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〈Original article〉

## Two New Records of *Peyssonnelia* Species and *Sonderophycus cauliferus* Comb. Nov. within the Family Peyssonneliaceae (Peyssonneliales) from Korea

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**Abstract** - Detailed morphological studies and molecular analyses based on plastid-encoded *rbcL* gene sequences were undertaken on *Peyssonnelia* species, a poorly known genus from Korea. We report new records for the Korean coast, *Peyssonnelia harveyana* and *P. rumoiana*. *Peyssonnelia harveyana* is chiefly characterized by *P. rubra*-type anatomy, closely packed perithallial filaments in firm matrix, hypothallial filaments arranged in parallel rows, thalli with appressed margins, hypobasal calcification, and unicellular rhizoids. *Peyssonnelia rumoiana* is principally characterized by two vegetative features, hypothallial filaments arranged in a polyflabellate layer, and perithallial filaments arising from the whole upper surface of each hypothallial cell (*Peyssonnelia rubra*-type anatomy). Our *rbcL* analyses revealed that *P. harveyana* and *P. rumoiana* were placed within a clade of *Peyssonnelia*. We also propose the new combination, *Sonderophycus cauliferus* comb. nov., for previous *Peyssonnelia caulifera*. Phylogenetic analyses revealed that our *S. cauliferus* was placed within a clade of *Sonderophycus*.

**Key words** : *Peyssonnelia rumoiana*, *Peyssonnelia harveyana*, *Sonderophycus cauliferus*, Rhodophyta

### INTRODUCTION

The Peyssonneliaceae (as the “Squamariaceae Zanardini”) was placed in the Gigartinales F. Schmitz (1892). Krayesky (2009) confirmed that the Peyssonneliaceae was a monophyletic group from Gigartinales sensu lato by *rbcL* and LSU rDNA data and he proposed a distinct order, Peyssonneliales. The order Peyssonneliales contains one family, Peyssonneliaceae and 13 genera. Among them, only five genera (*Cruoriella* P. Crouan & H. Crouan, *Peyssonnelia* Decaisne, *Polystrata* Heydrich, *Pulvinia* Hollenberg, *Sonderophycus* Denizot) have been recognized in Japan and

Korea (Nozawa 1968; Yoshida *et al.* 1990; Yoshida 1998; Kato *et al.* 2006; Kim *et al.* 2013).

Red algal genus *Peyssonnelia* (Peyssonneliaceae, Peyssonneliales) is the largest genus of non-coralline crustose Rhodophyta. It is established by Decaisne (1841) based on *P. squamaria* (S.G. Gmelin) Decaisne ex J. Agardh. *Peyssonnelia* is characterized by origin of erect perithallial filaments from the hypothallus, structure of the hypothallus and peri thallus, degree of adherence of the thallus to substratum, location of calcification when present, presence or absence of segmentation in rhizoids, development of sori, the developmental pattern of spermatangial filaments, origin of gonimoblast, position of tetrasporangia, and branching pattern of paraphyses (Kato and Masuda 2002). It cur-

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rently includes 81 species from tropical to warm-temperate regions in the world (Kato and Masuda 2002, 2003; Guiry and Guiry 2017). In Korea, eight species are known: *Peyssonnelia boergesenii* Weber-van Bosse, *P. caulifera* Okamura, *P. conchicola* Piccone & Grunow, *P. dubyi* P. Crouan & H. Crouan, *P. japonica* (Segawa) Yoneshigue, *P. orientalis* (Weber-van Bosse) Cormaci & G. Furnari, *P. rosenvingei* F. Schmitz, and *P. rubra* (Greville) J. Agardh (Kim *et al.* 2013). However, most of these species have been recorded as floristic list without taxonomic consideration.

Genus *Sonderophycus* (Peyssonneliaceae, Gigartinales) was established by Denizot (1968) based on *Sonderophycus australis* (Sonder) Denizot, currently synonymized to *S. capensis* (Montagne) M.J. Wynne. Kravesky *et al.* (2009) demonstrated that type species of *Sonderophycus capensis* (= *S. australis*) was distinct from *Peyssonnelia* clade in phylogenetic trees using *rbcL* and LSU rDNA data although they had common crustose thalli. In morphology, *Sonderophycus* also differs from *Peyssonnelia* in thallus structure, with longitudinal filaments that diverge to upper and lower surfaces (Womersley and Sinkora 1981).

We collected samples that fit the description of *Peyssonnelia* and *Sonderophycus* from east and south coasts of Korea. We observed morphology in detail and compared their phylogenetic relationship based on *rbcL* sequence analysis. In this study, we report *Sonderophycus cauliferus* comb. nov. and two new records of *Peyssonnelia* species from Korea.

## MATERIALS AND METHODS

### 1. Morphology

Plants were collected from the coasts of Korea. Fresh samples were removed from bedrock, shells, or seaweeds using a chisel and hammer. Samples were preserved in 4–5% formalin/seawater for morphological examination. Samples were completely decalcified in 0.6 M nitric acid for a minimum of 30 minutes, rinsed with distilled water, and stained using a 3 : 7 mixture of 1% aqueous aniline blue and glycerin for more than 30 minutes. For detailed morphology, sections of thallus (5–10 µm thickness) were prepared using an embedding matrix (O.C.T.; CellPath, Ltd., Newtown, Wales, UK) and a freezing microtome (Shandon

Cryotome FSE; Thermo Shandon, Ltd., UK). Photomicrographs were taken using an Olympus microscope (BX-51TRF; Olympus, Tokyo, Japan) and an Olympus DP71 camera. Permanent slides were mounted in karo syrup (70%). Representative specimens examined in this study are deposited in the National Institute of Biological Resources (NIBR) and herbarium of Chosun University (CUK), Korea.

### 2. Molecular study

Samples were cleaned in autoclaved seawater under a dissecting microscope and were preserved in silica gel for molecular analysis. Genomic DNA was extracted using a NucleoSpin Plant II Kit (Macherey-Nagel, Düren, Germany), following manufacturer's instructions. Polymerase chain reaction (PCR) was conducted in 30 µL reactions that contained 4–8 µL genomic DNA or 10 µL diluted genomic DNA (1 : 10), 1 µL 10 pmols forward and reverse primers, and HelixAmp Ready-2x-Go Series (NanoHelix Co., Ltd., Daejeon, Korea). The *rbcL* was amplified using primer combinations F57-R1150 and F993-RrbcStart (Freshwater and Rueness 1994) and purified with PCRquick-spin™ PCR product purification kit (iNtRON Biotechnology, Inc. Seongnam, Korea). Cycle sequencing was conducted with amplification primers. Sequences were determined by using an ABI Prism 3100 Genetic Analyzer (PE Applied Biosystems, Foster City, CA, USA). The *rbcL* sequences generated in this study were deposited in EMBL/GenBank under accession numbers MF668134 for *Peyssonnelia harveyana*, MF668135 for *P. rumoiana*, and MF668136 for *Sonderophycus cauliferus*. *Chondrus crispus* (U02984) was selected as outgroup. These sequences and other nucleotide sequences from GenBank were aligned using Clustal W (Thompson *et al.* 1994) and manually corrected using MEGA5 (Tamura *et al.* 2011). Maximum likelihood analyses were conducted in MEGA5, using 1000 bootstrap replications and the GTR +  $\Gamma$  + I model, and Bayesian inference was conducted using MrBayes 3.1.2 (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 +  $\Gamma$  + I evolutionary model and sampling and printing every 1000 generations. Summary trees were generated using a burn-in value of 800.

## RESULTS AND DISCUSSION

### 1. Phylogenetic analyses

The 1228 *rbcL* bp portion of the 1467-bp *rbcL* gene (83.7% sequenced) sequenced for *Peyssonnelia harveyana*, *P. rumoiana*, and *Sonderophycus cauliferus*. Phylogenetic analyses revealed that *P. harveyana* and *P. rumoiana* were placed within a clade of *Peyssonnelia* (Fig. 1). However, *S. cauliferus* (= *P. caulifera*) was placed within a clade of *Sonderophycus* (Fig. 1).

Phylogenetic analyses revealed *Peyssonnelia harveyana* differs from *P. orientalis* by 7.6–7.8% and *P. inamoena* by 8.1–8.3% gene sequence divergence respectively and *Peyssonnelia rumoiana* differs from *P. armorica* by 8.9–9.2% gene sequence divergence respectively. *Sonderophycus cauliferus* (= *P. caulifera*) differs from *S. coriaceus* by 9.5–9.8% gene sequence divergence respectively.

### 2. Morphological observations

Class Florideophyceae Cronquist, 1960 진정홍조강  
Order Peyssonneliales Kraysky, Fredericq & J. N.

Norris, 2009 바다표고목

Family Peyssonneliaceae Denizot, 1968 바다표고과

Genus *Peyssonnelia* Decaisne, 1841 바다표고속

*Peyssonnelia harveyana* P. Crouan & H. Crouan ex J. Agardh, 1851 붉은바다표고 (신칭) (Fig. 2A–D)

**Synonyms:** *Peyssonnelia adriatica* Hauk 1882, *Cruoriella adriatica* (Hauk) De Toni 1905.

**Material examined:** NIBRRD000000214 (deposited in the National Institute of Biological Resources), CUK13191, MF668134, Seongsan beach, Seongsan-eub, Seogyipo-si, Jeju, Korea (33°27'42.57"N, 126°56'20.93"E), July 7, 2014, Cho and Jeong, at 1 m depth by hand.

**Description:** Crustose thalli solitarily or gregariously growing on pebbles in the intertidal zone, closely adherent to substrata with appressed margins (Fig. 2A), purplish-red, 3–8 mm (partly collected) in diameter, 80–173 µm thick excluding nematocystia. Surface of each thallus with weakly marked concentric zones and faint radial lines. Hypothallial filaments arranged in parallel rows in surface view (Fig. 2B), 32–41 µm broad in radiating rows and 10–17 µm long. Perithallial filaments arising somewhat obliquely from the

whole upper surface of each hypothallial cell (the *P. rubra*-type anatomy), tightly packed in a firm matrix, 5–8 cells high in the center of the crust (Fig. 2C). The basal cells of the perithallial filaments wider than high, 29–38 µm broad by 12–20 µm high, boot-shaped producing two ascending filaments (one simple and one dichotomously branched filament) cells of ascending filament 4–20 µm broad by 3–12 µm high (Fig. 2C). Unicellular rhizoids produced frequently from the median region (rarely near the anterior end) of hypothallial cells, 3–12 µm broad by 32–95 µm long (Fig. 2D).

**Habitat:** Epilithic on the rock at intertidal zone.

**World Distribution:** Europe (Britain, France, Greece, Ireland, Italy, Portugal, Spain, Turkey), Atlantic Islands, South America (Argentina, Chile), Africa (Angola, Egypt, Tunisia), Indian Ocean Islands, Asia (Korea, Japan), Antarctic and the Sub Antarctic islands (Guiry and Guiry 2017).

**Identifier:** Tae Oh Cho and So Young Jeong.

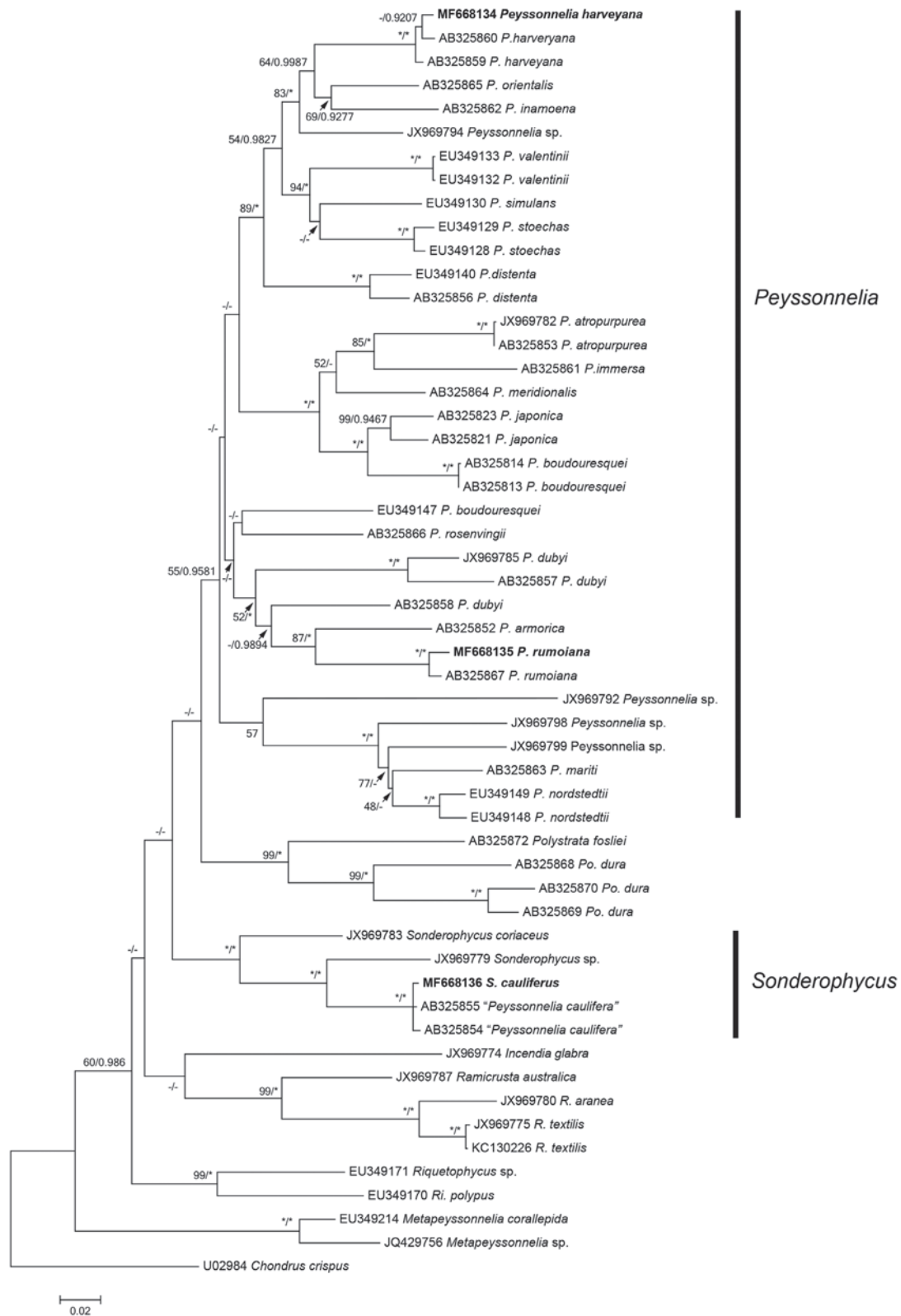
**Remarks:** *Peyssonnelia harveyana* was originally described by Crouan and Crouan (1851) from France. This species has been widely reported worldwide. Our collections match the protologue of *Peyssonnelia harveyana* in most characteristics from the British Isles (Irvine and Maggs 1983) and Japan (Kato *et al.* 2005). We add this species as *P. harveyana* in list of Korean macroalgal flora.

*Peyssonnelia rumoiana* Kato & Masuda, 2003

바위바다표고 (신칭) (Fig. 3A–E)

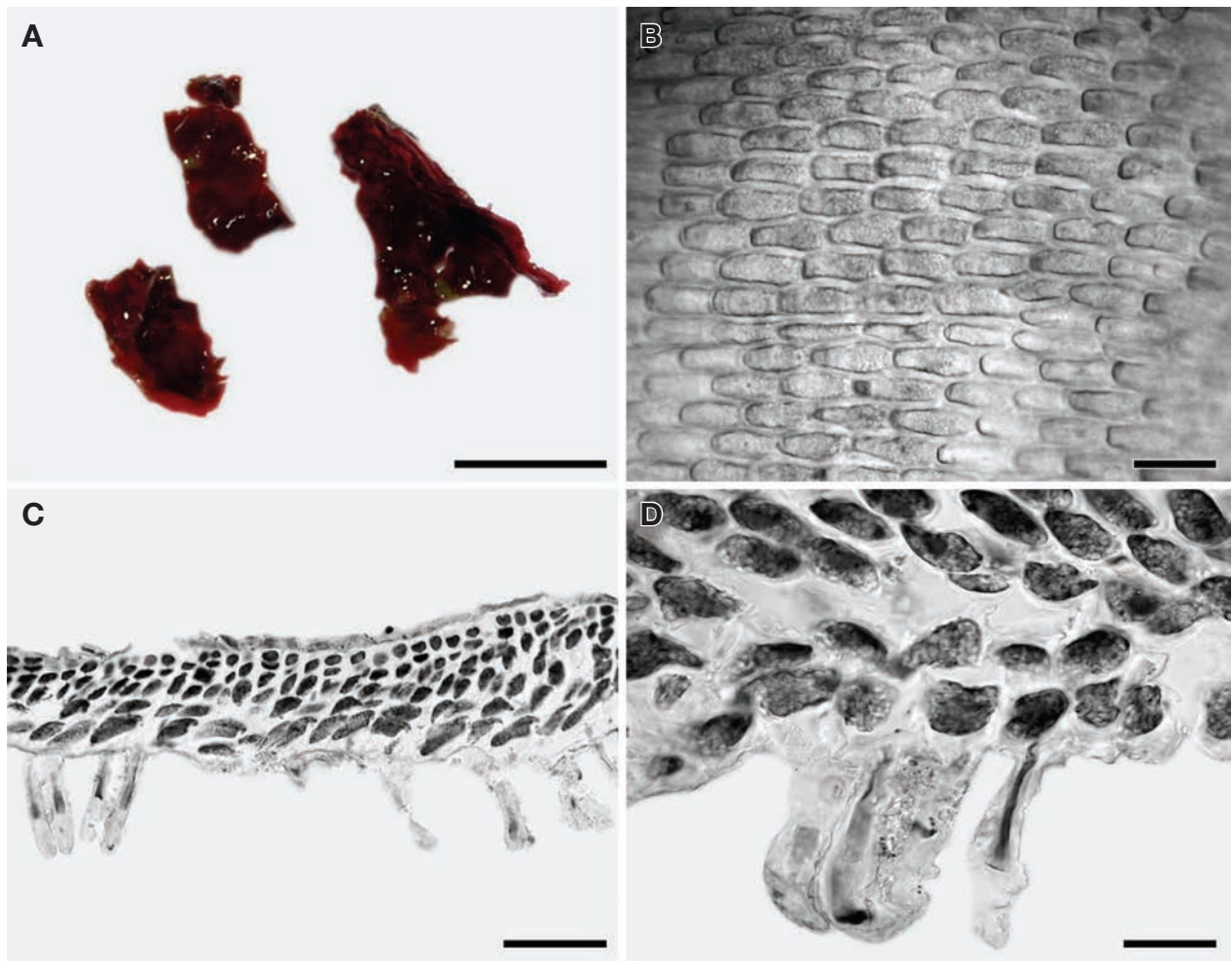
**Material examined:** NIBRRD000000213 (deposited in the National Institute of Biological Resources), CUK10494, MF668135, Gampo harbor, Gampo-eub, Gyeongju-si, Gyeongsangnam-do, Korea (35°48'05.36"N, 129°30'27.46"E), July 5, 2013, Cho and Jeong, at 1 m depth by hand.

**Description:** Crustose thalli solitarily growing on pebbles in the intertidal zone, circular in shape, completely adherent to the substrata with appressed margins (Fig. 3A), blood-red, 0.3–0.8 mm in diameter, 36–99 µm thick excluding nematocystia. Surface of each thallus with weakly marked concentric zones and faint radial lines. Hypothallial filaments comprising a polyflabellate layer (Fig. 3B), 16–21 µm broad in radiating rows and 8–11 µm long. Margin composing single cell (Fig. 3C). Perithallial filaments arising somewhat obliquely from the whole upper surface of each hypothallial cell (the *P. rubra*-type anatomy), tightly packed



**Fig. 1.** Phylogenetic tree of species of *Peyssonnelia* and *Sonderophycus* based on Maximum-likelihood (ML) analysis with *rbcL* sequences. Values above branches = ML bootstrap (BS) values in % > 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 (\*).





**Fig. 2.** *Peyssonnelia harveyana* P. Crouan & H. Crouan ex J. Agardh. A. Habit with typical crustose thalli; B. Hypothallial filaments arranged in a parallel layer; C. Cross section view showing perithallial filaments that arise from each cell of the hypothallus; D. Unicellular rhizoids developed from the median region or near the anterior end of each hypothallial cell. Scale bars: A = 5 mm; B = 40  $\mu$ m; C = 100  $\mu$ m; D = 25  $\mu$ m.

in firm matrix, 7–13 cells high in the center of the crust (Fig. 3D). Basal cells of the perithallial filaments wider than high 13–19  $\mu$ m broad by 7–11  $\mu$ m high, boot-shaped producing two ascending filaments (one simple and one dichotomously branched filament) cells of the ascending filament 3–6  $\mu$ m broad by 4–14  $\mu$ m high (Fig. 3D). Unicellular rhizoids produced frequently from the anterior region (rarely from median region) of hypothallial cells, 3–5  $\mu$ m broad by 8–15  $\mu$ m long (Fig. 3E).

**Habitat:** Epilithic on the rock at intertidal zone.

**World Distribution:** Korea, Japan (Guiry and Guiry 2017).

**Identifier:** Tae Oh Cho and So Young Jeong.

**Remarks:** *Peyssonnelia rumoiana* was first described by

Kato and Masuda (2003) from Ogon-miski, Rumoi, Hokkaido of Japan. We collected this species growing on a rock at the intertidal zone from Gampo on the eastern coast of Korea. Our morphological observations agree with the description of Kato and Masuda (2003). We add *P. rumoiana* in list of Korean algal flora.

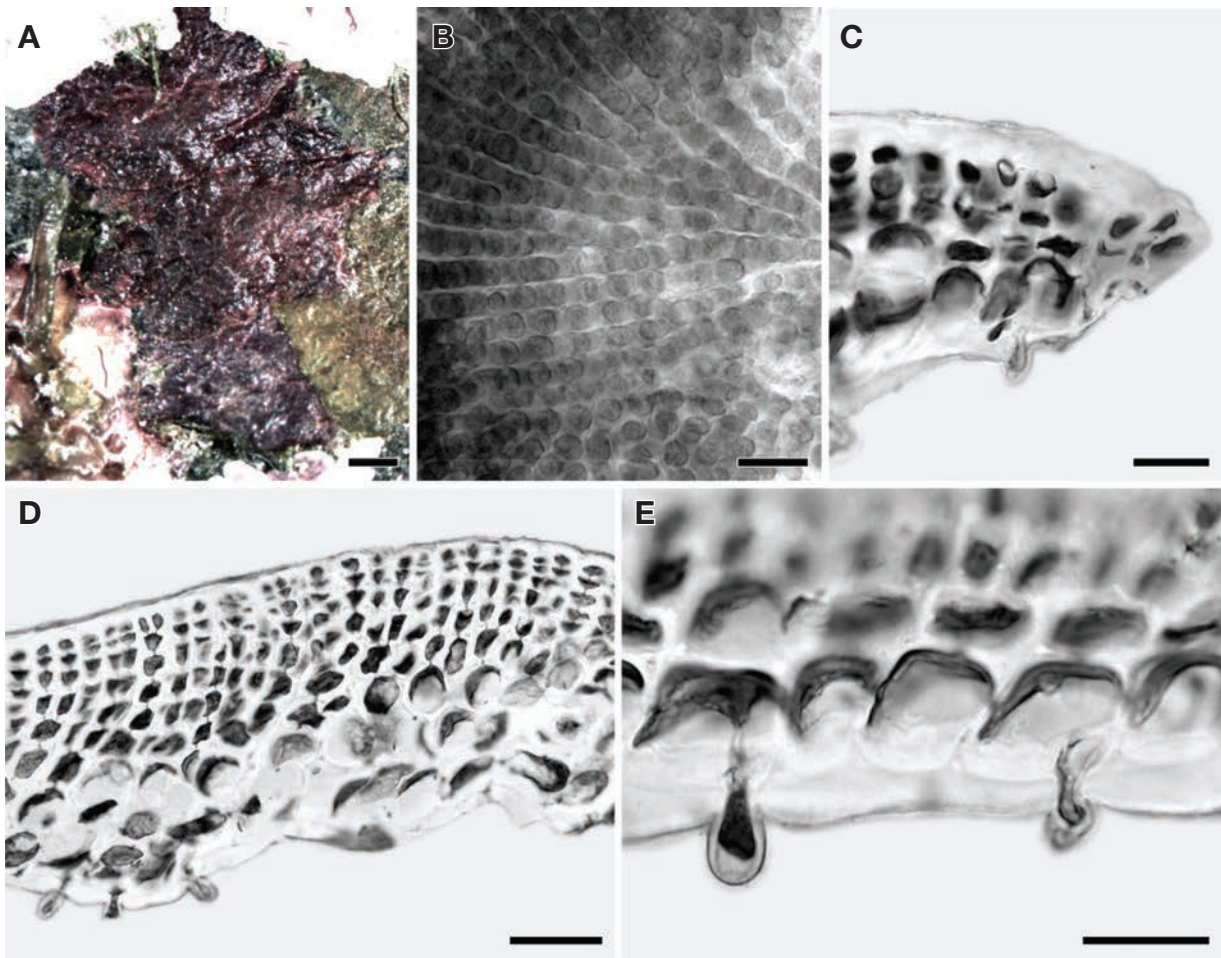
Genus *Sonderophycus* Denizot, 1968 아프리카바다표고속

*Sonderophycus cauliferus* (Okamura) S.Y. Jeong, B.Y.

Won & T.O. Cho comb. nov. 자루아프리카바다표고

(신칭) (Fig. 4A–E)

**Synonyms:** *Peyssonnelia caulifera* Okamura 1899.



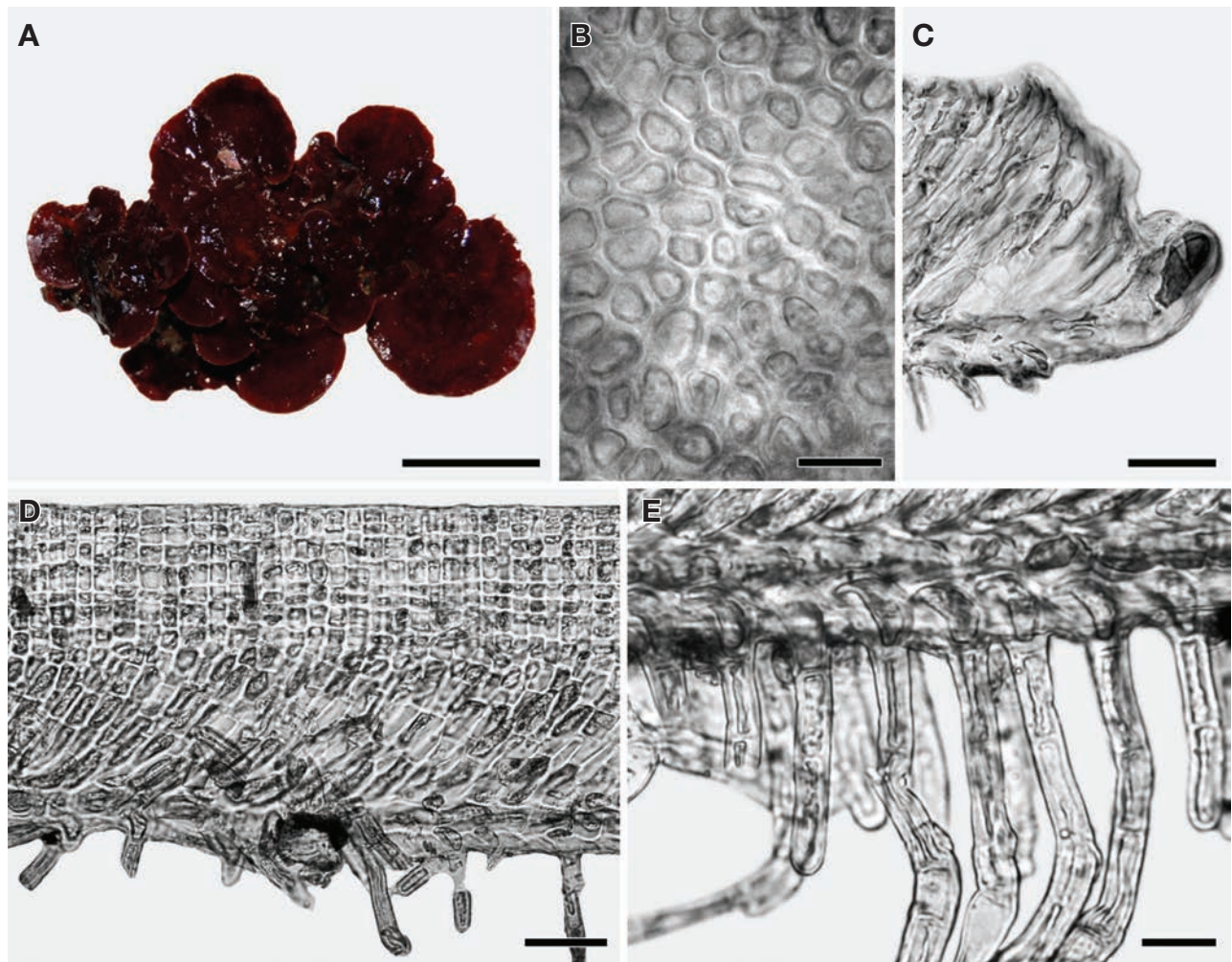
**Fig. 3.** *Peyssonnelia rumoiana* Kato & Masuda. A. Habit with typical crustose thalli; B. Hypothallial filaments arranged in a polyflabellate layer; C. Cross section view of marginal part; D. Cross section view of lower thallus showing perithallial filaments that arise from each cell of the hypothallus; E. Unicellular rhizoids developed from the near the anterior end of each hypothallial cell. Scale bars: A = 2 mm; B, C = 20  $\mu$ m; D = 25  $\mu$ m; E = 10  $\mu$ m.

**Material examined:** NIBRRD0000000216 (deposited in the National Institute of Biological Resources), CUK13194, MF668136, Geommeolae beach, Udo-myeon, Jeju-si, Jeju, Korea (33°29'49.56"N, 126°58'01.32"E), November 7, 2014, Cho and Jeong, at 15 m depth by scuba diving.

**Description:** Plants solitarily or gregariously growing on bottom (mollusk or other creeping algae) in subtidal zone, prostrate, thick, cartilaginous, crustose and lobed (Fig. 4A), dark red on upper surface, and pale-red on lower surface. Thalli loosely attached to substrata with detached margins, spreading from a stipe-like, dense interwoven mass of multicellular rhizoids beneath the thallus. Lamina proximally becoming wider and fan-like in appearance, forming layered foliose, to 0.8–1.5 cm long and to 1.1–1.7 cm broad,

and 103–183  $\mu$ m thick excluding nemathecium (Fig. 4A). Thallus internally composed of several cell rows that form the dorsal (Fig. 4B) and ventral surfaces. Dorsal surface cells isodiametric, 6–16  $\mu$ m in diameter (Fig. 4B). Margin composing one-celled apical cell (Fig. 3C). Internal structure of thallus composed of two layers: a ventral hypothallus and a dorsal perithallus. Basal layer of single cell layer on the lower surface produces perithallial cells and bent basal cells of rhizoids pointing slightly forward (Fig. 4D). Basal layer of perithallus of ascending filaments at angles of 30–40° in the lower and middle perithallus, with the apical portions of filaments in the upper perithallus bending to a 90° angle. Perithallial cells elongated in basal layer, isodiametric in upper layer. Basal cells of multicellular rhizoids





**Fig. 4.** *Sonderophycus cauliferus* (Okamura) S.Y. Jeong, B.Y. Won & T.O. Cho comb. nov. S.Y. Jeong, B.Y. Won & T.O. Cho. A. Habit with fan-shaped thalli; B. Isodiametric hypothallial filaments on ventral side; C. Cross section view of marginal part; D. Cross section view of lower thallus showing perithallial filaments that arise from each cell of the hypothallus; E. Unicellular rhizoids developed from the median region or near the anterior end of each hypothallial cell. Scale bars: A = 1 cm; B = 20 µm; C = 50 µm; D = 25 µm; E = 10 µm.

point forward and lie within the blade sheath, 5–12 µm broad by 37–89 µm long (Fig. 4E).

**Habitat:** Epilithic on the rock, epiphytic on other algae, epizoid on molluscs at subtidal zone.

**World Distribution:** Asia (Korea, Japan, Taiwan), South-east Asia (Vietnam), Pacific Islands (Guiry and Guiry 2017).

**Identifier:** Tae Oh Cho and So Young Jeong.

**Remarks:** *Peyssonnelia caulifera* is here transferred to the genus *Sonderophycus*. *Sonderophycus cauliferus* was originally reported in Japan and our sequences of *S. cauliferus* corresponded with *rbcL* data of Japanese samples. *Sonderophycus cauliferus* shares most of vegetative characters with *S. coriaceus*, however *S. cauliferus* differs from *S. coria-*

*ceus* in shape of ventral cells, with isodiametric cells on dorsal and ventral surface (Krayesky 2009). Reproduction thalli are not observed in this study.

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