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New record of *Codium lucasii* (Bryopsidales, Chlorophyta) in Korea

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Abstract

A prostrate species of *Codium* (Bryopsidales, Chlorophyta) was collected from Daejin on the eastern coast of Korea. This alga is morphologically characterized by a prostrate, adherent or pulvinate, dark green thallus that is tightly attached to substratum. The utricles are strongly grouped and cylindrical to slightly clavate. Their apex is rounded to capitated, and it frequently has an alveolate ornament. Hair scars are found in the upper portion of the utricle. The gametangia grow on a short pedicel in the upper part of the utricle. In the phylogenetic tree based on molecular data, this alga is placed in the same clade as *C. mozambiquense* in UPGMA analysis, and nests in a sister clade of *C. lucasii* subsp. *capense* and *C. mozambiquense* in ML and NJ analyses. However, the genetic distance between the sequences of the Korean alga and the two species is 1.3–1.9%, while that between the Korean alga and *C. lucasii* from Japan is 1.1% within intraspecific range. The divergence value between the Korean alga and *C. lucasii* from the type locality (Australia) is 2.7% considered to be interspecific range. As based on this genetic divergence value, the Korean alga together with Japanese *C. lucasii* can be separated from genuine *C. lucasii* from the type locality. However, the Korean alga is identified as *C. lucasii* until those entities are morphologically characterized in species level. This is the first record of *C. lucasii* in Korea

Key words: Chlorophyta, *Codium lucasii*, Korea, molecular analyses, morphology, new record

INTRODUCTION

Codium Stackhouse, which belongs to the order Bryopsidales J.H. Schaffner, is a green algal cosmopolitan genus distributed worldwide (Silva and Womersley 1956, Silva 1962). This genus is comprised of about 140 species that grow on rocky or sandy habitats in temperate and tropical seas (Guiry and Guiry 2015). The thalli show various forms such as, erect dichotomously branched, carpet-like, mat-like, spherical, pulvinate, and repent forms. However, the thallus structure is relatively simple, and it is composed of a medulla of a densely woven mass of coenocytic filaments surrounded by a cortex of utricles (Silva and Womersley 1956). In the past, the taxonomy of *Codium* was based mainly on the morphology (Schmidt 1923, Silva 1954, 1962, Oh et al. 1987), which made identification of the species in the genus difficult. Recently, molecular analyses have been used to improve the understanding of the taxonomy and phylogeny of the genus (Shimada et al. 2004, Verbruggen et al. 2007, Israel et al. 2010, Oliveira-Carvalho et al. 2012, Verbruggen et al. 2012, Lee and Kim 2015). Phylogenetic studies on *Codium* have been based on the *rbc*L gene that codes for the large subunit of the ribulose-1, 5-bisphosphate carboxylase–oxygenase enzyme (Shimada et al. 2004, González et al. 2012, Oliveira-Carvalho et al. 2012).

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Twenty species of *Codium* are currently recorded in the marine algal flora of Korea (Kim et al. 2013). Of these, four species have prostrate and adherent thallus. A *Codium* species, which shows prostrate and adherent or pulvinate form, was collected from Daejin Harbor on the eastern coast of Korea during a survey of indigenous species of marine algae. This alga is identified as *Codium lucasii* Setchell based on morphological and molecular analyses, and it is newly recorded in Korea here.

MATERIALS AND METHODS

Samples for the present study were collected from Daejin Harbor, Korea in January of 2015. All specimens were preserved in 5–10% formalin seawater. Species identification was based on thallus and utricle morphology (Silva and Womersley 1956). A portion of the sample was dried and preserved in silica gel. Total genomic DNA was extracted from silica gel-dried tissue samples.

Total genomic DNA was extracted from silica-gel preserved samples using the DNeasy Plant Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer's protocol. Concentrations of extracted DNA were assessed by using gel electrophoresis on a 1% agarose gel. Extracted DNA was used for the amplification of Ribulose-1, 5-bisphosphate carboxylase/oxygenase (*rbc*L) regions using published primers (Shimada et al. 2004, Verbruggen et al. 2007). PCR amplifications were performed in a TaKaRa PCR Thermal Cycler Dice (TaKaRa Bio Inc., Otsu, Japan) with an initial denaturation step at 94°C for 4 min followed by 35 cycles at 94°C for 30 s, 52°C for 1 min, and 72°C for 1 min and a final extension at 72°C for 7 min. The reaction volume was 20 μL, consisting of 20 ng of genomic DNA, 2 of 10x PCR buffer, 2 μL of 200 μM dNTP, 1 μL of each forward and reverse primer, and 0.5 μL of Taq polymerase (TaKaRa Bio Inc.). Amplifications were examined using gel electrophoresis in a 1% agarose. The PCR products were moved to Macrogen for sequencing (Macrogen, Seoul, Korea).

Sequences for the *rbc*L region were aligned using the ClustalW program within BioEdit (Hall 1999). Phylogenetic analyses were performed using the neighbor-joining (NJ), maximum-likelihood (ML) and unweighted pair group method using arithmetic averages (UPGMA) methods. Bootstrap values were calculated with 1,000 replications for NJ and ML. *rbc*L sequences of other *Codium* species were obtained from GenBank. *Bryopsis maxima* was used as an outgroup.

RESULTS AND DISCUSSION

Codium lucasii Setchell in Lucas 1935: 200-202

Korean name: Ju-reum-cheong-gak nom. nov.
(신칭: 주름청각)

Specimens examined: NIBRAL0000152590, NIBRAL0000152591, PKNU0000143983, PKNU0000143984, PKNU0000143985, PKNU0000143986 (Daejin Harbor: 30 January 2014).

Habitat: Epilithic near the intertidal zone.

Morphology: The thallus is prostrate, adherent or pulvinate, dark green, firm in texture, and tightly attached to the substratum. The size of the thallus is 3–8 cm in diameter (Fig. 1a-1d). The utricles are strongly grouped, cylindrical to slightly clavate, 70–120 μm in diameter and 450–910 μm long (Fig. 2a), and connected with each other by a siphon with a septum (Fig. 2a-2c). The apex of the utricle is rounded to capitated, and frequently alveolate (Fig. 2d). Its apical cell wall is 10–30 μm thick. One to three hair scars are found in the upper portion of the utricle (Fig. 2e). One to two gametangia are produced in the upper portion of the utricle. They are fusiform to ovoid and 70–100 μm in diameter and 120–250 μm long, and grow on a short pedicel (Fig. 2f).

Codium lucasii was originally described from Bondi, New South Wales, Australia by Setchell in Lucas (1935). This species is characterized by a prostrate, adherent or pulvinate, dark green thallus that is tightly attached to substratum (Lucas 1935, Shimada et al. 2007, the present study). The Korean alga collected from Daejin in the present study shares these features. According to Shimada et al. (2007), the morphology of the thallus and utricle apex are important criteria for identifying *Codium* species. *Codium lucasii* is similar to the four Korean species, *C. arabicum, C. coactum, C. dimorphum,* and *C. hubbsii,* in having a prostrate and adherent thallus (Lucas 1935, Oh et al. 1987, Shimada et al. 2007, Kim and Klochkova 2010, González et al. 2012, Lee and Kim 2015). Of these, *C. hubbsii* is the most similar to *C. lucasii* in showing frequently aveloate utricle apex (Dawson 1950, Shimada et al. 2007, Lee and Kim 2015). However, the species are distinguished from each other by the thallus texture and the shape of the utricle apex. The thallus of *C. lucasii* is firm in texture and has clavate and cylindrical utricles with a capitated apex, while that of *C. hubbsii* is soft in texture and has elongated cylindrical utricles with depressed-globose tips. These interspecific morphological differences between the two species are also supported by molecular data.

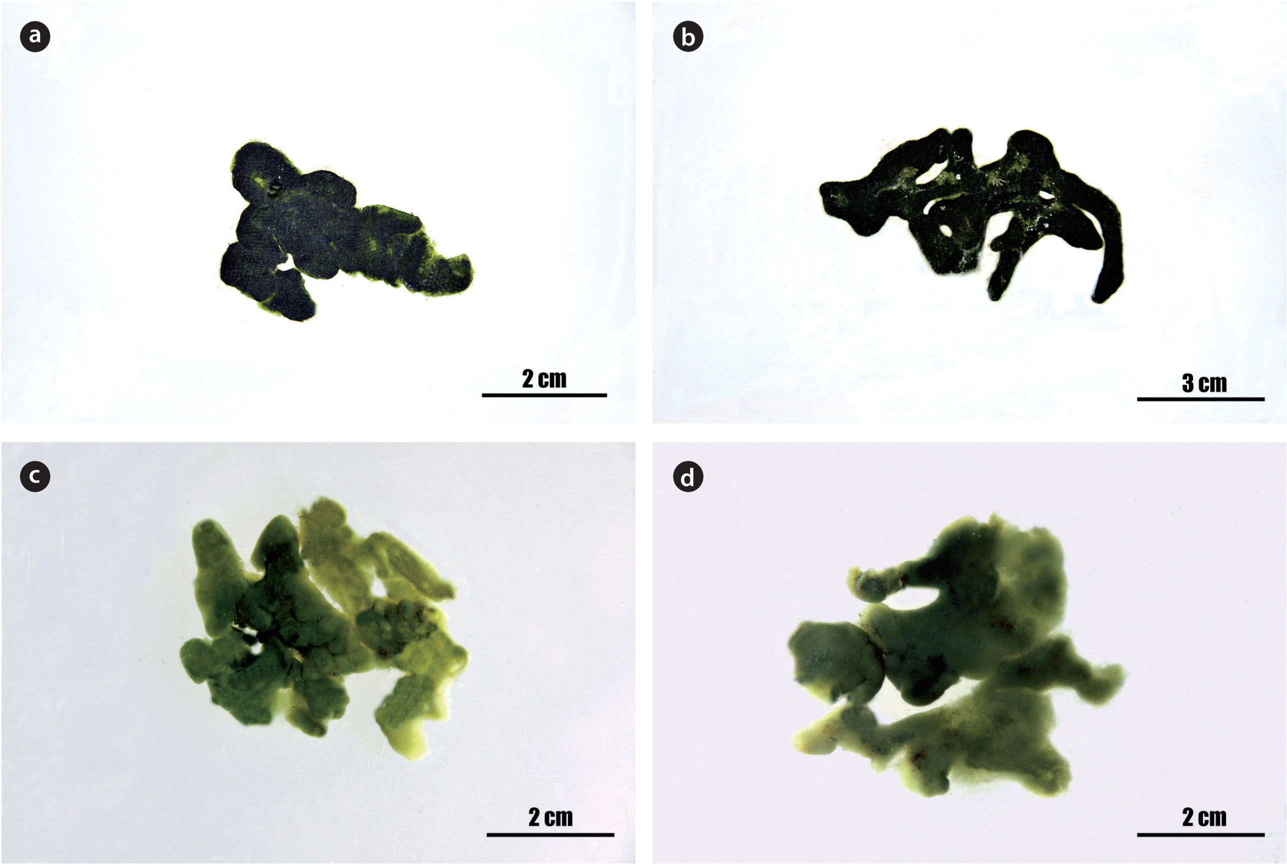

Fig. 1. *Codium lucasii* Setchell. (a, b) Herbarium specimen of *Codium lucasii*, (c, d) habit of vegetative plant. Scale bars are presented on the figures.

*rbc*L regions have been used to analyze *Codium* species in many studies (Shimada et al. 2004, González et al. 2012, Oliveira-Carvalho et al. 2012). In a phylogenetic tree based on the molecular data, the Korean alga collected from Daejin Harbor is placed in the same clade as *C. mozambiquense* in UPGMA analysis (Fig. 5), and nests in a sister clade of *C. lucasii* subsp. *capense* and *C. mozambiquense* in ML and NJ analyses (Figs. 3-4). However, the genetic distance between the sequences of the Korean alga and the two species is 1.3–1.9%, while that between the Korean alga and *C. lucasii* from Japan is 1.1%. The divergence value between the Korean alga and *C. lucasii* from the type locality (Australia) is 2.7%. It has been reported that the intra- and interspecific genetic distance ranges in *Codium* are 0.0–1.1% and 1.8–12.3%, respectively (Shimada et al. 2007). As based on this divergence value, sequence of the Korean alga falls into the intraspecific range of *C. lucasii* from Japan. It has been known that *C. lucasii* is a complex of many species level clusters, such as that from Australia (type locality), that from Japan, that from East Africa, and that from the Arabian Sea (Verbruggen et al. 2007; Verbruggen and Costa 2015). These geographically separated clusters with the genetic distance values suggest that the Korean alga together with Japanese *C. lucasii* can be separated from genuine *C. lucasii* from the type locality. However, we could not find significant differences in morphology between the entities during the present study. Therefore, the Korean alga is identified as *C. lucasii* until those entities are morphologically characterized in species level. This is the first record of *C. lucasii* in Korea.

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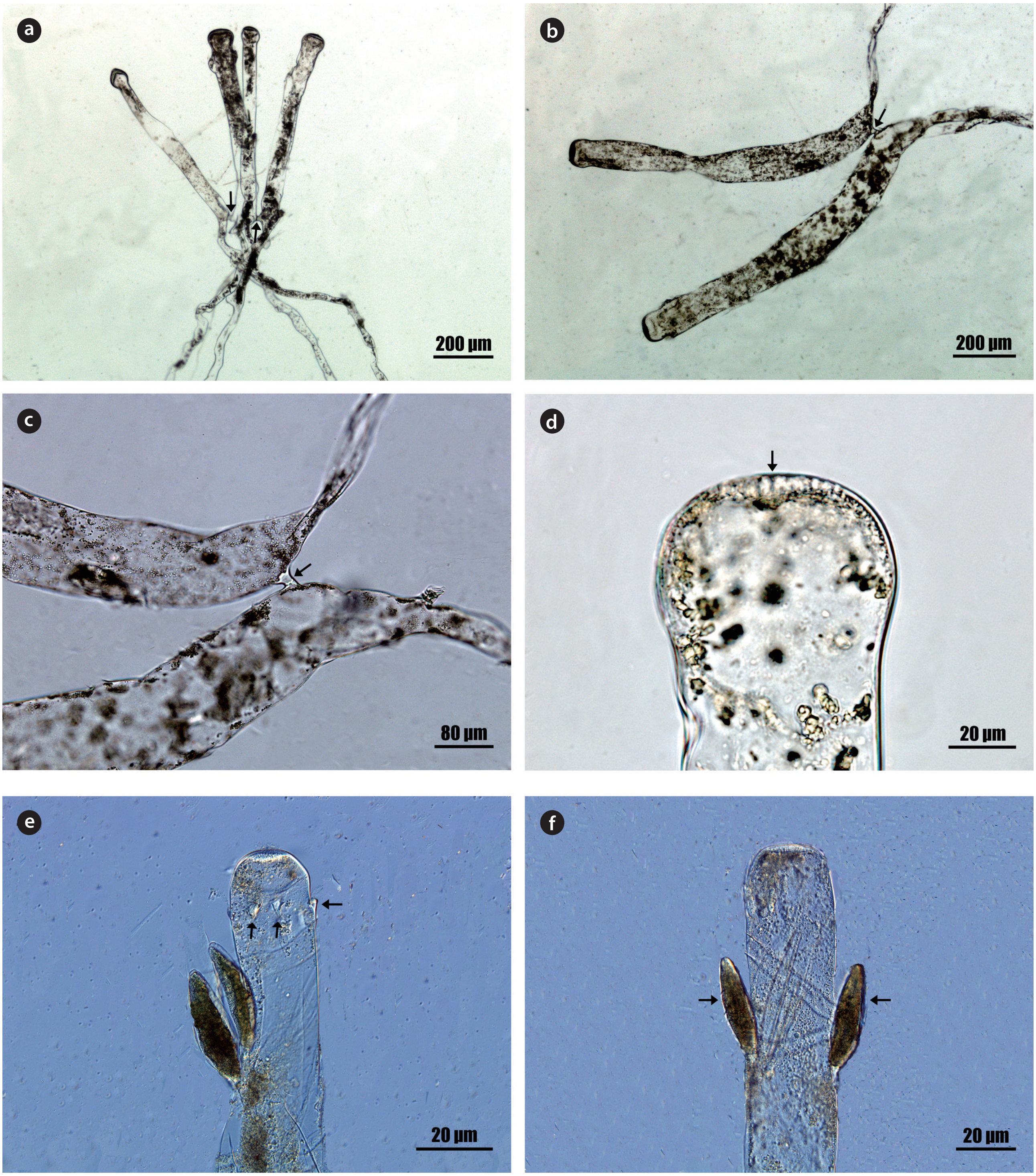

Fig. 2. *Codium lucasii* Setchell. (a-c) Cylindrical to slightly clavate utricles connected by siphon (arrows) with a septum, (d) apex of utricle with alveolate ornament (arrow), (e) apex of utricle with hair scars (arrows), (f) reproductive utricle with fusiform gametangia (arrows). Scale bars are presented on the figures.

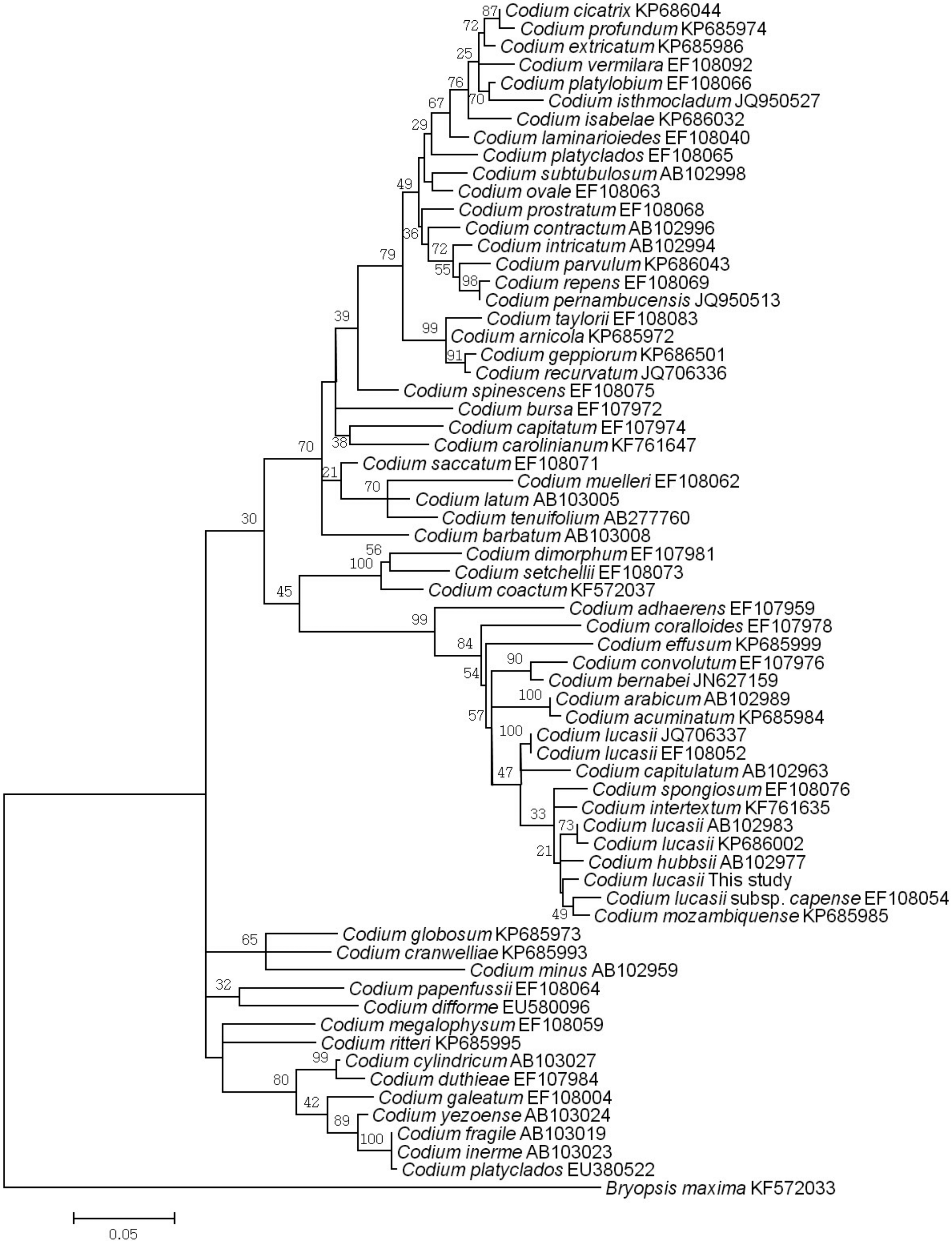

Fig. 3. Phylogenetic tree of *Codium* obtained from ML analysis based on *rbc*L sequences. Bootstrap percentages (1,000 replicates samples) are shown above branches. Scale bar, 0.05 substitutions/site.

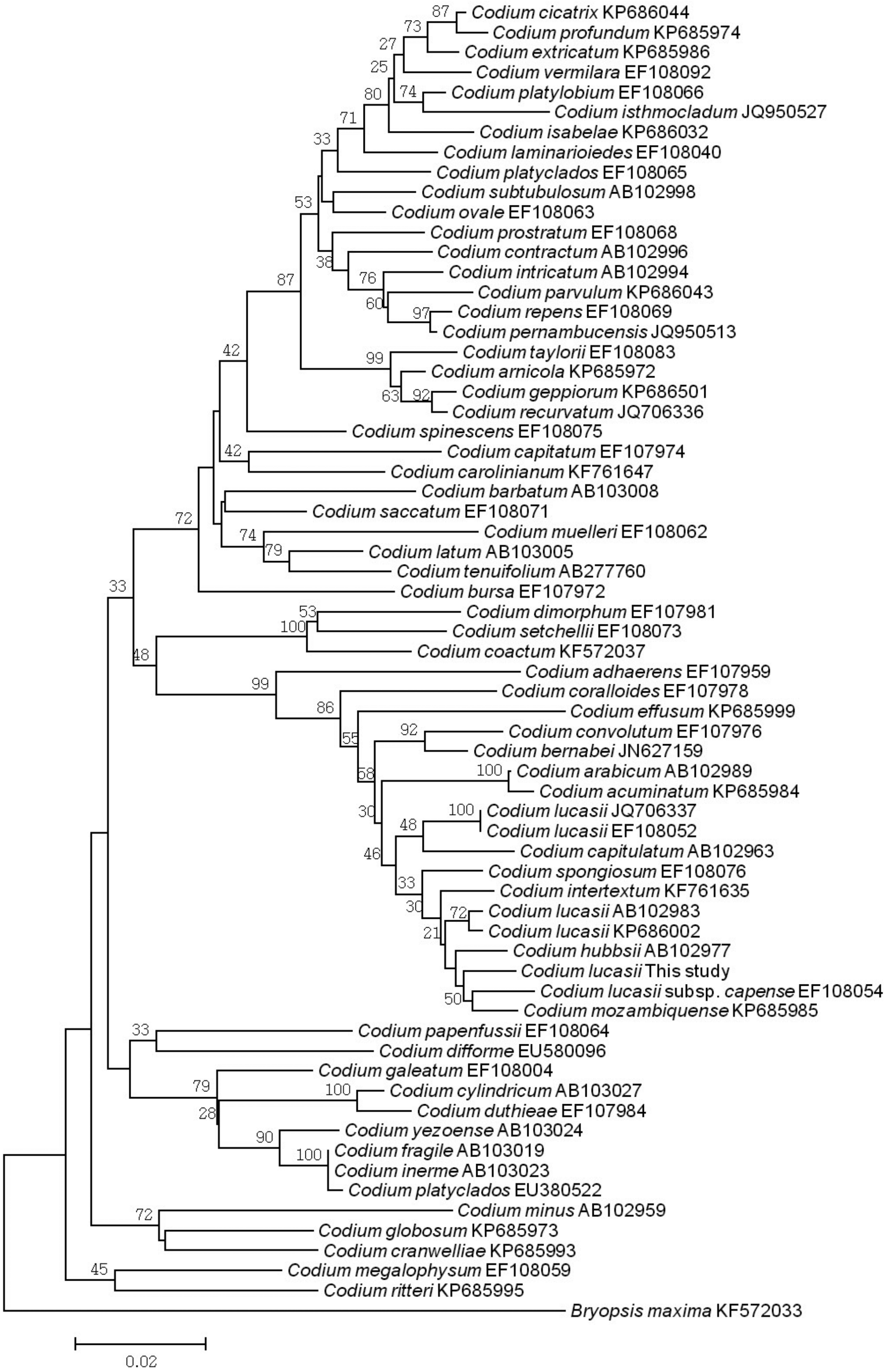

Fig. 4. Phylogenetic tree of *Codium* obtained from NJ analysis based on *rbc*L sequences. Bootstrap percentages (1,000 replicates samples) are shown above branches. Scale bar, 0.02 substitutions/site.

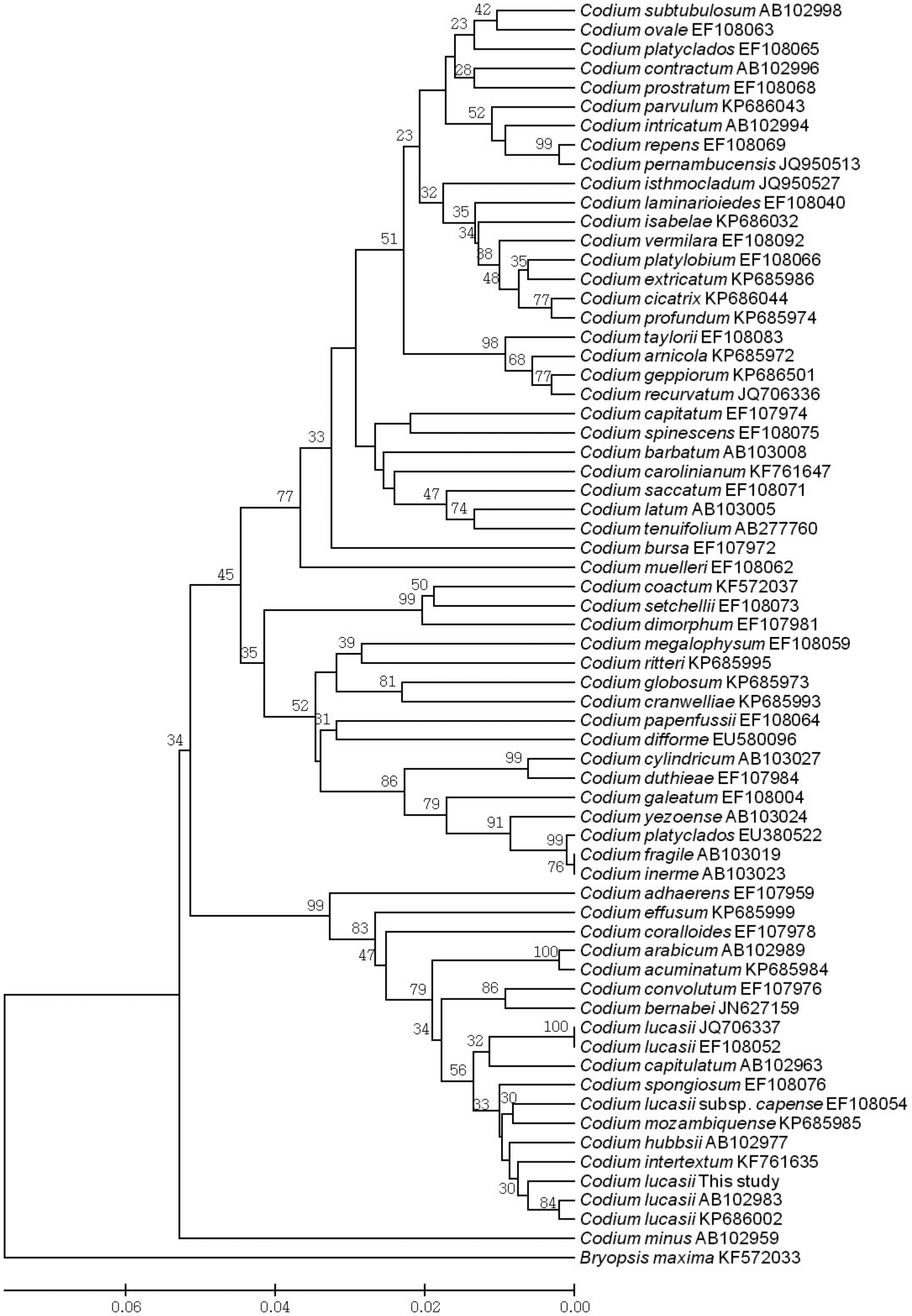

Fig. 5. Phylogenetic tree of *Codium* obtained from UPGMA analysis based on *rbc*L sequences. Bootstrap percentages (1,000 replicates samples) are shown above branches. Scale bar, 0.02 substitutions/site.

LITERATURE CITED

Dawson EY. 1950. Note on Pacific coast marine algae, IV. Am J Bot 37: 149-158.

González AV, Chacana ME, Silva PC. 2012. *Codium bernabei* sp. nov. (Bryopsidales, Chlorophyta), a coalescing green seaweed from the coast of Chile. Phycologia 51: 666-671.

Guiry MD, Guiry GM. 2015. AlgaeBase. World-wide electronic publication, National University of Ireland, Galway. http://www.algaebase.org/. Accessed 08 September 2015.

Hall TA. 1999. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucl Acids Symp Ser 41: 95-98.

Israel A, Einav R, Silva PC, Paz G, Chacana ME, Douek J. 2010. First report of the seaweed *Codium parvulum* (Chlorophyta) in Mediterranean waters: recent blooms on the northern shores of Israel. Phycologia 49: 107-112.

Kim GH, Klochkova TA. 2010. Bryopsidales. In: Algal Flora of Korea. Volume 1, Number 1. Chlorophyta: Ulvophyceae: Ulotrichales, Ulvales, Cladophorales, Bryopsidales. Marine Green Algae (Bae EH, Kim HS, Kwon CJ, Hwang IK, Kim GH, Klochkova TA, eds). National Institute of Biological Resources, Incheon, pp 157-209.

Kim HS, Boo SM, Lee IK, Sohn CH. 2013. National List of Species of Korea. Marine Algae, Jeonghaengsa, Seoul. (in Korean)

Lee HW, Kim MS. 2015. Species delimitation in the green algal genus *Codium* (Bryopsidales) from Korea using DNA barcoding. Acta Oceanol Sin 34: 114-124.

Lucas AHS. 1935. The marine algae of Lord Howe Island. Proc Linn Soc NSW 60: 194-232.

Oh YS, Lee YP, Lee IK. 1987. A taxonomic study on the genus *Codium*, Chlorophyta, in Cheju Island. Algae 2: 61-72.

Oliveira-Carvalho MF, Oliveira MC, Barreto-Pereira SM, Verbruggen H. 2012. Phylogenetic analysis of *Codium* species from Brazil, with the description of the new species *C. pernambucensis* (Bryopsidales, Chlorophyta). Eur J Phycol 47: 355-365.

Schmidt OC. 1923. Beiträge zur Kenntnis der Gattung *Codium* Stackhouse. Bibl Bot 91: 1-68.

Shimada S, Hiraoka M, Serisawa Y, Horiguchi T. 2004. Phylogenetic studies in the genus *Codium* (Chlorophyta) from Japan. Jpn J Phycol 52:137-141.

Shimada S, Ebata H, Horiguchi T, Kurihara A, Tanaka J. 2007. Molecular phylogenetic and morphological analyses of prostrate *Codium* (Chlorophyta) in Japan. J Jpn Bot 82: 190-204.

Silva PC. 1954. Phylogenetic significance of anatomical differences in *Codium*. Proc Eighth Int Bot Congr, pp 102-103.

Silva PC. 1962. Comparison of algal floristic patterns in the Pacific with those in the Atlantic and Indian Oceans, with special reference to *Codium*. Proc Ninth Pacific Sci Congr 4: 201-216.

Silva PC, Womersley HBS. 1956. The genus *Codium* (Chlorophyta) in southern Australia. Aust J Bot 4: 261-289.

Verbruggen H, Costa JF. 2015. Molecular survey of *Codium* species diversity in southern Madagascar. Cryptogam Algol 36: 171-187.

Verbruggen H, Leliaert F, Maggs CA, Shimada S, Schils T, Provan J, Booth D, Murphy S, De Clerck O, Littler DS, Littler MM, Coppejans E. 2007. Species boundaries and phylogenetic relationships within the green algal genus *Codium* (Bryopsidales) based on plastid DNA sequences. Mol Phylogenet Evol 44: 240-254.

Verbruggen H, Pauly K, De Clerck O. 2012. The new species *Codium recurvatum* from Tanzania. Eur J Phycol 47: 216-222.