Korean Journal of Environmental Biology

Note

https://doi.org/10.11626/KJEB.2024.42.2.187

Korean J. Environ. Biol.
42(2) : 187-192 (2024)
ISSN 1226-9999 (print)
ISSN 2287-7851 (online)

Pseudolithoderma subextensum (Sphacelariales, Phaeophyceae): a new record of crustose brown alga from Korea

Antony Otinga Oteng'o, Tae Oh Cho and Boo Yeon Won*

Department of Biological Science, Chosun University, Gwangju 61452, Republic of Korea

Contribution to Environmental Biology

- We present the inclusion of *Pseudolithoderma subextensum* as a novel addition to the catalog of macroalgal flora in Korea.
- *Pseudolithoderma subextensum* is located in the southern region of Korea.

***Corresponding author**
Boo Yeon Won
Tel. 062-230-7983
E-mail. giving_won@hanmail.net

Received: 20 May 2024
Revised: : 7 June 2024
Revision accepted: 14 June 2024

Abstract: *Pseudolithoderma subextensum* is a crustose brown algal species in the family Lithodermataceae and order Sphacelariales. This species is distributed in several regions across the world including, Europe, Western Atlantic, Middle East and Asia (Hong Kong and Japan). Recent floristic surveys along the Korean coastal shores have revealed new records of encrusting brown algae. In this study, we report *P. subextensum* as a new record from Korea. Morpho-anatomical and molecular studies on *Ralfsia*-like specimens from Korea identified some of them as *P. subextensum*. *Pseudolithoderma subextensum* is characterized by dark chestnut brown crust with a hypothallial basal layer and erect perithallial filaments, tufts of hairs occasionally arising from the basal layer, several discoid shaped chloroplasts per cell, plurangia arising terminally on erect filaments and without sterile cells, and unangia arising terminally on erect filaments, elongated cylindrical and without paraphyses. Phylogenetic analyses based on COI-5P (545bp) reveal that *P. subextensum* are nested within Lithodermataceae and forms the same clade with *P. roscoffense*. The genetic divergences for COI-5P between them is 24.5%.

Keywords: Phaeophyceae, *Pseudolithoderma subextensum*, Sphacelariales

1. INTRODUCTION

The crustose brown algal genus, *Pseudolithoderma* Svedelius is encompassed in the family Lithodermataceae Hauck and order Sphacelariales Migula (Guiry and Guiry 2024). *Pseudolithoderma* was described by Svedelius (Kjellman and Svedelius 1911) based on *Lithoderma fatiscens* sensu Kuckuck. This genus is characterized by following characters: 1) crustose thalli, relatively thin, horizontally expanded, attached firmly to the substratum, without rhizoids; 2) hypothallial basal layer of 1−3 cells and perithallial erect filaments simple or sparsely branched, strictly erect, loosely or firmly adjoined; 3) cells with several to many discoid chloroplasts without pyrenoids; 4) tufts of hairs occasionally arising from the basal layer; 5) plurangial reproductive structures arising terminally on erect filaments, without sterile cells; 6) unangia arise terminally on erect filament, without paraphyses (Kjellman and Svedelius 1911; Tanaka and Chihara 1981; Kaehler 1998; Poong 2014).

Currently, ten *Pseudolithoderma* species are recognized worldwide (Guiry and Guiry 2024), but none has been reported in Korea. *Pseudolithoderma subexten-*

sum (Waern) S. Lund was first described as *Lithoderma subextensum* from Öregrund, Sweden (Waern 1949). It is mainly characterized by a thin, dark chestnut brown crust; hypothallial basal layer giving rise to strictly erect perithallial filaments; tufts of hairs occasionally arising from the basal layer; several discoid or irregularly shaped chloroplasts per cell; plurangia arising terminally on erect filaments, uniseriate and branched, without sterile cells; unangia arising terminally on erect filaments, elongated and cylindrical (Waern 1949; Tanaka and Chihara 1981; Kaehler 1998; Poong 2014). Lund (1959), transferred this species to the genus *Pseudolithoderma* based on their plurangial reproductive organs borne terminally on erect filaments, that differentiates them from the *Lithoderma* which have plurangia borne both laterally and terminally on erect filaments.

The global distribution of *P. subextensum* includes Europe, Western Atlantic, Middle East and Asia (Hong Kong and Japan) (Tanaka and Chihara 1981; Kaehler 1998; Guiry and Guiry 2024). Despite the proximity of Korea to both Japan and Hong Kong, *P. subextensum* has not yet been reported in Korea. Most of the encrusting brown algae in Korea have been identified as species of the genera *Ralfsia* or *Neoralfsia* based on their morphology (Lee and Kang 1986; Lee and Kang 2002; Lee 2008; Keum 2010). However, in their recent floristic surveys, Oteng'o *et al.* (2020a, 2021, 2022, 2023a, 2023b) reported *Ralfsia longicellularis* as a new record, described four new *Endoplura* species, and erected a new genus *Sungminia* within Sungminiaceae and genus *Fissipedicella* from Korea.

Two unidentified samples of *Ralfsia*-like crusts were collected from intertidal areas along southern coast of Korea. In this study, we examined their morpho-anatomy and analyzed COI-5P sequences, identifying them as *Pseudolithoderma subextensum*.

2. MATERIALS AND METHODS

2.1. Morphological analyses

Samples were collected from intertidal areas along the coastline of Korea. Specimens were photographed using a waterproof digital camera (Nikon COOLPIX AW100; Nikon Corp., Tokyo, Japan) for their external morphological characteristics related to characters, including color, outline, and surface morphology. Prior to the analysis of morphological observation, the specimens were detached from the substrate using a single-edged blade. Squashed and microtome-sectioned preparations were prepared for each sample. For the microtome-sectioned preparations, samples were embedded in a matrix (OCT; CellPath Ltd., Newtown, Wales, UK) and sectioned (8−10 μm thickness) using a freezing microtome (Shandon Cryotome FSE; Thermo Shandon Ltd., Loughborough, UK). The sectioned and squashed samples were stained with a 1 : 1 mixture of aqueous aniline blue and acetic acid. Sections were mounted in 50% corn syrup and photographed using a DP-71 camera (Olympus, Tokyo, Japan) mounted on a BX-51TRF microscope (Olympus). Digitized images were edited for clarity using Adobe Photoshop software v.6.1 (Adobe Systems Inc., San Jose, CA, USA). Representative voucher specimens examined in this study were deposited in the herbarium of Chosun University (CUK) and the Marine Biodiversity Institute of Korea (MABIK).

2.2. Molecular analyses

Genomic DNA was extracted using the NucleoSpin Plant II Kit (Macherey-Nagel, Düren, Germany), following the manufacturer's instructions. The COI-5P region was amplified via polymerase chain reaction (PCR) with HelixAmp Ready-2x-Go premix from NanoHelix Co., Ltd. (Daejeon, Korea), adhering to the provided protocol. The primer sets for amplifying COI-5P were those specified in a previous study by Oteng'o *et al.* (2021). PCR was performed on a Veriti 96-well Thermal Cycler (Applied Biosystems, Waltham, MA, USA). The resulting PCR products were purified using the PCRquick-spin™ PCR Product Purification Kit (iNtRON Biotechnology, Inc., Seongnam, Korea). One COI-5P sequence from the brown crustose algae obtained in this study has been deposited in GenBank. The COI-5P sequence generated, along with those retrieved from GenBank, were aligned using Geneious Prime® (v.2023.0.1, Biomatters Ltd., Auckland, New Zealand). Phylogenetic analyses were conducted in Mega X (Kumar *et al.* 2018), employing the maximum likelihood method with the GTR+G+I model and 1,000 bootstrap replicates. Bayesian inference was executed using MrBayes 3.2.6 (Huelsenbeck and Ronquist 2001; Ronquist and Huelsenbeck 2003), with Markov chain Monte Carlo runs performed for 2,000,000 gen-

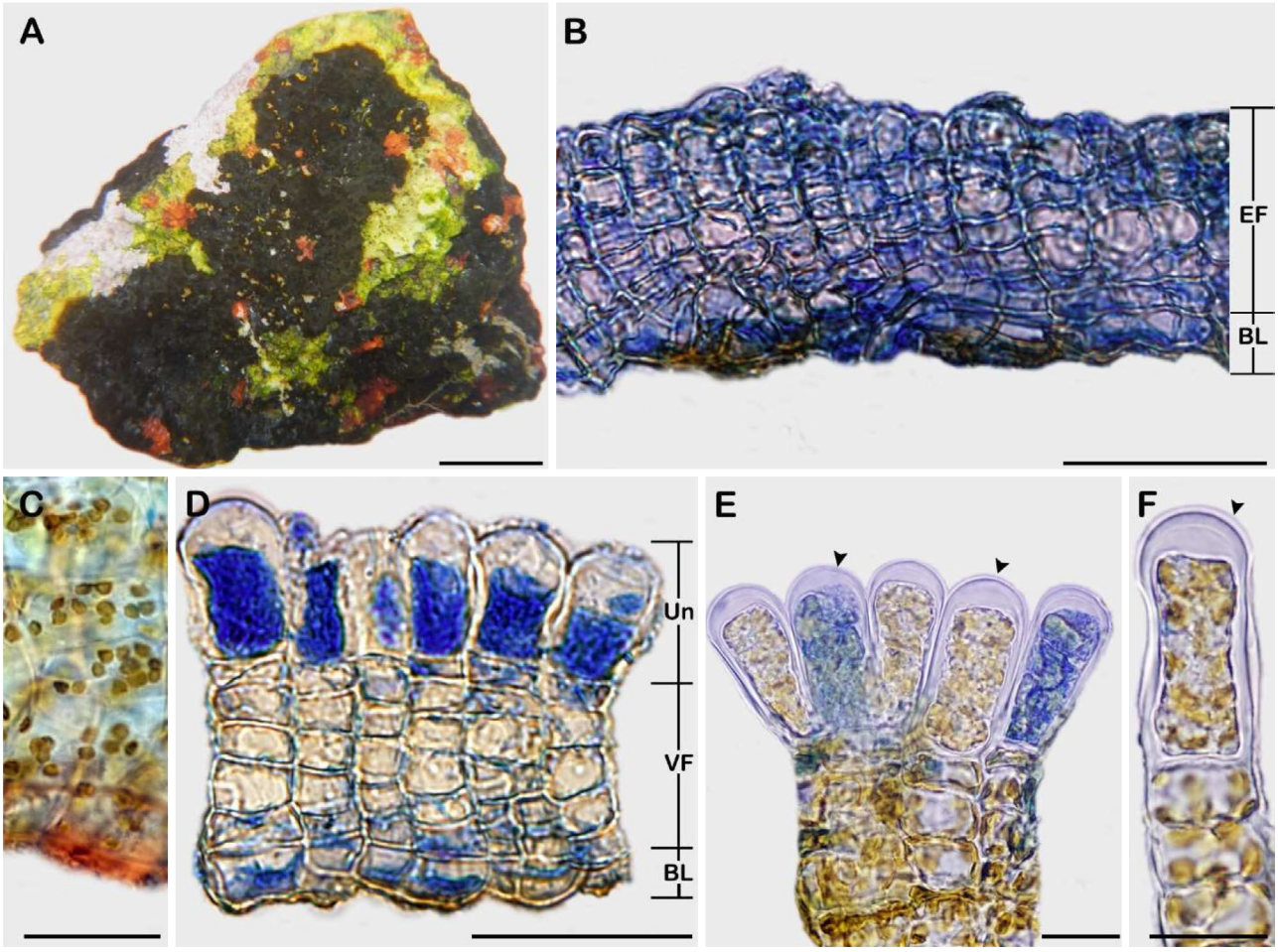

Fig. 1. Morphology of *Pseudolithoderma subextensum*. (A) Thalli on the rock forming epilithic dark chestnut brown crusts. (B) Longitudinal section views of vegetative thallus composed erect perithallial filaments (EF) and hypothallial basal layer (BL). (C) Erect perithallial filaments showing several discoid chloroplasts per cell. (D) Longitudinal section view of reproductive thallus showing unangia (Un) that are cylindrical to sub-globose, vegetative filaments (VF), and hypothallial basal layer (BL). (E and F) Unangial reproductive structure composed of cylindrical unangia (arrow heads) arising terminally on vegetative filaments. Scale bars represent: A, 1.0 cm; B and D, 50 μm; C, E and F, 20 μm.

erations. This involved one cold chain and three heated chains, using the GTR+Γ+I evolutionary model. Trees were sampled every 1,000 generations, and summary trees were generated with a burn-in value of 25%. Genetic distances were calculated using the p-distance method in Mega X (Kumar *et al.* 2018).

3. RESULTS

3.1. Morphological observations

Class Phaeophyceae F.R. Kjellman, 1891 갈조강
Order Sphacelariales Migula, 1908 갯쇠털목
Family Lithodermataceae Hauck, 1883
바위부과 (신칭)
Genus *Pseudolithoderma* Svedelius, 1911
헛바위부속 (신칭)

***Pseudolithoderma subextensum* (Waern) S. Lund, 1959** 헛바위부 (신칭) (Fig. 1A–F)
Basyonym. *Lithoderma subextensum* Waern
Holotype. UPS; Waern No. 1-2/49.
Type locality. Öregrund

Material examined. MABIK AL00100605 (=CUK 20782), Dolsan Port, Gunnae-ri, Dolsan-eup, Yeosu-si, Jeollanam-do, Korea (34°36′52.38″N, 127°43′09.55″E),

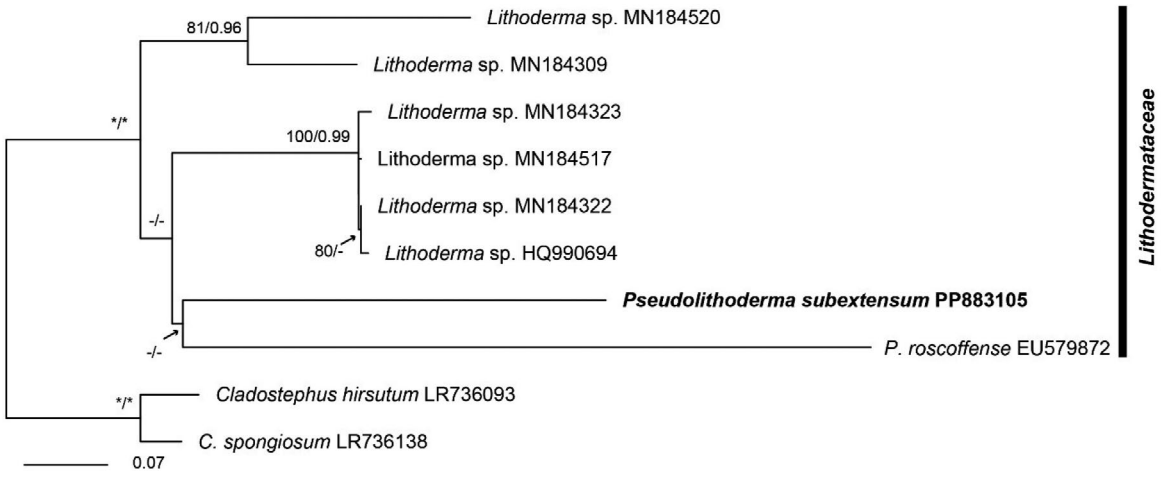

Fig. 2. Phylogenetic tree based on ML and Bayesian analysis with COI-5P sequences. Value above branches = Maximum likelihood bootstrap values in % ≥50/Bayesian posterior probabilities ≥0.75. Values lower than BS 50 or BPP 0.75 are indicated by hyphens (–). Values of BPP 1.00 or BS 100 are indicated by asterisks (*). Sequences generated in this study are highlighted in bold.

October 19, 2020, T.O. Cho and B.Y. Won, at 1 m depth by hand; CUK20783, Dolsan Port, Gunnae-ri, Dolsan-eup, Yeosu-si, Jeollanam-do, Korea (34°36′52.38″N, 127°43′09.55″E), October 19, 2020, T.O. Cho and B.Y. Won, at 1 m depth by hand.

Description. Thalli are epilithic crusts, dark chestnut brown to very dark brown, circular to irregular in outline and often confluent, without lighter margins, without growth lines and mostly smooth surface covered with thin gelatinous layer, 0.2–1.0 cm across, 65–180 µm thick, firmly attached to the substratum (Fig. 1A), and lacking rhizoids (Fig. 1B). The hypothallial basal layer is composed of 1–2 cell layers in which cells are 10–20 µm wide and have a width-to-length ratio of 1 : 0.3–0.8 and give rise to erect perithallial filaments (Fig. 1B). Erect perithallial filaments are sparsely branched, straight, and firmly adjoined with one another to form pseudoparenchymatous tissue. Cells of the erect perithallial filaments are 8–12 µm long and 7–16 µm wide. Chloroplast are discoid and several per cell (Fig. 1C). Hairs in tufts arising from the basal layer were rarely observed. Reproductive portions of the unangial sori form slightly elevated areas on the thalli (Fig. 1D). Unangia are cylindrical to sub-globose, 27–50 µm long, and 10–15 µm wide, terminally arising on erect filaments (Fig. 1E and F).

Habitat. This species has been confirmed on the southern coast (Dolsan), Korea. It grows on hard substrates such as pebbles and rocks in the intertidal zone.

World distribution. Europe, Western Atlantic, Middle East, Asia (Hong Kong and Japan) (Tanaka and Chihara 1981; Kaehler 1998; Guiry and Guiry 2024) and Korea (this study).

Identifier. Antony Otinga Oteng'o and Tae Oh Cho.

3.2. Phylogenetic analysis

The 545 base pair (bp) of the COI-5P region was sequenced from brown crustose samples collected in Korea. Phylogenetic tree was constructed by aligning the newly generated COI-5P sequences with those obtained from GenBank (Fig. 2). *Cladostephus hirsutum* (Linnaeus) Boudouresque & M. Perret-Boudouresque ex Heesch & al. and *C. spongiosum* (Hudson) C. Agardh were included as outgroup taxa. Phylogenetic analyses based on COI-5P sequences indicate that our Korean brown crustose algae are nested within the *Pseudolithoderma* clade of the Lithodermataceae (Fig. 2). There is a genetic divergence of 24.5% in the COI-5P (545 bp) between *Pseudolithoderma subextensum* and *P. roscoffense*.

4. DISCUSSION

Our morphological and molecular analyses indicate that our two unidentified brown crustose samples are *Pseudolithoderma subextensum* (Sphacelariales, Phae-

ophyceae). In Korea, there are six genera of crustose brown algae; *Endoplura* (Ralfsiaceae, Ralfsiales), *Fissipedicella* (Ralfsiaceae, Ralfsiales), *Neoralfsia* (Neoralfsiaceae, Ralfsiales), *Ralfsia* (Ralfsiaceae, Ralfsiales), *Petroderma* (Petrodermataceae, Ishigeales), and *Sungminia* (Sungminiaceae, Ralfsiales) (Oteng'o *et al.* 2020a, 2020b, 2021, 2022, 2023a, 2023b). *Pseudolithoderma* differs from *Endoplura*, *Fissipedicella*, *Neoralfsia*, *Ralfsia*, and *Sungmnia* by having thinner crustose thalli and cylindrical unangia. While *Petroderma* also has thin thalli, it is distinguished by cells with a single chloroplast and the presence of rhizoid, while *Pseudolithoderma* has cells with several chloroplasts and lacks rhizoids. These distinguishing characteristics are critical for accurate identification and classification within the diverse group of crustose brown algae found in Korea.

Pseudolithoderma subextensum (Waern) S. Lund was originally described by Waern (1949) from Sweden. It was characterized by simple erect threads, large chromatophores, thick gelatinous membrane, and elongated unilocular sporangia. Our Korean samples closely correspond to the majority of characteristics described in Waern's protologue from Öregrund, Sweden (Waern 1949), showing only unangial reproductive structures on the thalli and hairs arising from the basal layer. In contrast, Waern's protologue indicated that *P. subextensum* exhibited both unilocular and plurilocular reproductive structures on the same thalli, with hairs originating from the apices of erect filaments. However, Tanaka and Chihara (1981) pointed out that *P. subextensum* in Japan had only one type of reproductive organs born on per thallus and hairs arising from the basal layer. Similarly, Kaehler (1998) described *P. subextensum* as a new record in Hong Kong, based on the original description by Waern (1949) and the description from Japan by Tanaka and Chihara (1981), noting the presence of hair pits originating from the basal layer. These observations suggest variability in the reproductive structures and hair origin of *P. subextensum* across different geographical locations, highlighting the need for further taxonomic studies to understand its morphological diversity and distribution.

Our molecular analyses based on COI-5P indicate that *Pseudolithoderma subextensum* group together with *P. roscoffense*, although without robust support (Fig. 2). The genetic divergence between these two species is notably high at 24.5% for COI-5P sequences. In comparison, the divergence between our samples and other '*Lithoderma* sp.' sequences from GenBank ranges from 17.1% to 21.0%, which is lower than the divergence observed with *P. roscoffense*. Despite higher sequence similarities between our samples and '*Lithoderma* sp.', our morphological findings confirmed that our samples are actually *Pseudolithoderma*, not *Lithoderma*. Lund (1959) reestablished the genus *Pseudolithoderma* for species with terminal sporangia, a characteristic clearly observed in *Pseudolithoderma subextensum* (Fig. 1E and F).

In conclusion, our study makes an important contribution to the marine algal inventory of Korea by documenting the presence of the previously unreported crustose alga, *Pseudolithoderma subextensum* (Sphacelariales, Phaeophyceae). This discovery not only adds to the biodiversity records of the region but also underscores the importance of continued exploration and documentation of marine life. Consequently, we have provided new Korean names for the family, genus, and species to reflect this addition to the scientific and local community.

CRediT authorship contribution statement

AO Oteng'o: Formal analysis, Writing-original draft, Resources. **TO Cho**: Funding acquisition, Supervision. **BY Won**: Funding acquisition, Visualization.

Declaration of Competing Interest

The authors declare no conflicts of interest.

ACKNOWLEDGEMENTS

This study was supported by the Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education (2021R1I1A2059577), by the Ministry of Ocean and Fisheries (Marine Biotics Project, 20210469), and by the National Marine Biodiversity Institute of Korea (the management of Marine Fishery Bio-resources Center 2024) to Tae Oh Cho. This research was also supported by the Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education (2021R1

I1A1A01051909) to Boo Yeon Won.

REFERENCES

Guiry MD and GM Guiry. 2024. AlgaeBase. World-Wide Electronic Publication. National University of Ireland, Galway. http://www.algaebase.org. Accessed June 4, 2024.

Hauck F. 1885. Die Meeresalgen Deutschlands und Österreichs. pp. 513–575. In: Kryptogamen-Flora von Deutschland, Österreich und der Schweiz. Zweite Auflage (Rabenhorst L ed.). Vol.2. Verlag von Eduard Kummer. Leipzig, Germany.

Huelsenbeck JP and F Ronquist. 2001. MrBayes: Bayesian inference of phylogeny. Bioinformatics 17:754–755.

Kaehler S. 1998. The non-coralline epilithic encrusting algae of Hong Kong II: Additions and identification. Asian Mar. Biol. 15:1–17.

Keum YS. 2010. Sphacelariales, Cutleriales, Ralfsiales. pp. 73–108. In: Algal Flora of Korea (Shin S, ed.). Vol. 2. No. 2. *Heterokontophyta*: *Phaeophyceae*: *Ishigeales*, *Dictyotales*, *Desmarestiales*, *Sphacelariales*, *Cutleriales*, *Ralfisales*, *Laminariales*. National Institute of Biological Resources. Incheon, Korea.

Kjellman FR. 1891. Phaeophyceae (Fucoideae). pp. 176–181. In: Die natürlichen Pflanzenfamilien nebst ihren Gattungen und wichtigeren Arten, insbesondere den Nutzpflanzen, unter Mitwirkung zahlreicher hervorragender Fachgelehrten (Engler A and K Prantl, eds.). Teil 1, Abteilung 2. Verlag von Wilhelm Engelmann. Leipzig, Germany.

Kjellman FR and NL Svedelius. 1911. Lithodermataceae. pp. 173–176. In: Die natürlichen Pflanzenfamilien (Engler A and K Prantl, eds.). Nachträge zum 1 T., 2 Abt. Verlag von Wilhelm Engelmann. Leipzig, Germany.

Kumar S, G Stecher, M Li, C Knyaz and K Tamura. 2018. MEGA X: Molecular Evolutionary Genetics Analysis across computing platforms. Mol. Biol. Evol. 35:1547–1549. https://doi.org/10.1093/molbev/msy096

Lee IK and JW Kang. 1986. A check list of marine algae in Korea. Korean J. Phycol. 1:311–325.

Lee YP. 2008. Marine Algae of Jeju. Academy Publication. Seoul, Korea.

Lee YP and SY Kang. 2002. A Catalogue of the Seaweeds in Korea. Jeju National University Press. Jeju, Korea.

Lund S. 1959. The marine algae of East Greenland. I. Taxonomic Part. Medd. Grønl. 156:1–247.

Migula W. 1908–1909. Kryptogamen-Flora von Deutschland, Deutsch-Österreich und der Schweiz: im Anschluß an Thomé's Flora von Deutschland. Band II. Algen. 2. Teil. Rhodophyceae, Phaeophyceae, Characeae. Verlag Friedrich von Zezschwitz. Gera. pp. i–iv, 1–383, 122 (41 col.) pls.

Oteng'o AO and BY Won. 2020a. *Ralfsia longicellularis* (Ralfsiales, Phaeophyceae): A Far East Asian endemic brown alga from Korea. Korean J. Environ. Biol. 38:101–105. https://doi.org/10.11626/KJEB.2020.38.1.101

Oteng'o AO, BY Won and TO Cho. 2022. Proposal for the Sungminiaceae fam. nov. (Ralfsiales, Phaeophyceae) for *Sungminia* gen. nov. with three new species based on molecular and morphological analyses. J. Phycol. 58:719–728. https://doi.org/10.1111/jpy.13280

Oteng'o AO, BY Won and TO Cho. 2023a. *Endoplura gyeokpoensis* sp. nov. and *E. limpeticola* sp. nov. (Ralfsiales, Phaeophyceae), two new crustose brown algae from Korea. Phycologia 62:445–451. https://doi.org/10.1080/00318884.2023.2247713

Oteng'o AO, J Avila-Peltroche, GC Choi, TO Cho and BY Won. 2020b. New records of two brown algae, *Petroderma maculiforme* (Ishigeales, Phaeophyceae) and *Hincksia sordida* (Ectocarpales, Phaeophyceae) from Korea. Korean J. Environ. Biol. 38:416–423.

Oteng'o AO, TO Cho and BY Won. 2021. *Endoplura jejuensis* sp. nov. and *E. koreana* sp. nov. (Ralfsiales, Phaeophyceae) from Korea based on molecular and morphological analyses. Algae 36:155–163. https://doi.org/10.4490/algae.2021.36.7.22

Oteng'o AO, TO Cho and BY Won. 2023b. *Fissipedicella orientalis* gen. et sp. nov. (Ralfsiales, Phaeophyceae), a new crustose brown alga from Korea based on molecular and morphological analyses. Algae, 38:173-181.

Poong SW. 2014. Taxonomy and phylogeny of crustose brown algae (Phaeophyceae) from Malaysia and Lombok Island, Indonesia. Ph.D. Dissertation. University of Malaya. Kuala Lumpur, Malaysia.

Ronquist F and JP Huelsenbeck. 2003. MrBayes 3: Bayesian phylogenetic inference under mixed models. Bioinformatics 19:1572–1574. https://doi.org/10.1093/bioinformatics/btg180

Tanaka J and M Chihara. 1981. Taxonomic study of Japanese crustose brown algae (6). *Pseudolithoderma* (Lithodermataceae, Ralfsiales). J. Jpn. Bot. 56:377–381.

Waern M. 1949. Remarks on some Swedish *Lithoderma*. Sven. Bot. Tidskr. 43:633–670.

