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New records of two filamentous brown algae, *Acinetospora filamentosa* and *Microspongium stilophorae* from Korea

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Abstract: *Acinetospora filamentosa* and *Microspongium stilophorae* are reported as new records from South Korea based on morphological and molecular analyses. *A. filamentosa* is mainly characterized by having the sparsely branched erect filaments, the scattered meristematic zones, forming crampons, plurilocular sporangia on both prostrate filaments and lower part of erect filaments, and spherical to oval unilocular sporangia formed either sessile or with a pedicel. *M. stilophorae* is an epiphytic thalli mostly on *Stilophora* sp. It is characterized by prostrate filaments with irregular cells, short erect filaments with short ramuli, phaeophycean hairs, uniseriate plurilocular sporangia on the terminal part of erect filament. Our molecular analyses of *rbc*L and *cox*1 genes reveals that *A. filamentosa* and *M. stilophorae* are nested within the clades of *Acinetospora* and *Microspongium*, respectively.

Keywords: *Acinetospora filamentosa*, *Microspongium stilophorae*, Ectocarpales, Phaeophyceae

INTRODUCTION

The filamentous brown algal genus, *Acinetospora*, was described by Bornet in 1892. It is characterized by having sparsely branched uniseriate filaments forming entangled tufts, scattered meristematic zones, crampons, plurilocular sporangia (acinetosporangia), and monosporangia (Bornet 1892; Sauvageau 1899). Currently, four *Acinetospora* species are recognized from worldwide: *A. crinita* (Carmichael in Harvey) Sauvageau from Scotland (Womersley 1987), *A. nicholsoniae* Hollenberg from California, U.S.A. (Hollenberg 1971), *A. filamentosa* (Noda) Yaegashi from Japan (Noda 1970; Yaegashi *et al.* 2015), and *A. asiatica* Yaegashi, Yamagishi *et* Kogame from Japan (Yaegashi *et al.* 2015). Of them, *A. crinita* and *A. asiatica* have been reported in Korea (Kim 2010; Oteng'o *et al.* 2018).

The genus *Microspongium* was described by Reinke in 1888. It is characterized by pulvinate thalli with spongy-gelatinous structure, monostromatic base of apparently scattered filaments, branched and densely intricate erect filaments, 1−3 discoid phaeoplasts per cell, and plurilocular sporangia on terminal or lateral part of the erect filaments (Rienke 1888; Fletcher 1987; Peters 2003). Currently, six *Microspongium* species are recognized from worldwide: *M. alariae* (P. M. Pedersen) A. F. Peters from Greenland (Pedersen 1981; Peters 2003), *M. globosum* J. Rienke from Germany (Rienke 1888), *M. immersum* (Levring) P. M. Pedersen from Norway (Levring 1937; Athanasiadis 1996), *M. kuckuckianum* V. Schiffner from Adriatic Sea (Schiffner 1916), *M. radians* (M. Howe) A. F. Peters from Peru (Dawson *et al.* 1964) and *M. stilophorae* (P. L. Crouan & H. M. Crouan) Cormaci *et* G. Furnari from Adriatic Sea (Crouan and Crouan 1867; Hauck 1884). None of these has been reported in Korea.

We collected two unidentified filamentous brown algae from coast of Korea. We observed their detailed morphology and analysed molecular data based on *rbc*L and *cox*1 genes for their phylogenetic relationships. In this study, we add *Acinetospora filamentosa* and *Microspongium stilophorae* to the Korean marine algal inventory.

MATERIALS AND METHODS

1. Morphology

Samples of *Acinetospora filamentosa* were collected from west and south coasts of Korea. They were sorted into voucher herbarium specimens, silica gel samples, and formalin samples. Formalin samples were preserved in 4−5% formalin/seawater. A sample of *Microspongium stilophorae* was collected from east coast of Korea. It was isolated from *Dictyopteris pacifica* and cultured in provasoli enriched seawater (PES) medium in order to get enough material for morpho-anatomical and molecular analysis. 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 extracted using a NucleoSpin Plant II Kit (Macherey-Nagel, Düren, Germany). The extracted DNA was stored at −20°C and used to amplify *rbc*L and *cox*1. The *rbc*L gene was amplified using the primer combinations ND*rbc*L2-DRL1R and DRL2F-R3A (Kogame *et al.* 1999; Hwang *et al.* 2005) with HelixAmp Ready-2x-Go Series (NanoHelix Co., Ltd., Daejeon, Korea). The GazF2-GazR2 combination of primers used for *cox*1 (Saunders 2005; Lane *et al.* 2007). All PCR amplication were carried out with a Veriti 96-well Termal cycler (Applied Biosystem, ThermoFisher Scientific, USA). PCR products were purified using a PCRquick-spin™ PCR product purification kit (iNtRON Biotechnology, Inc, Seongnam, Korea). All *rbc*L and *cox*1 sequence data were compiled by the present study and obtained from GenBank and aligned with ClustalW (Thompson *et al.* 1994). New *rbc*L sequences obtained from *Acinetospora filamentosa* have been deposited in EMBL/GenBank under the accession numbers MN 052856 (CUK12425), MN052857 (CUK13048), MN 052858 (CUK12847) and MN052859 (CUK18942). *Asterocladon rhodochortonoides* and *A. interjectum* were selected as outgroup. *Microspongium stilophorae* sequence data are deposited in EMBL/GenBank under accession number MN052860 (CUK19276) for *cox*1. *Laminaria yezoensis* and *Saccharina groenlandica* were selected as outgroups.

Phylogenetic analyses were conducted using *MEGA* version 6.06 (Tamura *et al.* 2013). Maximum likelihood analyses were conducted using the GTR+G+I model, with 1,000 bootstrap replicates. Bayesian inference was performed using MrBayes 3.2.6 (Huelsenbeck and Ronguist 2001; Ronguist 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

***Acinetospora filamentosa* (Noda) Yaegashi, Uwai *et* Kogame, 2015** 실솜말 (신칭) (Figs. 1, 2)

Basionym: *Ectocarpus filamentosus* Noda 1970. Sci. Rep. Niigata Univ. Ser. D. 7: 27.
Heterotypic Synonym: *Ectocarpus ugoensis* Konno in Konno *et* Noda 1974. Sci. Rep. Niigata Univ. Ser. D. 11: 80.
Material examined: NIBROR0000001612 & CUK12425 (= MBRB0099TC12425) Chuja-hang, Chuja-myeon, Jeju-si, Jeju Special Self-govering Province, Korea (33° 57′44.69″N, 126°17′47.12″E), June 27, 2014, T. O. Cho, S. Y. Jeong, D. B. Mostajo, J. G. Lee and S. Y. Park, at 1 m depth by hand; CUK12847 (= MBRB0099TC12847), Daejin-hang, Daejin-dong, Donghae-si, Gangwon-do, Korea (37°34′ 47.64″N, 129°6′51.13″E), August 01, 2014, T. O. Cho, S. Y. Jeong, D. B. Mostajo, J. G. Lee and S. Y. Park, at 1 m depth by hand; CUK13048 (= MBRB0099TC13048), Biyang-do, Hanrim-eup, Jeju-si, Jeju Special Self-govering Province, Korea (33°24′ 21.9″N, 126°13′46.40″E), May 30, 2014, T. O. Cho, S. Y. Jeong, D. B. Mostajo and J. G. Lee, at 1 m depth by hand; CUK18942 (= MBRB0099TC18942), Mo-hang, Byeonsan-myeon, Buan-gu, Jeollabuk-do, Korea (35°34′ 58.49″N, 126°30′18.41″E), May 12, 2018, T. O. Cho and B. Y. Won, at 1 depth by hand.
Habitat: Epiphytic and saxicolous at the tide pool in intertidal zone.

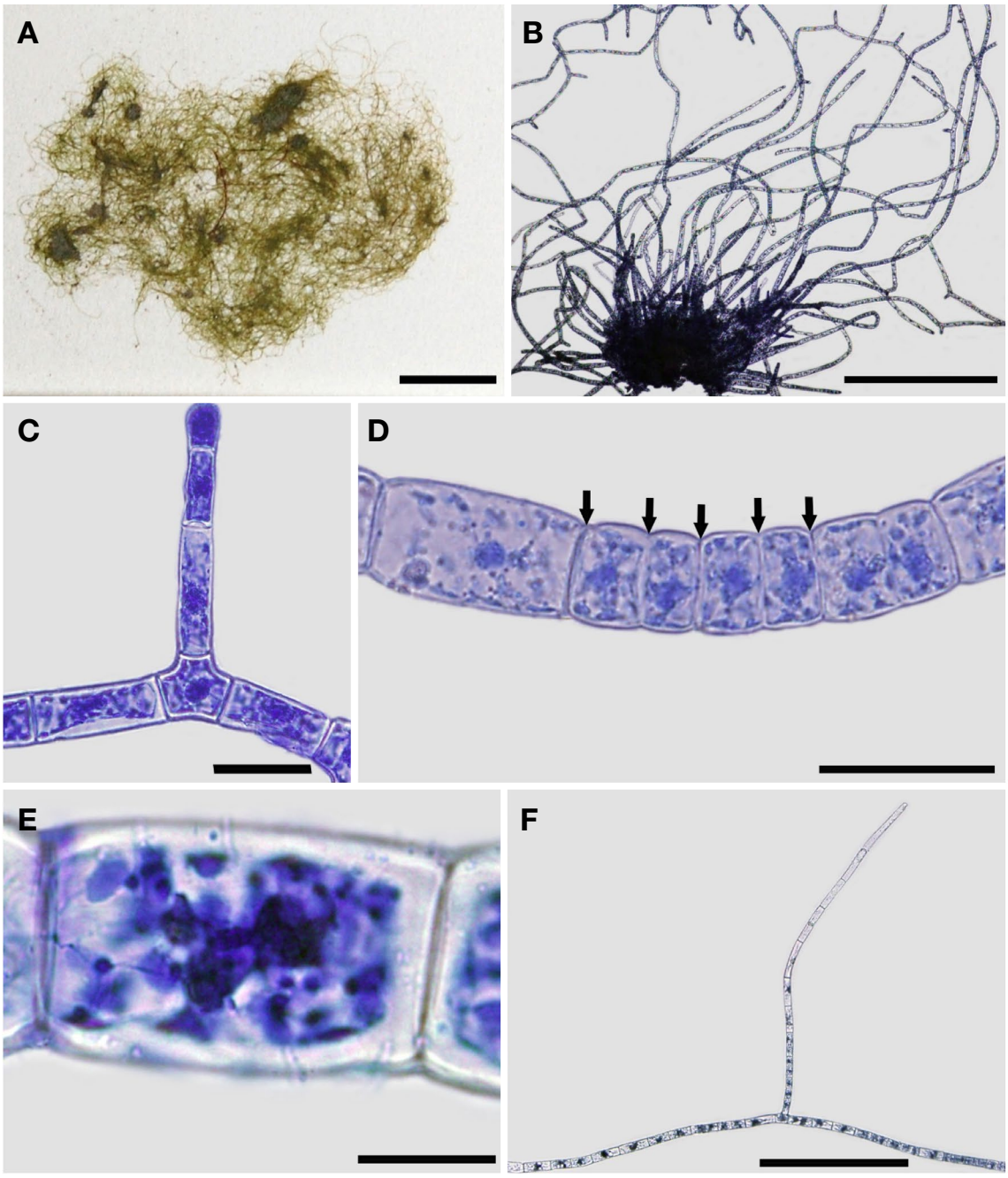

Fig. 1. *Acinetospora filamentosa* (CUK18942) from Mohang, Jeollabuk-do, Korea. A. Thalli forming entangled tufts; B. Erect filaments with crampons; C. Crampon on erect filament; D. Meristematic zone (arrows) on erect filaments; E. Cell with discoid chloroplasts; F. Phaeophycean hairs on erect filaments. Scale bars: A = 0.5 cm; B = 1 mm; F = 500 μm; C, D = 50 μm; E = 20 μm.

Morphological observation: Plants are uniseriate, forming entangled tufts (Fig. 1A) to 10 cm or more in length attached to rocks and other seaweeds (e.g. *Sargassum* spp.). Erect filaments are irregularly and sparsely branched at wide to right angles (Fig. 1B) and form straight to curved "crampons" composed of 2–5 cells (Fig. 1C). Meristematic zones (Fig. 1D) are scattered, consisting of short cells. Cells of erect filaments are 20–80 μm in length and 18–28 μm in width, containing many discoid chloroplasts (Fig. 1E) with pyrenoids. Phaeophycean hairs (Fig. 1F) are found later-

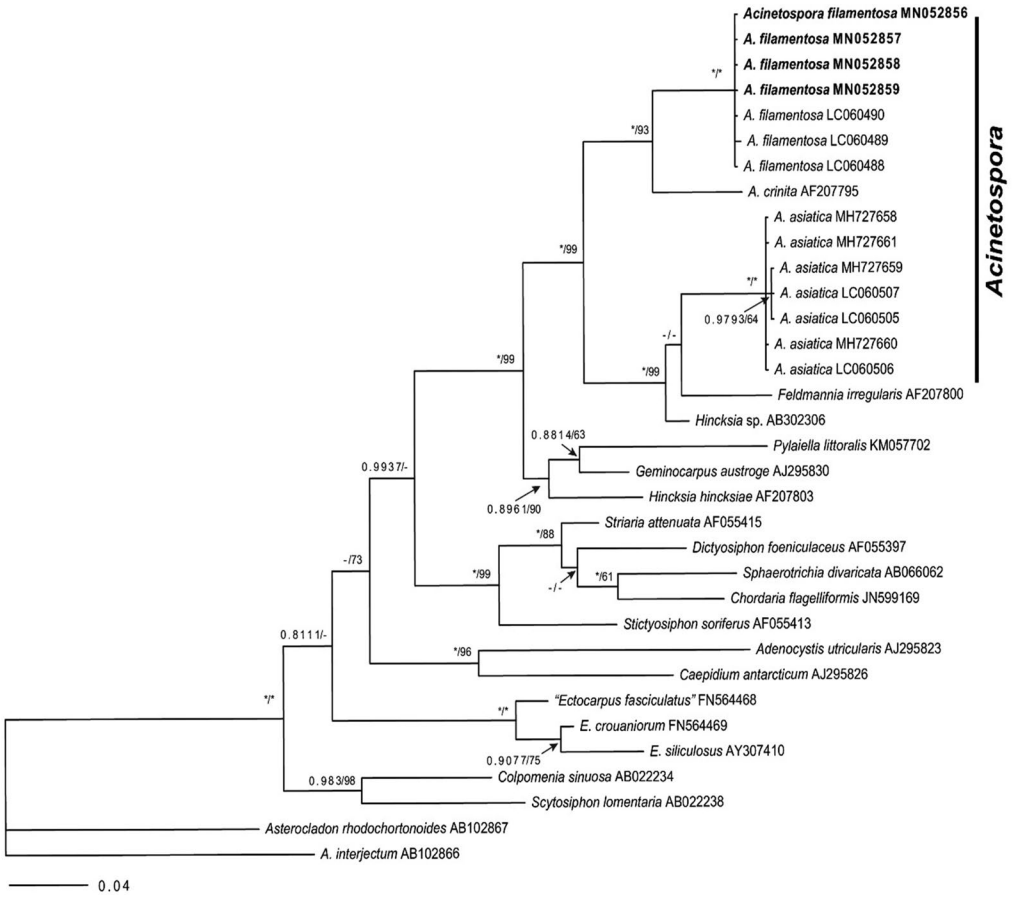

Fig. 2. Phylogenetic tree of *Acinetospora* species based on Bayesian and ML analysis with *rbc*L sequences. Value above branches = Bayesian posterior probabilities > 0.75/Maximum likelihood bootstrap values in % > 50. Values lower than BPP 0.75 or BS 50 are indicated by hyphens (−). Values of BPP 1.00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

ally or terminally on erect filaments. Unilocular sporangia are spherical to oval, 25−55 μm in length and 25−55 μm in width, sessile or on a pedicel, and are formed on erect filaments.

World distribution: Asia: Japan and Korea; Europe: Greece (Guiry and Guiry 2019).

Identifier: Tae Oh Cho and Antony Otinga Oteng'o.

Phylogenetic analyses: The 1326-nucleotide portion of *rbc*L was aligned for *Acinetosproa filamentosa*. Phylogenetic analyses revealed that our *Acinetospora* samples from Korea were placed within a clade of *Acinetospora filamentosa* in *rbc*L (Fig. 2). There was only 0−0.008% gene sequence divergence between Genbank and our collection of *Acinetospora filamentosa*. In addition, it revealed that *Acinetospora filamentosa* differs from *A. asiatica* by 4.4−4.7% and from *A. crinita* by 3.0% gene sequence divergence respectively.

Remarks: *Acinetospora filamentosa* was a new combination with *Ectocarpus filamentosus* as the basionym (Yaegashi *et al.* 2015). Our samples collected from Korea had vegetative morphology similar to that of *A. filamentosa*. Our molecular analyses based on *rbc*L gene show that our samples are nested in the clade of *A. filamentosa*. In this study, we report *A. filamentosa* as a new record from Korea and add this species in the list of Korean macroalgal flora.

***Microspongium* Reinke, 1888,** 점말속 (신칭)

***Microspongium stilophorae* (P. L. *et* H. M. Crouan) Cormaci *et* G. Furnari, 2012** 바늘점말 (신칭) (Figs. 3, 4)

Basionym: *Ectocarpus stilophorae* P. L. *et* H. M. Crouan, Florule du Finistére…., 1867: 161, Paris and Brest. x + 262 pp., 31 [+1] pls, frontispiece.

Homotypic synonym (s): *Streblonema stilophorae* (P. L. Crouan & H. M. Crouan) De Toni 1895; *Streblonema stilo-*

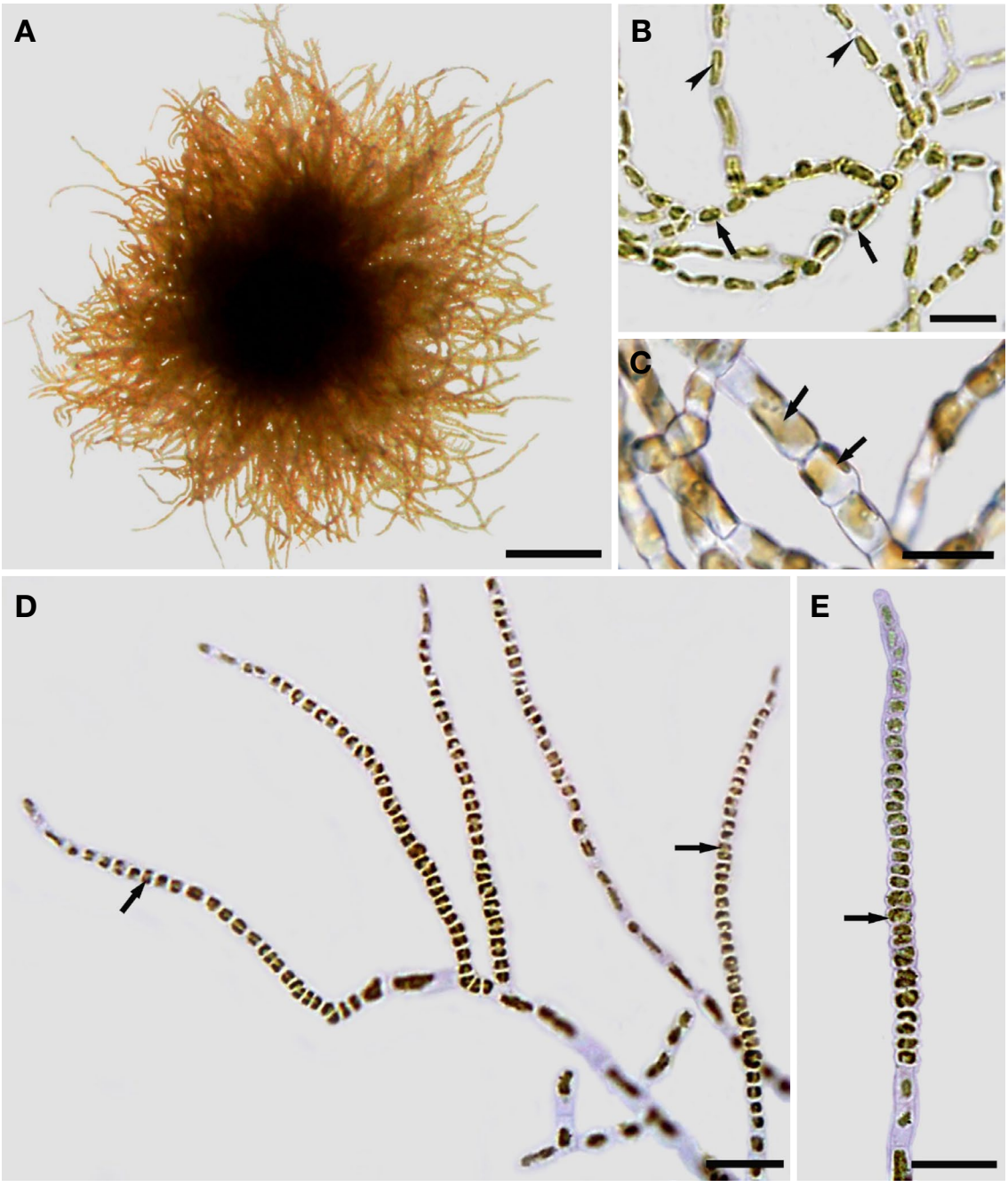

Fig. 3. *Microspongium stilophorae* (CUK19276) from Yangjeong-hang, Gyeongsangbuk-do, Korea. A. A 'ball-like' spongy spherical thallus; B. Prostrate filaments of irregular cells (arrows) and short erect filaments (arrowheads); C. Laminar- and lobate-shaped phaeoplasts (arrows); D. Uniseriate plurilocular sporangia (arrows) mostly on the terminal part of erect filaments. E. Uniseriate plurilocular sporangia. Scale bars: A = 250 μm; B–D, E = 20 μm.

phorae (P. L. Crouan & H. M. Crouan) Kylin 1908 (*comb. illeg.*).

Heterotypic synonym (s): *Ectocarpus stilophorae* f. *caespitosus* Rosenvinge 1893; *Ectocarpus stilophorae* v. *caespitosus* (Rosenvinge) L. Newton 1931; *Microspongium tenuissimum* (Hauck) A. F. Peters 2003; *Streblonema stilophorae* v. *caespitosum* (Rosenvinge) De Toni 1895; *Streblonema tenuissimum* Hauck 1884.

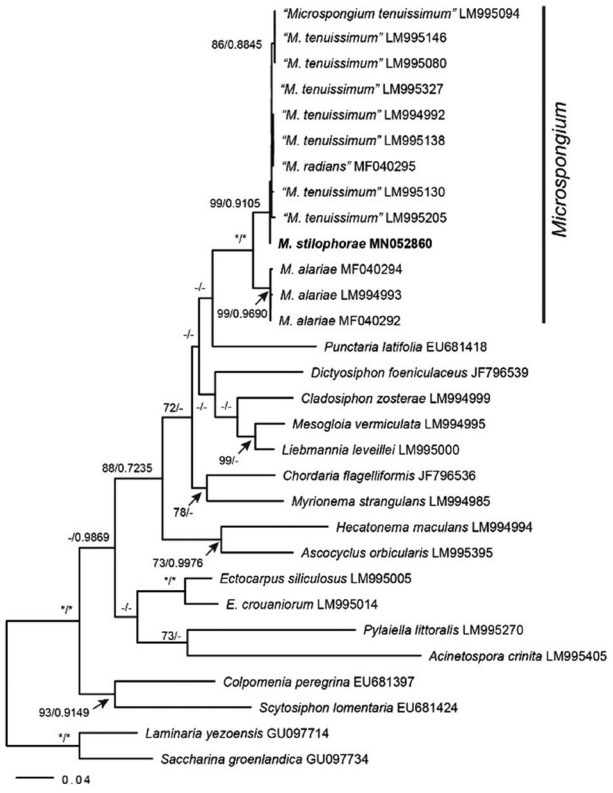

Fig. 4. Phylogenetic tree of *Microspongium* species based on ML and Bayesian analysis with *cox*1 sequences. Value above branches = Maximum likelihood bootstrap values in % > 50/ Bayesian posterior probabilities > 0.75. Values lower than BS 50 or BPP 0.75 are indicated by hyphens (–). Values of BS 100 or BPP 1.00 are indicated by asterisks (*).

Material examined: NIBROR0000001610 & CUK19276 (= MBRB0097TC19276) Yangjeong-hang, Uljin-eup, Uljin-gun, Gyeongsangbuk-do, Korea (37°00′59.15″N, 129°24′48.17″E), May 01, 2018, T. O. Cho and B. Y. Won, at 1 m depth by hand.

Habitat: Epiphytic on other seaweeds (e.g. on *Stilophora* sp., *Nemalion* sp. and *Dictyopteris pacifica*) at the tide pool in intertidal zone.

Morphological observation in culture: Cultured thallus isolated from *Dictyopteris pacifica* forms a spongy ball-like spherical tissue (Fig. 3A) formed by prostrate filaments of irregular cells in shape and size (Fig. 3B, arrows) and short erect filaments with short ramuli (Fig. 3B, arrowheads). Erect filaments are formed by cells 1–5 times longer than wide and 3–8 μm in diameter. The phaeoplasts (Fig. 3C, arrows) are one or two per cell. Phaeophycean hairs not frequent. Plurilocular sporangia lateral or mostly terminal, in uniseriate lodges, 3–8 μm wide (Fig. 3D, arrows).

World distribution: Arctic: Canada; Asia: Korea; Atlantic Islands: Iceland; Europe: Black Sea, Britain, Channel Islands, Faroe Islands, France, Ireland, Romania, Scandinavia and Spain (Guiry and Guiry 2019).

Identifier: Tae Oh Cho and Antony Otinga Oteng'o.

Phylogenetic analyses: The 613-nucleotide portion of *cox*1 was aligned for *Microspongium stilophorae*. Phylogenetic analyses revealed that our *Microspongium* sample from Korea was nested in a clade of *Microspongium stilophorae* based on *cox*1 (Fig. 4). In addition, it revealed that the gene sequence divergence between *M. stilophorae* (= "*M. tenuissimum*") and *M. alariae* is 2.9–3.6%. However, there was only 0.16–0.49% gene sequence divergence between Genbank and our collection of *M. stilophorae*.

Remarks: Morphologically, our *Microspongium* sample is matched into the description of *Microsporangium stilophorae*. *Microspongium tenuissimum* and *M. radians* were conspecific based on *cox*1 gene (Murúa *et al.* 2018). *Microspongium tenuissimum* is currently a synonym of *M. stilophorae* (Cormaci *et al.* 2012; Guiry and Guiry 2019). Our molecular data based on *cox*1 gene revealed that our Korean sample is nested in the same clade of *M. stilophorae*. In this study, we report *Microsporangium stilophorae* as a new record from Korea and add this species to the list of Korean macroalgal flora.

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