

New Records of Two unknown Micro-filamentous Endophytic Green Algae in Korea: *Phaeophila dendroides* and *Dilabifilum arthropyreniae*

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Abstract

Morphological and molecular information about two microfilamentous endophytic algae of the *Grateloupia lanceolata* or *Bryopsis* sp. from Korea is given. Of two endophytes, *Phaeophila dendroides* is endophytic in *Grateloupia lanceolata*. It is green in color and composed of uniseriate branched filaments with long setae. Each cell had several pyrenoids. Undulate or twisted *Phaeophila*-type hair developed from vegetative cells. *Dilabifilum arthropyreniae* is endophytic in *Bryopsis* sp. The frond of this species consists of frequently irregular, branching uniseriate filaments. Each cell had a single pyrenoid without hairs. Sporangia were not observed. The ends of the filaments were curved. In the phylogenetic tree, based on *tufA* and ITS sequences, these two species are nested in the same clade as *Phaeophila dendroides* and *Dilabifilum arthropyreniae*, respectively. In this study, these two species are newly recorded in the Korean marine algal flora, based on the morphological and molecular data.

Key words : New record, Korea, *Phaeophila dendroides*, *Dilabifilum arthropyreniae*, Endophytic, *tufA*, ITS, Molecular analysis

I. Introduction

Microfilamentous green algae grow on a variety of solid substrata. They also occur as epiphytes or endophytes of larger algae and seagrasses (Gunnarsson & Nielsen, 2016). A little more than 100 species of microfilamentous ulvophycean taxa are reported in AlgaeBase (Guiry & Guiry, 2016),

but the true number may be 200 or more species. This difference in number occurs because these algae are difficult to find and identify due to similarities in their appearances and a lack of information.

In Korea, only several microfilamentous endophytic green algae have been reported (Lee et

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※ This work was supported by a grant from National Institute of Biology Resources (NIBR), funded by the Ministry of Environment (MOE) of the Republic of Korea (NIBR201501204), and by a grant from the Marine Biotechnology Program funded by the Ministry of Oceans and Fisheries of the Korean Government.

al., 2013; Kim et al., 2014; Ogandaga et al., 2016). The presence of endophytic the *Ulvella* species was recorded in *Chondrus ocellatus* fronds in Korea by Lee et al. (2013), although epiphytic *U. viridis* was first reported on the fronds of *Griffithsia japonica* (Lee et al., 1998, Lee & Kang, 2002). Later, Kim et al. (2014) reported three endophytic filamentous algae (*Ulvella leptochaete*, *Blastophysa rhizopus*, and *Bolbocoleon piliferum*). Recently, Korean green endophyte *Ulvella ramosa* and Korean brown endophyte *Mikrosyphar zosterae* were added by Choi et al. (2015) and Ogandaga et al. (2016), respectively.

However, until now, we have very little information about Korean endophytes. We now have the opportunity to isolate endophytic algae from *Grateloupia lanceolata* and *Bryopsis* sp., which have been collected from the shore of Jeju Island and cultured in the laboratory. These species are newly recorded, and are based on morphological and molecular data in the present study.

II. Materials and Methods

The two host species, *Grateloupia lanceolata* and *Bryopsis* sp., were collected from Gujwa, Jeju Island, on July, 2015. Samples were stored in a cool box for transport to the laboratory, and then unialgal cultures were prepared by incubating specimens containing the target species in sections of host material. These were incubated in culture dishes with 150 mL of pasteurized seawater containing PES medium in 20°C, 100 $\mu\text{molm}^{-2}\text{s}^{-1}$, 33 psu, and 16:8h LD cycle.

Taxonomic data were obtained from unialgal culture specimens. Measurements are given as

length and diameter. Photographs were taken with a CCD camera (MicroPublisher 5.0, Qimaging, Canada) and a digital camera (TG-4, Olympus, Japan) attached to a microscope (DMLB, Leica, Germany). All specimens examined in this study are now deposited in the herbariums of the Department of Marine Biotechnology, Kunsan National University, Kunsan and of the National Institute of Biological Resources, Incheon, Korea.

Genomic DNA was extracted (Qiagen DNeasy Plant Mini Kit), and the plastid-encoded elongation factor *tufA* (Fama et al., 2002) and ITS (White et al., 1990) were amplified using published primers. Sequences were determined using an ABI 3130xl Genetic Analyzer (Applied Biosystems, USA), and were assembled in DNASIS[®]Max 3.0 (MiraiBio, USA). Phylogenetic analyses were performed using the maximum likelihood estimation method. Bootstrap values were calculated with 1,000 replications. The *tufA* and ITS sequences of other species were obtained from GenBank. *Ulothrix zonata* and *Desmochloris halophila* were used as outgroups.

III. Results and Discussion

***Phaeophila dendroides* (P. Crouan & H. Crouan) Batters: 1902: 13.**

Lectotype: CO (Brodie et al. 2007: 74).

Type locality: Rade de Brest, Finistère, France.

Korean name: Ggo-in-teol-sog-sal-i-mal nom. nov. (신칭: 꼬인털속살이말).

Specimens examined: NIBRCL0000112299 (Gujwa, Jeju: 13.v.2015), KSNU000010001-000010003 (Gujwa, Jeju: 13.v.2015).

Habitat: Epi/endophytic on other algae.

Morphology: Plants are endophytic in *Grateloupia lanceolata*. They are green color and composed

of uniseriate branched filaments with long setae. These plants formed cylindrical cells of the filaments, measured 5–19 µm in width, and were 8–10 times as long. Each cell had several pyrenoids. Undulate or twisted *Phaeophila*-type hair developed from vegetative cells ([Fig. 1]).

***Dilabifilum arthopyreniae* (Vischer & Klement)
Tschermak-Woess 1971: 452, 453**

Type: ?

Type locality: Macao.

Korean name: Sog-sal-i-ma-di-mal nom. nov. (신칭: 속살이마디말).

Specimens examined: NIBRCL0000112302 (Gujwa, Jeju: 13.v.2015), KSNU000010004-000010005 (Gujwa, Jeju: 13.v.2015).

Habitat: Epi/endophytic on other algae.

Morphology: Plants are endophytic in *Bryopsis* sp. The frond of this species consists of frequently irregular branching uniseriate filaments. The central cells were rounded (4–9 µm) and branched filaments were cylindrical cells (1.5–5 µm in width, and 3–5 times as long). Each cell had a single pyrenoid without hairs. Sporangia were not observed. The end of the filaments were curved ([Fig. 2]).

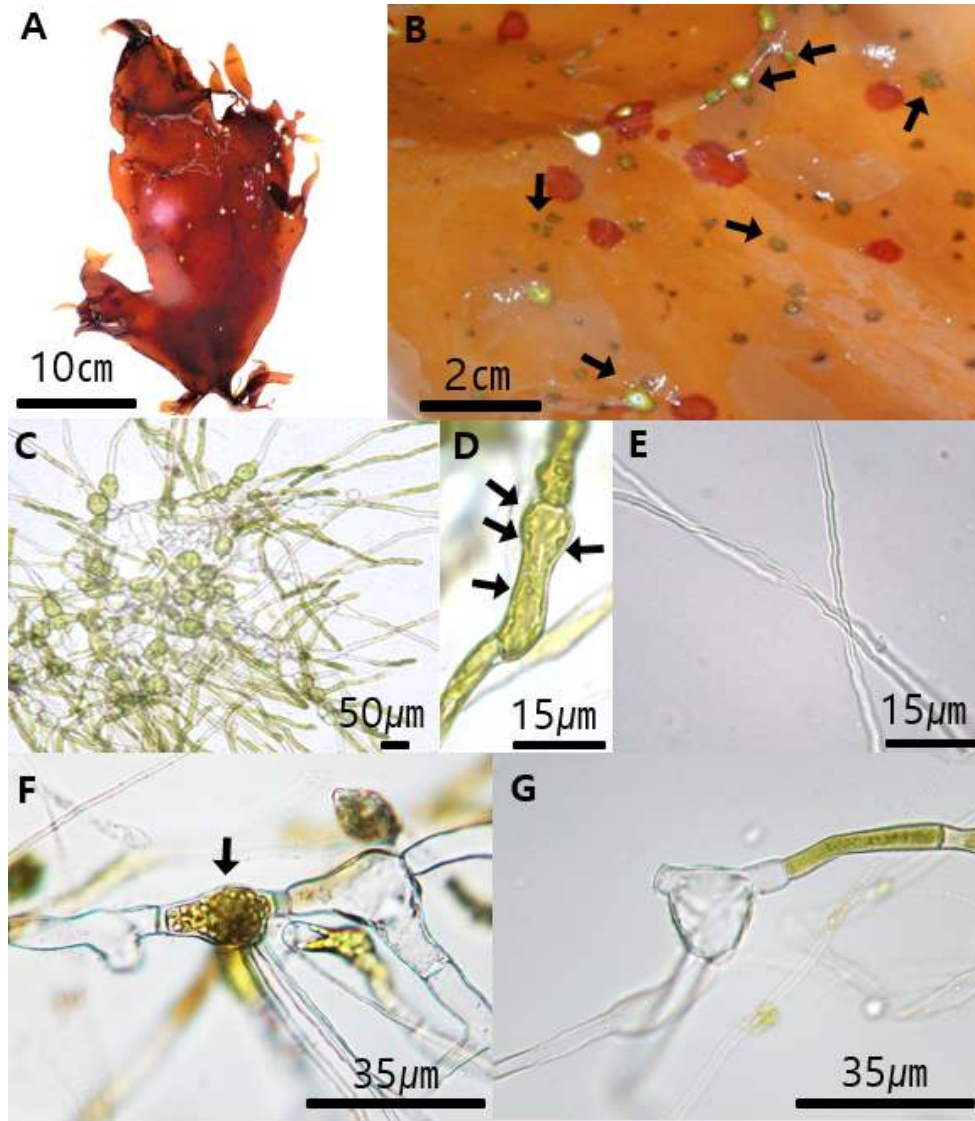
Phaeophila dendroides (Phaeophilaceae) was initially described as *Ochlochaete dendroides* by Crouan & Crouan (1852). Later this species was transferred to genus *Phaeophila* by Batters (1902). *P. dendroides* is a cosmopolitan species (Oliveira Filho & Ugadim, 1976; Kitayama & Garrigue, 1998; Brodie et al., 2007; Dawes & Mathieson, 2008). Moreover, this species has been reported from several Asian countries, including Japan (Kitayama & Yoshida, 1998) and China (Tseng, 2009; Phang et al., 2016). However, its occurrence has not been reported in Korea. According to

studies of many researchers (Oliveira Filho & Ugadim, 1976; Kitayama & Yoshida, 1992; Kitayama & Garrigue, 1998), this species has a chaetophoraleous appearance, in that it has characteristically twisted setae. Several traits (form of cells and twisted setae) of our specimens fit well with previous other description of this species <Table 1>. In a molecular analysis based on the *tufA* sequence ([Fig. 3]), this Korean alga nests in the same clade as *Phaeophila dendroides* from plants on dead leaves of *Zostera marina* of France (O’Kelly et al., 2004). The genetic distance between both sequences within the clade was calculated as 0.4 %. This species is here newly recorded in the Korean algal flora.

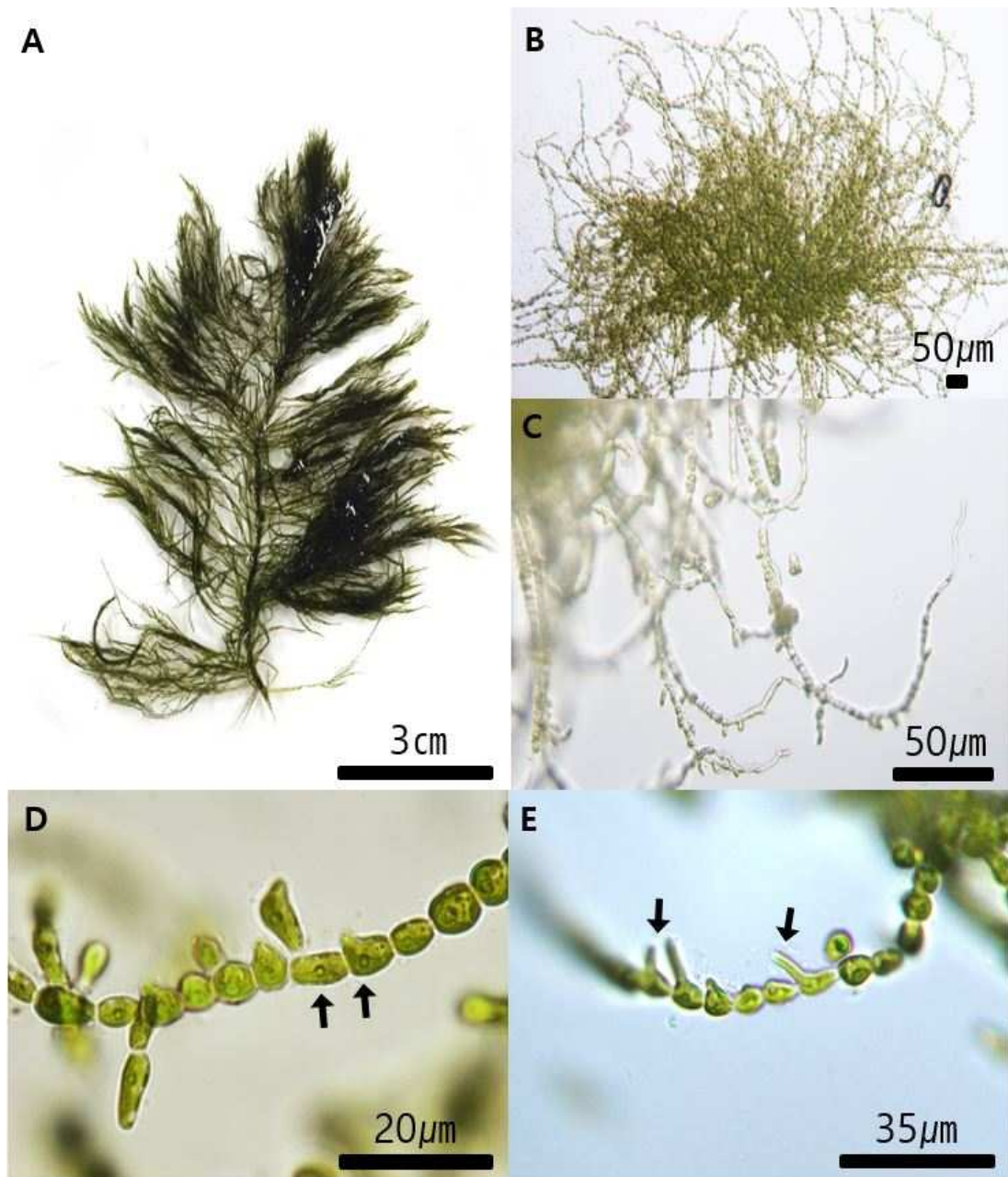
Dilabifilum was originally considered a member of Chaetophoraceae (Tschermak-Woess, 1971). A reappraisal of *Dilabifilum* Tschermak-Woess (Johnson & John, 1990) reviews the complicated taxonomic history of the genus (Broady & Ingerfeld, 1993). The systematic position of *Dilabifilum*, is uncertain and referred to “*Ulvales incertae sedis*” by Guiry & Guiry (2016). However, Gunnarsson & Nielsen (2016) suggested recently that it is referred to the family Kornmanniaceae, based on morphological similarity with *Pseudoclonium* and the molecular data obtained by Thüs et al. (2011). Several traits (form of cells and width) of our specimen closely corresponded to a previously described species, *Dilabifilum arthopyreniae* <Table 2>. However, we did not observe sporangia. In a molecular analysis based on the ITS sequence ([Fig. 4]), this Korean alga nests in the same clade as *Dilabifilum arthopyreniae* from plants of the original isolate (467-2) obtained from the Culture Collection of Algae (SAG), University of Göttingen, Germany. Most *D. arthopyreniae* have been reported in Europe

New records of two unknown micro-filamentous endophytic green algae in Korea: *Phaeophila dendroides* and *Dilabifilum arthropyreniae*

(Vischer, 1953; Ettl & Gärtner, 1995; Gunnarsson & Nielsen, 2016). In Asia, this species has been reported in Japan (Ihda et al., 1996). This species is here newly recorded in the Korean algal flora.

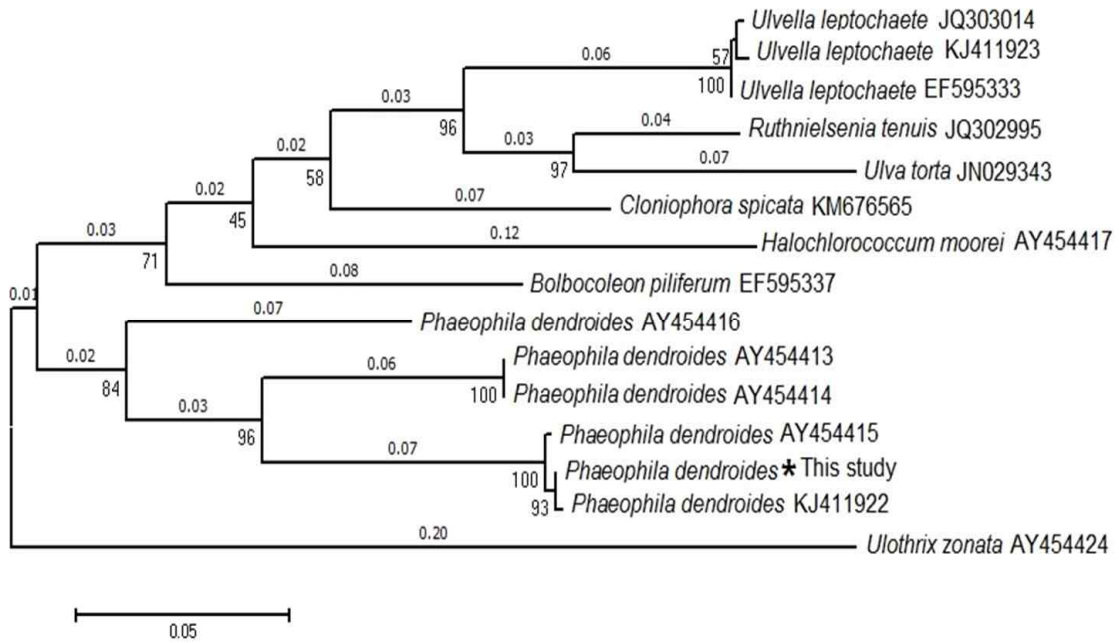


[Fig. 1] Host *Grateloupia lanceolata* fronds (A-B), and details of *Phaeophila dendroides* culture specimens (C-G). A, Host species, *G. lanceolata*; B, An infected *G. lanceolata* by *P. dendroides* (arrows); C, Parts of host vegetative plant; D, Chloroplast with several pyrenoids (arrows); E, Twisted *Phaeophila*-type hairs; F, Mature sporangium (arrow); G, Empty sporangium.

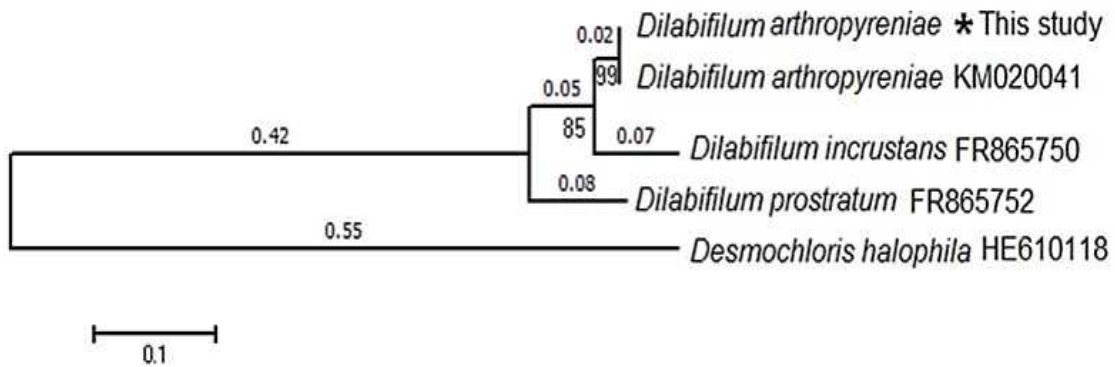


[Fig. 2] Host *Bryopsis* sp. frond (A), and details of *Dilabifilum arthropyreniae* culture specimens (B-E). A, Host species, *Bryopsis* sp.; B, Parts of vegetative plant; C, Curved filaments; D, Chloroplast with single pyrenoid (arrows); E, Distal branches (arrows). Note the shape of growth.

New records of two unknown micro-filamentous endophytic green algae in Korea: *Phaeophila dendroides* and *Dilabifilum arthropyreniae*



[Fig. 3] Phylogenetic relationships of the green endophyte, *Phaeophila dendroides* found in this study among other endophytic species. The tree was obtained with a maximum likelihood analysis of a *tuA* dataset, using a species of *Ulothrix zonata* as an outgroup. Numbers above branches reflect bootstrap support based on 1,000 replications.



[Fig. 4] Phylogenetic relationships of the green endophyte, *Dilabifilum arthropyreniae* found in this study among other endophytic species. The tree was obtained with a maximum likelihood analysis of an ITS dataset, using a species of *Desmochloris halophila* as an outgroup. Numbers above branches reflect bootstrap support based on 1,000 replications.

<Table 1> Comparison of some traits of several *Phaeophila dendroides* species

Traits	Oliveira Filho & Ugadim (1974)	Kitayama & Yoshida (1992)	Kitayama & Garrigue (1998)	Albis-Salas & Gavio (2015)	This study
Form of cells	Irregular	Narrow cylindrical, rather straight	Cylindrical or irregular	Cylindrical	Cylindrical or irregular
Twisted setae	Yes	Yes	Yes	No data	Yes
Width (μm)	12-20	10-27	10-25	4.5-5	5-19
Length (μm)	36-48	44-200	No data	17.5-27.5	60-210
Host	Various algae	<i>Hyalosiphonia caespitosa</i>	<i>Dictyota</i> spp.	<i>Hydrolithon farinosum</i> , <i>Pneophyllum fragile</i>	<i>Grateloupia lanceolata</i>

<Table 2> Comparison of some traits of several *Dilabifilum arthropyreniae* species

Traits	Johnson & John (1990)	Gunnarsson & Nielsen (2016)	This study
Form of cells	Cylindrical	Cylindrical	Rounded (central cell) or cylindrical (filaments)
Width (μm)	3.5-7	3.5-5	4-9
Length (times)	6-8 times	2-8 times	3-5 times (filaments)
Sporangia	Observed	Observed	Not observed
Host	No data	Wood	<i>Bryopsis</i> sp.

References

- Albis-Salas, M. & Gavio, B.(2015). Notes on the marine algae of the international biosphere reserve seaflower, Caribbean Colombia IV: New records of the macroalgal epiphytes on the seagrass *Thalassia testudinum*. Boletín de Investigaciones Marinas Costeras 44: 55~70.
- Batters, E. A. L.(1902). A catalogue of the British marine algae being a list of all the species of seaweeds known to occur on the shores of the British Islands, with the localities where they are found. Journal of Botany, British and Foreign 40 (Supplement): 1~107.
- Broady, P. A. & Ingerfeld, M.(1993). Three new species and a new record of Chaetophoracean (Chlorophyta) algae from terrestrial habitats in Antarctica. European Journal of Phycology 28: 25~31.
- Brodie, J. · Maggs, C. A. & John, D. M.(2007). Green seaweeds of Britain and Ireland. pp. [i-v], vi-xii, 1-242, 101 figs. London: British Phycological Society.
- Choi, H. G. · Kim, C. · Kim, Y. S. · Lee, S. J. · Park, M. A. & Nam, K. W.(2015). Phenology of host *Chondrus ocellatus* with filamentous green endophyte infection. Ocean Science Journal 50: 519~527.
- Crouan, P. L. & Crouan, H. M.(1852). Algues marines du Finistère, Brest 404p.
- Dawes, C. J. & Mathieson, A. C.(2008). The Seaweeds of Florida. pp. [i]- viii, [1]-591, [592], pls I-LI. Gainesville, Florida: University Press of Florida.
- Ettl, H. & Gärtner, G.(1995). Syllabus der Boden-, Luft- und Flechtenalgen. pp. i-vii, 1-721. Stuttgart: Gustav Fischer.
- Famà, P. · Wysor, B. · Kooistra, W. H. C. F. & Zuccarello, G. C.(2002). Molecular phylogeny of

- genus *Caulerpa* (Caulerpales, Chlorophyta) inferred from chloroplast *tufA* gene. *Journal of Phycology* 38: 1040-1050.
- Guiry, M. D. & Guiry, G. M.(2016). *AlgaeBase*. World-wide electronic publication, National University of Ireland, Galway. <http://www.algaebase.org>; searched on 26 December 2016.
- Gunnarsson, K. & Nielsen, R.(2016). Culture and field studies of Ulvellaceae and other microfilamentous green seaweeds in subarctic and arctic waters around Iceland. *Nova Hedwigia*, 103: 17-46.
- Ihda, T. · Nakano, T. & Iwatsuki, Z.(1996). *Dilabifilum arthopyreniae* (Chlorophyta) newly found in Japan. *Nova Hedwigia* 63: 195-202.
- Johnson, L. R. & John, D. M.(1990). Observations on *Dilabifilum* (Class Chlorophyta, order Chaetophorales sensu stricto) and allied genera. *British Phycological Journal*, 25: 53-61.
- Kitayama, T. & Garrigue, C.(1998). Marine algal endophyte and epiphytes new to New Caledonia. *Bulletin of the National Science Museum of Tokyo. Serie B* 24: 93-101.
- Kitayama, T. & Yoshida, T.(1992). First report of *Phaeophila dendroides* (P. Crouan et H. Crouan) Batters (Ulvophyceae, Chlorophyta) from Japan. *Japanese Journal of Phycology* 40: 47-50.
- Lee, H. B. · Kim, J. I. · Lee, J. W. & Oh, B. G.(1998). Notes on little-known algae in Korea (I). *Algae* 13: 165-172.
- Lee, S. J. · Park, M. A. · Ogandaga, C. A. M. · Park, S. K. · Kim, H. · Kim, Y. S. & Choi, H. G.(2013). A study on the growth and disease of *Chondrus ocellatus* in Korea. *Journal of Fish Pathology* 26: 265-274.
- Lee, Y. P. & Kang, S. Y.(2002). *A Catalogue of the Seaweeds in Korea*. Jeju: Jeju National University Press.
- Ogandaga, C. A. M, · Choi, H. G. · Kim, J. K. & Nam, K. W.(2016). Growth responses of *Chondrus ocellatus* Holmes (Gigartinales, Rhodophyta) to two endophytes, *Mikrosyphar zosterae* Kuckuck (Ectocarpales, Ochrophyta) and *Ulvella ramosa* (N. L. Gardner) R. Nielsen (Ulvales, Chlorophyta) in culture. *Algae* 31: 363-371.
- O'Kelly, C. J. · Wysor, B. & Bellows, W. K. (2004). Gene sequence diversity and the phylogenetic position of algae assigned to the genera *Phaeophila* and *Ochlochaete* (Ulvophyceae, Chlorophyta). *Journal of Phycology* 40: 789-799.
- Oliveira Filho, E. D. & Ugadim, Y.(1974). New references of benthic marine algae to Brazilian flora. *Bol Bot Univ São Paulo* 2: 71-91.
- Phang, S. M. · Yeong, H. Y. · Ganzon-Fortes, E. T. · Lewmanomont, K. · Prathep, A. · Hau, L. N. · Gerung, G. S. & Tan, K. S.(2016). Marine algae of the South China Sea bordered by Indonesia, Malaysia, Philippines, Singapore, Thailand and Vietnam. *Raffles Bulletin of Zoology* 34: 13-59.
- Thüs, H. · Muggia, L. · Pérez-Ortega, S. · Favero-Longo, S. E. · Joneson, S. · O'Brien, H. · Nelsen, M. P. · Duque-Thüs, R. · Grube, R. · Friedl, T. · Brodie, J. · Andrew, C. J. · Lücking, R. · Lutzoni, F. & Gueidan, C.(2011). Revisiting photobiont diversity in the lichen family Verrucariaceae (Ascomycota). *European Journal of Phycology*, 46: 399-415.
- Tschermak-Woess, E.(1971). Über wenig bekannte und neue Flechtengenonidien V Der Phycobiont von *Verrucaria aquatilis* und die Fortpflanzung von *Pseudopleurococcus arthopyreniae*. *Österreichische Botanische Zeitschrift* 118: 443-455.
- Tseng, C. K.(2009). *Seaweeds in Yellow Sea and Bohai Sea of China*. pp. [2], i-xi, 1-453. Beijing: Science Press.
- Vischer, W.(1953). Ober primitivste Landpflanzen. *Ber. Schweiz. bot. Ges.*, 63: 169-193.
- White, T. J. · Bruns, T. · Lee, S. & Taylor, J. W.(1990). Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. *PCR protocols: a guide to methods and applications*, 18: 315-322.
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- Recieved : 03 January, 2017
 - Revised : 13 January, 2017
 - Accepted : 18 January, 2017