Korean Journal of Environmental Biology

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https://doi.org/10.11626/KJEB.2020.38.3.416

Korean J. Environ. Biol.
38(3) : 416-423 (2020)
ISSN 1226-9999 (print)
ISSN 2287-7851 (online)

New records of two brown algae, *Petroderma maculiforme* (Ishigeales, Phaeophyceae) and *Hincksia sordida* (Ectocarpales, Phaeophyceae) from Korea

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Received: 4 August 2020
Revised: 7 September 2020
Revision accepted: 9 September 2020

Abstract: The genus *Petroderma* never been reported from the coast of Korea. In this study, our collection from Chaeseokang is matched with *P. maculiforme* morphologically. *Petroderma maculiforme* is characterized by having small irregular light to dark brown crusts, a basal layer of irregularly shaped cells giving rise to erect parallel filaments which easily separate with pressure, single chromatophore per cell, small spherical to cylindrical unilocular sporangia in a terminal position, and plurilocular sporangia narrower than erect filaments or wider and shorter than erect filaments in a terminal position. In addition, *Hineksia sordida* was also collected from Korea. It is mostly epiphytic and characterized by uniseriate filamentous thalli forming loose tangled masses, sparse and spiral branching, some long lateral branches, rhizoids occurring throughout the plant, plurilocular and unilocular sporangia scattered on separate plants. Our molecular analyses based on the *rbc*L gene reveal that our samples of *P. maculiforme* and *H. sordida* are nested within the clades of *Petroderma* and *Hincksia*, respectively. Therefore *P. maculiforme* and *H. sordida* are reported as new records from Korea based on morphological and molecular analyses.

Keywords: *Hincksia sordida*, *Petroderma maculiforme*, Phaeophyceae, *rbc*L, taxonomy

INTRODUCTION

The crustose brown algal genus, *Petroderma*, was described by Kuckuck in 1897. It is characterized by having small dark brown confluent spots, single-layer basal cells giving rise to erect filaments that easily separate by pressure, one plate-shaped chromatophore per cell, unilocular and plurilocular sporangia arising from the transformation of the top vegetative cells, unilocular sporangia on erect filaments without paraphyses, successive production of unilocular sporangia on the same filament results in collar-like remnants of old sporangial walls along the filament, multirow erect filament plurilocular sporangia on a terminal position (Kuckuck 1897; Waern 1949; Boraso de Zaixso 2013). Currently, three *Petroderma* species are recognized from worldwide (Guiry and Guiry 2020): *P. maculiforme* (Wollny) Kuckuck from Helgoland Island, German (Kuckuck 1897; Athanasiadis 1996), *P. steinitzii* Rayss et Dor (1963) from Eilat, Israel, and *P. vietnamensis* Pham-Hoàng (1969) from Vietnam. None of these has been reported in Korea even if *P. maculiforme* has a wide distribution range.

The genus *Hincksia* was first described by Gray (1864) and characterized by thalli having secundly branched frond, intercalary growth, discoid chloroplast with a pyrenoid, unilocular and plurilocular sporangia produced from the adaxial side of the lateral branches (Gray 1864; Womers-

ley 1987; Guiry and Guiry 2020). Currently, twenty-nine *Hincksia* species are recognized from worldwide (Guiry and Guiry 2020). Of them, *H. granulosa* (Smith) P.C. Silva, *H. onslowensis* (Amsler & Kapraun) P.C. Silva, *H. ovata* (Kjellman) P.C. Silva (Silva *et al.* 1996), *H. sandriana* (Zanardini) P.C. Silva, and *H. secunda* (Kützing) P.C. Silva have been reported in Korea (Kim 2010; Guiry and Guiry 2020).

We collected some unidentified crustose and filamentous brown alga along the coasts of Korea. We observed their detailed morphology and analyzed the phylogenetic relationship based on the *rbc*L. We add *Petroderma maculiforme* and *Hincksia sordida* to the Korean marine algal inventory.

MATERIALS AND METHODS

1. Morphology

Samples of *Petroderma maculiforme* were collected from the west (Byeonsan) coast of Korea. They were sorted into voucher specimens, air-dried, and preserved in silica gel for morphological and molecular analyses. For morphology, samples were detached from substrate by use of a single-edged blade, then embedded in a matrix (O.C.T., CellPath, Ltd., Newtown, Wales, UK) and sectioned (8–10 μm thickness) using a freezing microtome (Shandon Cryotome FSE, Thermo Shandon, Ltd., Loughborough, UK), stained in a 1 to 1 mixture of aqueous aniline blue and acetic acid. Samples of *Hincksia sordida* were collected from southeastern (Gampo) coast of Korea. They were sorted into voucher herbarium specimens, silica gel samples, and formalin samples. Formalin samples were preserved in 4–5% formalin/seawater. Silica gel samples were used for molecular analyses. Formalin sample was stained with aniline blue and morphological observations carried out. Photomicrographs were taken using an Olympus DP71 camera mounted on an Olympus microscope (BX51TRF; Olympus, Tokyo, Japan) and a digital camera (Nikon D40; Nikon, Japan). Representative voucher specimens examined in this study are deposited in the herbarium of Chosun University (CUK) and the National Institute of Biological Resources (NIBR), Korea.

2. Molecular study

Genomic DNA was 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. 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). PCR amplification was carried out with a Veriti 96 well Thermal cycler (Applied Biosystems, Foster city, USA). PCR products were purified using a PCRquick-spinTM PCR product purification kit (iNtRON Biotechnology, Inc, Seongnam, Korea). New *rbc*L sequences obtained from *Petroderma maculiforme* and *Hincksia sordida* and been deposited in EMBL/GenBank under the accession numbers MT023108 (CUK-19777) and MT469950 (CUK18158) respectively. For *P. maculiforme* molecular analysis, eighteen *rbc*L sequences (1069 bp) including sequences from GenBank and two outgroup taxa were aligned using ClustalW (Thompson *et al.* 1994). *Canistrocarpus cervicornis* (Kützing) De Paula and De Clerck and *Dictyota dichotoma* (Hudson) J.V. Lamouroux have been chosen as outgroup. For *H. sordida*, twenty-three *rbc*L sequences (1300 bp) were aligned using ClustalW. *Hincksia granulosa* and *H. sandriana* were included in this study for molecular analyses as MT569437 (CUK18909) and MT469949 (CUK18929) respectively. *Asterocladon rhodochortonoides* and *A. interjectum* selected as outgroups. Phylogenetic analyses were conducted using raxmlGUI1.5b2 (Silvestro and Michalak 2012). 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 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

Family Petrodermataceae Silberfeld, F. Rousseau *et* Reviers, 2014 납작패과(신칭)
Genus *Petroderma* Kuckuck, 1897 납작패속(신칭)

Petroderma maculiforme **(Wollny) Kuckuck, 1897: 382**
원반납작패(신칭) (Figs. 1, 2)

Basionym: *Lithoderma maculiforme* Wollny 1881. Hedwigia. 20: 31.
Heterotypic Synonym: *Lithoderma lignicola* Kjellman 1883. Vega-expeditionens Vetenskapliga Iakttagelser. 3: 318.

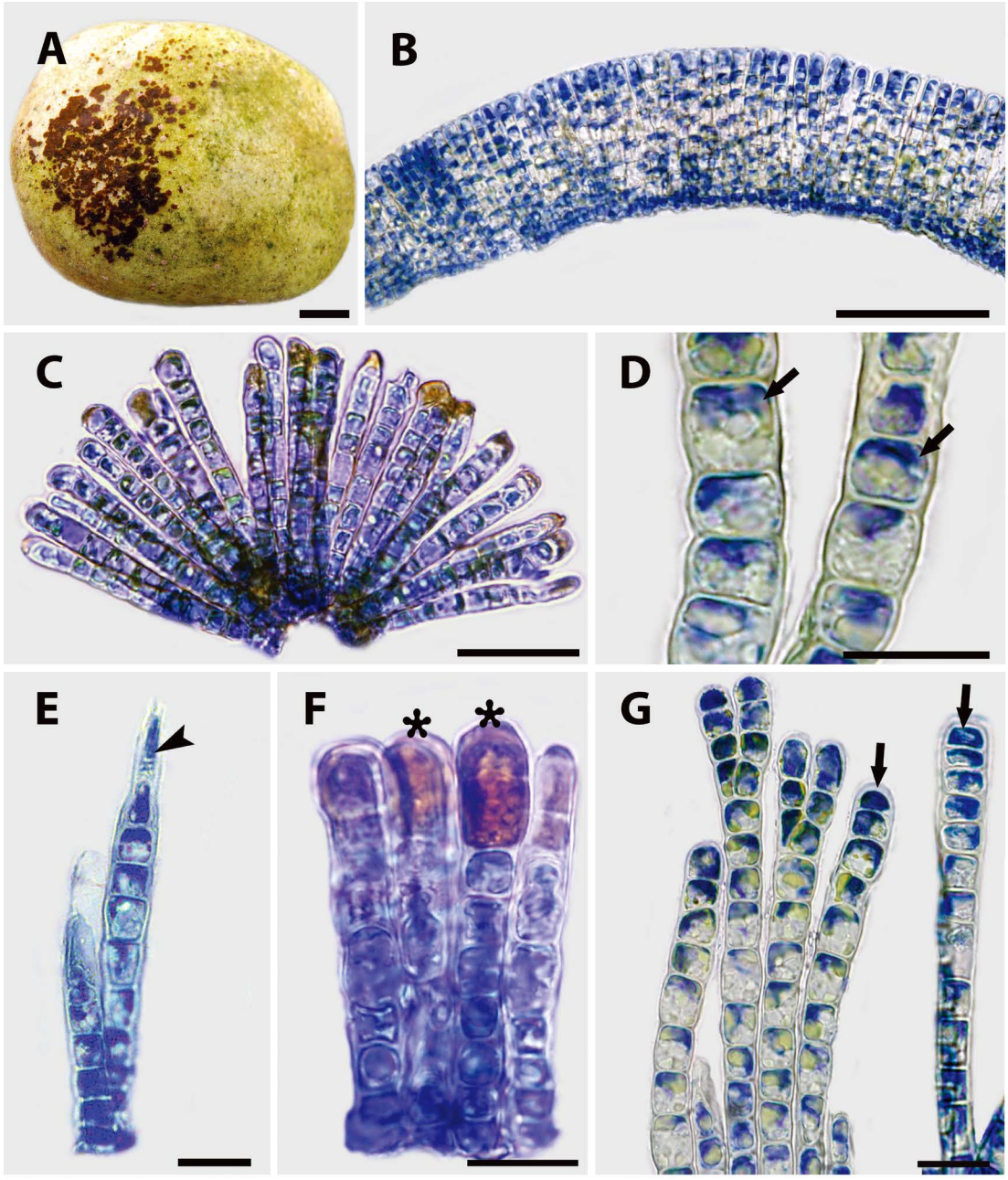

Fig. 1. *Petroderma maculiforme* CUK19777 (GenBank number: MT023108) from Korea. A. Dark brown crustose thalli on a pebble; B. Radial longitudinal section showing monostromatic basal layer which gives rise to erect parallel filaments; C. Erect filaments easily separated with pressure; D. Erect filament showing one parietal chromatophore (arrows) per cell; E. Erect filament having hair (arrowhead) on terminal part; F. Unilocular sporangia (asterisks) on terminal of erect filaments without associated paraphyses; G. Plurilocular sporangial initials (arrows) on terminal of erect filaments. Scale bars: A = 1 cm; B = 100 μm; C = 50; D–G = 20 μm.

Material examined. CUK19777 (=MBRB0104TC19777S1) & NIBROR0000001764 (National Institute of Biological Resources); Chaeseokang, Byeongsan-myeon, Buan-gun, Jeollabuk-do, Korea (35°37′38.18″N, 126°28′05.52″E); July 26, 2019; T.O. Cho and B.Y. Won.

Morphological observations. Plants are small irregular epilithic crusts (Fig. 1A), light to dark brown color, a few millimeters to 1 cm diam., gelatinous but adherent

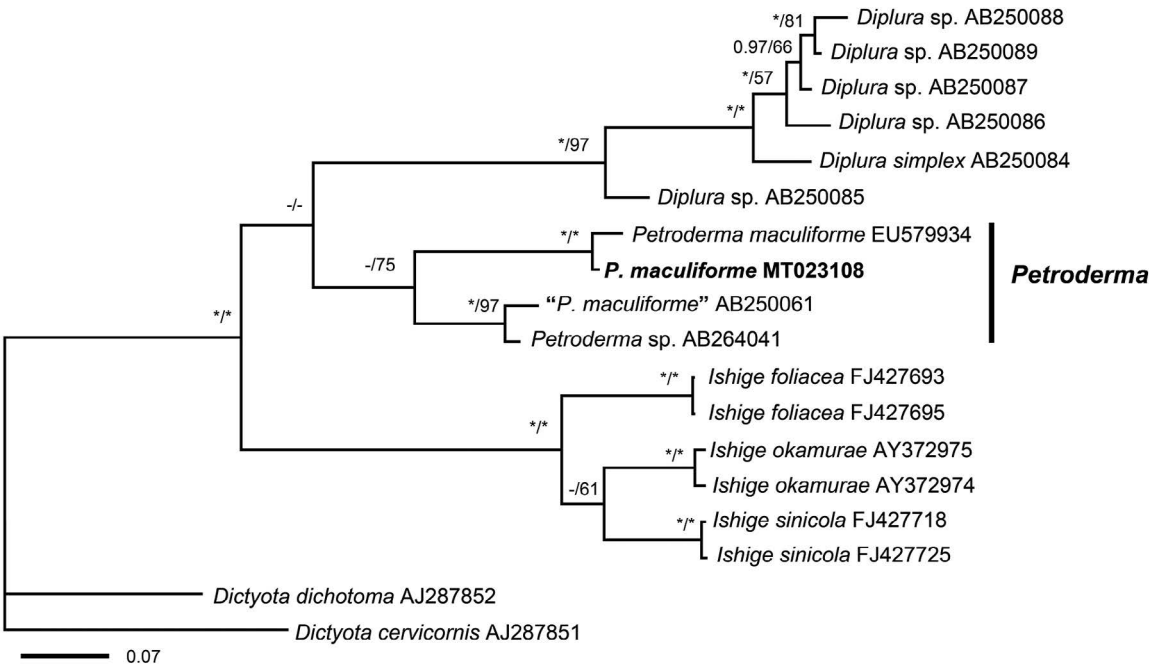

Fig. 2. Phylogenetic tree for the *Petroderma* and other species of Ishigeales based on Bayesian and RAxML analyses with *rbc*L sequences. The value above branches = Bayesian posterior probabilities > 0.75, Maximum likelihood bootstrap values in % > 50. Values lower than BPP 0.75 or BS 50 are indicated by hyphens (-). Values of BPP 1.00 or BS 100 are indicated by asterisks (*).

to the substratum. Short multicellular rhizoids were rarely observed. Thalli are 139−175 μm high, composed of a monostromatic basal layer and erect filaments (Fig. 1B), easily separate with pressure (Fig. 1C). They are mostly composed of up to 11 cells. Each cell contains a single parietal chromatophore (Fig. 1D). Hairs are infrequent, developed from terminal of erect filaments, and not arranged in clusters (Fig. 1E). Unilocular sporangia are on the terminal of erect filaments, spherical to cylindrical, 20−35 μm long, 10−15 μm wide, without associated paraphyses (Fig. 1F). Plurilocular sporangia (Fig. 1G) are on terminal of erect filaments, without associated paraphyses. Some plurilocular sporangia are elongate uniseriate with occasional oblique or longitudinal partitions and not wider than erect filaments while others are short wider than erect filaments and may be branched.

Habitat. Epilithic at the intertidal zone.

World Distribution. North America: Alaska, California, Maine, New Hampshire, Oregon, and Washington; Arctic: Svalbard (Spitsbergen). Europe: Britain, Denmark, Germany, Helgoland, Ireland, Norway, Scandinavia, and Spain. Atlantic Islands: Greenland and Iceland. Central America: Mexico. South America: Argentina; Asia: Russia (Far East). Antarctic: King George Islands (Guiry and Guiry 2020).

Phylogenetic analyses. The 1069-nucleotide portion of *rbc*L was aligned for *Petroderma maculiforme*. Phylogenetic analyses revealed that our *Petroderma* sample from Korea was nested in a clade of *Petroderma maculiforme* (Fig. 2) and it revealed that the gene sequence divergence between California and Korean samples of *Petroderma maculiforme* is 1.0%.

Remarks. Sample of *Petroderma maculiforme* from Korea is matched into original description of *P. maculiforme* (Wollny) Kuckuck. Our *P. maculiforme* differs from *P. steinitzii* by having a monostratos basal disc giving rise to assimilatory filaments and uni- or plurilocular sporangia or hairs developed terminally on assimilatory filaments. *Petroderma steinitzii* has a three-layer basal disc and unilocular sporangia, plurilocular sporangia, or hairs developed from cells of basal disc (Rayss and Dor 1963). Also, although *Petroderma maculiforme* has more similarities with *P. vietnamensis* in having a monostratos basal disc giving rise to assimilatory filaments, it differs from *P. vietnamensis* by being epilithic and having branched assimilatory filaments (Kuckuck 1897; Edelstein and McLachlan 1969; Boraso de Zaixso 2013). *Petroderma vietnamensis* has unbranched (simple) assimilatory filaments and smaller unilocular sporangia (Pham-Hoàng 1969).

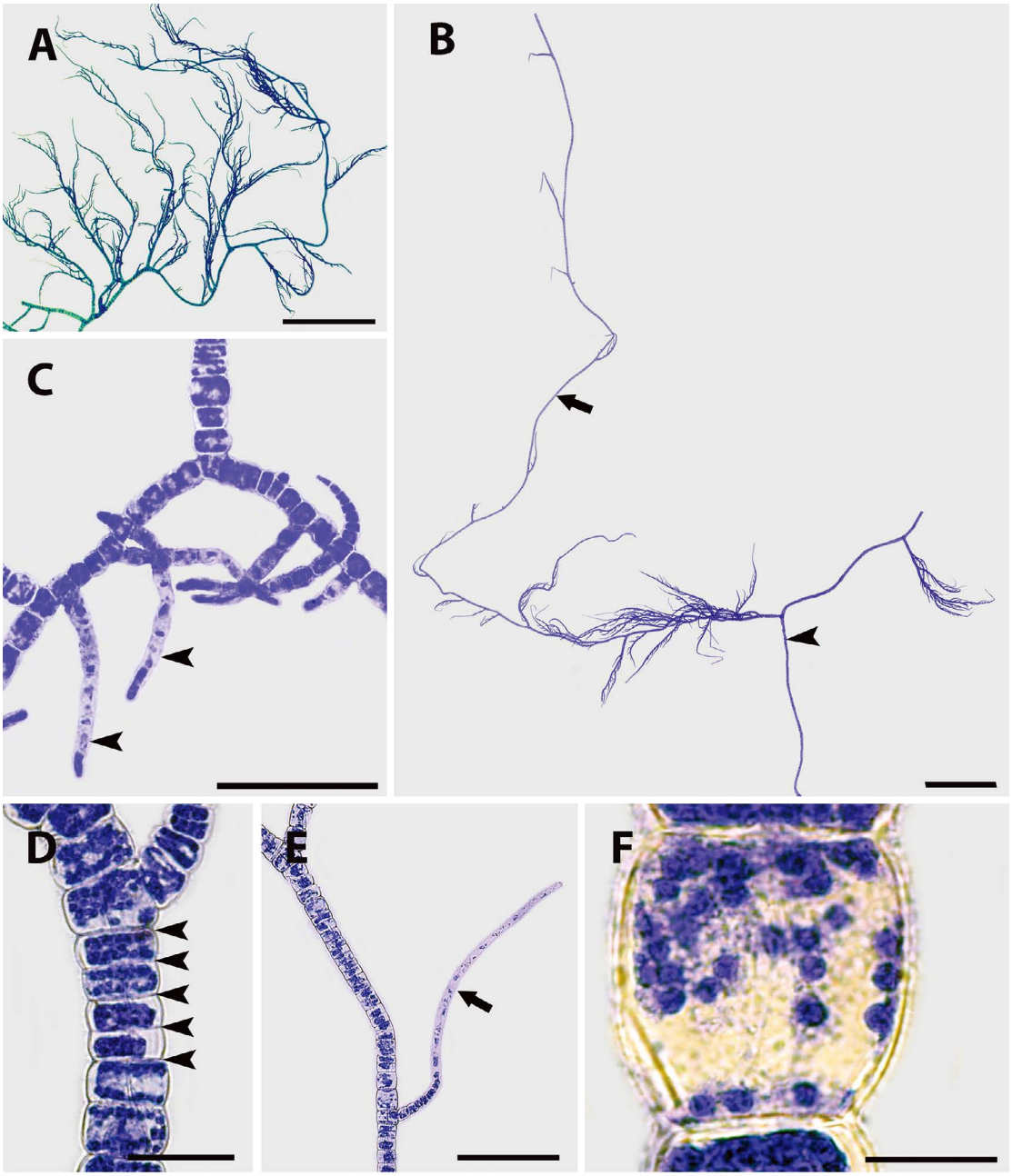

Fig. 3. *Hincksia sordida* CUK18158 (GenBank number: MT469950) from Korea. A. Thallus with irregularly branched laterals. B. Main filament (arrowhead) with long lateral (arrow). C. Rhizoids (arrowheads) occur on the main filament throughout the plant. D. Meristematic region (arrowheads) at the base of lateral branch. E. Pseudohair (arrow). F. Numerous discoid chloroplasts within a cell of main filament. Scale bars: A, B = 1.0 mm; C, E = 200 μm; D = 50 μm; F = 20 μm.

Our molecular analysis based on *rbc*L gene reveals that sample from Korea is placed in the same clade with *Petroderma maculiforme* from California, USA (EU579934, Bittner *et al.* 2008). Although there was no *rbc*L sequence from the type locality (Helgoland) in GenBank, Our *Petroderma* sample from Korea is identified as *Petroderma maculiforme* because *P. maculiforme* from California was recognized into identical species with type locality based on ITS1 sequences (Peters and Moe 2001). In this study, we report *Petroderma maculiforme* as a new record from Korea

and add this species to the list of Korean macroalgal flora based on morphological and molecular analyses.

***Hincksia sordida* (Harvey) P.C. Silva, 1987**
깃대긴털실말(신칭) **(Figs. 3, 4)**

Basionym: *Ectocarpus sordidus* Harvey 1859: 294
Homotypic synonym(s): *Ectocarpus sordidus* Harvey 1859; *Giffordia sordida* (Harvey) M.N. Clayton 1974.

Material examined. CUK18158 (=MBRB0107TC18158H1) & NIBROR0000001765 (National Institute of Biological Resources); Gampo, Gampo-eup, Gyeongju-si, Gyeongsangbuk-do, Korea (35°48′23.74″N, 129°30′22.48″E); July 09, 2017; T.O. Cho and S.Y. Jeong.
Morphological observations. Plants are epiphytic (on larger algae or seagrasses), free-floating, or rarely epilithic, forming more or less extensive, entangled, 5–30 cm long, with sparsely branching laterals (Fig. 3A). Main filaments are spirally or irregularly branched with many short and curved laterals, 31–52 μm wide (Fig. 3B). Rhizoids are occurring throughout the plant (Fig. 3C). Growth is from scattered meristematic regions in axes and main branches (Fig. 3D). Branchlets are tapered and terminated into pseudohairs (Fig. 3E), 8–15 μm wide. Vegetative cells have a few or inconspicuous physodes and numerous discoid plastids (Fig. 3F) with one(-2) pyrenoid. Plurilocular sporangia are rare, scattered or occasionally grouped, sessile (or rarely pedicellate), conical, 52–68 μm long and 22–38 μm wide. Unilocular sporangia are rare, on separate plants, scattered, sessile, ovoid, 39–44 μm long, and 24–32 μm wide.
Habitat. Epiphytic on larger algae (e.g. *Sagarssum* spp.) or seagrasses and confined to calm rock pools or to sheltered bays and inlets.
World distribution. Asia: China, Australia, and New Zealand (Guiry and Guiry 2020).
Phylogenetic analyses. The 1300-nucleotide portion of *rbc*L was aligned for *Hincksia sordida*. Phylogenetic analyses revealed that our sample of *Hincksia sordida* from Korea was nested within a clade of *Hincksia* (Fig. 4). *Hincksia hincksiae* was a sister species with *Hincksia sordida* and the gene sequence divergence between them was is 3.6%.
Remarks. Morphologically, our *Hincksia* samples are matched into the description of *Hincksia sordida*. *Hincksia sordida* is morphologically distinct from other reported *Hincksia* species from Korea. *Hincksia sordida* is mainly distinguished by having many short curved laterals and rhizoids scattered on the main axis and large branches (Womersley

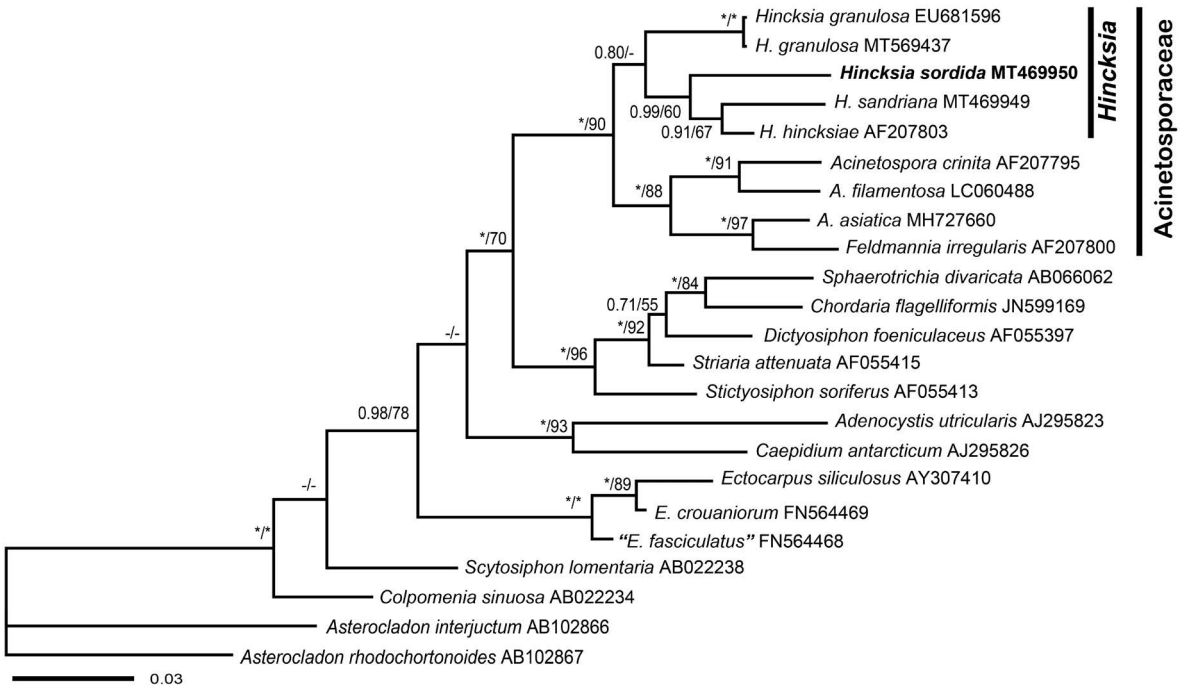

Fig. 4. Phylogenetic tree for the *Hincksia* and other species of Ectocarpales based on Bayesian and RAxML analysis with *rbc*L sequences. The value above branches = Bayesian posterior probabilities > 0.75, Maximum likelihood bootstrap values in % > 50. Values lower than BPP 0.75 or BS 50 are indicated by hyphens (-). Values of BPP 1.00 or BS 100 are indicated by asterisks (*).

1987). Molecular data based on *rbc*L gene revealed that *Hincksia sordida* from Korean is nested in the same clade of the genus *Hincksia* and distinguished from congeners, *H. granulosa*, *H. sandriana*, and *H. hincksiae*. In this study, we report *Hincksia sordida* as a new record from Korea and add this species to the list of Korean macroalgal flora based on morphological and molecular analyses.

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 (NIBR201501204 and NIBR 20161204) and by a grant from the research fund of Chosun University 2018. 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 (2019R1F1A1060346) and a grant from Marine Biotechnology Program (20170431) funded by Ministry of Oceans and Fisheries of Korean Government to Tae Oh Cho.

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