australis*, and *Splachnidium rugosum*, which are chosen based on previous data (Peters & Ramírez 2001). All sequences were edited with SeqPup (Gilbert 1995), and aligned visually. A total of 1467 positions except *Analipus* (1367 bp) was included for the phylogenetic analyses, which were conducted with PAUP* v4.0b8 (Swofford 2001).

Maximum likelihood (ML) phylogenetic analysis was done with the *rbc*L data. The MODELTEST (v3.04, Posada & Crandall 1998) chose the general time - reversible (GTR, Rodriguez et al. 1990) + proportion of invariable sites (I) + the shape of parameter of the gamma distribution (Γ), as the best-fit model for our data. Tree likelihoods were estimated using a heuristic search with 10 random addition sequence replicates, and the tree bisection-reconnection (TBR) branch swapping. We did Bayesian analysis (MrBayes v2.0, Huelsenbeck & Ronquist 2001), which is considered analogous to maximum likelihood bootstrap analysis (Huelsenbeck et al. 2001; Larget & Simon 1999). The GTR model and site specific (SS) gamma parameter for each codon site were used in this analysis. Markov chain Monte Carlo (MCMC) was initiated from a random tree and run for 500,000 generations, with tree sampling done every 100 cycles. A consensus tree was made with 4,510 MCMC trees after convergence, which requires 49,100 generations.

Maximum parsimony (MP) analysis was conducted using heuristic search algorithm with the following options: 10 random additions, TBR branch-swapping, MulTrees, and branches with a maximum length of zero collapsed to yield polytomies. Minimum evolution (ME) distance analysis (Rzhetsky & Nei 1992), using Kimura-two parameter model, was conducted. Ten heuristic searches with random-addition-sequence starting trees and TBR branch rearrangement were conducted to find the optimal minimum evolution tree. Bootstrap analyses were undertaken with 1,000 replicates using the same parameters for MP and ME analysis.

Table 1. Taxa, collection site or data source, and GenBank accession number of both the *rbc*L and Rubisco spacer. Taxonomy in the Ectocarpales follows Peters and Ramírez (2001). [1]*rbc*L only; [2]Rubisco spacer only.

Taxon	Collection site or data source	GenBank accession number
Ectocarpales		
Acinetosporaceae		
Geminocarpus austro-georgiae Skottsberg	Peters & Ramírez (2001)	AJ295830
Pylaiella littoralis (L.) Kjellman	Assali et al. (1990)	X55372
Adenocystaceae		
Adenocystis utricularis (Bory) Skottsberg	Peters & Ramírez (2001)	AJ295823[1]
	Cho et al. (2001)	AF385856[2]
Caepidium antarcticum J. Agardh	Peters & Ramírez (2001)	AJ295826
Utriculidium durvillei Skottsberg	Peters & Ramírez (2001)	AJ295835
Chordariaceae		
Asperococcus fistulosus (Hudson) Hooker	Port Erin, Isle of Man, Great Britain	AY095321
Chordaria flagelliformis (O.F. Müller) C. Agardh	Avacha Bay, Kamchatka, Russia	AY095324
Coelocladia arctica Rosenvinge	Siemer et al. (1998)	AF055395
Delamarea attenuata (Kjellman) Rosenvinge	Siemer et al. (1998)	AF055396
Dictyosiphon foeniculaceus (Hudson) Greville	Siemer et al. (1998)	AF055397
Elachista fucicola (Velley) Areschoug	Siemer et al. (1998)	AF055398[1]
Giraudia sphacelarioides Derbès et Solier	Siemer et al. (1998)	AF055399[1]
Hummia onusta (Kützing) Fiore	Siemer et al. (1998)	AF055402[1]
Isthmoplea sphaerophora (Harvey) Kjellman	Siemer et al. (1998)	AF055403[1]
Myriotrichia clavaeformis Harvey	Siemer et al. (1998)	AF055408
Punctaria latifolia Greville	Hoedong, Jindo, Korea	AY095322
P. plantaginea (Roth) Greville	Siemer et al. (1998)	AF055410
Sphaerotrichia divaricata (C. Agardh) Kylin	Siemer et al. (1998)	AF055412[1]
Striaria attenuata (Greville) Greville	Siemer et al. (1998)	AF055415
Ectocarpaceae		
Ectocarpus siliculosus (Dillwyn) Lyngbye	Valentin & Zetsche (1990)	X52503
Scytosiphonaceae		
Chnoospora implexa J. Agardh	Kogame et al. (1999)	AB022231[1]
Colpomenia bullosa (Saunders)	Kogame et al. (1999)	AB022236[1]
Yamada in Yamada et Kinoshita	Cho et al. (2001)	AF385835[2]
C. peregrina (Sauvageau) Hamel	Kogame et al. (1999)	AB022235[1]
	Cho et al. (2001)	AF385837[2]
C. phaeodactyla Wynne et J.N. Norris	Kogame et al. (1999)	AB022237[1]
C. sinuosa (Mertens ex Roth)	Kogame et al. (1999)	AB022234[1]
Derbès et Solier in Castagne	Cho et al. (2001)	AF385839[2]
Hydroclathrus clathratus (C. Agardh) Howe	Kogame et al. (1999)	AB022233[1]
	Cho et al. (2001)	AF385855[2]
Myelophycus cavus J. Tanaka et Chihara	Daedoryedo, Sinan, Korea	AY095315[2]
	Gotdo, Taean, Korea	AY095316[2]
	Seokhwangdo, Sinan, Korea	AY095317[2]
	Woongdo, Taean, Korea	AY095319
M. simplex (Harvey) Papenfuss	Daesado, Wando, Korea	AY095318[2]
	Seongsan, Jeju, Korea	AY095320

Petalonia binghamiae (J. Agardh) Vinogradova	Kogame et al. (1999)	AB022244[1]
	Cho et al. (2001)	AF385840[2]
P. fascia (O.F. Müller) Kuntze	Kogame et al. (1999)	AB022243[1]
	Cho et al. (2001)	AF385844[2]
P. zosterifolia (Reinke) Kuntze	Kogame et al. (1999)	AB022242[1]
Rosenvingea intricata (J. Agardh) Boergesen	Kogame et al. (1999)	AB022232[1]
Scytosiphon canaliculatus (Setchell et Gardner) Kogame	Kogame et al. (1999)	AB022239[1]
Scytosiphon dotyi Wynne	Cho et al. (2001)	AF385851[2]
S. gracilis Kogame	Kogame et al. (1999)	AB022240[1]
	Cho et al. (2001)	AF385852[2]
S. lomentaria (Lyngbye) Link	Kogame et al. (1999)	AB022238[1]
	Cho et al. 2001 (2001)	AF385853[2]
S. tenellus Kogame	Kogame et al. (1999)	AB022241[1]
Incertae sedis		
Asterocladon lobatum Müller et al.	Peters & Ramírez (2001)	AJ295824[1]
Asteronema ferruginea (Harvey) Delépine et Asensi	Peters & Ramírez (2001)	AJ295818[2]
Asteronema rhodochortonoides (Boergesen) Müller et Padori	Peters & Ramírez (2001)	AJ295825
Desmarestiales		
Desmarestia aculeata (L.) Lamouroux	Draisma et al. (2001)	AJ287847
Ralfsiales		
Analipus japonicus (Harvey) Wynne	Boiler Bay, Oregon, USA	AY095323
Scytothamnales		
Scytothamnus australis (J. Agardh) Hooker et Harvey	Peters & Ramírez (2001)	AJ295833
Splachnidium rugosum (L.) Greville	Peters & Ramírez (2001)	AJ295834

Table 2. Comparison of the two *Myelophycus* species.

	M. cavus	*M. simplex*
Thallus shape	twisted in the upper part	straight
Length	5-15 cm	up to 30 cm
Width	1-1.5 mm	up to 4 mm
Rhizoid	a mat of filaments	discoid
Medulla	hollow when mature	solid
Cortex	2-4 cells thick	10 or more cells thick
Paraphyses	uniseriate	biseriate
Plurilocular zoidangium	80-100 μm long	140-200 μm long
Unilocular sporangium	40-55 μm long	80-110 μm long
	20-25 μm in diameter	40-50 μm in diameter
References	Tanaka & Chihara (1984)	Wynne (1969)
	Yoshida (1998)	Kawai et al. (1994)
	This study	Yoshida (1998)

In the second data set, a total of 32 Rubisco spacer sequences from 31 taxa (Table 1) including published data were included for analyses. The size and variation of the Rubisco spacer sequences were used for comparing *Myelophycus* with relatives. Only unambiguous positions (362 nt) were used, including the 3' end of *rbc*L region (246 nt), for MP and ME analyses, using the same parameters mentioned above.

Finally, *rbc*L (1467 nt) and Rubisco spacer (116 nt) data sets were concatenated from 29 taxa. GTR + I (0.57267) + Γ (1.04887) model was used in ML analysis. Because of the non-coding region of the Rubisco spacer sequences, GTR + I + Γ parameter was used in Bayesian analysis. The Shimodaira-Hasegawa (SH) test (Shimodaira & Hasegawa 1999) was employed to compare statistically alternative phylogenetic hypotheses focused on the positions of *Myelophycus*. The SH test was conducted using PAUP* v4.0b8, with resampling estimated log-likelihood (RELL) optimization, and 100,000 bootstrap replicates.

Results

Myelophycus cavus J. Tanaka *et* Chihara (1984): 152 Figs 2-9

Type: TNS-AL-35769.

Type locality: Shimoda, Shizuoka prefecture, Japan.

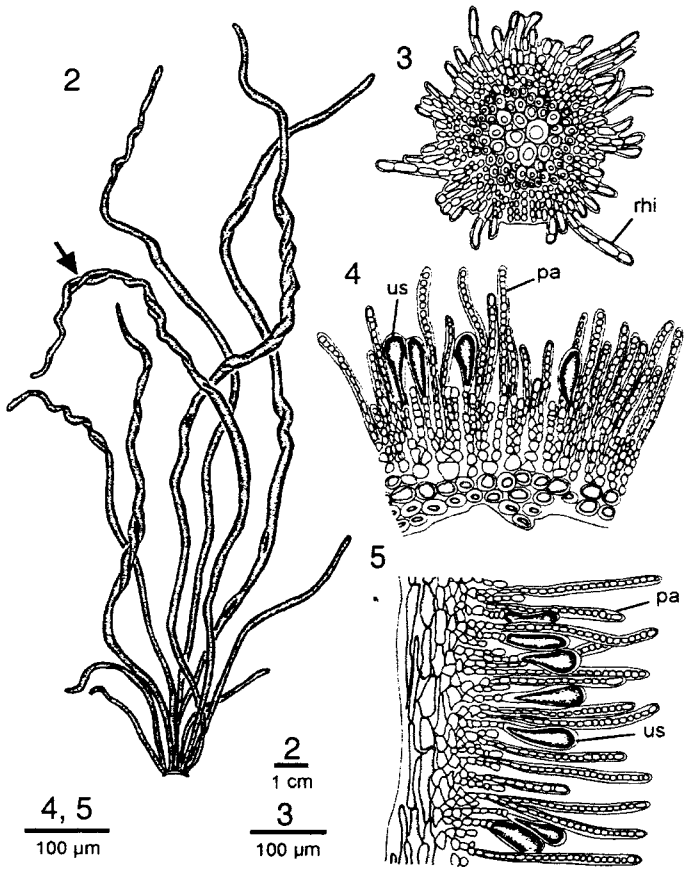
Etymology: *Myelophycus* gets its gender from the last word 'phycus'. Although phycus is a neuter word in Greek, it is traditionally treated as a masculine word in Botanical Latin. Therefore *Myelophycus* is a masculine word and the gender correction of the epithet '*cavum*' (neuter) to '*cavus*' (masculine) is necessary.

Distribution: Japan and Korea.

Representative specimens examined: west coast; in front of Power Plant of Boryong (Boo & Seo, CNUK 004276-004277, 13.vi.1992), Dakseom, Taean (Boo & Seo, CNUK 007354, 15.vi.2000), Gotdo, Taean (G.Y. Cho, CNUK 005016, 15.vi.2000), Woongdo, Taean (Boo, G.Y. Cho & Seo, CNUK 007355-007356, 12.vi.2000); Bigeumdo, Sinan (T.O. Cho, CNUK 004523, 007539, 25.vii.2000), Daedoryedo, Sinan (G.Y. Cho, CNUK 005015, 27.vi.2001), Seokhwangdo, Sinan (T.O. Cho, CNUK 005017, 10.vii.2001). south coast; Naedo, Wando (Lee & Shin, CNUK 005210, 005234-005235, 005311, 12.vi.1999), Sodarangdo, Wando (T.O. Cho, CNUK 005329, 005412, 12.vi.1999), Bogildo, Wando (T.O. Cho, CNUK 007537, 26.vii.2000).

Morphology: Thalli were erect, caespitose, unbranched, cylindrical to flattened, hollow, twisted, 5-15 cm long and up to 1.5 mm in diameter (Fig. 2). The texture was soft and the colour was yellowish to brown in young stages and dark brown later. Rhizoids (Fig. 3) were developed from outer cortical cells near the base of thallus and produced a mat of filaments.

In the base of the thallus, the tissue was parenchymatous and composed of medulla and cortex, which were arranged compactly (Fig. 3); The medulla was composed of 1-2 layers of cells, which were thick-walled, 30-40 μm in diameter, and lacked plastids. The cortex consisted of 1-2 layers of cells, which were 3-4 μm in diameter, and contained 1-2 plastids per cell.

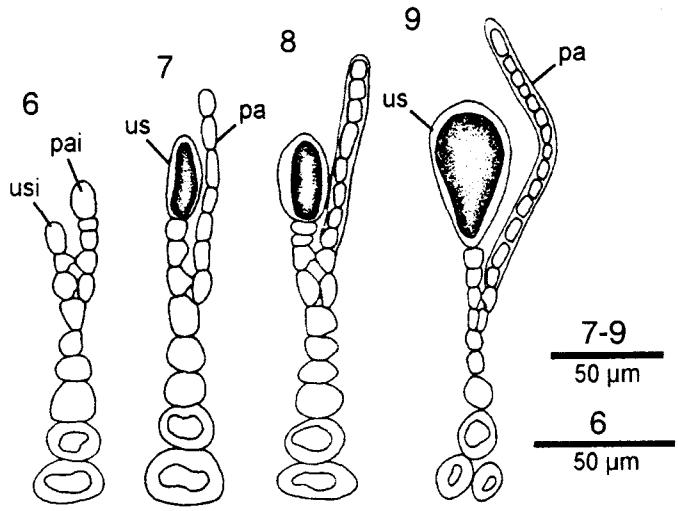


Figs 2-5. *Myelophycus cavus*. – 2. Habit of unisporangial thallus showing twisted axes (arrow). 3. Cross section of thallus base showing rhizoids (rhi) developed from cortical cells. 4. Cross section of central middle sector of erect axis, with unilocular sporangia (us) and paraphyses (pa) borne on the upper cortical cell. 5. Longitudinal section of the same region as in Fig. 4.

In the middle part of the thallus, the central medulla disintegrated while the parenchymatous tissue was composed of an outer medulla and a cortex. The outer medulla was 30-60 µm thick, and consisted of 2-4 layers of longitudinally elongated cells without plastids. The cortex (Figs 4-5) was 30-50 µm thick, consisted of 3-4 layers of angular cells, 10-14 µm in diameter, each of which contained one plate-like plastid per cell in the young thallus; later plastids were irregular in shape and numbered 1-2 in each cell.

Unilocular sporangia (Figs 6-9), developed on the terminal cells of the cortex, were ellipsoid to ovoid, c. 50 µm long × c. 29 µm in diameter.

Paraphyses (Figs 4-5, 9) were uniseriate, composed of 14-22 cells, 140-210 µm long, with an apical cell (5-8 µm long).



Figs 6-9. *Myelophycus cavus*. – 6-9. Developmental stages of unilocular sporangia (us) and paraphyses (pa) from the upper cortical cells.

Plurilocular sporangial thalli were not found.

Myelophycus cavus thalli occurred often mixed with *M. simplex*. Both species were abundant in summer.

ANALYSIS OF *RBC*L AND RUBISCO SPACER SEQUENCES: Sequences of the entire *rbc*L from *Myelophycus cavus*, *M. simplex*, *Asperococcus fistulosus*, *Chordaria flagelliformis*, and *Punctaria latifolia*, and partial *rbc*L from *Analipus japonicus* were determined during this study. The length of the *rbc*L gene was 1467 bp throughout, but the first 100 bp in *A. japonicus* were not determined because of use of another primer. The *rbc*L sequences between *Myelophycus cavus* from Woongdo, Taean and *M. simplex* from Seongsan, Jeju differed by 32 nucleotides or 2.22% pairwise divergence, which is in the range of 0.5-5.9% described among scytosiphonacean members (Kogame et al. 1999).

The ML analysis showed that both *Myelophycus* species formed a monophyletic group together with the members of the Scytosiphonaceae. There was strong support of ME bootstrap and Bayesian posterior probability (Fig. 10). Within the scytosiphonacean clade, *Myelophycus* strongly clustered with *Colpomenia*, *Petalonia*, and *Scytosiphon*, while it was separated from the group of *Chnoospora*, *Hydroclathrus*, *Rosenvingea*, and *Colpomenia sinuosa*. The members of the Ectocarpales sensu lato, including *Asteronema* and *Asterocladon*, formed a strong monophyletic group and were subdivided into six subclades, represented by *Scytosiphon*, *Chordaria*, *Pylaiella*, *Adenocystis*, *Ectocarpus*, and *Asterocladon*. However, relationships of *Coelocladia* and *Isthmoplea* were still unresolved. *Analipus* came out as more related to scytothamnalean members, but there was no bootstrap support. The MP analysis recovered 132 most parsimonious trees (length = 1583; CI = 0.415; RI = 0.589), and showed a topology similar with the ML tree, and also with the ME tree (trees not shown).

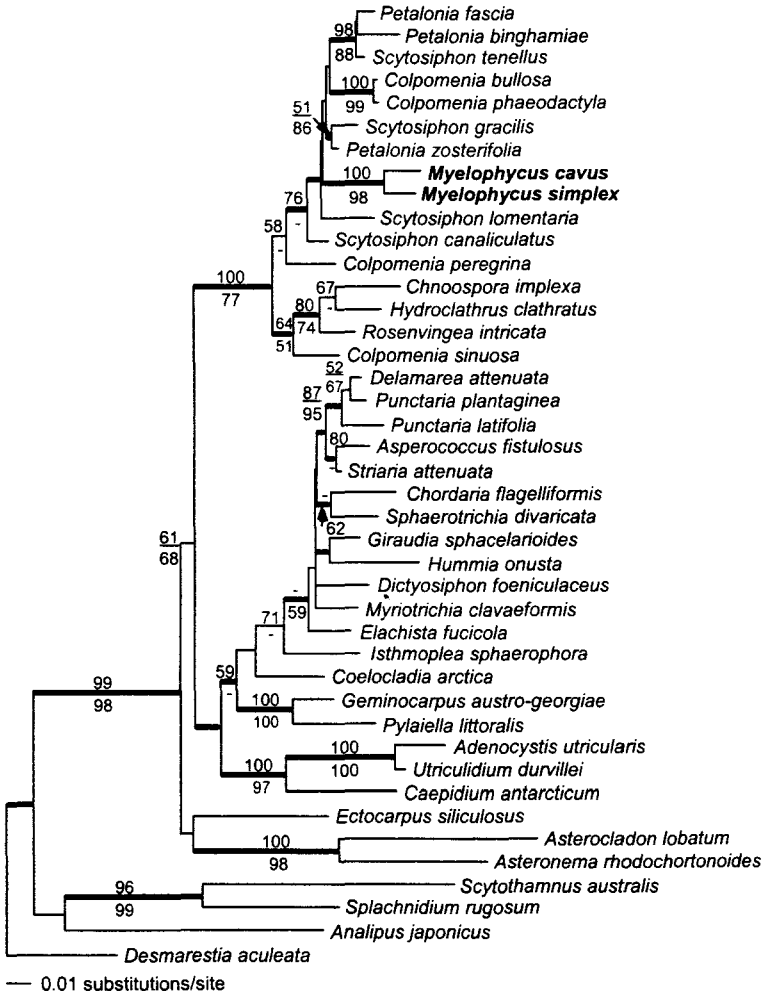


Fig. 10. Maximum likelihood tree for *Myelophycus* and relatives estimated from *rbc*L sequence data (GTR + I + Γ model, -Log likelihood = 9745.20; I = 0.5984; Γ = 0.9113; A ↔ C = 1.227, A ↔ G = 4.55; A ↔ T = 1.207; C ↔ G = 1.314; C ↔ T = 10.16; and G ↔ T = 1). Thicker branches represent the posterior probabilities (>95%) from Bayesian analysis. Bootstrap values (>50%) are given above (MP) and below (ME) branches. The alternative hypothetical topologies of the *rbc*L tree are shown with numbered arrows, indicating the constrained monophyly with *Myelophycus* for Shimodaira-Hasegawa test.

The Rubisco spacer was 186 bp long in both species of *Myelophycus*. It was 143 bp in *Analipus japonicus*, 180 bp in *Asperococcus fistulosus*, 181 in *Chordaria flagelliformis*, and 183 bp in *Punctaria latifolia*. The size of the Rubisco spacer of *Myelophycus* is similar to those (183- 203 bp) of the scytosiphonacean algae except that of *Petalonia binghamiae* (150 bp) (Cho et al. 2001) and comparable with Rubisco

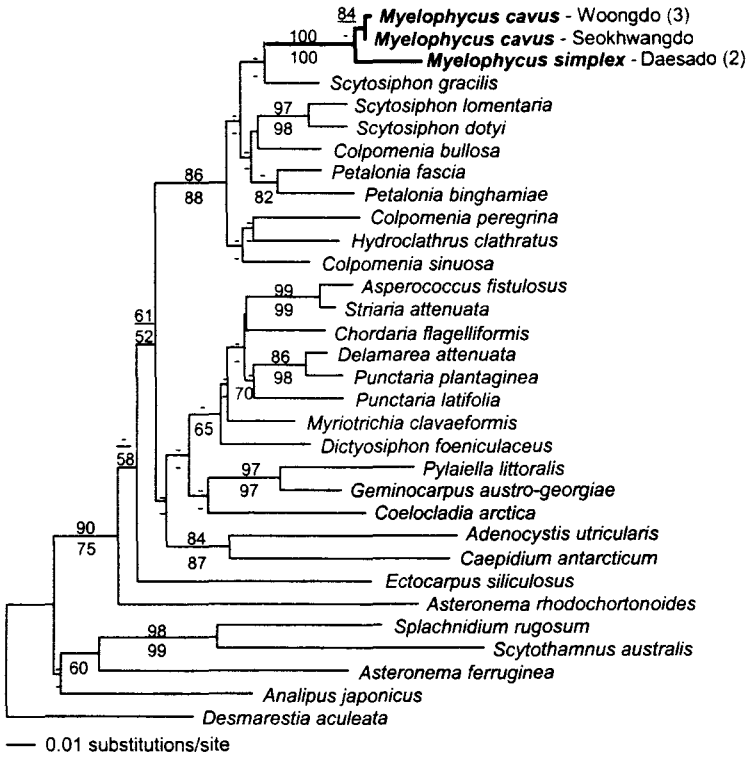


Fig. 11. Optimal minimum evolution tree of the Rubisco spacer sequences for *Myelophycus* and relatives. Bootstrap values (>50%) are plotted on this ME tree and are given above (MP) and below (ME) branches.

spacer length in the ectocarpalean members (Peters & Ramírez 2001). Intraspecific variation of the Rubisco spacer among 4 samples of *M. cavus* was 0.27% and sequences of our two *M. simplex* samples were identical. Interspecific divergence between *M. cavus* and *M. simplex* was 6.38%, which is higher than that reported within a genus in other brown algae (Lee et al. 1999).

ME analysis of the Rubisco spacer sequences (Fig. 11) showed a very similar topology to the trees from the *rbc*L. A clade of the *Myelophycus* species including six populations showed monophyly within the scytosiphonacean clade, which was moderately supported (86% of MP, 88% of ME). This ME topology is consistent with the MP tree.

When the concatenated data was used for ML analysis, *Myelophycus* was nested within the scytosiphonacean clade with high bootstrap value and posterior probability (Figure 12). Tree topology was basically the same as the *rbc*L tree, however it showed more support for each node (i.e. 100% of MP and ME bootstrap for the clade including *Myelophycus*).

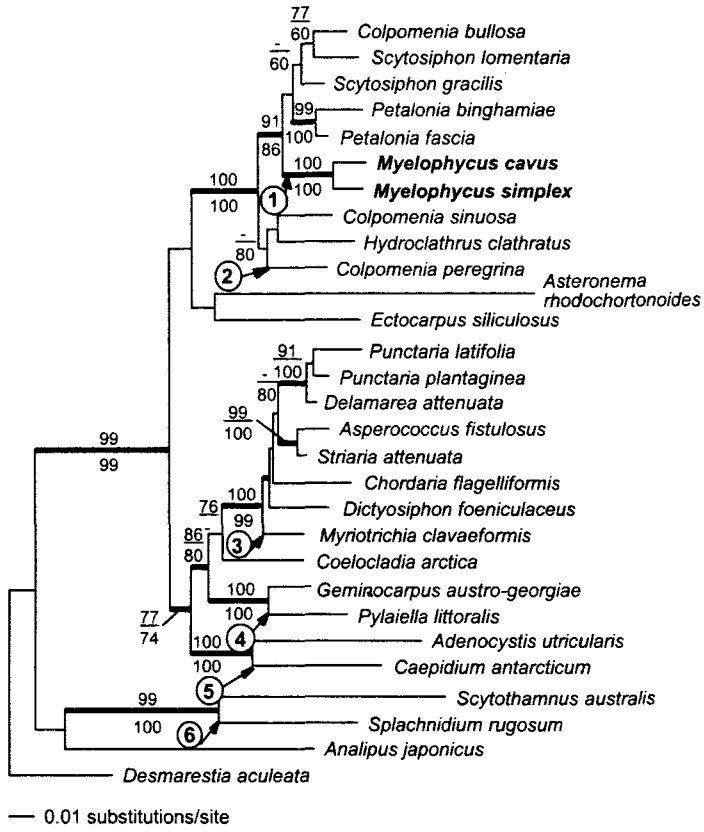


Fig. 12. Maximum likelihood tree for *Myelophycus* and relatives estimated from the combined *rbc*L and Rubisco spacer data (GTR + I + Γ model, -Log likelihood = 9393.00; I = 0.57267, Γ = 1.04887; A ↔ C = 1.667, A ↔ G = 4.798, A ↔ T = 1.527, C ↔ G = 1.162, C ↔ T = 9.514, and G ↔ T = 1). Presentation order of bootstrap values and posterior probability is the same as in the *rbc*L tree.

Table 3. Shimodaira-Hasegawa test results for comparisons of alternative hypotheses.

Hypothesis	-ln *L*	Difference -ln *L*	*P*
1	10456.83	(best)	
2	10499.95	43.13	0.2546
3	10726.96	270.14	0.0000*
4	10723.73	266.90	0.0000*
5	10688.96	232.13	0.0000*
6	10839.44	382.62	0.0000*

*$P < 0.05$

The Shimodaira-Hasegawa test shows that the best position of *Myelophycus* is in the Scytosiphonaceae (Table 3, ②- in Fig. 12). The alternative topologies (③- ⑥ positions in Fig. 12) that forced it into a clade with any of the three major groups in the tree and with the taxa of the *incertae sedis* are significantly worse than the best tree.

Discussion

Our recent collections of *Myelophycus cavus* from Korea correspond in their habit and structure of cortex, medulla, and unilocular sporangia to the description of Tanaka & Chihara (1984). However, thalli bearing plurilocular sporangia were not collected and it is not known whether they occur in other seasons. In *Myelophycus*, the plurilocular-sporangial thallus is the dioecious gametophyte, which is isomorphic to the sporophyte bearing unilocular sporangia (Kawai et al. 1994). *Myelophycus cavus* is separable from *M. simplex* in morphology and anatomy, as seen in Table 2, and by interspecific divergences in the Rubisco spacer (6.38%) and *rbc*L sequences (2.23%), respectively. This is the first report on the occurrence of *M. cavus* outside Japan, recognized by both morphological and molecular data. Since *M. cavus* occurs mixed with *M. simplex*, *M. cavus* plants may be misidentified as old thalli of *M. simplex*. Detailed observations of field-collected material will probably enable a more realistic evaluation of distribution of *M. cavus* to be made in the Northwest Pacific.

Our phylogenetic analyses of the *rbc*L, Rubisco spacer, and combined *rbc*L + Rubisco spacer sequence datasets support the classification of the Ectocarpales into five families, as proposed by Peters & Ramírez (2001) on the basis of plastid structure, life histories, and *rbc*L sequences. The Scytosiphonaceae, of which a single member was included in the above analyses (Peters & Ramírez 2001), forms a well-supported monophyletic group in the present study. Although three new sequences were included in our study, the Chordariaceae are still not resolved. Increased taxon sampling and analyses of other genes, nuclear as well as plastid, will reconcile uncertainty in internal branches of the broad family.

The main finding of our study is that *Myelophycus* together with the Scytosiphonaceae consistently form a monophyletic clade with strong support of MP bootstrap and Bayesian posterior probability (Figs 10-12). The fact that *Myelophycus* should be placed in the Scytosiphonaceae is statistically demonstrated by the Shimodaira-Hasegawa test, as seen in Table 3. These results corroborate the suggestion of Kawai et al. (1994) that *Myelophycus* compares well with members of the Scytosiphonaceae, as discussed below. The scytosiphonacean clade consists of two groups; the first contains most *Colpomenia* species, all tested species of *Petalonia* and *Scytosiphon* as well as species of *Myelophycus*. All these species have unilocular zoidangia on their sporophytes (Kogame et al. 1999). The interrelationships within this group, however, are less well resolved. Inclusion of *Colpomenia peregrina*, with both plurilocular and unilocular zoidangia on sporophytes, was also less supported by bootstrap support and Bayesian posterior probability. The sister group to the above clade includes species of *Chnoospora*, *Hydroclathrus*, *Rosenvingea*, and also *Colpomenia sinuosa*, which all have both unilocular and plurilocular zoidangia on their sporophytes (Kogame et al. 1999).

similar to an isomorphic pattern of life history and might be indicative of the evolutionary processes of life history patterns in the Scytosiphonaceae. There may be a transformation of life history within the lineage consisting of *Myelophycus* and the Scytosiphonaceae, changing from an isomorphic pattern to a heteromorphic type or vice versa.

The strong monophyly of the Scytosiphonaceae including *Myelophycus* in the present study concludes that parenchymatous tissue, a single plate-like plastid with one pyrenoid per cell, and hormosirene are synapomorphic characters for *Myelophycus* and the rest of the Scytosiphonaceae. Therefore, we propose to emend the concept of that family to contain also isomorphic life cycles in addition to heteromorphic life histories.

Scytosiphonaceae Farlow (1881, p. 62) emend. G.Y. Cho et Boo

Thalli unbranched, erect, bladelike to globular, hollow or entire. Growth apical or later intercalary. Plastid plate-like, single per cell, each with a single pyrenoid. Gametophyte dioecious. Gametophytic thallus erect, parenchymatous, bearing plurilocular sporangia. Sporophytic thallus prostrate to erect, parenchymatous to pseudoparenchymatous, having unilocular or plurilocular sporangia. Life history isomorphic to heteromorphic. Main sexual pheromone hormosirene.

The emended Scytosiphonaceae includes *Myelophycus* as well as the scytosiphonacean genera included in the present study. In addition, *Iyengaria* Boergesen and *Jolyna* Guimarês, as currently recognized, and *Melanosiphon* Wynne, as suggested by Tanaka & Chihara (1984) and Kawai et al. (1994), may also be placed in the family. Phylogenies of these three genera are beyond the present study.

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Our phylogenetic analyses show that the *Myelophycus* species do not group with members of the Dictyosiphonales (i.e. *Asperococcus fistulosus* and *Punctaria* spp.). Thus *Myelophycus* cannot be classified in the Punctariaceae (as in Kjellman 1893; Tanaka & Chihara 1984) or the Asperococcaceae (Setchell & Gardner 1925; Yoshida 1998). Our trees also confirm that the newly instated family Chordariaceae, having discoid plastids and a heteromorphic life history with macroscopic sporophyte and microscopic gametophyte (Peters & Ramírez 2001), cannot contain the genus *Myelophycus*. Our plastid sequence data show that *Analipus japonicus* must be placed outside the Ectocarpales, as in published SSU rDNA sequences by Tan & Druehl (1994). *Analipus* Kjellman is placed in the order Ralfsiales (Nakamura 1972), which differs from the Ectocarpales in having discal-type development and lacking pyrenoids (see Tan & Druehl 1994). However, the concept of the Ralfsiales is controversial (Kawai 1989; Tan & Druehl 1994).

The controversy on the familial position of *Myelophycus* appears to begin with different interpretations of the structure of its thallus: parenchymatous or pseudoparenchymatous tissue. From studies on comparative morphology of *M. cavus* and *M. simplex* in culture, Tanaka & Chihara (1984) concluded that both species had multiseriate thalli of parenchymatous tissue with a single apical cell (see their p. 159, figs 16, 18 and 23 and p. 160, figs 26, 31). Our observation of medulla and cortex in the hollow thallus of *M. cavus* agrees with that of Tanaka & Chihara (1984). As in *Myelophycus*, thalli of the Scytosiphonaceae are parenchymatous (Bold & Wynne 1985, p. 307-308). However, observation of preserved material of *M. simplex* from Japan made Wynne (1969) consider that the medulla was pseudoparenchymatous.

The number and shape of plastids are used as diagnostic characters for classification of the ectocarpalean families (Peters & Ramírez 2001). According to Kawai (1992), a single plastid (plate-like) with a pyrenoid in each cortical cell is the most primitive, whereas cell containing many plastids without pyrenoids is a derived condition. Plastids of species in *Myelophycus* and the Scytosiphonaceae have the same primitive type (Wynne 1969; Kawai et al. 1994), although a few plastids per cell occur in mature thalli of *M. cavus* (Tanaka & Chihara 1984). We did not study the genus *Melanosiphon* Wynne, which has a single plate-like plastid with one pyrenoid in each cell (Wynne 1969; Tanaka & Chihara 1984; Kawai et al. 1994).

The nature of the sexual pheromone is reported to reflect phylogenetic relationships (Maier 1995). Hormosirene is present in the Scytosiphonaceae (Maier & Müller 1986) and is also the major pheromone (88.5%) of *Myelophycus simplex* among dictyopterene A (2.6%), ectocarpene (5.5%) and dictyotene (3%) (Kawai et al. 1994).

The life history pattern is important for classification of the ectocapalean families (Peters & Ramírez 2001). *Myelophycus* has an isomorphic life history alternating between a dioecious gametophyte and a sporophyte, both being erect (Kawai et al. 1994), while the Scytosiphonaceae have a heteromorphic life cycle with an erect gametophyte and a prostrate sporophyte (Kogame et al. 1999). However, Kogame (2001) considered that the occurrence of a zygotic erect thallus in artificial cultures of *Petalonia fascia* (Kogame 1997) and *Chnoospora imflexa* (Kogame 2001) was

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