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A new record of brown algae, *Papenfussiella densa* from Dok-do, Korea

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Abstract: *Papenfussiella densa* was described as *Papenfussiella kuromo* f. *densa* from Japan by Inagaki in 1958. *P. densa* has been recognized as an endemic and independent species based on the molecular analyses of type material without detailed morphological observations. In this study, *Papenfussiella densa* is reported as a new record from Dok-do, South Korea, based on morphological and molecular analyses. *Papenfussiella densa* is mainly characterized as having narrow, branched, slimy, and tomentose thalli with branchlets, partially hollow in the medulla of the middle part. The molecular analyses of the chloroplast *rbc*L-*rbc*S DNA sequence of the *Papenfussiella densa* sample from Korea revealed that it matched that of *P. densa* from Japan and was nested in the clade of *Papenfussiella*. There was only a 0.02% gene sequence divergence between the Korean and Japanese samples. We report *P. densa* as a new record from Korea and add this species to the list of Korean macroalgal flora.

Keywords: morphology, Chordariaceae, *Papenfussiella densa*, chloroplast *rbc*L-*rbc*S DNA, Phaeophyceae

INTRODUCTION

The brown algal genus, *Papenfussiella*, was described by Kylin (Kylin 1940). It is characterized by having multiaxial, terete thalli without transition zones between their medullary and cortical tissues (Kylin 1940). They generally have long and short assimilatory filaments and form rhizoidal filaments from the base of assimilatory filament (Kylin 1940). Currently, eleven *Papenfussiella* species are reported worldwide (Kylin 1940; Levring 1941; Inagaki 1958; Womersley and Bailey 1987; Kawai *et al.* 2016). Among them, *P. extensa* Womersley & A. Bailey, *P. gracilis* Kylin, *P. laxa* Kylin, *P. lutea* Kylin, *P. moseleyi* Levring, and *P. tristanensis* Kylin, are distributed in Southern hemisphere, and *P. callitriche* (Rosenvinge) Kylin, *P. densa* H. Kawai & T. Hanyuda, *P. kuromo* (Yendo) Inagaki, *P. iemasae* H. Kawai, and *P. shikokuensis* H. Kawai, K. Miyoshi & T. Hanyuda in Northern hemisphere (Kawai *et al.* 2016). Until now, only *Papenfussiella kuromo* has been reported in Korea.

Papenfussiella densa was described as *Papenfussiella kuromo* f. *densa* by Inagaki in 1958. Kawai *et al.* (2016) reappraised as an independent species, *P. densa* after comparison of type materials based on molecular analyses (Kawai *et al.* 2016). However, Kawai *et al.* (2016) could not be examined the detailed morphological characters of *P. densa* because he did not have fresh materials from the field and it was difficult to examine the anatomy using the original voucher specimens collected by Inagaki. Although *Papenfussiella densa* is accepted as valid species, its detailed characters have not been described.

Dok-do is an island located 200 km east coast of the Korea Peninsula. The currents affecting Dok-do are known

as the high-temperature and high-salt Tsushima warm current, North Korean cold current and the Liman current (Yoon *et al.* 2007). Its alga flora study was begun in 1960 and its marine vegetation was similar to that of the south coast although it located in the East Sea geographically (Kang 1966).

We collected an unidentified brown alga from Dok-do, Ulleung-gun, Gyeongsangbuk-do, Korea. We observed the detailed morphology and analyzed molecular data based on the chloroplast *rbc*L-*rbc*S DNA gene for the phylogenetic relationships. In this study, we add this unidentified brown alga as *Papenfussiella densa* to the Korean marine algal inventory.

MATERIALS AND METHODS

1. Morphology

Samples of unidentified brown alga were collected from Dok-do in the East Sea of Korea. They were sorted into voucher herbarium specimens, silica gel samples, and formalin samples. Formalin samples were preserved in 4−5% formalin/seawater. Photomicrographs were 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 chloroplast *rbc*L-*rbc*S DNA. The *rbc*L-*rbc*S DNA was amplified using a pair of RS1 + RS2 primer was used for each reaction (RS1: 50GCC AAA TGC ACC AACTTC TT 30, RS2: 50AGA CCC CAT AAT TCC C 30) (Yoon and Boo 1999) with HelixAmp Ready-2x-Go Series (NanoHelix Co., Ltd., Daejeon, Korea). All PCR amplifications were carried out with a Veriti 96 well Thermal cycler (Applied Biosystem). PCR products were purified using a PCRquick-spin™ PCR product purification kit (iNtRON Biotechnology, Inc, Seongnam, Korea). All *rbc*L-*rbc*S DNA sequence data were compiled by the present study and obtained from GenBank and aligned with ClustalW (Thompson *et al.* 1994). New *rbc*L-*rbc*S DNA sequences obtained from *Papenfussiella densa* have been deposited in EMBL/GenBank under the accession numbers MT000921 (CUK9549) and from *Papenfussiella kuromo* under the Accession numbers MT000919and MT000920 (CUK9587 and CUK18784). *Proselachista taeniaeformis* were selected as outgroup.

Phylogenetic analyses were conducted using raxmlGUI1.5b2 (Silvestro and Michalak 2012). Maximum likelihood analyses were conducted using the GTR + G + I model, with 1000 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

***Papenfussiella densa* H. Kawai & T. Hanyuda**
독도연두털말(신칭) **(Figs. 1, 2)**

Heterotypic Synonyms.
Papenfussiella kuromo f. *densa* Inagaki, 1958
Papenfussiella kuromo f. *gracilis* Inagaki, 1958
Holotype. SAP 058308.
Material examined. CUK9549 (= MBRB0098TC9549 H1-H3) & NIBROR0000001762 (National Institute of Biological Resources (NIBR) Dongdo, Dok-do, Ulleung-gun, Gyeongsangbuk-do, Korea (37°14′24.37″N, 131°52′5″E), 95 April 22, 2013, T. O. Cho, at 1m depth by hand.
Habitat. epilithic or epiphytic in shallow and calm waters.
Morphological observation. Plants are olive or greenish-brown in color, erect, cylindrical, solitary to caespitose, highly branched, slimy, and tomentose. The main branches are 10−30 cm long, 614 ± 174 μm in diameter (Fig. 1A), and slightly narrower towards the apices (Fig. 1B). Branches are sparse or abundant with branchlets, irregularly alternately or laterally divided in 1−4 order (Fig. 1A−E). Branches are composed of medullary filaments and cortical pigmented assimilatory hairs (Fig. 1G−I). Each apical filament is extended to the assimilating hair at the apex of the young branch (Fig. 1A), and the assimilating hairs on the old branch gradually become progressively shorter (Fig. 1E). Medullar is composed of medullary filaments, hollow

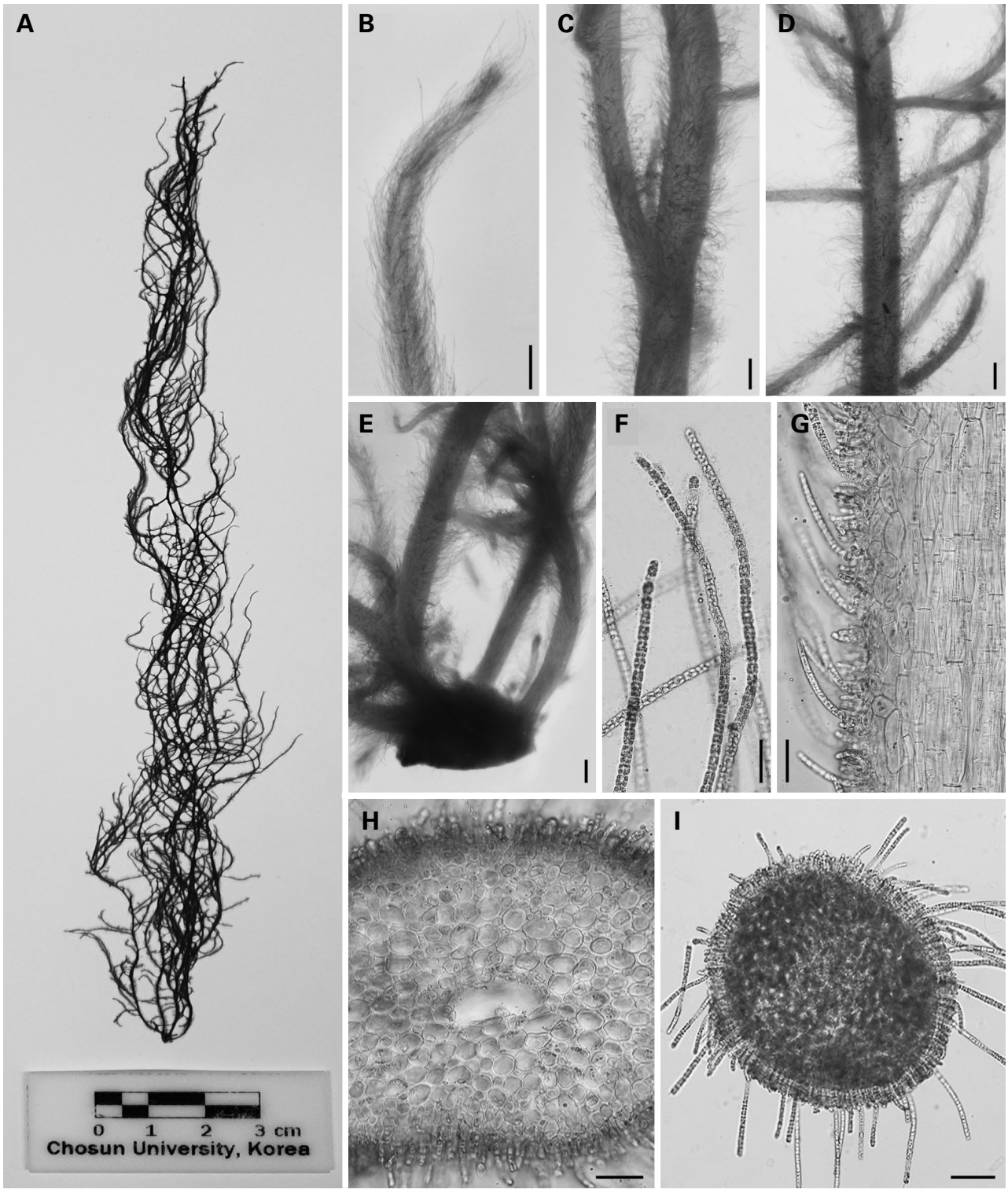

Fig. 1. *Papenfussiella densa* (CUK9549) from Dok-do, Ulleung-gun, Gyeongsangbuk-do, Korea. A. Gross morphology of representative specimens. B. Apex with assimilatory filaments. C & D. Middle of the thallus showing branches and branchlets. E. Basal part of the plant showing thalli from holdfast. F. Long assimilatory filaments. G. Longitudinal section view of the thallus. H. Cross-sectional view of the middle part of the thallus showing partial hollow in the medulla. I. Cross-sectional view of the basal part of the thallus showing long and short assimilatory filaments. Scale bars: A = 3 cm; B = 300 mm; C–E = 500 μm; F–H = 50 μm; I = 100 μm.

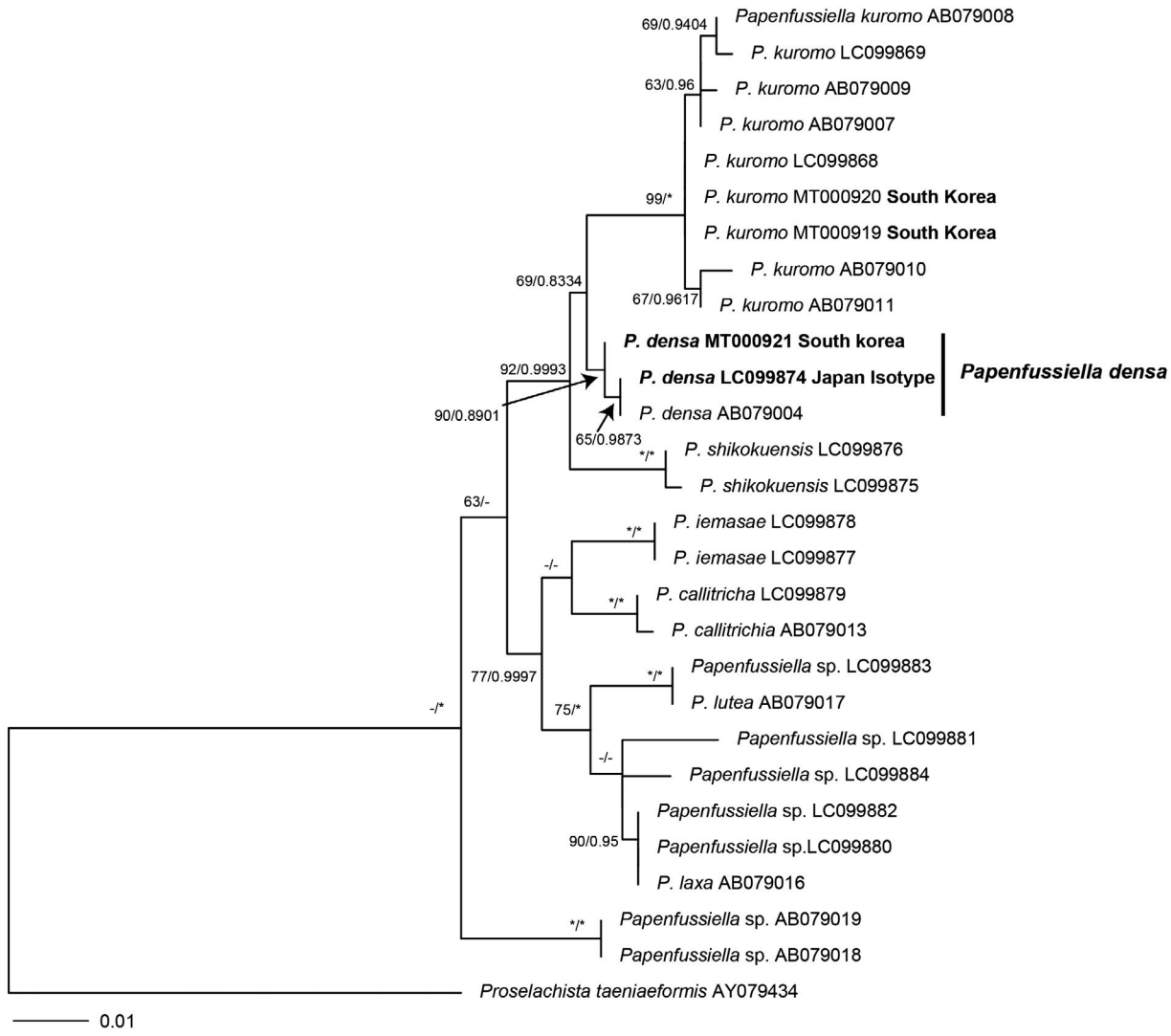

Fig. 2. Phylogenetic tree of *Papenfussiella* species based on ML and Bayesian analysis of *rbc*L-*rbc*S gene sequences. Value above the branches are maximum-likelihood bootstrap (BS) values with >50% Bayesian posterior probabilities (BPP) >0.75. Values lower than BS 50 or BPP 0.75 are indicated by hyphens (–). Values of BS 100 or BPP 1.00 are indicated by asterisks (*).

in the middle part of the thallus (Fig. 1H), and not hollow in the basal part (Fig. 1I). The medullary filaments are multiaxial, composed of a bundle of many parallel longitudinal cell lines (Fig. 1G), tightly compact pseudo-parenchymatous (Fig. 1H). The subcortical layer is absent between the medullary layer and cortical ones (Fig. 1H). There are two different forms of assimilating filament: the long ones are not imbedded in gelatinous substance, elongated like hairs, about 112±95 mm long, somewhat thicker in the middle portion, but in age often filling off (Fig. 1F, G, I); the short one imbedded in gelatinous substance, considerably shorter than former, composed of 3–6 or more cells, about 1.7± 1.3 mm long, club-shaped (Fig. 1G–I).

World distribution. Japan and Korea.

Identifier. Boo Yeon Won.

Phylogenetic analyses. The 466-nucleotide of the *rbc*L-*rbc*S gene was aligned for *P. densa*. Phylogenetic analyses revealed that our *Papenfussiella densa* sample from Dok-do, Korea was placed within a clade *P. densa* in the *rbc*L-*rbc*S gene (Fig. 2). There was only a 0.02% gene sequence divergence between Genbank and our collection of *P. densa*. *Papenfussiella densa* was a sister group of *Papenfussiella kuromo*. Also, *P. densa* differs from *P. callitricha* by 2.8–3.2%, from *P. iemasae* by 3.0–3.4%, from *P. kuromo* by 1.5–2.4%,

from *P. laxa* by 2.9–3.2%, from *P. lutea* by 3.3–3.7% and from *P. shikokuensis* by 1.9–2.2% gene sequence divergence respectively.

Remarks. *Papenfussiella densa* was a new species based on *Papenfussiella kuromo* f. *densa* (Inagaki 1958; Kawai *et al.* 2016). It has been reported as endemic species only from Japan (Kawai *et al.* 2016). *Papenfussiella densa* sample from Korea matched with *P. densa* from Japan and was nested in the clade of *Papenfussiella*. Although it was not recognized in the original description, partially hollow in the medulla of middle thallus may be a diagnostic character for this species. In this study, *P. densa* is reported as a new record from Korea and add this species in the list of Korean macroalgal flora.

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