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[Note]

First Record of *Cladosiphon umezakii* (Ectocarpales, Phaeophyceae) in Korea

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Recently described new species, *Cladosiphon umezakii* Ajisaka (Ectocarpales, Phaeophyceae) is reported in Korea based on morphology and plastid *rbc*L sequences. *Cladosiphon umezakii* occurred on low intertidal to subtidal zone along the south and southeast coasts in Korea. Thalli are gold to dark brown, erect, cylindrical, irregularly branched, and very mucoid. Unilocular zoidangium is developed at the base of assimilatory filaments. Assimilatory filaments are very long. Eight specimens of the species collected from Korea clustered with those of *C. umezakii* in Japan in *rbc*L tree. The species showed a sister relationship with *C. okamuranus*. The occurrence of this warm water species is thought an example of northward migration of marine algae caused by global warming. Because *C. umezakii* is of the economical importance of the genus as foods and medicines, the study on mass culture of the species is necessary in Korea.

Key Words: Chordariaceae, *Cladosiphon*, *C. umezakii*, morphology, *rbc*L

INTRODUCTION

The genus *Cladosiphon* Kützing (1843) is classified into the Chordariaceae in Ectocarpales *sensu lato* and includes 13 species in the world (Sansón *et al.* 2006; Ajisaka *et al.* 2007). Although most of the species of *Cladosiphon* have been reported in Europe and Africa (Guiry and Guiry 2009), only two species, *C. okamuranus* Tokida and *C. umezakii* Ajisaka, are reported in Japan (Tokida 1942; Ajisaka *et al.* 2007). *Cladosiphon okamuranus* is very famous for foods in Japan as well as a resource of fucoidan (Nagaoka *et al.* 1999) and cultivate massively in Okinawa. Recently *C. umezakii* has been established as a new species based on morphological and molecular data in Japan (Ajisaka *et al.* 2007). This species is annual, growing on lower intertidal to subtidal regions along the south part of Japan. *Cladosiphon umezakii* is distinguished by long assimilatory filaments up to 840 μm from the other taxa of the genus (Ajisaka *et al.* 2007). It is most similar with *C. filum* (Harvey) Kylin from Australia having long assimilatory filaments up to 800 μm, however it differs from *C. umezakii* on the basis of its habitus (mainly epiphytic) and cell number of assimilatory filaments (Womersley 1987; Ajisaka *et al.* 2007).

In this study, we encountered *Cladosiphon umezakii* on the southern coast of Korea. Both morphological characters and plastid *rbc*L regions of the species confirmed its identity. Two slimy chordariacean species, *Tinocladia crassa* (Suringar) Kylin and *Papenfussiella kuromo* (Yendo) Inagaki in Korea were also included for comparison.

MATERIALS AND METHODS

Morphology

Thalli of *Cladosiphon umezakii* were collected from the subtidal zone at Gijang, Busan (24 May 2008) and Yeochori, Chujado (4 July 2008) for the morphology observation. Fresh material was used for cross section by hand with a blade under the microscope. Photographs were taken with a digital camera attached to Vanox light microscope (Olympus, Tokyo). All specimens collected in this study are deposited at the Non-vascular Plant Collections Storage of National Institute of Biological Resources (NIBR) in Incheon and the herbarium of Chungnam National University (CNUK) in Daejeon, Korea (Table 1).

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Table 1. Specimen information of *Cladosiphon umezakii* used in this study

Species	Collection site/date	Voucher number	GenBank accession no.
Cladosiphon umezakii	Gijang, Busan, Korea/24 May 2008	GYB54	FJ805434
	Guryongpo, Pohang, Korea/ 26 Jun. 2008	GYB86	FJ805435
	Mukri, Chujado, Jeju, Korea/26 May 2005	CNUK PE512	FJ805436
	Seokduri, Chujado, Jeju, Korea/4 Jul. 2008	GYB105	FJ805437
	Yechori, Chujado, Jeju, Korea/25 May 2005	CNUK PE513	FJ805438
	Yechori, Chujado, Jeju, Korea/4 Jul. 2008	GYB102-104	FJ805439-FJ805441
Papenfussiella kuromo	Gijang, Busan, Korea/24 May 2008	GYB60	FJ805442
Tinocladia crassa	Gampo, Gyeongju, Korea/26 Jun. 2008	GYB87, 100	FJ805443, FJ805444

Plastid *rbc*L analysis

Total genomic DNAs were extracted from silica gel-preserved dry materials. Approximately 0.05g of dried thallus was ground by TissueLyser (QIAGEN, Austin, USA) as 30/s frequencies for 3 min. Grounded powder was used for the DNA extraction procedure using DNeasy Plant Mini Kit (QIAGEN, Hilden, Germany), following the manufacturer's protocol. Plastid *rbc*L regions were amplified using the primer sets, PRB-F0/R1A, PRB-F2/R2, PRB-F3/R3 (Kogame *et al.* 1999), and RS1/ RS2 (Yoon and Boo 1999). PCR amplifications were carried out using the AccuPower® PCR premix (Bioneer, Daejeon, Korea) on GeneAmp PCR System 9700 (Applied Biosystems, Foster City, USA). The steps and cycles of the amplification were as followings: an initial denaturation at 95°C for 4 min; 30 cycles of denaturation at 95°C for 30 sec, annealing at 47 or 50°C for 30 sec, and extension at 72°C for 1 min; and a final extension at 72°C for 7 min. The sequencing reactions of the *rbc*L region were performed using ABIPRISM BigDyeTM Terminator Cycle Sequencing Kits and its florescent signal was detected by ABI PRISM 3730XL Analyzer (Applied Biosystems, Foster City, USA). The *rbc*L sequences were double checked using Chromas 1.45 (McCarthy 1996) and collated using the multisequence editing program SeAl 2.0 (Rambaut 2002), and were aligned visually with previously published data (Valentin and Zetsche 1990; Siemer *et al.* 1998; Kogame *et al.* 1999; Kim and Kawai 2002; Cho *et al.* 2003, 2004; Peters 2003; Cho and Boo 2006; Ajisaka *et al.* 2007).

Bayesian analysis was performed using the GTR + I + G model for *rbc*L using MrBayes v. 3.1.2 (Huelsenbeck and Ronquist 2001), because the program can be set as 6 parameter model only. The analysis was conducted from a random starting tree, and the program was set to perform two independent runs with four chains of Markov chain Monte Carlo iterations simultaneously for 2 million generations with trees sampled every 100th generation, respectively. We harvested trees after 1,000,000 generations for *rbc*L because the average standard deviation of split frequencies reached and kept around 0.02. The last trees for each data set were combined to produce a 50% majority rule tree.

RESULTS AND DISCUSSION

***Cladosiphon umezakii* Ajisaka 2007: 197.**

Figs 1-7.

Type: SAP 100676 (collected in 23 January 1977).

Type locality: Sabiura, Kushimoto, Wakayama Prefecture, Japan.

Distribution: South and southeast coasts of Korea, Japan (Ajisaka *et al.* 2007).

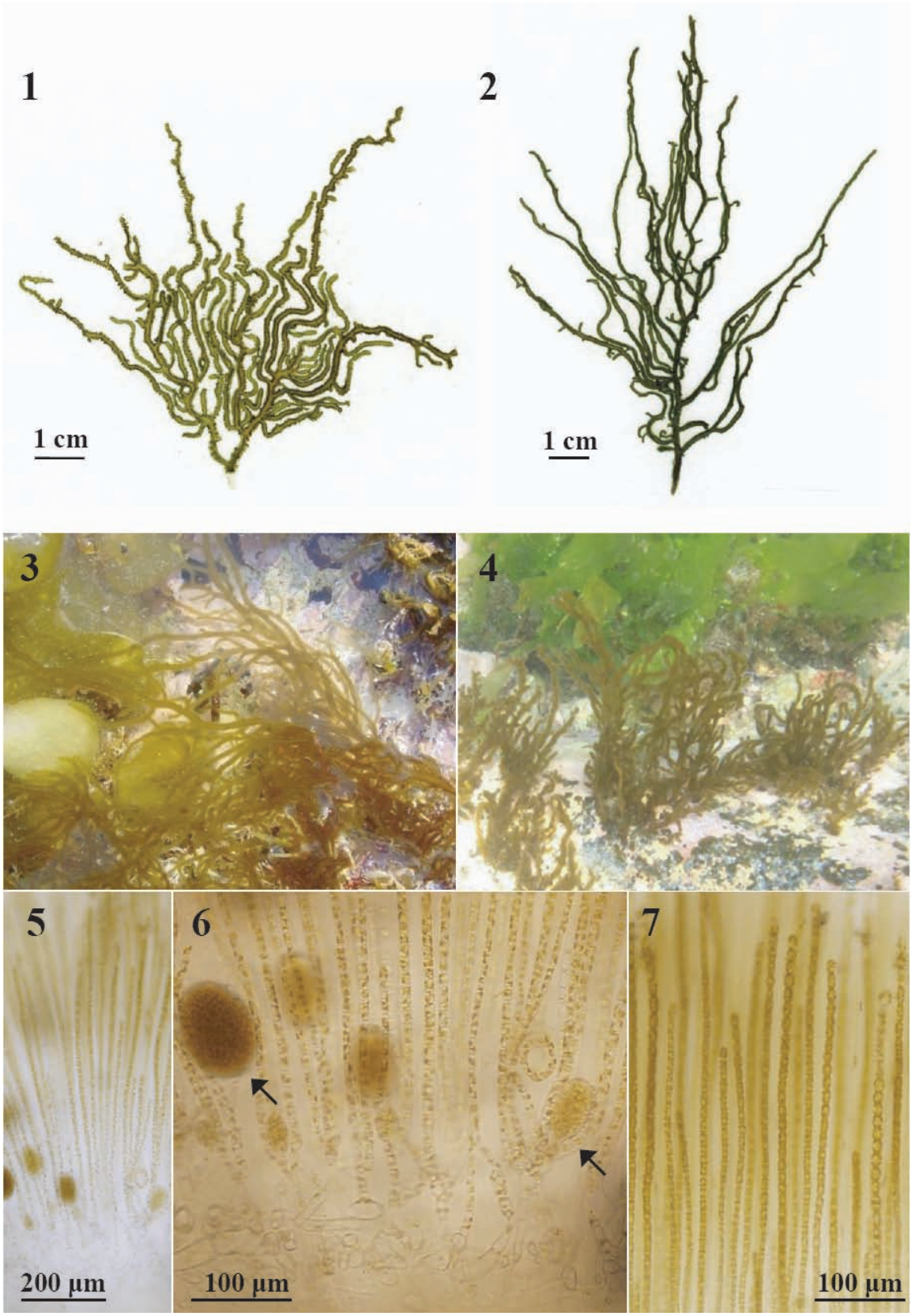
Korean name: 미끌큰실말 (Mi-kkeul-keun-sil-mal, nom. nov).

Specimens examined: Gijang, Busan (Cho, NIBRAL0000105733-7, 24 v 2008); Gampo, Gyeongju (Cho and Bae, NIBRAL0000107187-99, 26 vi 2008), Guryongpo, Pohang (Cho and Bae, NIBRAL0000107185-6, 26 vi 2008), Mukri, Chujado (Boo, Hwang, and Hong, CNUK PE512, 26 v 2005), Seokduri, Chujado (Cho and Kim, NIBRAL0000107178-84, 4 vii 2008), Yechori, Chujado (Boo, Hwang, and Hong, CNUK PE513, 25 v 2005; Cho and Kim, NIBRAL0000107165-76, 4 vii 2008).

Habitat: Growing on lower intertidal and subtidal zone (Figs 3-4).

Morphology: Thalli are gold to dark brown, erect, cylindrical, irregularly branched, very mucoid, attached to rocks with a small discoid holdfast, up to 12 cm long (Fig. 2) and 0.1-0.3 mm in width. Unilocular zoidangium is developed at the base of assimilatory filaments, elliptical, 50-120 μm long, 25-80 μm in diameter; plurilocular zoidangia were not found. The morphological features of *Cladosiphon umezakii* from Korea were identical to those of the species in Japan (Ajisaka *et al.* 2007).

A total of 1467 bp were newly determined from 8 spec-



Figs 1-7. *Cladosiphon umezakii* from Korea. Figs 1 & 2. Herbarium specimens collected from Gijang, Busan (24 May 2008) and Yechori, Chujado (4 July 2008). Figs 3 & 4. Habitat of *C. umezakii* in Chujado (23 May 2005) and Gampo, Gyeongju (26 June 2008). Figs 5 & 6. Longitudinal section showing medullary cells, subcortical cells, and unilocular zoidangium (arrow) with assimilatory filaments. Fig. 7. Assimilatory filaments.

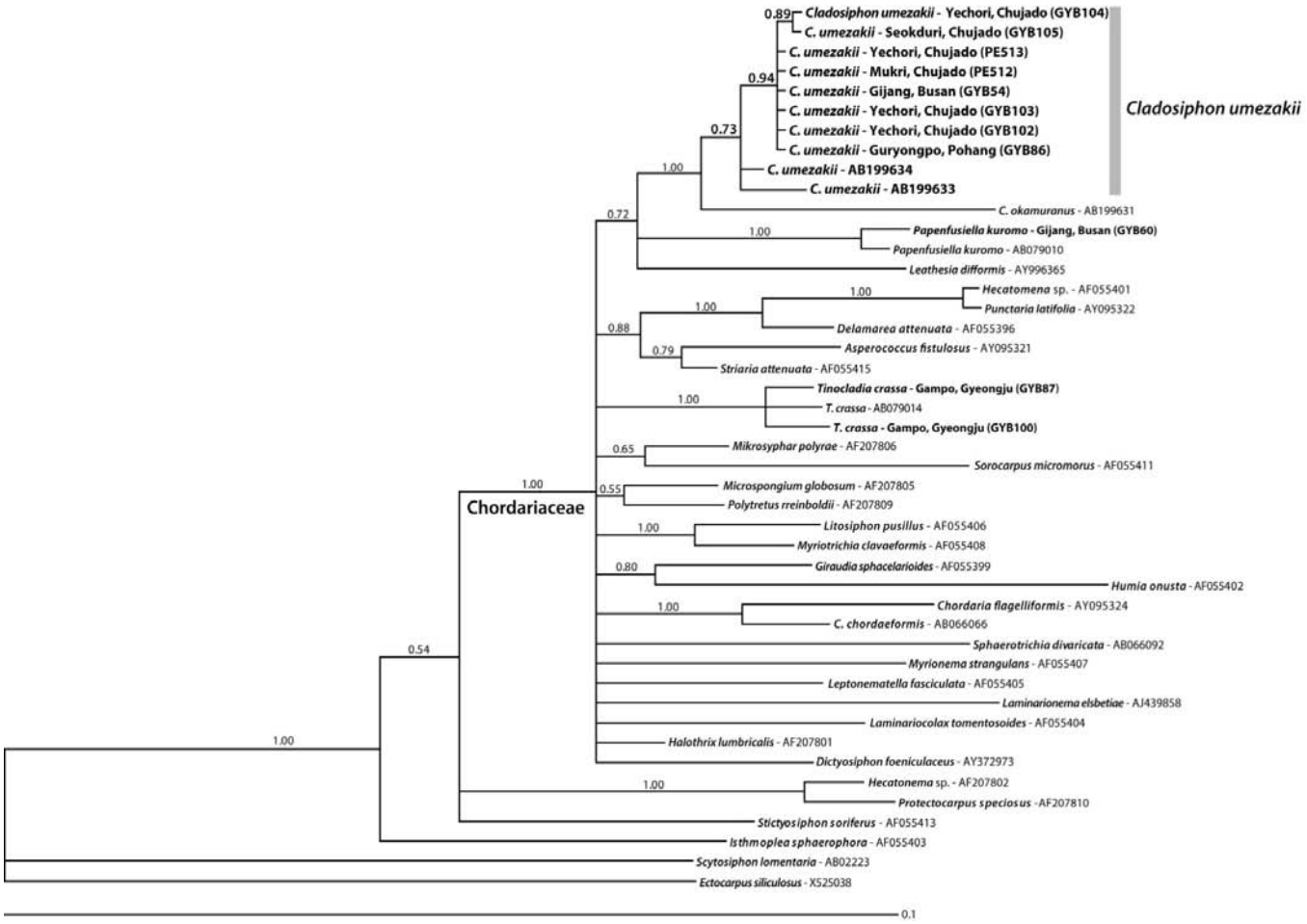


Fig. 8. Bayesian tree for *Cladosiphon umezakii* and putative relatives estimated from *rbc*L sequences. Bayesian posterior probability values are shown on the branches. Bold cases indicate specimens analyzed in this study.

imens of *Cladosiphon umezakii* and two putative species, *Papenfussiella kuromo* and *Tinocladia crassa*, collected in Korea (Table 1). Eight specimens of *Cladosiphon umezakii* from Korea clustered in a group with 0.94 Bayesian posterior probability value (BPP) and formed a monophyletic group with the Japanese material (0.73 BPP). Korean and Japanese *Cladosiphon* species formed a monophyletic group with 1.0 BPP (Fig. 8).

The Korean *Cladosiphon umezakii* differed by 0-1 bp each other, and differed by 1-3 bp (0.07-0.2%) from specimens of the species in Japan. Between two Japanese samples, 2 (0.14%) bp were different in *rbc*L. Korean *C. umezakii* had 36-37 bp (2.5%) difference with *C. okamuranus*. These sequence divergences in *rbc*L region are very small compared to other ectocarpalean species such like *Colpomenia peregrina*. Although *C. peregrina* specimens were collected from worldwide, they showed 25 bp (1.7%) *rbc*L divergences (Cho *et al.* 2005). According to the ITS region of *Cladosiphon umezakii* which had collected in Japan, sequence divergence differed by 3-45 bp (c.a. 4%) on 1055-1062 bp size of ITS 1 + 2 region excluding 5.8S (Ajisaka *et al.* 2007).

Morphologically similar species *Tinocladia crassa* and *Papenfussiella kuromo* from Korea clustered with specimens of these two species in Japan, respectively. These species occurred together with *Cladosiphon umezakii* in the south part of Korea. However *Tinocladia crassa* and *Papenfussiella kuromo* distributed more commonly on the south and east coasts of Korea than *Cladosiphon umezakii*.

In Korea and Japan, *Cladosiphon* species occur mainly along the warm-temperate seas (e.g., *Cladosiphon okamuranus* from Okinawa; Yoshida 1998). Based on the studies of cultivation of *C. okamuranus*, the best growth of its gametophytes is known at 25°C water temperature and the development of zoospore from both unilocular and plurilocular sporangia are suitable at 20-30°C (Shinmura 1974, 1975). These reports indicate that *C. okamuranus* is a warm water species. *Cladosiphon umezakii* also occurs in

warm-temperate waters in Korea; from the South Sea to southern part of East Sea (up to Guryongpo, Pohang; N36°00′12.4″, E129°34′33.3″) as similar in Japan (Kyushu, Shikoku and southeast part of Honshu; Ajisaka *et al.* 2007). As the sea water temperature is getting increased along the Korean coasts (National Oceanographic Research Institute Reports), the occurrence of this warm water species, *C. umezakii*, might be considered as an example of northward migration of marine algae caused by global warming. Because of the economical importance of *Cladosiphon* species as foods and medicines, further study to explore the possibility for mass culture of *C. umezakii* is necessary in Korea.

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