



PROPOSAL FOR THE SUNGMINIACEAE FAM. NOV. (RALFSIALES, PHAEOPHYCEAE) FOR *SUNGMINIA* GEN. NOV. WITH THREE NEW SPECIES BASED ON MOLECULAR AND MORPHOLOGICAL ANALYSES¹


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Based on morphological and molecular analyses, five families have been recognized within the crustose brown algal order Ralfsiales. Our morphological and molecular sequence data were used to assess the establishment and phylogenetic relationship of *Sungminia* gen. nov. Phylogenies based on *rbcL* and concatenated *rbcL* and COI-5P genes support the recognition of *Sungminia* composed of three distinct lineages, *Sungminia gladiata* sp. nov., *S. pyriformis* sp. nov., and *S. asiatica* sp. nov. We consider that the *Sungminia* group is clearly distinct at the family level and propose to place *Sungminia* in a new family, the Sungminiaceae fam. Nov. Our phylogenetic analyses show that the Sungminiaceae forms a strongly supported monophyletic clade with probable sister relationship to the Mesosporaceae. The Sungminiaceae is characterized by perithallial erect filaments moderately adhered, the rod-shaped perithallial erect filaments, plurangia terminated with single sterile cell, and unangia terminally inserted on 1–2 celled stalk that is lateral-basal or sessile to a paraphysis.

Key index words: COI-5P; phylogeny; Ralfsiales; *rbcL*; *Sungminia asiatica* sp. nov.; *Sungminia* gen. Nov.; *Sungminia gladiata* sp. nov.; *Sungminia pyriformis* sp. nov.; Sungminiaceae fam. Nov.; taxonomy

Abbreviations: BI, Bayesian inference; BS, Bootstrap; COI-5P, 5' region of the cytochrome oxidase subunit 1 gene; CUK, herbarium of Chosun

University; GTR, General Time Reversible; MABIK, Marine Biodiversity Institute of Korea; ML, Maximum Likelihood; PP, Posterior probability; RAxML, Randomized Axelerated Maximum Likelihood

The brown algal order Ralfsiales was first described based on morphological taxonomy (Nakamura 1972). After numerous studies questioned the validity of this order (Tanaka and Chihara 1980, 1982, Nelson 1982, Kawai 1989, Silva and de Reviers 2000), the Ralfsiales has been recognized as a distinct taxonomic group by Lim et al. (2007) based on morphology and molecular data (*rbcL* gene sequences). The Ralfsiales has been recognized by emended morphological characters: (i) discoid early development of the thallus, (ii) one to several plate- or cup-shaped chloroplasts without pyrenoids, (iii) plurangia with sterile terminal cell (s) and terminal unangia and (iv) presence of crustose gametophytic or sporophytic stages in their life history (Lim et al. 2007). The classification system of the Ralfsiales at the familial level has been added and revised several times (Tanaka and Chihara 1982, Lim et al. 2007, León-Alvarez et al. 2017, Parente et al. 2021).

The principal morphological characters delineating families within the Ralfsiales are the number of plastids per cell, distinct delineation of cortex and medulla, the number of sterile cells on plurangia, and paraphyses in unangia (Tanaka and Chihara 1982, Lim et al. 2007, León-Alvarez et al. 2017, Parente et al. 2021). However, due to a lack of robust taxonomic characteristics, especially in vegetative samples, it is difficult to identify crustose

¹Received 24 April 2022. Accepted 15 July 2022.

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Editorial Responsibility: C. Schneider (Associate Editor)

[Correction added on 19 September 2022, after first online publication: The copyright line was changed.]

species correctly and recognize their taxonomic placement within the Ralfsiales based on traditional morpho-anatomical characteristics (Parente and Saunders 2019). Recently, molecular analyses have been used to infer phylogenetic relationships among genera within the Ralfsiales and to circumscribe their higher-level rank (Lim et al. 2007, León-Alvarez et al. 2017, Parente et al. 2021). The *rbcl* gene has been considered to have the most suitable resolution for discerning the ordinal and familial phylogenetic relations within the Phaeophyceae (Sasaki et al. 2001, Kawai and Sasaki 2004, Draisma et al. 2010). Currently, five families have been recognized within the Ralfsiales based on morphological and molecular analyses: Hapalospongiaceae, Mesosporaceae, Neoralfsiaceae, Pseudoralfsiaceae, and Ralfsiaceae (Farlow 1881, Lim et al. 2007, Poong et al. 2013, 2014, 2017, León-Alvarez et al. 2017, Parente et al. 2021).

Currently, 10 species within the Ralfsiales have been reported from Korea (Lee and Kang 1986, Lee and Kang 2001, Keum 2010, Oteng'o and Won 2020, Oteng'o et al. 2021). Although most crustose brown algal species have been known as species of *Ralfsia* and *Neoralfsia* based on their morphology (Lee and Kang 1986, Lee and Kang 2001, Lee 2008, Keum 2010), Oteng'o et al. (2021) recently described two new *Endoplura* species, *E. jejuensis* and *E. koreana*, within the Ralfsiaceae from Korea based on morphological and molecular analyses. We collected some unidentified samples of *Ralfsia*-like crustose brown algae along the coastlines of Korea from 2017 to 2020. Using molecular and morphological analyses, we analyze these crusts in comparison with known members of the Ralfsiales to elucidate their taxonomy and relationships.

MATERIALS AND METHODS

Specimen collections. Samples were collected from intertidal areas along the east, west, and south coastlines of Korea from 2017 to 2020. Vouchers were air-dried with fragments preserved in silica gel for molecular and anatomical analyses. Representative voucher specimens examined in this study were deposited in the herbarium of Chosun University (CUK) and Marine Biodiversity Institute of Korea (MABIK), Korea.

DNA extractions, PCR amplification, and sequencing. Genomic DNA was extracted using the NucleoSpin Plant II Kit (Macherey-Nagel, Düren, Germany) according to the manufacturer's protocol. The extracted DNA was stored at -20°C and used to amplify the *rbcl* and COI-5P genes. All polymerase chain reactions (PCRs) were performed using the HelixAmp Ready-2x-Go premix (NanoHelix Co., Ltd., Daejeon, Korea) following the manufacturer's protocol. The *rbcl* gene was amplified and sequenced in two reactions using the primer pairs ND*rbcl*2-DRL1R and DRL2F-R3A (Kogame et al. 1999, Hwang et al. 2005). The mitochondrial COI-5P gene was amplified and sequenced using GWSFn and GWSRx (Saunders and McDevit 2012). The PCR amplification for the *rbcl* gene was performed as described by Oteng'o et al. (2021) while that for the COI-5P gene was performed as described by Saunders and McDevit (2012). The additional *rbcl* gene and COI-5P sequences used in the phylogenetic

analysis were selected from GenBank (Table S1 in the supporting information).

Sequence and phylogenetic analyses. We analyzed the data sets of the *rbcl* and the concatenated *rbcl* and COI-5P gene sequence data including our sequences and sequences from members of all five extant families within the Ralfsiales to resolve phylogenetic relationships within the order (Table S1). The 34 *rbcl* and 27 COI-5P gene data sets were aligned using ClustalW (Thompson et al. 1994). *Sargassum muticum* and *Tilopteris mertensii* were selected as outgroups. Before performing the phylogenetic analyses, both the best model of nucleotide or protein evolution and the best combination of partitions were computed using PartitionFinder 2.1.1 (Lanfear et al. 2017). Maximum likelihood (ML) analysis was estimated by the General Time-Reversible (GTR) + Γ + I model with 1000 bootstrap (BS) replications using RAxMLGUI v1.5 (Silvestro and Michalak 2012). 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 2,000,000 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 25%. Phylogenetic trees inferred from concatenated *rbcl* and COI-5P gene sequence data sets (Table S1) were constructed to delimit the species, generic, and family boundaries within the Ralfsiales. The phylogenetic trees were constructed using the ML method in RAxML and MrBayes and expressed using FigTree ver. 1.4.0 (Rambaut 2012).

Morphological methods. To document their external morphology related to characters, including color, outline, and surface, the fresh samples were photographed with a waterproof digital camera (Nikon COOLPIX AW100, Nikon Corp., Japan). The samples were detached from the substrate using a single-edged blade before the analysis of anatomical characters. 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). 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 with a DP-71 camera (Olympus, Tokyo, Japan) mounted on a BX-51TRF microscope (Olympus). Digitized images were edited for clarity using Adobe Photoshop software ver. 6.1 (Adobe Systems Inc., San Jose, CA, USA).

RESULTS

Sungminia Oteng'o, Won & T.O. Cho gen. nov.

Description: Dimerous thalli are crusts, mucilaginous, firmly attached to substratum without rhizoids. They are composed of hypothallial basal layer and perithallial erect filaments. The hypothallial basal layer is formed by 1–2 prostrate filaments that are composed of cuboid to elongated cells which give rise to perithallial erect filaments. Perithallial erect filaments are straight or slightly curved, simple or sparsely branched, moderately adhering, and rod-shaped perithallial erect filaments. Apical vegetative cells are larger than the other cells. Tufts of hair in pits arise from the hypothallial basal layer or form lower to middle

portions of perithallial erect filaments. Chloroplasts are one per cell without pyrenoid. Plurangia are uniseriate or biseriata and intercalary with a terminal sterile cell. Unangial reproductive thalli produce filaments of two types, both originating from a perithallial erect cell that branches to give rise to a paraphysis and shorter stalk, which supports a terminal unangium. Unangia are sessile and lateral-basal to a paraphysis or terminally inserted on 1–2 celled stalk lateral-basal to a paraphysis. Paraphyses are of clavate shape and composed of 7–20 celled rod-shaped cells.

Etymology: The name “*Sungminia*” is in honor of Prof. Sung-Min Boo of Chungnam National University, Daejeon, Korea, for his outstanding contributions to macroalgal taxonomy and phylogeny.

Type species: *Sungminia gladiata*

***Sungminia gladiata* Oteng’o, Won & T.O. Cho sp. nov.**

Description: Dimerous thalli are small and irregularly expanded epilithic crusts, light to olive brown in color, mucilaginous, firmly attached to the substratum, lacking rhizoids, and growing to 2.3 cm in diameter and 207–456 μm thick (Fig. 1A). Hypothallial basal layers are formed by 1–2 prostrate filaments that are composed of horizontally elongated cells 7–17 μm long and 5–12 μm wide that give rise to erect perithallial filaments. Erect perithallial filaments are straight, sparsely branched below middle portions, rod-shaped, and laterally adjoined (Fig. 1B) but can be partially separated with pressure. Apical vegetative cells are 1.2–2.0 times larger than other cells of erect perithallial filaments, 9–22 μm long and 4–12 μm wide (Fig. 1C). Tufts of hairs in pits arise from the hypothallial basal layer or the lower to middle portions of erect perithallial filaments (Fig. 1D). There is one chloroplast per cell without a pyrenoid. Plurangia are uniseriate, 60–107 μm long and 3–8 μm wide, intercalary, and terminated by single sword-shaped sterile cell measuring 14–25 μm long and 1–8 μm wide (Fig. 1, E and F). Unangial reproductive thalli produce filaments of two types, both originating from erect perithallial cells that branch to give rise to a paraphysis and shorter stalk cell that supports a terminal unangium. Unangia are oblong to obovoid, terminally produced on 1–2 celled stalks, 41–70 μm long and 7–30 μm wide (Fig. 1, G and H). Paraphyses are clavate, 104–162 μm long and 4–21 μm wide, composed of 12–20 cells, and distinguished from erect perithallial filaments by their club shape and having rod-shaped cells while the latter have cells that are mostly wider than long (Fig. 1H).

Holotype (designated here): MABIK AL00084654 (= CUK19619B), *leg.* T.O. Cho, S.Y. Jeong, J. Avila, A. Oteng’o, and G.C. Choi, 01 May 2019, Seongsan Ilchul Peak, Jeju Island, Korea, 33°27’38.2” N, 126°56’05.2” E, 1–2 m depth.

Isotypes: CUK19594B, CUK19600B, CUK19607B, CUK19630B, CUK19631B.

Etymology: Named for sterile cells that are sword-shaped (= *gladiata* L. f., straight or slightly curved with parallel edges and acute apices) and terminate the plurangia.

Other specimen examined: CUK19816B, *leg.* T.O. Cho & B.Y. Won, 01 August 2019, Oeyeondo, Chungcheongnam-do, Korea, 36°13’43.4” N, 126°04’29.1” E, 1–2 m depth.

Habitat and distribution in Korea: This species is presently known from two locations, Seongsan Ilchul Peak (Jeju Island) and Oeyeondo (Chungcheongnam-do), growing on rocks and pebbles in the intertidal zone. Plurangia and unangia are found on separate thalli.

***Sungminia asiatica* Oteng’o, Won & T.O. Cho sp. nov.**

Description: Dimerous thalli are irregularly expanded, epilithic crusts, brown to yellowish brown in color, mucilaginous, firmly attached to the substratum, lacking rhizoids, and 0.3–3.3 cm in diameter and 90–611 μm thick (Fig. 2A). The monostromatic hypothallial basal layer is composed of horizontally elongated cells that are 5–16 μm long and 3–12 μm wide and give rise to erect perithallial filaments (Fig. 2, B and E). Perithallial filaments are straight to slightly curved, sparsely branched throughout, rod-shaped, and laterally adjoined (Fig. 2, B and E) but can be partially separated with pressure. Apical vegetative cells are 1.8–2.5 times larger than other cells of erect perithallial filaments, 10–22 μm long and 4–10 μm wide (Fig. 2C). Tufts of hairs in pits arise from the hypothallial basal layer or the lower to middle portions of erect perithallial filaments (Fig. 2D). There is one chloroplast per cell without a pyrenoid. Plurangia are uniseriate or biseriata, 42–62 μm long and 3–8 μm wide, intercalary, terminated by single dome-shaped sterile cells measuring 11–16 μm long and 4–7 μm wide (Fig. 2, E and F). Unangial reproductive thalli produce filaments of two types, both originating from erect perithallial cells that branch to give rise to paraphyses and shorter stalk cells that support terminal unangia. Unangia are oblong to obovoid, sessile, and lateral-basal to a paraphysis or terminally produced on 1–2 celled stalks that are 43–94 μm long and 7–45 μm wide (Fig. 2, G and H). The paraphyses are clavate, 89–157 μm long and 2–18 μm wide, composed of 10–16 cells, and distinguished from erect perithallial filaments by their club shape and having rod-shaped cells, while the latter have cells mostly wider than long (Fig. 2H).

Holotype (designated here): MABIK AL00084653 (= CUK19812B), *leg.* T.O. Cho & B.Y. Won, (01 August 2019), Oeyeondo, Chungcheongnam-do, Korea, 36°13’43.4” N, 126°04’29.1” E, 1–2 m depth.

Etymology: Named for its geographical distribution in Asia including Korea, its type locality.

Other specimens examined: CUK19175C, T.O. Cho & B.Y. Won, 08 October 2018, Gijang, Busan, Korea,

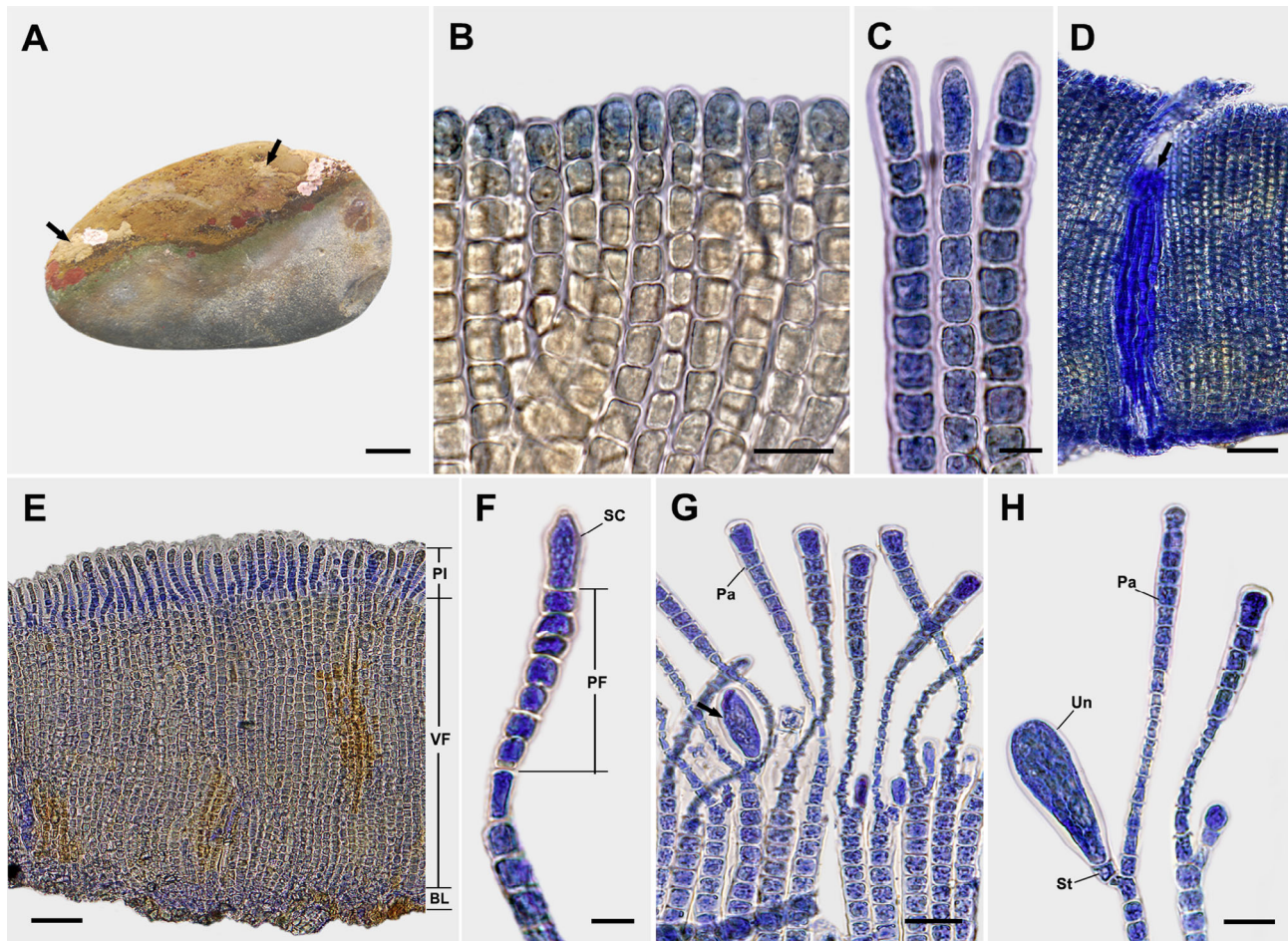


FIG. 1. *Sungminia gladiata* Oteng'o, Won & T.O. Cho sp. nov. (A) Thalli on the rock forming small irregular epilithic olive-brown crusts (arrows). Scale bar = 2 cm. (B) Longitudinal section view of vegetative thallus showing laterally adjoined erect perithallial filaments. Scale bar = 25 μ m. (C) Apical cells of vegetative erect perithallial filaments. Scale bar = 10 μ m. (D) Tufts of hairs (arrow) in pits developed from hypothallial basal layer or the lower portions of erect perithallial filaments. Scale bar = 50 μ m. (E) Longitudinal section view of reproductive thallus showing plurangia (Pl) and vegetative filaments (VF) developed from the hypothallial basal layer (BL). Scale bar = 50 μ m. (F) Plurangium composed of plurangial filament (PF) and terminal sword-shaped sterile cell (SC). Scale bar = 10 μ m. (G) Longitudinal section view of reproductive thallus showing unangia (arrow) and paraphyses (Pa). Scale bar = 25 μ m. (H) Unangium (Un) on stalk (St) with paraphysis (Pa). Scale bar = 20 μ m.

35°15'29.6" N, 129°14'06.7" E, 1–2 m depth; CUK20636A, CUK20631A&B, CUK20656, T.O. Cho, J. Avila, A. Oteng'o & G.C. Choi, 12 August 2020, Dumunjin Port, Baekryeong Island, Korea, 37°58'34.5" N, 124°37'04.2" E, 1–2 m depth; CUK20874, T.O. Cho & B.Y. Won, 28 November 2020, Daechon Port, Chungcheongnam-do, Korea, 36°19'43.9" N, 126°30'14.3" E, 1–2 m depth.

Habitat and distribution in Korea: Known from several locations, Daechon Port and Oeyeondo (Chungcheongnam-do), Dumunjin Port (Baekryeong Island), and Gijang (Busan), growing on rocks and pebbles in the intertidal zone. Plurangia and unangia are found on separate thalli.

***Sungminia pyriformis* Oteng'o, Won & T.O. Cho sp. nov.**

Description: Dimerous thalli are light to yellowish brown crusts that are mucilaginous, firmly attached

to the substratum without rhizoids, and 0.2–1.3 cm in diameter and 86–243(–392) μ m thick (Fig. 3A). Hypothallial basal layers are formed by one to several prostrate filaments that are composed of horizontally elongated cells 6–16 μ m long and 3–10 μ m wide that give rise to erect perithallial filaments (Fig. 3B). Erect perithallial filaments are straight to slightly curved, simple, rod-shaped, and laterally adjoined (Fig. 3B) but can be partially separated with pressure. Apical vegetative cells are 1.8–2.4 times larger than other cells of erect perithallial filaments, 12–23 μ m long and 5–12 μ m wide (Fig. 3C). Tufts of hairs arise in pits from the hypothallial basal layer or the lower to middle portions of erect perithallial filaments (Fig. 3D). There is one chloroplast per cell without a pyrenoid. Plurangia are uniseriate or biseriata, 31–48 μ m long and 5–10 μ m wide, intercalary, and terminated by single pyriform

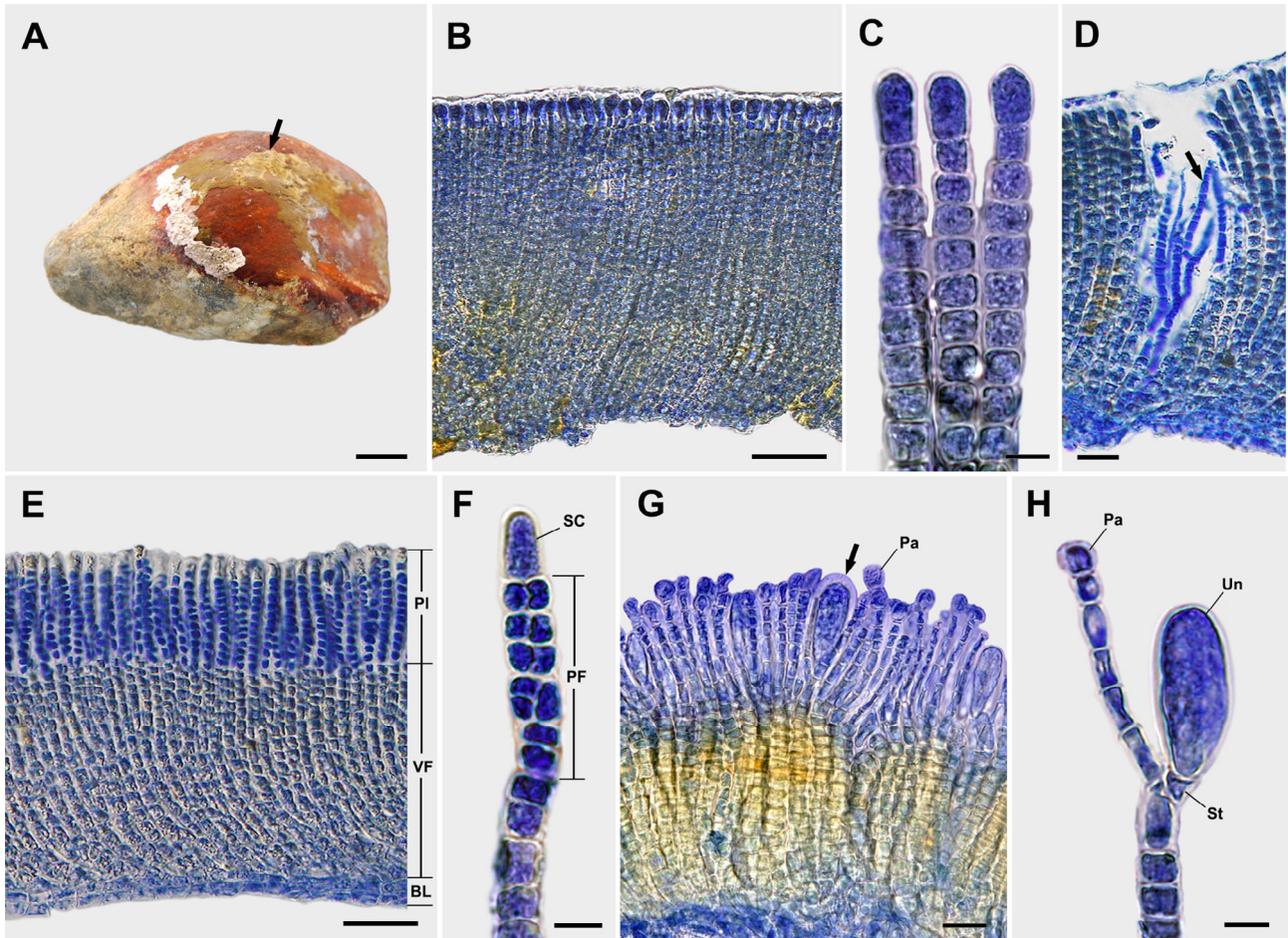


FIG. 2. *Sungminia asiatica* Oteng'o, Won & T.O. Cho sp. nov. (A) Thalli on the rock forming an irregular yellowish-brown crust (arrow). Scale bar = 2 cm. (B) Longitudinal section view of vegetative thallus showing laterally adjoined erect perithallial filaments. Scale bar = 50 μ m. (C) Apical cells of vegetative erect perithallial filaments. Scale bar = 10 μ m. (D) Tufts of hairs (arrow) in pits developed from hypothallial basal layer or the lower to middle portions of erect perithallial filaments. Scale bar = 25 μ m. (E) Longitudinal section view of a reproductive thallus showing plurangia (Pl) and vegetative filaments (VF) developed from the hypothallial basal layer (BL). Scale bar = 50 μ m. (F) Plurangium composed of plurangial filament (PF) and terminal dome-shaped sterile cell (SC). Scale bar = 10 μ m. (G) Longitudinal section view of reproductive thallus showing unangia (arrow) and paraphyses (Pa). Scale bar = 25 μ m. (H) Unangium (Un) on stalk (St) with paraphysis (Pa). Scale bar = 10 μ m.

sterile cells measuring 16–26 μ m long and 5–15 μ m wide (Fig. 3, E and F). Unangial reproductive thalli produce filaments of two types, both originating from an erect perithallial cell that branches to give rise to a paraphysis and shorter stalk cell that supports a terminal unangium. Unangia are obovoid and terminally produced on 1–2 celled stalks, 80–131 μ m long and 7–34 μ m wide (Fig. 3, G and H). Paraphyses are clavate, 171–268 μ m long and 6–26 μ m wide, composed of 7–10 cells, and distinguished from erect perithallial filaments by their clavate shape and having rod-shaped cells, while the latter have cells mostly wider than long (Fig. 3H).

Holotype (designated here): MABIK AL00084652 (= CUK19694A), leg. T.O. Cho & B.Y. Won, 06 June 2019, Saegdal-dong, Jeju Island, Korea, 33°14'28.6" N, 126°23'52.6" E, 1–2 m depth.

Isotypes: CUK19697B.

Etymology: Named for the pear-shaped sterile cells (=pyriformis, L. f.)

Other specimen examined: CUK18425, T.O. Cho, S.Y. Jeong, J. Avila & A. Oteng'o, 03 November 2017, Dala Park, Gyeongsangnam-do, Korea, 34°46'11.4" N, 128°23'51.5" E, 1–2 m depth.

Habitat and distribution in Korea: This species is presently known from two locations, Dala Park (Gyeongsangnam-do) and Saegdal-dong (Jeju Island), growing on rocks and pebbles in the intertidal zone. Plurangia and unangia are found on separate thalli.

Phylogenetic analyses. A total of 15 sequences from the *rbcL* (1279 bp) and COI-5P (657 bp) loci were obtained from samples collected from Korea (Table S1). Phylogenetic analyses of *rbcL* gene data set and concatenated data sets showed similar topologies in ML and Bayesian analyses (Fig. 4, Fig. S1 in

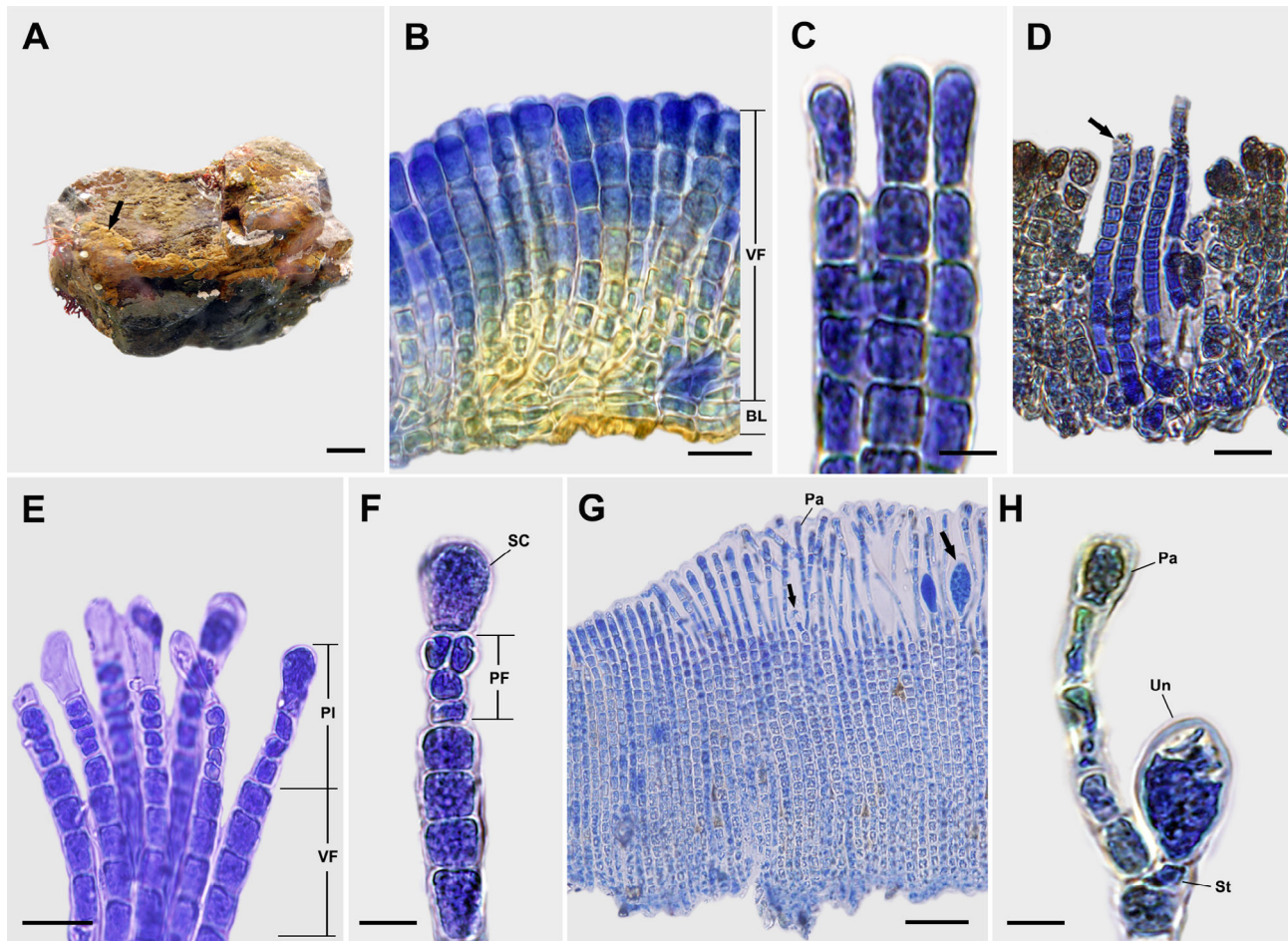


FIG. 3. *Sungminia pyriformis* Oteng'o, Won & T.O. Cho sp. nov. (A) Thallus on the rock forming irregular epilithic yellowish-brown crusts (arrow). Scale bar = 2 cm. (B) Longitudinal section view of vegetative thallus showing laterally adjoined perithallial erect filaments composed of vegetative filaments (VF) and hypothallial basal layer (BL). Scale bar = 25 μ m. (C) Apical cells of vegetative erect perithallial filaments. Scale bar = 10 μ m. (D) Tufts of hairs (arrow) in pits developed from the hypothallial basal layer or the lower portions of erect perithallial filaments. Scale bar = 20 μ m. (E) Longitudinal section view of reproductive thallus showing plurangia (PI) and vegetative filaments (VF). Scale bar = 20 μ m. (F) Plurangium composed of plurangial filament (PF) and terminal pyriform sterile cell (SC). Scale bar = 10 μ m. (G) Longitudinal section view of reproductive thallus showing young and mature unangia (arrows) and paraphyses (Pa). Scale bar = 50 μ m. (H) Unangium (Un) on stalk (St) with paraphysis (Pa). Scale bar = 10 μ m.

the Supporting Information). *Sungminia* gen. nov. formed a monophyletic clade with strong support (100%/1.00 for ML and BPP) with three distinct new species described below (*Sungminia asiatica* sp. nov., *S. gladiata* sp. nov., and *S. pyriformis* sp. nov.; Fig. 4). *Sungminia pyriformis* was a sister clade to *S. gladiata* in the *rbcl* tree (Fig. 4), while it was nested sister to *S. asiatica* in concatenated tree (Fig. S1). These three *Sungminia* species were resolved with full support as monophyletic from the remaining genera within the Ralfsiales (Fig. 4). Sequence divergences between *Sungminia* gen. nov. and other genera within the Ralfsiales were 9.6–13.9% for the *rbcl* gene and 30.0–35.4% for the COI-5P gene (Table 1). Interspecific sequence divergences between *S. asiatica* and *S. gladiata* were 3.0% for *rbcl* and 9.3% for COI-5P; between *S. asiatica* and *S. pyriformis*, they were 2.8–2.9% for *rbcl* and 8.9% for COI-5P; between *S. pyriformis* and *S.*

gladiata, they were 2.2–2.4% for *rbcl* and 8.8% for COI-5P.

DISCUSSION

We propose a new genus, *Sungminia* gen. nov., containing three new species based on morphological and molecular analyses. The sequences of *rbcl* and COI-5P from the specimens collected from Korea were nested as a distinct clade, *Sungminia*, composed of *S. gladiata*, *S. asiatica*, and *S. pyriformis*. *Sungminia* is characterized by (i) thalli with perithallial erect filaments moderately adhered, (ii) the rod-shaped perithallial erect filaments, (iii) hairs in tufts in pits, (iv) single chloroplasts per cell without pyrenoid, (v) plurangia with one apical sterile cell and (vi) unangia are sessile or terminally inserted on a stalk lateral-basal to a paraphysis. *Sungminia gladiata*

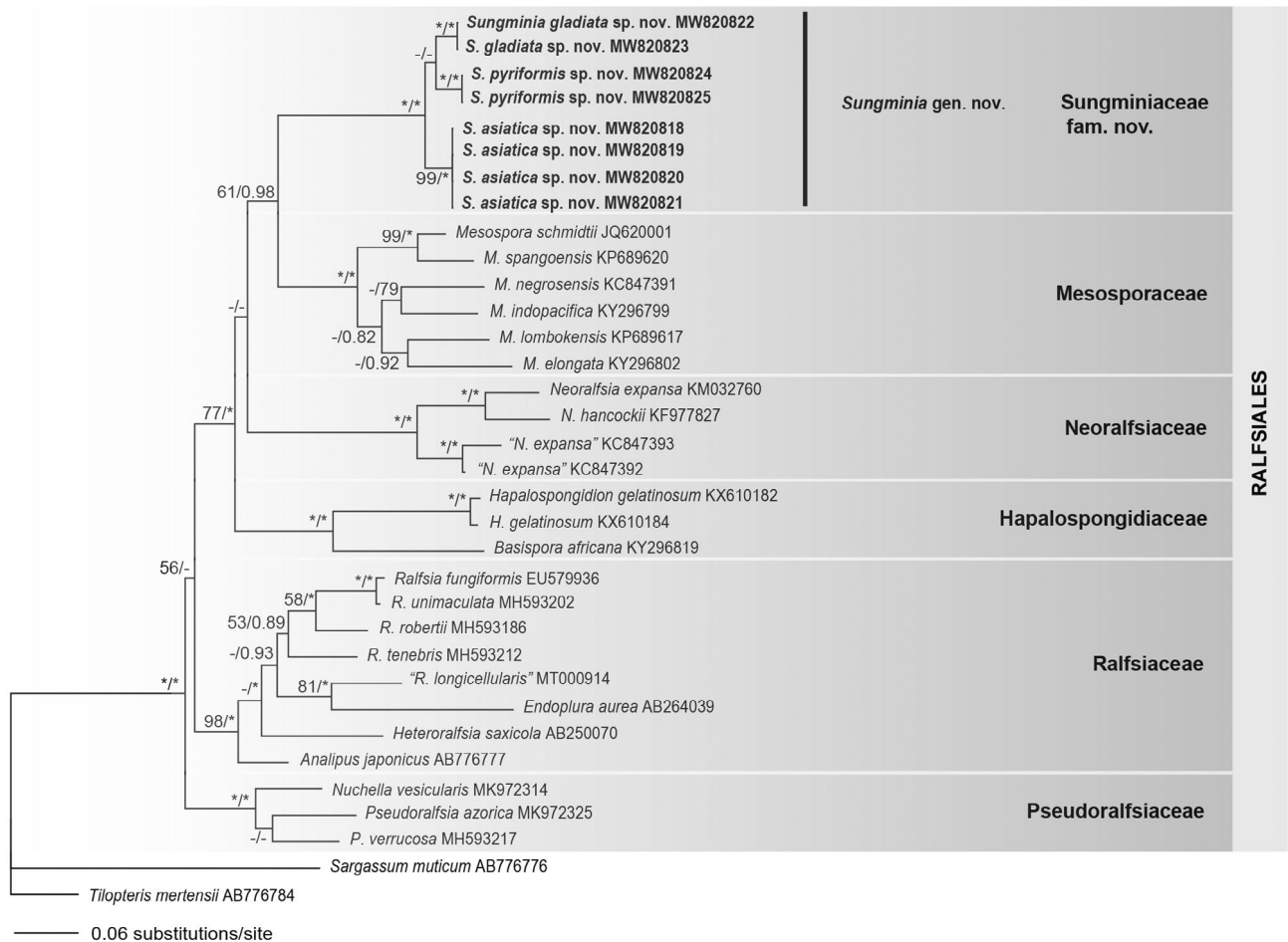


FIG. 4. Maximum likelihood tree based on *rbcL* gene sequence alignment for the Sungminiaceae fam. Nov. and other Ralfsiales species. The value above branches = Maximum likelihood bootstrap values in % > 50, Bayesian posterior probabilities >0.75. Values lower than BS 50 or BPP 0.75 are indicated by hyphens (-). Values of BS 100 or BPP 1.00 are indicated by asterisks (*). Taxa in bold text are described in this paper.

sp. nov. is distinguished from the other two species by having long uniseriate plurangia terminated with sword-shaped sterile cells. *Sungminia pyriformis* sp. nov. is distinguished from the other two species by having simple perithallial erect filaments and plurangia with terminal pyriform sterile cells. *Sungminia asiatica* sp. nov. differs from the former two new species by having a monostromatic hypothallial basal layer, and plurangia terminated by dome-shaped sterile cells. The shape of sterile cells may be recognized one of key characteristics for distinguishing each species of this genus: sword-shaped in *S. gladiata*, pear-shaped in *S. pyriformis*, and dome-shaped in *S. asiatica*.

The Ralfsiales is an order of crustose brown algae containing five families: Hapalospongidiaceae, Mesosporaceae, Neoralfsiaceae, Pseudoralfsiaceae, and Ralfsiaceae (Farlow 1881, Tanaka and Chihara 1982, Lim et al. 2007, León-Alvarez et al. 2017, Parente et al. 2021). Molecular analyses of crustose brown algae have expanded our understanding of

the phylogenetic affinities between related families of the Ralfsiales and have helped us infer their taxonomic positions. Our phylogenetic analyses of *rbcL* and concatenated *rbcL* and COI-5P gene sequences show that *Sungminia* forms a strongly supported monophyletic clade and a probable sister relationship with the Mesosporaceae. Our multigene sequence analyses revealed the genetic differences in *rbcL* gene sequences (9.8–11.8%) and COI-5P gene (30.0–34.3%) between *Sungminia* and *Mesospora*, while in *rbcL* gene sequences (9.6–13.9%) and COI-5P gene (31.5–35.4%) between *Sungminia* and other genera within the Ralfsiales, respectively. As a comparison, families recognized within the Ralfsiales differed by $\geq 9.7\%$ in the *rbcL* gene (León-Alvarez et al. 2017, Parente et al. 2021). We propose that *Sungminia* can be recognized as the basis for a new, higher-level taxon within the Ralfsiales.

While molecular data can provide compelling evidence for erecting a new taxon, they should not be the sole basis for determining higher-level

TABLE 1. Gene sequence divergences (*rbcL*/COI-5P) among families including Sungminiaceae fam. Nov. within Ralfsiales.

% gene sequence divergence	1	2	3	4	5	6
1 Sungminiaceae fam. Nov.	—	—	—	—	—	—
2 Mesosporaceae	9.8–11.8/30.0–34.3	—	—	—	—	—
3 Hapalospongiaceae	11.9–13.0/32.8–34.3	10.9–12.8/23.2–26.2	—	—	—	—
4 Neoralfsiaceae	10.4–13.9/32.9–34.1	10.9–13.7/22.8–24.7	11.3–14.0/21.2–22.2	—	—	—
5 Ralfsiaceae	9.6–13.5/31.5–34.4	9.1–12.8/22.2–26.8	11.3–13.6/20.2–21.9	10.0–13.6/20.0–23.5	—	—
6 Pseudoralfsiaceae	10.4–12.6/33.3–35.4	9.4–11.1/23.8–28.8	11.8–13.8/22.2–24.8	11.1–13.5/22.2–25.0	7.5–11.1/21.9–25.6	—

classification to the exclusion of vegetative and reproductive morphology. We have revisited the morphological descriptions and compared the most common characteristics of *Sungminia* with genera of the other families in the Ralfsiales (Table 2). *Sungminia* and *Mesospora* (Mesosporaceae) share morphological features of the absence of medullary layer and rhizoids, and cells with a single chloroplast lacking pyrenoids. However, *Sungminia* is distinguished from *Mesospora* by having unangia developed on short 1–2 celled stalk-like filaments, paraphyses in unangial reproductive thalli, and erect perithallial filaments moderately adhered. *Sungminia* is also distinguished from *Hapalospongidion* (Hapalospongiaceae) which has unangia developed on long 7–19 (–31) celled stalk-like filaments without paraphyses (León-Alvarez et al. 2017), from *Neoralfsia* (Neoralfsiaceae) which has distinct delineation of cortex and medulla and rhizoids on the base of thallus (Lim et al. 2007), and from *Pseudoralfsia* (Pseudoralfsiaceae) and the crustose genera (*Ralfsia*, *Endoplura*, and crustose phase of *Heteroralfsia*) (Ralfsiaceae) which have firmly adhering erect filaments (Farlow 1881, Hollenberg 1969, Parente et al. 2021; Table 2).

The reproductive structure of unangia has been used to distinguish the families within the Ralfsiales (Tanaka and Chihara 1982, Lim et al. 2007, León-Alvarez et al. 2017, Parente et al. 2021). There are two types: unangia with and without paraphyses. Unangia without paraphyses have been known to occur in the Hapalospongiaceae and Mesosporaceae (Tanaka and Chihara 1982, León-Alvarez et al. 2017). However, in the Hapalospongiaceae, unangia originate on the stalk-like filaments laterally developed from hypothallial basal layer, whereas in the Mesosporaceae, unangia originate on the stalk-like filaments laterally developed from a basal cell of perithallial erect filament. Unangia with paraphyses have been known to occur in the Neoralfsiaceae, Pseudoralfsiaceae, and Ralfsiaceae (Hollenberg 1969, Lim et al. 2007, Parente et al. 2021) along with the Sungminiaceae, proposed below. However, unangia in the Sungminiaceae differ from unangia in the Neoralfsiaceae (3–6 celled), Pseudoralfsiaceae (1–3 celled), and Ralfsiaceae (1–6 celled) in that they develop from 1–2 celled short stalk-like filaments (Table 2). Also, because the detailed morphologies of unangia (e.g., stalk-like filaments, paraphyses, position of unangia) vary within families, the combinations of these characteristics may be important in taxonomy for each group of different levels within the Ralfsiales. Our morpho-anatomical observations and phylogenetic analyses support monophyly of the *Sungminia* clade within the order Ralfsiales as a probable sister taxon to the Mesosporaceae. We consider that the *Sungminia* group is clearly distinct at the family level and therefore propose to place the *Sungminia* in a new family designated here:

TABLE 2. Comparison of morphological features among *Sungminia* of Sungminiaceae fam. Nov. and genera of the other families within Ralfsiiales.

Taxa/Characteristics	Sungminiaceae fam. Nov. (<i>Sungminia</i> gen. Nov.)	Hapalospongidiaceae (<i>Hapalospongidium</i>)	Mesosporaceae (<i>Mesospora</i>)	Neoralisiaceae (<i>Neoralisia</i>)	Pseudoralisiaceae (<i>Pseudoralisia</i> , <i>Nuchella</i>)	Ralfsiaceae (<i>Ralfsia</i> , <i>Endophara</i> , <i>Heteroralisia</i>)
Erect perithallial erect filaments	Moderately adhered	Loosely adhered	Loosely adhered	Firmly adhered	Firmly adhered	Firmly adhered
Shape of erect perithallial filaments	Rod-shaped	Tapered downward	Tapered downward	Tapered downward	Rod-shaped	Tapered upward or rod-shaped
Distinct delineation of cortex and medullar layer	No	No	No	Yes	No	No/yes
Form of hairs	Tuft	Single/tuft	Tuft	Hair pit	Hair pit	–
No. of plastids per cell	One	One to several	One to several	One	One	One to several
No. of sterile cell on plurangia	One	One to several	One or three	One	One or two	One to several
Unangia	Sessile or on stalk-like filament	On stalk-like filament	On stalk-like filament	On stalk-like filament	Sessile or on stalk-like filament	Sessile or on stalk-like filament
No. of cells in stalk-like filaments	1–2	7–19 (31)	5–12	3–6	1–3	1–6
Paraphyses with unangium	Present	Absent	Absent	Present	Present	Present
No. of cells in paraphyses	7–20	–	–	–	Up to 13	(6)–8–12
Rhizoids	Absent	Absent	Absent	Present	Present or absent	Present or absent
Reference	This study	León-Alvarez et al. 2017	Weber-van Bosse 1911, Tanaka and Chihara 1982	Lim et al. 2007	Parente et al. 2021	Farlow 1881, Hollenberg 1969

Sungminiaceae Oteng'o, Won & T.O. Cho fam. Nov.

Description: Dimerous thalli are mucilaginous crusts firmly attached to the substratum without rhizoids. A hypothallial basal layer with one to several cells gives rise to erect perithallial filaments. Erect perithallial filaments are moderately adjoined and rod-shaped, and their and terminal cells are larger than other cells. Tufts of hair arise in pits arise from the hypothallial basal layer or form in lower to middle portions of erect perithallial filaments Chloroplasts are one per cell and lack a pyrenoid. Plurangia are intercalary and terminated with single sterile cell. Unangial reproductive thalli produce filaments of two types, both originating from an erect perithallial cell that branches to give rise to a paraphysis and shorter stalk that supports a terminal unangium. Unangia are sessile or lateral-basal to a paraphysis or terminally produced on 1–2 celled stalk or lateral-basal to a paraphysis. Paraphyses are distinguished from erect perithallial filaments by their clavate shape and having rod-shaped cells.

Type genus: *Sungminia* Oteng'o, Won & T.O. Cho gen. Nov.

In our study, we confirmed that all of the known species in the Sungminiaceae are presently distributed only along the coastline of Korea. Future studies based on more extensive collections worldwide are necessary to understand the geographic ranges of species in the Sungminiaceae and to further investigate the relationships of this distinct group of crustose algae.

We thank to Dr. Craig Schneider for many constructive suggestions to improve the manuscript. This study was supported by the Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education (2021R111A2059577), by the Ministry of Ocean and Fisheries (MarineBiotics Project, 20210469), and by the National Marine Biodiversity Institute of Korea (2022M01100) 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 (2021R111A1A01051909) to Boo Yeon Won.

AUTHOR CONTRIBUTIONS

A.O. Oteng'o: Conceptualization (equal); data curation (equal); formal analysis (equal); methodology (equal); writing – original draft (equal). **B.Y. Won:** Data curation (equal); formal analysis (equal); project administration (equal); software (equal); writing – review and editing (equal). **T.O. Cho:** Funding acquisition (equal); project administration (equal); supervision (equal); writing – review and editing (equal).

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Supporting Information

Additional Supporting Information may be found in the online version of this article at the publisher's web site:

Figure S1. Maximum likelihood tree based on concatenated DNA sequence (plastid *rbcL* and mitochondrial COI-5P) alignments for the Sungminiaceae fam. Nov. and other Ralfsiales species. The 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 BS 100 or BPP 1.00 are indicated by asterisks (*). Taxa in bold text are described in this paper.

Table S1. List of specimen information used in molecular analyses (*rbcL*, COI-5P). “–” indicates no sequence obtained.