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A new record of *Feldmannia chitonicola* from Korea based on laboratory culture and molecular data

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Abstract: *Feldmannia chitonicola* is reported as a new record from Korea based on morphological studies in laboratory-cultured materials and molecular analyses. *F. chitonicola* is mainly characterized by a small size (1–2 mm), erect filaments mostly unbranched, plurilocular sporangia produced on both prostrate and laterally on the basal part of erect filaments, and 2 types (lanceolate and ovate) of sporangia. In our cultures, sporangia production was slower at 10°C than in 16°C and 20°C. Our molecular analyses of *rbc*L and *cox*1 genes supported its independence from other congeners reported for Korea. This is the first report of *F. chitonicola* for western Pacific.

Keywords: *Feldmannia chitonicola*, *cox*1, Ectocarpales, Phaeophyceae, *rbc*L

INTRODUCTION

The filamentous brown algal genus, *Feldmannia*, was described by G. Hamel (1939) based on *F. lebelii*. It is characterized by having basal meristematic zones in axial filaments, branches below meristematic zones and plurilocular sporangia developing from below meristems (Kim and Lee 1994; Abbott and Huisman 2004; Kim 2010). Currently, 28 species of *Feldmannia* have been known around the world (Guiry and Guiry 2019). Of them, six species have been reported in Korea (Kim 2010): *F. lebelii* (Areschoug ex Crouan and Crouan) Hamel, *F. globifera* (Montagne) Hamel 1939, *F. irregularis* (Kützing) Hamel, *F. rhizoidea* Hollenberg and Abbott, *F. indica* (Sonder) Womersley and Bailey, and *F. mitchelliae* (Harvey) Kim.

Feldmannia chitonicola (De A. Saunders) Levring was originally described as *Ectocarpus chitonicolus* De A. Saunders from California based on a small specimen growing on the back of a chiton (Saunders 1898). It is characterized by having small size (1–2 mm), epizoic habitat, mostly unbranched erect filaments, plurilocular sporangia produced laterally on basal part of erect filaments and occasionally on prostrate filaments, sessile or short pedicellate plurilocular sporangia, and 2 types (big lanceolate and small ovate) of plurilocular sporangia (Saunders 1898; Setchell and Gardner 1925; Abbott and Hollenberg 1976). *Feldmannia chitonicola* has been reported only from eastern Pacific, in California (Abbott and Hollenberg 1976), Chile (Ramirez and Santelices 1991) and Peru (Acleto 1980).

We collected unidentified tiny filamentous brown algae from west and east coasts of Korea and cultured them. We observed their detailed morphology and developmental pattern in laboratory cultures. We also analysed molecular data based on *rbc*L and *cox*1 genes for their phylogenetic relationships with others in their respective genera and a few selected species within the Ectocarpales. We identify them as *Feldmannia chitonicola* and add it to the Korean marine algal inventory, representing the first report of this species in western Pacific.

MATERIALS AND METHODS

1. Culture and Morphological studies

Two unialgal isolates of filamentous brown algae were established from samples collected from west and east coasts of Korea in November, 2017 and February, 2019. Young germlines were inoculated in Petri dishes (60 mm diameter × 15 mm depth) containing PES medium (Provasoli 1968). Cultures were kept at 10°C, 16°C and 20°C under 20–30 μmol photons m^{-2} s^{-1} white fluorescent light and 14 : 10-h light/dark photoperiod. Medium was renewed weekly.

After culture process, thalli were sorted into voucher herbarium specimens, silica gel samples, and formalin samples (4–5% formalin/seawater). Fresh cultured materials were used for morphological analysis. Photomicrographs were taken using an Olympus BX51TRF microscope (Olympus, Tokyo, Japan) and an Olympus DP71 camera. At least 25 individuals were selected for the determination of quantitative characters and their means and standard deviations were calculated. For developmental study, a Leica DMi8 inverted microscope (Leica, Wetzlar, Germany) equipped with a Leica DFC450C camera was used. The percentage of fertile plants and different morphologies of sporangia at different temperatures were calculated based on at least 50 plants or sporangia, respectively. Permanent slide specimens were mounted by 70% karo syrup and the examined representative specimens 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 *rbc*L and *cox*1. Polymerase chain reaction (PCR) was carried out with a Veriti 96-well Termal cycler (Applied Biosystem). The *rbc*L gene was amplified using the primer combinations NDrbcL2-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 was used for *cox*1 (Saunders 2005; Lane *et al.* 2007). PCR products were purified using a PCRquick-spinTM PCR product purification kit (iNtRON Biotechnology, Inc, Seongnam, Korea). Determination of the nucleotide sequence or sequencing was performed by Macrogen Inc., Seoul, South Korea. DNA sequence data (*rbc*L and *cox*1) were compiled by the present study and obtained from GenBank and aligned with ClustalW (Thompson *et al.* 1994). New sequences obtained from *Feldmannia chitonicola* have been deposited in EMBL/GenBank under the accession numbers MN092346 (CUK18833) and MN092347 (CUK19719) for *rbc*L, and MN092344 (CUK 18833) and MN092345 (CUK19719) for *cox*1.

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. A 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

***Feldmannia chitonicola* (De A. Saunders) Levring, 1960**
전복솜털 (신칭) (Figs. 1–3)

Type locality: Pacific Grove, California, USA
Habitat: Epiphytic on *Sargassum thunbergii*.
Material examined: NIBROR0000001611 & CUK18833 (= MBRB0100TC18833), Chaesokgang, Byeonsan-myeon, Buan-gun, Jeollabuk-do, Korea (35°37′27.04″N, 126°27′56.88″E), November 17, 2017, T. O. Cho and B. Y. Won, at 1 m depth by hand; CUK19719 (=MBRB 0100TC19719), Guryong Pohang, Guryong Pohang-eup, Pohang-si, Gyeongsangbuk-do, Korea (35°58′48.53″N, 129°34′17.33″E), February 02, 2019, T. O. Cho and B. Y. Won, at 1 m depth by hand.
World distribution: Korea, USA, Chile, Peru (Guiry and Guiry 2019).

Culture studies

Culture strains produced plurilocular sporangia after 10–12 days in culture. Spores were settled without showing any signs of sexuality (Fig. 1A). They developed a "germ tube" (Fig. 1B) which septated by cross-wall and later became rhizoid (Fig. 1C). The first erect filament emerged on the opposite side of the rhizoid, forming a heterotrichous thallus after 7 days in culture (Fig. 1D). Additional erect

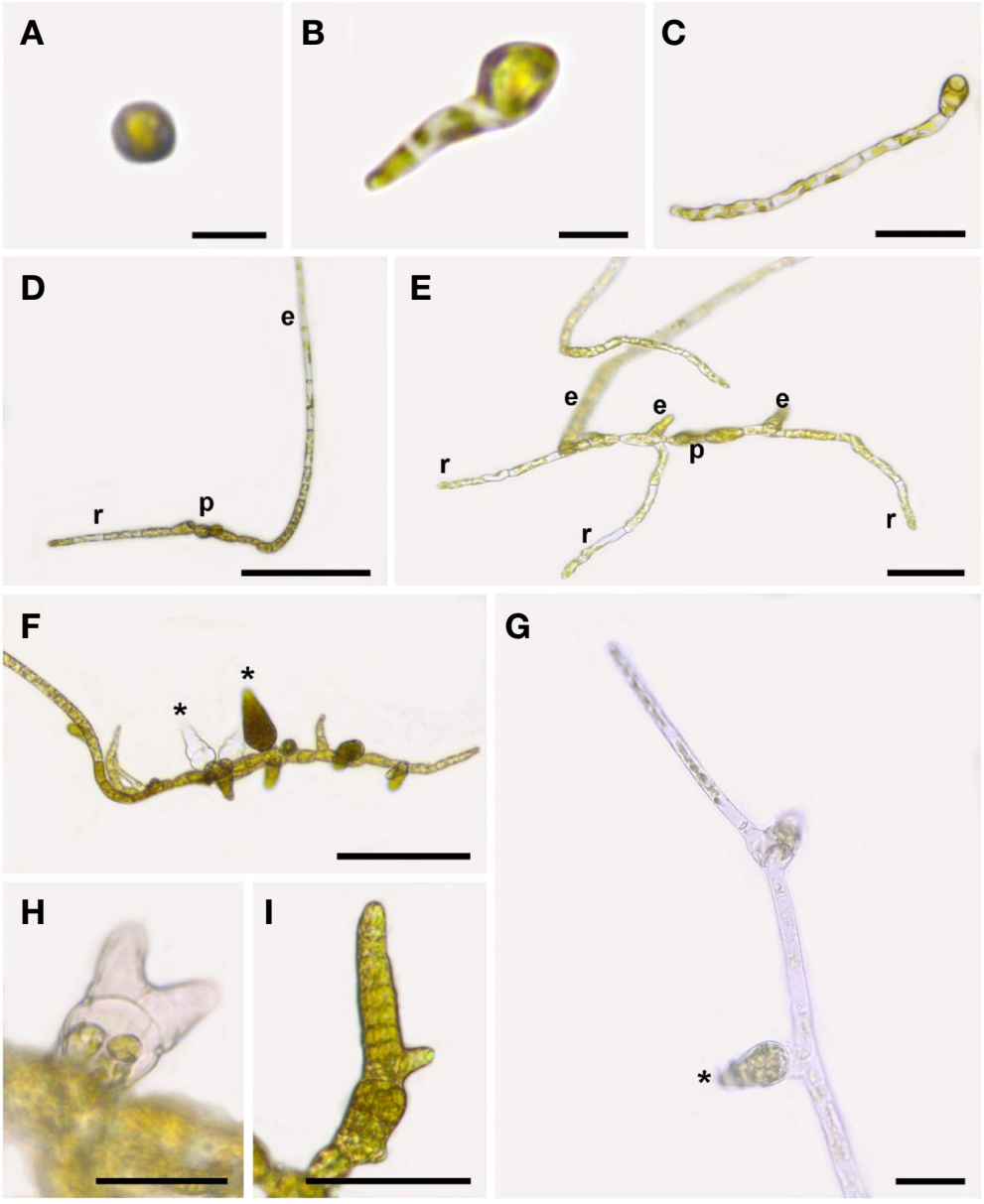

Fig. 1. Development of *Feldmannia chitonicola* in laboratory culture. A. Settled spore; B, C. Germination of zoospore showing the development of rhizoid; D. Heterotrichous thallus with rhizoid (r), prostrate part (p), and erect filament (e) after 7 days in culture; E. Additional erect filaments (e) and rhizoids (r) from prostrate part (p); F. Plurilocular sporangia (asterisks) on prostrate filament after 10 days in culture; G. Secondary meristem on end portion of erect filament with small plurilocular sporangium (asterisk); H. Branched plurilocular sporangium; I. Plurilocular sporangium with short outgrowths. Scale bars: A, B = 10 μm; C, G, H, I = 50 μm; D, F = 200 μm; E = 100 μm.

and rhizoidal filaments arose from the prostrate part during the next days (Fig. 1E). The plants produced plurilocular sporangia in the prostrate part after 10 days in culture (Fig. 1F), and then below the meristem near the base of the erect filament after 12 days in culture. The upper filaments did not show branches in older thalli, but they developed long pseudohairs and some of them produced secondary meristems on terminal portion with small plurilocular sporangia and rhizoids (Fig. 1G).

The developmental pattern of thalli was not affected according to different temperatures but the production of plurilocular sporangia was slower at 10°C than in 16°C and

Table 1. Effect of temperature on percentages of fertile plants and different morphologies of plurilocular sporangia for two strains of *Feldmannia chitonicola* from Korea. Percentages were calculated based on at least 50 plants or plurilocular sporangia, respectively.

Strain	Temperature (°C)	Fertile plants (%)	Plurilocular sporangia shape (%)		
			Ovate	Lanceolate	Branched or with outgrowths
TC18833 (western coast)	20	41.9	16.1	72.6	11.3
	16	49.1	90.7	9.3	0
	10	PD	NS	NS	NS
TC19719 (eastern coast)	20	81.0	69.3	17.3	13.3
	16	73.2	92.9	1.0	6.1
	10	42.9	100.0	0.0	0

PD: Poor development; NS: No sporangia

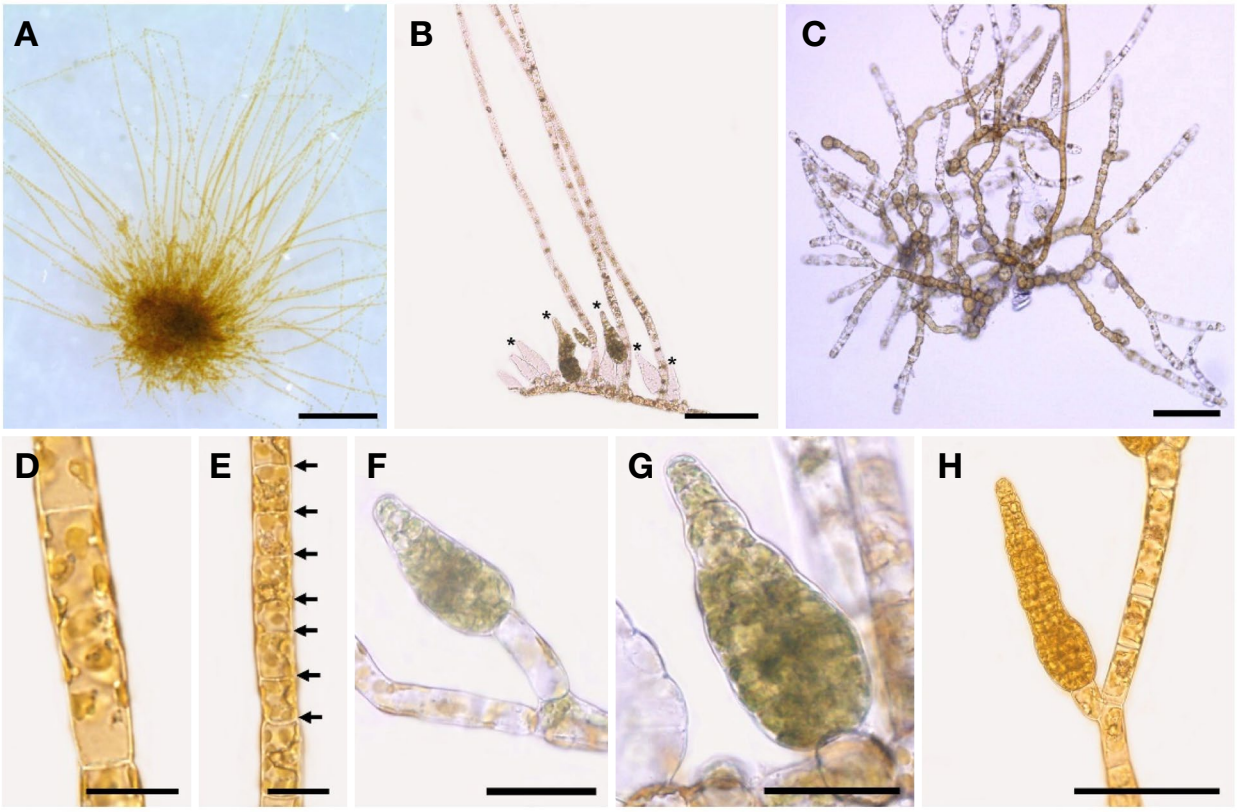

Fig. 2. Development of *Feldmannia chitonicola*. A. Cultured thallus showing long unbranched filaments; B. Thallus with plurilocular sporangia (asterisks) on both prostrate and erect filaments; C. Thallus of numerous prostrate branched filaments; D. Cells of erect filament showing discoid chloroplasts; E. Meristematic zones (arrows) composed of small cells on erect filament; F. Pedicellate plurilocular sporangium on prostrate filament; G. Sessile plurilocular sporangium on prostrate filament; H. Elongated plurilocular sporangia on erect filament. Scale bars: A = 500 μm; B = 250 μm; C = 200 μm; D, E = 25 μm; F, G = 50 μm; H = 100 μm.

20°C (Table 1). Few irregular morphologies of plurilocular sporangia were observed at 20°C and 16°C (Fig. 1H, I). Direct-monophasic life history by plurilocular sporangia was repeated during four generations in our culture. Unilocular sporangia were not found in all the conditions tested.

Morphological observations

Plants are forming small tufts, 1.2–2.1 mm tall (Fig. 2A), with numerous uniseriate prostrate filaments, and irregularly branched. (Fig. 2C). Uniseriate erect filaments are mostly unbranched or sometimes sparingly branched near

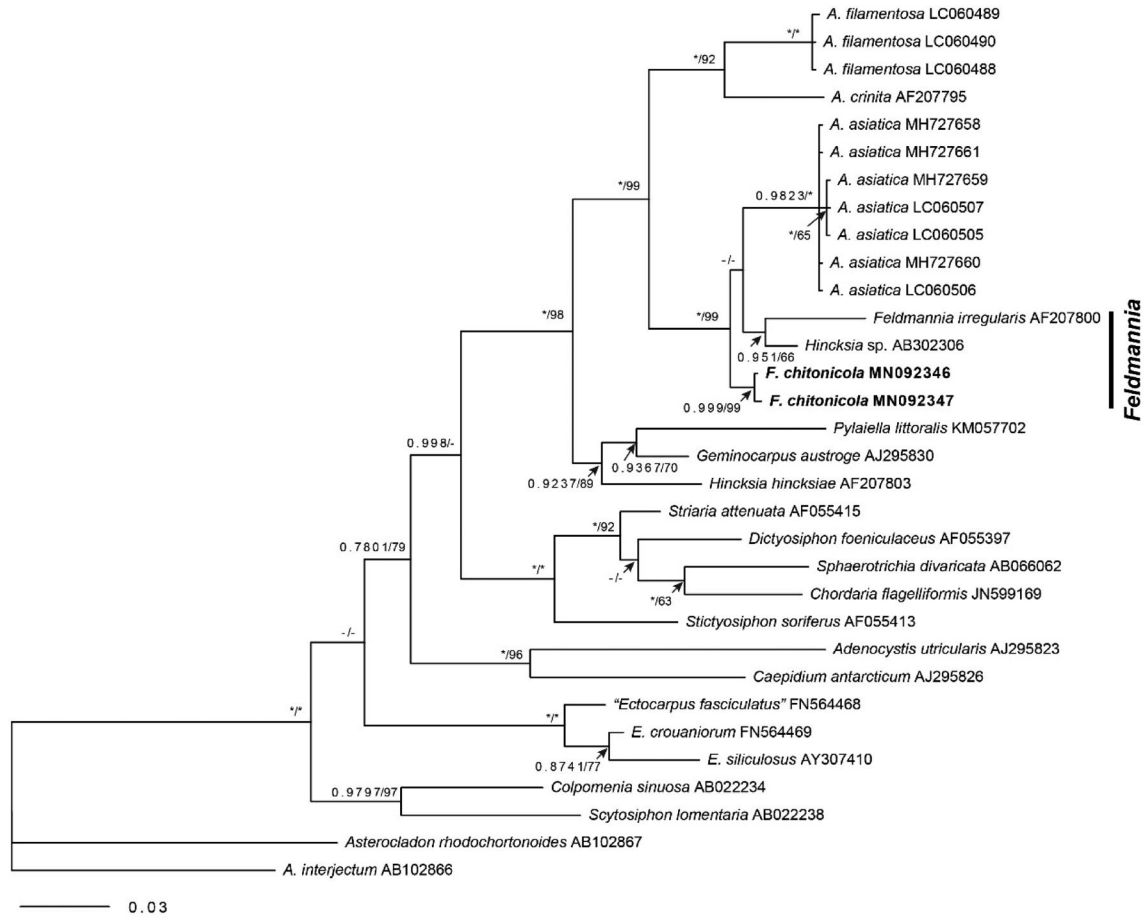

Fig. 3. Phylogenetic tree of *Feldmannia* species based on Bayesian and ML analysis with *rbc*L sequences. Value above branches = Bayesian posterior probabilities > 0.75/Maximum likelihood bootstrap values in % > 50. Values lower than BPP 0.75 or BS 50 are indicated by hyphens (–). Values of BPP 1.00 or BS 100 are indicated by asterisks (*).

base, and slightly attenuated upward or forming long pseudohairs (Fig. 2B). Erect filaments are composed of rectangular cells with 19.3 ± 3.1 μm in width and with numerous discoid chloroplasts and not constricted at the septum (Fig. 2D). Meristematic zones are composed of small-sized cells on erect filaments (Fig. 2E). There are various in the size of cells according to the position: 3.3 ± 0.8 longer than broad in apical and medium parts, 2.1 ± 0.5 longer than broad in basal part, and 1.1 ± 0.3 longer than broad in meristematic zone. Plurilocular sporangia are produced as sessile and on one (or two)-celled pedicels both on erect and prostrate filaments. Plurilocular sporangia are developed below meristematic zone of erect filament (Fig. 2B). There are 2 types of plurilocular sporangia: large lanceolate (Fig. 2H) and small ovate shapes (Fig. 2F, G). Plurilocular sporangia are (42)84–192 μm × 26–58 μm in size. Unilocular sporangia not observed.

Phylogenetic analysis

The 1326-nucleotide portion of *rbc*L and 613-nucleotide portion of *cox*1 was aligned for *Feldmannia chitonicola*. The phylogenetic trees were obtained from the alignment of the *rbc*L sequences newly generated and downloaded from GenBank. *Asterocladon rhodochortonoides* and *A. interjectum* tree were selected as outgroups. Our samples were nested within a clade of *Feldmannia* (Fig. 3). Gene sequence divergence differed from *F. irregularis* by 2.3–2.4%, while from *Acinetospora filamentosa* and *A. crinita* by 3.9–4.0%.

DISCUSSION

Our brown algal collections from Korea matched with

the diagnosis of *Feldmannia chitonicola* described from the type locality in thallus size, the lanceolate and ovate shapes of plurilocular sporangia, and the development of plurilocular sporangia on both prostrate and basal part of erect filaments. These samples are nested within a clade of *Feldmannia* in phylogenetic trees based on *rbc*L and *cox*1 genes (Fig. 3). We add *F. chitonicola* in the list of Korean macroalgal flora. This is also the first record of *F. chitonicola* in western Pacific.

Although *Feldmannia chitonicola* closely resembles to *F. irregularis*, in some morphological features as the size of thalli, unbranched erect filament, and terminal hyaline pseudo-hairs, *F. chitonicola* always develops plurilocular sporangia first on the prostrate filaments while *F. irregularis* only on the erect filaments in both wild and culture materials (Kim and Lee 1994). *Feldmannia chitonicola* may be distinguished from *F. irregularis* by the presence of reproductive organs in prostrate filaments, sporangia shape, and molecular data.

The secondary meristems are the upper part regions producing plurilocular sporangia and rhizoids in erect filaments. The presence of a secondary meristem has been known only from culture materials of *F. globifera* and *F. irregularis*. The upper filaments of *Feldmannia chitonicola* in older thalli developed the secondary meristems with small plurilocular sporangia and rhizoids. These secondary meristems may be attributed to the contact of the filaments with the culture dish, which seems to stimulate the development of new meristems or rhizoids (Clayton 1974; Kim and Lee 1994).

The temperature has been reported as one of important culture conditions for the production of plurilocular sporangia in filamentous brown algae (Clayton 1974). In this study, the production of plurilocular sporangia was increased under higher temperatures (Table 1) and this matched with the Clayton's result. Also, our result shows that temperature can determine the morphological types of plurilocular sporangia: ovate plurilocular sporangia in lower temperatures and elongated plurilocular sporangia in higher temperatures (Table 1). These effects of different temperatures on the morphologies of plurilocular sporangia have not been reported in *Feldmannia* species.

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REFERENCES

Abbott IA and GJ Hollenberg. 1976. Marine Algae of California. Stanford University Press, Stanford.

Abbott IA and JM Huisman. 2004. Marine Green and Brown Algae of the Hawaiian Islands. Bishop Museum Press, Honolulu.

Acleto C. 1980. Notas sobre las algas marinas del Perú I. Nuevos registros. Publ. Mus. Hist. Natur. Serie Botánica 30:1–33.

Clayton MN. 1974. Studies on the development, life history and taxonomy of Ectocarpales (Phaeophyta) in southern Australia. Aust. J. Bot. 22:743–813.

Guiry MD and GM Guiry. 2019. AlgaeBase. World-wide electronic publication, National University of Ireland, Galway. http://www.algaebase.org; searched on 18 June 2019.

Hamel G. 1939. Sur la classification des Ectocarpales. Bot. Notiser 1939:65–70.

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 and IK Lee. 1994. Morphotaxonomic studies on the Korean Ectocarpaceae (Phaeophyta) III. Genus *Feldmannia* Hammel, specially referred to morphogenesis and phylogenetic relationships among related genera. Algae 9:153–168.

Kim HS. 2010. Ectocarpaceae, Acinetopsoraceae, Chordariaceae. pp. 5–153. 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 *rbc*L, partial *rbc*S, and partial LSU nrDNA. Phycologia 38:496–502.

Lane CE, SC Lindstrom and GW Saunders. 2007. A molecular

assessment of north east Pacific *Alaria* species (Laminariales, Phaeophyceae) with reference to the utility of DNA barcoding. Mol. Phylogenet. Evol. 44:634–648.

Provasoli L. 1968. Media and prospects for the cultivation of marine algae. pp. 63–75. In Cultures and Collections of Algae (Watanabe A and A Hattori eds.). Proceedings of the U.S.–Japan Conference, Hakone.

Ramirez ME and B Santelices. 1991. Catálogo de las algas marinas bentónicas de la costa temperada del Pacífico de Sudamérica. Monografías Biológicas 5:1–437.

Ronquist F and JP Huelsenbeck. 2003. MrBayes 3: Bayesian phylogenetic inference under mixed models. Bioinformatics 19:1572–1574.

Saunders DA. 1898. Phycological memoirs. Proceedings of the California Academy of Sciences, Series 3. Botany 1:147–168.

Saunders GW. 2005. Applying DNA barcoding to red macroalgae: a preliminary appraisal holds promise for future applications. Philos. Trans. R. Soc. B-Biol. Sci. 360:1879–1888.

Setchell WA and NL Gardner. 1925. The marine algae of the Pacific coast of North America. Part III. Melanophyceae. Univ. Calif. Publ. Bot. 8:383–898.

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.

