

〈Original article〉

New Records of Two Filamentous Brown Algae, *Acinetospora asiatica* and *Botrytella reinboldii* from Korea

Antony Otinga Oteng'o, Jose Avila-Peltroche, So Young Jeong,
Boo Yeon Won and Tae Oh Cho*

Department of Life Science, Chosun University, Gwangju 61452, Republic of Korea

Abstract - *Acinetospora asiatica* and *Botrytella reinboldii* are reported as two new records from S. Korea based on morphological studies and molecular analyses. *A. asiatica* is mainly characterized by the presence of sparsely branched uniseriate filaments with diffused meristematic zones, the formation of crampons at right angles, and the formation of plurilocular sporangia on both prostrate and erect filaments. *B. reinboldii* is characterized by the presence of irregularly alternating branched uniseriate filaments attached by rhizoids, and single or clustered plurilocular sporangia with a single opening. Molecular analyses of *rbcL* gene revealed that *A. asiatica* and *B. reinboldii* are placed within each clade of *Acinetospora* and *Botrytella*, respectively.

Keywords : *Acinetospora asiatica*, *Botrytella reinboldii*, Ectocarpales, Phaeophyceae

INTRODUCTION

The filamentous brown algal genus *Acinetospora* is described as plants that possess sparsely branched uniseriate filaments forming entangled tufts, plurilocular sporangia (acinetosporangia) which produce acinetospores, monosporangia, scattered meristematic zones, and crampons (Bornet 1892; Sauvageau 1899). Currently, four *Acinetospora* species are recognized: *A. crinita* (Carmichael in Harvey) Sauvageau (1899), *A. nicholsoniae* Hollenberg (1971), *A. filamentosa* (Noda) Yaegashi, Uwai *et* Kogame (2015), and *A. asiatica* Yaegashi, Yamagishi *et* Kogame (2015). Of them, only *A. crinita* has been reported in Korea (Kim 2010).

The genus *Botrytella* is characterized by heterotrichous thallus organization arising from a basal disc, the erect filaments with sympodial branching, diffuse intercalary meristem, and sub-globular to irregular larger plurilocular sporangia arising terminally or serially on the adaxial side of the laterals (Womersley 1987; Kim 1996; Guiry and Guiry 2018).

Currently, four *Botrytella* species are recognized around the world; *Botrytella micromora* Bory (1822), *B. parva* (Takamatsu) H. S. Kim (1996), *B. pacifica* (Hollenberg) G. I. Hansen (1997) and *B. reinboldii* (Reinke) Kornmann & Sahling (1988). Of them, the three species have been reported in Korea except *B. reinboldii* (Kim 2010).

We collected two filamentous brown algae from coast of Korea that fit the description of *Acinetospora asiatica* and *Botrytella reinboldii*. We observed their detailed morphology and analysed molecular data based on *rbcL* gene for their phylogenetic relationships with others in their respective genera and a few selected species within the Ectocarpales. In this study, we add *Acinetospora asiatica* and *Botrytella reinboldii* to the Korean marine algal inventory.

MATERIALS AND METHODS

1. Morphology

Plants were collected from coasts of Korea and sorted into voucher herbarium specimens, silica gel samples for molec-

* Corresponding author: Tae Oh Cho, Tel. 062-230-7161,
Fax. 062-230-7161, E-mail. tocho@chosun.ac.kr

ular analysis, and formalin samples for morphological analysis. Formalin samples were preserved in 4–5% formalin/seawater. Photomicrographs taken using an Olympus BX51TRF microscope (Olympus, Tokyo, Japan) and an Olympus DP71 camera. Permanent slides were mounted in 70% karo syrup. Representative specimens examined in this study were deposited in the herbarium of Chosun University (CUK) and National Institute of Biological Resources (NIBR), Korea.

2. Molecular study

Genomic DNA was manually extracted from silica-gel samples using a NucleoSpin Plant II Kit (Macherey-Nagel, Düren, Germany). The extracted DNA was stored at -20°C and used to amplify *rbcL*. Polymerase chain reaction (PCR) was conducted in 30 μL reactions that contained 3.2 μL genomic DNA or 3.2 μL diluted genomic DNA (1 : 10), 1 μL 10 pmols forward and reverse primers, and 15 μL HelixAmp Ready-2x-Go Series (NanoHelix Co., Ltd., Daejeon, Korea). The *rbcL* gene was amplified using the primer combinations NDRbcL2-DRL1R and DRL2F-R3A (Kogame *et al.* 1999; Hwang *et al.* 2005). The PCR product was purified with PCR quick-spinTM PCR product purification kit (iNtRON Biotechnology, Inc. Seongnam, Korea). Determination of the nucleotide sequence or sequencing performed by Macrogen Inc., Seoul, South Korea.

RbcL sequence data were compiled by the present study and obtained from GenBank and aligned with ClustalW (Thompson *et al.* 1994). New *rbcL* sequences obtained from *Acinetospora asiatica* have been deposited in EMBL/GenBank under the accession numbers MH727658, MH727659, MH727660, and MH727661. Also *Botrytella reinboldii* sequence data are deposited in EMBL/GenBank under accession number MH727662 for *rbcL*. Phylogenetic analyses were conducted using MEGA version 6.06 (Tamura *et al.* 2013). Maximum likelihood analyses were conducted using the GTR + G model, with 1,000 bootstrap replicates. A bayesian inference was performed using MrBayes 3.2.6 (Huelsenbeck and Ronquist 2001; Ronquist and Huelsenbeck 2003). Markov chain Monte Carlo runs were conducted for 2 million generations, each with one cold chain and three heated chains using the GTR + Γ + I evolutionary model and sampling and printing every 1,000 generations. Summary trees were generated using a burn-in value of 800.

RESULTS AND DISCUSSION

1. Morphological observations

Acinetospora asiatica Yaegashi, Yamagishi *et* Kogame, 2015

아시아숨말 (신칭) (Figs. 1–2)

Type locality: Oshoro, Otaru, Hokkaido, Japan.

Material examined: MBRB0063TC13380 (voucher specimens deposited in the National Marine Biodiversity Institute of Korea for MH727658 GenBank accession number), Byeonsan-myeon, Buan-gun, Jeollabuk-do, Korea ($35^{\circ}37'41.87''\text{N}$, $126^{\circ}28'04.60''\text{E}$), January 09, 2015, T. O. Cho and B. Y. Won, at 1 m depth by hand; MBRB0063TC14114 (voucher specimens deposited in the National Marine Biodiversity Institute of Korea for MH727659 GenBank accession number), Hupo-hang, Hupo-myeon, Uljin-gun, Gyeongsangbuk-do, Korea ($36^{\circ}40'42.90''\text{N}$, $129^{\circ}27'02.96''\text{E}$), January 23, 2015, T. O. Cho, S. Y. Jeong, J. G. Lee, S. Y. Park, J. B. Chai and H. U. Jany, at 1 m depth by hand; MBRB0063TC17307 (voucher specimens deposited in the National Marine Biodiversity Institute of Korea for MH727660 GenBank accession number), Hongdo-hang, Hongdo-ri, Heuksan-myeon, Sinan-gun, Jellanam-do, Korea ($34^{\circ}41'02.15''\text{N}$, $125^{\circ}11'35.50''\text{E}$), May 21, 2016, T. O. Cho, S. Y. Jeong, D. B. Mostajo and A. Jose, at 1 m depth by hand; NIBROR0000001359 (deposited in the National Institute of Biological Resources), MBRB0063TC18922 (voucher specimens deposited in the National Marine Biodiversity Institute of Korea for MH727661 GenBank accession number), Jaranman-ro, Hai-myeon, Goseong-gun, Gyoungsangnam-do, Korea ($34^{\circ}54'19.08''\text{N}$, $128^{\circ}09'08.43''\text{E}$), May 05, 2018, T. O. Cho and B. Y. Won, at 1 m depth by hand.

Description: Plants forming entangled tufts (Fig. 1A) on rocks and other seaweeds (e.g. *Sargassum* spp.), sparsely branched uniseriate filaments (Fig. 1B), and up to 30 cm high. Erect filaments have meristematic zones composed of small sized cells (Fig. 1C) and crampons formed on erect filaments at right angles (Fig. 1D). Cells of erect filaments 20–77 μm in length, 18–30 μm in width, and contain many discoid chloroplasts (Fig. 1E). Erect filaments produced from prostrate filaments (Fig. 1F), and tapered slightly in upper part to form pseudohairs (Fig. 1G). Plurilocular zoidangia produced on

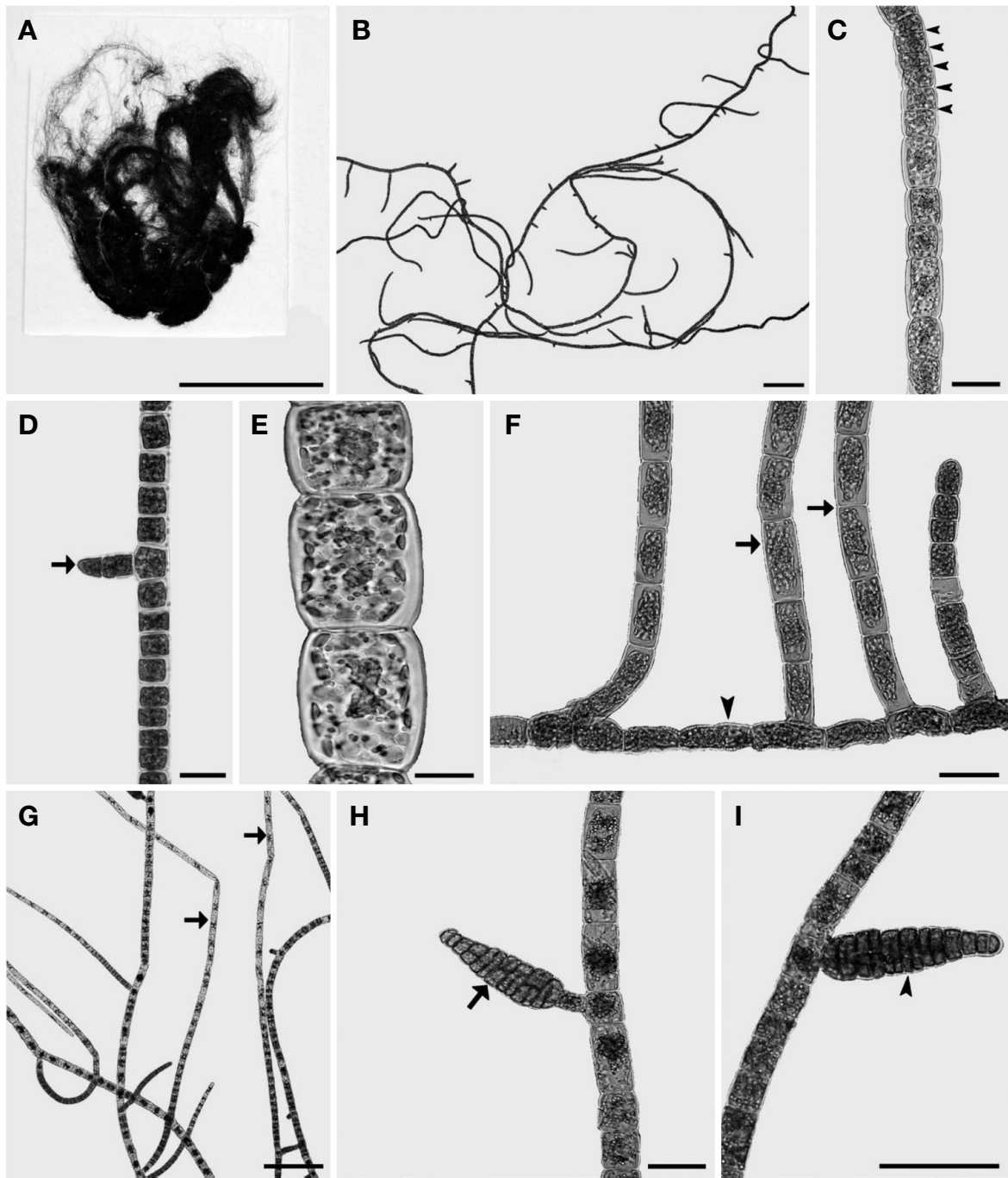


Fig. 1. *Acinetospora asiatica* Yaegashi, Yamagishi *et* Kogame. A. Specimen with filamentous entangled tufts; B. Thallus with Sparsely branched uniseriate filaments; C. Meristematic zones (arrow heads) composed of small cells; D. Formation of crampon (arrow) on erect filaments at right angles; E. Cells with discoid chloroplasts; F. Erect filaments (arrows), prostrate filaments (arrowhead); G. Pseudohair (arrows) in the upper part of erect filaments; H. Pedicellate plurilocular zoidangia (arrow); I. Sessile plurilocular zoidangia (arrow). Scale bars: A = 2.0 cm; B = 500 μ m; C, D, F, H = 50 μ m; E = 20 μ m; G = 200 μ m; I = 100 μ m.

both prostrate and erect filaments, ectocarpoid, 90–135 μ m in length, 25–40 μ m in width, with one- or two-celled pedicels (Fig. 1H) or sessile (Fig. 1I). Unilocular sporangia were

not found.

Habitat: Epiphytic and saxicolous at the tide pool in intertidal zone.

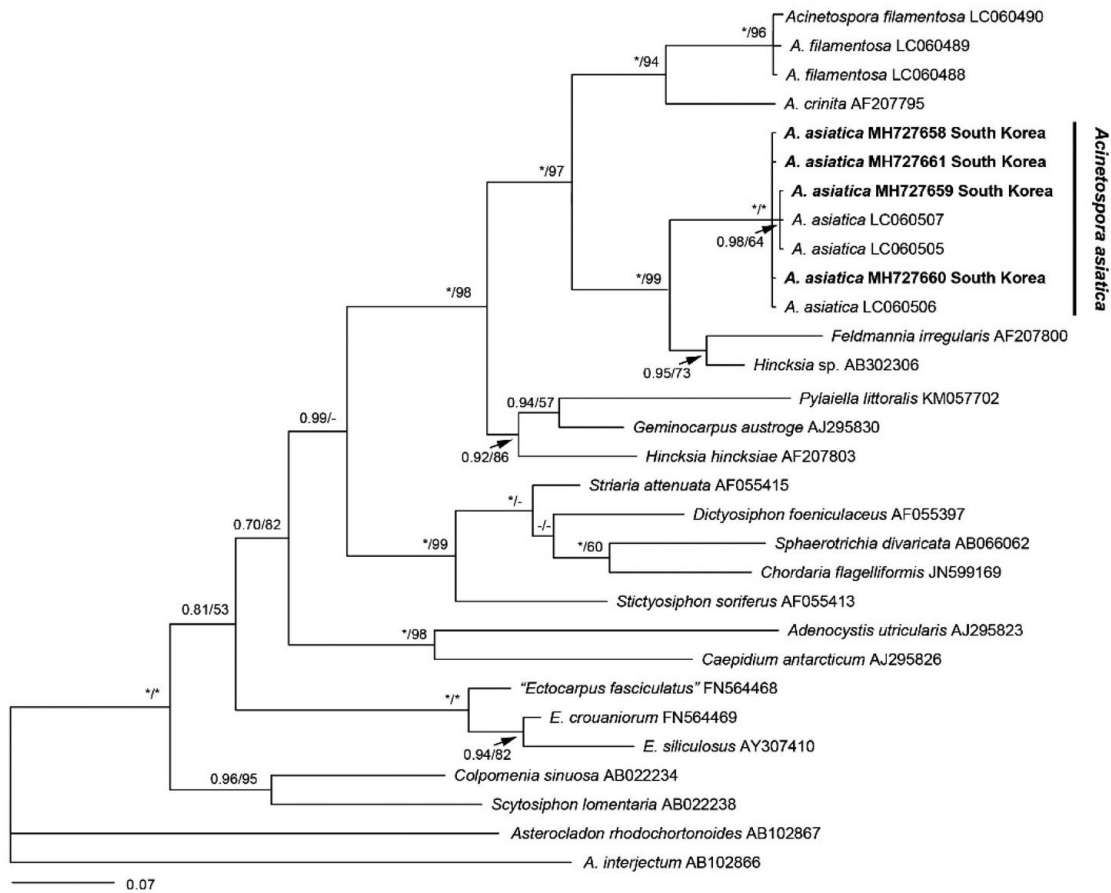


Fig. 2. Phylogenetic tree of *Acinetospora* species based on Bayesian and ML analysis with *rbcL* sequences. Value above the branches = Bayesian posterior probabilities > 0.75/Maximum likelihood bootstrap values in % > 50. Values lower than BPP 0.75 or BS 50 are indicated by hyphens (-). Values of BPP 1.00 or BS 100 are indicated by asterisks (*).

World distribution: Korea, Japan.

Identifier: Tae Oh Cho.

Phylogenetic analyses: The 1326-nucleotide portion of *rbcL* was aligned for *Acinetospora asiatica*. The phylogenetic trees were obtained from the alignment of the *rbcL* sequences newly generated and downloaded from GenBank. *Asterocladon rhodochortonoides* and *A. interjectum* were selected as outgroup. Phylogenetic analyses revealed that *Acinetospora* collected from South Korea was placed within a clade of *Acinetospora asiatica* (Fig. 2). In addition, *Acinetospora asiatica* collected from South Korea differs from *A. crinita* by 4.2–5.2% and from *A. filamentosa* by 4.7–4.9% gene sequence divergence respectively. However, there was only 0–0.02% gene sequence divergence between Genbank and our collection of *Acinetospora asiatica*.

Remarks: *Acinetospora asiatica* was recently described as

a new species from Japan (Yaegashi *et al.* 2015). Our collections match the diagnosis of *Acinetospora asiatica* as described from the type locality. Molecular data based on *rbcL* gene match with the Japanese samples. In this study, we report *A. asiatica* as a new record from Korea and add this species in the list of Korean macroalgal flora.

***Botrytella reinboldii* (Reinke) Kornmann & Sahling, 1988**

가지바다오디 (신칭) (Figs. 3–4)

Type locality: Helgoland, Germany.

Material examined: MBRB0061TC10574, Beakpo village, Dolsan-eup, Yeosu-si, Jeollanam-do, Korea (34°37'15.03"N, 127°47'25.70"E), January 18, 2014, T. O. Cho, at 1 m depth by hand; NIBROR0000001360 (deposited in the National

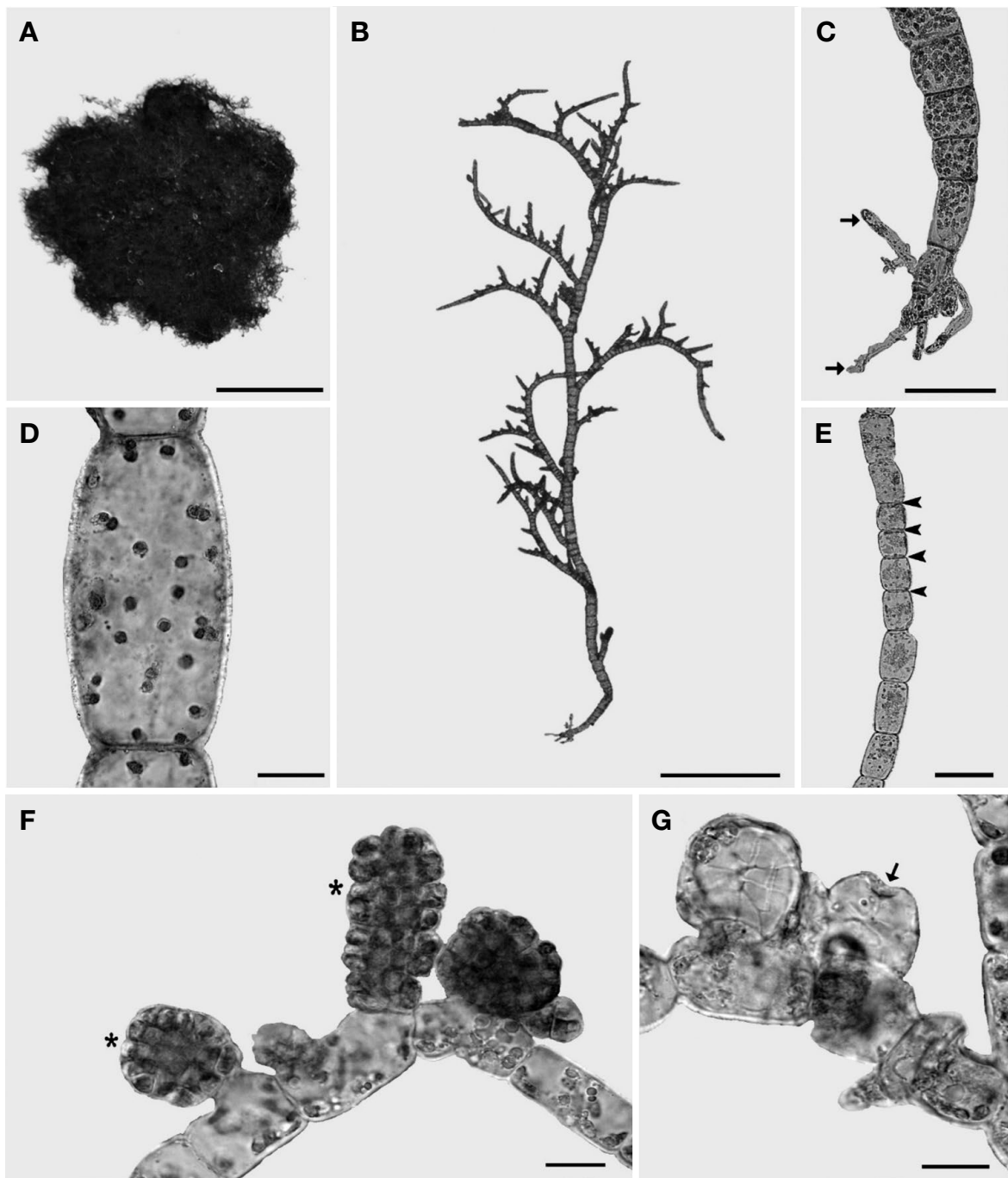


Fig. 3. *Botrytella reinboldii* (Reinke) Kornmann & Sahling. A. Specimen with filamentous entangled tufts; B. A uniseriate filament showing irregular alternative branching pattern; C. Rhizoids (arrows) near the base; D. Cells with discoid chloroplasts; E. Meristematic zones (arrowheads) composed of small cells; F. Sessile plurilocular zoidangia of various sizes (asterisks); G. Empty plurilocular sporangium with an anterior opening (arrow) after the release of spores. Scale bars: A = 0.5 cm; B = 0.5 mm; C, E = 100 μ m; D = 20 μ m; F, G = 20 μ m.

Institute of Biological Resources), MBRB0061TC18903 (voucher specimens deposited in the National Marine Biodiversity Institute of Korea for MH727662 GenBank acces-

sion number), Aninjin hang, Gangdong-myeon, Gangneung-si, Gangwon-do, Korea (37°44'15.81"N, 128°59'19.55"E), January 05, 2018, T. O. Cho and B. Y. Won, at 1 m depth by

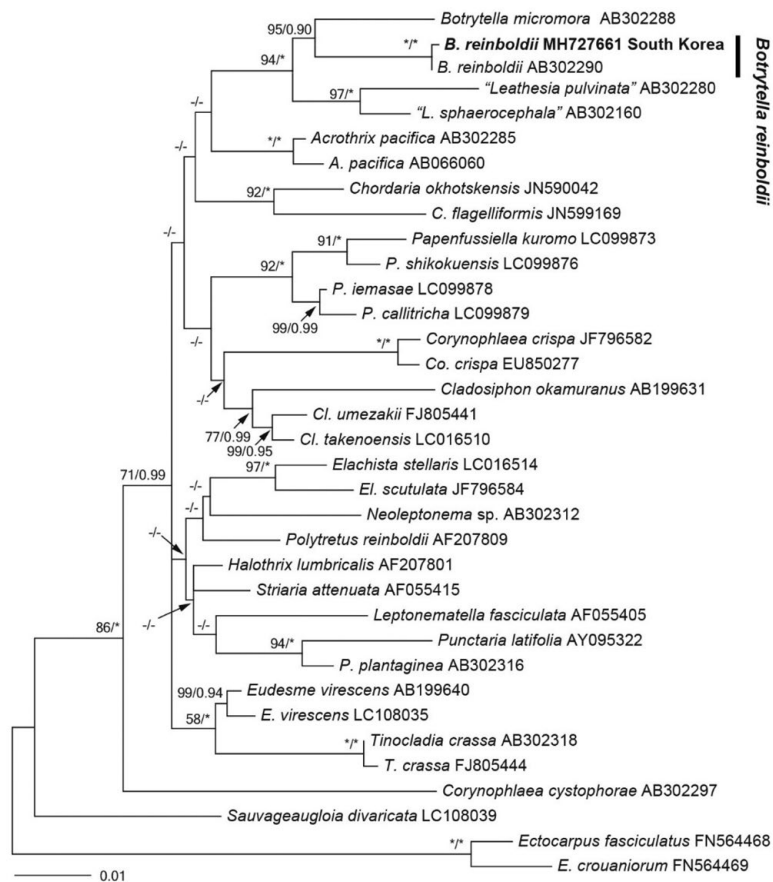


Fig. 4. Phylogenetic tree of *Botrytella* species based on ML and Bayesian analysis with *rbcL* sequences. Value above the branches = Maximum likelihood bootstrap values in % > 50/Bayesian posterior probabilities > 0.75. Values lower than BS 50 or BPP 0.75 are indicated by hyphens (-). Values of BS 100 or BPP 1.00 are indicated by asterisks (*).

hand.

Description: Plant yellowish brown, epiphytic (e.g. on *Sargassum* spp.), forming tufts (Fig. 3A), up to 10 cm in length, 8 μ m in diameter, irregularly alternating branched uniseriate filaments (Fig. 3B), and attached by rhizoids (Fig. 3C). Cells cylindrical, slightly constricted at the septum, and with numerous discoid chloroplasts (Fig. 3D). Filaments 30 μ m in diameter, tapered slightly in upper part, and bear diffuse meristem (Fig. 3E). Plurilocular sporangia sessile or stalked with various shapes and sizes, single on the lower thalli, grape-like clusters on laterals of upper thalli (Fig. 3F), release spores through one opening located anteriorly on the plurilocular sporangium (Fig. 3G). Unilocular sporangia not observed.

Habitat: Epiphytic on other seaweeds (e.g. on *Sargassum* spp.) at the tide pool in intertidal zone.

World distribution: Korea, Japan (Guiry and Guiry 2018).

Identifier: Tae Oh Cho and So Young Jeong.

Phylogenetic analyses: The 1122-bp portion of *rbcL* was aligned for *Botrytella reinboldii*. *Ectocarpus fasciculatus* and *E. crouaniorum* were selected as outgroup. Phylogenetic analyses revealed that *Botrytella reinboldii* from South Korea was nested in a clade of *Botrytella reinboldii* (Fig. 4). The gene sequence divergence between *Botrytella reinboldii* and *B. micromora* is 3% and there is no difference within *Botrytella reinboldii*.

Remarks: Morphologically, our samples closely match the description of *B. reinboldii* described by Kornmann and Sahling (1988). Also, molecular data based on *rbcL* gene revealed that our samples are placed within the clade of the *Botrytella* and specifically very close to the Japanese *B. reinboldii* (GenBank accession number: AB302290). In this study, we report *B. reinboldii* as a new record from Korea and add this species to the list of Korean macroalgal flora.

ACKNOWLEDGEMENTS

This study was supported by a grant from the National Institute of Biological Resources (NIBR), funded by the Ministry of Environment (MOE) of the Republic of Korea (NIBR 201801205). This research was also supported by Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education, Science and Technology (2015R1D1A1A01058359) and a grant from Marine Biotechnology Program (PJT200669 and 20170431) funded by Ministry of Oceans and Fisheries of Korean Government to Tae Oh Cho.

REFERENCES

- Bornet E. 1892. Note sur quelques *Ectocarpus*. B. Soc. Bot. Fr. 38:353–372.
- Bory de Saint Vincent JBM. 1822. Dictionnaire classique d'histoire Naturelle. Paris.
- Guiry MD and GM Guiry. 2018. AlgaeBase. World-wide electronic publication, National University of Ireland, Galway. <http://www.algaebase.org>; searched on 15 August 2018.
- Hansen GI. 1997. A revised checklist and preliminary assessment of the macrobenthic marine algae and seagrasses of Oregon. pp. 175–200. In Conservation and Management of Native Flora and Fungi (Kaye TN, A Liston, RM Love, DL Luoma, RJ Meinke and MV Wilson eds.). Native Plant Society of Oregon.
- Hollenberg GJ. 1971. Phycological notes. V. New species of marine algae from California. Phycologia 10:11–16.
- Hwang IK, HS Kim and WJ Lee. 2005. Polymorphism in the brown alga *Dictyota dichotoma* (Dictyotales, Phaeophyceae) from Korea. Mar. Biol. 147:999–1015.
- Huelsenbeck JP and F Ronquist. 2001. MrBayes: Bayesian inference of phylogeny. Bioinformatics 17:754–755.
- Kim HS. 1996. Morphotaxonomic studies on the Korean Ectocarpaceae (Phaeophyta). IV. *Botrytella parvus* (Takamatsu) comb. nov., life history and morphogenesis based on light-temperature gradient culture. Algae 11:45–57.
- Kim HS. 2010. Ectocarpaceae, Acinetosporaceae, Chordariaceae. pp. 3–137. In Algal Flora of Korea. Volume 2, Number 1. Heterokontophyta: Phaeophyceae: Ectocarpales. Marine Brown Algae I (Kim HS and SM Boo eds.). National Institute of Biological Resources, Incheon.
- Kogame K, T Horiguchi and M Masuda. 1999. Phylogeny of the order Scytosiphonales (Phaeophyceae) based on DNA sequences of *rbcL*, partial *rbcS*, and partial LSU nrDNA. Phycologia 38:496–502.
- Kornmann P and PH Sahling. 1988. The disentanglement of the *Botrytella* (*Sorocarpus*) complex (Ectocarpaceae, Phaeophyta). Helgoland. wiss. Meer. 42:1–12.
- Ronquist F and JP Huelsenbeck. 2003. MrBayes 3: Bayesian phylogenetic inference under mixed models. Bioinformatics 19:1572–1574.
- Sauvageau C. 1899. Les *Acinetospora* et la Sexualité des Tilopteridacées. Jour. De Bot. 13:107–127.
- Tamura K, G Stecher, D Peterson, A Filipski and S Kumar. 2013. MEGA6: Molecular Evolutionary Genetics Analysis Version 6.0. Mol. Biol. Evol. 30:2725–2729.
- Thompson JD, DG Higgins and TJ Gibson. 1994. Clustal W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. Nucleic Acids Res. 22:4673–4680.
- Womersley HBS. 1987. The Marine Benthic Flora of Southern Australia. Part II. South Australian Government Printing Division, Adelaide.
- Yaegashi K, Y Yamagishi, S Uwai, T Abe, WJE Santiañez and K Kogame. 2015. Two species of the genus *Acinetospora* (Ectocarpales, Phaeophyceae) from Japan: *A. filamentosa* comb. nov. and *A. asiatica* sp. nov. Bot. Mar. 58:331–343.

Received: 21 August 2018

Revised: 3 September 2018

Revision accepted: 4 September 2018