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Taxonomy and Phylogeny of Creeping Brown Algae, Ralfsiales (Phaeophyceae)

Graduate School of Chosun University

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글로벌바이오융합학과

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ABSTRACT

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The order Ralfsiales is one of the twenty recognized brown algal orders in class Phaeophyceae. Members of the Ralfsiales are distinguished by their: 1) discoid early development of the thallus; 2) one to several plate- or cup-shaped chloroplasts without pyrenoids; 3) plurangia with sterile terminal cell(s) and terminal unangia; and 4) presence of creeping (crustose) gametophytic or sporophytic stage(s) in the life history. The Ralfsiales thalli are predominantly crusts with simple morphology. It is difficult to correctly identify creeping species in this order based on traditional morpho-anatomical characteristics because they lack robust taxonomic characters, especially for sterile samples. But recently, DNA sequencing of COI-5P and *rbc*L genes and phylogenetic studies have provided a family to species level resolution in the taxonomy of this group. Currently, there are 5 families, 10 genera and 41 species recognized worldwide. In Korea, 3 families, 5 genera and 8 species have been reported. In this study, I focused on the taxonomy of order Ralfsiales in Korea using the contemporary molecular and morphological approaches. A total of 700 creeping brown algal specimens were collected from 2013 to 2021 at different localities along the Korean coastal shore and Vladivostok, Russia. I generated a set of morpho-anatomical characters useful for species identification by sequencing morphologically variable specimens and matching morphological features to their respective clades in the molecular data. Phylogenetic analyses of COI-5P and *rbc*L gene sequence data sets demonstrated that there are several new taxa of the order Ralfsiales in Korea. These included 1 new family, 4 new genera, 13 new species, and 1 new combination. The study also uncovered 2 unreported genera (*Endoplura* and *Mesospora*) in Korea. The new taxa are listed in this study: 1) new family Sungminiaceae, fam. nov., 2) 4 new genera; *Sungminia* gen. nov., *Neoralfsiopsis* gen. nov., *Fissipedicella* gen. nov., *Ramipedicella* gen. nov., 3) 13 new species; *Sungminia asiatica* sp. nov., *Sungminia gladiata* sp. nov., *Sungminia pyriformis* sp. nov., *Mesospora bifurcata* sp. nov., *Neoralfsiopsis jejuensis* sp. nov., *Endoplura geojensis* sp. nov., *Endoplura gyeokpoensis* sp. nov., *Endoplura jejuensis* sp. nov., *Endoplura koreana* sp. nov., *Endoplura limpeticola* sp. nov., *Fissipedicella orientalis* sp. nov., *Ralfsia claviformis* sp. nov., *Ramipedicella microloba* sp. nov. Therefore, through this study, 18 new taxa belonging to the order Ralfsiales, one new combination, and two Korean new records were recognized.

초록

포복형 갈조류 바위딱지목(Ralfsiales)의 계통분류에 관한 연구

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갈조강(Phaeophyceae)은 20 개 목(order)들로 구성되어져 있다. 이들 중 하나이다. 바위딱지목(Ralfsiales)은 1) 엽체의 원반(discoid) 형의 초기 발달, 2) 피레노이드가 없는 하나 또는 여러 개의 판 또는 컵모양의 엽록체, 3) 말단에 불염세포를 갖는 plurangia 와 말단 위치한에 있는 unangia, 4) 생활사 중 포복(creeping /crustose) 형태의 배우체와 포자체가 있다는 것으로 다른 목들과 구분된다. 바위딱지목(Ralfsiales)에 속하는 종들은 단단한 기질에 부착하여 포복을 이루고 단순한 형태를 갖고 있기 때문에 종 동정에 어려움이 큰 해조류 분류군들로 알려져 있다. 특히 생식기를 갖지 않는 영양식물체들은 기존의 해부형태학적(morpho-anatomical) 연구만으로 종 동정을 명확히 하기가 어려운 특성을 나타낸다. 하지만, 최근 DNA 염기서열 분자분석은 이들 형태적 분류의 어려움을 겪는 분류군들에 대한 동정과 계통분류에 많은 정보를 제공하고 있다. 본 연구는

바위딱지목(Ralfsiales)을 대상으로 형태 및 분자분석에 기초하여 종을 동정하고 계통을 분석하고자 수행 되었다. 현재, 바위딱지목(Ralfsiales)은 전세계에 5 과, 10 속, 41 종이 알려지고 있고, 한국에는 3 과, 5 속, 8 종이 보고되고 있다. 본 연구를 통해 2013 년부터 2021 년까지 우리나라 전 연안에서 700 여개의 샘플들을 채집하였으며, 각 종들의 유용한 형태형질 파악을 위해 외부 및 내부 해부학적 관찰들을 이루었고, 분자계통분석은 명확한 특성이 파악된 개체들을 대상으로 다양한 표본에서 *rbc*L 과 COI-5P 의 DNA 염기서열들을 생성하여 GenBank 에 등록하고 기존의 유사 분류군들의 GenBank 염기서열들과 비교분석 하였다. 이러한 형태학적, 분자적 연구를 통하여 1 개의 신과(new family), 4 개의 신속(new genera), 13 개의 신종(new species)을 인식 하였고, 이외에도 1 개의 신조합명(new combination)과 2 개의 미기록속(*Endoplura, Mesospora*)을 확인하였다. 본 연구를 통해 새로이 추가되는 바위딱지목(Ralfsiales) 내의 신분류군은 목록은 1) 신과 Sungminiaceae fam. nov. 2) 신속 *Sungminia* gen. nov., *Neoralfsiopsis* gen. nov., *Fissipedicella* gen. nov., *Ramipedicella* gen. nov., 3) 신종 *Sungminia asiatica* sp. nov., *Sungminia gladiata* sp. nov., *Sungminia pyriformis* sp. nov., *Mesospora bifurcata* sp. nov., *Neoralfsiopsis jejuensis* sp. nov., *Endoplura geojensis* sp. nov., *Endoplura gyeokpoensis* sp. nov., *Endoplura jejuensis* sp. nov., *Endoplura koreana* sp. nov., *Endoplura limpeticola* sp. nov., *Fissipedicella orientalis* sp. nov., *Ralfsia claviformis* sp. nov., *Ramipedicella microloba* sp.

nov.이다. 따라서, 본 연구를 통해 바위딱지목(Ralfsiales)에 속한 신분류군 18 개와 신조합 1 개 및 한국 미기록 2 개가 인식되었다.

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I. INTRODUCTION

1. Taxonomic history of the order Ralfsiales Nakamura ex P.-E. Lim and H. Kawai

The order Ralfsiales Nakamura ex P.-E. Lim and H. Kawai was first proposed on the basis of genus *Ralfsia* to accommodate the creeping (crustose) brown algal families Lithodermataceae Hauck, Nemodermataceae Feldmann and Ralfsiaceae Farlow (Nakamura 1972). This order was mainly characterized by: 1) one plate-shaped, parietal chloroplast without pyrenoids in each cell; 2) isomorphic life history with creeping thalli; and 3) discoid in early development of the thallus. Distingiushing the three families was mainly based on their reproductive morphology. Lithodermataceae and Nemodermataceae have terminal unangia and plurangia and intercalary unangia and lateral plurangia respectively, but Ralfsiaceae was identified by having lateral unangia and intercalary plurangia. Nakamura (1972) also implied on the possibility of transfering Lithodermataceae and Nemodermataceae into the family Ralfsiaceae.

Nakamura (1972) provided no diagnosis, thus the name failed to meet the requirement of the International Code of Botanical Nomenclature, and therefore the order was not valid (see McNeill et al. 2006: Art 36.2 of the International Code of Nomenclature for algae, fungi and plants). Although the newly proposed order Ralfsiales was recognized by some authors, numerous studies challenged its validity. For instance, the reliability of three diagnostic characters for the Ralfsiales, single parietal chloroplast without pyrenoid, isomorphic life history and discoid early development of thallus, as proposed by Nakamura, was questioned (Wynne and Loiseaux 1976). Nelson (1982) did not accept the order Ralfsiales due to qualifications or exceptions in all the three diagnostic characters prompting her to conclude that the order was unwarranted and instead proposed including all creeping brown algae in Ralfsiaceae within order Ectocarpales. Some authors recognized the order but emended the distinguishing features to exclude the number of chloroplasts (Tanaka and Chihara 1980a, 1981b, 1982, Silva and de Reviers 2000). Also, *Gobia saxicola* Okamura and Yamada originally described under the Chordariaceae having a creeping gametophytic stage in its life history was later transferred to Ralfsiaceae (Ralfsiales) as *Heteroralfsia saxicola* (Okamura and Yamada) Kawai, thus including both isomorphic and heteromorphic life history patterns in the Ralfsiales (Kawai 1989) instead of only isomorphic life history with creeping thalli as proposed earlier on by Nakamura. The families Mesosporaceae and Ralfsiaceae were placed in order Chordariales instead of Ralfsiales (Phillips and Price 1997). Hardy and Guiry (2003) also recognized the order Ralfsiales, and transferred the genera *Sorapion*, *Stragularia* and *Symphyocarpus* to the family Scytosiphonaceae in order Ectocarpales. Costello et al. (2021) recognized the order Ralfsiales as distinct group while placing families Lithodermataceae and Scytosiphonaceae in the Ectocarpales.

In the 1990s to mid-2000s, DNA sequencing technology led to a number of phylogenetic studies of the Phaeophyceae using several molecular markers (Tan and Druehl 1994, Siemer et al. 1998, Boo et al. 1999, Rousseau and de Reviers 1999, Draisma et al. 2001, Sasaki et al. 2001, Cho et al. 2004, Kawai and Sasaki 2004, Kawai et al. 2005). For instance, molecular phylogeny based on 18S rDNA revealed that the order Ectocarpales was polyphyletic with *Analipus japonicus* (Harvey) Wynne closely related with *Ralfsia fungiformis* (Gunnerus) Setchell and Gardner and distant from Ectocarpales (Tan and Druehl 1994). In comparison with other DNA markers, plastid ribulose-1,5-bisphosphate carboxylase large subunit (*rbc*L) gave the best resolution for discerning ordinal and familial phylogenies in the Phaeophyceae (Draisma et al. 2001, Sasaki et al. 2001, Kawai and Sasaki 2004). Molecular tools have helped to resolve the confusion and contradictions that came up in the past regarding the validity of the order Ralfsiales and its recognized families (e.g., Fletcher 1978, Nelson 1982). Phylogeny based on *rbc*L and *cox*1 genes demonstrated that the Lithodermataceae are distantly related to Ralfsiales (Bittner et al. 2008). Phillips et al. (2008) establish order Nemodermatales for family Nemodermataceae based on *rbc*L and LSU rDNA phylogeny.

Ralfsiales as currently recognized, was validated and amended by Lim et al. (2007) based on the *rbc*L gene sequence data of *Ralfsia fungiformis*. Ralfsiales are now distinguished from other orders of Phaeophyceae Kjellman by their: 1) discoid early development of the thallus; 2) one to several plate- or cup-shaped chloroplasts without pyrenoids; 3) plurangia with sterile terminal cell(s) and terminal unangia; and 4) presence of creeping gametophytic or sporophytic stage(s) in the life history (Lim et al. 2007). The order Ralfsiales currently constitutes five recognized families: Hapalospongidiaceae Reyes Gómez and León-Alvarez, Mesosporaceae J. Tanaka and Chihara, Neoralfsiaceae P.-E. Lim and H. Kawai, Pseudoralfsiaceae Parente, Fletcher and G.W. Saunders and Ralfsiaceae W.G. Farlow (Guiry and Guiry 2021).

1.1. Taxonomic history of the family Hapalospongidiaceae Reyes Gómez and León-Álvarez

The family Hapalospongidiaceae Reyes Gómez and León-Alvarez was recently proposed based on morphological and molecular (*rbc*L and *cox*1 genes) analyses of *Hapalospongidion gelatinosum* De A. Saunders from California (León-Álvarez et al. 2017). Hapalospongidiaceae is a monogeneric family identified by the characteristics of its type genus, *Hapalospongidion* Saunders. The genus *Hapalospongidion* (derived from Greek words: *apalos* meaning soft; and *spongos* meaning “a sponge”) was established by Saunders (1889) to accommodate *H. gelatinosum*. *Hapalospongidion* is mainly characterized by a cushion-like creeping and gelatinous thallus, with an orbicular and confluent outline, irregular or eroded, firmly attached to the substratum without rhizoids, basal layer composed of two to several cells, giving rise to free long erect filaments, mostly unbranched composed of 40–85 cells, embedded in gelatinous matrix, curving upwards, single or tufts hairs arising from the basal or in the middle part of erect filaments, one to several plastids per cell without pyrenoid, intercalary plurangia uniseriate to multiseriate with one to few terminal sterile cells, unangia arise from the transformation of terminal cell of the shorter erect filament.

Hapalospongidion saxigenum and *H. durvillaeae* Lindauer from New Zealand were added to the genus (Lindauer 1949), but later transferred to the genus *Basispora* by John and Lawson (1974). Later, *H. capitatum* Womersley from Australia was described (Womersley 1987). Sophiammal and Panikkar (2009) described *H. thirumullavaramense* P. Sophiammal Nettar and M. V. N. Panikkar from Kerala, India. Hollenberg (1942) transferred *Ralfsia pangoensis* Setchell to *Hapalospongidion* as *H. pangoense* (Setchell) Hollenberg. *Mesospora schmidtii* Weber-van Bosse was transferred to *Hapalospongidion* as *H. schmidtii* (Weber-van Bosse) P. C. Silva (Silva et al. 1996). León-Álvarez and González-González (1993) transferred *Mesospora macrocarpa* (Feldmann) Hartog and *Mesospora vanbosseae* Borgesen to genus *Hapalospongidion* as *H. macrocarpum* (Feldmann) León-Alvarez and González-González and *H. vanbosseae* (Borgesen) D. León-Alvarez and J. González-González, respectively.

Currently, six species are recognized in the genus *Hapalospongidion*: *H. capitatum*, *H. gelatinosum*, *H. macrocarpum*, *H. saxigenum*, *H. thirumullavaramense* and *H. vanbosseae* (Guiry and Guiry 2021). *H. capitatum* is identified by its crusts having long erect capitate filaments (the uppermost two or three cells are enlarged) of up to 60 cells, multiseriate plurangia, and the absence of both unangia and hairs (Womersley 1987). *H. gelatinosum* characterized by gelatinous crusts, with long filaments (of more 40; up to 60 cells), terminal unangia on stalklike filaments, uniseriate to biseriate intercalary plurangia (Saunders 1889). *H. macrocarpum* characterized by having free filaments, with very large terminal unangia, 200–300 µm long and 6–37 µm wide, while *H. vanbosseae* mainly identified by crusts with a basal layer giving rise to sparse and dichotomously branched free erect filaments composed of 20–30 cells, hairs present, intercalary plurangia with irregularly divided locules (León-Álvarez and González-González 1993). Buchanan (2005), commented that *H. saxigenum* description is consistent and identical with that of *H. gelatinosum*. Therefore, to resolve this, a molecular and morphological study of authentic specimens of *H. gelatinosum* and *H. saxigenum* is required. *H.*

thirumullavaramense is characterized by having longest erect filaments (up to 85 cells), uniseriate intercalary plurangia (Sophiammal Nettar and Panikkar 2009).

1.2. Taxonomic history of the family Mesosporaceae J. Tanaka and Chihara

The family Mesosporaceae was first described based on the morphology of *Mesospora schmidtii* Weber-van Bosse (Tanaka and Chihara 1982). *Mesospora* is the type genus of this family. The genus *Mesospora* was erected with *M. schmidtii* from Indonesia as type species, under the family Ralfsiaceae (Weber-van Bosse 1911, 1913). The genus *Mesospora* was characterized by having mucilaginous pseudoparenchymatous thalli wholly adherent to the substratum without rhizoids, loosely adherent and clavate erect filaments consisting up to 30 cells, intercalary plurangia inserted near the apex of erect filaments, and by stalked unangia borne laterally at the base of erect filaments, without paraphyses (Weber-van Bosse 1911, 1913). Family Mesosporaceae encompasses two genera, *Mesospora* Weber-van Bosse as the type genus, and *Basispora* D. M. John and G. W. Lawson (Guiry and Guiry 2021).

John and Lawson (1974) described the genus *Basispora* based on *B. africana* John and Lawson from Komenda, Ghana as the type species. This genus is mainly characterized by gelatinous crusts with three to several layers of prostrate filaments from which assurgent filaments arise, free and closely packed, consisting up to 40 cells, distinctly clavate towards the upper part, several discoid chloroplasts per cell, terminal unangia on long stalks composed of 4–15 cells from near the base of the simple, often assurgent free vegetative filaments, plurilocular reproductive structures were unknown. *Basispora* was erected under the family Ralfsiaceae and distinguished from other members by having terminal unangia on long stalks composed of 4–15 cells. Concurrently, *H. saxigenum* and *H. durveillaeae* were transferred to *Basispora* as *B. saxigena* (Lindauer) John and Lawson and *B. durvillaeae* (Lindauer) John and Lawson, respectively (John and Lawson 1974). While citing epiphytic nature of *B. durvillaeae* on *Durvillaea antarctica*, South (1974) erected the genus *Herpodiscus* with *H. durvillaeae* (Lindauer) South as the type species under the family Elachistaceae. Currently, one species, *B. africana* is recognized (Guiry and Guiry 2021).

Tanaka and Chihara (1982) transferred *Ralfsia pangoensis* Setchell to *Mesospora* as *M. pangoensis* (Setchell) Chihara and J. Tanaka. *M. negrosensis* J. A. West and H. P. Calumpong was described from Negros Oriental, Philippines (West and Calumpong 1996). Poong et al. (2013) described *M. elongata* S. W. Poong, P. E. Lim and S. M. Phang from Okinawa, Japan. *M. indopacifica* and *M. lombokensis* were both described from Lombok Island, Indonesia (Poong et al. 2017).

Some authors pointed out the close morphological relationship among three genera, *Basispora*, *Hapalospongidion* and *Mesospora* (John and Lawson 1974, Tanaka and Chihara 1982, Womersley 1987). For instance, Womersley (1987) regarded the three genera to be closely related based on chloroplast number and position of unangia and thus, suggesting *Basispora* and *Mesospora* to be synonyms of *Hapalospongidion*. Womersley (1987) pointed out the transfer of both *M. macrocarpa* and *M. vanbosseae* to *Hapalospongidion* by León-Álvarez and González-González (1993) and *M. schmidtii* to *Hapalospongidion* by Silva et al. (1996). Both *Mesospora* and *Hapalospongidion* shared similarities of having 1–3 chloroplasts per cell and unangia terminal on vegetative filaments which may or may not have branched close to the base (Womersley 1987). Moreover, Womersley continued by stating that *Basispora* shared similarities with *Hapalospongidion* in possessing several chloroplasts per cell and terminal unangia on long stalks arising from near the base of laterally free erect filaments. Thus he retained the genus *Basispora* and *Mesospora* in *Hapalospongidion* in the family Ralfsiaceae. On the contrary, a molecular study base on *rbc*L gene for member of Mesosporaceae and Ralfsiaceae proved that the two families are distinct (Lim et al. 2007). Moreover, in the phylogenetic analysis based on *rbc*L for the Ralfsiales, *Basispora africana* was nested in clade of the family Hapalospongidiaceae while Mesosporaceae remained as a distinct monogeneric family (Poong et al. 2017). Following the phylogenetic result, Poong et al. (2017), suggested that more studies need to be carried out with type material of *Basispora africana* in order to have substantive conclusion on the phylogenetic position of this genus. Furthermore, considering the number of chloroplasts per cell for *Mesospora*, several authors agree on the genus having one chloroplast per cell (Weber-van Bosse 1911, 1913, Tanaka and Chihara 1982, Poong et al. 2013, 2014, 2017). Therefore, the current recognized species in the family Mesosporaceae are *B. africana* under *Basispora* and *M. elongata*, *M. indopacifica*, *M. lombokensis*, *M. negrosensis*, *M. pangoensis* and *M. schmidtii* under *Mesospora* (Guiry and Guiry 2021).

1.3. Taxonomic history of the family Neoralfsiaceae P.-E. Lim and H. Kawai

The family Neoralfsiaceae P.-E. Lim and H. Kawai was established based on morphological and molecular analyses of *Ralfsia expansa* (J. Agardh) J. Agardh (now *Neoralfsia expansa*) in relation to other Ralfsiales taxa (Lim et al. 2007). It was erected to accommodate a new genus *Neoralfsia* P.-E. Lim and H. Kawai which is nested in a distinct clade separate from other families in the order Ralfsiales. Neoralfsiaceae is distinguished by expanded creeping thalli that are attached to the substratum with many rhizoids and later tending to be free, pseudoparenchymatous, tightly adjoined and composed of obvious cortical and medullary layers with hair pits, medullary vegetative filaments form unilateral to bilaterally symmetrical structure, single chloroplast per cell, unangia on stalks with paraphysis, intercalary plurangia terminated by a sterile cell (Lim et al. 2007).

Agardh (1847) described *Myrionema expansum* J. Agardh from Veracruz, Mexico under the genus *Myrionema* Greville. Later, he transferred this species to the genus *Ralfsia* Berkeley as *Ralfsia expansa* (J. Agardh) J. Agardh (Agardh 1848). *Ralfsia expansa* specimens reported from the Asian region (Indonesia, Japan and Malaysia) were characterized by having bilaterally symmetrical thalli with distinct cortical and medullary layers, unangia inserted on three to six-celled stalk and biseriate plurangia terminated by a sterile cell (Weber-van Bosse 1913, Tanaka and Chihara 1980b, Lim et al. 2017). These specimens had a high similarity with *R. hancockii* E. Y. Dawson from Mexico (Dawson 1944). Specimens of *R. expansa* reported from the Danish West Indies (present day U.S.A. Virgin Islands) contrasted the Asian ones by mainly having unilaterally symmetrical thalli and with sessile unangia, or unangia on a single-celled stalk (Børgesen 1912, 1914). Many authors recognize *R. expansa* based on Børgesen's morphotype because Agardh's description was short and based on sterile samples while that of Børgesen included detailed reproductive morphology (León-Álvarez et al. 2014b). But, neither *R. expansa* sensu Tanaka and Chihara nor *R. expansa* sensu Børgesen can be attributed to *R. expansa* (J. Agardh) J. Agardh unless they are matched both molecularly and morphologically to the latter.

Lim et al. (2007) established a new genus, *Neoralfsia* based on morphological and molecular analyses. The authors subsequently transferred *R. expansa* to the new genus as *Neoralfsia expansa* (J. Agardh) Lim and Kawai but failed to cite the original publication of the basionym thus the combination was not valid. The combination, *Neoralfsia expansa* (J. Agardh) Lim and Kawai ex Kraft was also invalid because, Kraft (2009: 87) cited the entire pagination of Agardh's (1847) paper where besides *M. expansum* (the intended basionym) he described more species. The combination, *Neoralfsia expansa* (J. Agardh) Lim and Kawai ex Cormaci and G. Furnari is valid because unlike the previous two, accurate citations for the basionym and homotypic synonym were provided (Cormaci et al. 2012). During the Allan Hancock's Pacific expeditions, *Ralfsia hancockii* E. Y. Dawson was describe from San Jose del Cabo, Mexico (Dawson 1944). León-Álvarez et al. (2014a) transferred *Ralfsia hancockii* to *Neoralfsia* as *N. hancockii* (E. Y. Dawson) D. León-Alvarez and M. L. Nunez-Reséndiz based of morphology and molecular studies. Therefore, two species, *Neoralfsia expansa* and *N. hancockii* are recognized in the family Neoralfsiaceae (Guiry and Guiry 2021).

1.4. Taxonomic history of the family Pseudoralfsiaceae Parente, Fletcher and G.W. Saunders

The family Pseudoralfsiaceae Parente, Fletcher and G.W. Saunders was established based on morphological and molecular analyses of *Ralfsia verrucosa* (Areschoug) Areschoug (now *Pseudoralfsia verrucosa*) in relation to other Ralfsiales taxa (Parente et al. 2021). It was erected to accommodate two new genera, *Nuchella* and *Pseudoralfsia* Parente, Fletcher and G.W. Saunders which formed distinct lineages nested in a distinct clade separate from other families in the order Ralfsiales. Pseudoralfsiaceae is mainly characterized by circular to irregular crusts, with or without rhizoids and with or without superimposed thalli, erect filaments are straight or curved upwards (rarely curved downwards), cells with a single chloroplast without obvious pyrenoids, hair pits are frequent, arising from the middle and/or lower cells of erect filaments, unangia are sessile, or on 1, rarely 2–3 pedicel cells, on the terminal part of erect filaments, with 1–2 paraphyses, sori of plurangia are non-adventitious, with synchronized and non-synchronized development of the plurangia, plurangia are uniseriate or biseriate, intercalary on the erect filaments and terminated by 1, occasionally 2, sterile cells (Parente et al. 2021).

The type genus of family Pseudoralfsiaceae is *Pseudoralfsia*. It is distinguished by its thalli having a circular or indefinite outline, with or without rhizoids and with or without superimposed thalli, erect filaments are straight or curved upwards (rarely curving downwards), cells are with a single chloroplast without obvious pyrenoids, hair pits are frequent and arising from the lower cells of erect filaments, unangia are sessile or on pedicels of 1, rarely 2–3 cells, on the terminal part of erect filaments, with 1–2 paraphyses, sori of plurangia are non-adventitious with a synchronized development, plurangia are uniseriate or at times biseriate, intercalary in the erect filaments terminated by a sterile cell (Parente et al. 2021). The type species of this genus is *Pseudoralfsia verrucosa* (Areschoug) Parente, Fletcher and G.W. Saunders. The type species was first described as *Cruoria verrucosa* Areschoug from Bohuslän, Western Götaland, Sweden (Areschoug 1843). Areschoug (in Fries 1845) transferred this species to the genus *Ralfsia* as *Ralfsia verrucosa* (Areschoug) Areschoug. Subsequently, he described two varieties, *R. verrucosa* var. *lignicola* (Areschoug 1847) and *R. verrucosa* var. *cochlearum* (Areschoug 1876). Parente et al. (2021) transferred this species to and new genus *Pseudoralfsia* in a new family Pseudoralfsiaceae as *Pseudoralfsia verrucosa.* Another species described under *Pseudoralfsia* is *P. azorica* Parente, Fletcher and G.W. Saunders from São Miguel. Azores, Portugal (Parente et al. 2021). *P. verrucosa* differs from *P. azorica* by having upwardly curved erect vegetative filaments and with superimposed thalli while the latter mostly has strictly erect vegetative filaments without superimposed thalli (Parente et al. 2021).

The second genus in family Pseudoralfsiaceae is *Nuchella*. This is a monotypic genus with *Nuchella vesicularis* Parente, Fletcher and G.W. Saunders from Gaia, São Felix da Marinha, Portugal, as the type species. The genus *Nuchella* is characterized by crusts with circular outline, without superimposed thalli, sometimes with rhizoids, erect filaments slightly curving upward, cells with a single chloroplast, without pyrenoids and typically with numerous vesicles, hair pits are frequent, arising from both lower and middle cells of the erect filaments, plurangia are embedded in concave, non-adventitious sori with non-synchronous development, plurangia are uniseriate or biseriate, intercalary in erect filaments and terminated by 1, occasionally 2, sterile cell (Parente et al. 2021).

Three species are recognized in the family Pseudoralfsiaceae, *N. vesicularis* under *Nuchella* and *P. verrucosa* and *P. azorica* under the genus *Pseudoralfsia* (Guiry and Guiry 2021).

1.5. Taxonomic history of the family Ralfsiaceae W.G. Farlow

The family Ralfsiaceae W.G. Farlow was erected based on the morphology of *Ralfsia deusta* (C. Agardh) Berkeley (now *Ralfsia fungiformis*) from Iceland (Farlow 1881). It was erected to accommodate genus *Ralfsia*. Family Ralfsiaceae is distinguished by encrusting thalli with rounded to irregular outline, with smooth to convoluted surface, developing usually from a discoid germination stage, basal layer composed of radiating appressed filaments, one to several cells thick, usually without rhizoids, giving rise to simple or slightly branched erect or assurgent filaments, chloroplasts are single or few per cell and usually without pyrenoids, life history is supposedly diplohaplontic and isomorphic, but probably more usually direct from the sporophyte, sori of unangia or plurangia on separate individuals, reproductive structures are lateral, terminal, or intercalary on reproductive filament and usually associated with surrounding paraphyses or vegetative filaments (Farlow 1881, Loiseaux 1968, Abbott and Hollenberg 1976, Womersley 1987, Norris 2010).

Although Nakamura (1972) placed the family Ralfsiaceae in his newly established order Ralfsiales, several authors disagreed with this treatment. For instance, Wynne (1981), Nelson (1982), Scagel et al. (1989), Schneider and Searles (1991), and Silva et al. (1996), placed Ralfiaceae in the order Ectocarpales, and Loiseaux (1968) argued that members of Ralfsiaceae were indeed Myrionemataceae. But from the findings of rDNA analyses, Tan and Druehl (1994) concluded that family Ralfsiaceae did not belong in order Ectocarpales. Lim et al. (2007) *rbc*L molecular phylogeny resolved the Ralfsiaceae in order Ralfsiales.

Currently, Ralfsiaceae is comprised of four recognized genera: *Analipus* Kjellman, *Endoplura* Hollenberg, *Heteroralfsia* Kawai and *Ralfsia* Berkeley (Smith and Sowerby 1843, Kjellman 1889, Hollenberg 1969, Kawai 1989, Silberfeld et al. 2014, Guiry and Guiry 2021). The genus *Analipus* was established based on *Analipus fusiformis* Kjellman [now *Analipus filiformis* (Ruprecht) Papenfuss] from Bering Island, in the Bering Sea (Kjellman 1889). The genus is mainly characterized by plants having rhizomatous perennial basal disc and annual erect thallus arising from the disc, erect thalli are caespitose, simple or branched, hollow, yellowish brown to dark brown, up to 40 cm in height, unangia and plurangia are formed on separate thalli, each cell contain a single parietal cup-shaped chloroplast without pyrenoid and a lot of physodes, except for inner cells which contain several discoid chloroplasts per cell, plurangia are uniseriate or biseriate and terminated by 1–3 sterile cells, unangia are formed from the basal part of assimilatory filaments, ovoid or ellipsoid, among assimilatory filaments, plants are isomorphic with alteration of generation between unangial plants and dioecious plurangial plants (Kjellman 1889, Nakahara 1984).

Analipus filiformis was first described as *Haplosiphon filiformis* Ruprecht from the Sea of Okhotsk, and was mainly identified by having no laterals on its erect axes (Ruprecht 1850). Kjellman (1889) erected the genus *Analipus* based on *Analipus fusiformis*, the same species describe earlier by Ruprecht. Later, Papenfuss (1967) transferred *Haplosiphon filiformis* to the genus *Analipus* as *A. filiformis*. *Halosaccion japonicum* Harvey was described from "Hakodadi" the present Hakodate, Hokkaido, Japan (Harvey 1857). *Halosaccion japonicum* was later transferred to *Analipus* as *A. japonicus* (Harvey) M. J. Wynne mainly distinguished from *A. filiformis* by having numerous lateral branchlets on long main axes (Wynne 1971). Yendo (1913) described *Chordaria gunjii* Yendo from Shimushu Island, Kurile Islands. This species was later transferred to the genus *Analipus* as *A. gunjii* (Yendo) Kogame and Yoshida mainly identified by having a dicoid holdfast which is different from the other two species that are branched (Yoshida 1997). Three species are recognized in the genus *Analipus*, *A. filiformis*, *A. gunjii* and *A. japonicus* (Guiry and Guiry 2021).

Hollenberg (1969) proposed the genus *Endoplura* with *Endoplura aurea* Hollenberg from Orange County, CA, USA as the type species. *Endoplura* is distinguished from other genera of Ralfsiaceae by epilithic or epizoic plants forming thin irregular creeping thalli, firmly attached to the substratum without rhizoids, with firmly adjoined erect filaments from an indistinct hypothallial layer, cells with several to many chloroplasts, plurangia in irregular sori, initially uniseriate, ultimately becoming biseriate to quadriseriate, and terminated by a sterile portion consisting of 2–8 cells, unangia are sessile, on the terminal part of vegetative filaments, with 1–2 paraphyses (Hollenberg 1969, Oteng'o et al. 2021). The genus *Endoplura* has remained monotypic for a long period since it was established (Hollenberg 1969, Abbott and Hollenberg 1976). In recent time, two species, *Endoplura jejuensis* and *E. koreana* A. O. Oteng'o, T. O. Cho and B. Y. Won have been described from Korea based on molecular and morphological analyses (Oteng'o et al. 2021). Currently, three *Endoplura* species are recognized worldwide, *E. aurea*, *E. jejuensis* and *E. koreana* (Guiry and Guiry 2021, Oteng'o et al. 2021). *E. aurea* is mainly identified by its thick and broad thalli without hairs (Hollenberg 1969). Both *E. jejuensis* and *E. koreana* have thin thalli with hairs but they differ in the latter having 2–8 sterile cells and 1–2 paraphyses per unangia while the former has 2–4 sterile cells with only 1 paraphysis per unangium (Oteng'o et al. 2021).

Genus *Heteroralfsia* was established based on phenology, morphology and life history studies of *Saundersella saxicola* (Okamura and Yamada) Inagaki from Kamome-jima, Aomori, Japan (Kawai 1989). This genus is characterized by having annual erect thalli during summer and creeping portion probably perennial. Erect thalli are epilithic on gregarious creeping basal portion, simple, cylindrical attenuated towards the base, hollow, slightly slimy, elastic, yellowish to reddish brown. Creeping portion is composed of basal cell layer giving rise to closely packed, parallel, erect filaments. The erect portions of thalli are multiaxial and pseudoparenchymatous, with medullary filaments densely packed and simple near the base, rather loose and connected with each other here and there in the middle and upper parts, resulting in the formation of a reticular medullary layer. Surface of young erect thallus is covered with cuticle, shed following the development of the multicellular paraphyses. Hairs lacking its own basal meristematic region present. Sessile unangia, obovoid, formed on the basal cell of assimilatory filaments. Cells contain physodes, single cup-shaped chloroplast without pyrenoid, which often become fragmented later. In culture, *Ralfsia*-like creeping thalli form intercalary plurangia terminated by a sterile cell while erect filamentous thalli develop on the creeping thalli and form multicellular assimilatory filaments and unangia (Kawai 1989). Yamada (1928) originally described *Gobia saxicola* Okamura and Yamada in the genus *Gobia* (Rienke 1889) based on its morphological similarity with *Gobia simplex* Setchell and Gardner. Later, this species was transferred to genus *Saundersella* as *S. saxicola* (Okamura and Yamada) Inagaki (Inagaki 1958). Kawai (1989) established the genus *Heteroralfsia* based on *Saundersella saxicola*, thus the combination *Heteroralfsia saxicola* (Okamura and Yamada) H. Kawai. Currently, only one species, *H. saxicola* is recognized in *Heteroralfsia* (Guiry and Guiry 2021).

The genus *Ralfsia* was originally described by Berkeley (in Smith and Sowerby 1843) based on *Zonaria deusta* C. Agardh from Iceland. *Ralfsia deusta* (C.Agardh) Berkeley was typified with material from Berwick, Scotland. Currently, *R. deusta* is a synonym of *R. fungiformis* (Gunnerus) Setchell and N. L. Gardner, which has its type locality in Iceland (Gunnerus 1772, Silva 1996–2018). *Ralfsia* is the type genus of family Ralfsiaceae. *Ralfsia* is characterized by having encrusting thalli with flat and expanded more or less circular outline, attached to the substratum with or without rhizoids, erect vegetative filaments tightly adherent, straight or curved, branched or simple, arising from a hypothallial layer of one to several prostrate filaments, chloroplasts are mostly plate-shaped, one per cell, unangia are sessile or pedicellate, associated with multicellular paraphysis, plurangial reproductive structures are intercalary, arising from sub-terminal cells of vegetative filaments, terminated by one or more sterile cells (Setchell and Gardner 1924, Hamel 1931-1939, Hollenberg 1969, Abbott and Hollenberg 1976, Tanaka and Chihara 1980b, Fletcher 1987, Womersley 1987, Parente and Saunders 2019, Oteng'o and Won 2020).

Ralfsia has sixteen recognized species: *R. australis* Skottsberg, *R. confusa* Hollenberg, *R. endopluroides* J. Tanaka and Chihara, *R. fungiformis* (Gunnerus) Setchell and N. L. Gardner, *R. hesperia* Setchell and N. L. Gardner, *R. huanghaiensis* Li Xiyi and Li Junfeng, *R. integra* Hollenberg, *R. longicellularis* Perestenko, *R. lucida* S. Lund, *R. ovata* Rosenvinge, *R. pacifica* Hollenberg, *R. pedicellata* J. Tanaka and Chihara, *R. pusilla* (Strömfelt) Foslie, *R. robertii* M. I. Parente and G. W. Saunders, *R. tenebris* M. I. Parente and G. W. Saunders and *R. unimaculata* M. I. Parente and G. W. Saunders (Guiry and Guiry 2021). *R. australis* is mainly characterized by having a scaly surface, without hairs, mostly uniseriate plurangia Skottsberg (1921). *R. confusa* identified by its light brown colored crusts with strict erect filaments, sessile unangia or unangia on 1–3 (–6) celled-stalks and mostly uniseriate plurangia, while *R. pacifica* characterized by assurgent erect filaments, one chloroplast per cell, unangia mostly sessile at the base of paraphyses and uniseriate plurangia terminated by a sterile cell (Hollenberg 1969). *R. endopluroides* is mainly characterized by erect filaments slightly curving upwards, sessile unangia and intercalary plurangia composed of 2 parallel rows of plurangial reproductive filaments terminated by 3 sterile cells, while *R. pedicellata* distinguished by its straight simple or sparsely erect filaments, unangia on 1-celled stalks and plurangia composed of 2 rows of plurangial reproductive filaments terminated by 2 sterile cells (Tanaka and Chihara 1981a). *R. fungiformis* is mainly distinguished by its thick thallus with bilaterally symmetrical erect filaments and notably imbricate lobes while *R. hesperia* mainly characterized thalli nearly similar to those of *R. verrucosa*, but differing in having fleshy, thicker and smaller diameter crusts, with larger unangia and longer paraphyses than the latter (Setchell and Gardner 1924). *R. huanghaiensis* is distinguished by thalli with erect filaments forming a bilateral symmetry, large elongate unangia in a stalk cell, and uniseriate plurangia terminated by a sterile cell (Xiyi and Junfeng 1993). *R. integra* mainly identified by it large single centrally located reproductive sorus (Hollenberg 1969). *R. longicellularis* is distinguished from congeners by its long cells of the upwardly curved filaments and unangia on multicellular stalks (Perestenko 1980). *R. ovata* identified by its small ovate unilocular sporangia laterally positioned in the middle of paraphyses (Rosenvinge 1893). *R. lucida* distinguished by its unangia which may be terminal, lateral or intercalary position (Lund 1967). *R. pusilla* is identified as epiphytic on other algae and seagrasses, and has loosely adhering erect filaments with unangia accompanied with paraphyses (Foslie 1892). *R. robertii* is identified by having upward and downward curving erect filaments producing a symmetrical thallus with uniseriate plurangia mostly terminated with one sterile cell, rarely two, while *R. tenebris* is identified by its dark colored crust with strictly straight erect filaments having mostly quadrate cells, and uniseriate plurangia with one sterile cell, and *R. unimaculata* with a crust with an internal vegetative morphology resembling that of *R. fungiformis* but differing from the latter by crusts not forming imbricate lobes (Parente and Saunders 2019).

In retrospect, the first species in genus *Ralfsia* was described as *Fucus fungiformis* Gunnerus from Iceland (Gunnerus 1776). Later, Berkeley proposed the genus *Ralfsia* was based on *Zonaria deusta* C. Agardh from Iceland, a similar species as Gunneru's specimens (Smith and Sowerby 1843). Following the priority rule, (Setchell and Gardner 1924) proposed the combination *Ralfsia fungiformis* (Gunnerus) Setchell and N. L. Gardner. Areschoug (in Fries 1845) transferred *Cruoria verrucosa* Areschoug to *Ralfsia*, as *R. verrucosa* (Areschoug) Areschoug. *R. verrucosa* is presently recognized as *Pseudoralfsia verrucosa* (Areschoug) Parente, Fletcher and G. W. Saunders (Parente et al. 2021). *Myrionema expansum* J. Agardh was transferred to *Ralfsia* as *R. expansa* (J. Agardh) J. Agardh (Agardh 1848). This name is currently regarded as a synonym of *Neoralfsia expansa* (Cormaci et al. 2012). Kützing (1859) described a new species, *Ralfsia major* Kützing from South Africa, but this name is currently synonymized to a red algal species *Sonderopelta capensis* (Montagne) A. D. Krayesky (Krayesky et al. 2009). Crouan and Crouan (1867) described two species and one combination in the genus *Ralfsia* as *R. disciformis*, *R. extensa* P. Crouan and H. Crouan, and *R. clavata* (Harvey) P. Crouan and H. Crouan. But currently the three names are synonyms, the former one as *Pseudolithoderma extensum* (P. Crouan and H. Crouan) S. Lund (Lund 1959) and the latter two as *Stragularia clavata* (Harvey) Hamel (Hamel 1939). Gobi (1878) transferred *Lithoderma fatiscens* Areschoug to *Ralfsia* as *R. fatiscens* (Areschoug) Gobi, but currently the name *Lithoderma fatiscens* Areschoug is maintained (De Toni 1895). *R. spongiocarpa* Batters was described under *Ralfsia* (Batters 1888), but synonymized to *Stragularia spongiocarpa* (Batters) Hamel (Hamel 1939). Foslie (1892) proposed the combination *Ralfsia pusilla* (Strömfelt) Foslie which is recognized to date. Rosenvinge (1893) describe the recognized species, *Ralfsia ovata* Rosenvinge. *R. bornetii* Kuckuck was described under *Ralfsia* (Kuckuck 1894), but synonymized to *Stragularia clavata* (Harvey) Hamel (Hamel 1939). Subsequently, *R. ceylanica* Harvey ex E.S. Barton (Barton 1903) was described but recently synonymized to *Lobophora ceylanica* (Harvey ex Barton) C. W. Vieira, De Clerk and Payri (Vieira et al. 2016). *Ralfsia australis* Skottsberg was described from Falkland Islands (Skottsberg 1921), and is a recognized species in this genus. *Ralfsia pangoensis* Setchell was described from Tutuila Island, Samoa (Setchell 1924), but is now synonymized to *Mesospora pangoensis* (Setchell) Chihara and J. Tanaka (Tanaka and Chihara 1982). Skottsberg was described from Falkland Islands (Skottsberg 1921), and is a recognized species in this genus. Setchell and Gardner (1924) proposed the combination *Ralfsia fungiformis* (Gunnerus) Setchell and N. L. Gardner and described *Ralfsia hesperia* Setchell and N. L. Gardner, and both are recognized names to date. They also described *Ralfsia californica* Setchell and N. L. Gardner in the same publication but this name was synonymized to *petalonia fascia* (O. F. Müller) Kuntze (Kuntze 1898). *Ralfsia fluviatilis* Areschoug was described by Areschoug, but is now referred as a synonym of the fresh water encrusting brown alga, *Heribaudiella fluviatilis* (Areschoug) Svedelius (Svedelius

1930). Feldmann (1931) described *R. macrocarpa* Feldmann from Algeria but this algae is currently recognized as *Hapalospongidion macrocarpum* (Feldmann) León-Alvarez and González-González (León-Álvarez and González-González 1993). *Ralfsia pacifica* Hollenberg was describe from Corona del Mar, Orange Co., California U.S.A. and is currently a recognized species (Smith 1944). Dawson (1944) describe *R. hancockii* E. Y. Dawson form San Jose del Cabo, but this name is now synonymized to *Neoralfsia hancockii* (E. Y. Dawson) D. León-Alvarez and M. L. Nunez-Reséndiz (León-Álvarez et al. 2014a). *Ralfsia occidentalis* Hollenberg was describe from Braithwaite Bay, I. Soccoro, Is. Revilla Gigedo, Mexico, by Hollenberg (in Taylor 1945) but this species is currently recognized by the name *R. pacifica*. Kylin (1947) described *Ralfsia tenuis* Kylin which is currently a synonym of *Stragularia clavata. R. lucida* S. Lund was describe from Helsingör and is currently a recognized species in this genus (Lund 1967). Subsequently, two more recognized species, *Ralfsia confusa* and *R. integra* Hollenberg from Orange County, California, U.S.A were described (Hollenberg 1969). Perestenko (1980) described *Ralfsia longicellularis* Perestenko from Peter the Great Bay, Russia, a recognized species in this genus. Two more recognized species, *Ralfsia endopluroides* and *R. pedicellata* J. Tanaka and Chihara from Japan were described (Tanaka and Chihara 1981a). Xiyi and Junfeng (1993) described *Ralfsia huanghaiensis* Li Xiyi and Li Junfeng from Heishijiao of Dalian region, China. This name is currently flagged as accepted (Guiry and Guiry 2021). Parente and Saunders (2019) described three species, *Ralfsia robertii*, *R. tenebris* and *R. unimaculata* M. I. Parente and G. W. Saunders from British Columbia, Canada and all are recognized species in the genus *Ralfsia*.

Currently, there are thirty four species names in the genus *Ralfsia*, of which sixteen are recognized species and eighteen are synonyms. Out of the eighteen synonyms, seven are synonyms for species in other families within order Ralfsiales while eleven are synonyms of taxa outside order Ralfsiales (Guiry and Guiry 2021). Synonyms often arise in this group due to insufficient robust taxonomic characters particularly for sterile specimens (Parente and Saunders 2019) and contradictory morphological features sometimes assigned to some species by different authors (Hamel 1931–1939, Taylor 1957, Fletcher 1987, Womersley 1987). In light of their research findings, Parente and Saunders (2019) suggested that species presently assigned to *Ralfsia* (i.e. *R. australis* Skottsberg, *R. confusa* Hollenberg, *R. endopluroides* J. Tanaka and Chihara, *R. hesperia* Setchell and N. L. Gardner, *R. huanghaiensis* Li Xiyi and Li Junfeng, *R. integra* Hollenberg, *R. longicellularis* Perestenko, *R. lucida* S. Lund, *R. ovata* Rosenvinge, *R. pacifica* Hollenberg, *R. pedicellata* J. Tanaka & Chihara and *R. pusilla* (Strömfelt) Foslie) require molecular and morphological re-examination to accurately verify their taxonomic ranks.

2. Taxonomic history of the order Ralfsiales in Korea

The first report of order Ralfsiales in Korea was in a floristic list that included the family Ralfsiaceae with two genera, *Analipus* with *A. japonicus* (Harvey) Wyyne and *Ralfsia* with *R. clavata*, *R. confusa*, *R. fungiformis*, *R. integra* and *R. verrucosa* (Lee and Kang 1986). Subsequently, Lee and Kang (2001) published a catalogue of seaweeds in Korea with the three genera, *Analipus*, *Heteroralfsia* and *Ralfsia* in two different families and two different orders. *A. japonicus* under family Heterochordariaceae Setchell and Gardner in the order Chordariales Setchell and Gardner, and *Heteroralfsia saxicola*, *Ralfsia clavata*, *R. confusa*, *R. fungiformis*, *R. integra* and *R. verrucosa* under family Ralfsiaceae in the order Ralfsiales. But currently, genus *Analipus* is in the family Ralfsiaceae (Lim et al. 2007, Silberfeld et al. 2014) while *R. verrucosa* is in the genus *Pseudoralfsia*, a member of family Pseudoralfsiaceae (Parente et al. 2021). *Ralfsia expansa* was reported from Jeju Island (Lee 2008). Currently *R. expansa* is a synonym of *Neoralfsia expansa* (Lim et al. 2007). Keum (2010) reported only two species, *R. fungiformis* and *R. verrucosa*, while citing that *R. clavata*, *R. confusa* and *R. integra* could not be collected in that particular study. *Ralfsia longicellularis* was reported in the West and South

Sea (Oteng'o and Won 2020). Oteng'o et al. (2021) described two *Endoplura* species, *E. jejuensis* and *E. koreana*. *Pseudoralfsia verrucosa* was reported from Sogul-do, Sinan-gun, in the west-southern coast (Ryu and Kim 2021). Most of the reports were based on morphology of the plants (Lee and Kang 1986, 2001, Lee 2008, Keum 2010, Ryu and Kim 2021). There are few studies combining both molecular and morphological analyses of the members of the order Ralfsiales in Korea (Oteng'o and Won 2020, Oteng'o et al. 2021).

Based on the current systematics of order Ralfsiales in AlgaeBase (Guiry and Guiry 2021), three families are recognized in Korea. Family Neoralfsiaceae with *Neoralfsia expansa* (reported as *R. expansa*), family Pseudoralfsiaceae with *Pseudoralfsia verrucosa*, and family Ralfsiaceae with *Analipus japonicus*, *Endoplura jejuensis*, *E. koreana*, *Heteroralfsia saxicola*, *Ralfsia confusa*, *R. fungiformis*, *R. integra* and *R. longicellularis* (Oteng'o and Won 2020, Guiry and Guiry 2021, Oteng'o et al. 2021, Ryu and Kim 2021).

3. Morphological frameworks in the order Ralfsiales

Most of the taxa in the order Ralfsiales undergo isomorphic life history with diplohaplontic phases (alternating haploid and diploid stages) in which both gametophytes and sporophytes possess pseudoparenchymatous (haplostichous) thalli construction (Lobban and Wyyne 1981). Exceptions are two taxa; *Analipus* which undergo a diplohaplontic life history with isomorphic annual erect sporophytes and gametophyptes with perennial creeping holdfasts (Kjellman 1889, Nelson 1980); and *Heteroralfsia* having a diplohaplontic life history with heteromorphic creeping gametophytes and erect sporophytic thalli (Kawai 1989). In addition to the life history pattern, criteria such us mode of growth is important when generally classifying the algae (Draisma et al. 2001). The substrate is also an important point to consider as some species in this group of algae grown only on certain substrate. Although most are epilithic, some species are epizoic while others are epiphytic (Abbot and Hollenberg 1976, Lobban and Wyyne 1981).

Vegetative morphology

When considering the taxonomy of creeping brown algae in the order Ralfsiales, a number of vegetative morpho-anatomical features have been employed by various authors (Saunders 1889, Weber-van Bosse 1911, Hollenberg 1969, Perestenko 1980, Tanaka and Chihara 1980a, 1980b, 1982, Lim et al 2007, Poong et al 2013, 2017, León-Álvarez et al. 2017, Parente et al. 2021). These features may be divided into two categories, external morphological features and internal morpho-anatomy. Characters related to the external morphology include: color of crust, presence or absence of dark or lighter margin; surface texture, presence or absence of growth line, ridges and warts; presence or absence of lobes and microlobes; outline shape, diameter, and thickness of the crust. Characters related to the internal morpho-anatomy include: presence or absence of superimposed thalli; number of cell layers in the basal disc (layer); organisation of vertical vegetative filaments (strictly straight vs. unilateral symmetry vs. bilateral symmetry, degree of lateral cohesion between filaments, presence or absence of striations in filaments, demarcation of cortical and medullary layers); presence or absence and point of insertion of hair; presence or absence of rhizoids; cell size and shape; absence or presence and number of cell inclusions (e.g. vesicles); number and morphology of plastids; and presence or absence of pyrenoids.

It is very important to be cogniscent to the fact that some characters such as color maybe used to distinguish among some species in order Ralfsiales, for example between *Ralfsia confusa* and *R. tenebris* (Parente and Saunders 2019). However, some authors have disputed characters such as lateral dimension, crust color and thickness as not reliable for use as diagnostic features (Wilce et al. 1970, Poong 2014, Poong et al, 2017). I partly agree with these authors. As much as some feature may be subject to variation with their envrironmental conditions and age, in my view it will be good to handle a feature and generally compare among taxa at a taxonomic rank higher than species. For example, some taxa such as *Ralfsia longicellularis* generally have thicker and broader thalli as compared to others like *Endoplura jejuensis* which have thinner and smaller crusts. The thickness in this case could generally be used to hint at a possibility of a specimen being in certain taxon but not definitive. Also, color, may be applied in the same way as some taxons generally have darker thalli as compared to others which have lighter and some even yellow. This may be a criterion used for sorting or even making sure one collects the right target of samples in the field. Therefore as much as some characters may not be used definitively to asign specimens to their correct taxa, they may play a big role in guiding the collection and sorting processes.

Reproductive morphology

Reproductive morphology is crusial in the taxonomy of member of order Ralfsiales. Lack of robust taxonomic characters makes it challenging to delimit or assign a samples to its correct taxon especially for sterile sample (Parente et al. 2019). This makes reproductive morphology pivotal in the taxonomy of this group. It is therefore important that the reproductive morphology is well understood and documented. Descriptive terminology of the position of reproductive structures are key to accurate identification of a specimen (León-Álvarez et al. 2005). There are two types of reproductive structures, plurangial reproductive structures and unangial reproductive structures. Characters related to the external morphology include: structure of reproductive sorus (difused vs combined, elevated bumps vs flat surface). Characters related to the internal morphology include: size, shape and position of reproductive structures inside sori; presence or absence of paraphyses; presence or absence of soral filaments (reproductive filaments that bear unangia); absence or presence of unangial stalks and number of stalk cells; and number of sterile cells terminating the plurangia (Tanaka and Chihara 1980 a, 1980b, Anderson 1988, León-Álvarez et al. 2005, Lim et al. 2007).

4. Objectives of the study

Taxonomically, creeping brown algae are widely considered as one of the most difficult and challenging group in the Phaeophyceae. Plants with creeping thalli spread across several taxa in different orders, including the order Ralfsiales. Considering the Ralfsiales, some authors recommended that some species currently assigned to genus *Ralfsia*, for example, *R. bornetii, R. clavata, R. disciformis* and *R. pusilla* are better assigned to the genus *Stragularia* Strömfelt, and *R. californica* is considered to represent creeping phases in the life histories of members of family Scytosiphonaceae (Wynne 1969, Tanaka and Chihara 1980b, Fletcher 1987). Also, some authors pointed out that the entire order Ralfsiales is understudied by contemporary taxonomists (Poong et al. 2013, 2017, León-Álvarez et al. 2017, Parente and Saunders 2019). This situation is aggravated by insufficient robust taxonomic characters especially in the case of sterile specimens, and this notwithstanding, taxonomic confusion or "stalemate" brought about when contradictory morphological features are assigned to the same species by different authors (Taylor 1957, Fletcher 1987, Womersley 1987, Parente and Saunders 2019). Fortunately, recent assessments of species diversity has benefited from advent of DNA molecular barcodes to the taxonomist's toolbox. The combination of molecular (*rbc*L and COI-5P genes) and morphological analyses in taxonomy of the Ralfsiales has helped in the accurate identification of taxa not only at species level but also generic and family levels (Lim et al. 2007, Poong et al. 2013, 2014, 2017, León-Álvarez et al. 2014a, 2014b, 2017, Parente and Saunders 2019, Oteng'o and Won 2020, Oteng'o et al. 2021, Parente et al. 2021).

Korea is known as a potential hotspot of seaweed diversity for the species and generic level (Yang et al. 2020), but there is little or no monographic studies on order Ralfsiales to fully expand our knowledge of species diversity of this group in Korea. In this light, the aim of this study was to provide a taxonomic account of the creeping species of the order Ralfsiales (Phaeophyceae) in Korea using both molecular (*rbc*L and COI-5P genes) and morphological analyses following the research framework in figure 1. My choice for the two molecular makers is informed by previous studies: plastid-encoded *rbc*L gene is one of the mostly widely used molecular marker with a high resolving power in phylogenetic studies of brown algae, this notwithstanding, it has a relatively slow evolutionary rate (compared to mitochondrial-encoded molecular markers like COI-5P) and generally with unambiguous alignment, thus making it important for familial and ordinal levels phylogenetic inferences (Poong 2014); the mitochondrial-encoded COI-5P marker, on the other hand has a faster mutation rate thus making it very helpful for species level phylogenetics (Riisberg et al. 2009, Trobajo et al. 2010).

Part 1 is showing detailed vegetative and reproductive morphological analyses of species in the order Ralfsiales. Creeping brown algal specimens from Korea are analyzed and order Ralfsiales revised.

This part provides morphological analyses in four categories. Category one provides a revision of the family Mesosporaceae by morphological analyses. A new species is described in the genus *Mesospora*, as *M. bifurcata* sp. nov.

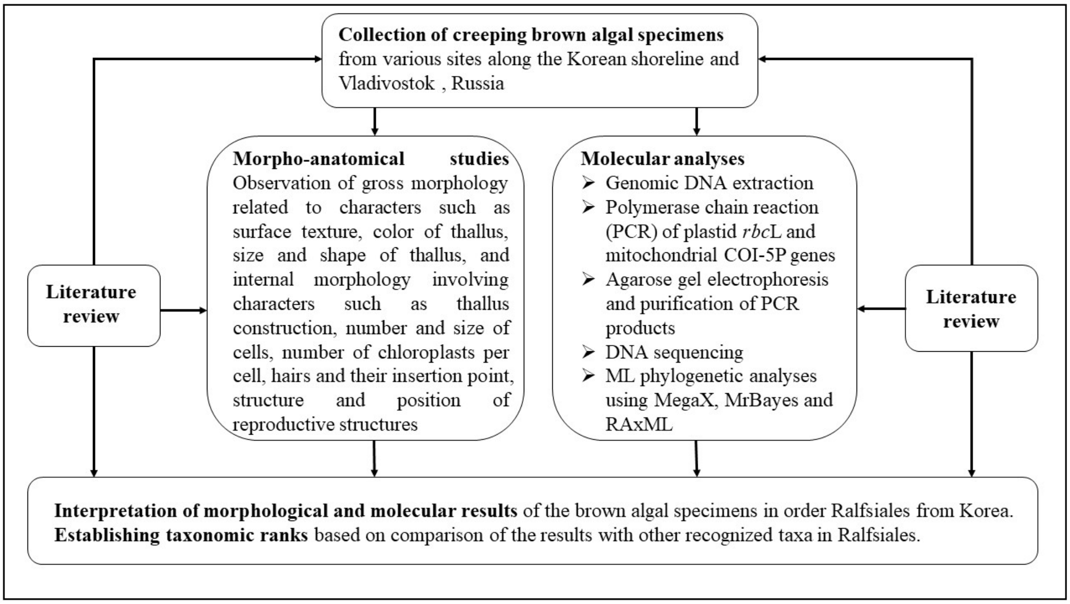
Category two provides a reappraisal of classification of the family Neoralfsiaceae based on detailed morphological observations. These analyses characterized a new genus *Neoralfsiopsis* gen. nov. and the recognition of *Ralfsia expansa* (=*Neoralfsia expansa*) as true and/or same to *Neoralfsia expansa* from type locality in Veracruz, Mexico.

Category three gives a revision of the family Ralfsiaceae by detailed morphological analyses. These analyses characterized 2 new genera *Fissipedicella* and *Ramipedicella* gen. nov., and 9 species with recognition of 8 new species and 1 combination.

Category four provides a revision of the order Ralfsiales based detailed morphological observations. These analyses characterized a new family Sungminiaceae fam. nov., a new genus *Sungminia* gen. nov., and recognition of 3 new species.

Part 2 is showing the phylogenetic relationship among species of the order Ralfsiales. Phylogenetic relationships between Korean creeping Ralfsiales specimens and already recognized taxa in order Ralfsiales are elucidated based on *rbc*L and COI-5P gene sequence data sets.

This part provides molecular analyses in three categories: phylogenetic analyses of the order Ralfsiales based on *rbc*L gene sequence data; phylogenetic analyses of the order Ralfsiales based on COI-5P gene sequence data; and phylogenetic analyses of the order Ralfsiales based on concatenated *rbc*L and COI-5P gene sequence data sets.



Collection of creeping brown algal specimens from various sites along the Korean shoreline and Vladivostok , Russia

Morpho-anatomical studies
Observation of gross morphology related to characters such as surface texture, color of thallus, size and shape of thallus, and internal morphology involving characters such as thallus construction, number and size of cells, number of chloroplasts per cell, hairs and their insertion point, structure and position of reproductive structures

Molecular analyses
- Genomic DNA extraction
- Polymerase chain reaction (PCR) of plastid *rbc*L and mitochondrial COI-5P genes
- Agarose gel electrophoresis and purification of PCR products
- DNA sequencing
- ML phylogenetic analyses using MegaX, MrBayes and RAxML

Interpretation of morphological and molecular results of the brown algal specimens in order Ralfsiales from Korea. **Establishing taxonomic ranks** based on comparison of the results with other recognized taxa in Ralfsiales.

Fig. 1. Outline of research approach in this study

II. MATERIALS AND METHODS

1. Specimen collection

A total of 700 creeping (encrusting) brown algal specimens were collected at the intertidal zone (c. 1 m depth) mainly along the Korean coastal shores and a few from the shores of Vladivostok, Russia (Table 1). The samples were collected along the rocky shores during surveys focused on assessing creeping brown algal biodiversity in the period spanning 2013–2021. Specimens of encrusting brown seaweeds were collected by hand with a chisel and hammer or taken as pebbles or boulders, shell substrata and basiphyte (such as *Padina*) containing the creeping algae. Samples were cleaned with a brush, air-dried for two to three days and preserved in zipper bag with silica gel. Voucher specimens are deposited in the herbarium of Chosun University, Korea (CUK) and the Marine Biodiversity Institute of Korea (MABIK), Korea.

Table 1. Sample and gene sequence information for species included in morphological and molecular studies.

Species	**Voucher No.**	**Collection details**	**GenBank accession number**		**References**
			***rbc*L**	**COI-5P /*cox*1**	
Outgroup					
Syringoderma abyssicola (Setchell & N.L. Gardner) Levring	KU-758	Kobe University macroalgal culture collection (KU-MACC)	AB899294	AB899185	Kawai et al. 2015
Tilopteris mertensii (Turner) Kützing	-	Helgoland, Germany. (D. G. Müller culture)	AB045260	-	Sasaki et al. 2001
″	FRA0109	Culture strain	-	EU681430	Silberfeld et al. 2010
Ingroup (Ralfsiales)					
Hapalospongidiaceae					
Hapalospongidion gelatinosum De A. Saunders	FCME:PTM10056/ M17H	Cabo Corrientes, Jalisco, Mexico 16. Apr. 2015	KX610190	-	León-Álvarez et al. 2017

Table 1. (continued)

Species	**Voucher No.**	**Collection details**	**GenBank accession number**		**References**
			***rbc*L**	**COI-5P /*cox*1**	
Hapalospongidion gelatinosum De A. Saunders	FCME:PTM9576/VR30H	Las Cuatas, Guerrero, Mexico 06. Feb. 2013	KX610184	KX765493	León-Álvarez et al. 2017
″	FCME:PTM9657	El Palmar, Guerrero, Mexico 04. Feb. 2013	-	KX765489	León-Álvarez et al. 2017
Mesosporaceae					
Basispora africana D. M. John & G. W. Lawson	BM000770078/A1241	Komenda, Ghana	KY296819	-	Poong et al. 2017
Mesospora bifurcata A. O. Oteng'o, B. Y. Won & T. O. Cho sp. nov.	CUK19739	Igeumpo Beach, Geomundo, Samsan-myeon, Yeosu-si, Jeollanam-do, Korea 10. Jul. 2019	O	O	This study
″	CUK19741	Igeumpo Beach, Geomundo, Samsan-myeon, Yeosu-si, Jeollanam-do, Korea 10. Jul. 2019	O	O	This study
Mesospora elongata Poong, Lim & Phang	KU-d11206/FUS3	Fusaki, Ishigaki Island, Okinawa Prefecture, Japan	JQ620003	JQ620011	Poong et al. 2013

Table 1. (continued)

Species	**Voucher No.**	**Collection details**	**GenBank accession number**		**References**
			***rbc*L**	**COI-5P /*cox*1**	
Mesospora elongata Poong, Lim & Phang	PSM12214/NIP66	Nipah, Lombok Island, Indonesia	JQ620005	JQ620013	Poong et al. 2013
Mesospora indopacifica Poong, Lim & Phang	PSM12212/LEN41	Lendang Luar, Lombok Island, Indonesia	KP689604	KP689621	Poong et al. 2017
″	PSM12319/KEM111	Pantai Kemasik, Terengganu, Malaysia	KP689605	KP689622	Poong et al. 2017
Mesospora lombokensis Poong, Lim & Phang	PSM12239/LAB74	Labuhan Pandan, Lombok Island, Indonesia	KP689614	KP689631	Poong et al. 2017
″	PSM12242/LAB83	Labuhan Pandan, Lombok Island, Indonesia	KP689617	KP689634	Poong et al. 2017
Mesospora negrosensis J. West & H. Calumpong	PSM12326/CHD120	Terengganu, Pantai Chendering, Malaysia 16. Feb. 2012	KC847391	KC847370	Poong et al. 2014
″	PSM12183/MER14	Johor, Palau Merambong, Malaysia	KC847390	KC847369	Poong et al. 2014

Table 1. (continued)

Species	**Voucher No.**	**Collection details**	**GenBank accession number**		**References**
			***rbc*L**	**COI-5P /*cox*1**	
Mesospora. pangoensis (Setchell) Chihara & Tanaka	UC221298	Pago Pago Harbor, Tutuila Island, American Samoa	KP689620	KP689637	Poong et al. 2017
Mesospora schmidtii Weber Bosse	PSM12235/BAT43	Batukijok, Lombok Island, Indonesia	JQ620001	JQ620009	Poong et al. 2013
〃	PSM12187/MER18	Johor, Palau Merambong, Malaysia	KY296807	KY296829	Poong et al. 2017
Neoralfsiaceae					
Neoralfsia expansa (J. Agardh) P.-E. Lim & H. Kawai *ex* Cormaci & G. Furnari	FCME:GM651	Morro de la Mancha, Veracruz, Mexico 29. Apr. 2010	KM032760	-	León-Álvarez et al. 2014b
〃	FCME:GM649	Morro de la Mancha, Veracruz, Mexico 18. May. 2010	KM032758	-	León-Álvarez et al. 2014b

Table 1. (continued)

Species	**Voucher No.**	**Collection details**	**GenBank accession number**		**References**
			***rbc*L**	**COI-5P /*cox*1**	
Neoralfsia expansa (J. Agardh) P.-E. Lim & H. Kawai *ex* Cormaci & G. Furnari	KU-d2243/RspG	Hyogo, Igumi, Japan	AB250079	-	Lim et al. 2007
〃	RspK	Hyogo, Takeno, Oura, Japan	AB250083	-	Lim et al. unpublished
〃	CUK19705A	Saegdal-dong, Seogwipo-si, Jeju-do, Korea. 06. Jun. 2019	O	-	This study
〃	CUK20038	Bomok Port, Bomok-dong, Seogwipo-si, Jeju-do, Korea. 26. Oct. 2019	O	O	This study
〃	CUK20756A	Seongsan Ilchul Peak, Seongsan-eup, Seogwipo-si, Jeju-do, Korea. 17. Aug. 2020	O	O	This study
Neoralfsia hancockii (E.Y. Dawson) D. León-Álvarez & M.L. Núñez -Reséndiz	FCME:PTM9167	Baja California, Mexico 30. Sept. 2009	KF977827	-	León-Álvarez et al. 2014a

Table 1. (continued)

Species	**Voucher No.**	**Collection details**	**GenBank accession number**		**References**
			***rbc*L**	**COI-5P /*cox*1**	
Neoralfsia hancockii (E.Y. Dawson) D. León-Álvarez & M.L. Núñez -Reséndiz	FCME:PTM9165	La Palmilla, Baja California, Mexico 30. Sept. 2009	KF977828	-	León-Álvarez et al. 2014a
Neoralfsiopsis jejuensis A. O. Oteng'o, B. Y. Won & T. O. Cho sp. nov.	CUK19507C	Seongsan Ilchul Peak, Seongsan-eup, Seogwipo-si, Jeju-do, Korea. 19. Apr. 2019	O	O	This study
″	CUK19606B	Seongsan Ilchul Peak, Seongsan-eup, Seogwipo-si, Jeju-do, Korea. 01. May. 2019	O	O	This study
Pseudoralfsiaceae					
Nuchella vesicularis Parente, Fletcher & G. W. Saunders	PG071357	Reis Magos, Madeira, Portugal 31. Jul. 2007	MK972314	MK972258	Parente et al. 2021
″	MP13N	South Devon, Wembury, United Kingdom. 02. Jan. 2002	-	MK972255	Parente et al. 2021
Pseudoralfsia azorica Parente, Fletcher & G.W. Saunders	MD0001334d	Azores S. Miguel, Caloura, Portugal 08. Oct. 2009	MK972325	MK972309	Parente et al. 2021

Table 1. (continued)

Species	**Voucher No.**	**Collection details**	**GenBank accession number**		**References**
			***rbc*L**	**COI-5P /*cox*1**	
Pseudoralfsia verrucosa (Areschoug) Parente, Fletcher & G.W. Saunders	NOR01	Marine Station, Norwegian west coast, Norway 16. Sept. 2010	MH593217	MH593177	Parente et al. 2021
″	GWS030522	Gwaii Haanas, Gordon Islands (Lagoon), British Columbia, Canada. 07. Jun. 2012	MK972312	-	Parente et al. 2021
″	GWS022408	Between 1st and 2nd Beach, English Bay, Vancouver, British Columbia, Canada 20. May. 2010	-	MK972285	Parente et al. 2021
Ralfsiaceae					
Analipus japonicus (Harvey) M. J. Wynne	KU-MACC:KU-883	Oshoro, Hokkaido Pref., Japan	AB776777	AB776658	Kawai et al. 2015
″	GWS035897	Woodruff Bay (NE Beach), Gwaii Haanas, British Columbia, Canada 20. Aug. 2013	MH593219	MH593180	Parente and Saunders 2019
″	-	-	-	MZ156065	Starko et al. 2021

Table 1. (continued)

Species	**Voucher No.**	**Collection details**	**GenBank accession number**		**References**
			***rbc*L**	**COI-5P /*cox*1**	
Endoplura aurea Hollenberg	KU-d2273/KU1465	Inubouzaki, Chiba Pref., Japan	AB264039	-	Lim et al. 2007
Endoplura geojensis A. O. Oteng'o, B. Y. Won & T. O. Cho sp. nov.	CUK18789C	Waheon, Irun-myeon, Geoje-si, Gyeongsangnam-do, Korea 16. Mar. 2018	O	O	This study
″	CUK20066C	Hwasam-ri, Yongnam-myeon, Tongyeong-si, Gyeongsangnam-do, Korea 28. Oct. 2019	O	O	This study
″	CUK20228	Seongu-ri, Nam-myeon, Namhae-gun, Gyeongsangnam-do, Korea. 31. Mar. 2020	O	O	This study
Endoplura gyeokpoensis A. O. Oteng'o, B. Y. Won & T. O. Cho sp. nov.	CUK19785C	Gyeokpo Port, Byeongsan-myeon, Buan-gun, Jeollabuk-do, Korea. 26. July. 2019	O	O	This study
″	CUK20923B	Gyeokpo Port, Byeongsan-myeon, Buan-gun, Jeollabuk-do, Korea. 09. May. 2021	O	O	This study

Table 1. (continued)

Species	**Voucher No.**	**Collection details**	**GenBank accession number**		**References**
			***rbc*L**	**COI-5P /*cox*1**	
Endoplura jejuensis A. O. Oteng'o, T. O. Cho & B. Y. Won	MABIK AL00080969 (CUK19630A)	Seongsan Ilchul Peak, Seongsan-eup, Seogwipo-si, Jeju-do, Korea. 01. May. 2019	MW627308	MW627316	Oteng'o et al. 2021
″	CUK20756C	Seongsan Ilchul Peak, Seongsan-eup, Seogwipo-si, Jeju-do, Korea. 17. Aug. 2020	MW627309	MW627317	Oteng'o et al. 2021
Endoplura koreana A. O. Oteng'o, T. O. Cho & B. Y. Won	CUK18755B	Pado-ri, Sowon-myeon, Taean-gun, Chungcheongnam-do, Korea. 03. Mar. 2018	MW627310	MW627318	Oteng'o et al. 2021
″	CUK19175A&B	Gijang, Ilgwang-myeon, Gijang-gun, Busan, Korea 08. Oct. 2018	MW627311	MW627319	Oteng'o et al. 2021
″	CUK19814C	Oeyeondo, Ocheonmyeon, Boryeong-si, Chungcheongnam-do, Korea 01. Aug. 2019	MW627312	MW627320	Oteng'o et al. 2021
″	CUK20574	Chosa-ri, Uisin-myeon, Jindo-gun, Jeollanam-do, Korea 24. Jul. 2020	MW627313	MW627321	Oteng'o et al. 2021

Table 1. (continued)

Species	**Voucher No.**	**Collection details**	**GenBank accession number**		**References**
			***rbc*L**	**COI-5P /*cox*1**	
Endoplura koreana A. O. Oteng'o, T. O. Cho & B. Y. Won	MABIK AL00080968 (CUK20628)	Dumunjin Port, Baekryeongdo, Incheon, Korea. 12. Aug. 2020	MW627314	MW627322	Oteng'o et al. 2021
Endoplura koreana A. O. Oteng'o, T. O. Cho & B. Y. Won	CUK20669	Gobong Port, Baekryeongdo, Incheon, Korea 12. Aug. 2020	MW627315	MW627323	Oteng'o et al. 2021
Endoplura limpeticola A. O. Oteng'o, B. Y. Won & T. O. Cho sp. nov.	CUK20582	Jeongdo-ri, Wando-eup, Wando-gun, Jeollanam-do, Korea. 05. Aug. 2020	O	O	This study
″	CUK20823	Jeongdo-ri, Wando-eup, Wando-gun, Jeollanam-do, Korea. 15. Nov. 2020	O	O	This study
Fissipedicella orientalis A. O. Oteng'o, B. Y. Won & T. O. Cho sp. nov.	CUK18754	Pado-ri, Sowon-myeon, Taean-gun, Chungcheongnam-do, Korea. 03. Mar. 2018	O	O	This study
″	CUK19178A	Gijang, Ilgwang-myeon, Gijang-gun, Busan, Korea 08. Oct. 2018	O	O	This study

Table 1. (continued)

Species	**Voucher No.**	**Collection details**	**GenBank accession number**		**References**
			***rbc*L**	**COI-5P /*cox*1**	
Fissipedicella orientalis A. O. Oteng'o, B. Y. Won & T. O. Cho sp. nov.	CUK19509C	Seongsan Ilchul Peak, Seongsan-eup, Seogwipo-si, Jeju-do, Korea. 19. Apr. 2019	O	O	This study
″	CUK19707B	Yeraehaean-ro, Seogwipo-si, Jeju-do, Korea. 06. Jun. 2019	O	O	This study
″	CUK20041D	Bomok Port, Bomok-dong, Seogwipo-si, Jeju-do, Korea 26. Oct. 2019	O	O	This study
″	CUK20066A	Hwasam-ri, Yongnam-myeon, Tongyeong-si, Gyeongsangnam-do, Korea 28. Oct. 2019	O	O	This study
″	CUK20332	Jeongdo-ri, Wando-eup, Wando-gun, Jeollanam-do, Korea. 08. May. 2020	O	O	This study
″	CUK20771	Dolsan Port, Dolsan-eup, Yeosu-si, Jeollanam-do, Korea 17. Oct. 2020	O	O	This study

Table 1. (continued)

Species	Voucher No.	Collection details	GenBank accession number		References
			*rbc*L	COI-5P /*cox*1	
Heteroralfsia saxicola (Okamura & Yamada) Kawai	KU-882	Ohma, Aomori Pref., Japan	AB250070	-	Lim et al. 2007
〃	-	Ohma, Aomori Pref., Japan	AB264040	-	Lim et al. unpublished
Ralfsia claviformis A. O. Oteng'o, B. Y. Won & T. O. Cho sp. nov.	CUK18162C	Cheongsapo, Jung-dong, Haeundae-gu, Busan, Korea 08. Jul. 2017	O	O	This study
〃	CUK18403B	Chaeseokgang, Buan-gun, Jeollabuk-do, Korea 17. Nov. 2017	O	O	This study
〃	CUK18412	Geumgap beach, Uisin-myeon, Jindo-gun, Jeollanam-do, Korea. 21. Oct. 2017	O	O	This study
〃	CUK18423A	Dala park, Sanyang-eup, Tongyeong-si, Gyeongsangnam-do, Korea 03. Nov. 2017	O	O	This study
〃	CUK19698C	Saegdal-dong, Seogwipo-si, Jeju-do, Korea. 06. Jun. 2019	O	O	This study

Table 1. (continued)

Species	**Voucher No.**	**Collection details**	**GenBank accession number**		**References**
			***rbc*L**	**COI-5P /*cox*1**	
Ralfsia claviformis A. O. Oteng'o, B. Y. Won & T. O. Cho sp. nov.	CUK19816A	Oeyeondo, Ocheonmyeon, Boryeong-si, Chungcheongnam-do, Korea 01. Aug. 2019	O	O	This study
″	CUK20576	Jeongdo-ri, Wando-eup, Wando-gun, Jeollanam-do, Korea. 05. Aug. 2020	O	-	This study
Ralfsia fungiformis (Gunnerus) Setchell & N. L. Gardner	LLG0038/LBC75	Cap du Bon Désir, Québec, Canada. 21. Sep. 2004	EU579936	EU681419	Bittner et al. 2008
″	GWS012423	Bay of Funfy, Lepreau exposed biodiversity site, New Brunswick, Canada. 28. May. 2009	MH277273	MH309979	Bringloe and Saunders unpublished
Ralfsia robertii Parente & Saunders	GWS017821	Right side, Gloucester, Folly Cove, Massachusetts, U.S.A 13. Apr. 2010	MH593186	HM915088	Parente and Saunders 2019

Table 1. (continued)

Species	**Voucher No.**	**Collection details**	**GenBank accession number**		**References**
			***rbc*L**	**COI-5P /*cox*1**	
Ralfsia robertii Parente & Saunders	GWS014034	Bay of Funfy, Lepreau exposed biodiversity site, New Brunswick, Canada. 07. Sept. 2010	MH593215	-	Parente and Saunders 2019
″	GWS003097	Bay of Funfy, Lepreau exposed biodiversity site, New Brunswick, Canada 07. Oct. 2005	-	MH593179	Parente and Saunders 2019
Ralfsia tenebris Parente & Saunders	GWS019661	Opening of Grappler Inlet, Bamfield, British Columbia, Canada. 03. Jun. 2010	MH593212	HQ919317	Parente and Saunders 2019
″	GWS014321	Scotts Bay, Bamfield, British Columbia, Canada 25. Jun. 2011	MH593185	-	Parente and Saunders 2019
″	GWS014341	Black Fish I., near Helby L., Bamfield, British Columbia, Canada. 25. Jun. 2011	-	MH593163	Parente and Saunders 2019

Table 1. (continued)

Species	**Voucher No.**	**Collection details**	**GenBank accession number**		**References**
			***rbc*L**	**COI-5P /*cox*1**	
Ralfsia unimaculata Parente & Saunders	GWS021014	NW of Masset, Haida Gwaii, Between Wiah Point & Cape Edensaw (#2), British Columbia, Canada 08. Jun. 2010	MH593202	HQ990670	Parente and Saunders 2019
〃	GWS021144	Masset inlet, Haida Gwaii, Haida Gwaii, Cowley Islands, British Columbia, Canada 09. Jun. 2010	MH593196	-	Parente and Saunders 2019
Ramipedicella longicellularis A. O. Oteng'o, B. Y. Won & T. O. Cho comb. nov.	CUK18820	Jeongdo-ri, Wando-eup, Wando-gun, Jeollanam-do, Korea 01. Apr. 2018	O	-	This study
〃	CUK19221	Pado-ri, Sowon-myeon, Taean-gun, Chungcheongnam-do, Korea 03. Nov. 2018	O	O	This study
〃	CUK19269	Chaeseokgang, Buan-gun, Jeollabuk-do, Korea 26. Jan. 2019	O	-	This study

Table 1. (continued)

Species	Voucher No.	Collection details	GenBank accession number		References
			*rbc*L	COI-5P /*cox*1	
Ramipedicella longicellularis A. O. Oteng'o, B. Y. Won & T. O. Cho comb. nov.	CUK20024	Ulitsa Leytenanta Shmidta, Vladivostok, Russia 10. Oct. 2019	O	-	This study
″	CUK20617A	Chunghwa-dong Port, Baekryeongdo, Incheon, Korea 12. Aug. 2020	O	O	This study
″	CUK20650B	Dumunjin Port, Baekryeongdo, Incheon, Korea. 12. Aug. 2020	O	-	This study
″	CUK20664C	Jinchon-ri 40-41 Sagot Beach, Baekryeongdo, Incheon, Korea 12. Aug. 2020	O	-	This study
Ramipedicella microloba A. O. Oteng'o, B. Y. Won & T. O. Cho sp. nov.	CUK13559	Bangeojin, Bangeo-dong, Dong-gu, Ulsan, Korea 22. Jan. 2015	O	-	This study
″	CUK18395	Daecheon Beach, Sinheuk-dong, Boryeong-si, Chungcheongnam-do, Korea 18. Nov. 2017	O	-	This study

Table 1. (continued)

Species	**Voucher No.**	**Collection details**	**GenBank accession number**		**References**
			***rbc*L**	**COI-5P /*cox*1**	
Ramipedicella microloba A. O. Oteng'o, B. Y. Won & T. O. Cho sp. nov.	CUK18428A	Dala park, Sanyang-eup, Tongyeong-si, Gyeongsangnam-do, Korea 03. Nov. 2017	O	-	This study
″	CUK18731	Cheongsapo, Jung-dong, Haeundae-gu, Busan, Korea 01. Mar. 2018	O	-	This study
″	CUK18737A	Yeonhwa-ri, Gijang- eup, Gijang-gun, Busan, Korea 01. Mar. 2018	O	-	This study
″	CUK18883	Bangjukpo, Dolsan-eup, Yeosu-si, Jeollanam-do, Korea 29/Apr/2018	O	-	This study
″	CUK18972	Seosang, Seo-myeon, Namhae-gun, Gyeongsangnam-do, Korea. 18. May. 2018	O	O	This study
″	CUK19150	Chaeseokgang, Byeonsan-myeon, Buan-gun, Jeollabuk-do, Korea 03. Oct. 2018	O	-	This study

Table 1. (continued)

Species	**Voucher No.**	**Collection details**	**GenBank accession number**		**References**
			***rbc*L**	**COI-5P /*cox*1**	
Ramipedicella microloba A. O. Oteng'o, B. Y. Won & T. O. Cho sp. nov.	CUK19218A	Pado-ri, Sowon-myeon, Taean-gun, Chungcheongnam-do, Korea 03. Nov. 2018	O	-	This study
″	CUK19794	Gyeokpo Port, Byeonsan-myeon, Buan-gun, Jeollabuk-do, Korea 26. Jul. 2019	O	-	This study
″	CUK19823	Oeyeon-do, Ocheon-myeon, Boryeong-si, Chungcheongnam-do, Korea 01. Aug. 2019	O	-	This study
″	CUK20165	Hwasam-ri183-7, Yongnam-myeon, Tongyeong-si, Gyeongsangnam-do, Korea 08. Feb. 2020	O	O	This study
″	CUK20589	Jeongdo-ri, Wando-eup, Wando-gun, Jeollanam-do, Korea. 05. Aug. 2020	O	O	This study

Table 1. (continued)

Species	Voucher No.	Collection details	GenBank accession number		References
			***rbc*L**	**COI-5P /*cox*1**	
Ramipedicella microloba A. O. Oteng’o, B. Y. Won & T. O. Cho sp. nov.	CUK20613A	Chunghwa-dong Port, Baekryeongdo, Incheon, Korea 12. Aug. 2020	O	O	This study
″	CUK20649	Dumunjin Port, Baekryeongdo, Incheon, Korea 12. Aug. 2020	O	-	This study
″	CUK20676A	Gobong Port, Baekryeongdo, Incheon, Korea 12. Aug. 2020	O	-	This study
Sungminiaceae A. O. Oteng’o, B. Y. Won & T. O. Cho fam. nov.					
Sungminia asiatica A. O. Oteng’o, B. Y. Won & T. O. Cho sp. nov.	CUK19175C	Gijang, Ilgwang-myeon, Gijang-gun, Busan, Korea 08. Oct. 2018	O	O	This study
″	CUK19812B	Oeyeondo, Ocheon-myeon, Boryeong-si, Chungcheongnam-do, Korea 01. Aug. 2019	O	O	This study

Table 1. (continued)

Species	**Voucher No.**	**Collection details**	**GenBank accession number**		**References**
			***rbc*L**	**COI-5P /*cox*1**	
Sungminia asiatica A. O. Oteng'o, B. Y. Won & T. O. Cho sp. nov.	CUK20636A	Dumunjin Port, Baekryeongdo, Incheon, Korea 12. Aug. 2020	O	O	This study
″	CUK20874	Daecheon Port, Sinheuk-dong, Boryeong-si, Chungcheongnam-do, Korea 28. Nov. 2020	O	O	This study
Sungminia gladiata A. O. Oteng'o, B. Y. Won & T. O. Cho sp. nov.	CUK19619B	Seongsan Ilchul Peak, Seongsan-eup, Seogwipo-si, Jeju-do, Korea 01. May. 2019	O	O	This study
″	CUK19816B	Oeyeondo, Ocheonmyeon, Boryeong-si, Chungcheongnam-do, Korea 01. Aug. 2019	O	O	This study
″	CUK20249	Gujora Beach, Irun-myeon, Geoje-si, Gyeongsangnam-do, Korea. 01. Apr. 2020	O (P3)	O	This study

Table 1. (continued)

Species	**Voucher No.**	**Collection details**	**GenBank accession number**		**References**
			***rbc*L**	**COI-5P /*cox*1**	
Sungminia gladiata A. O. Oteng'o, B. Y. Won & T. O. Cho sp. nov.	CUK20926D	Gyeokpo Port, Byeongsan-myeon, Buan-gun, Jeollabuk-do, Korea 09. May. 2021	O	O	This study
Sungminia pyriformis A. O. Oteng'o, B. Y. Won & T. O. Cho sp. nov.	CUK18425	Dala park, Sanyang-eup, Tongyeong-si, Gyeongsangnam-do, Korea 03. Nov. 2017	O	-	This study
〃	CUK19694A	Saegdal-dong, Seogwipo-si, Jeju-do, Korea 06. Jun. 2019	O	O	This study

O, represent sequence without genbank accession number; -, represent no sequence

2. Morphological examination

Morphological observations were fulfilled at two levels: 1) external or surface morphology; and 2) internal morphology. Before the samples were processed for storage, surface view was done and photographs taken under water. Then vouchers were air-dried and preserved in silica gel for molecular and internal morphological analyses. Specimens were identified on the basis of the following morphological features: erect vs creeping thalli, color, lighter vs darker margins (present/absent), thalli shape (outline), smooth vs rough surface, growth lines (present/absent), thalli size (diameter and thickness), rhizoids (present/absent), delimitation of cortex and medulla, number of basal cell layers, thallus construction/arrangement of vegetative erect filaments (straight/unilateral/bilateral; tightly or firmly adhering/moderately adhering/free or loosely adhered), branching of the erect filaments, cell size (length and width), chloroplast shape and number per cell, hairs (present/absent; origin), life history pattern (isomorphic vs heteromorphic), plurangial position, number of sterile cells terminating the plurangia, number of plurangial reproductive filaments, number of unangial stalk cells, position of the unangia, paraphyses (present/absent; number per unangium), soral filaments (present/absent) (Agardh 1848, Weber-van Bosse 1913, Hollenberg 1969, Tanaka and Chihara 1980a, b, c, 1981a, b, c, 1982, Anderson et al. 1988, Kawai 1989, León-Álvarez and Norris 2005, Lim et al. 2007, Kawai et al. 2016, Poong et al. 2017, Parente et al. 2021). Newly proposed features observed in this study include: branching of plurangial reproductive structures, number of unangia per stalk and unangial stalk morphology (simple/branched and cleaved/uncleaved).

2.1. Photography

To document their external morphology related to characters, including color, outline, and surface morphology, the fresh samples were cleaned with a brush and photographed inside an aquarium with a waterproof digital camera (Nikon COOLPIX AW100; Nikon Corp., Tokyo, Japan). Digitized images were edited for clarity using the Adobe Photoshop software ver. 6.1 (Adobe Systems Inc., San Jose, CA, USA).

2.2. Light microscopy (LM)

The samples were flooded with autoclaved sea water for about 20 minutes and then detached from the substrate using a single-edged blade before the analysis of internal morphological characters. Squashed and microtome-sectioned preparations were made 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). The digitized images obtained were edited for clarity using the Adobe Photoshop software ver. 6.1 (Adobe Systems Inc., San Jose, CA, USA).

Preparation of solutions for staining

1) Aniline blue solution

Materials: aniline blue (Sigma-Aldrich 415049-25G), distilled water, reagent bottle

Procedure:

(1) Place 50 ml of distilled water into bottle

(2) Dissolve 0.5 g of aniline blue

(3) Shake vigorously

2) 1:1 mixture of Aniline blue - Acetic acid

Materials: aniline blue solution, acetic acid, 100 ml reagent bottle

Procedure:

1. Pour 50 ml of aniline blue solution in the 100 ml reagent bottle
2. Add 50 ml of acetic acid into reagent bottle
3. Shake vigorously

3. Molecular analyses

3.1. DNA extraction, PCR amplification, purification, and sequencing

DNA was extracted from 70 silica gel-dried representative specimens. For molecular analyses, the samples were cleaned with brush and autoclaved seawater under a dissecting microscope, then only small fragments of material were scooped from approximately the upper two third layer of crust. Samples were prepare using clean materials and in batches of 5 at a time to avoid the risk of contamination.

Samples were ground with sand for DNA extraction. Genomic DNA was extracted with the NucleoSpin Plant II Kit (Macherey-Nagel, Düren, Germany), following the manufacturer's protocol. The extracted DNA was stored at -20°C and used to amplify the *rbc*L and COI-5P genes. Polymerase chain reaction (PCR) was performed with a final volume 30 µl using 3.2–4.5 µl of genomic DNA, 1 µl of 10 pmol forward and reverse primers and the HelixAmp Ready-2x-Go premix (NanoHelix Co., Ltd., Daejeon, Korea). PCR products were purified with PCRquick-spin PCR product purification kit (iNtRON Biotechnology, Inc. Seongnam, Korea) and sequenced at Macrogen (Seoul, Korea). Sequences were determined for both forward and reverse strands using an ABI prism 3100 Genetic Analyzer (Life Technologies, Seoul, Korea). Sequences generated in the study and others obtained from GenBank were aligned with ClustalW (Thompson et al. 1994) and corrected manually using MEGA X (Kumar et al. 2018).

Mitochondrial-encoded cytochrome c oxidase subunit I (COI-5P) sequence analyses

COI-5P from newly collected samples encompassing 27 taxa of the order Ralfsiales (Table 1) was sequenced in both directions (5' and 3') and analyzed in this study using primer combinations GazF2-GazR2 and GWSFn-GWSRx. The choice of a primer combination depended on the level of success the primer produced in PCR during the initial tests in a taxon. *Syringoderma abyssicola* (Setchell and N.L. Gardner) Levring (AB899185) and *Tilopteris mertensii* (Turner) Kützing (EU681430) were selected as outgroups for this study. The PCR conditions for the primer combination GazF2-GazR2 were as described by Lane et al. (2007) with some modifications: initial denaturation at 94°C for 4 min, followed by 38 cycles of 1 min denaturation at 94°C, 30 s annealing at 54°C and 1 min extension at 72°C, followed a final extension at 72°C for 7 min and then storage at 4°C. The thermal profile for PCR amplification using primer combination GWSFn-GWSRx as described by Saunders and McDevit (2012) consisted of an initial denaturation at 95°C for 2 min, followed by 5 cycles of 30 s denaturation at 95°C, 30 s annealing at 45°C, and 1 min extension at 72°C, followed by 35 cycles of 30 s denaturation at 95°C, 30 s annealing at 46.5°C and 1 min extension at 72°C, followed by an additional 7 min at 72°C and storage at 4°C. The COI-5P gene region was amplified using the GazF2-GazR2 (Lane et al. 2007) or GWSFn-GWSRx (Saunders and McDevit 2012) primer combinations and cycle sequences using the same primers used for amplification (Table 2).

Chloroplast encoded Ribulose bisphosphate carboxylase large chain (rbcL) sequence analyses

*Rbc*L only from newly collected samples encompassing 27 taxa of the order Ralfsiales (Table 1) was sequenced in both directions (5' and 3') and analyzed in this study using available *rbc*L primers (Table 2). *Syringoderma abyssicola* (Setchell and N.L. Gardner) Levring (AB899294) and *Tilopteris mertensii* (Turner) Kützing (AB045260) were selected as outgroups for this study. The PCR conditions for the *rbc*L gene were as described by Oteng'o et al. (2021) as follows: initial denaturation at 94°C for 4 min; 35 cycles of denaturation at 94°C for 30 s, annealing at 40°C for 30 s, and extension at 72°C for 1 min; final extension at 72°C for 10 min and storage at 4°C. The partial *rbc*L locus was amplified using the ND*rbc*L2-DRL1R (Daugbjerg and Andersen 1997, Hwang et al. 2005) combination for front fragment and DRL2F-R3A (Kogame et al. 1999, Hwang et al. 2005) combination for back fragment and cycle sequences done using the same primers used for amplification (Table 2).

3.2. Phylogenetic analyses

Three data sets were used for the phylogenetic analyses: 33 taxa for COI-5P, 36 taxa for *rbc*L, and 36 taxa for the concatenated COI-5P and *rbc*L sequence data sets. Before performing phylogenetic analyses for the protein-coding data sets, *rbc*L and COI-5P, both the best model of nucleotide and the best combination of partitions by codon position 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 1,000 bootstrap (BS) replications using RAxMLGUI v1.5 (Silvestro and Michalak 2012) and MEGA X (Kumar et al. 2018). 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 *rbc*L, COI-5P and concatenated *rbc*L and COI-5P sequence datasets (Tables 1) were constructed to delimit the species, generic and family boundaries within the order Ralfsiales. The phylogenetic trees were constructed using the ML and MrBayes and expressed using FigTree ver. 1.4.0 (Rambaut 2012) and Adobe illustrator program.

Table 2. Primer sequences used in amplification and sequencing reactions of COI-5P and *rbc*L.

Primer	**Primer sequences**	**References**
COI-5P		
GazF2	CCAACCAYAAAGATATWGGTAC	Saunders 2005, Lane et al. 2007
GazR2	GGATGACCAAARAACCAAAA	Lane et al. 2007
GWSFn	TCAACAAAYCAYAAAGATATYGG	Saunders and McDevit 2012
GWSRx	ACTTCTGGRTGICCRAARAAYCA	Saunders and McDevit 2012
***rbc*L**		
ND*rbc*L2	AAAAGTGACCGTTATGAATC	Daugbjerg and Andersen 1997
DRL1R	CCRGAAAGACCTAATTTAGG	Hwang et al. 2005
DRL2F	TACGTTTAGAAGATATGAGAAT	Hwang et al. 2005
R3A	GTAATATCTTTCCATAAATCTAA	Kogame et al. 1999

III. RESULTS AND DISCUSSION

PART 1. MORPHOLOGY OF SPECIES IN THE ORDER RALFSIALES

1. Family Mesosporaceae J. Tanaka & Chihara 1982

Type genus: *Mesospora* Weber Bosse 1911

1.1. Genus Mesospora Weber Bosse 1911

1.1.1. *Mesospora bifurcata* A. O. Oteng'o, B. Y. Won & T. O. Cho sp. nov. (Fig. 2)

Diagnosis: Plants epilithic, mucilaginous crusts, dark greenish brown, circular to irregular outline, smooth surface, up to 2.5 cm across, 285–696 μm thick, firmly attached to the substratum, without rhizoids; vegetative erect filaments composed of 20–60 cells, infrequent dichotomously branched, straight, loosely held in mucilaginous matrix, taper downwards, and arise from a one to two-layered hypothallial layer; chloroplasts one per cell; hairs tufts arise from basal disc; intercalary plurangial reproductive structures composed of a row of bifurcated or unbranched uniseriate reproductive filaments, each terminated by 1–2 sterile cells; sessile unangia borne laterally at the middle of the parent filament, without paraphyses.

Holotype: CUK19741. Voucher specimen was deposited in the herbarium of Chosun University, Korea (CUK).

Type locality: Igeumpo Beach, Geomun Island, Samsan-myeon, Yeosu-si, Jeollanam-do, Korea; 34°03′19.47″N, 127°17′40.10″E; intertidal; collected by A. O. Oteng'o; 10 July 2019.

Isotype: CUK19738, **CUK19739**, **CUK19740**, **CUK19742** & **CUK19754**. Voucher specimens were deposited in the herbarium of Chosun University, Korea (CUK).

Specimens examined: CUK19738, **CUK19739**, **CUK19740**, **CUK19741**, **CUK19742** & **CUK19754** (Igeumpo Beach, Geomun Island, Samsan-myeon, Yeosu-si, Jeollanam-do, Korea, collected by A. O. Oteng'o, 10 July 2019).

Etymology: The specific epithet, "*bifurcata*", refers to the dichotomously branched plurangial reproductive structures.

Vegetative morphology: Thalli are epilithic mucilaginous crusts, dark greenish brown (dark brown when dry), circular to irregular in outline and often confluent, with smooth surface, up to 2.5 cm across, 285–696 µm thick, firmly attached to the substratum by the entire lower surface, and lacking rhizoids (Fig. 2A). Vegetative thallus is composed of a basal disc of prostrate filaments, erect filaments and hairs. Basal disc consists of one to two layers of creeping filaments in which cells are 7–12 µm wide and have a width to length ratio of 1:1.4–2 (Fig. 2B). Erect filaments arising from the basal disc are mostly simple, sometimes dichotomously branched in the mid to lower parts of thallus, straight, tapering towards the lower part of thallus, held loosely in a mucilaginous matrix and separate easily under slight pressure, composed of 20–60 cells (Figs 2C, D). Cells of the erect filaments are cylindrical to somewhat barrel-shaped, 8–18 µm long and 5–12 µm wide and with thick cell wall. Chloroplasts are mostly parietal, one per cell. Tuft of hairs arise from the basal disc and are randomly scattered on the thallus (Fig. 2E). Data on measured characters are summarized in Table 3.

Reproductive morphology: Reproductive portions of plurangia and unangia are on same or different thalli. Plurangial reproductive structures are 40–82 µm long, composed of a row of bifurcated or unbranched uniseriate reproductive filaments, rarely two rows of reproductive filaments, each terminated by 1–2 sterile cells (Fig. 2F). Unangia are sessile, borne laterally at the middle of the parent filament and surrounding erect filaments, without paraphyses (Fig. 2G). Unangia observed were young and immature, therefore sizes not given. Data on measured characters are summarized in Table 3.

Habitat and Phenology: Plants grow from the high to the low intertidal zone. They are found attached on hard substrates such as pebbles and rocks (epilithic) in sheltered areas. Plants with both mature plurangial reproductive structures and immature unangia were collected in July.

Distribution in Korea: Geomun Island, South sea (Fig. 3).

Remarks: *Mesospora bifurcata* sp. nov. is newly describe in Korea based on detailed morphological studies and molecular analyses. This new species is recognized as a *Mesospora* member by having epilithic, mucilaginous and pseudoparenchymatous thalli wholly adherent to the substratum, loosely adherent erect filaments arising from a basal disc of prostrate filaments, one chloroplast per cell, intercalary plurangia inserted near the apex of erect filaments, and by unangia borne laterally to the erect filaments. Diagnostic features delimiting this as a new species in *Mesospora* are greenish to dark brown mucilaginous crust thalli of up to 2.5 cm across; vegetative erect filaments composed of 20–60 cells, infrequent dichotomously branched, straight, loosely held in mucilaginous matrix; intercalary plurangial reproductive structures composed of a row of bifurcated or unbranched uniseriate reproductive filaments, each terminated by 1–2 sterile cells; sessile unangia borne laterally at the middle of the parent filament, without paraphyses.

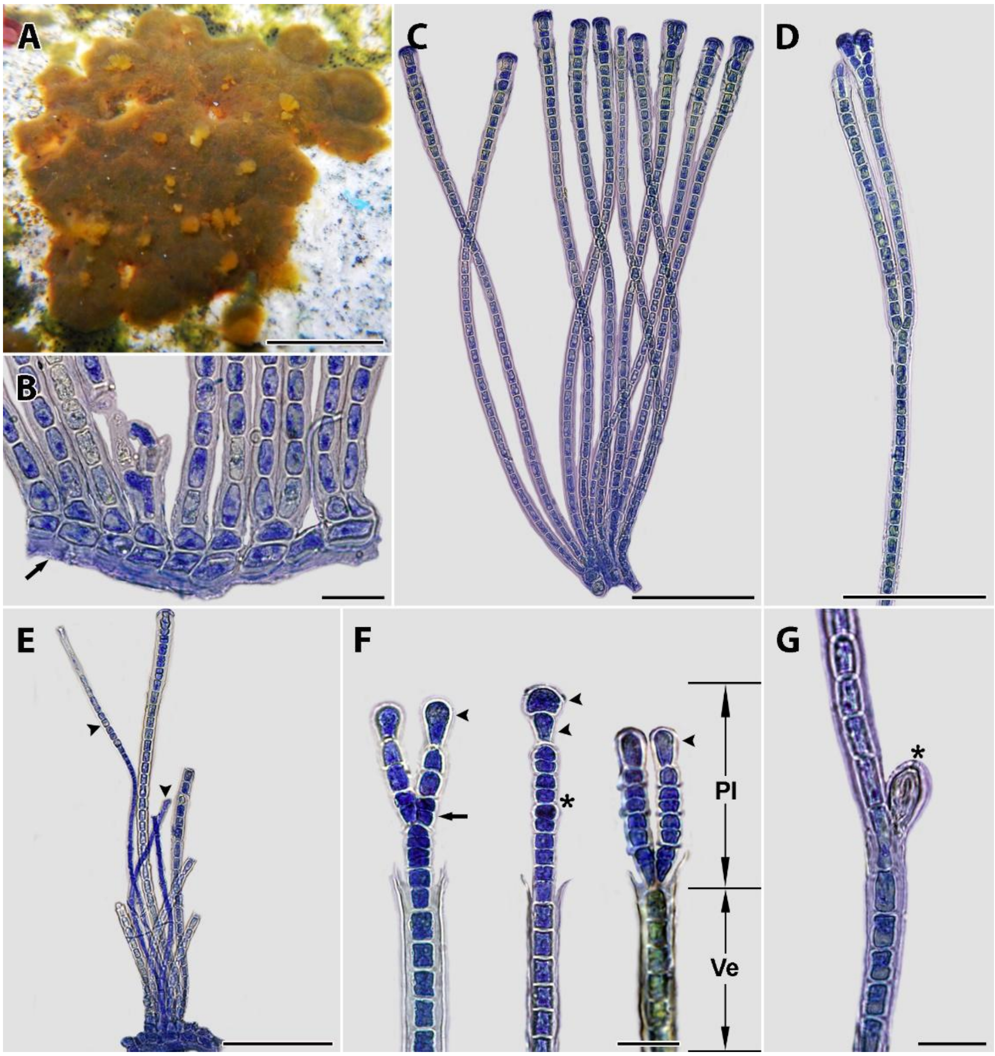


Fig. 2. Morphological characteristics of *Mesospora bifurcata* sp. nov.: (A) Thalli with dark greenish brown crusts and hair tufts as yellowish spots on the surface (CUK19741). (B) Radial section view of creeping vegetative thallus showing free erect filaments arising from a monostromatic hypothallial layer (arrow) (CUK19738). (C) A section of thallus showing free erect vegetative filaments (CUK19741). (D) Dichotomously branched erect vegetative filament (CUK19738). (E) Hairs (arrowhead) arise from the basal disc (CUK19754). (F) Plurangia arise from subterminal cells of erect vegetative filaments (Pl, plurangia; Ve, vegetative filaments), and are terminated by 1–2 sterile cells (arrowheads). Plurangial reproductive structures form uniseriate bifurcated rows (arrow), uniseriate row (asterisk), or rarely two uniseriate rows on a vegetative filament (unmarked) (CUK19739). (G) Unangium (asterisk) sessile, borne laterally at the mid portion of erect vegetative filament without a paraphysis (CUK19738). Scale bars represent: A, 0.5 cm; C, D & E, 100 μm; B, F & G, 20 μm.

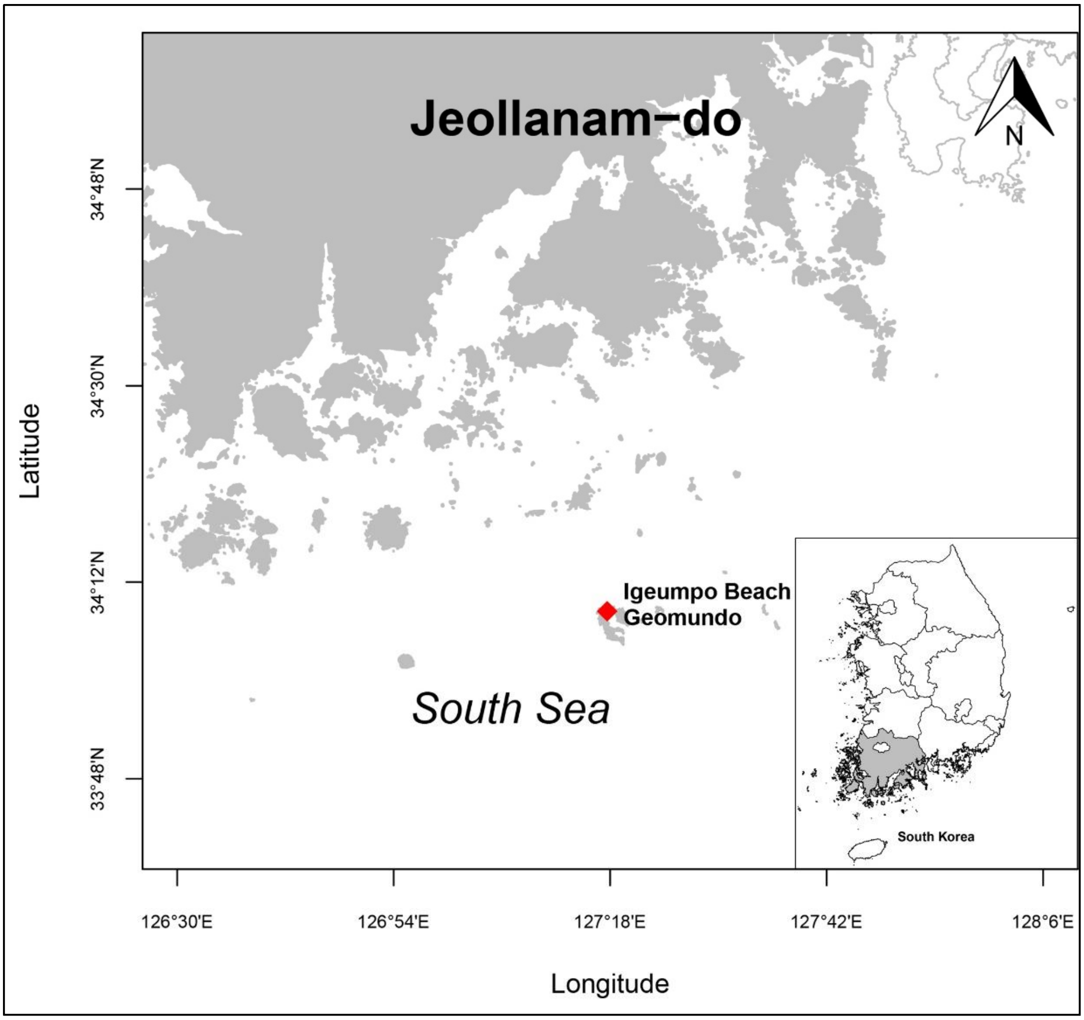


Fig. 3. Distribution of *Mesospora* species in Korea. *Mesospora bifurcata* sp. nov. (red).

Table 3. Comparison of morphological features between seven *Mesospora* species.

Characters	***M. bifurcata***	***M. elongata***	***M. indopacifica***	***M. lombokensis***	***M. negrosensis***	***M. pangoensis***	***M. schmidtii***
Substrate	Epilithic	Epilithic	Epilithic	Epilithic	Epilithic	Epilithic	Epilithic
Crust color	Dark greenish brown (dark brown when dry)	–	Greenish to dark brown	Light to dark brown	–	Dark brown to black	Light or pale brown
Lighter margins	Absent	–	–	–	–	–	–
Crust outline	Circular to irregular, confluent	–	Initially circular, later irregular, confluent	Circular, confluent	–	Circular	Circular to irregular, confluent
External growth lines or ridges	Absent	–	–	–	–	–	–

Table 3. (continued)

Characters	***M. bifurcata***	***M. elongata***	***M. indopacifica***	***M. lombokensis***	***M. negrosensis***	***M. pangoensis***	***M. schmidtii***
Surface	Smooth	–	–	–	–	–	Very smooth
Diameter of thalli	Up to 2.5 cm	–	–	–	–	1–7 cm	Up to 2 cm
Thickness of thalli	285–696 μm	150–250 μm	Up to 220 μm	150–230 μm	Usually <200 μm	200 (145–210[1]) μm	100–190
Basal cell layer	1–2	Generally 2	2 or 3	1–3	2–4	2–3	1–4, usually 2
Basal cell length	7–12 μm	–	3.4–10.9 μm	–	6–8 μm	7–10 μm[1]	4 μm
Basal cell width	10–24 μm	–	7.3–25.1 μm	–	11–13 μm	11–18 μm[1]	8–10 μm
Rhizoids	Absent	Absent	Absent	Absent	Absent	Absent	Absent

Table 3. (continued)

Characters	***M. bifurcata***	***M. elongata***	***M. indopacifica***	***M. lombokensis***	***M. negrosensis***	***M. pangoensis***	***M. schmidtii***
Organization and symmetry of erect filaments	Loosely associated and adjoined at basal portion, straight	Loosely associated and joined at basal portion	Basal closely packed and terminal loosely associated, straight	All laterally cohesive except in reproductive portion, assurgent	Lower half closely packed, rest loosely associated	Loosely associated, straight	Loosely associated and joined at basal portion, straight
Erect filament branching	Sparse dichotomous branching in lower and mid parts	–	–	–	Simple	Simple	Mostly simple
No. of cells per erect filament	20–60	Up to 30	6–18	ca. 20	Up to 20	18–20	8–20*

Table 3. (continued)

Characters	***M. bifurcata***	***M. elongata***	***M. indopacifica***	***M. lombokensis***	***M. negrosensis***	***M. pangoensis***	***M. schmidtii***
Erect filament cell length	8–18 µm	–	–	–	–	8–14 µm	6–19 µm
Erect filament cell width	5–12 µm	–	–	–	–	8–11 µm	5–12 µm
Chloroplasts / cell	1/cell	1/cell	–	–	1/cell	–	1/cell
Phaeophycean hairs	Form tufts	–	–	–	–	–	Form tufts
Origin of hairs	Basal disc	–	–	–	–	–	Basal disc
Plurangia and unangia	On same or different thalli	–	–	–	–	–	–

Table 3. (continued)

Characters	***M. bifurcata***	***M. elongata***	***M. indopacifica***	***M. lombokensis***	***M. negrosensis***	***M. pangoensis***	***M. schmidtii***
Length of plurangia	40–82 µm	(13.8) 22.1–49.9 µm	27.5–59.2 µm	14.9–58.1 µm	Variant 1: 11.6–17.5 µm Variant 2: 18.3–62.1 µm	25–38[1] µm	12.4–52.6 µm
Plurangial filaments	1	1, later 2	1, later 2	1, later 2	1, sometimes 2	2[1]	1, later 2
Bifurcate plurangia	Present	Absent	Absent	Absent	Absent	Absent	Absent
No. of sterile cells	1–2	2–4	2 or 3 (Seldom 1)	1	1–3	1–3 (generally 2[1])	2–3
Unangial shape	Young structures	–	–	–	–	Ellipsoid to obovate	Elliptical to obovate
Unangial length	Young structures	25.9–76.7 µm	37.1–70.7 µm	29.9–88.3 µm	15.9–99.6 µm	100–120 (70–150[1]) µm	24.2–143.2 (80–220[1]) µm

Table 3. (continued)

Characters	***M. bifurcata***	***M. elongata***	***M. indopacifica***	***M. lombokensis***	***M. negrosensis***	***M. pangoensis***	***M. schmidtii***
Unangial width	Young structures	11.5–26.9 µm	18.8–37.4 µm	9.1–33.8 µm	7.3–54.5 µm	60–100 (20–60[1]) µm	14.7–54.8 µm
Unangial insertion position on erect filament	Lateral to middle of erect filament	Terminally on stalk, lateral and basal of erect filament	Terminally on stalk, lateral and middle of erect filament	Terminally on stalk, lateral and middle of erect filament	Terminally on stalk, lateral and basal of erect filament	Terminally on stalk, lateral and middle of erect filament	Terminally on stalk, lateral and basal of erect filament
Unangial stalk	Absent (sessile)	Present	Present	Present	Present	Present	Present
No. of stalk cells	0	Up to 10	1 or 2	1–3	1–2, sometimes sessile	3 to 5 (2 to 3[1])	Up to 4
Paraphysis	Absent	Absent	Absent	Absent	Absent	Absent	Absent

Table 3. (continued)

Characters	***M. bifurcata***	***M. elongata***	***M. indopacifica***	***M. lombokensis***	***M. negrosensis***	***M. pangoensis***	***M. schmidtii***
Reference	Oteng'o et al. unpublished	Poong et al. 2013, 2014, 2017	Poong et al. 2017	Poong et al. 2017	West and Calumpong 1996; Poong et al. 2013	Setchell 1924; Poong et al. 2013, 2017[1]	Weber-van Bosse 1911, 1913; Tanaka and Chihara 1982[1]; Poong et al. 2013

2. Family Neoralfsiaceae P.-E. Lim & H. Kawai 2007

Type genus: *Neoralfsia* P.-E. Lim & H. Kawai 2007

2.1. Genus *Neoralfsia* P.-E. Lim & H. Kawai 2007

2.1.1. *Neoralfsia expansa* (J. Agardh) P.-E. Lim & H. Kawai ex Cormaci & G. Furnari 2012 (Fig. 4)

Basionym: *Myrionema expansum* J. Agardh 1847: 7

Homotypic synonym: *Ralfsia expansa* (J. Agardh) J. Agardh 1848: 63

Type locality: Veracruz, Mexico (Silva et al. 1996: 573).

Specimens examined: CUK19510A, **CUK19512A**, **CUK19516** (Seongsan Ilchul Peak, Seongsan-eup, Seogwipo-si, Jeju Island, Korea, collected by T. O. Cho & B. Y. Won, 19 April 2019), **CUK19592A**, **CUK19594A**, **CUK19609A**, **CUK19617A**, **CUK19634A**, **CUK19635A**, **CUK19636A**, **CUK19638A**, **CUK19640A**, **CUK19641A**, **CUK19642A**, **CUK19700A**, **CUK19702A**, **CUK19705A** (Seongsan Ilchul Peak, Seongsan-eup, Seogwipo-si, Jeju Island, Korea, collected by T. O. Cho & S. Y. Jeong, J. Avila, A. O. Oteng'o & G. C. Choi, 01 May 2019), **CUK20037**, **CUK20038**, **CUK20040B**, **CUK20041A** (Bomok Port, Bomok-dong, Seogwipo-si, Jeju Island, Korea, collected by T. O. Cho & B. Y. Won, 26 October 2019), **CUK20752A** (Gapari, Daejeong-eup, Seogwipo-si, Jeju Island, Korea, collected by T. O. Cho & B. Y. Won, 18 August 2020), **CUK20756A**, **CUK20757A** (Seongsan Ilchul Peak, Seongsan-eup, Seogwipo-si, Jeju Island, Korea, collected by T. O. Cho & B. Y. Won, 17 August 2020).

Vegetative morphology: Thalli are mostly epilithic expanded crusts, light to yellowish brown (olive to dark brown when dry), circular to irregular in outline and often confluent, seldom with lobes, with smooth to warty surface, with conspicuous growth lines, up to 7.5 cm across and 228–1349 µm thick, first attached firmly to the substratum by entire under surface and sometimes by means of many rhizoids, later tending to be entirely free (Fig. 4A). Vegetative thallus is composed of two distinct parts, medullary and cortical layers, with filaments tightly adjoined to form pseudoparenchymatous tissue (Fig. 4B). Medullary layer showing a symmetrically bilateral structure is composed of upwardly and downwardly curved filaments arising from the central part of the thallus, whose cells are 13–118 µm long and 6–40 µm wide. Upwardly curved filaments taper downwards to the central part of the thallus. Terminal cells of the downwardly curved filaments are spheroidal and give rise to multicellular rhizoids (Fig. 4C). Cortical layer is 26–67 µm thick, arising from the apical cells of the medulla, composed of simple or sometimes branched erect filaments slightly tapering towards the surface, with cells 4–11 µm long and 4–10 µm wide. Vertical growth of thalli is by growth and transformation of old cortical cells to form new medullary and cortical layers, then old cortical cells transform into medullary cells and merge with old medulla forming a thicker medulla and a new cortex (Fig. 4D). Tuft of hairs are frequent and arise from the middle part of the thallus (Fig. 4E). Chloroplasts are plate-shaped, single per cell. Data on measured characters are summarized in Table 4.

Reproductive morphology: Reproductive portions of unangial sori form elevated and irregularly confluent areas scattered throughout the thallus (Fig. 4F). Unangia are long and ellipsoidal or clavate or obovate, 70–106 µm long and 8–33 µm wide, sessile or borne on 1–3-celled stalks, with a paraphysis (Fig. 4G). Paraphyses are long and clavate, composed of 8–15 cells, whose size is 134–205 µm long with terminal cells being 9–14 µm wide and 1–1.2 times as long as the width and the basal cells being 10–14 µm wide and 3.1–8 times as long as the width (botuliform or sausage-shaped cells). Plurangial reproductive structures were not observed. Data on measured characters are summarized in Table 4.

Habitat and Phenology: Plants grow from the high to the low intertidal zone. They are found attached on hard substrates such as pebbles and rocks (epilithic) in sheltered to wave-exposed areas. Plants with mature unangial reproductive structures were collected in August.

Distribution in Korea: Jeju Island (Fig. 6).

Remarks: The genus *Neoralfsia* was recently described to accommodate *Ralfsia expansa* specimens from Japan and Malaysia (Lim et al. 2007). The genus is mainly identified by: expanded crusts, tightly attached to the substratum with many rhizoids and later tending to be free, with pseudoparenchymatous tightly adjoined vegetative filaments composed of obvious cortical and medullary layers with hair pits; medullary vegetative filaments are unilateral to bilateral; unangia with pedicels and paraphyses; intercalary plurangia with a single sterile terminal cell (Lim et al. 2007). León-Álvarez et al. (2014a, 2014b), pointed out that many authors identified this species as *R. expansa sensu* Børgesen which is mainly characterized by unilateral symmetry and unangia on a single-celled stalk or sessile. But Tanaka and Chihara (1980 a, 1980b) described the reproductive characteristics of *R. expansa* from various Islands of Japan and generated a different interpretation of the species than the description in Børgesen (1912). The Japanese *R. expansa sensu* Tanaka and Chihara is mainly characterized by unilateral to bilateral symmetry and unangia with multicellular stalks. Based on these differences and *rbc*L molecular analyses, León-Álvarez et al. (2014b) suggested that Asian specimens of *Neoralfsia expansa* (=*R. expansa*) are a different species in a different genus within family Neoralfsiaceae yet to be described. In this study, I observed Korean specimens of *R. expansa* (= *Neoralfsia expansa*) having mostly bilateral symmetry, seldom unilateral, and sessile unangia or on multicellular stalks composed of 1–3 cells. These characters overlap those of *R. expansa sensu* Børgesen and *R. expansa sensu* Tanaka and Chihara.

2.2. Genus *Neoralfsiopsis* A. O. Oteng'o, B. Y. Won & T. O. Cho gen. nov.

Diagnosis: Plants epilithic, crusts, circular to irregular outline, smooth to warty surface, firmly attached to the substratum, with rhizoids; thalli composed of three distinct parts, basal disc, medullary layer and cortex, with filaments firmly adhering; medullary layer composed of filaments that arise from the basal disc and curve upwards to form unilateral symmetry; cortical layer arise from terminal cells of medullary filaments, composed of simple or branched erect filaments; hair in tufts, arise from the basal disc; chloroplasts single per cell.

Thalli are epilithic crusts, circular to irregular in outline, with smooth to warty surface, firmly attached to the substratum, with multicellular rhizoids. Thalli are composed of three distinct parts, a basal disc of prostrate filaments and medullary and cortical layers, with filaments tightly adjoined to form pseudoparenchymatous tissue. Medullary layer is composed of filaments that arise from the basal disc and curve upwards forming a unilateral symmetry and are generally tapered downwards. Cortical layer arises from the terminal cells of upwardly curved medullary filaments, composed of simple or sometimes branched erect filaments. Hair in tufts, arising from the basal disc. Chloroplasts are single per cell.

Type species: *Neoralfsiopsis jejuensis* A. O. Oteng'o, B. Y. Won & T. O. Cho sp. nov.

Etymology: The alga is named in reference to its closed resemblance to *Neoralfsia* by having a clear delimitation of cortex and medulla.

2.2.1. *Neoralfsiopsis jejuensis* A. O. Oteng'o, B. Y. Won & T. O. Cho sp. nov. (Fig. 5)

Diagnosis: Plants epilithic, crusts, brown to dark brown, lighter margins, circular to irregular outline, smooth to warty surface with small lobes, up to 1.5 cm across, 152–862 μm thick, firmly attached to the substratum, with rhizoids; thalli composed of three distinct layers, basal, medullary and cortical layers, with filaments firmly adhering to form pseudoparenchymatous tissue; basal layer consist three to several layers of creeping filaments, lower cells give rise to multicellular rhizoids; medullary layer arise from basal disc, taper downwards and curve upwards to form unilateral symmetry; cortical layer arise from terminal cells of medullary filaments, composed of simple or branched erect filaments; hair in tufts, arise from the basal disc; chloroplasts single per cell.

Holotype: CUK19606B. Voucher specimen was deposited in the herbarium of Chosun University, Korea (CUK).

Type locality: Seongsan Ilchul Peak, Seongsan-eup, Seogwipo-si, Jeju Island, Korea; 33°27′38.08″N, 126°56′04.73″E; intertidal; collected by A. O. Oteng'o & T. O. Cho; 01 May 2019.

Isotype: CUK19596B & **CUK19641B**. Voucher specimens were deposited in the herbarium of Chosun University, Korea (CUK).

Specimens examined: CUK19507C (Seongsan Ilchul Peak, Seongsan-eup, Seogwipo-si, Jeju Island, Korea, collected by T. O. Cho & B. Y. Won, 19 April 2019).

Etymology: The specific epithet, "*jejuensis*" is derived from its type locality "Jeju" in Korea where the alga grows naturally.

Vegetative morphology: Thalli are epilithic crusts, brown to dark brown with lighter margins, circular to irregular in outline and sometimes confluent, with smooth to rough surface with warts and small lobes, up to 1.5 cm across, 152–862 µm thick, firmly attached to the substratum by the entire lower surface, with multicellular rhizoids (Fig. 5A & B). Thalli are composed of three distinct layers, basal, medullary and cortical layers, with filaments tightly adjoined to form pseudoparenchymatous tissue (Fig. 5C). Basal layer consists of three to several layers of creeping filaments in which cells are 10–29 µm wide and with a length to width ratio of 1:0.8–6, the lower cells give rise to multicellular rhizoids (Fig. 5D). Medullary layer shows a symmetrically unilateral structure, composed of upwardly curved filaments whose cells are 15–112 µm long and 8–27 µm wide. Medullary filaments taper downwards to the cells of the basal layer. Cortical layer is 25–94 µm thick, arising from the terminal cells of the medulla, composed of simple or sometimes branched erect filaments slightly tapering towards the surface, with cells 5–10 µm wide and 1–1.1 times as long as the width. Vertical growth of thalli is by growth and transformation of old cortical cells to form new medullary and cortical layers, then old cortical cells transform into medullary cells and merge with old medulla to form a thicker medulla and a new cortex (Fig. 5E). Tuft of hairs arise from the basal disc and are randomly scattered on the thallus (Fig. 5F). Chloroplasts are plate-shaped, single per cell. Reproductive structures were not observed. Data on measured characters are summarized in Table 4.

Habitat and Phenology: Plants attached, on the high to the low intertidal zone in sheltered to wave-exposed areas. They are epilithic on rocks or on small boulders and pebbles. Plants collected both in April and May were all sterile.

Distribution in Korea: Jeju Island (Fig. 6).

Remarks: *Neoralfsiopsis jejuensis* sp. nov. is newly describe in Korea based on detailed morphological studies and molecular analyses. In this study, we established a new genus *Neoralfsiopsis* based on morphological and molecular evidences. The genus *Neoralfsiopsis* is mainly characterized by epilithic crusts, with three distinct layers: basal layer, medulla and cortex, and with hair tufts arising from the basal layer and single chloroplast per cell. *Neoralfsiopsis* differs from *Neoralfsia* by the latter having thalli with two distinct parts and hairs arising from the middle part of the thallus. The new species, *Neoralfsiopsis jejuensis*, is matched well with the diagnostic characters of *Neoralfsiopsis*. *Ne. jejuensis* is characterized by having brown to dark brown epilithic crusts thalli of up to 1.5 cm across, with circular to irregular in outline, smooth to warty surface, firmly attached to the substratum with multicellular rhizoids; thalli composed of three distinct parts, a basal disc, medullary and cortical layers, with filaments tightly adjoined to form pseudoparenchymatous tissue; medulla composed of filaments that arise from the basal disc and curve upwards forming a unilateral symmetry; cortex arises from terminal cells of medullary filaments, composed of simple or sometimes branched erect filaments; hair in tufts, arising from the basal disc; chloroplasts single per cell.

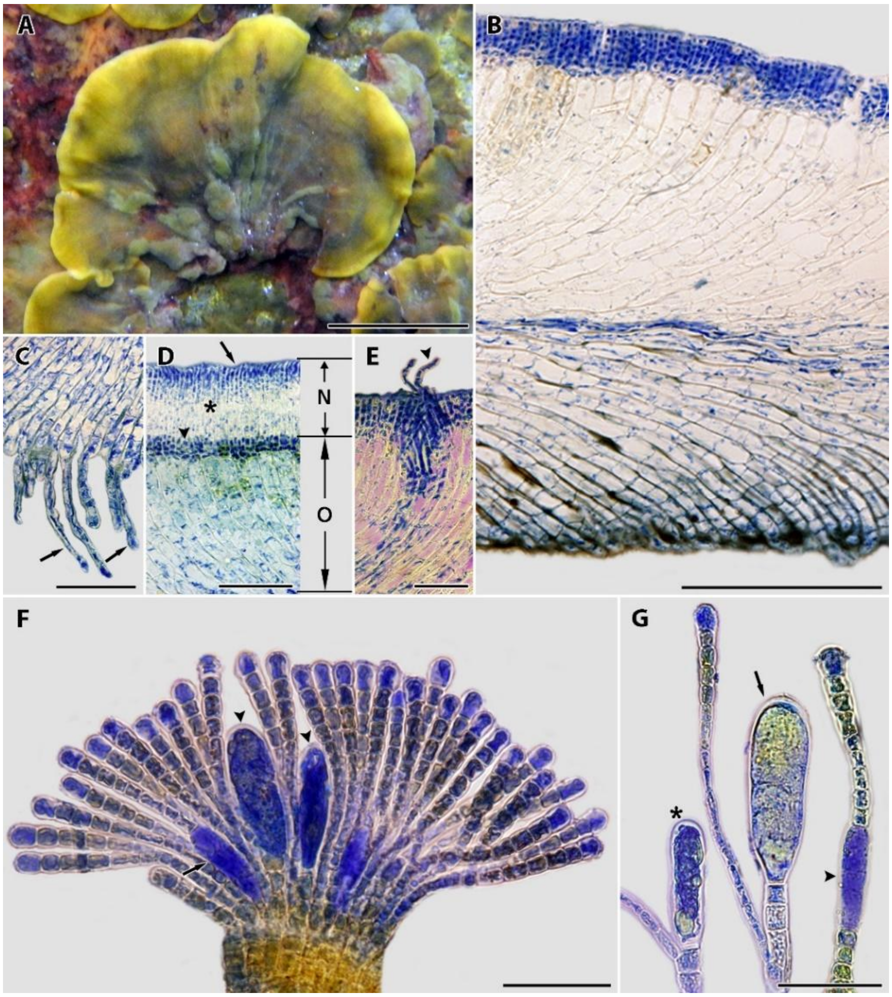


Fig. 4. Morphological characteristics of *Neoralfsia expansa*.: (A) Thalli with light to yellowish brown and expanded crusts on a pebble (CUK20756A). (B) Radial section view of creeping vegetative thallus showing two distinct layers (dark staining cortex and a medulla that does not stain well). The medulla shows a symmetrically bilateral structure composed of upwardly and downwardly curved filaments arising from the central part of the thallus (CUK19512A). (C) Multicellular rhizoids (arrows) on the undersurface of thallus (CUK19510A). (D) A section of crusts showing vertical growth zone composed of a stratified thallus (O, old thallus; N, new thallus layer). Old cortical cells (arrowhead) grow and give rise to new medullary layer (asterisk) and a new cortex (arrow) (CUK19512A). (E) Hair tuft (arrowhead) arising from the middle part of the thallus (CUK19512A). (F) Radial section view of creeping reproductive thallus with sorus bearing unangia (arrowheads) and paraphyses with boluliform cells (arrow) (CUK20756A). (G) Unangia sessile (asterisk) or on 1–3-celled stalks (arrow) and paraphyses with boluliform cells (arrowhead) (CUK20756A). Scale bars represent: A, 0.5 cm; B, 200 μm; C & D, 100 μm; E, F & G, 50 μm.

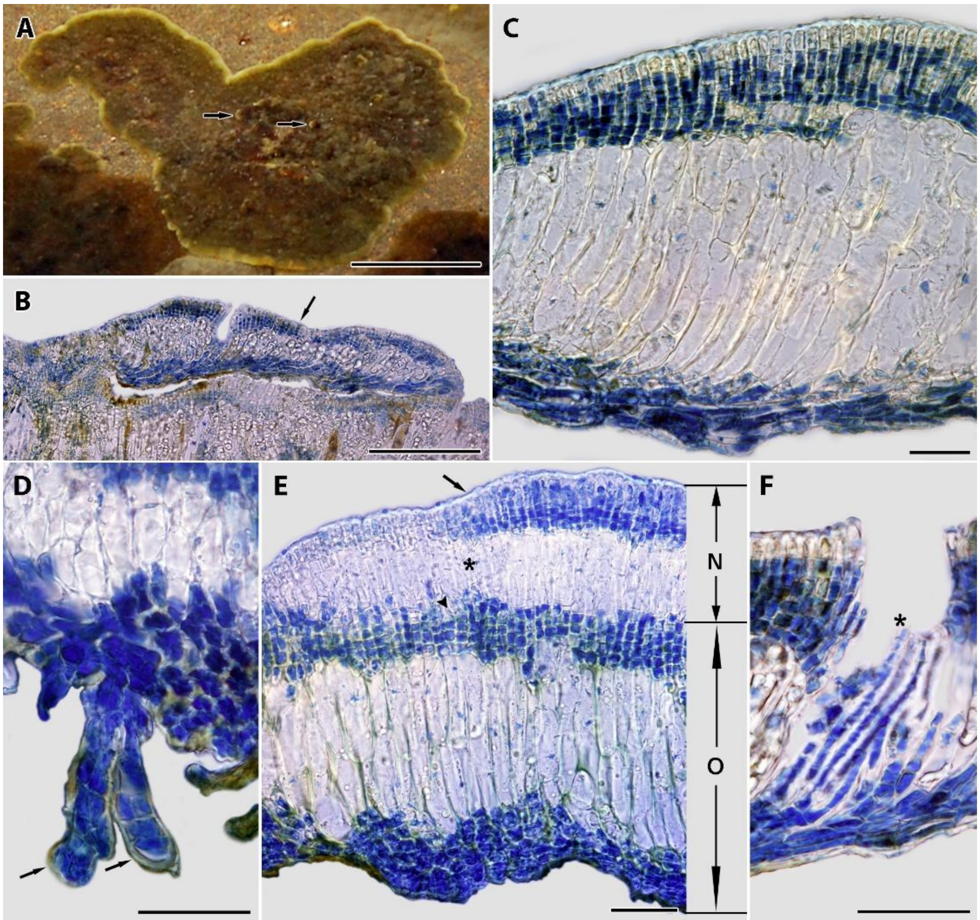


Fig. 5. Morphological characteristics of *Neoralfsiopsis jejuensis* sp. nov.: (A) Thallus with dark brown and lighter margins, with small lobes (arrows) on the surface and forming irregular crust on a pebble (CUK19606B). (B) Radial section view of creeping vegetative thallus showing a small lobe (arrow) arising from the surface of the thallus (CUK19596B). (C) Radial section view of crust showing the vegetative thallus composed of three distinct layers: basal layer, medulla (unstained), and cortex (CUK19642C). (D) Multicellular rhizoids (arrows) on the undersurface of thallus (CUK19507C). (E) A section of crusts showing vertical growth zone composed of a stratified thallus (O, old thallus; N, new thallus layer). Old cortical cells (arrowhead) grow and give rise to new medullary layer (asterisk) and a new cortex (arrow) (CUK19594B). (F) Hair tuft (asterisk) arising from the basal layer (CUK19596B). Scale bars represent: A, 0.5 cm; B, 200 μm; C, D, E & F, 50 μm.

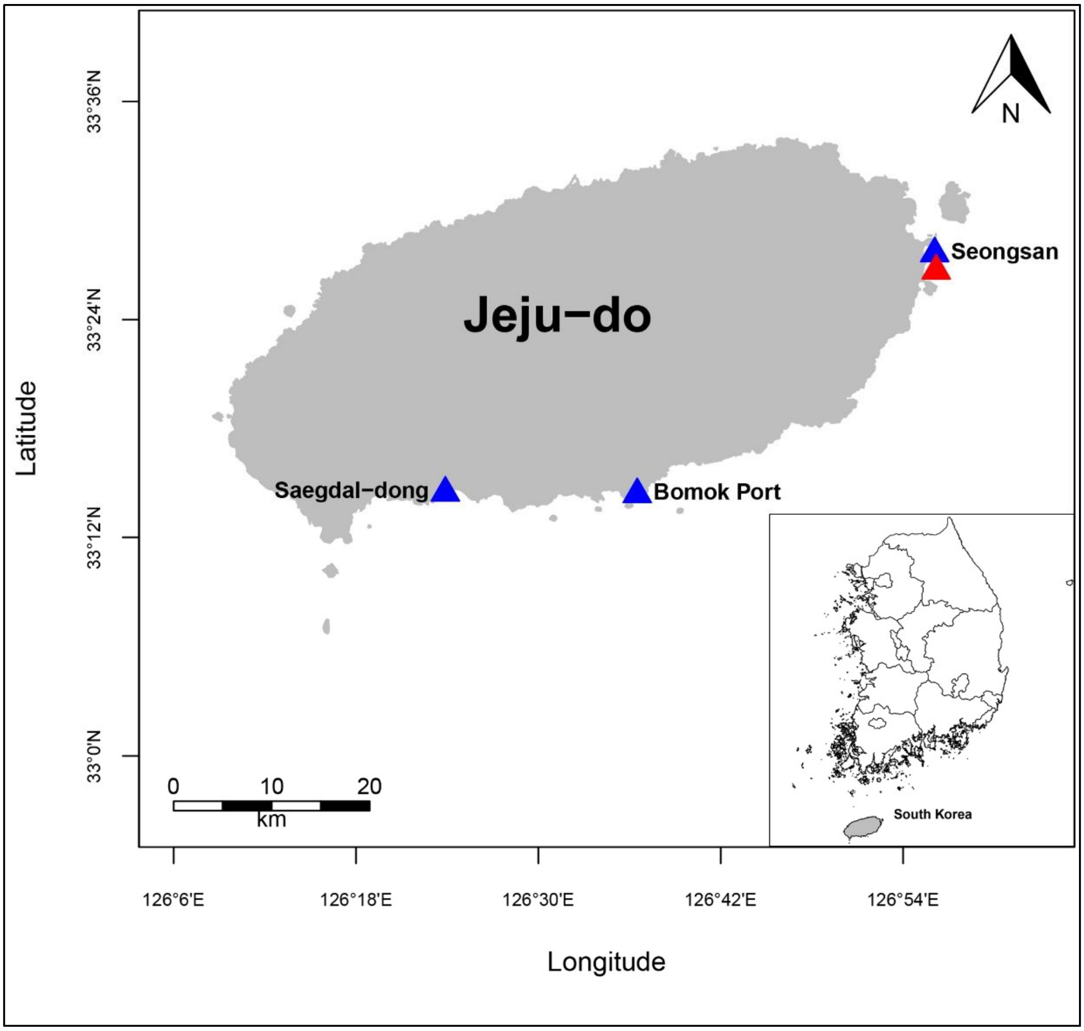


Fig. 6. Distribution of the Neoralfsiaceae species in Korea. *Neoralfsia expansa* (blue), *Neoralfsiopsis jejuensis* (red).

Table 4. Comparizon of morphological features between two genera in family Neoralfsiaceae.

Characters	***Neoralfsia* (Type genus)**	***Neoralfsiopsis* gen. nov.**
Substrate	Epilithic	Epilithic
Crust color	Light to yellowish brown (olive to dark brown when dry)	Dark brown
Lighter margins	Present	Present
Crust outline	Circular to irregular, confluent	Circular to irregular, confluent
External growth lines or ridges	Present	Absent
Surface	Smooth at first, later become uneven	Smooth to rough with warts
Lobes	Present or absent	Present, small (microlobes)
Diameter of thalli	Up to 7.5 cm	Up to 1.5 cm
Thickness of thalli	228–1349 µm	152–862 µm
Thallus parts	2	3
Basal cell layer	Absent	Three to several

Table 4. (continued)

Characters	***Neoralfsia* (Type genus)**	***Neoralfsiopsis* gen. nov.**
Basal cell length	–	5–12 µm
Basal cell width	–	10–29 µm
Rust-red color underside of crusts	–	Present
Rhizoids	Present, multicellular	Present, multicellular
Delimitation of cortex and medulla	Yes	Yes
Medulla cell length	13–118 µm	15–112 µm
Medulla cell width	6–40 µm	8–27 µm
Organization and symmetry of medullary filaments	Firmly adhering, curved upwards and downwards (bilateral symmetry)	Firmly adhering, curved upwards (unilateral symmetry)
Branching of medullary filaments	Branched	Branched
Cortical layer thickness	50–70 µm	25–94 µm
Cortical cell length	4–11 µm	5–11 µm

Table 4. (continued)

Characters	***Neoralfsia* (Type genus)**	***Neoralfsiopsis* gen. nov.**
Cortical cell width	4–10 µm	5–10 µm
Organization and symmetry of cortical filaments	Firmly adhering, straight	Firmly adhering, straight
Branching of cortical filaments	Sparsely branched	Sparsely branched
Chloroplasts / cell	1 / cell	1 / cell
Phaeophycean hairs	Form tufts, frequent	Form tufts
Origin of hairs	Middle part of thallus	Basal disc
Plurangia and unangia sori	On separate thalli (sometimes on same thallus[1])	Not observed
Length of plurangia	40–65 µm[1]	–
Plurangial filaments	1–2[1]	–
No. of sterile cells	1	–
Unangial shape	Ellipsoid, clavate, obovate	–

Table 4. (continued)

Characters	***Neoralfsia* (Type genus)**	***Neoralfsiopsis* gen. nov.**
Unangial length	70–106 µm	–
Unangial width	8–33 µm	–
Unangial position	Terminal	–
Unangial stalks	Present	–
No. of stalk cells	0–3 (3–6[1])	–
No. of unangia per stalk or erect filament	1	–
Paraphysis	Present	–
Paraphysis length	134–205 µm	–
Paraphysis width	9–14 µm	–
Basal cell measurement	10–14 µm wide, 3.1–8 times as long as the width (botuliform / sausage-shaped)	–

Table 4. (continued)

Characters	***Neoralfsia* (Type genus)**	***Neoralfsiopsis* gen. nov.**
No. of cell per paraphysis	8–15	–
Paraphysis shape	Clavate	–
Soral filaments	Absent	–
Reference	Tanaka and Chihara 1980b[1], León-Álvarez 2005, Lim et al 2007, Oteng'o et al. unpublished	Oteng'o et al. unpublished

3. Family Ralfsiaceae W. G. Farlow 1881

Type genus: *Ralfsia* Berkeley 1843

3.1. Genus *Endoplura* Hollenberg 1969

3.1.1. *Endoplura geojensis* A. O. Oteng'o, B. Y. Won & T. O. Cho sp. nov. (Fig. 7)

Diagnosis: Plants epilithic, encrusting, yellowish olive to dark brown, almost circular in outline, often confluent, with inconspicuous growth lines and ridges, up to 1.5 cm across, 68–316 µm thick, firmly attached to the substratum, without rhizoids; vegetative erect filaments sparsely branched, straight or upwardly curved, firmly adjoined with one another, arise from hypothallial layer composed of one to several cell layers; chloroplasts several per cell; hairs single or in tufts arise from mid to apical cells of erect filaments; intercalary plurangia composed of two to five separate parallel reproductive filaments terminated by 2–3 sterile cells; unangia sessile, on terminal part of erect filaments, with a single paraphysis, some unangia born on soral filaments.

Holotype: CUK18789A&C. Voucher specimen was deposited in the herbarium of Chosun University, Korea (CUK).

Type locality: Waheon, Irun-myeon, Geoje-si, Gyeongsangnam-do, Korea; 34°48′45.36″N, 128°42′25.43″E; intertidal; collected by T. O. Cho & B. Y. Won; 16 March 2018.

Specimens examined: CUK20066C (Hwasam-ri, Yongnam-myeon, Tongyeong-si, Gyeongsangnam-do, Korea, collected by T. O. Cho, 28 October 2019), **CUK20228** (Seongu-ri, Nam-myeon, Namhae-gun, Gyeongsangnam-do, Korea, collected by T. O. Cho & B. Y. Won, 31 March 2020), **CUK20239** (Gujora Beach, Irun-myeon, Geoje-si, Gyeongsangnam-do, Korea, collected by T. O. Cho & B. Y. Won, 01 April 2020) .

Etymology: The specific epithet, "*geojensis*" is derived from its type locality "Geoje" in Korea, where the alga grows naturally.

Vegetative morphology: Thalli are epilithic crusts, yellowish olive to dark brown (shinny dark brown when dry), without lighter margins, mostly circular outline but often confluent, with inconspicuous radial lines, 0.1–1.5 cm across, 68–316 µm thick, firmly attached to the substratum, sometimes with rust-red undersurface, and lacking rhizoids (Fig. 7A). Vegetative erect filaments are sparsely branched, straight or upwardly curved, slightly tapering towards the surface, firmly adjoined with one another to form pseudoparenchymatous tissue, and arise from a hypothallial layer of one to several cell layers in which cells are 4–14 µm wide and have a width to length ratio of 1:1.4–3 (Fig. 7B). Cells of the erect filaments are 6–14 µm long and 5–14 µm wide and contain granular storage substances. Chloroplasts are irregularly discoid, several per cell. Phaeophycean hairs are either single or in tufts and arise from the mid to apical cells of erect filaments (Fig. 7C). Data on measured characters are summarized in Table 5.

Reproductive morphology: Reproductive portions of plurangial and unangial sori form elevated and irregularly confluent areas on the same or different thalli (Figs 7D & F). Plurangial reproductive structures are 48–130 µm long and composed of 2–5 separate parallel reproductive filaments with 2–3 terminal sterile cells (Fig 7E). Unangia are 37–92 µm long and 5–28 µm wide, clavate to obovoid to oblong, sessile, on the terminal part of erect filaments, and accompanied by one paraphysis. Paraphyses are 81–182 µm long and 3–16 µm wide, composed of 7–16 cells. Some unangia are borne along the length of soral filaments, with some in lateral and others in intercalary position. Soral filaments are 80–183 µm long and 2–15 µm wide, composed of 7–15 cells. (Fig 7G). Data on measured characters are summarized in Table 5.

Habitat and Phenology: Plants grow in the intertidal zone where they are found attached on hard substrates such as pebbles and rocks (epilithic) in sheltered to wave-exposed areas.

Plurangia and unangia bearing plants were collected on March and April while those without reproductive structures (sterile) were collected in October.

Distribution in Korea: Gyeongsangnam-do, South coast (Fig. 12).

Remarks: *Endoplura geojensis* sp. nov. is newly described from Korea based on detailed morphological studies and molecular analyses. This new species is recognized as an *Endoplura* member by having intercalary plurangia composed of 2–5 parallel separate reproductive filaments terminated by 2–3 sterile cells and several to many chloroplasts per cell. Diagnostic features delimiting this as a new species in *Endoplura* are small and yellowish olive to dark brown crust thalli of up to 1.5 cm across, almost circular in outline, with inconspicuous growth lines and ridges, hairs arise from the mid to apical cells of erect filaments, plurangia terminated by 2–3 sterile cells, and unangia sessile with single paraphysis and some borne on soral filaments. *Endoplura geojensis* resembles *E. gyeokpoensis*, *E. jejuensis*, *E. koreana* and *E. limpeticola* in having hairs and relatively small crusts of less than 3 cm across. However, *E. geojensis* is mainly distinguished from all the four mentioned *Endoplura* species by having unangial reproductive structures borne on soral filaments. *E. geojensis* differs from the type species, *E. aurea*, by having smaller thalli of up to 1.5 cm diameter and presence of hairs while the latter have bigger thalli of up to 10 cm or more broad and hairs absent.

3.1.2. *Endoplura gyeokpoensis* A. O. Oteng'o, B. Y. Won & T. O. Cho sp. nov. (Fig. 8)

Diagnosis: Plants epilithic, encrusting, olive to light brown, light margin, irregular outline, smooth surface, less than 1 cm across, 57–322 μm thick, firmly attached to the substratum, sometimes with rust-red undersurface, lacking rhizoids; vegetative erect filaments infrequently branched, straight or upwardly curved, firmly adjoined with one another, and arise from a hypothallial layer of one to several cell layers; chloroplasts several per cell; hairs tufts arise from the basal disc and lower parts of erect filaments; intercalary plurangial reproductive structures composed of two to four separate parallel reproductive filaments terminated by 2–3 sterile cells.

Holotype: CUK20923B. Voucher specimen was deposited in the herbarium of Chosun University, Korea (CUK).

Type locality: Gyeokpo Port, Byeongsan-myeon, Buan-gun, Jeollabuk-do, Korea; 35°37′27.30″N, 126°27′57.30″E; intertidal; collected by T. O. Cho & B. Y. Won; 09 May 2021.

Specimens examined: CUK19785C (Gyeokpo Port, Byeongsan-myeon, Buan-gun, Jeollabuk-do, Korea, collected by T. O. Cho & B. Y. Won, 26 July 2019), **CUK20350B** (Gyeokpo Port, Byeongsan-myeon, Buan-gun, Jeollabuk-do, Korea, collected by T. O. Cho, J. Avila, A. O. Oteng'o & G. C. Choi, 26 June 2020).

Etymology: The name "*gyeokpoensis*" is derived from its type locality "Gyeokpo" in Korea where the alga grows naturally.

Vegetative morphology: Thalli are epilithic crusts, olive to light brown (shiny brown when dry), with a lighter margin, irregular in outline, with a smooth surface, less than 1 cm across, 57–322 µm thick, firmly attached to the substratum, sometimes with a rust-red undersurface, and lacking rhizoids (Fig. 8A). Vegetative erect filaments are infrequently branched, straight or upwardly curved, tapering towards the surface, firmly adjoined with one another to form pseudoparenchymatous tissue, and arise from a hypothallial layer of one to several cell layers in which cells are 4–11 µm wide and have a width to length ratio of 1:1.2–3 (Fig. 8B). Cells of the erect filaments are 4–21 µm long and 4–13 µm wide and contain granular storage substances. Chloroplasts are disc-shaped to irregularly discoid, several per cell. Tufts of hairs are sparse and arise from the basal disc and the lower parts of erect filaments (Fig. 8C). Data on measured characters are summarized in Table 5.

Reproductive morphology: Reproductive portions of plurangial sori form small elevated areas on the thalli (Fig. 8D). Plurangial reproductive structures are 45–126 µm long and composed of two to four separate parallel reproductive filaments with 2–3 terminal sterile cells (Fig. 8E). Unangial reproductive structures were not observed. Data on measured characters are summarized in Table 5.

Habitat and Phenology: Plants grow in the intertidal zone. They are found attached on hard substrates such as pebbles and rocks (epilithic) in sheltered areas. Plants with plurangial reproductive structures were collected on May and June. Samples collected in July were without reproductive structures.

Distribution in Korea: Jeollabuk-do, West coast (Fig. 12).

Remarks: In the present study, we newly describe *Endoplura gyeokpoensis* sp. nov. from Korea based on detailed morphological studies and molecular analyses. This new species is recognized as an *Endoplura* member by having intercalary plurangial reproductive structures composed of 2–4 separate filaments terminated by 2–3 sterile cells and several to many chloroplasts per cell. Diagnostic features delimiting this as a new species in *Endoplura* are small olive to light brown creeping thalli with light margins, irregular outline, smooth surface, less than 1 cm across, firmly attached to the substratum and without rhizoids, hairs tufts arise from the basal disc and lower parts of erect filaments, intercalary plurangia composed of two to four separate parallel reproductive filaments terminated by 2–3 sterile cells. *Endoplura gyeokpoensis* differs from *E. aurea*, the type species, by its small crust and presence of hairs while the latter has larger crusts of 10 cm or more in diameter and the absence of phaeophycean hairs. *E. geojensis* is distinguished from *E. gyeokpoensis* by having soral filaments while the later has no soral filaments. *E. gyeokpoensis* differs from *E. limpeticola* by having relatively thinner epilithic crusts, without rhizoids as compared to the latter having epizoic crusts with rhizoids. *E. koreana* mainly differs from *E. gyeokpoensis* by having larger crusts of up to 3 cm across and plurangia terminated by 2–8 sterile cells. Although *E. jejuensis* and *E. gyeokpoensis* both have crusts of same size, the former differs from the latter by having crusts more circular in outline, with inconspicuous growth lines and without lighter margins as compared to *E. gyeokpoensis* which has crust with irregular outline, no growth lines and lighter margins.

3.1.3. *Endoplura jejuensis* A. O. Oteng'o, T. O. Cho & B. Y. Won sp. nov. (Fig. 9)

Diagnosis: Plants epilithic, encrusting, light to dark brown, almost circular in outline, often confluent, with inconspicuous growth lines and ridges, less than 1 cm across, 63–317 µm thick, firmly attached to the substratum, without rhizoids; vegetative erect filaments infrequently branched, straight or upwardly curved, firmly adjoined with one another, and arise from an indistinct hypothallial layer; chloroplasts several per cell; hairs tufts arise from basal disc; intercalary plurangial reproductive structures composed of two to four separate parallel reproductive filaments terminated by 2–4 sterile cells; unangial reproductive structures sessile, on terminal part of erect filaments, with a single paraphysis.

Holotype: MABIK AL00080969. Voucher specimen was deposited in the herbarium of the National Marine Biodiversity Institute of Korea (MABIK).

Type locality: Seongsan Ilchul Peak, Seongsan-eup, Seogwipo-si, Jeju Island, Korea; 33°27′38.08″ N, 126°56′05.73″ E; intertidal; collected by A. O. Oteng'o & T. O. Cho; 01 May 2019.

Isotype: CUK19592C, **CUK19607A**, **CUK19600** & **CUK19631A**. Voucher specimens were deposited in the herbarium of Chosun University, Korea (CUK).

Specimens examined: CUK20756C, **CUK20757B&C**, **CUK20759C&E**, **CUK20761A&B** (Seongsan Ilchul Peak, Seongsan-eup, Seogwipo-si, Jeju, Korea, collected by T. O. Cho & B. Y. Won, 17 August 2020).

Etymology: The name "*jejuensis*" is derived from its type locality "Jeju" in Korea where the alga grows naturally.

Vegetative morphology: Plants are epilithic creeping thalli, light to dark brown (shiny brown when dry), almost circular in outline at all stages but often confluent, with inconspicuous growth lines and ridges, less than 1 cm across, 63–317 μm thick, firmly attached to the substratum, sometimes with a rust-red undersurface, and lacking rhizoids (Fig. 9A). Vegetative erect filaments are infrequently branched, straight or upwardly curved, tapering towards the surface, firmly adjoined with one another to form pseudoparenchymatous tissue, and arise from an indistinct hypothallial layer in which cells are 3–9 μm wide and have a width to length ratio of 1 : 1.8–3 (Fig. 9B). Cells of the erect filaments are 4–20 μm long and 3–11 μm wide and contain granular storage substances. Chloroplasts are disc-shaped to irregularly discoid, several to many per cell. Tufts of hairs are sparse and arise from the basal disc (Fig. 9C). Data on measured characters are summarized in Table 5.

Reproductive morphology: Reproductive portions of plurangial and unangial sori form elevated and irregularly confluent areas on different thalli (Fig. 9D & F). Plurangial reproductive structures are 31–98 μm long and composed mostly of two to four separate parallel reproductive filaments with 2–4 terminal sterile cells (Fig. 9E). Unangial reproductive structures are 61–116 μm long and 10–37 μm wide, oblong to obovoid, sessile, on terminal erect filaments; each sporangium accompanied by a single paraphysis (Fig. 9G). Paraphyses are present, each consisting of 7–12 cells, 73–155 μm long, and 3–14 μm wide. Data on measured characters are summarized in Table 5.

Habitat and Phenology: Plants grow from the high to the low intertidal zone. They are found attached on hard substrates such as pebbles and rocks (epilithic) in sheltered to wave-exposed areas. Plants with plurangial reproductive structures were collected on May and those with unangial reproductive structures in August.

Distribution in Korea: Jeju Island (Fig. 12).

Remarks: In the present study, we newly describe *Endoplura jejuensis* sp. nov. from Jeju Island, Korea based on detailed morphological studies and molecular analyses. This new species is recognized as an *Endoplura* member by having intercalary plurangial reproductive structures composed of 2–4 separate filaments terminated by 2–5 sterile cells and several to many chloroplasts per cell. Diagnostic features delimiting this as a new species in *Endoplura* are small and light to dark brown crust thalli of less than 1 cm, almost circular in outline, with inconspicuous growth lines and ridges, tufts of hairs arising from the basal disc, plurangia terminated by 2–4 sterile cells, and unangia each with a single paraphysis. *Endoplura jejuensis* differ from the type species, *E. aurea*, by having smaller thalli of less than 1 cm diameter and presence of hairs while the latter have bigger thalli of up to 10 cm or more broad and hairs absent. *E. jejuensis* resembles *E. geojensis*, *E. gyeokpoensis*, *E. koreana* and *E. limpeticola* by having hairs and relatively small crusts of less than 3 cm broad. However, *E. geojensis* is distinguished from *E. jejuensis* by having unangial reproductive structures borne on soral filaments. *E. gyeokpoensis* differs from *E. jejuensis* by its thalli being irregular in outline and by having a smooth surface while the latter has almost circular thalli with inconspicuous growth lines and ridges. *E. jejuensis* is distinguished from *E. koreana* by tufts of hairs arising from the basal disc, plurangia terminated by 2–4 sterile cells, and unangia each with a single paraphysis while the latter has hair tufts arising from both basal disc and apical cell of erect filaments, plurangia terminated by 2–8 sterile cells, and unangia with one to two paraphyses. *E. limpeticola* differs from *E. jejuensis* in it being entirely epizoic on limpets and the presence of multicellular rhizoids on the undersurface.

3.1.4. *Endoplura koreana* A. O. Oteng'o, T. O. Cho & B. Y. Won sp. nov. (Fig. 10)

Diagnosis: Plants epilithic or epizoic, encrusting, olive or yellowish-brown to brown, irregular or circular in outline, often confluent, with or without lighter margins, mostly with growth lines and ridges, up to 3 cm across, 60–314 µm thick, firmly attached to the substratum, without rhizoids; vegetative erect filaments sparsely branched, straight or upwardly curved, firmly adjoined with one another, and arise from an indistinct hypothallial layer; chloroplasts several per cell; phaeophycean hair tufts arise from the basal disc and apical parts of erect filaments; plurangial reproductive structures composed of two to five separate parallel reproductive filaments terminated by 2–8 sterile cells; unangial reproductive structures sessile, on terminal part of erect filaments, with 1–2 paraphyses.

Holotype: MABIK AL00080968. Voucher specimen was deposited in the herbarium of the National Marine Biodiversity Institute of Korea (MABIK).

Type locality: Dumunjin Port, Baekryeongdo, Incheon, Korea; 37°58′34.79″ N, 124°37′03.66″ E; intertidal; collected by A. O. Oteng'o & T. O. Cho; 12 August 2020.

Isotype: CUK20630, **CUK20635**, **CUK20640** & **CUK20644**. Voucher specimens were deposited in the herbarium of Chosun University, Korea (CUK).

Specimens examined: CUK18755B (Pado-ri, Sowon-myeon, Taean-gun, Chungcheongnam-do, Korea, collected by T. O. Cho & B. Y. Won, 03 March 2018), **CUK19175A&B** (Gijang, Ilgwang-myeon, Gijang-gun, Busan, Korea, collected by T. O. Cho & B. Y. Won, 08 October 2018), **CUK19809B**, **CUK19814C**, **CUK19818**, **CUK19824** (Oeyeondo, Ocheonmyeon, Boryeong-si, Chungcheongnam-do, Korea, collected by T. O. Cho & B. Y. Won, 01 August 2019), **CUK20574** (Chosa-ri, Uisin-myeon, Jindo-gun, Jeollanam-do, Korea, collected by T. O. Cho & B. Y. Won, 24 July 2020), **CUK20665**, **CUK20667**, **CUK20669**, **CUK20674**, **CUK20679**, **CUK20680**, **CUK20681** (Gobong Port, Baekryeongdo, Incheon, Korea, collected by A. O. Oteng'o & T. O. Cho, 12 August 2020).

Etymology: The specific epithet "*koreana*" is derived from the country name "Korea" where the alga grows naturally.

Vegetative morphology: Thalli are epilithic crusts, olive or yellowish-brown to brown (shiny olive to dark brown when dry), irregular or circular in outline and often confluent, with or without lighter margins, mostly with growth lines and ridges, up to 3 cm across, 60–314 µm thick, firmly attached to the substratum, with patches of a rust-red undersurface, and without rhizoids (Fig. 10A). Vegetative erect filaments are sparsely branched, straight or upwardly curved, tapering towards the surface, firmly adjoined with one another to form pseudoparenchymatous tissue, and arise from an indistinct hypothallial layer in which cells are 3–11 µm wide and have a width to length ratio of 1 : 1.2–3 (Fig. 10B). Cells of the erect filaments are 4–20 µm long and 4–12 µm wide and contain granular storage substances. Chloroplasts are disc-shaped to irregularly discoid, several to many per cell. Phaeophycean hairs form either singly or in tufts and arise from the basal disc and apical parts of erect filaments (Fig. 10C). Data on measured characters are summarized in Table 5.

Reproductive morphology: Reproductive portions of plurangial and unangial sori form the elevated, extensive, and irregularly confluent areas on the same or different thalli (Fig. 10D & F). Plurangial reproductive structures are 37–105 µm long, composed of 2–4 (5) separate parallel reproductive filaments terminated by 2–8 sterile cells (Fig. 10E). Unangial reproductive structures are 50–158 µm long and 10–37 µm wide, oblong to obovoid to ellipsoid, sessile, present on terminal erect filaments, and accompanied by 1–2 paraphyses (Fig. 10G). Paraphyses are present, 6–12 cells, 70–178 µm long, and 4–16 µm wide. Data on measured characters are summarized in Table 5.

Habitat and Phenology: Plants grow from the high to the low intertidal zone. They are found attached on hard substrates such as rocks, small boulders or pebbles (epilithic) and shells of molluscs (epizoic) in sheltered to wave-exposed areas. Plants with both plurangial and unangial reproductive structures were collected in March, August and October while those with only unangia were collected in August.

Distribution in Korea: West coast (Baekryeongdo, Taean, and Oeyeondo), South coast (Busan and Jindo) (Fig. 12).

Remarks: *Endoplura koreana* sp. nov. is newly described from Korea based on detailed morphological studies and molecular analyses. This new species is suggested in a position within *Endoplura* by having intercalary plurangial reproductive structures composed of 2–5 separate parallel reproductive filaments terminated by 2–8 sterile cells and by several to many chloroplasts per cell. *Endoplura koreana* sp. nov. is distinguished from other *Endoplura* species by having encrusting epilithic or epizoic thalli, olive or yellowish-brown to brown, mostly with growth lines and ridges, up to 3 cm across, without rhizoids, with phaeophycean hairs arising from the basal disc and apical parts of erect filaments, plurangial reproductive structures composed of two to five separate parallel reproductive filaments terminated by 2–8 sterile cells, unangial reproductive structures sessile, on terminal part of erect filaments, with 1–2 paraphyses.

3.1.5. *Endoplura limpeticola* A. O. Oteng'o, B. Y. Won & T. O. Cho sp. nov. (Fig. 11)

Diagnosis: Plants epizoic crusts on limpets, olive to yellowish-brown, irregular or circular outline, often confluent, darker margins, growth lines, ridges and few warts on the surface, up to 2.2 cm across, 115–751 μm thick, firmly attached to the substratum, rust-red undersurface, numerous multicellular rhizoids; vegetative erect filaments sparsely branched, upwardly curved, firmly adjoined with one another, arise from an indistinct hypothallial layer; chloroplasts several per cell; phaeophycean hairs arise from basal disc and lower parts of erect filaments; intercalary plurangia mostly composed of 2 separate parallel reproductive filaments terminated by 2–3 sterile cells.

Holotype: CUK20823. Voucher specimen was deposited in the herbarium of Chosun University, Korea (CUK).

Type locality: Jeongdo-ri, Wando-eup, Wando-gun, Jeollanam-do, Korea; 34°17′45.98″ N, 126°42′05.26″ E; intertidal; collected by T. O. Cho, A. O. Oteng'o & G. C. Choi; 15 November 2020.

Isotype: CUK20818, **CUK20819**, **CUK20820**, **CUK20821**, **CUK20822**, **CUK20824**, **CUK20825**, **CUK20826**, **CUK20827** & **CUK20831**. Voucher specimens were deposited in the herbarium of Chosun University, Korea (CUK).

Specimens examined: CUK20582, **CUK20583**, **CUK20584**, **CUK20586**, **CUK20587** (Jeongdo-ri, Wando-eup, Wando-gun, Jeollanam-do, Korea, collected by T. O. Cho, J. Avila, A. O. Oteng'o & G. C. Choi, 05 August 2020).

Etymology: The name "*limpeticola*" is derived from its preferred substrata, limpets, on which the alga attaches and grows naturally.

Vegetative morphology: Thalli are epizoic crusts on limpets, olive to yellowish-brown (shiny dark brown when dry), irregular or circular in outline and often confluent, with darker margins, with growth lines, ridges and few warts on the surface, up to 2.2 cm across, 115–751 µm thick, firmly attached to the substratum, with a rust-red undersurface, and with numerous multicellular rhizoids (Fig. 11A & B). Vegetative erect filaments are sparsely branched, upwardly curved, tapering towards the surface, firmly adjoined with one another to form pseudoparenchymatous tissue, and arise from an indistinct hypothallial layer in which cells are 4–10 µm wide and have a width to length ratio of 1:1.2-3 (Fig. 3C). Cells of the erect filaments are 5–17 µm long and 4–11 µm wide and contain granular storage substances. Chloroplasts are disc-shaped to irregularly discoid, several per cell. Phaeophycean hairs form tufts and arise from the basal disc and lower parts of erect filaments (Fig. 11D). Data on measured characters are summarized in Table 5.

Reproductive morphology: Reproductive portions of plurangial sori form elevated and irregularly confluent areas on the thalli (Fig. 11E). Plurangial reproductive structures are 57–165 µm long, mostly composed of 2 separate parallel reproductive filaments terminated by 2–3 sterile cells (Fig. 11F). Unangial reproductive structures were not observed. Data on measured characters are summarized in Table 5.

Habitat and Phenology: Plants grow in the low intertidal zone. They are found attached on shells of limpets (epizoic) in sheltered to wave-exposed areas. Plants with mature plurangial reproductive structures were collected on November and those with young (immature) plurangia collected on August.

Distribution in Korea: South sea, Wando (Fig. 12).

Remarks: In the present study, we newly describe *Endoplura limpeticola* sp. nov. from Wando, Korea based on detailed morphological studies and molecular analyses. This new species is recognized as an *Endoplura* member by having intercalary plurangial reproductive structures composed of 2 separate reproductive filaments terminated by 2–3 sterile cells and several chloroplasts per cell. Diagnostic features delimiting this as a new species in *Endoplura* are small olive to yellowish-brown epizoic crusts of up to 2.2 cm across, 115–751 μm thick, circular to irregular in outline, with darker margins, with growth lines, ridges and few warts on the surface, with rust-red undersurface and numerous multicellular rhizoids, tufts of hairs arising from the basal disc and lower parts of erect filaments, intercalary plurangia mostly composed of 2 separate parallel reproductive filaments terminated by 2–3 sterile cells. *Endoplura limpeticola* is probably closely related to *E. jejuensis.* Phylogenetically it is nested in a clade sister to *E. jejuensis* and the genetic distance between the two species is the least among the *Endoplura* species (Tables 16 & 17). *E. limpeticola* differ not only from *E. jejuensis* but all other *Endoplura* species by being epizoic on limpets, and having the thickest thalli up to 751 μm, with multicellular rhizoids on the undersurface.

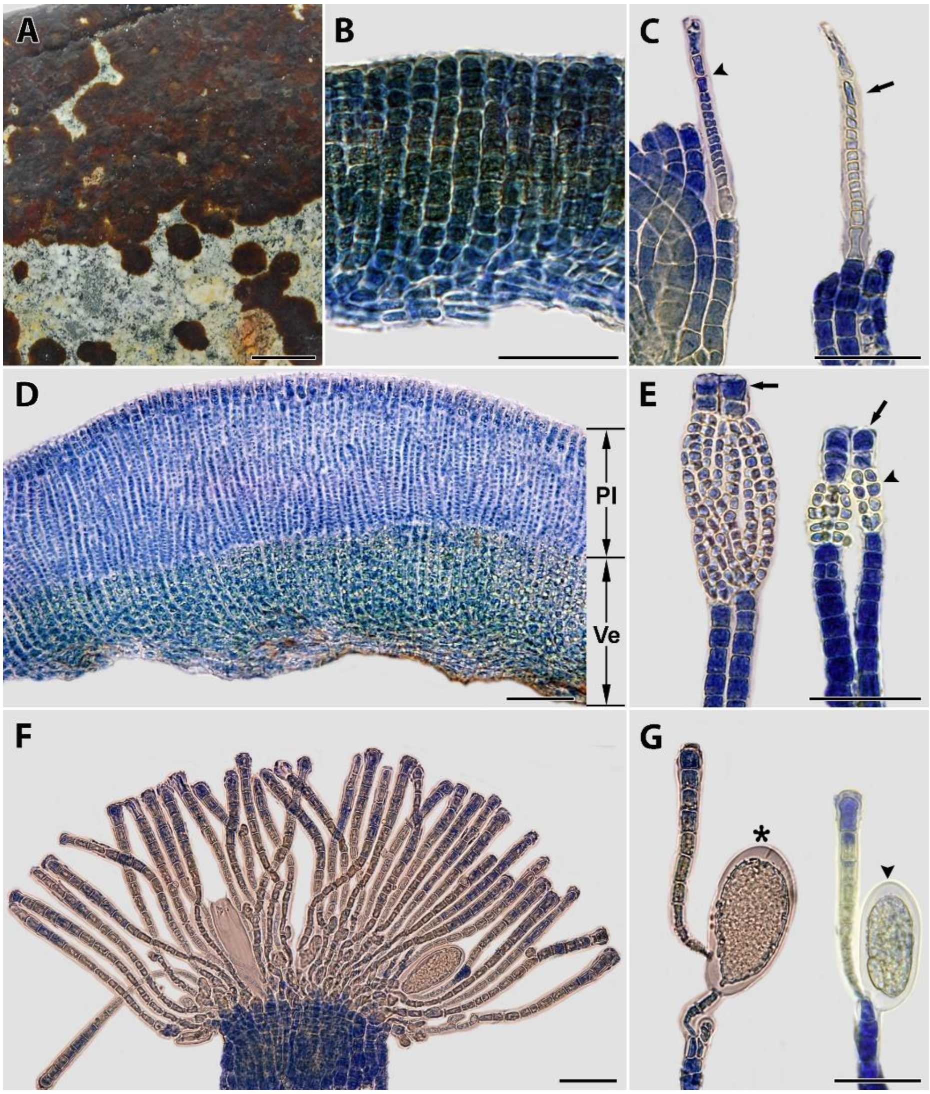


Fig. 7. Morphological characteristics of *Endoplura geojensis* sp. nov.: (A) Holotype specimen showing dark brown and almost circular crusts on a rock (CUK18789A and CUK18789C). (B) Radial longitudinal section of crust showing upwardly curved erect vegetative filaments arising from a hypothallial layer (CUK18789C). (C) Hairs arise from the mid (arrowhead) to apical (arrow) cells of erect vegetative filaments (CUK18789C). (D) A portion of thallus with sorus bearing plurangial reproductive structures (Pl, plurangial sorus; Ve, vegetative filaments) (CUK20239). (E) Plurangia with 2–5 separate parallel reproductive filaments (arrowhead) terminated by 2–3 sterile cells (arrows) (CUK18789C). (F) Sorus with soral filaments bearing unangia (CUK18789A). (G) Unangium (asterisk) born intercalary on soral filament. Sessile unangium (arrowhead) with a paraphysis (CUK18789A).
Scale bars represent: A, 1 cm; B, C, D, E, F & G, 50 μm.

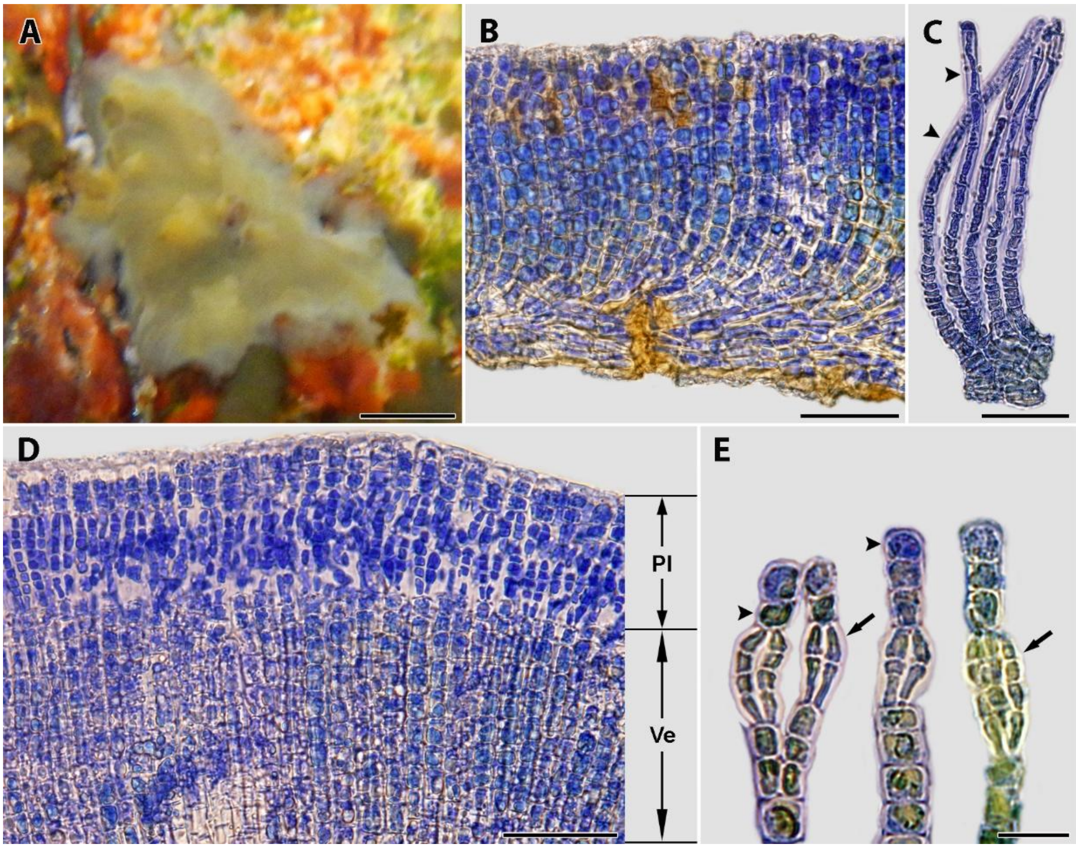


Fig. 8. Morphological characteristics of *Endoplura gyeokpoensis* sp. nov.: (A) Thalli with olive to light brown crust and irregular in outline, with a lighter margin (CUK20923B). (B) Radial section view of creeping vegetative thallus showing upwardly curved erect filaments arising from the hypothallial layer. (C) Hairs (arrowhead) developed from the basal layer and basal part of erect filaments (CUK19785C). (D) Radial section view of creeping reproductive thallus with sorus bearing plurangial reproductive filaments (Pl) on vegetative filaments (Ve) (CUK20923B). (E) Plurangia with two to four separate parallel reproductive filaments (arrow) terminated by 2–3 sterile cells (arrowheads). Scale bars represent: A, 0.1 cm; B, C & D, 50 μm; E, 20 μm.

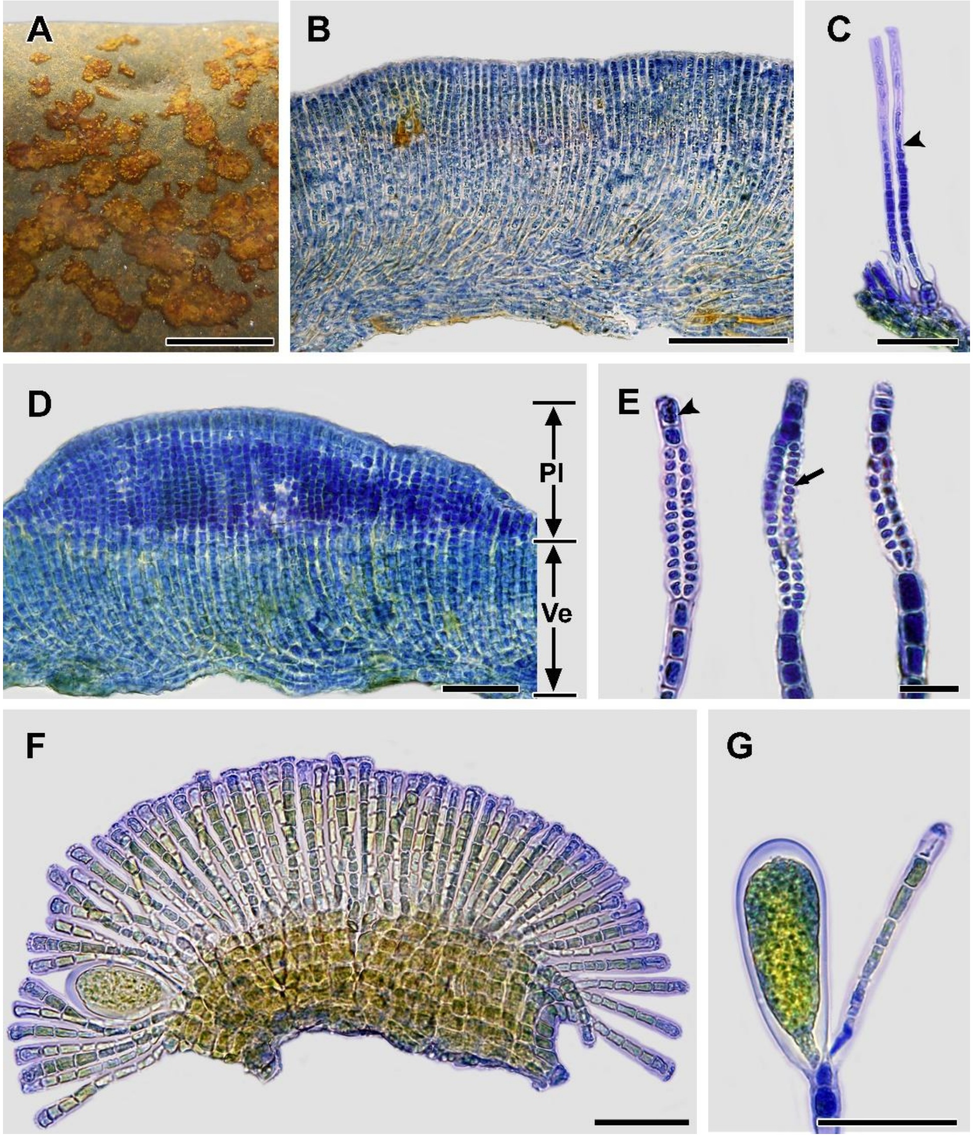


Fig. 9. Morphological characteristics of *Endoplura jejuensis* sp. nov.: (A) Thalli with light to dark brown and almost circular crusts on a pebble (CUK19600). (B) Radial section view of creeping vegetative thallus showing upwardly curved erect filaments arising from indistinct hypothallial layer (CUK19630A). (C) Hairs (arrowhead) developed from the basal disc. (D) Radial section view of creeping reproductive thallus with sorus bearing plurangial reproductive filaments (Pl) on vegetative filaments (Ve) (CUK19600). (E) Plurangial reproductive structures with two separate parallel reproductive filaments (arrow) terminated by 2–4 sterile cells (arrowhead) (CUK19631A). (F) Radial section view of creeping reproductive thallus with sorus bearing unangia (CUK20757C). (G) Unangium sessile with a paraphysis (CUK20756C). Scale bars represent: A, 1 cm; B, 100 µm; C, D, F & G, 50 µm; E, 20 µm.

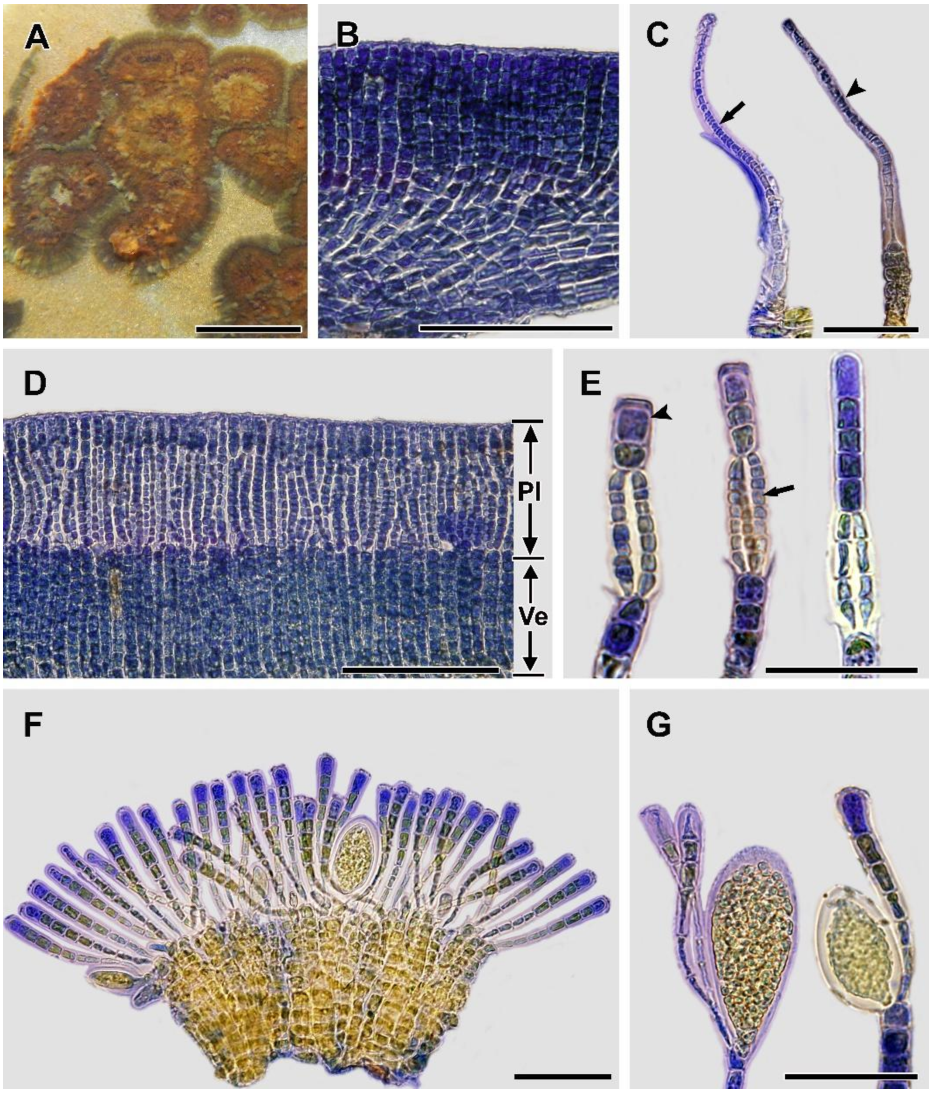


Fig. 10. Morphological characteristics of *Endoplura koreana* sp. nov.: (A) Thalli with brown and circular to irregular crusts with lighter margins on a pebble (CUK20628). (B) Radial section view of creeping vegetative thallus showing upwardly curved erect filaments arising from indistinct hypothallial layer (CUK19175). (C) Hair (arrow) developed from the basal disc and hair (arrowhead) developed from apical cell of erect filaments. (D) Radial section view of creeping reproductive thallus with sorus bearing plurangial reproductive filaments (Pl) on vegetative filaments (Ve) (CUK19175). (E) Plurangial reproductive structures with two to four separate parallel reproductive filaments (arrow) terminated by 2–5 sterile cells (arrowhead) (CUK20644). (F) Radial section view of creeping reproductive thallus with sorus bearing unangia (CUK20669). (G) Unangium sessile with one (right) or two (left) paraphyses (CUK20628). Scale bars represent: A, 1 cm; B & D, 100 µm; C & E–G, 50 µm.

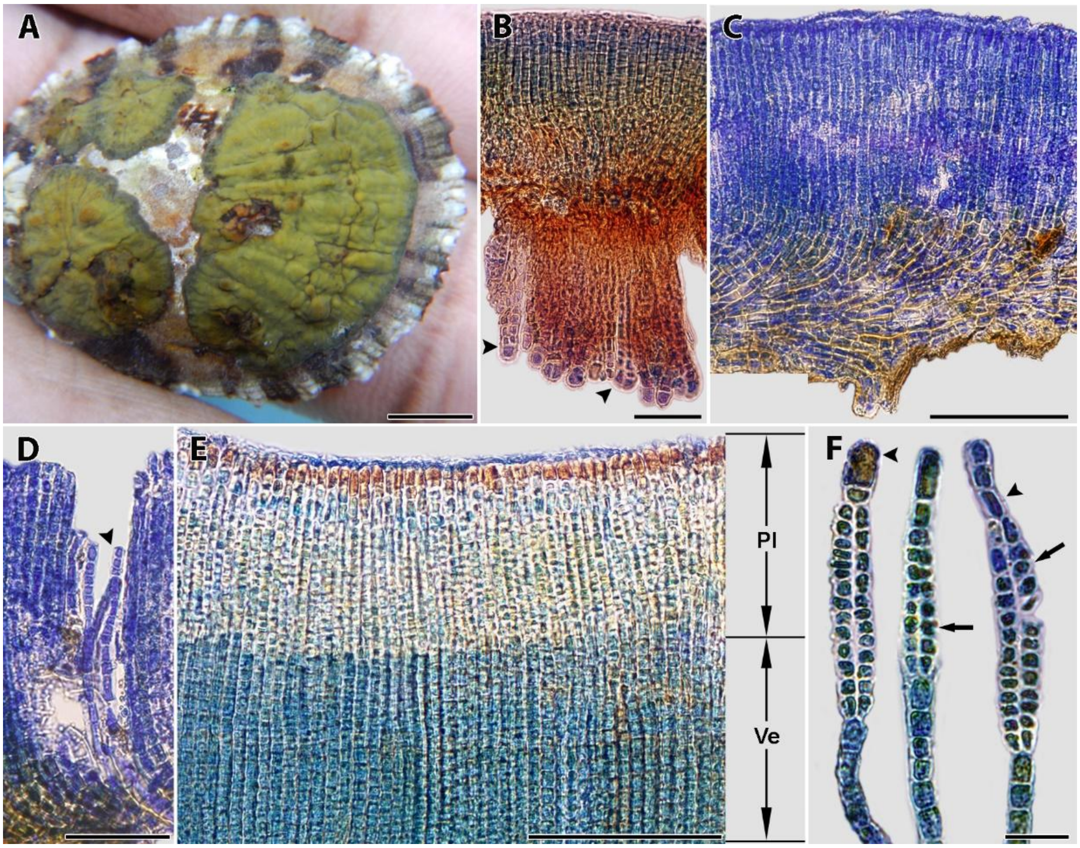


Fig. 11. Morphological characteristics of *Endoplura limpeticola* sp. nov.: (A) Thalli with olive to yellowish-brown and circular to irregular crusts with darker margins on a limpet (CUK20818). (B) Radial section view of the Holotype specimen showing creeping vegetative thallus with multicellular rhizoids (arrowheads) on the undersurface (CUK20823). (C) Radial section showing creeping vegetative thallus with upwardly curved erect filaments arising from indistinct hypothallial layer. (D) Hair (arrowhead) developed from the basal layer. (E) Radial section view of creeping reproductive thallus with sorus bearing plurangial reproductive filaments (Pl) on vegetative filaments (Ve). (F) Plurangia with two separate parallel reproductive filaments (arrow) terminated by 2–3 sterile cells (arrowhead). Scale bars represent: A, 0.5 cm; C & E, 100 µm; B & D, 50 µm; F, 20 µm.

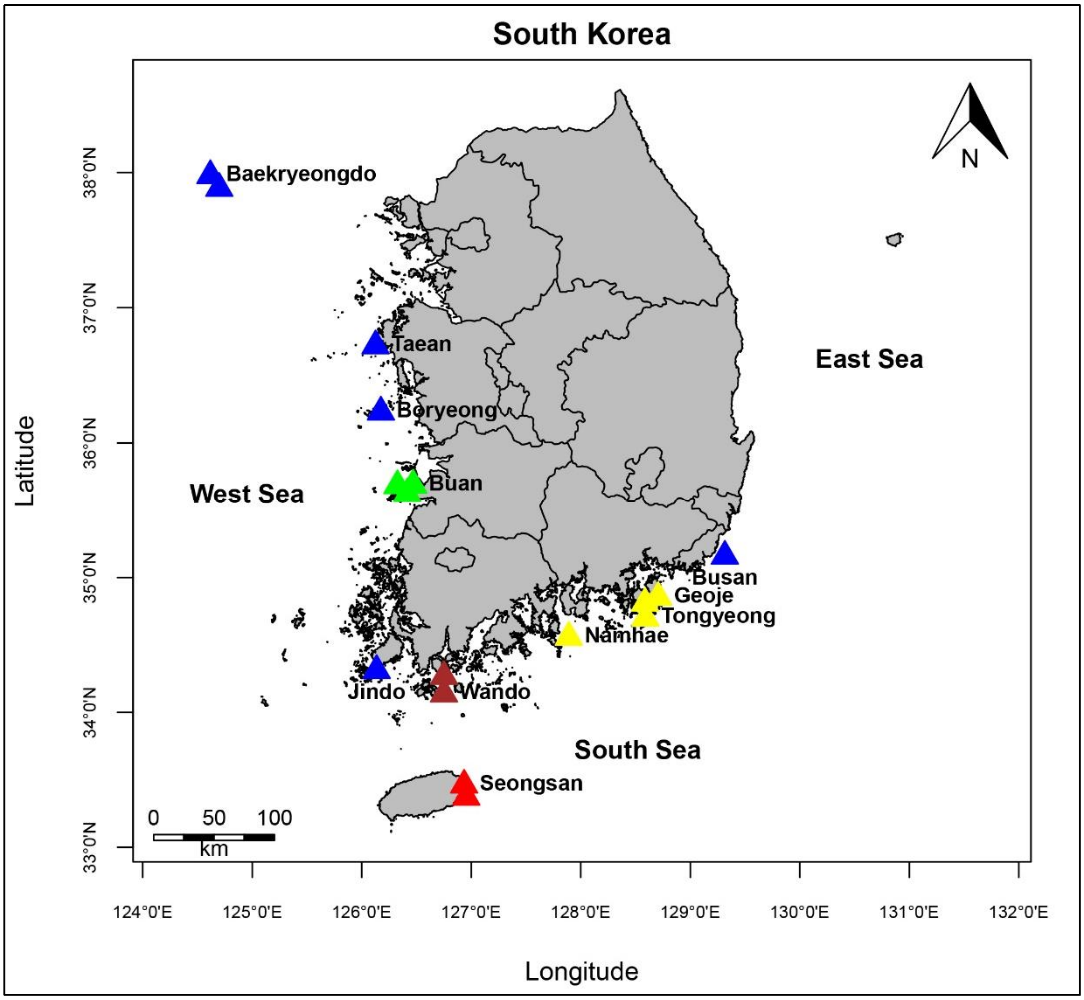


Fig. 12. Distribution of *Endoplura* species in Korea. *Endoplura geojensis* sp. nov. (yellow), *Endoplura gyeokpoensis* sp. nov. (green), *Endoplura jejuensis* sp. nov. (red), *Endoplura koreana* sp. nov. (blue), *Endoplura limpeticola* sp. nov. (brown).

Table 5. Comparison of morphological features between five *Endoplura* species.

Characters	***E. geojensis***	***E. gyeokpoensis***	***E. jejuensis***	***E. koreana***	***E. limpeticola***
Substrate	Epilithic	Epilithic	Epilithic	Epilithic or epizoic	Epizoic (on limpets)
Crust color	Yellowish olive to dark brown (shinny dark brown when dry)	Olive to light brown (shiny brown when dry)	Light to yellowish brown (shiny brown when dry)	Light to yellowish or dark brown (shiny olive to yellowish brown when dry)	olive to yellowish-brown (shiny dark brown when dry)
Margins	Not lighter, not darker	Lighter	Not lighter, not darker	Seldom lighter, not darker	Darker
Crust outline	Circular, confluent	Irregular	Almost circular	Circular or irregular, confluent	Circular or irregular, confluent
External growth lines or ridges	Inconspicuous	Absent	Indistinct growth lines or ridges	Present or absent	Present
Surface	Smooth	Smooth	Smooth	Smooth to irregular, occasionally warty	Smooth to irregular, occasionally warty

Table 5. (continued)

Characters	***E. geojensis***	***E. gyeokpoensis***	***E. jejuensis***	***E. koreana***	***E. limpeticola***
Diameter of thalli	0.1–1.5 cm	Less than 1 cm	Less than 1 cm	Up to 3 cm	Up to 2.2 cm
Thickness of thalli	68–316 µm	57–322 µm	63–317 µm	80–320 µm	115–751 µm
Basal cell layer	One to several	One to several	One to several	One to several	One to several
Basal cell length	4–14 µm	4–11 µm	3–9 µm	3–11 µm	4–10 µm
Basal cell width	7–20 µm	6–24 µm	5–33 µm	4–33 µm	8–27 µm
Rhizoids	Absent	Absent	Absent	Absent	Present
Organization and symmetry of erect filaments	Firmly adhering, straight or curved upwards	Firmly adhering, straight or curved upwards	Firmly adhering, straight or curved upwards	Firmly adhering, straight or curved upwards	Firmly adhering, curved upwards
Erect filament branching	sparsely branched	sparsely branched	sparsely branched	sparsely branched	sparsely branched

Table 5. (continued)

Characters	***E. geojensis***	***E. gyeokpoensis***	***E. jejuensis***	***E. koreana***	***E. limpeticola***
Erect filament cell length	6–14 µm	4–21 µm	4–20 µm	4–20 µm	5–17 µm
Erect filament cell width	5–14 µm	4–13 µm	3–11 µm	4–12 µm	4–11 µm
Chloroplasts / cell	Several to many	Several to many	Several to many	Several to many	Several to many
Phaeophycean hairs	Single or in tufts	Form tufts	Form tufts	Form tufts	Form tufts
Origin of hairs	Mid to apical cell of erect filament	Basal disc and lower part of erect filament	Basal disc	Basal disc and apical cell of erect filament	Basal disc and lower part of erect filament
Plurangia and unangia sori	On different thalli	Probably on different thalli	On different thalli	On same or different thalli	Probably on different thalli

Table 5. (continued)

Characters	***E. geojensis***	***E. gyeokpoensis***	***E. jejuensis***	***E. koreana***	***E. limpeticola***
Length of plurangia	48–130 µm	45–126 µm	31–98 µm	37–105 µm	57–165 µm
Plurangial filaments	2–5	2–4	Mostly 2, up to 4	1–4	Mostly 2
No. of sterile cells	2–3	2–3	2–4	2–8	2–3
Unangial shape	Clavate, obovoid, oblong	Not observed	Oblong, obovoid	Oblong, obovoid, ellipsoid	Not observed
Unangial length	37–92 µm	–	61–116 µm	50–158 µm	–
Unangial width	5–28 µm	–	10–37 µm	10–37 µm	–
Unangial position / No. of paraphyses	Sessile, Terminal / 1 paraphysis	–	Sessile, Terminal / 1 paraphysis	Sessile, Terminal / 1–2 paraphyses	–

Table 5. (continued)

Characters	***E. geojensis***	***E. gyeokpoensis***	***E. jejuensis***	***E. koreana***	***E. limpeticola***
Paraphysis length	81–182 µm	–	73–155 µm	70–178 µm	–
Paraphysis width	3–16 µm	–	3–14 µm	4–16 µm	–
No. of cells in paraphysis	7–16	–	7–12	6–12	–
Soral filaments	Present	Absent	Absent	Absent	Absent
Soral filaments length	80–183 µm	–	–	–	–
Soral filaments width	2–15 µm	–	–	–	–
No. of cells in Soral filaments	7–15	–	–	–	–
Reference	Oteng'o et al. unpublished	Oteng'o et al. unpublished	Oteng'o et al. 2021	Oteng'o et al. 2021	Oteng'o et al. unpublished

3.2. Genus *Fissipedicella* A. O. Oteng'o, B. Y. Won & T. O. Cho gen. nov.

Diagnosis: Epilithic crusts, almost circular to irregular outline, firmly attached to the substratum, without rhizoids; unistratose thalli, with hypothallial layer of one to several cell layers, perithalial layer of erect vegetative filaments; erect vegetative filaments branched, straight or upwardly curved, tapering towards the surface, firmly adjoined with one another, arise from hypothallial layer; chloroplasts one per cell; hairs in hair pits arise from lower to mid cells of erect filaments; intercalary plurangia composed of one to two rows of plurangial reproductive filaments terminated by 1–3 sterile cells; unangia on pedicels of vertically to obliquely cleaved cells, with paraphyses.

Crusts are epilithic, circular to irregular in outline, firmly attached to the substratum without rhizoids. Thalli are unistratose, composed of a hypothallial layer of one to several prostrate filaments and a perithallial layer of erect filaments. Hypothallial layer is several cells thick and gives rise to erect filaments. Erect filaments are branched, straight or upwardly curved, tapering towards the surface, firmly adjoined with one another. Chloroplasts are parietal, one per cell. Hairs pits are frequent, arising from lower to mid cells of erect filaments.

Plurangia are intercalary, forming one to two rows of plurangial reproductive filaments terminated by 1–3 sterile cells. Unangia are on pedicels of vertically or obliquely cleaved cells, with paraphyses.

Type species: *Fissipedicella orientalis* A. O. Oteng'o, B. Y. Won & T. O. Cho sp. nov.

Etymology: "*Fissipedicella*" is derived from the Latin words fissus (split or cloven or cleave or cleft) and pedicellus (stalk bearing a spore-producing body).

3.2.1. *Fissipedicella orientalis* A. O. Oteng'o, B. Y. Won & T. O. Cho sp. nov. (Figs.13 & 14)

Diagnosis: Plants epilithic crusts, greenish or reddish-brown to dark brown, circular to irregular outline, without lighter margins, with inconspicuous growth lines, 0.2–4.5 cm across, 85–421 μm thick, firmly attached to the substratum, with rust-red undersurface, without rhizoids; vegetative erect filaments branched, straight or upwardly curved, tapering towards the surface, firmly adjoined with one another, and arise from a hypothallial layer composed of one to several cell layers; chloroplasts parietal, one per cell; phaeophycean hairs in tufts, arise from the basal to mid parts of erect filaments; intercalary plurangia, 43–202 μm long, composed of 1–2 rows of reproductive filaments terminated by 1–3 sterile cells; unangia 47–116 μm long and 7–40 μm wide, on pedicels of 1–6 vertically or obliquely cleaved cells, on terminal part of erect filaments, with a paraphysis.

Holotype: CUK18754. Voucher specimen was deposited in the herbarium of Chosun University, Korea (CUK).

Type locality: Pado-ri, Sowon-myeon, Taean-gun, Chungcheongnam-do, Korea; 36°43′05.65″ N, 126°07′33.79″ E; intertidal; collected by B. Y. Won & T. O. Cho; 03 March 2018.

Isotype: CUK18765. Voucher specimens were deposited in the herbarium of Chosun University, Korea (CUK).

Specimens examined: CUK18821, **CUK18822**, **CUK18825** (Jeongdo-ri, Wando-eup, Wando-gun, Jeollanam-do, Korea, collected by T. O. Cho & B. Y. Won, 01 April 2018), **CUK19178A** (Gijang, Ilgwang-myeon, Gijang-gun, Busan, Korea, collected by T. O. Cho & B. Y. Won, 08 October 2018), **CUK19219B** (Pado-ri, Sowon-myeon, Taean-gun, Chungcheongnam-do, Korea, collected by T. O. Cho & B. Y. Won, 03 November 2018), **CUK19509C**, **CUK19510C**, **CUK19512B**, **CUK19519** (Seongsan Ilchul Peak, Seongsan-eup, Seogwipo-si, Jeju-do, Korea, collected by T. O. Cho & B. Y. Won, 19 April 2019), **CUK19606C** (Seongsan Ilchul Peak, Seongsan-eup, Seogwipo-si, Jeju-do, Korea, collected by T. O. Cho, S. Y. Jeong, J. Avila, A. O. Oteng'o & G. C. Choi, 01 May 2019), **CUK19696**, **CUK19699**, **CUK19703**, **CUK19706**, **CUK19707B**, **CUK19709**, **CUK19710** (Yeraehaean-ro, Seogwipo-si, Jeju-do, Korea, collected by T. O. Cho & B. Y. Won, 06 June 2019), **CUK20040D**, **CUK20041D** (Bomok Port, Bomok-dong, Seogwipo-si, Jeju-do, Korea, collected by T. O. Cho & B. Y. Won, 26 October 2019), **CUK20066A** (Hwasam-ri, Yongnam-myeon, Tongyeong-si, Gyeongsangnam-do, Korea, collected by T. O. Cho, 28 October 2019), **CUK20331**, **CUK20332** (Jeongdo-ri, Wando-eup, Wando-gun, Jeollanam-do, Korea, collected by T. O. Cho, J. Avila, A. O. Oteng'o & G. C. Choi, 08 May 2020), **CUK20753A**, **CUK20754**, **CUK20755**, **CUK20759E**, **CUK20760A** (Seongsan Ilchul Peak, Seongsan-eup, Seogwipo-si, Jeju-do, Korea, collected by T. O. Cho & B. Y. Won, 17 August 2020), **CUK20771**, **CUK20772** (Dolsan Port, Dolsan-eup, Yeosu-si, Jeollanam-do, Korea, collected by T. O. Cho & B. Y. Won, 17 October 2020).

Etymology: The specific epithet "*orientalis*" is derived from the region name "Orient" (Far East Asia) where the alga grows naturally.

Vegetative morphology: Thalli are epilithic crusts, greenish or reddish-brown to dark brown (very dark brown when dry), circular to irregular in outline and often confluent, without lighter margins, with inconspicuous growth lines and mostly smooth surface, 0.2–4.5 (–7.5) cm across, 85–421 μm thick, firmly attached to the substratum, with rust-red undersurface, without rhizoids (Fig. 13A). Erect filaments are branched, straight or upwardly curved, tapering towards the surface, firmly adjoined with one another to form pseudoparenchymatous tissue, and arise from a hypothallial layer composed of one to several cell layers in which cells are 4–12 μm wide and have a width to length ratio of 1 : 1.8–3.8 (Fig. 13B). Cells of the erect filaments are 4–25 μm long and 4–16 μm wide. Chloroplasts are parietal, one per cell (Fig. 13C). Phaeophycean hairs form tufts and arise from the basal to mid parts of erect filaments (Fig. 13D). Data on measured characters are summarized in Table 6.

Reproductive morphology: Reproductive portions of plurangial and unangial sori form flat or slightly elevated areas on the same or different thalli (Fig. 14A & C). Plurangia are intercalary, 43–202 μm long, composed of 1–2 rows of reproductive filaments terminated by 1–3 sterile cells (Fig. 14B). Unangia are 47–116 μm long and 7–40 μm wide, obovoid to ellipsoid to oblong, on pedicels of 1–6 vertically or obliquely cleaved cells, on terminal part of erect filaments, accompanied by a paraphysis (Fig. 14D). Paraphyses are present, 5–10 cells, 92–148 μm long, and 2–12 μm wide. Data on measured characters are summarized in Table 6.

Habitat and Phenology: Plants grow from the high to the low intertidal zone. They are found attached on hard substrates such as rocks and pebbles (epilithic) in sheltered to wave-exposed areas. Plants with both plurangial and unangial reproductive structures were collected in March to June and November.

Distribution in Korea: Jeju Island, East coast (Busan), West coast (Taean) and South coast (Tongyeong, Wando and Yeosu) (Fig. 15).

Remarks: *Fissipedicella orientalis* sp. nov. is newly described in Korea based on detailed morphological studies and molecular analyses. In this study, we established a new genus *Fissipedicella* based on morphological and molecular evidences. The genus *Fissipedicella* is characterized by Epilithic crusts, almost circular to irregular in outline, firmly attached to the substratum, without rhizoids, unistratose thalli, with a hypothallial layer of one to several cell layers giving rise to a perithalial layer of erect vegetative filaments, erect vegetative filaments branched, straight or upwardly curved, tapering towards the surface, firmly adjoined with one another, chloroplasts are single per cell, hairs in hair pits arising from lower to mid cells of erect filaments, intercalary plurangia composed of one to two rows of plurangial reproductive filaments terminated by 1–3 sterile cells, unangia on pedicels of vertically to obliquely cleaved cells, with paraphyses. The new species, *Fissipedicella orientalis* is matched well with diagnostic characters of *Fissipedicella*. *Fissipedicella orientalis* is characterized by having epilithic crusts, greenish or reddish-brown to dark brown, circular to irregular in outline, without lighter margins, 0.2–7.5 cm across, 85–421 μm thick, firmly attached to the substratum, without rhizoids, erect filaments are branched, straight or upwardly curved, tapering towards the surface and firmly adjoined with one another to form pseudoparenchymatous tissue, and arise from a hypothallial layer composed of one to several cell layers, chloroplasts are one per cell, hairs form tufts and arise from the basal to mid parts of erect filaments, intercalary plurangia, 43–202 µm long, composed of 1–2 rows of reproductive filaments terminated by 1–3 sterile cells and unangia 47–116 µm long and 7–40 µm wide, on pedicels of 1–6 vertically or obliquely cleaved cells, on terminal part of erect filaments, accompanied by a paraphysis.

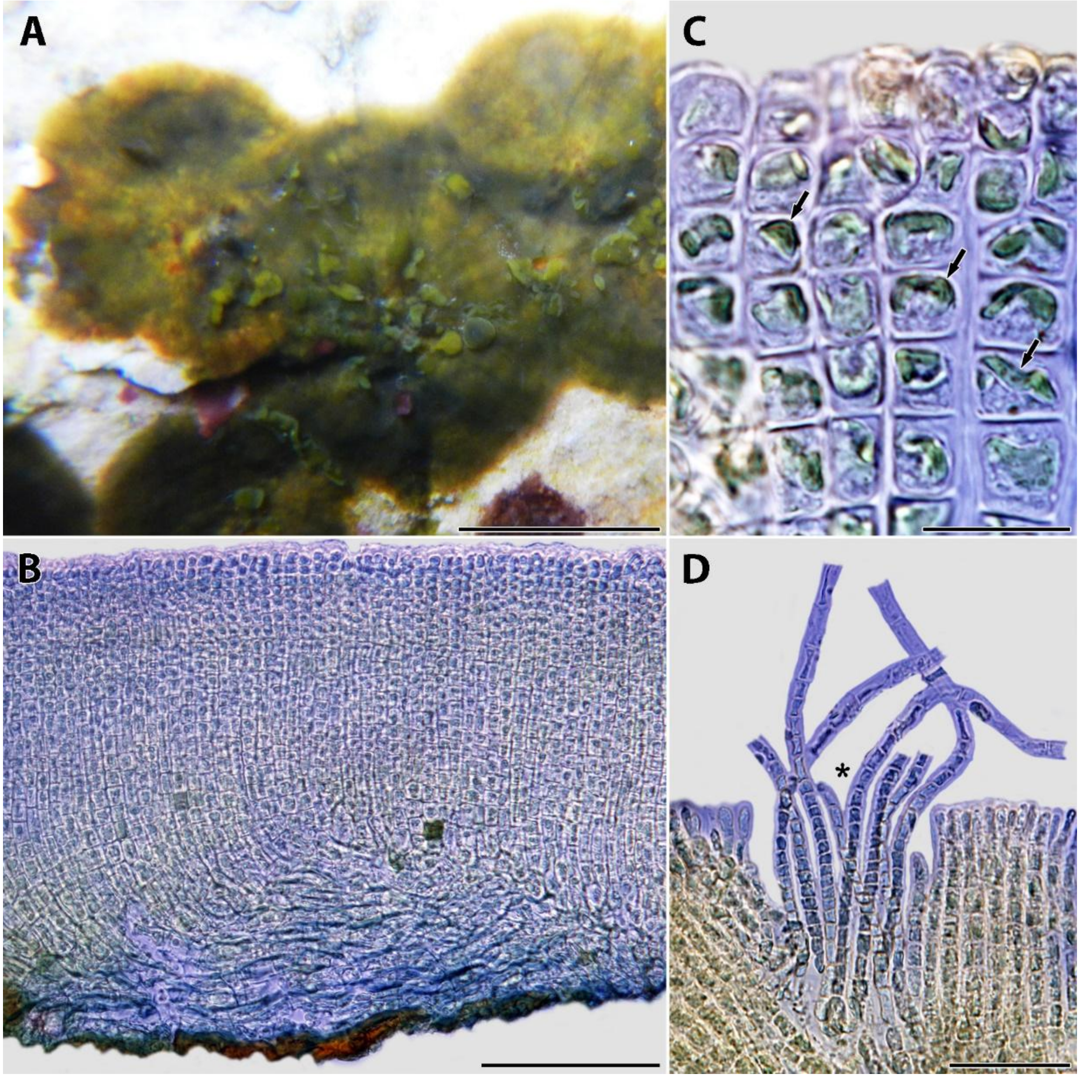


Fig. 13. Vegetative morphology of *Fissipedicella orientalis* sp. nov.: (A) Thalli with reddish to greenish-brown and almost circular confluent crusts on a pebble (CUK20332). (B) Radial section view of creeping vegetative thallus showing upwardly curved erect filaments arising from hypothallial layer (CUK19219B). (C) Parietal chloroplasts (arrows), mostly on the upper part of the cells (CUK19707B). (D) Hairs (asterisk) developed from the mid portion of the erect filaments (CUK19524). Scale bars represent: A, 0.5 cm; B, 100 μm; D, 50 μm; C, 20 μm.

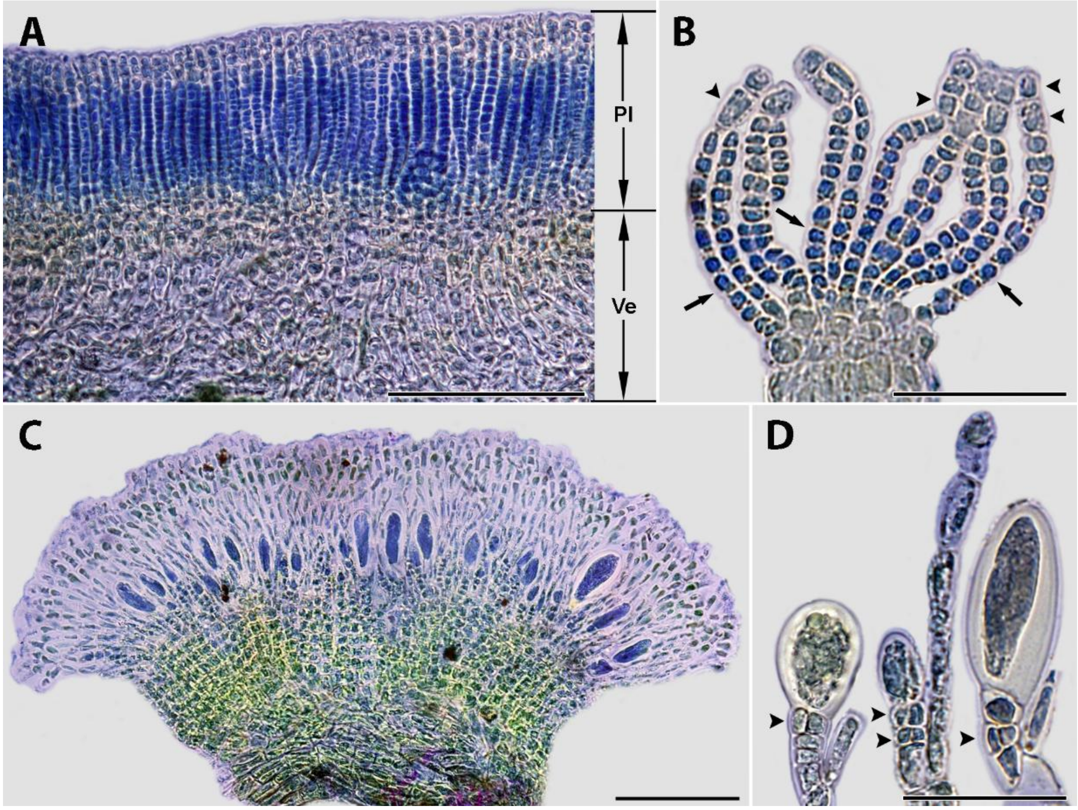


Fig. 14. Reproductive morphology of *Fissipedicella orientalis* sp. nov.: (A) Radial section view of creeping reproductive thallus with sorus bearing plurangial reproductive filaments (Pl) on vegetative filaments (Ve) (CUK19219B). (B) Plurangial reproductive structures with one to two reproductive filaments (arrow) terminated by 1–3 sterile cells (arrowhead) (CUK18754). (C) Radial section view of creeping reproductive thallus with sorus bearing unangia (CUK18754). (D) Unangia on stalks with cleaved stalk cells (arrowheads) (CUK18754). Scale bars represent: A & C, 100 µm; B & D, 50 µm.

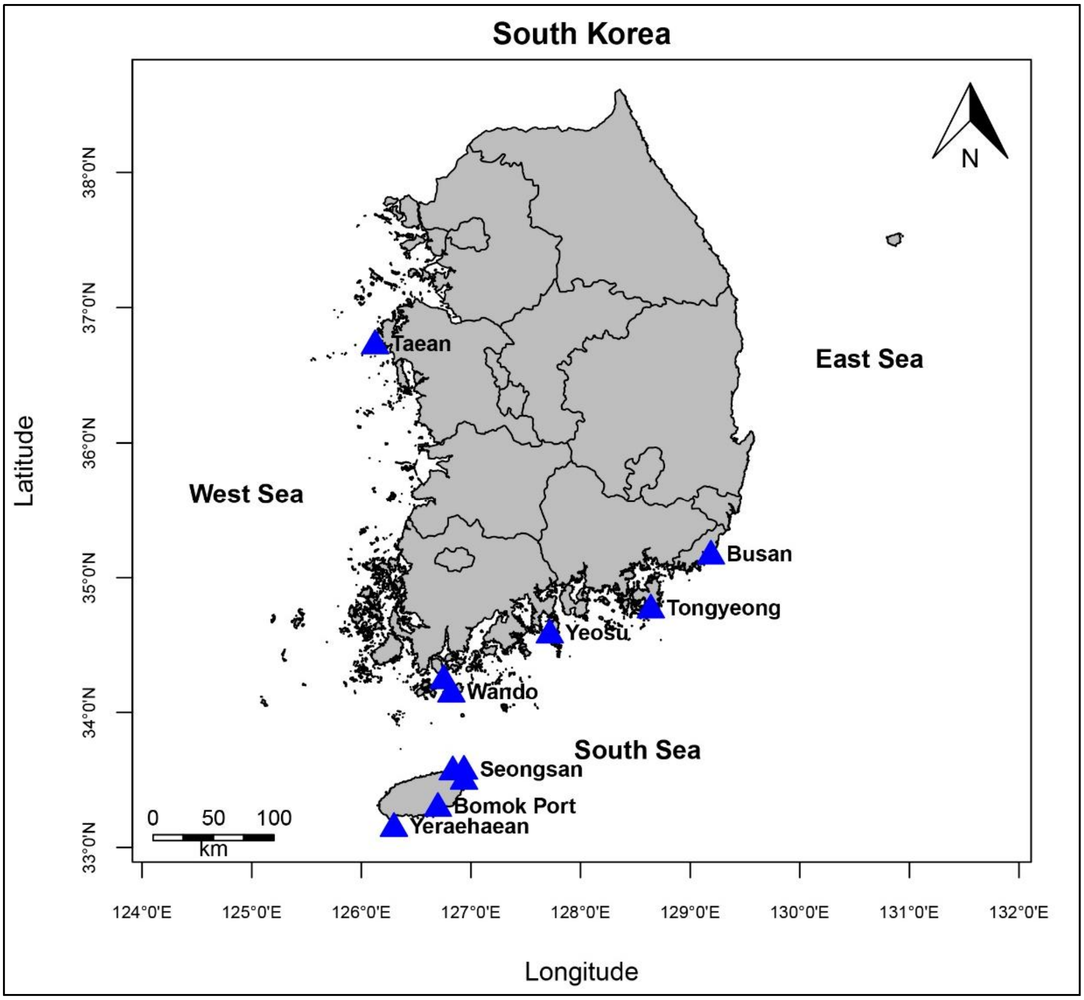


Fig. 15. Distribution of *Fissipedicella* species in Korea. *Fissipedicella orientalis* sp. nov. (blue).

Table 6. Morphological comparison between *Fissipedicella* and other genera in family Ralfsiaceae.

Features	***Analipus***	***Endoplura***	***Fissipedicella***	***Heteroralfsia***	***Ralfsia***	***Ramipedicella***
Life history	Haplodiplontic, isomorphic	Haplodiplontic, isomorphic	Haplodiplontic, isomorphic	Haplodiplontic, heteromorphic	Haplodiplontic, isomorphic or direct	Haplodiplontic, isomorphic
Sporophyte	Erect, tough	Creeping, tough	Creeping, tough	Erect, tough	Creeping, tough	Creeping, tough
Holdfast	Creeping	–	–	Creeping	–	–
Substrate	Epilithic	Epilithic or epizoic	Epilithic	Epilithic or epizoic	Epilithic or epiphytic or epizoic	Epilithic or epizoic
Unistratose / multistratose thalli	–	Unistratose	Unistratose	–	Unistratose or multistratose	Multistratose
Symmetry / asymmetry of thallus	Unilateral	Straight or unilateral	Straight or unilateral	Unilateral	Straight or unilateral or bilateral	Unilateral

Table 6. (continued)

Features	***Analipus***	***Endoplura***	***Fissipedicella***	***Heteroralfsia***	***Ralfsia***	***Ramipedicella***
Stratified erect filaments	–	Absent	Absent	–	Present or absent	Present
Rhizoids	Present	Present or absent	Absent	Present	Present or absent	Present
Cells of basal layer	–	Quadrate to horizontally elongated	Quadrate to horizontally elongated	–	Quadrate to horizontally elongated	Horizontally elongated
Cells of erect filaments	–	Quadrate to vertically elongated	Quadrate to vertically elongated	–	Quadrate to vertically elongated	Vertically elongated
Chloroplasts	Single to many/cell	Several/cell	Single/cell	Single/cell	Single/cell	Single/cell
Hairs	Present	Present or absent	Present	Present	Absent	Present or absent

Table 6. (continued)

Features	***Analipus***	***Endoplura***	***Fissipedicella***	***Heteroralfsia***	***Ralfsia***	***Ramipedicella***
Plurangia and unangia sori	On different thalli	On same or different thalli	On same or different thalli	On different thalli	On same or different thalli	On different thalli
Plurangial filaments	1–2	2–5	1–2	1	1–2	1–2
No. of sterile cells	1–3	2–8	1–3	1	1 (to 6)	1–4
Unangial position	Terminal/lateral	Terminal	Terminal	Terminal	Terminal	Terminal
Unangial stalk	–	Absent	Present	Absent	Present or absent	Present
No. of unangia per stalk or erect filament	–	1	1	1	1	1 to several
No. of unangial stalk cells	–	0	1–6	0	0–2	0–4

Table 6. (continued)

Features	***Analipus***	***Endoplura***	***Fissipedicella***	***Heteroralfsia***	***Ralfsia***	***Ramipedicella***
Branched / unbranched stalk	–	–	Unbranched	–	Unbranched	Branched
Cleaved / uncleaved stalk cells	–	–	Cleaved	–	Uncleaved	Uncleaved
Paraphysis	Present	Present	Present	Present	Present	Present
No. of paraphyses per unangium	–	1–2	1	–	1	1
Soral filaments	Absent	Present or absent	Absent	Absent	Absent	Absent
Reference	Kjellman 1889, Wynne 1971, Nelson 1980, Lim et al. 2007	Hollenberg 1969, Tanaka and Chihara 1981b, Lim et al. 2007, Oteng'o et al. 2021	Oteng'o et al. unpublished	Kawai 1989, Lim et al. 2007	Hollenberg 1969, Womersley 1987, Tanaka and Chihara 1980b, Parente and Saunders 2019, Oteng'o et al. unpublished	Oteng'o et al. unpublished

3.3. Genus *Ralfsia* Berkeley 1843

3.3.1. *Ralfsia claviformis* A. O. Oteng'o, B. Y. Won & T. O. Cho sp. nov. (Fig. 16)

Diagnosis: Plants epilithic crusts, olive to greenish brown, irregular outline, without lighter margins, with coriaceous surface, without growth lines and ridges, up to 8 cm across, 109–812 µm thick, firmly attached to the substratum, with patches of rust-red undersurface, without rhizoids; hypothallial layer with one to three cell layers, cells rectangular to cuboidal; vegetative erect filaments sparsely branched, strictly straight, not tapered, firmly adjoined with one another, with cells consistently cuboidal to slightly rectangular; chloroplasts parietal, one per cell; phaeophycean hairs rarely observed, arise from lower to mid part of erect filaments; intercalary plurangia, 70–204 µm long, composed of 1–2 rows of reproductive filaments each terminated by 2–8 sterile cells, sterile cells 7–27 µm long, 3–16 µm wide, form clavate-shaped structure; unangia 46–72 µm long and 7–20 µm wide, sessile, on terminal part of erect filaments, at the base of a paraphysis.

Holotype: CUK18423A. Voucher specimen was deposited in the herbarium of Chosun University, Korea (CUK).

Type locality: Dala park, Sanyang-eup, Tongyeong-si, Gyeongsangnam-do, Korea; 34°46′63.75″N, 128°24′18.56″E; intertidal; collected by T. O. Cho, S. Y. Jeong, J. Avila, A. O. Oteng'o & E. C. Shin; 03 November 2017.

Specimens examined: CUK18162C, **CUK18163C**, **CUK18164C** (Cheongsapo, Jung-dong, Haeundae-gu, Busan, Korea, collected by T. O. Cho & S. Y. Jeong, 08 July 2017), **CUK18403B** (Chaeseokgang, Buan-gun, Jeollabuk-do, Korea, collected by T. O. Cho & B. Y. Won, 17 November 2017), **CUK18412**, **CUK18413**, **CUK18414** (Geumgap beach, Uisin-myeon, Jindo-gun, Jeollanam-do, Korea, collected by T. O. Cho, 21 October 2017),

CUK18423A (Dala park, Sanyang-eup, Tongyeong-si, Gyeongsangnam-do, Korea, collected by T. O. Cho, S. Y. Jeong, J. Avila, A. O. Oteng'o & E. C. Shin, 03 November 2017), **CUK19698C**, **CUK19701** (Saegdal-dong, Seogwipo-si, Jeju-do, Korea, collected by T. O. Cho & B. Y. Won, 06 June 2019), **CUK19813C**, **CUK19814A**, **CUK19816A** (Oeyeon-do, Ocheon-myeon, Boryeong-si, Chungcheongnam-do, Korea, collected by T. O. Cho & B. Y. Won, 01 August 2019)**, CUK20567**, **CUK20571**, **CUK20572** (Chosa-ri, Uisin-myeon, Jindo-gun, Jeollanam-do, Korea, collected by T. O. Cho & B. Y. Won, 24 July 2020)**, CUK20576** (Jeongdo-ri, Wando-eup, Wando-gun, Jeollanam-do, Korea, collected by T. O. Cho, J. Avila, A. O. Oteng'o & G. C. Choi, 05 August 2020)**, CUK20919B** (Jeongdo-ri, Wando-eup, Wando-gun, Jeollanam-do, Korea, collected by T. O. Cho & B. Y. Won, 08 May 2021)**.**

Etymology: The name "*claviformis*" is derived from the clavate-shaped structure of sterile cells that terminate the plurangia.

Vegetative morphology: Thalli are epilithic crusts, olive to greenish brown (brown when dry), irregular in outline and often confluent, with coriaceous surface, without lighter margins, without growth lines and ridges, up to 8 cm across (Fig. 16A). Crusts are 109–812 µm thick, firmly attached to the substratum, with patches of rust-red undersurface, without rhizoids (Fig. 16B). Vegetative erect filaments are sparsely branched, strictly straight, not tapered, firmly adjoined with one another to form pseudoparenchymatous tissue, and arise from a hypothallial layer composed of one to three cell layers in which cells are rectangular to cuboidal, 4–11 µm long and 11–26 µm wide (Fig. 16B). Cells of the erect filaments are consistently cuboidal to slightly rectangular, 7–14 µm long and 7–13 µm wide and contain light, intracellular vesicles (physodes). Chloroplasts are parietal and one per cell (Fig. 16C). Phaeophycean hairs arise from lower to mid part of erect filaments, were rarely observed. Data on measured characters are summarized in Table 7.

Reproductive morphology: Reproductive portions of sori form small elevated patches, mostly with plurangial reproductive structures (Fig. 16D), rarely with both plurangia and unangia (Fig. 16E). Unangia are 46–72 µm long and 7–20 µm wide, sessile, obovoid, on terminal part of erect filaments, at the base of a paraphysis (Fig. 16E). Plurangia are intercalary, composed of one to two rows of reproductive filaments arising from subterminal cells of erect filaments, mostly uniseriate, 70–204 µm long, each terminated by 2–8 sterile cells (Fig. 16F). Sterile cells are 7–27 µm long, 3–8 µm (basal) and 6–16 µm (apical) wide, forming a clavate-shaped structure (Fig. 16G). Data on measured characters are summarized in Table 7.

Habitat and Phenology: Plants grow from the high to the low intertidal zone. They are found attached on hard substrates such as pebbles and rocks (epilithic) in sheltered to wave-exposed areas. Plants with mostly plurangial reproductive structures were collected in October and November. Unangial reproductive structures were rarely observed and were found in the sori of the holotype specimen. The sori of holotype specimen were predominantly with plurangial reproductive structures.

Distribution in Korea: Jeju Island, East coast (Busan), West coast (Boryeong and Buan) and South coast (Jindo, Tongyeong and Wando) (Fig. 17).

Remarks: In the present study, we newly describe *Ralfsia claviformis* sp. nov. from Korea based on detailed morphological studies and molecular analyses. This new species is recognized as a member of *Ralfsia*. Berkeley in Smith and Sowerby (1843) described the genus *Ralfsia* as plants having creeping fronds, more or less squamæform and coriaceous, discrete and orbicular in juvenile plants, often irregular in older thalli, loosely to strongly attached to substratum either with or without rhizoid; one to several cells thick hypothallial layer giving rise to either straight or assurgent firmly adjoined erect filaments; one chloroplast per cell, without obvious pyrenoid; hairs infrequent in hair-pits; unangia in sori, borne at the base of loosely associated clavate, multi-cellular, simple paraphyses, with or without stalk cells; intercalary plurangia terminated by one to several sterile cells. *Ralfsia claviformis* is matched well with the diagnostic characters of *Ralfsia*. *Ralfsia claviformis* is characterized by having epilithic crusts, olive to greenish brown, irregular outline, with coriaceous surface, up to 8 cm across and 109–812 μm thick, firmly attached to substratum, without rhizoids; hypothallial layer give rise to sparsely branched straight vegetative erect filaments; one chloroplasts per cell; phaeophycean hairs rarely observed; intercalary plurangia, 70–204 μm long, composed of 1–2 rows of reproductive filaments each terminated by 2–8 sterile cells, sterile cells form clavate-shaped structure; unangia 46–72 μm long and 7–20 μm wide, sessile, on terminal part of erect filaments, at the base of a paraphysis. Although *R. claviformis* thallus construction closely resembles that of *R. confusa* and *R. tenebris*, the former has thicker thalli as compared to the latter two (Table 7). *Ralfsia claviformis* is the only one species which has plurangia composed of 1–2 rows of reproductive filaments each terminated by sterile cells that form clavate-shaped structures among the *Ralfsia* species.

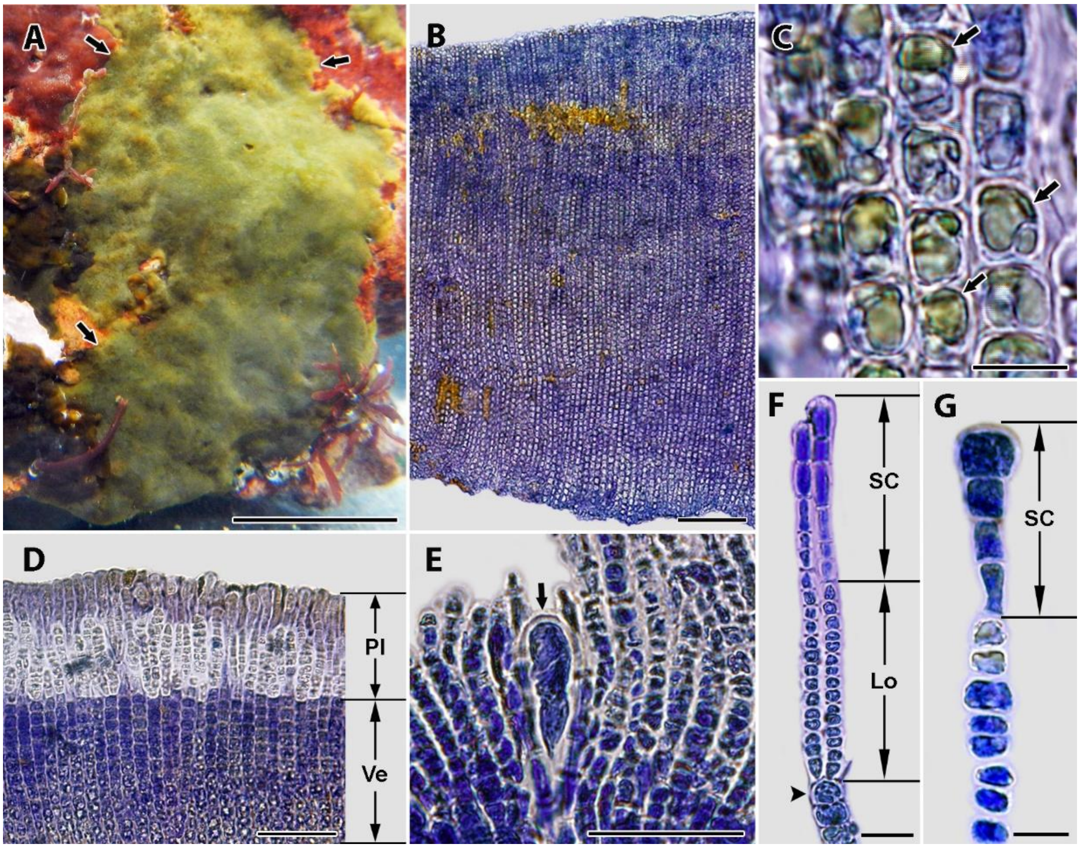


Fig. 16. Morphological characteristics of *Ralfsia claviformis* sp. nov.: (A) Olive to greenish brown crusts (arrows) on a pebble (CUK20567). (B) Radial longitudinal section of crust showing vegetative filaments strictly straight and perpendicular to the hypothallial layer (CUK18412). (C) Parietal chloroplasts (arrows) (CUK20576). (D) Radial section view of creeping reproductive thallus with sorus bearing plurangial reproductive filaments (Pl) on vegetative filaments (Ve) (CUK18403B). (E) Holotype specimen with obovoid unangium (arrow) amidst plurangial reproductive organs (CUK18423A). (F) Two rows of plurilocular reproductive organs on a subterminal vegetative cell (arrowhead) (Lo, plurangial locules; SC, sterile cells) (CUK18423A). (G) Clavate-shaped sterile cells (SC) (CUK18403B). Scale bars represent: A, 1 cm; B, 100 µm; D & E, 50 µm; F, 20 µm; C & G, 10 µm.

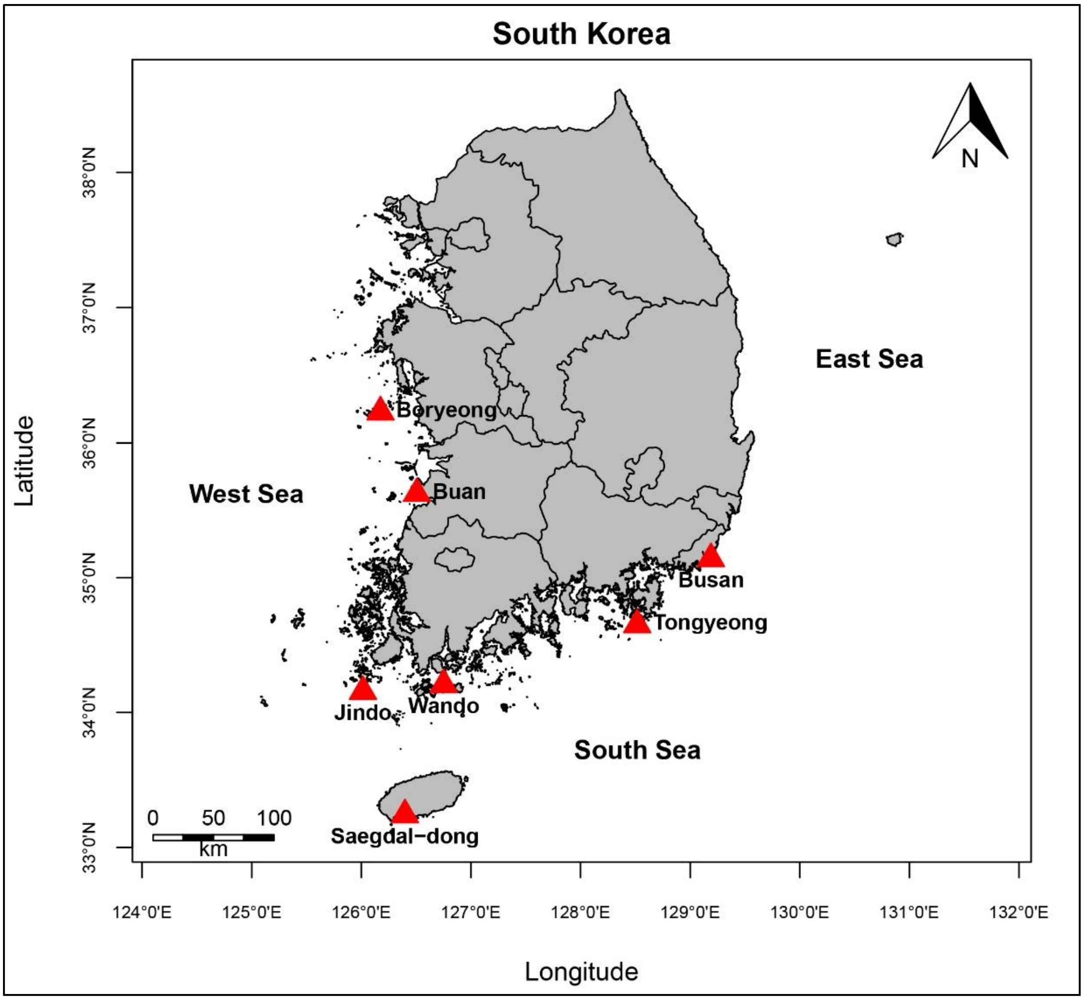


Fig. 17. Distribution of *Ralfsia* species in Korea. *Ralfsia claviformis* sp. nov. (red).

Table 7. Morphological comparison between four *Ralfsia* species.

Characters	***R. claviformis***	***R. confusa***	***R. fungiformis* (Type species)**	***R. tenebris***
Substrate	Epilithic	Epilithic	Epilithic	Epilithic
Crust color	Olive to greenish brown (brown when dry)	Light to medium dark brown	Light to dark brown or olive (black when dry)	Dark brown to dark olive
Lighter margins	Absent	–	Present	Absent
Crust outline	Irregular	Irregular	Circular	Irregular
External growth lines or ridges	Absent	Absent	Present	Absent
Surface	Smooth	Smooth	Irregular, occasionally warty	Smooth or slightly warty
Lobes	Absent	Absent	Present, prominent	Absent
Diameter of thalli	Up to 8.0 cm	0.5–1.0 cm	Up to 5.0 cm or more	Up to 6.0 cm
Thickness of thalli	109–812 μm	150–250 μm	500–1200 μm	Up to 250 μm
Unistratose /multistratose	Unistratose	–	Unistratose	Unistratose

Table 7. (continued)

Characters	***R. claviformis***	***R. confusa***	***R. fungiformis* (Type species)**	***R. tenebris***
Delimitation of cortex and medulla	Absent	Absent	Present	Absent
Medulla cell length	–	–	12–60 μm	–
Medulla cell width	–	–	8–12 μm	–
Cortex cell length	–	–	4–12 μm	–
Cortex cell width	–	–	4–8 μm	–
Basal cell layer	One to three	One to several	One to several	One to two
Basal cell length	4–11 μm	9–12 μm	–	7–10 μm
Basal cell width	11–26 μm	13–24 μm	–	7–18 μm
Rust-red color underside of crusts	Occasionally form patches	Present	Absent	Occasional
Rhizoids	Absent	Absent	Present, numerous unicellular and multicellular	Absent

Table 7. (continued)

Characters	***R. claviformis***	***R. confusa***	***R. fungiformis* (Type species)**	***R. tenebris***
Organization and symmetry of erect filaments	Firmly adhering, straight	Firmly adhering, strictly straight	Firmly adhering, curved upwards and downwards	Firmly adhering, straight not curving
Erect filament branching	sparsely branched	–	Branched	–
Erect filament cell length	7–14 µm	5–6 µm	–	2–12 µm
Erect filament cell width	7–13 µm	5–6 µm	–	4–10 µm
Chloroplasts / cell	1 / cell	1 / cell	1 / cell	1 / cell
Phaeophycean hairs	Rarely observed	Frequent in pits (?)*	Absent	Hair pits rare
Origin of hairs	Lower to mid cell of erect filament	–	–	Mid cell of erect filament

Table 7. (continued)

Characters	***R. claviformis***	***R. confusa***	***R. fungiformis* (Type species)**	***R. tenebris***
Plurangia and unangia sori	On separate thalli or occasionally same thalli	On separate thalli	On same or different thalli	–
Length of plurangia	70–204 µm	30–60 µm	90–180 µm	Up to 60.5 µm
Plurangial filaments	1–2	Mostly 1	1	1, occasionally 2
No. of sterile cells	2–8, form clavate-shaped structure	1	3–6	1
Unangial shape	Obovoid	Slightly clavate	Clavate, pyriform	–
Unangial length	46–72 µm	70–90 µm	Up to 180 µm	–
Unangial width	7–20 µm	20–25 µm	36 µm	–
Unangial position	Terminal	Terminal	Terminal	–

Table 7. (continued)

Characters	***R. claviformis***	***R. confusa***	***R. fungiformis* (Type species)**	***R. tenebris***
Unangial stalks	Absent	Present	Absent	–
No. of stalk cells	0	0–6	0	–
No. of unangia per stalk or erect filament	1	1	1	–
Paraphysis	Present	Present	Present	–
Paraphysis length	71–104 µm	75–100 µm	165–200 µm	–
Paraphysis width	4–6 µm	(ca.3–) 6–8 µm	–	–
No. of cell per paraphysis	9–14	10–12	5–7	
Paraphysis shape	Slightly clavate	Clavate	Clavate	–
Soral filaments	Absent	–	–	–

Table 7. (continued)

Characters	***R. claviformis***	***R. confusa***	***R. fungiformis* (Type species)**	***R. tenebris***
Reference	Oteng'o et al. unpublished	Hollenberg 1969, Parente and Saunders 2019	Hollenberg 1969, Tanaka and Chihara 1980b, Lim et al 2007, Parente and Saunders 2019	Parente and Saunders 2019

*In Hollenberg's (1969) original description of *R. confusa* hair pits were frequent, however Parente and Saunders (2019) did not find hair pits when re-examining the type material.

3.4. Genus *Ramipedicella* A. O. Oteng'o, B. Y. Won & T. O. Cho gen. nov.

Diagnosis: Epilithic or epizoic crusts, circular to irregular outline, firmly attached to the substratum, with or without rhizoids; multistratose thalli with erect filaments frequently stratified; hypothallial layer several cells thick, give rise to firmly adjoined and usually assurgent erect filaments, cells of basal to apical portions of thalli consistently longer than wide with width to length ratio of 1:1–16; chloroplasts one per cell; hairs present or absent; plurangial reproductive structures uniseriate, occasionally biseriate, subterminal on erect filaments, terminated by one to several sterile cells; unangia one to several, on branched stalks or sessile, with paraphysis.

Crusts are epilithic or epizoic, circular to irregular outline, firmly attached to the substratum with or without rhizoids. Thalli are multistratose with youngest plant layer at the top. Crusts are seemingly perennial, with erect filaments frequently stratified, owing to different growth rates attributed to seasonal changes. Hypothallial layer is several cells thick, giving rise to firmly adjoined and usually assurgent erect filaments. Cells of basal to apical portions of thalli are consistently longer than wide with width to length ratio of 1:1–16. Chloroplasts are one per cell. Phaeophycean hairs are present or absent.

Sori form elevated patches. Plurangial reproductive structures are uniseriate, occasionally biseriate, subterminal on erect filaments, terminated by one to several sterile cells. One to several unangia are on branched stalks or sessile, with paraphysis.

Type species: *Ramipedicella microloba* A. O. Oteng'o, B. Y. Won & T. O. Cho sp. nov.

Etymology: "*Ramipedicella*" is derived from the Latin words *ramus* (branch) and *pedicellus* (stalk bearing a spore-producing body).

3.4.1. *Ramipedicella microloba* A. O. Oteng'o, B. Y. Won & T. O. Cho sp. nov. (Figs. 18 & 19)

Diagnosis: Plants thick epilithic or epizoic crusts, light to dark brown, with lighter margins, circular to irregular outline, with growth lines, with microlobes mostly towards the center, up to 15 cm across, 300–1650 μm thick, firmly attached to substratum, with rust-red undersurface, with multicellular rhizoids; plant multistratose owing to superimposed thalli composed of 1–4 plant layers; horizontal rows give rise to frequently stratified, firmly adherent, upwardly curved and tapering vertical filaments, cells with width to length ratio of 1:1–16; chloroplasts parietal, one per cell; numerous hairpits; intercalary plurangia, 65–153 μm long, uniseriate, occasionally biseriate, terminated by 1–4 sterile cells; unangia 90–180 μm long and 20–46 μm wide, one to several, sessile or on 1–3(–4)-celled branched stalks, on the terminal part of erect filaments, with paraphysis; paraphyses 114–363 μm long and 3–16 μm wide, clavate-shaped, composed of 14–20 cells.

Holotype: CUK20165. Voucher specimen was deposited in the herbarium of Chosun University, Korea (CUK).

Type locality: Hwasam-ri183-7, Yongnam-myeon, Tongyeong-si, Gyeongsangnam-do, Korea; 34°51′08.07″N, 128°27′03.36″E; intertidal; collected by B. Y. Won & T. O. Cho; 08 February 2020.

Isotype: CUK20166. Voucher specimens were deposited in the herbarium of Chosun University, Korea (CUK).

Specimens examined: CUK13559 (Bangeojin, Bangeo-dong, Dong-gu, Ulsan, South Korea, collected by T. O. Cho, S. Y. Jeong, J. G. Lee, S. Y. Park, J. B. Chai & H. U. Jang, 22 January 2015), **CUK18162A**, **CUK18164A** (Cheongsapo, Jung-dong, Haeundae-gu, Busan, Korea, collected by T. O. Cho & S. Y. Jeong, 08 July 2017), **CUK18395**, **CUK18396**, **CUK18397**, **CUK18398**, **CUK18399** (Daecheon Beach, Sinheuk-dong, Boryeong-si, Chungcheongnam-do, Korea, collected by T. O. Cho & B. Y. Won, 18 November 2017), **CUK18400**, **CUK18401**, **CUK18402**, **CUK18404**, **CUK18405** (Chaeseokgang, Buan-gun, Jeollabuk-do, Korea, collected by T. O. Cho & B. Y. Won, 17 November 2017), **CUK18428A**, **CUK18432**, (Dala park, Sanyang-eup, Tongyeong-si, Gyeongsangnam-do, Korea, collected by T. O. Cho, S. Y. Jeong, J. Avila, A. O. Oteng'o & E. C. Shin, 03 November 2017), **CUK18440A**, **CUK18445A**, **CUK18449**, **CUK18452**, **CUK18457**, **CUK18458**, **CUK18459**, **CUK18460** (Yeonhwa-ri, Gijang- eup, Gijang-gun, Busan, Korea, collected by T. O. Cho, S. Y. Jeong, J. Avila, A. O. Oteng'o & E. C. Shin, 04 November 2017), **CUK18729B**, **CUK18730**, **CUK18731** (Cheongsapo, Jung-dong, Haeundae-gu, Busan, Korea, collected by T. O. Cho & B. Y. Won, 01 March 2018), **CUK18737A**, **CUK18738B** (Yeonhwa-ri, Gijang- eup, Gijang-gun, Busan, Korea, collected by T. O. Cho & B. Y. Won, 01 March 2018), **CUK18751**, **CUK18753**, **CUK18755A, CUK18768** (Pado-ri, Sowuon-myeon, Taean-gun, Chungcheongnam-do, Korea, collected by T. O. Cho & B. Y. Won, 01 March 2018), **CUK18823** (Jeongdo-ri, Wando-eup, Wando-gun, Jeollanam-do, Korea, collected by T. O. Cho & B. Y. Won, 01 April 2018), **CUK18883** (Bangjukpo, Dolsan-eup, Yeosu-si, Jeollanam-do, Korea, collected by T. O. Cho & B. Y. Won, 29 April 2018), **CUK18972** (Seosang, Seo-myeon, Namhae-gun, Gyeongsangnam-do, Korea, collected by T. O. Cho & B. Y. Won, 18 May 2018), **CUK19150** (Chaeseokgang, Byeonsan-myeon, Buan-gun, Jeollabuk-do, Korea, collected by T. O. Cho & B. Y. Won, 03 October 2018), **CUK19215A**, **CUK19217A**, **CUK19218A**, **CUK19219A**, **CUK19222A**, **CUK19225A** (Pado-ri, Sowon-myeon, Taean-gun, Chungcheongnam-do, Korea, collected by T. O. Cho & B. Y. Won, 03 November 2018), **CUK19794** (Gyeokpo Port, Byeonsan-myeon, Buan-gun, Jeollabuk-do, Korea, collected by T. O. Cho & B. Y. Won, 26 July 2019), **CUK19813A, CUK19823** (Oeyeon-do, Ocheon-myeon, Boryeong-si, Chungcheongnam-do, Korea, collected by T. O. Cho & B. Y. Won, 01 August 2019), **CUK20066D** (Hwasam-ri, Yongnam-myeon, Tongyeong-si, Gyeongsangnam-do, Korea, collected by T. O. Cho, 28 October 2019), **CUK20165**, **CUK20166** (Hwasam-ri183-7, Yongnam-myeon, Tongyeong-si, Gyeongsangnam-do, Korea, collected by T. O. Cho & B. Y. Won, 08 February 2020), **CUK20578**, **CUK20585**, **CUK20589** (Jeongdo-ri, Wando-eup, Wando-gun, Jeollanam-do, Korea, collected by T. O. Cho, J. Avila, A. O. Oteng'o & G. C. Choi, 05 August 2020), **CUK20613A** (Chunghwa-dong Port, Baekryeongdo, Incheon, Korea, collected by T. O. Cho, J. Avila, A. O. Oteng'o & G. C. Choi, 12 August 2020), **CUK20649** (Dumunjin Port, Baekryeongdo, Incheon, Korea, collected by T. O. Cho, J. Avila, A. O. Oteng'o & G. C. Choi, 12 August 2020), **CUK20666C**, **CUK20673A**, **CUK20676A**, **CUK20677** (Gobong Port, Baekryeongdo, Incheon, Korea, collected by T. O. Cho, J. Avila, A. O. Oteng'o & G. C. Choi, 12 August 2020).

Etymology: The specific epithet "*microloba*" is derived from micro-lobe structures found on the surface of thalli.

Vegetative morphology: Plants are thick epilithic or epizoic crusts, light to dark brown (almost black in dry state) with lighter margins, circular to irregular in outline, with external concentric zones and radial lines, with microlobes mostly towards the center making the surface uneven, up to 15 cm across, 300–1650 μm thick (Fig. 18A & B). Young thalli grow typically as distinct crusts but older ones are multistratose owing to superimposed thalli composed of 1–4 plant layers (Fig. 18C). Crusts are seemingly perennial, with erect filaments frequently stratified, with rows of short cells alternating with rows of elongated cells, presumably a result of periods of active growth followed by reduced growth and or reproduction (Fig. 18C). Thalli form unilateral symmetry with undersurface rust-red (Fig. 18D), and irregularly distributed with multicellular rhizoids 6–15 μm wide (Fig. 18E). Cells of creeping portion are 7–15 μm width, with a ratio of width to length 1:1–12, forming horizontal rows. Horizontal rows give rise to firmly adherent and upwardly tapering vertical filaments. Cells of vertical filaments are 3–12 μm width, with a ratio of width to length 1:1–16. Chloroplasts are parietal, single per cell, mostly on the apical part of the cells. Hairs pits are numerous, scattered on the surface of the thalli, and arise from middle cells of erect filaments (Figs. 18F & G). Data on measured characters are summarized in Table 8.

Reproductive morphology: Plurangial and unangial reproductive portions of sori form elevated bumps on the surface of separate thalli (Figs. 19A & B). Plurangia are 65–153 µm long, uniseriate, occasionally biseriate, terminated by 1–4 sterile cells. Sterile cell are 4–12 µm width and 1.5–4 times as long as the width (Fig. 19C). Unangia are 90–180 µm long and 20–46 µm wide, oblong, one to several, sessile or on 1–3(–4)-celled branched stalks, on the terminal part of erect filaments, and accompanied with paraphysis. Paraphyses are 114–363 µm long and 3–16 µm wide, clavate-shaped, composed of 14–20 cells (Fig. 19D). Data on measured characters are summarized in Table 8.

Habitat and Phenology: Plants grow from the high to the low intertidal zone. They are found attached on hard substrates such as small boulders or pebbles, rocks (epilithic) and seldom on shells of molluscs (epizoic) in sheltered to wave-exposed areas. Plants with plurangial and unangial reproductive structures were collected in January and February.

Distribution in Korea: East coast (Busan and Ulsan), West coast (Baekryeongdo, Boryeong, Buan and Taean) and South coast (Namhae, Tongyeong, Wando and Yeosu) (Fig. 22).

Remarks: *Ramipedicella microloba* sp. nov. is newly described from Korea based on detailed morphological studies and molecular analyses. In this study, we established a new genus *Ramipedicella* based on morphological and molecular evidences. The genus *Ramipedicella* is characterized by Epilithic or epizoic crusts, circular to irregular outline, firmly attached to the substratum, with or without rhizoids, multistratose thalli with erect filaments frequently stratified, hypothallial layer give rise to firmly adjoined and usually assurgent erect filaments, cells of basal to apical portions of thalli consistently longer than wide with width to length ratio of 1:1–16, chloroplasts one per cell, with or without hairs, intercalary plurangia terminated by one to several sterile cells, one to several unangia sessile or on branched stalks, with paraphysis. The new species, *Ramipedicella microloba* is matched well with diagnostic characters of

Ramipedicella. *Ra. microloba* is characterized by having thick epilithic or epizoic crusts, light to dark brown, with lighter margins, circular to irregular outline, with growth lines, with micro-lobes mostly towards the center of plant, up to 15 cm across, 300–1650 µm thick, firmly attached to substratum, with multicellular rhizoids, plant is multistratose owing to superimposed thalli composed of 1–4 plant layers, horizontal rows give rise to frequently stratified, firmly adherent, upwardly curved and tapering vertical filaments, cells with width to length ratio of 1:1–16, chloroplasts parietal, one per cell, numerous hair pits, intercalary plurangia 65–153 µm long, uniseriate, occasionally biseriate, terminated by 1–4 sterile cells, unangia 90–180 µm long and 20–46 µm wide, one to several, sessile or on 1–4-celled branched stalks, on the terminal part of erect filaments, with clavate-shaped paraphysis.

3.4.2. *Ramipedicella longicellularis* (Perestenko) A. O. Oteng'o, B. Y. Won & T. O. Cho comb. nov. (Figs. 20 & 21)

Basionym: *Ralfsia longicellularis* Perestenko.

Homotypic synonym: *Ralfsia longicellularis* Perestenko 1980: 193.

Type locality: Peter the Great Bay, Russia (Perestenko 1980: 193).

Specimens examined: CUK10442 (Jeongdo-ri, Wando-eup, Wando-gun, Jeollanam-do, Korea, collected by T. O. Cho, 24 October 2013), **CUK17342**, (Sa-ri, Heuksan-myeon, Sinan-gun, Jeollanam-do, Korea, collected by T. O. Cho, S. Y. Jeong, B. M. Danilo, S. Y. Park & J. Avila, 20 May 2016), **CUK18752, CUK18766** (Pado-ri, Sowon-myeon, Taean-gun, Chungcheongnam-do, Korea, collected by T. O. Cho & B. Y. Won, 03 March 2018), **CUK18819**, **CUK18820**, **CUK18824** (Jeongdo-ri, Wando-eup, Wando-gun, Jeollanam-do, Korea, collected by T. O. Cho & B. Y. Won, 01 April 2018), **CUK19216**, **CUK19221**, **CUK19224** (Pado-ri, Sowon-myeon, Taean-gun, Chungcheongnam-do, Korea, collected by T. O. Cho & B. Y. Won, 03 November 2018), **CUK19269**, **CUK19270** (Chaeseokgang, Buan-gun, Jeollabuk-do, Korea, collected by T. O. Cho & B. Y. Won, 26 January 2019), **CUK20022**, **CUK20023**, **CUK20024**, **CUK20025**, **CUK20026**, **CUK20027**, **CUK20028**, **CUK20029**, **CUK20030**, **CUK20031**, **CUK20032** (Ulista Leytenanta Shmidta, Vladivostok, Russia, collected by T. O. Cho & B. Y. Won, 10 October 2019), **CUK20611A**, **CUK20616A**, **CUK20617** (Chunghwa-dong Port, Baekryeongdo, Incheon, Korea, collected by T. O. Cho, J. Avila, A. O. Oteng'o & G. C. Choi, 12 August 2020), **CUK20650B**, **CUK20653B**, **CUK20659** (Dumunjin Port, Baekryeongdo, Incheon, Korea, collected by T. O. Cho, J. Avila, A. O. Oteng'o & G. C. Choi, 12 August 2020), **CUK20664A**, **CUK20664C** (Sagot Beach, Jinchon-ri 40-41, Baekryeongdo, Incheon, Korea, collected by T. O. Cho, J. Avila, A. O. Oteng'o & G. C. Choi, 12 August 2020).

Vegetative morphology: Thalli form thick epilithic crusts, dark brown (almost black in the dry state), with generally a smooth surface, with or without external concentric zones and radial lines, may have overlapping lobes at the margins, several centimeters across, 410–1200 µm thick (Fig. 20A). Old thalli are multistratose and composed of 1–3 plant layers. Crusts are perennial, with erect filaments frequently stratified, with rows of short cells alternating with rows of elongated cells (Fig. 19B). Thalli form unilateral symmetry (Fig. 20C), with rust-red undersurface irregularly distributed with multicellular rhizoids 8.4–14 µm wide (Fig. 20D). In the creeping and ascending parts of the filaments, the cells are curved, often with oblique partitions, 8.5–11 (–14) µm width, with a ratio of width to length 1:1.5–7, forming horizontal rows. The lower layer of creeping and ascending filaments is 0.3 times the thickness of the thallus in young thalli. The cells of vertical filaments are cylindrical 4–8.5 µm width, with a ratio of width to length 1:1–10. Chloroplasts are parietal, single per cell, mostly on the apical part of the cell. Phaeophycean hairs were not observed. Data on measured characters are summarized in Table 8.

Reproductive morphology: Plurangial and unangial reproductive portions of sori, form elevated bumps on the surface of separate thalli (Fig. 21A & B). Plurangia are 90–265 µm long, uniseriate, occasionally biseriate, terminated by 1–2 sterile cells. Sterile cells are 5–8 µm width and 1.8–3 times as long as the width (Fig. 21C). Unangia are 65–92 µm long and 22.5–31 µm wide, oblanceolate to oblong, sessile or on 1–2(–3)-celled branched stalks, one to several per stalk, on the terminal part of erect filaments, with paraphysis. Paraphyses are 190–210 µm long, composed of 10–13 cells. Apical cells of paraphyses are 8.5–10 µm wide (Fig. 21D). Data on measured characters are summarized in Table 8.

Habitat and Phenology: Plants grow from the high to the low intertidal zone. They are found attached on hard substrates such as rocks and small boulders or pebbles (epilithic) in sheltered to wave-exposed areas. Plants with plurangial plurangial reproductive structures were collected from the Korean coast in March. Unangia bearing plants were collected from Vladivostok, Russia, in October.

Distribution in Korea: West coast (Baekryeongdo, Buan, Sinan and Taean) and South coast (Wando) (Fig. 22).

Remarks: *Ramipedicella longicellularis* comb. nov. was originally described as *Ralfsia longicellularis* by Perestenko (1980). This species was characterized by having encrusting dark brown thalli (almost black in the dry state), with uneven surface, easily detached from the substratum, several centimeters across, 770–1200 µm thick, with rhizoids 8.4–14 um wide; in the creeping and ascending parts of the filaments, the cells are curved and often with oblique partitions, 8.5–14 µm width, with a ratio of width to length 1:1.5–7, forming horizontal rows; cells of vertical filaments are cylindrical 5.5–8.5 um width, with a ratio of width to length 1:1.5–10, the lower layer of creeping and ascending filaments is 0.3 times the thickness of the thallus; unangia are 22.5–31 x 65–92 µm, narrowly ovate, on multicellular stalks, with paraphysis; paraphyses of 10–13 cells, 190–210 µm long, with apical cells 8.5–10 µm wide; hairs unknown. Although Perestenko (1980) did not give a detailed morphology of unangial stalks and the number of chloroplasts per cell, our morphological analyses of *Ralfsia longicellularis* samples recently collected from Vladivostok, Russia revealed them having branched unangial stalks and single chloroplast per cell. In this study, we established a new genus *Ramipedicella* based on morphological and molecular evidences. The genus *Ramipedicella* is characterized by Epilithic or epizoic crusts, circular to irregular outline, firmly attached to the substratum, with or without rhizoids, multistratose thalli with erect filaments frequently stratified, hypothallial layer give rise to firmly adjoined and usually assurgent erect filaments, cells of basal to apical portions of thalli consistently longer than wide with width to length ratio of 1:1–16, chloroplasts one per cell, with or without hairs, intercalary plurangia terminated by one to several sterile cells, one to several unangia sessile or on branched stalks, with paraphysis. *Ramipedicella longicellularis* corresponds well to the generic characteristics of *Ramipedicella*. *Ra. longicellularis* differs from *Ra. microloba* by having generally smooth crusts, absence of phaeophycean hairs, plurangia terminated by 1–2 apical sterile cells and smaller mostly oblanceolate unangia.

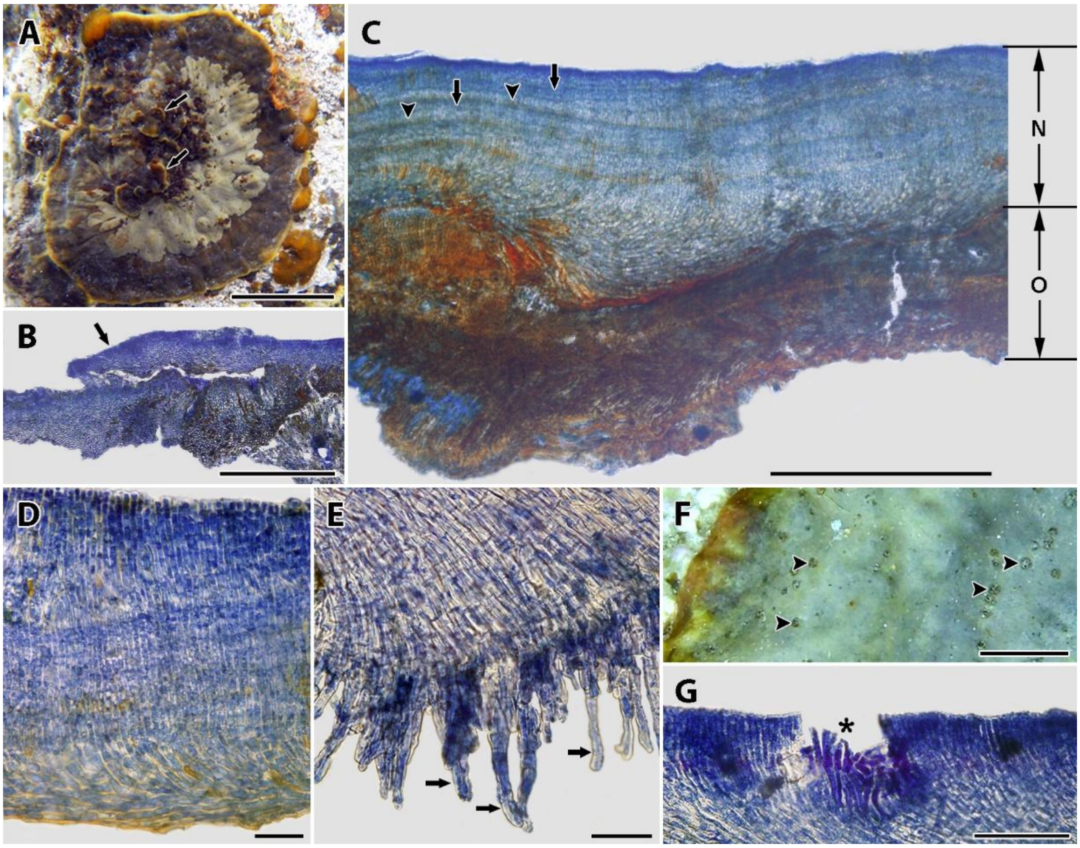


Fig. 18. Vegetative morphology of *Ramipedicella microloba* sp. nov.: (A) Holotype specimen with dark brown crust with light margins and small lobes (arrows) towards the central portion (CUK20165). (B) Radial longitudinal section showing a small lobe (arrow) (CUK20165). (C) A section of thallus showing superimposed portions (N, new layer; O, old layer). Filaments stratified, rows of short cells (arrowheads) alternate with rows of elongated cells (arrows) (CUK18458A). (D) Unilaterally symmetrical thallus with cells longer than wide (CUK18972). (E) Multicellular rhizoids (arrows) on the undersurface (CUK18883). (F) Numerous hair pits (arrowheads) on surface of the crust (CUK20165). (G) Radial longitudinal section showing a hair pit (asterisk). Scale bars represent: A, 1 cm; F, 1 mm; B & C, 500 µm; G, 100 µm; D & E, 50 µm.

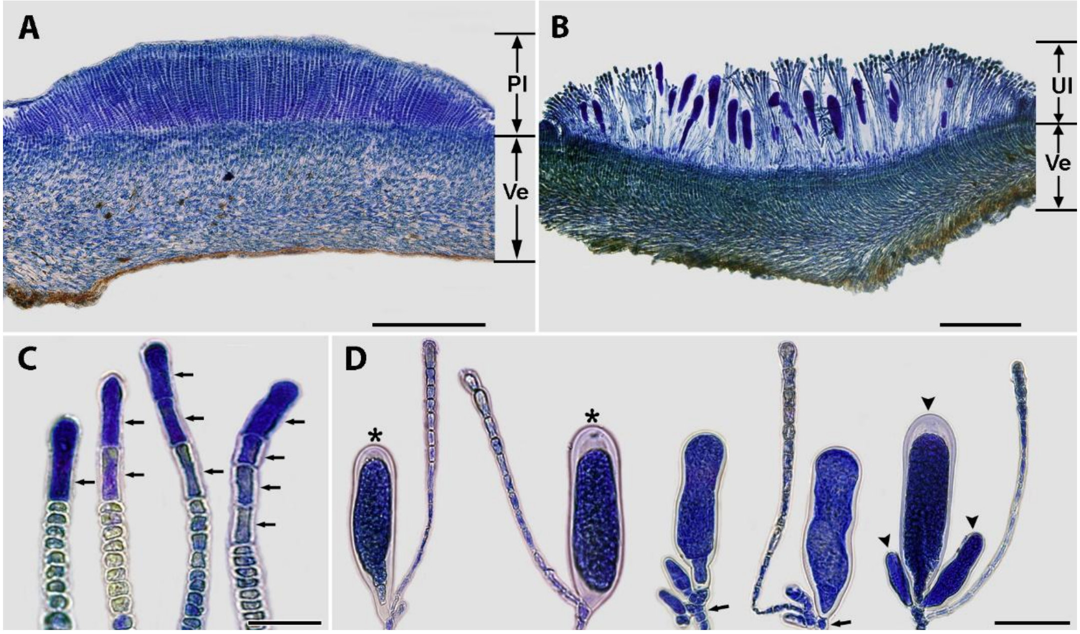


Fig. 19. Reproductive morphology of *Ramipedicella microloba* sp. nov.: (A) Syntype specimen, radial section view of creeping reproductive thallus with sorus bearing plurangial reproductive filaments (Pl) on vegetative filaments (Ve) (CUK20166). (B) Radial section view showing a portion of reproductive thallus with sorus bearing unangial reproductive structures (Ul) on vegetative filaments (Ve) (CUK13559). (C) One to four sterile cells (arrows) terminating plurangial reproductive structures (CUK20165). (D) Sessile unangia (asterisks), branched stalks (arrows) bearing unangia, several unangia (arrowheads) per stalk (CUK13559). Scale bars represent: A & B, 200 µm; D, 50 µm; C, 20 µm.

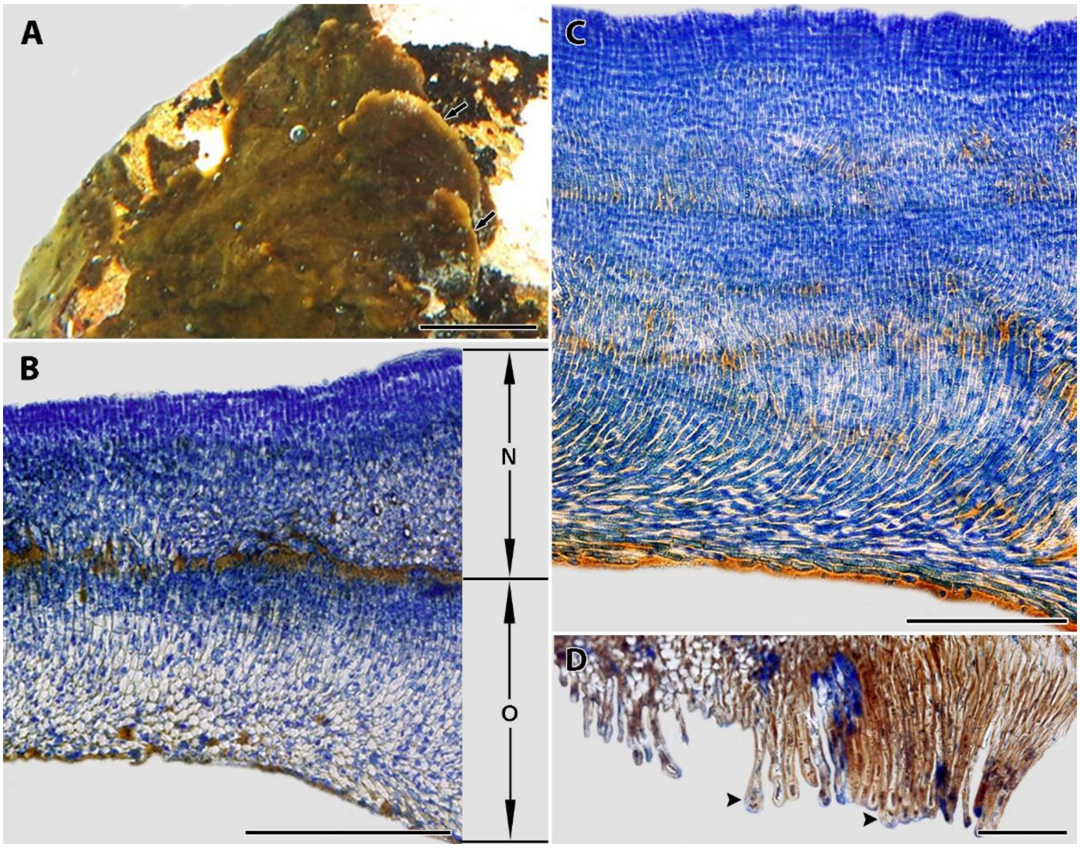


Fig. 20. Vegetative morphology of *Ramipedicella longicellularis* comb. nov.: (A) Dark brown crust with smooth surface and few small lobes on the margins (CUK18820). (B) A section of thallus showing superimposed portions (N, new layer; O, old layer) (CUK20024). (C) Unilaterally symmetrical thallus with cells longer than wide and a rust-red undersurface (CUK18752). (D) Multicellular rhizoids (arrowheads) on the undersurface of thallus (CUK20024). Scale bars represent: A, 1 cm; B, 200 µm; C & D, 100 µm.

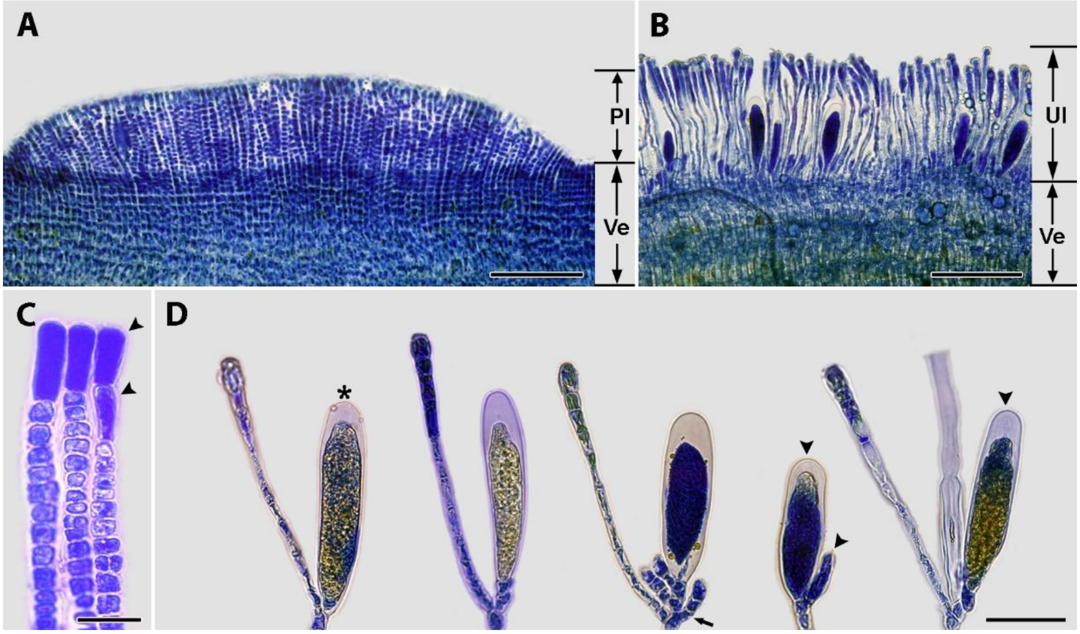


Fig. 21. Reproductive morphology of *Ramipedicella longicellularis* comb. nov.: (A) Radial section view of creeping reproductive thallus with sorus bearing plurangial reproductive filaments (Pl) on vegetative filaments (Ve) (CUK18752). (B) Radial section view showing a portion of reproductive thallus with sorus bearing unangial reproductive structures (Ul) on vegetative filaments (Ve) (CUK20024). (C) One to two sterile cells (arrowheads) terminate plurangial reproductive structures (CUK18820). (D) Sessile unangia (asterisk), branched stalk (arrow) bearing unangia, two unangia (arrowheads) per stalk (CUK20025). Scale bars represent: A & B, 100 µm; D, 50 µm; C, 20 µm.

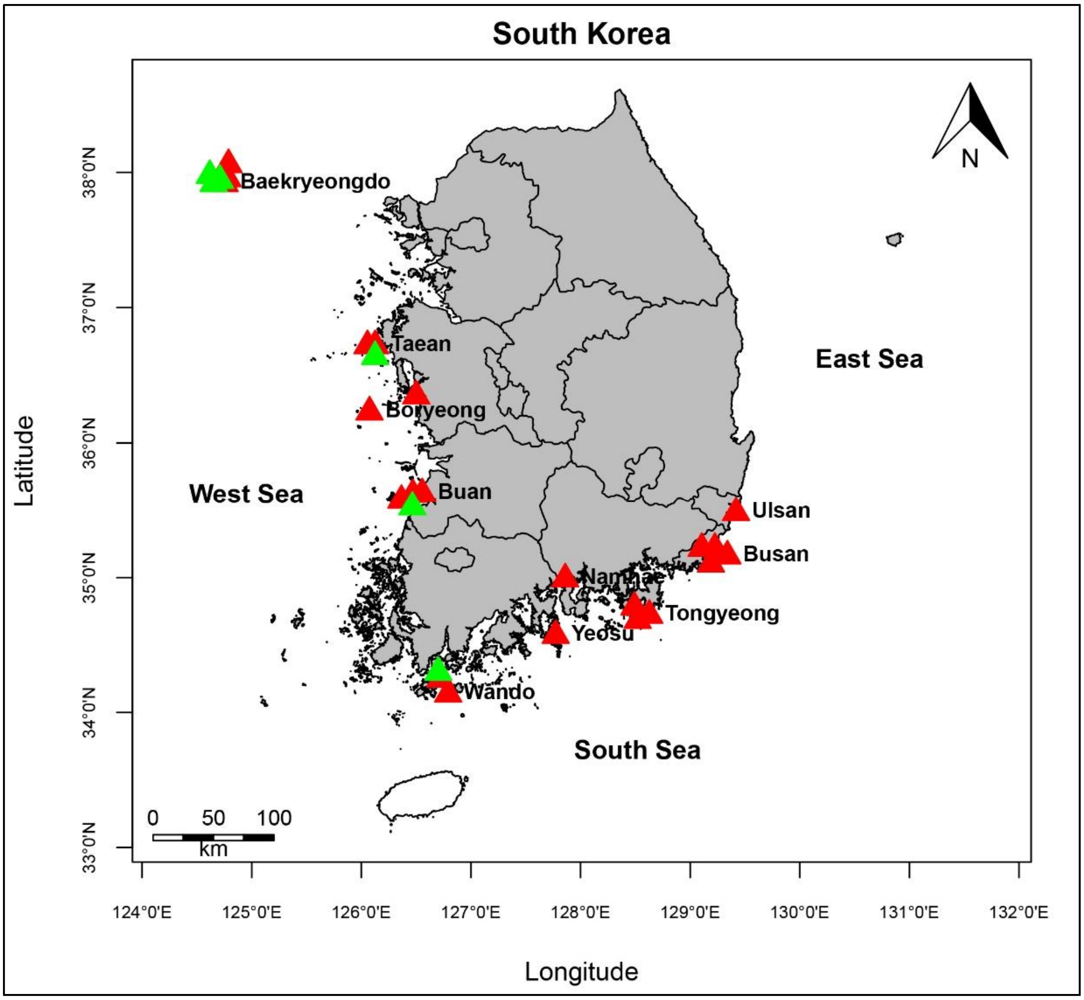


Fig. 22. Distribution of *Ramipedicella* species in Korea. *Ramipedicella microloba* sp. nov. (red), *Ramipedicella longicellularis* comb. nov. (green).

Table 8. Morphological comparison between *Ramipedicella* species.

Characters	***Ra. longicellularis***	***Ra. microloba***
Substrate	Epilithic	Epilithic or epizoic
Crust color	Dark brown (almost black in the dry state)	Light to dark brown (almost black in dry state)
Margins	Mostly lighter	Lighter
Crust outline	Circular to irregular, confluent	Circular to irregular, confluent
External growth lines or ridges	Present or absent	Present
Surface	Generally smooth	Rough towards center of thallus
Lobes	May be present, few mostly at the margins	Microlobes mostly towards center of thallus
Diameter of thalli	Up to 10 cm or more	Up to 15 cm
Thickness of thalli	410–1200 µm	300–1650 µm
No. of layers in a superimposed thallus	1–3	1–4

Table 8. (continued)

Characters	***Ra. longicellularis***	***Ra. microloba***
Basal cell layer	Several	Two to several
Basal cell length	12–79 µm	18–98 µm
Basal cell width	8.5–11 (14) µm	7–15 µm
Rhizoids	Present, multicellular	Present, multicellular
Organization and symmetry of erect filaments	Firmly adhering, unilateral	Firmly adhering, unilateral
Stratified erect filaments	Present	Present
Erect filaments branching	Branched	Branched
Erect filament cell length	4–85.5 µm	3–79 µm
Erect filament cell width	3–8.5 µm	2–12 µm
Chloroplasts	1 / cell	1 / cell
Phaeophycean hairs	Not observed	Form tufts in pits

Table 8. (continued)

Characters	***Ra. longicellularis***	***Ra. microloba***
Origin of hairs	Not observed	Lower to mid part of erect filament
Plurangia and unangia sori	On different thalli	On different thalli
Length of plurangia	90–265 µm	65–153 µm
Plurangial filaments	1, occasionally 2	1, occasionally 2
No. of sterile cells	1–2	1–4
Sterile cell length	8–24 µm	6–37 µm
Sterile cell width	5–8 µm	4–12 µm
Unangial position	Terminal	Terminal
Unangial shape	Oblanceolate to oblong	Oblong
Unangial length	65–92 µm	90–180 µm
Unangial width	22.5–31 µm	20–46 µm
Branched unangial stalks	Present	Present

Table 8. (continued)

Characters	***Ra. longicellularis***	***Ra. microloba***
No. of cells per stalk	0–3	0–4
No. of unangia per stalk or erect filament	1 to several	1 to several
Paraphysis	Present	Present
Paraphysis length	190–210 µm	114–363 µm
Paraphysis width	2–11 µm	3–16 µm
No. of cells in paraphysis	10–13	14–20
Soral filaments	Absent	Absent
Reference	Perestenko 1980, Oteng'o and won 2020, Oteng'o et al. unpublished	Oteng'o et al. unpublished

4. Family Sungminiaceae A. O. Oteng'o, B. Y. Won & T. O. Cho fam. nov.

Diagnosis: Plants gelatinous epilithic crusts, firmly attached to the substratum, without rhizoids; hypothallial layer of one to several cell layers, erect filaments moderately adjoined and partially separate with pressure, not tapered, with large apical vegetative cells; hairs in tufts; chloroplasts one per cell, without obvious pyrenoid; diplohaplontic isomorphic life history; intercalary plurangia terminated by a sterile cell; unangia on the terminal part of erect filaments, with a paraphysis.

Thalli are gelatinous crusts, firmly attached to substratum without rhizoids. Hypothallial layer with one to several cell layers that give rise to erect filaments. Erect filaments are moderately adjoined and partially separate with pressure, not tapered. Apical cells of erect filaments are larger than other cells. Hairs form in tufts. Chloroplasts are one per cell, without obvious pyrenoid.

Life history supposedly diplohaplontic and isomorphic. Plurangia are intercalary, terminated by a sterile cell. Unangia are on the terminal part of erect filaments, with associated paraphyses. Summarized comparison of Sungminiaceae with other families in the Ralfsiales in Table 9.

Type genus: *Sungminia* A. O. Oteng'o, B. Y. Won & T. O. Cho gen. nov.

Etymology: The family name "Sungminiaceae" is in honor of Professor Sung-Min Boo for his outstanding contributions in the taxonomy of seaweeds not only in the Eastern Asia region but also worldwide.

4.1. Genus *Sungminia* A. O. Oteng'o, B. Y. Won & T. O. Cho gen. nov.

Diagnosis: Irregular epilithic crusts, gelatinous, firmly attached to the substratum, without rhizoids; basal layer one to several horizontally elongated cells, erect filaments straight or slightly curved, simple or sparsely branched, moderately adjoined and partially separates with pressure, not tapered, with large apical cells; hairs in tufts, arise from basal layer or basal to mid portions of erect filaments; chloroplasts one per cell, without obvious pyrenoid; intercalary plurangia composed of one to two reproductive filaments terminated by a sterile cell; unangia sessile or on stalks, on terminal part of erect filaments, with a paraphysis.

Plants with irregularly expanded epilithic crusts, gelatinous and firmly attached to substratum without rhizoids. Thalli are composed of a basal layer consisting of one to several horizontally elongated cells that give rise to erect filaments. Erect filaments are straight or slightly curved, simple or sparsely branched, moderately adjoined and partially separates with pressure, not tapered. Apical cells of erect filaments are larger than other cells. Hairs in tufts, arising from basal layer or basal to mid portions of erect filaments. Chloroplasts are one per cell, without obvious pyrenoid.

Plurangial reproductive structures are uniseriate or biseriate, intercalary with a terminal sterile cell. Unangia are sessile or on stalks, on the terminal part of erect filaments, with associated paraphysis.

Type species: *Sungminia gladiata* A. O. Oteng'o, B. Y. Won & T. O. Cho sp. nov.

Etymology: The name "*Sungminia*" is in honor of Professor Sung-Min Boo for his outstanding contributions in the taxonomy of seaweeds not only in the Eastern Asia region but also worldwide.

4.1.1. *Sungminia asiatica* A. O. Oteng'o, B. Y. Won & T. O. Cho sp. nov. (Fig. 23)

Diagnosis: Plants irregular epilithic crusts, brown to yellowish brown, without lighter margins, with smooth surface, 0.3–3.3 cm across, 90–611 μm thick, gelatinous, firmly attached to the substratum, without rhizoids; hypothallial layer monostromatic, erect filaments straight to slightly curved, sparsely branched, not tapered, moderately adjoined, partially separate with pressure, with apical vegetative cells larger than other cells; hairs sparse, arise from basal layer;

chloroplasts one per cell, without pyrenoid; intercalary plurangia uniseriate or biseriate, terminated by a rectangular sterile cell, with rounded apices; unangia sessile or on stalks of 1–2 cells, on terminal part of erect filaments, with associated paraphyses.

Holotype: CUK19812B. Voucher specimen was deposited in the herbarium of Chosun University, Korea (CUK).

Type locality: Oeyeondo, Ocheon-myeon, Boryeong-si, Chungcheongnam-do, Korea; 36°13′43.30″N, 126°04′29.16″E; intertidal; collected by T. O. Cho & B. Y. Won; 01 August 2019.

Specimens examined: CUK19175C (Gijang, Ilgwang-myeon, Gijang-gun, Busan, Korea, collected by T. O. Cho & B. Y. Won, 08 October 2018), **CUK20631A, B & C, CUK20636A & D** (Dumunjin Port, Baekryeongdo, Incheon, Korea, collected by T. O. Cho J. Avila, A. O. Oteng'o & G. C. Choi, 12 August 2020), **CUK20874** (Daecheon Port, Sinheuk-dong, Boryeong-si, Chungcheongnam-do, Korea, collected by T. O. Cho & B. Y. Won, 28 November 2020).

Etymology: The specific epithet, "*asiatica*" is derived from the geographical region "Asia", where the alga grows naturally.

Vegetative morphology: Plants form irregularly expanded epilithic crusts, brown to yellowish brown, without lighter margins, with smooth surface, 0.3–3.3 cm across, 90–611 μm thick, gelatinous and firmly attached to the substratum, without rhizoids (Fig. 22A). Hypothallial layer is monostromatic, composed of horizontally elongated cells which are 5–16 μm long, 3–12 μm wide and give rise to erect filaments. Erect filaments are straight to slightly curved, sparsely branched, not tapered, moderately adjoined (Fig. 23B) and partially separate with pressure (Fig. 23C). Apical vegetative cells are 10–22 μm long and 4–10 μm wide, 1.8–2.5 times larger than other cells of the erect filaments (Fig. 23D). Hairs are sparse, arise from basal layer. Chloroplasts are one per cell, without pyrenoid. Data on measured characters are summarized in Table 10.

Reproductive morphology: Plurangial reproductive structures are uniseriate or biseriate, 42–62 µm long and 3–8 µm wide, intercalary, terminated by a single sterile cell. Sterile cells are 11–16 µm long and 4–7 µm wide, rectangular with rounded apices (Fig. 23E). Unangia are 43–94 µm long and 7–45 µm wide, obovoid to oblong, sessile or on stalks of 1–2 cells, on the terminal part of erect filaments, with associated paraphyses (Fig. 23F). Paraphyses are clavate-shaped, 89–157 µm long and 2–18 µm wide, composed of 10–16 cells. Data on measured characters are summarized in Table 10.

Habitat and Phenology: Plants grow in the intertidal zone where they are found attached on hard substrates such as pebbles and rocks (epilithic) in sheltered to wave-exposed areas. Plurangia and unangia bearing plants were collected in October while those bearing only unangial reproductive structures were collected in August.

Distribution in Korea: East coast (Busan) and West coast (Baekryeongdo and Boryeong) (Fig. 26).

Remarks: *Sungminia asiatica* sp. nov. is newly described in Korea based on detailed morphological studies and molecular analyses. In this study, we established a new family Sungminiaceae with the characteristics of a new genus *Sungminia*. This new species is recognized as a *Sungminia* member by having Irregular epilithic crusts, firmly attached to the substratum, without rhizoids, erect filaments moderately adjoined and partially separates with pressure and with large apical cells, hairs in tufts, chloroplasts one per cell, without pyrenoid, intercalary plurangia composed of one to two reproductive filaments terminated by a sterile cell, unangia sessile or on stalks, on terminal part of erect filaments, with a paraphysis. Diagnostic features delimiting this as a new species in *Sungminia* are irregular crusts, brown to yellowish brown, 0.3–3.3 cm across, 90–611 µm thick, erect filaments sparsely branched, intercalary plurangia uniseriate or biseriate, terminated by a rectangular sterile cell with rounded apices, unangia sessile or on stalks of 1–2 cells, on terminal part of erect filaments, with associated paraphyses.

4.1.2. *Sungminia gladiata* A. O. Oteng'o, B. Y. Won & T. O. Cho sp. nov. (Fig. 24)

Diagnosis: Small irregular epilithic crusts, light to olive brown, without lighter margins, with smooth surface, a few millimeters to 2.3 cm across, 207–456 μm thick, firmly attached to substratum, without rhizoids; hypothallial layer consist 1–2 layers of cells, erect filaments straight, sparsely branched, not tapered, moderately adjoined, partially separate with pressure, with large apical cells; tufts of hairs arise from the basal to mid of erect filaments; chloroplasts one per cell, without pyrenoid; intercalary plurangia uniseriate, terminated by single gladiatus sterile cell; unangia on stalks of 1–2 cells, on terminal part of erect filaments, with associated paraphysis.

Holotype: CUK19619B. Voucher specimen was deposited in the herbarium of Chosun University, Korea (CUK).

Type locality: Seongsan Ilchul Peak, Seongsan-eup, Seogwipo-si, Jeju-do, Korea; 33°27′38.08″N, 126°56′05.73″E; intertidal; collected by T. O. Cho, S. Y. Jeong, J. Avila, A. O. Oteng'o & G. C. Choi, 01 May 2019.

Isotype: CUK19594B, **CUK19600B**, **CUK19631B**. Voucher specimens were deposited in the herbarium of Chosun University, Korea (CUK).

Specimens examined: CUK19816B (Oeyeon-do, Ocheon-myeon, Boryeong-si, Chungcheongnam-do, Korea, collected by T. O. Cho & B. Y. Won, 01 August 2019), CUK20926D (Gyeokpo Port, Byeongsan-myeon, Buan-gun, Jeollabuk-do, Korea, collected by T. O. Cho & B. Y. Won, 09 May 2021).

Etymology: The specific epithet, "*gladiata*" is derived from the Latin word *gladiatus* (shaped like a sword), in reference to the shape of sterile cells of the alga.

Vegetative morphology: Plants are small irregularly expanded epilithic crusts, light to olive brown, without lighter margins, with smooth surface, a few millimeters to 2.3 cm across, 207–

456 µm thick, gelatinous and firmly attached to the substratum, without rhizoids (Fig. 24A). Hypothallial layer consists of 1–2 layers of horizontally elongated cells which are 7 – 17 µm long, 5 – 12 µm wide and give rise to erect filaments. Erect filaments are straight, sparsely branched, not tapered, moderately adjoined (Fig. 24B) and partially separate with pressure (Fig. 24C). Apical vegetative cells are 9–22 µm long and 4–12 µm wide, 1.2–2 times larger than other cells of the erect filaments (Fig. 24D). Tufts of hairs arise from the basal to mid portions of erect filaments. Chloroplasts are one per cell and without obvious pyrenoid. Data on measured characters are summarized in Table 10.

Reproductive morphology: Plurangial reproductive structures are uniseriate, 60–107 µm long and 3–8 µm wide, intercalary, terminated by a single gladiatus (sword-shaped) sterile cell. Sterile cells are 14–25 µm long and 1–8 µm wide (Fig. 24E). Unangia are 41–70 µm long and 7–30 µm wide, oblong to obovoid, on stalks of 1–2 cells, on the terminal part of erect filaments, with associated paraphysis (Fig. 24F). Paraphyses are clavate-shaped, 104–162 µm long and 4–21 µm wide, composed of 12–20 cells. Data on measured characters are summarized in Table 10.

Habitat and Phenology: Plants grow from the high to the low intertidal zone. They are found attached on hard substrates such as rocks and pebbles (epilithic) in sheltered to wave-exposed areas. Plurangia bearing plants were collected in May while those bearing unangial reproductive structures were collected in August.

Distribution in Korea: Jeju Island and West coast (Boryeong and Buan) (Fig. 26).

Remarks: *Sungminia gladiata* sp. nov. is newly described in Korea based on detailed morphology and molecular analyses. In this study, we established a new family Sungminiaceae with the characteristics of a new genus *Sungminia*, of which *Sungminia gladiata* is the type species. This new species, *Sungminia gladiata* is characterized by having small irregular epilithic crusts, light to olive brown, with smooth surface, a few millimeters to 2.3 cm across, 207–456 µm thick, firmly attached to substratum and without rhizoids, hypothallial layer consisting of 1–2 layers of cells, erect filaments straight, sparsely branched, not tapered, moderately adjoined, partially separate with pressure, with large apical cells, tufts of hairs arise from the basal to mid of erect filaments, intercalary plurangia uniseriate, terminated by single gladiatus sterile cell; unangia on stalks of 1–2 cells, on terminal part of erect filaments, with associated paraphysis.

4.1.3. *Sungminia pyriformis* A. O. Oteng'o, B. Y. Won & T. O. Cho sp. nov. (Fig. 25)

Diagnosis: Plants irregular encrusting thalli, light to yellowish brown, without lighter margins, with smooth surface, 0.2–1.3 cm across, 86–392 μm thick, firmly attached to substratum without rhizoids; hypothallial layer composed of one to several cell layers, erect filaments are straight to slightly curved, simple, not tapered, moderately adjoined, partially separate with pressure, with large apical vegetative cells; tufts of hairs arise from basal part of erect filaments; chloroplasts are one per cell, without pyrenoid; intercalary plurangial reproductive structures uniseriate or biseriate, terminated by a pyriform sterile cell; unangia on stalks of 1–2 cells, on terminal part of erect filaments, with associated paraphyses.

Holotype: CUK19694A. Voucher specimen was deposited in the herbarium of Chosun University, Korea (CUK).

Type locality: Saegdal-dong, Seogwipo-si, Jeju-do, Korea; 33°14′28.58″N, 126°23′52.62″E; intertidal; collected by T. O. Cho & B. Y. Won; 06 June 2019.

Isotype: CUK19697B. Voucher specimens were deposited in the herbarium of Chosun University, Korea (CUK).

Specimens examined: CUK18425 (Dala park, Sanyang-eup, Tongyeong-si, Gyeongsangnam-do, Korea, collected by T.O.Cho, S.Y.Jeong, J. Avila A.O. Oteng'o & E.C.Shin, 03 November 2017).

Etymology: The specific epithet, "*pyriformis*" is derived from the Latin words *pyrus* (a pear) and *forma* (shape), in reference to the pear-shaped sterile cells that terminate plurangial reproductive structures.

Vegetative morphology: Plants form irregular crusts, light to yellowish brown, without lighter margins, with smooth surface, 0.2–1.3 cm across, 86–392 µm thick, gelatinous and firmly attached to the substratum without rhizoids (Fig. 25A). Hypothallial layer is composed of one to several horizontally elongated cell layers in which cells are 6–16 µm long, 3–10 µm wide and give rise to erect filaments. Erect filaments are straight to slightly curved, simple, not tapered, moderately adjoined (Fig. 25B) and partially separate with pressure (Fig. 25C). Apical vegetative cells are 12–23 µm long and 5–12 µm wide, 1.8–2.4 times larger than other cells of the erect filaments (Fig. 25D). Tufts of hairs arise from the basal part of erect filaments. Chloroplasts are one per cell and without pyrenoid. Data on measured characters are summarized in Table 10.

Reproductive morphology: Plurangial reproductive structures are uniseriate or biseriate, 31–48 µm long and 5–10 µm wide, intercalary, with a pyriform terminal sterile cell. Sterile cells are 16–26 µm long and 5–15 µm wide (Fig. 25E). Unangia are 80–131 µm long and 7–34 µm wide, obovoid, on stalks of 1–2 cells, on the terminal part of erect filaments, with associated paraphyses (Fig. 25F). Paraphyses are clavate-shaped, 171–268 µm long and 6–26 µm wide, composed of 7–10 cells. Data on measured characters are summarized in Table 10.

Habitat and Phenology: Plants grow from the high to the low intertidal zone. They are found attached on hard substrates such as pebbles or boulders and rocks (epilithic) in sheltered to wave-exposed areas. Plants bearing plurangial and unangial reproductive structures were collected in June while those bearing unangial reproductive structures were collected in November.

Distribution in Korea: Jeju Island and South coast (Tongyeong) (Fig. 26).

Remarks: *Sungminia pyriformis* sp. nov. is newly described from Korea based on detailed morphological studies and molecular analyses. This new species is recognized as a member of *Sungminia* by having irregular epilithic crusts, firmly attached to the substratum, without rhizoids, erect filaments moderately adjoined and partially separates with pressure and with large apical cells, hairs in tufts, chloroplasts one per cell, without pyrenoid, intercalary plurangia composed of one to two reproductive filaments terminated by a sterile cell, unangia sessile or on stalks, on terminal part of erect filaments, with a paraphysis. *Sungminia pyriformis* is mainly distinguished from other Sungminia species by having simple erect filaments, intercalary plurangia composed of one to two reproductive filaments terminated by a pyriform sterile cell, and with largest unangia.

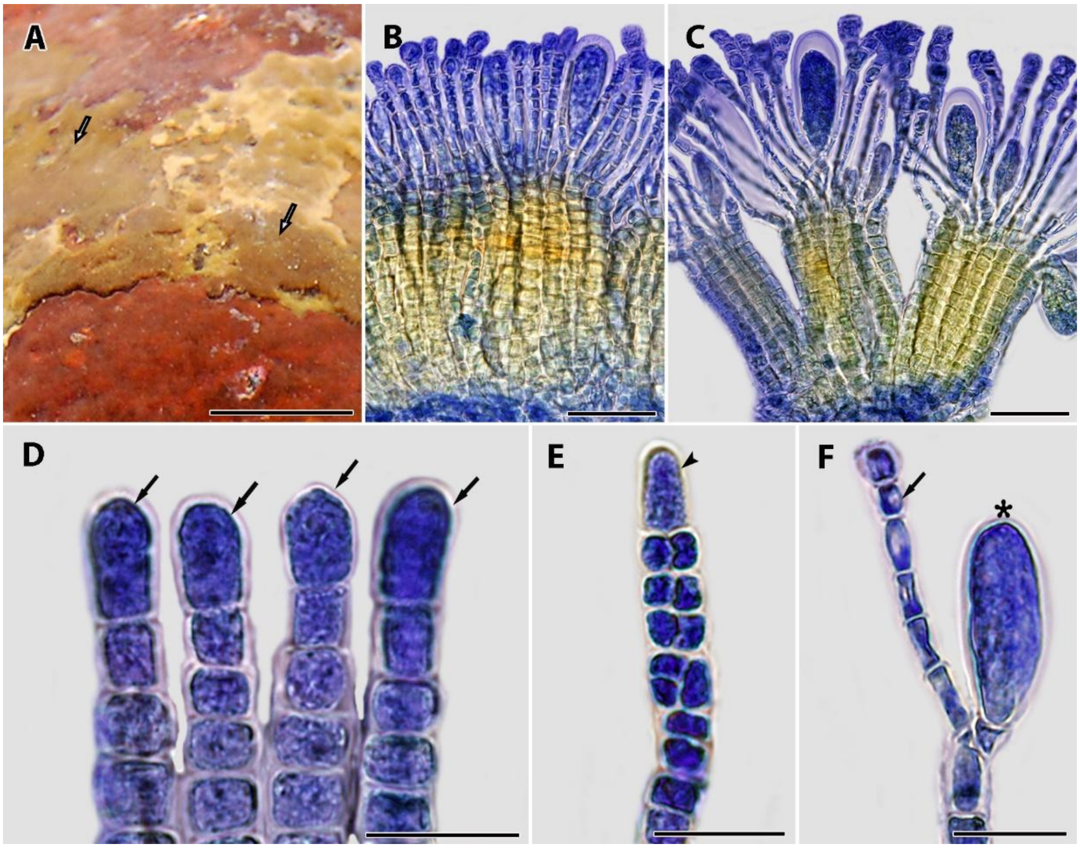


Fig. 23. Morphological characteristics of *Sungminia asiatica* sp. nov.: (A) Irregular yellowish brown crusts (arrows) growing among creeping red algae (CUK19812B). (B) Radial longitudinal section showing laterally adjoined erect filaments (CUK19175C). (C) Erect filaments partially separated with pressure. (D) Apical cells (arrows) of erect filaments are larger than other cells within the filaments (CUK19812B). (E) Biseriate plurangium terminated by a rectangular sterile cell with rounded apex (arrow) (CUK19175C). (F) Unangium (asterisk) on a 1-celled pedicel on the terminal part of erect filaments, with associated paraphysis (arrow) (CUK19175C). Scale bar = 25 µm. Scale bars represent: A, 0.5 cm; B & C, 50 µm; F, 25 µm; D & E, 20 µm.

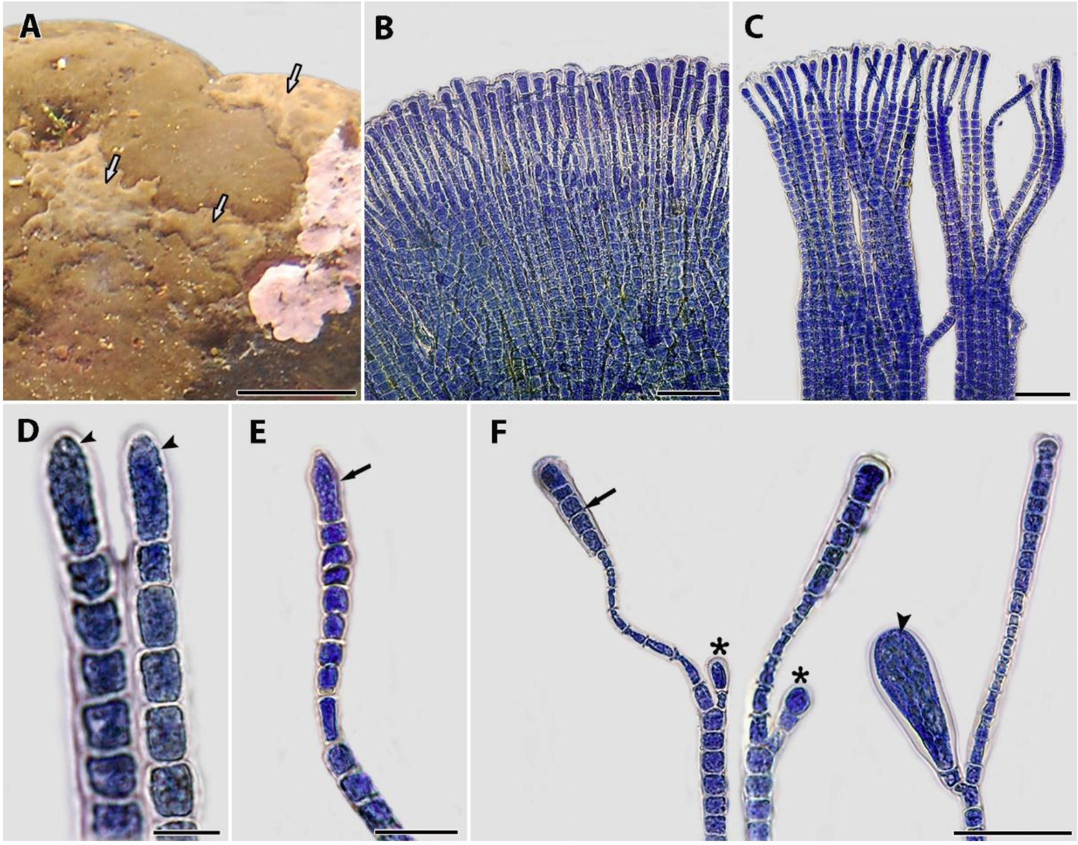


Fig. 24. Morphological characteristics of *Sungminia gladiata* sp. nov.: (A) Plants forming small irregular epilithic olive-brown crusts (arrows) (CUK19816B). (B) Radial longitudinal section showing laterally adjoined erect filaments (CUK19816B). (C) Erect filaments partially separated with pressure. (D) Apical vegetative cells (arrowheads) are larger than other cells of erect filaments (CUK19631B). (E) Uniseriate plurangium terminated by a gladiatus sterile cell (arrow) (CUK19600B). (F) Young uniangia (asterisks) on pedicel of 1-2 cells on the terminal part of erect filaments, with associated paraphysis (arrow), and obovate mature unangium (arrowhead) (CUK19816B). Scale bars represent: A, 1 cm; B, C & F, 50 µm; D & E, 20 µm.

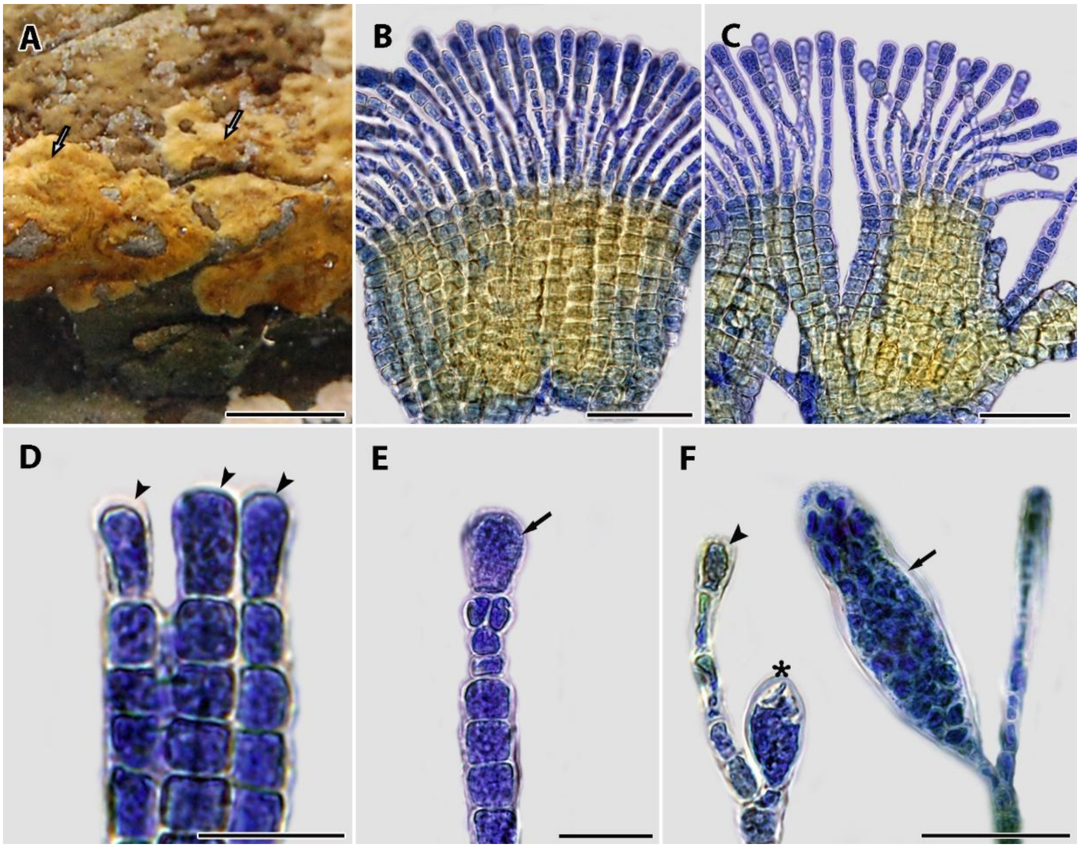


Fig. 25. Morphological characteristics of *Sungminia pyriformis* sp. nov.: (A) Yellowish brown epilithic crusts (arrows) (CUK19694A). (B) Radial longitudinal section showing laterally adjoined erect filaments (CUK18425). (C) Erect filaments partially separated with pressure. (D) Larger apical cells (arrowheads) of erect filaments (CUK19694A). (E) Plurangium terminated by a pyriform sterile cell (arrow) (CUK19697B). (F) Young unangium (asterisks) on a 1-celled pedicel on the terminal of erect filaments, with associated paraphysis (arrowhead), and mature unangium (arrow) about to release its content (CUK19694A). Scale bars represent: A, 0.5 cm; B, C & F, 50 µm; D, 25 µm; E, 10 µm.

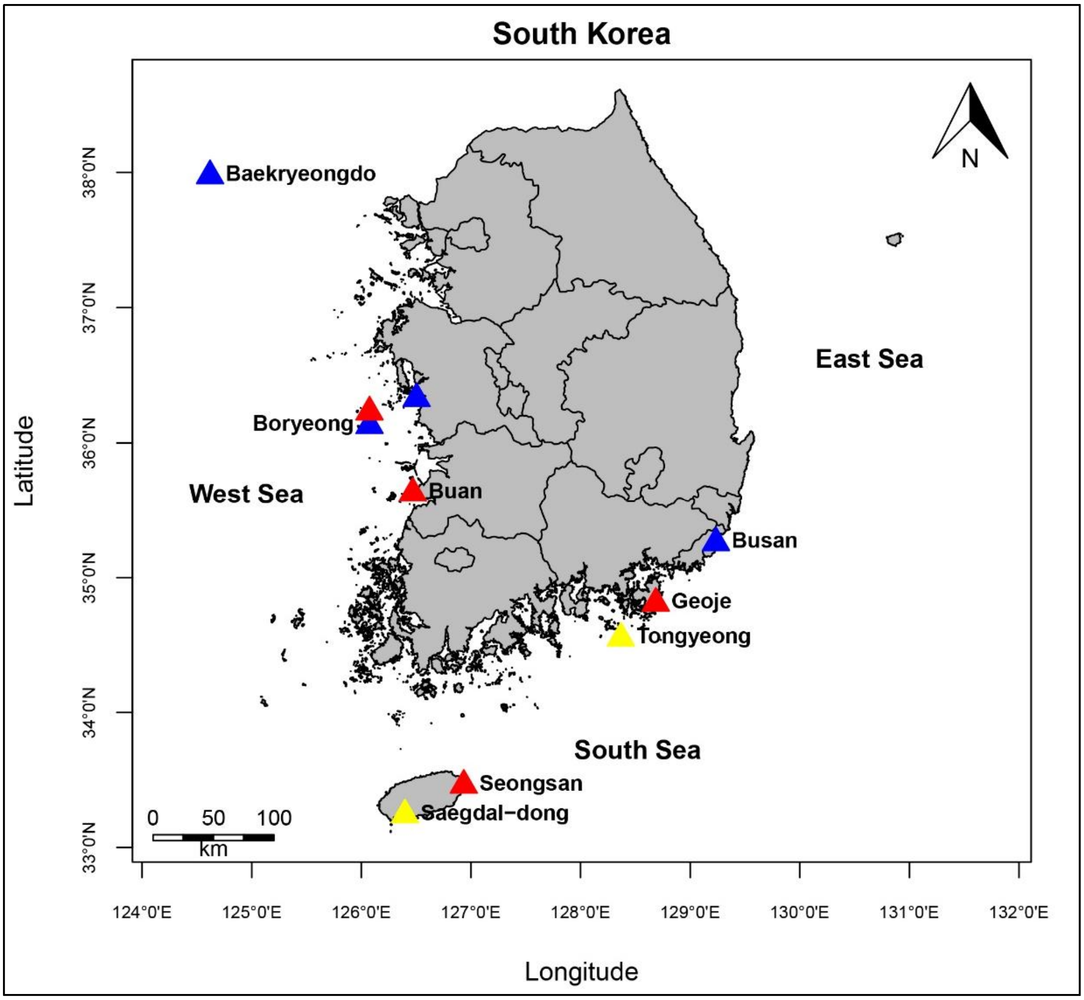


Fig. 26. Distribution of *Sungminia* species in Korea. *Sungminia asiatica* sp. nov. (blue), *Sungminia gladiata* sp. nov. (red), *Sungminia pyriformis* sp. nov. (yellow)..

Table 9. Morphological comparison between six families in the order Ralfsiales.

Characters	**Hapalospongidiaceae**	**Mesosporaceae**	**Neoralfsiaceae**	**Pseudoralfsiaceae**	**Ralfsiaceae**	**Sungminiaceae**
Life history	Isomorphic	Isomorphic	Isomorphic	Isomorphic	Isomorphic and Heteromorphic	Isomorphic
Thallus construction	Erect filaments free, straight, tapering downwards	Erect filaments free, straight, tapering downwards	Erect filaments firmly adhering, unilateral to bilaterally symmetrical, medullary filaments tapering downwards	Erect filaments firmly adhering, typically unsymmetrical, tapering upwards	Erect filaments firmly adhering, straight or unilateral or bilaterally symmetrical, tapering upwards	Erect filaments laterally adhering and partially separate with pressure, straight, not tapered
Superimposed thalli	Absent	Absent	Absent	Present or absent	Present or absent	Absent

Table 9. (continued)

Characters	**Hapalospongidiaceae**	**Mesosporaceae**	**Neoralfsiaceae**	**Pseudoralfsiaceae**	**Ralfsiaceae**	**Sungminiaceae**
Size of apical cells of erect filaments	Same size as subapical cells and larger than cells below them	Same size as subapical cells and larger than cells below them	Smaller than cells below them	Smaller than cells below them	Smaller than cells below them	Larger than cells below them
Rhizoids	Absent	Absent	Present or absent	Present or absent	Present or absent	Absent
Cortex & medulla	Absent	Absent	Present	Absent	Present or absent	Absent
Plastids per cell	1 to several	1	1	1	1 or several	1
Hairs	Present	Present	Present	Present (numerous)	Present or absent	Present
Plurangia	Intercalary	Intercalary	Intercalary	Intercalary	Intercalary	Intercalary

Table 9. (continued)

Characters	**Hapalospongidiaceae**	**Mesosporaceae**	**Neoralfsiaceae**	**Pseudoralfsiaceae**	**Ralfsiaceae**	**Sungminiaceae**
No. of sterile cells	1–7	2–3	1	1–2	1 or several	1
Unangial position	Terminal on erect filament	Terminal on stalk inserted laterally on erect filament	Terminal on stalk inserted terminally on erect filament	Terminal on stalk inserted terminally on erect filament	Terminal on stalk inserted terminally on erect filament	Terminal on stalk inserted terminally on erect filament
Paraphyses (No./unangium)	Absent	Absent	Present (1)	Present (1–2)	Present (1–2)	Present (1)
Reference	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, Oteng'o et al. 2021	Oteng'o et al. unpublished

Table 10. Morphological comparison between *Sungminia* species.

Characters	***S. asiatica***	***S. gladiata***	***S. pyriformis***
Substrate	Epilithic	Epilithic	Epilithic
Crust color	Brown, yellowish-brown to yellow	Light to olive-brown	Light to yellowish-brown
Margins	Not lighter, not darker	Not lighter, not darker	Not lighter, not darker
Crust outline	Irregular	Irregular	Irregular
External growth lines or ridges	Absent	Absent	Absent
Surface	Smooth	Smooth	Smooth
Diameter of thalli	0.3–3.3 cm	Up to 2.3 cm	0.2–1.3 cm
Thickness of thalli	90–611 µm	207–456 µm	86–243 (392) µm
Basal cell layer	One	One to two	One to several
Basal cell length	3–12 µm	5–12 µm	3–10 µm

Table 10. (continued)

Characters	***S. asiatica***	***S. gladiata***	***S. pyriformis***
Basal cell width	5–16 µm	7–17 µm	6–16 µm
Rhizoids	Absent	Absent	Absent
Organization and symmetry / asymmetry of erect filaments	Straight to slightly curved upwards, laterally adhering and partially separate with pressure	Straight to slightly curved upwards, laterally adhering and partially separate with pressure	Straight to slightly curved upwards, laterally adhering and partially separate with pressure
Erect filament tapering	Not tapered	Not tapered	Not tapered
Erect filaments branching	Sparsely branched	Sparsely branched	Not branched (simple)
Erect filament cell length	4–12 µm	4–12 µm	5–12 µm
Erect filament cell width	5–12 µm	5–12 µm	7–12 µm

Table 10. (continued)

Characters	***S. asiatica***	***S. gladiata***	***S. pyriformis***
Apical cell length	10–22 µm	9–22 µm	12–23 µm
Apical cell width	4–10 µm	4–12 µm	5–12 µm
Chloroplasts / cell	1	1	1
Phaeophycean hairs	Present	Present	Present
Origin of hairs	Basal disc	Basal to mid part of erect filament	Lower part of erect filament
Plurangia and unangia sori	On different thalli	On different thalli	On different thalli
Length of plurangia	42–62 µm	60–107 µm	31–48 µm
Plurangial filaments	1–2	1	1–2
No. of sterile cells	1	1	1
Sterile cell length	11–16 µm	14–25 µm	31–48 µm

Table 10. (continued)

Characters	***S. asiatica***	***S. gladiata***	***S. pyriformis***
Sterile cell width	4–7 µm	1–8 µm	5–10 µm
Sterile cell shape	Rectangular with rounded apices	Gladiatus (shaped like roman sword)	Pyriform (pear-shaped)
Unangial shape	Oblong to obovoid	Oblong to obovoid	Obovoid
Unangial length	43–94 µm	41–70 µm	80–131 µm
Unangial width	7–45 µm	7–30 µm	7–34 µm
Unangial position / No. of stalk cells	Terminal / 0–2	Terminal / 1–2	Terminal / 1–2
No. of paraphyses per unangium	1	1	1
Paraphysis length	89–157 µm	104 – 162 µm	171–268 µm
Paraphysis width	2–18 µm	4 – 21 µm	6–26 µm

Table 10. (continued)

Characters	***S. asiatica***	***S. gladiata***	***S. pyriformis***
No. of cells in paraphysis	10–16	12–20	7–10
Soral filaments	Absent	Absent	Absent
Reference	Oteng’o et al. unpublished	Oteng’o et al. unpublished	Oteng’o et al. unpublished

PART 2. PHYLOGENETIC RELATIONSHIP AMONG SPECIES IN THE ORDER RALFSIALES

1. Phylogenetic analyses based on *rbc*L, COI-5P and concatenated *rbc*L and COI-5P gene sequence data

1.1. Family Mesosporaceae J. Tanaka & Chihara 1982

1.1.1. Genus *Mesospora* Weber Bosse 1911

I sequenced a 662–bp portion of COI-5P and a 1315–bp portion of *rbc*L for Ralfsiales species from Korea. In addition, sequences of other recognized species belonging to the order Ralfsiales were downloaded from GenBank (Table 1). Phylogenetic trees based on ML (in MEGA and RAxML) and Bayesian inference (BI) for COI-5P, and ML (in RAxML) and BI for *rbc*L and concatenated alignement of *rbc*L and COI-5P sequence data were obtained with *Syringoderma abyssicola* (Setchell and N.L. Gardner) Levring and *Tilopteris mertensii* (Turner) Kützing selected as out group taxa. The phylogenetic trees obtained from the ML analyses of COI-5P sequence data were largely congruent and both resolved a section of our Korean *Mesospora* samples into a distinct and well supported clade in the genus *Mesospora*, Mesosporaceae (Fig. 27). COI-5P sequence data analyses using MrBayes gave a phylogenetic tree (not shown) with a slightly different topology with our Korean *Mesospora* samples nested in a clade sister to *Sungminia* and outside the genus *Mesospora*. Both phylogenetic trees obtained from RAxML and MrBayes analyses of *rbc*L sequence data were congruent and resolved the Korean *Mesospora* samples in a distinct and well supported clade in genus *Mesospora*, family Mesosporaceae (Fig. 28). Although the phylogenetic tree obtained from MrBayes analysis of the concatenated alignment of *rbc*L and COI-5P sequence data had collapsed branch between Korean *Mesospora* samples, the Mesosporaceae and Sungminiaceae (Fig 29), the tree obtained from RAxML (not shown) resovled Korean *Mesospora* samples in a well supported clade within the Mesosporaceae. Sequence divergence values between our Korean *Mesospora* samples and other *Mesospora* species is 20.4–23.8% for COI-5P and 10.4–12.4% for *rbc*L gene (Tables 11 & 12 respectively). The genetic divergence are sufficient to warrant recognition of Korean *Mesospora* samples as a new species. Therefore, the samples are tentatively recognized as a new species, *Mesospora bifurcata* sp. nov., within the *Mesospora* mainly based morphology, gene sequence divergence and largely the *rbc*L phylogeny.

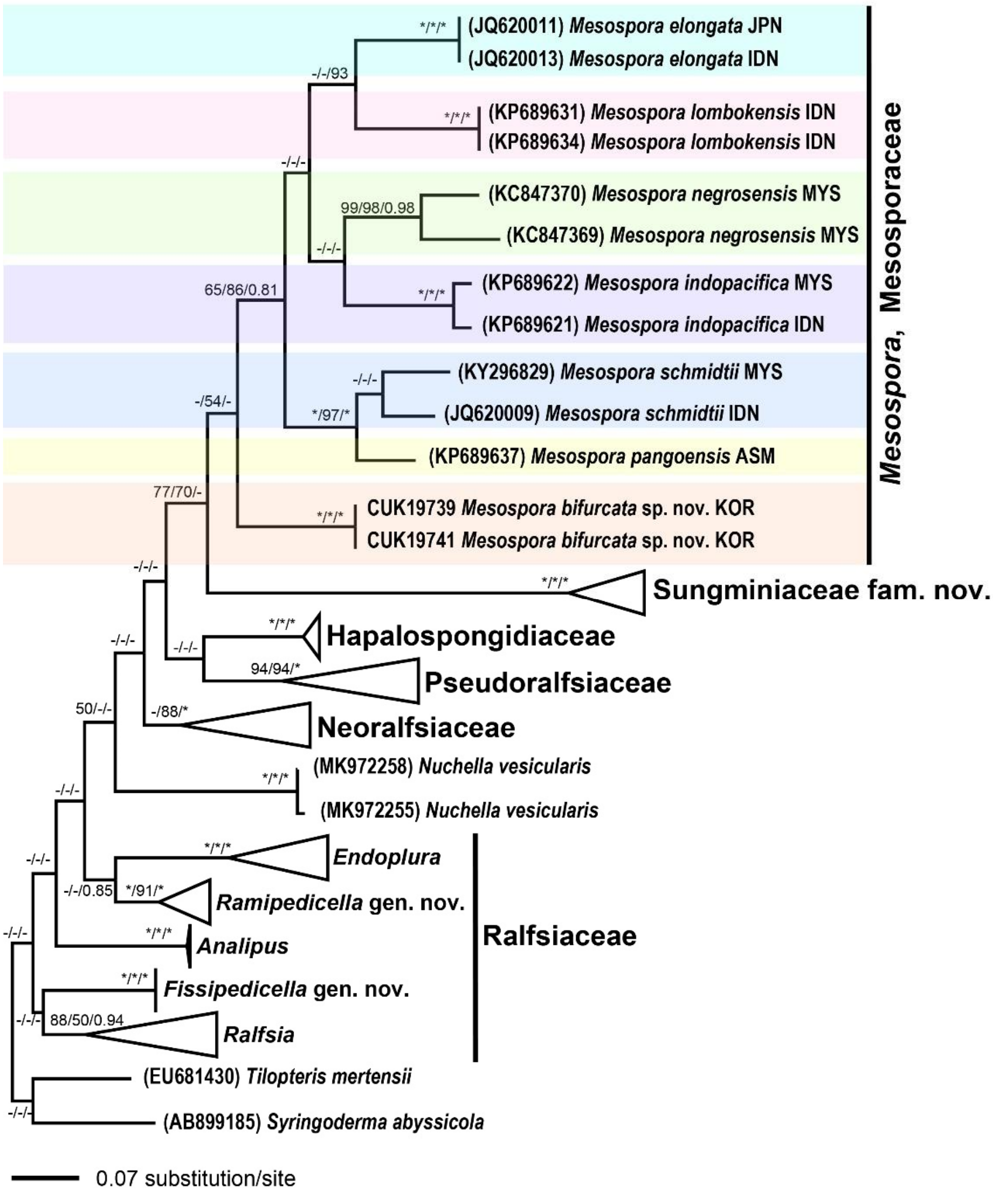


Fig. 27. Phylogenetic tree of COI-5P sequence data from *Mesospora bifurcata* and other Ralfsiales species. A phylogenetic analysis was performed using MEGA X, RAxMLGUI v1.5 and MrBayes 3.2.6 methods for Maximum likelihood (ML) analysis (for first two methods) and Bayesian inference. The first two values above branches = ML bootstrap (BS) values in % >50, and the last value = bayesian posterior probability (BPP) >0.75. Values lower than BS 50 or BPP 0.75 are indicated by hyphens (-). Values of bootstrap (BS) 100 or BPP 1.00 are indicated by asterisks (*). Branch lengths are proportional to substitution rate. (ASM, American Samoa; IDN, Indonesia; JPN, Japan; KOR, Korea; MYS, Malaysia).

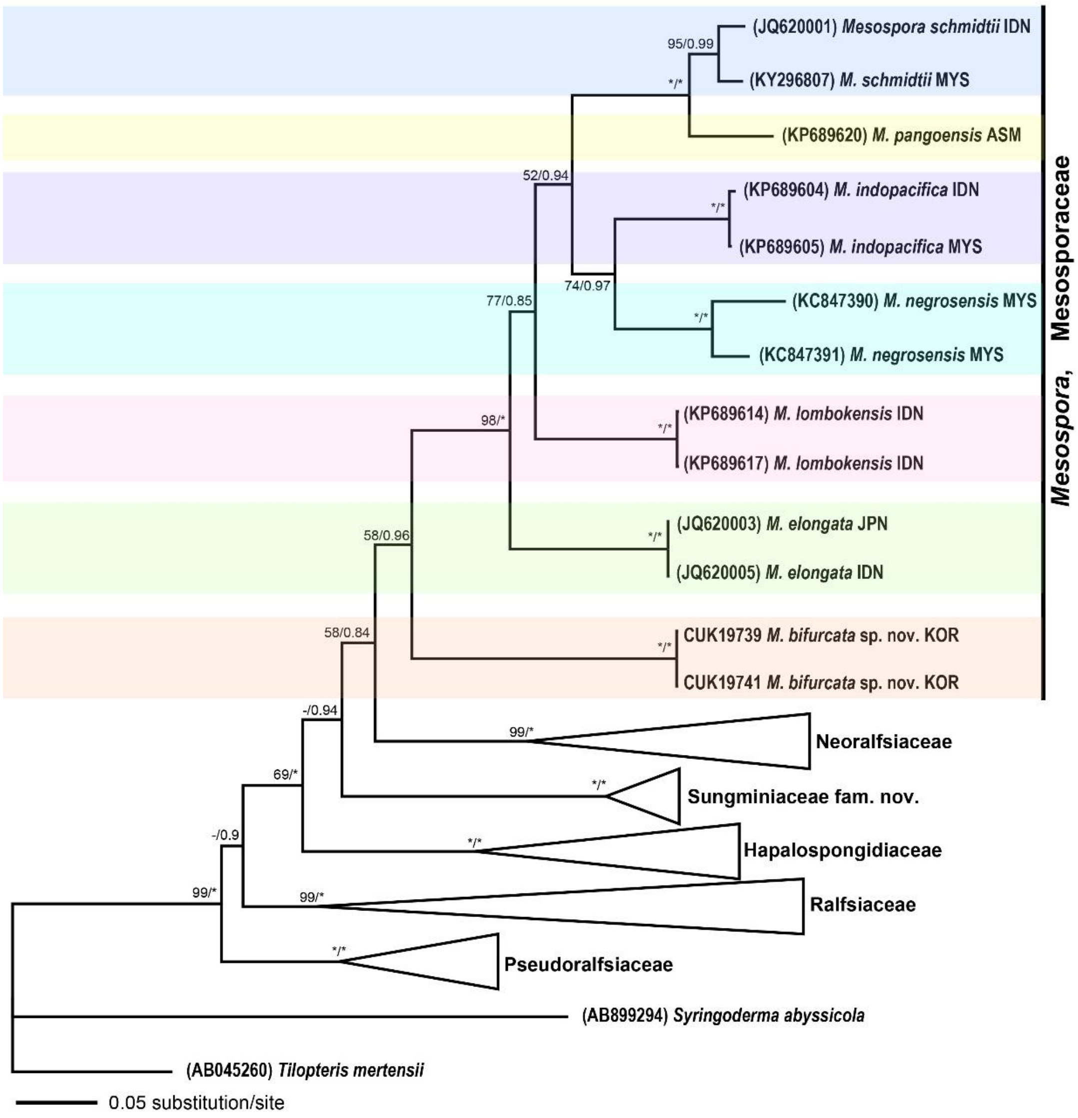


Fig. 28. Phylogenetic tree of *rbc*L gene sequence data from *Mesospora bifurcata* and other Ralfsiales species. A phylogenetic analysis was performed using RAxMLGUI v1.5 and MrBayes 3.2.6 methods for Maximum likelihood (ML) analysis and Bayesian inference. The value above branches = maximum likelihood 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 bootstrap (BS) 100 or BPP 1.00 are indicated by asterisks (*). Branch lengths are proportional to substitution rate. (ASM, American Samoa; IDN, Indonesia; JPN, Japan; KOR, Korea; MYS, Malaysia).

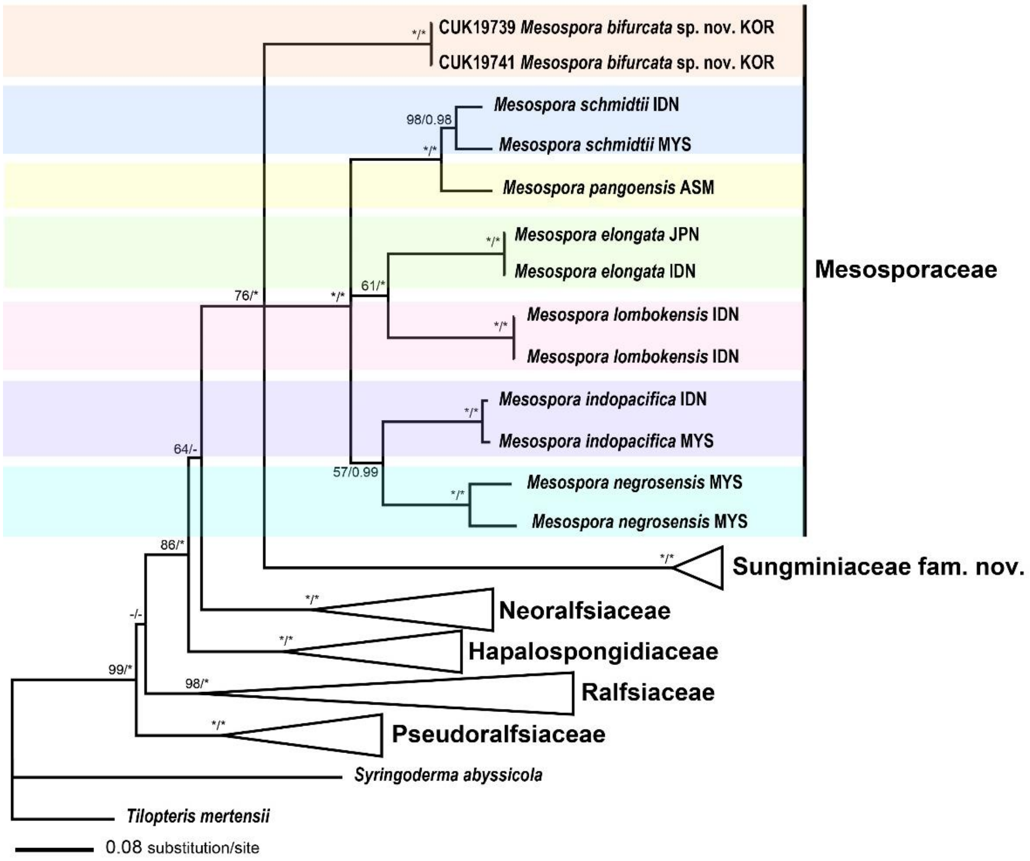


Fig. 29. Phylogenetic tree of concatenated *rbc*L and COI-5P sequence data from *Mesospora bifurcata* and other Ralfsiales species. A phylogenetic analysis was performed using RAxMLGUI v1.5 and MrBayes 3.2.6 methods for Maximum likelihood (ML) analysis and Bayesian inference. The value above branches = maximum likelihood 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 bootstrap (BS) 100 or BPP 1.00 are indicated by asterisks (*). Branch lengths are proportional to substitution rate. (ASM, American Samoa; IDN, Indonesia; JPN, Japan; KOR, Korea; MYS, Malaysia).

Table 11. Gene sequence divergences (%) between *Mesospora* species using COI-5P sequence data.

	M. bifurcata	***M. elongata***	***M. indopacifica***	***M. lombokensis***	***M. negrosensis***	***M. pangoensis***	***M. schmidtii***
M. bifurcata	–						
M. elongata	21.9-22.0	–					
M. indopacifica	23.0-23.2	20.9-21.1	–				
M. lombokensis	23.8	19.0-19.2	19.6-19.8	–			
M. negrosensis	21.7-22.5	20.4-21.7	20.6-22.2	22.8-23.3	–		
M. pangoensis	22.2	18.6-18.7	21.0	21.8	19.8-21.4	–	
M. schmidtii	20.4-20.8	19.6-21.7	19.6-22.5	21.2-23.0	20.8-22.5	11.7-14.2	–

Table 12. Gene sequence divergences (%) between *Mesospora* species using *rbc*L sequence data.

	M. bifurcata	***M. elongata***	***M. indopacifica***	***M. lombokensis***	***M. negrosensis***	***M. pangoensis***	***M. schmidtii***
M. bifurcata	–						
M. elongata	11.7	–					
M. indopacifica	11.5-11.7	8.7	–				
M. lombokensis	10.4	7.8	8.3	–			
M. negrosensis	11.6-12.2	8.6-9.1	5.6-7.2	7.7-8.0	–		
M. pangoensis	11.4	10.5	8.1-8.4	9.8	8.5-9.8	–	
M. schmidtii	12-12.4	9.1-9.8	7.4-7.8	8.6	8.2-9.2	4.1-4.3	–

1.2. Family Neoralfsiaceae P.-E. Lim & H. Kawai 2007

1.2.1. Genus *Neoralfsia* P.-E. Lim & H. Kawai 2007

León-Álvarez et al. (2014b) molecular analyses based on *rbc*L led to the conclusion that Asian *N. expansa* specimens were a different genus and species in family Neoralfsiaceae yet to be described. I analyzed the *rbc*L gene sequences of *N. expansa* from the type locality, Mexico (KM032758 and KM032760), and from Japan (AB250079 and AB250083), and those of *N. hancockii* (KF977827 and KF977828). BLAST results for the front segments (1–573 bp) of *N. expansa* from Mexico and *N. hancockii* sequences showed 96.16% identity with *Asterocladon lobatum* D. G. Müller, E. R. Parodi and A. F. Peters and 99.65% identity with *Feldmannia chitonicola* (De A. Saunders) Levring, respectively. This prompted me to use only the back segments (574–1278 bp) of the *rbc*L gene loci which showed more similarity with sequences of other taxa in order Ralfsiales. I sequenced a 662–bp portion of COI-5P and a 1315–bp portion of *rbc*L for Ralfsiales species from Korea. In addition, sequences of other recognized species belonging to the order Ralfsiales were downloaded from GenBank (Table 1). Phylogenetic trees based on ML (in MEGA and RAxML) and Bayesian inference (BI) for COI-5P, and ML (in RAxML) and BI for *rbc*L and concatenated alignement of *rbc*L and COI-5P sequence data were obtained with *Syringoderma abyssicola* (Setchell and N.L. Gardner) Levring and *Tilopteris mertensii* (Turner) Kützing as out group taxa. All the three phylogenetic trees obtained from the ML and BI analyses of COI-5P sequence data were largely congruent and resolved the Korean *N. expansa* samples into a distinct and well supported clade as genus *Neoralfsia* in the family Neoralfsiaceae (Fig. 30). In the *rbc*L Phylogeny based on BI analysis, *N. expansa* was nested in an unsupported clade within which two lineages were accommodated (Figs 31). Korean and Japanese *N. expansa* were nested in lineage A, while the Mexican *N. expansa* nested in lineage B. In the ML phylogenetic tree based on *rbc*L sequence data (not shown), there are two distinct and well supported clades, one with Asia *N. expansa* and another one with the Mexican *Neoralfsia* (both Mexican *N. expansa* and *N. hancockii*). The phylogeny based on both BI and ML for concatenated alignment were largely congruent to their *rbc*L counter parts (Fig. 32). The gene sequence divergence values for *rbc*L between Korean and Japanese *N. expansa*, Korean and Mexican *N. expansa*, and Korean *N. expansa* and *N. hancockii* were 0.1–0.4%, 1.4–1.7% and 1.7–1.9%, respectively (Table 13). León-Álvarez et al. (2014b) suggested that Asian specimens of *N. expansa* were a distinct genus and species yet to be described, but our results revealed insufficient *rbc*L sequence divergence to warrant delimitation of Asian *N. expansa* as a new genus and species. I tentatively maintain them as *N. expansa* until more studies including type specimen or authentic specimens of *N. expansa* from type locality are included in the analyses to make a substantive conclusion whether the two lineages are two independent species or two subspecies. This is because *N. expansa* clade has no support and formed two distinct lineages (A & B) in the BI phylogeny. Morphologically, Asian *N. expansa* (lineage A) is characterized by crusts mostly with a bilateral symmetry and unangia either sessile or on stalks composed of 1–3 cells as observed in this study, or unangia on stalks having 3–6 cells as observed by Tanaka and Chihara (1980b). The Mexican *N. expansa* (lineage B) is characterized by crusts mainly with a unilateral symmetry and unangia either sessile or on 1-celled stalks (León-Álvarez and González-González 2003). Although *N. hancockii* is also characterized by crusts mainly with unilateral symmetry, it differs from Mexican *N. expansa* by having unangia on 2–6-celled stalks (Dawson 1944, León-Álvarez and González-González 2003, León-Álvarez et al. 2014a). The number of cells in unangial stalk seems to form an overlap in the *Neoralfsia*, therefore in my view, molecular data is crusial in separating or identifying the species in this genus.

1.2.2. Genus *Neoralfsiopsis* A. O. Oteng'o, B. Y. Won & T. O. Cho gen. nov.

I sequenced a 662–bp portion of COI-5P and a 1315–bp portion of *rbc*L for Ralfsiales species from Korea. In addition, sequences of other recognized species belonging to the order Ralfsiales were downloaded from GenBank (Table 1). Phylogenetic trees based on ML (in MEGA and RAxML) and Bayesian inference (BI) for COI-5P, and ML (in RAxML) and BI for *rbc*L and concatenated alignement of *rbc*L and COI-5P sequence data were obtained. *Syringoderma abyssicola* (Setchell and N.L. Gardner) Levring and *Tilopteris mertensii* (Turner) Kützing were selected as out group taxa. The COI-5P phylogenetic trees obtained from all the three analytical methods were largely congruent with all resolving a section of our unidentified Korean Neoralfsiacean samples into a distinct and well supported clade in Neoralfsiaceae but outside the genus *Neoralfsia* (Fig. 30). The phylogenies of *rbc*L and concatenated alignments of *rbc*L and COI-5P for both ML and BI analyses were largely congruent with the unidentified Neoralfsiacean samples nested in distinct and well supported clade in the family Neoralfsiaceae (Figs 31 & 32). Sequence divergence values between the unidentified Neoralfsiacean specimens and genus *Neoralfsia* were 17.9% for COI-5P and 9.4–10.8% for *rbc*L (Tables 14 & 15 respectively). The phylogeny and genetic divergence values are sufficient to warrant recognition of the Neoralfsiacean specimens as a new genus and species, *Neoralfsiopsis jejuensis* gen. *et* sp. nov., within the family Neoralfsiaceae.

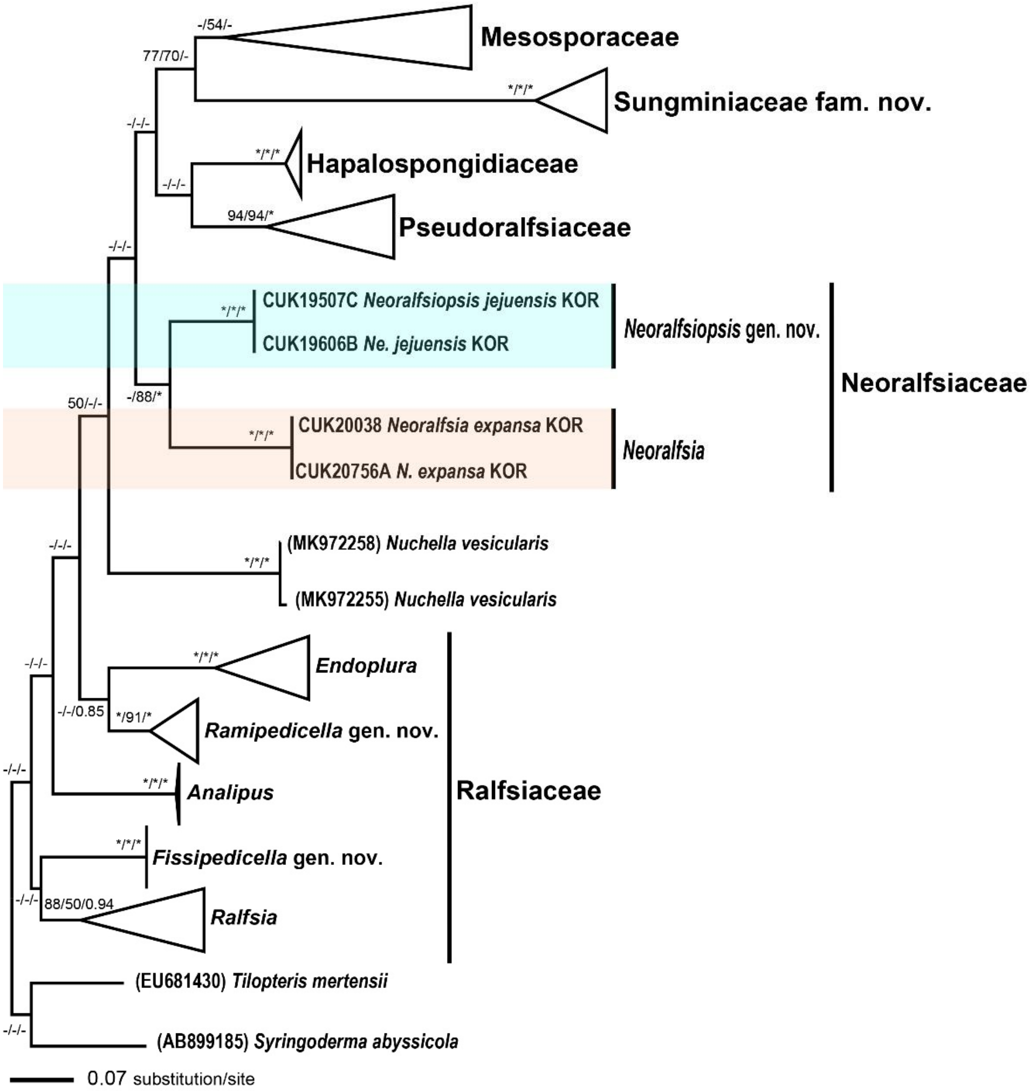


Fig. 30. Phylogenetic tree of COI-5P sequence data from the Neoralfsiaceae and other Ralfsiales species. A phylogenetic analysis was performed using MEGA X, RAxMLGUI v1.5 and MrBayes 3.2.6 methods for Maximum likelihood (ML) analysis (for first two methods) and Bayesian inference. The first two values above branches = ML bootstrap (BS) values in % >50, and the last value = bayesian posterior probability (BPP) >0.75. Values lower than BS 50 or BPP 0.75 are indicated by hyphens (-). Values of bootstrap (BS) 100 or BPP 1.00 are indicated by asterisks (*). Branch lengths are proportional to substitution rate. (KOR, Korea).

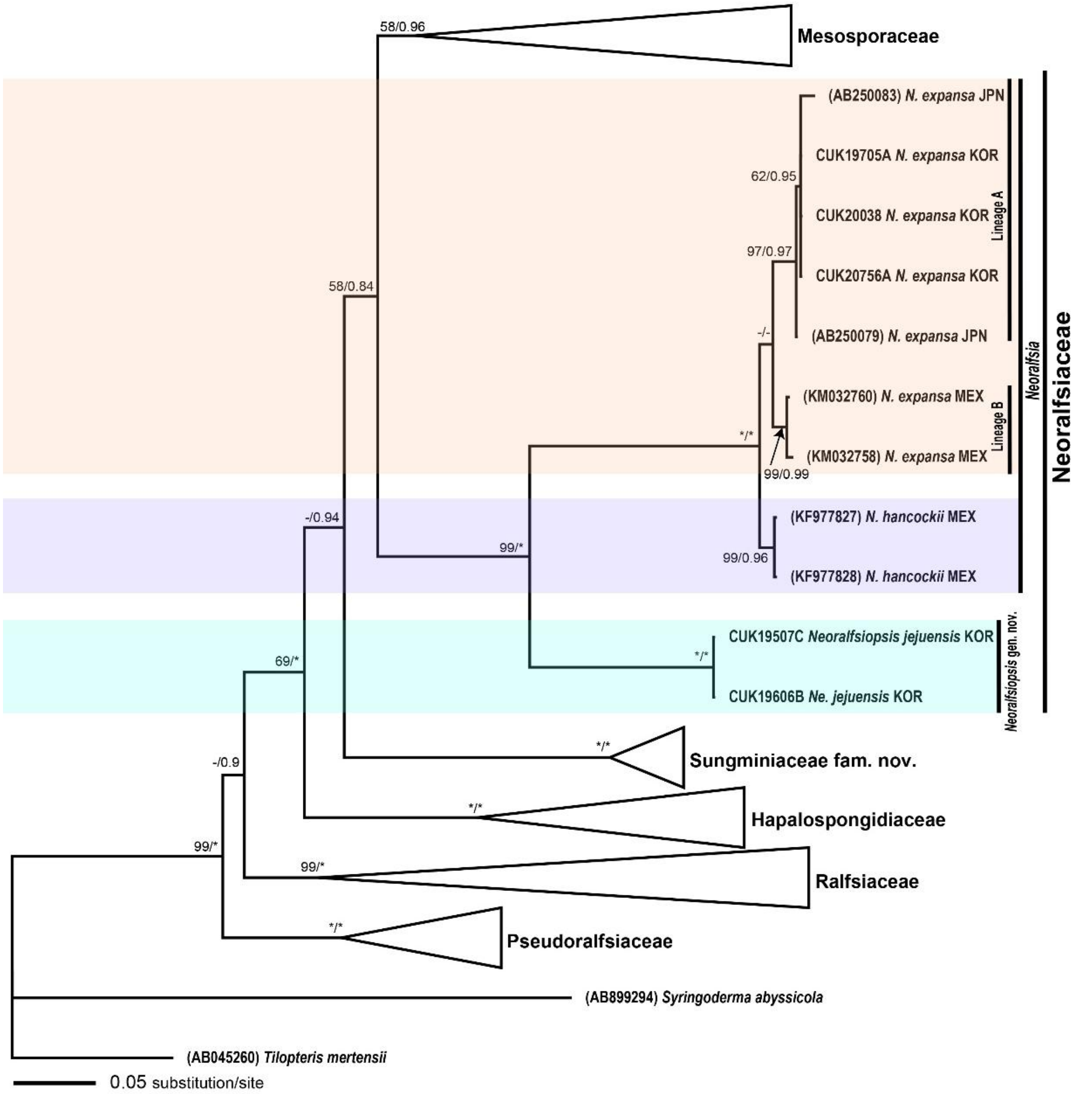


Fig. 31. Phylogenetic tree of *rbc*L gene sequence data from the Neoralfsiaceae and other Ralfsiales species. A phylogenetic analysis was performed using RAxMLGUI v1.5 and MrBayes 3.2.6 methods for Maximum likelihood (ML) analysis and Bayesian inference. The value above branches = maximum likelihood 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 bootstrap (BS) 100 or BPP 1.00 are indicated by asterisks (*). Branch lengths are proportional to substitution rate. (JPN, Japan; KOR, Korea; MEX, Mexico).

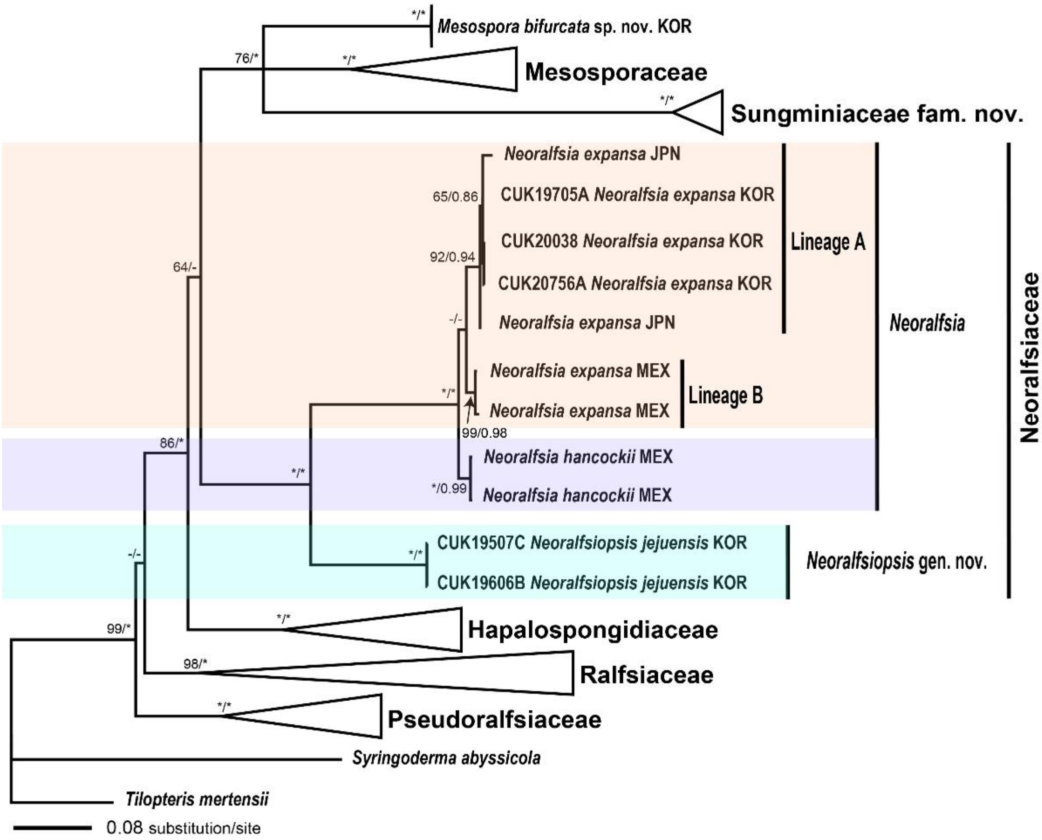


Fig. 32. Phylogenetic tree of concatenated *rbc*L and COI-5P sequence data from the Neoralfsiaceae and other Ralfsiales species. A phylogenetic analysis was performed using RAxMLGUI v1.5 and MrBayes 3.2.6 methods for Maximum likelihood (ML) analysis and Bayesian inference. The value above branches = maximum likelihood 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 bootstrap (BS) 100 or BPP 1.00 are indicated by asterisks (*). Branch lengths are proportional to substitution rate. (JPN, Japan; KOR, Korea; MEX, Mexico).

Table 13. Gene sequence divergences (%) between *Neoralfsia* species using *rbc*L sequence data.

	***N. expansa* JPN**	***N. expansa* KOR**	***N. expansa* MEX**	***N. hancockii* MEX**
***N. expansa* JPN**	0.5			
***N. expansa* KOR**	0.1-0.4	–		
***N. expansa* MEX**	1.4-1.7	1.5-1.7	0.2	
***N. hancockii* MEX**	1.7-1.9	1.9	1.4-1.5	–

(JPN, Japan; KOR, Korea; MEX, Mexico).

Table 14. Gene sequence divergences (%) between genera of Neoralfsiaceae using COI-5P sequence data.

	Neoralfsia	***Neoralfsiopsis***
Neoralfsia	–	
Neoralfsiopsis	17.9	–

Table 15. Gene sequence divergences (%) between genera of Neoralfsiaceae using *rbc*L sequence data.

	Neoralfsia	***Neoralfsiopsis***
Neoralfsia	–	
Neoralfsiopsis	9.4-10.8	–

1.3. Family Ralfsiaceae W. G. Farlow 1881

1.3.1. Genus *Endoplura* Hollenberg 1969

A 662–bp portion of COI-5P and a 1315–bp portion of *rbc*L for Ralfsiales species from Korea were sequenced. In addition, sequences of other recognized species belonging to this order were downloaded from GenBank (Table 1). Phylogenetic trees based on ML (in MEGA and RAxML) and Bayesian inference (BI) for COI-5P, and ML (in RAxML) and BI for *rbc*L and concatenated alignement of *rbc*L and COI-5P sequence data were obtained. *Syringoderma abyssicola* (Setchell and N.L. Gardner) Levring and *Tilopteris mertensii* (Turner) Kützing were selected as out group taxa. The COI-5P phylogenetic trees obtained from all the three analytical methods were largely congruent with all resolving Korean *Endoplura* specimens into a distinct and well supported clade with five lineages nested in the Ralfsiaceae (Fig. 33). The phylogenies of *rbc*L and concatenated alignments of *rbc*L and COI-5P sequence data for both ML and BI analyses were largely congruent with the Korean *Endoplura* specimens nested also in a distinct and well supported clade with five lineages within the family Ralfsiaceae (Figs 34 & 35). The lineages were distinct from one another as well as from the Japanese "*E. aurea*". Sequence divergences between *Endoplura* species ranged from 9.4% to 13.6% for COI-5P and 2.0% to 9.7% for *rbc*L gene (Tables 16 & 17 respectively). The molecular phylogeny and genetic divergence values present sufficient evidence to warrant recognition of the five *Endoplura* lineages as five new species, *Endoplura geojensis* sp. nov., *E. gyeokpoensis* sp. nov., *E. jejuensis* sp. nov., *E. koreana* sp. nov. and *E. limpeticola* sp. nov.

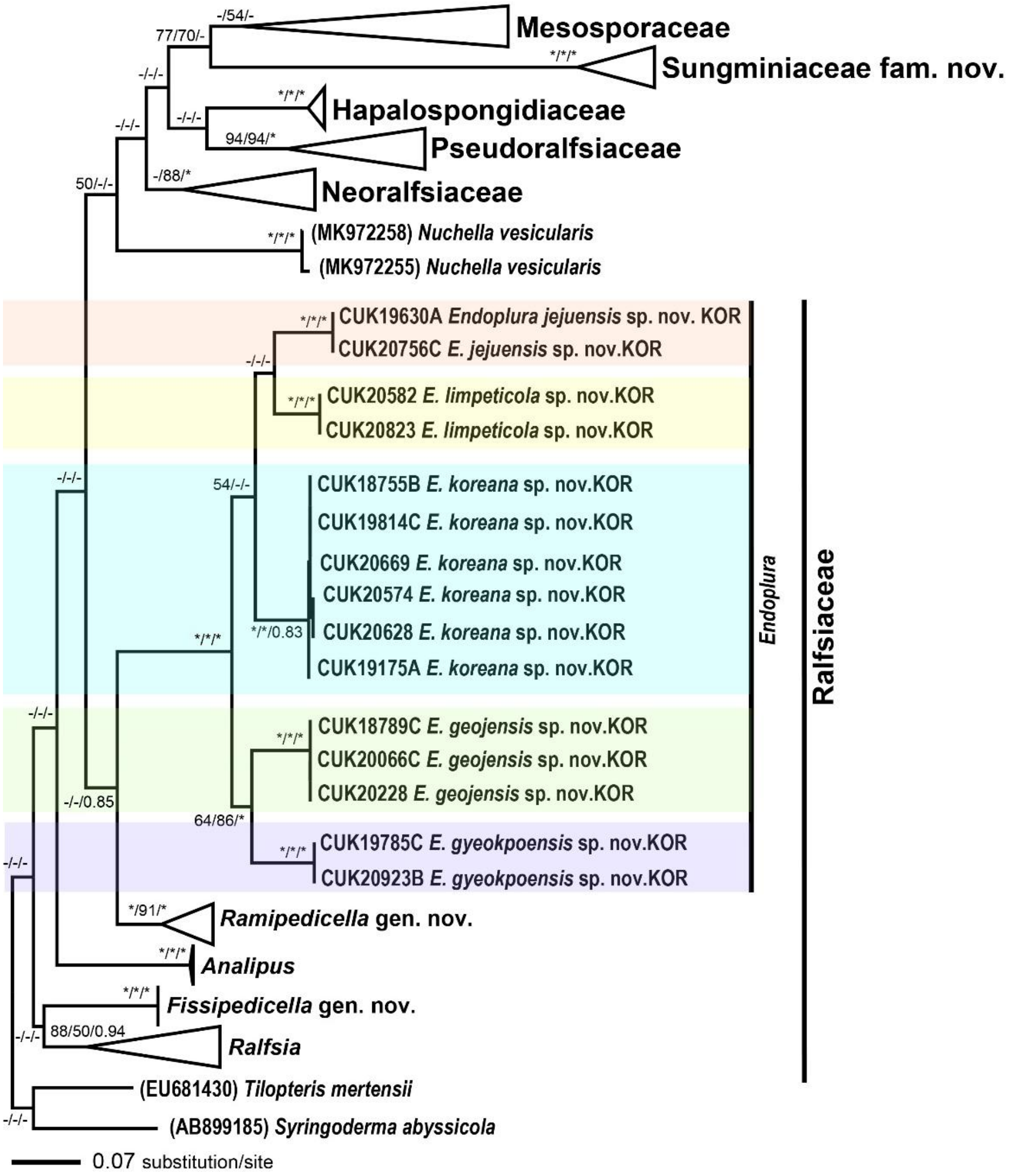


Fig. 33. Phylogenetic tree of COI-5P sequence data from the *Endoplura* and other Ralfsiales species. A phylogenetic analysis was performed using MEGA X, RAxMLGUI v1.5 and MrBayes 3.2.6 methods for Maximum likelihood (ML) analysis (for first two methods) and Bayesian inference. The first two values above branches = ML bootstrap (BS) values in % >50, and the last value = bayesian posterior probability (BPP) >0.75. Values lower than BS 50 or BPP 0.75 are indicated by hyphens (-). Values of bootstrap (BS) 100 or BPP 1.00 are indicated by asterisks (*). Branch lengths are proportional to substitution rate. (KOR, Korea).

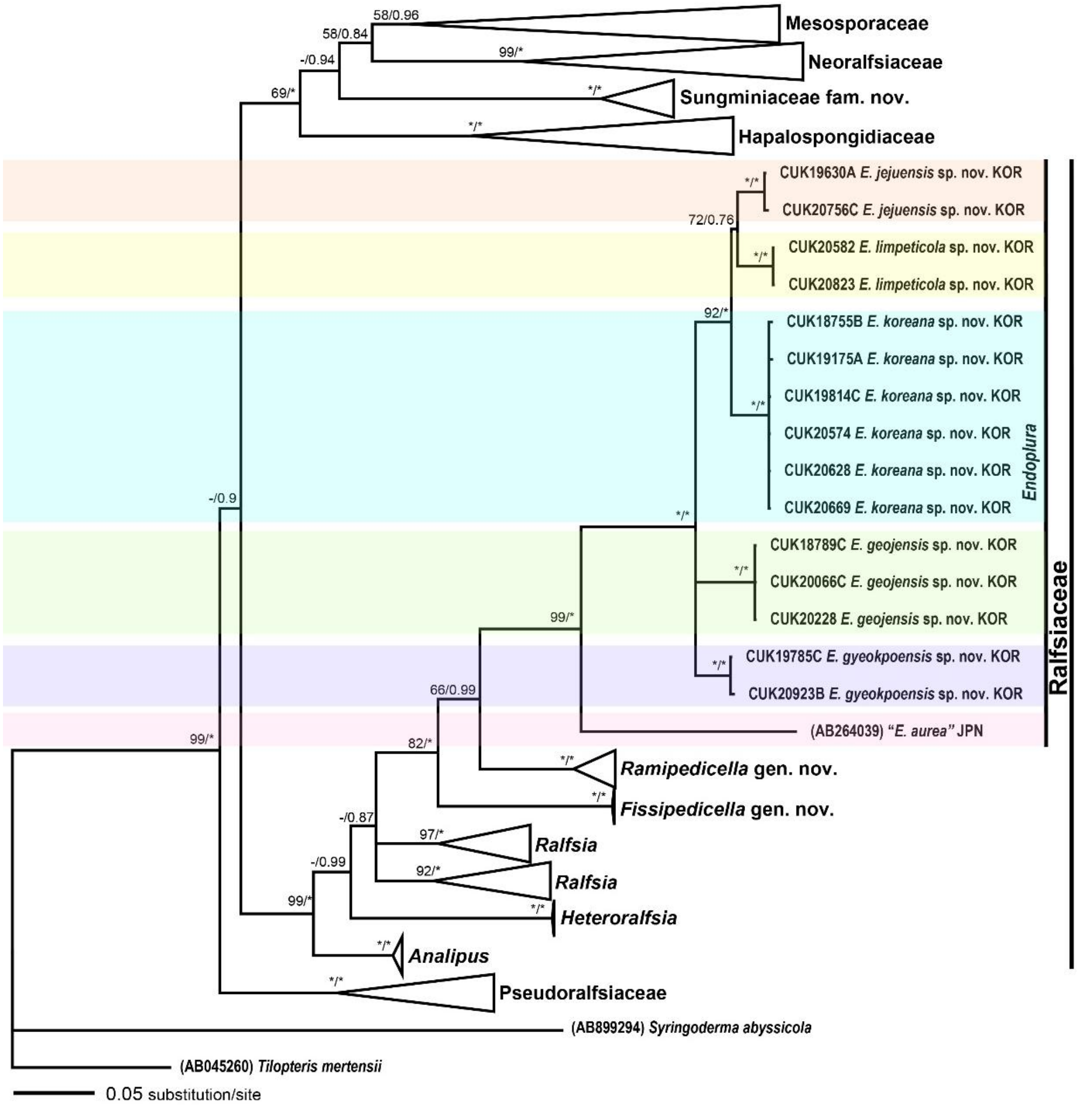


Fig. 34. Phylogenetic tree of *rbc*L gene sequence data from *Endoplura* and other Ralfsiales species. A phylogenetic analysis was performed using RAxMLGUI v1.5 and MrBayes 3.2.6 methods for Maximum likelihood (ML) analysis and Bayesian inference. The value above branches = maximum likelihood bootstrap (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 bootstrap (BS) 100 or BPP 1.00 are indicated by asterisks (*). Branch lengths are proportional to substitution rate. (JPN, Japan; KOR, Korea).

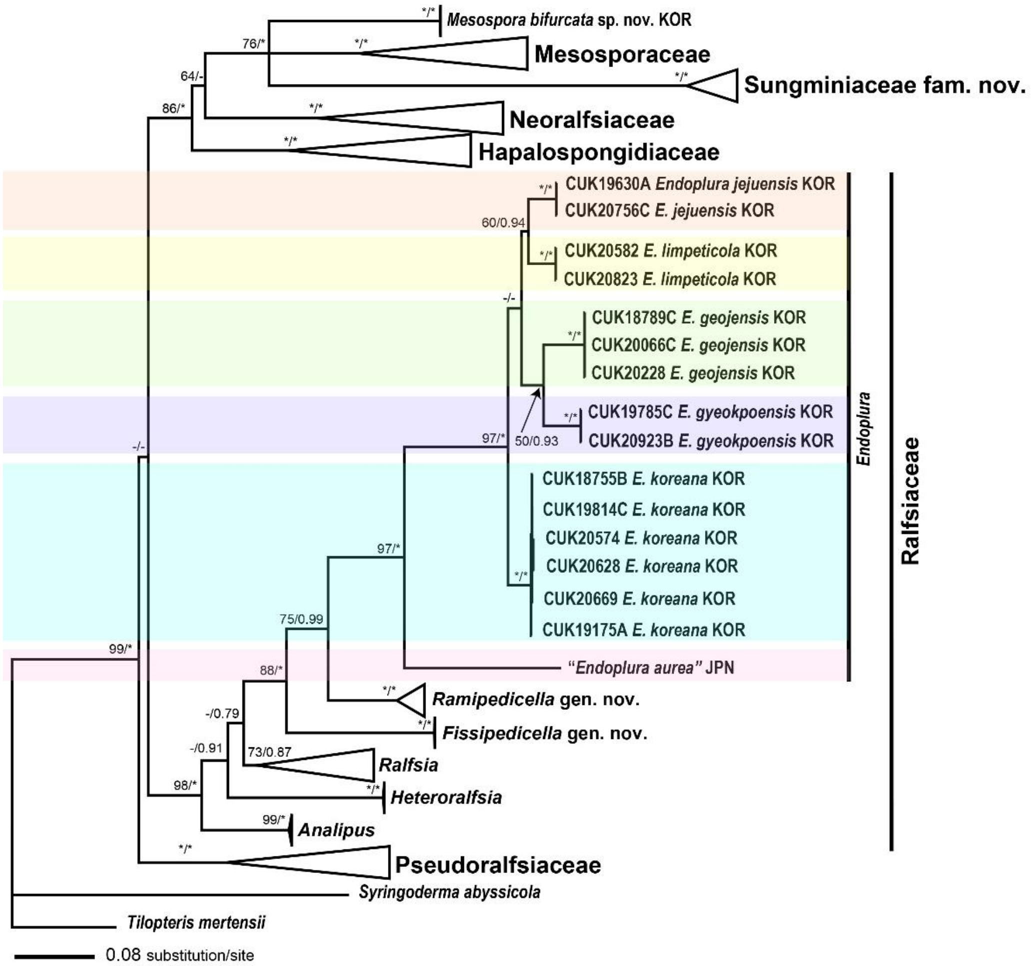


Fig. 35. Phylogenetic tree of concatenated *rbc*L and COI-5P sequence data from *Endoplura* and other Ralfsiales species. A phylogenetic analysis was performed using RAxMLGUI v1.5 and MrBayes 3.2.6 methods for Maximum likelihood (ML) analysis and Bayesian inference. The value above branches = maximum likelihood 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 bootstrap (BS) 100 or BPP 1.00 are indicated by asterisks (*). Branch lengths are proportional to substitution rate. (JPN, Japan; KOR, Korea).

Table 16. Gene sequence divergences (%) between *Endoplura* species using COI-5P sequence data.

	E. geojensis	*E. gyeokpoensis*	*E. jejuensis*	*E. koreana*	*E. limpeticola*
E. geojensis	–				
E. gyeokpoensis	11.2	–			
E. jejuensis	13.1	13.3	–		
E. koreana	13.1-13.6	13.3-13.6	11.7-11.8	–	
E. limpeticola	11.7	13.1	9.4	10.2-10.7	–

Table 17. Gene sequence divergences (%) between *Endoplura* species using *rbc*L sequence data.

	“E. aurea”	***E. geojensis***	***E. gyeokpoensis***	***E. jejuensis***	***E. koreana***	***E. limpeticola***
“E. aurea”	–					
E. geojensis	9.2	–				
E. gyeokpoensis	8.6-8.7	2.2-2.5	–			
E. jejuensis	9.3	2.9	2.5-2.7	–		
E. koreana	9.6-9.7	3.3-3.4	2.5.2.8	2.1-2.3	–	
E. limpeticola	9.6	3.8	2.4-2.5	2.0	2.4-2.5	–

1.3.2. Genus *Fissipedicella* A. O. Oteng'o, B. Y. Won & T. O. Cho gen. nov.

I sequenced a 662–bp portion of COI-5P and a 1315–bp portion of *rbc*L for Ralfsiales species from Korea. In addition, sequences of other recognized species belonging to the order Ralfsiales were downloaded from GenBank (Table 1). Phylogenetic trees based on ML (in MEGA and RAxML) and Bayesian inference (BI) for COI-5P, and ML (in RAxML) and BI for *rbc*L and concatenated alignement of *rbc*L and COI-5P sequence data were obtained with *Syringoderma abyssicola* (Setchell and N.L. Gardner) Levring and *Tilopteris mertensii* (Turner) Kützing selected as out group taxa. The phylogenetic trees obtained from the ML analyses of COI-5P sequence data were largely congruent and both resolved a section of the Korean specimens of unidentified creeping Ralfsiales species into a distinct and well supported clade in the family Ralfsiaceae (Fig. 36). COI-5P sequence data analyses using MrBayes gave a phylogenetic tree (not shown) with a slightly different topology with a collapsed branch for the unidentified creeping Ralfsiales species and other genera in family Ralfsiaceae. The phylogenetic trees of *rbc*L and concatenated alignments of *rbc*L and COI-5P sequence data for both ML and BI analyses were largely congruent with the unidentified creeping Ralfsiales samples nested in distinct and well supported clade in the family Ralfsiaceae (Figs 37 & 38). The genetic divergence values between the unidentified creeping Ralfsiales specimens and other recognized genera in the family Ralfsiaceae ranged from 17.4% to 20.9% for COI-5P and from 7.6% to 11.9% for *rbc*L gene (Tables 18 & 19 respectively). These molecular phylogeny and genetic divergence values are sufficient to warrant recognition of the section of unidentified creeping Ralfsiales specimens from Korea as a genus and new species, *Fissipedicella orientalis* gen. *et* sp. nov.

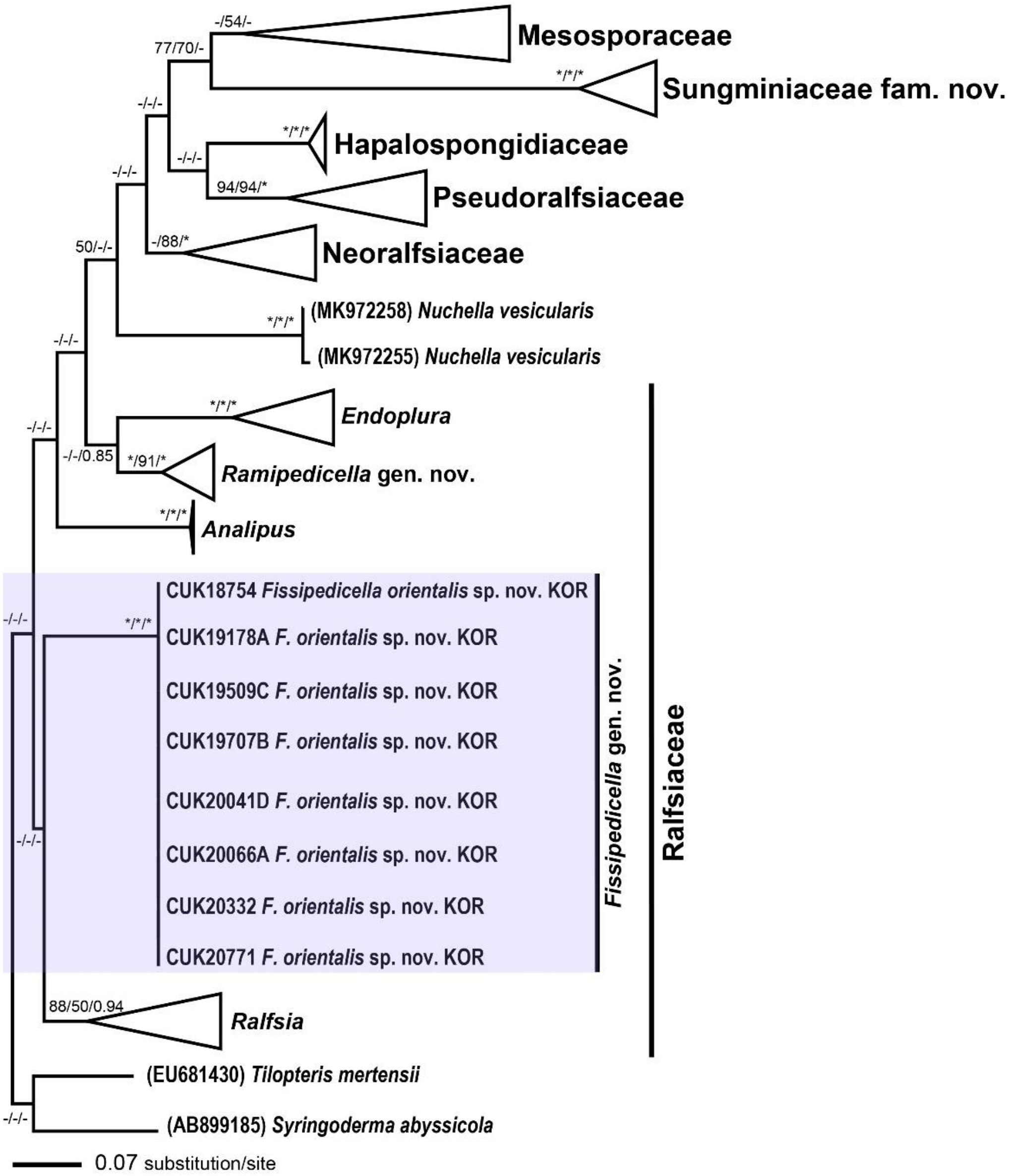


Fig. 36. Phylogenetic tree of COI-5P sequence data from *Fissipedicella* and other Ralfsiales species. A phylogenetic analysis was performed using MEGA X, RAxMLGUI v1.5 and MrBayes 3.2.6 methods for Maximum likelihood (ML) analysis (for first two methods) and Bayesian inference. The first two values above branches = ML bootstrap (BS) values in % >50, and the last value = bayesian posterior probability (BPP) >0.75. Values lower than BS 50 or BPP 0.75 are indicated by hyphens (-). Values of bootstrap (BS) 100 or BPP 1.00 are indicated by asterisks (*). Branch lengths are proportional to substitution rate. (KOR, Korea).

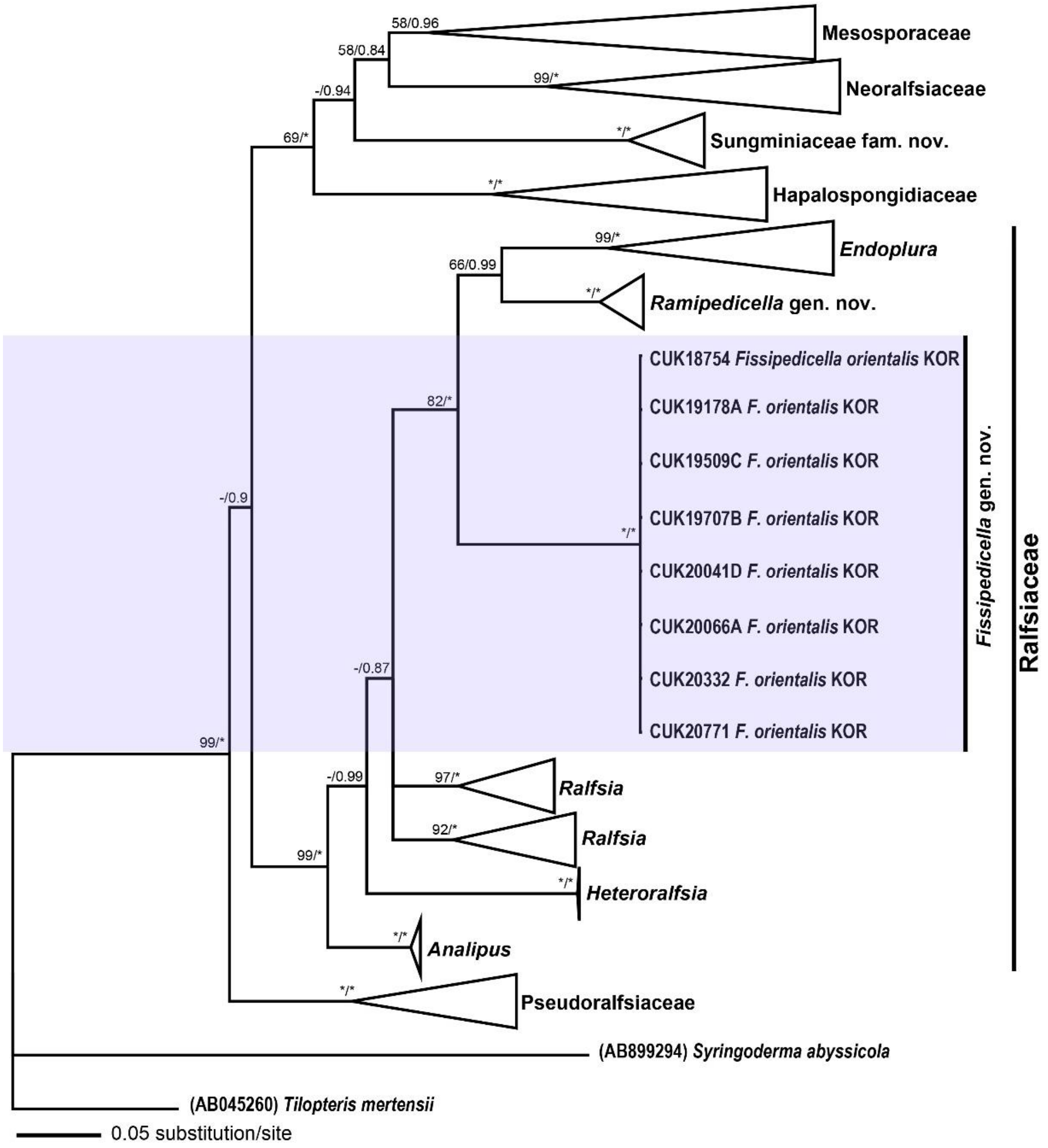


Fig. 37. Phylogenetic tree of *rbc*L gene sequence data from *Fissipedicella* and other Ralfsiales species. A phylogenetic analysis was performed using RAxMLGUI v1.5 and MrBayes 3.2.6 methods for Maximum likelihood (ML) analysis and Bayesian inference. The value above branches = maximum likelihood bootstrap (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 bootstrap (BS) 100 or BPP 1.00 are indicated by asterisks (*). Branch lengths are proportional to substitution rate. (KOR, Korea).

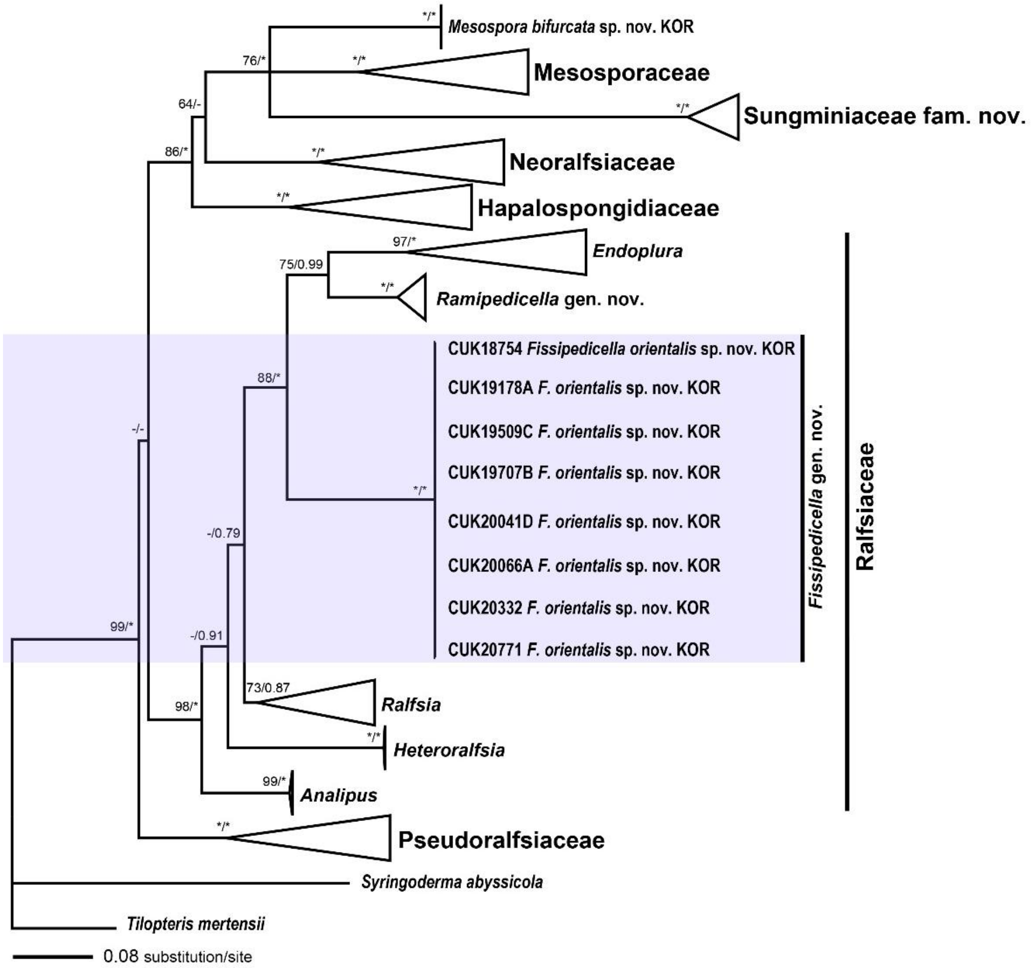


Fig. 38. Phylogenetic tree of concatenated *rbc*L and COI-5P sequence data from the *Fissipedicella* and other Ralfsiales species. A phylogenetic analysis was performed using RAxMLGUI v1.5 and MrBayes 3.2.6 methods for Maximum likelihood (ML) analysis and Bayesian inference. The value above branches = maximum likelihood 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 bootstrap (BS) 100 or BPP 1.00 are indicated by asterisks (*). Branch lengths are proportional to substitution rate. (KOR, Korea).

Table 18. Gene sequence divergences (%) between *Fissipedicella* and other genera in family Ralfsiaceae using COI-5P sequence data.

	Analipus	***Endoplura***	***Fissipedicella***	***Ralfsia***	***Ramipedicella***
Analipus	–				
Endoplura	19.3-22.5	–			
Fissipedicella	19.5-20.3	20.3-20.9	–		
Ralfsia	17.4-20.4	19.5-23.6	17.4-19.5	–	
Ramipedicella	18.2-19.3	17.9-20.6	17.9-18.8	15.7-20.3	–

Table 19. Gene sequence divergences (%) between *Fissipedicella* and other genera in family Ralfsiaceae using *rbc*L sequence data.

	Analipus	***Endoplura***	***Fissipedicella***	***Heteroralfsia***	***Ralfsia***	***Ramipedicella***
Analipus	–					
Endoplura	9.2-10.6	–				
Fissipedicella	8.5-8.8	9.6-11.9	–			
Heteroralfsia	7.6	11.1-12.2	9.1	–		
Ralfsia	6.3-7.2	9.1-11.6	8.2-9.5	7.7-9.6	–	
Ramipedicella	8.5-9.4	8.6-10.4	7.6-8.0	8.2-8.5	7.6-9.3	–

1.3.3. Genus *Ralfsia* Berkeley 1843

A 662–bp portion of COI-5P and a 1315–bp portion of *rbc*L for Ralfsiales species from Korea were sequenced. In addition, sequences of other recognized species belonging to this order were downloaded from GenBank (Table 1). Phylogenetic trees based on ML (in MEGA and RAxML) and Bayesian inference (BI) for COI-5P, and ML (in RAxML) and BI for *rbc*L and concatenated alignement of *rbc*L and COI-5P sequence data were obtained. *Syringoderma abyssicola* (Setchell and N.L. Gardner) Levring and *Tilopteris mertensii* (Turner) Kützing were selected as out group taxa. The phylogenetic trees obtained from the three analyses for COI-5P sequence data were largely congruent with Korean *Ralfsia* specimens resolved into a distinct and well supported clade within the genus *Ralfsia*, Ralfsiaceae (Fig. 39). Although the genus *Ralfsia* did not resolve well in the *rbc*L phylogeny based on BI analysis, the Korean *Ralfsia* specimens resolved into a distinct and well supported clade sister to *R. tenebris* (Fig. 40). The genus *Ralfia* resolved well in the ML phylogenetic tree (not shown) for *rbc*L also with the Korean *Ralfsia* specimens nested in a distinct and well supported clade sister to *R. tenebris*. Both phylogenetic trees of concatenated alignment of *rbc*L and COI-5P sequence data were congruent with one another as well as with COI-5P trees and the Korean *Ralfsia* specimens nested in a distinct and well supported clade (Fig. 41). Sequence divergence values between the Korean specimens of *Ralfsia* species and congeners ranged from 11.3% to 16.6% for COI-5P and 3.7% to 8.3% for *rbc*L gene (Tables 20 & 21 respectively). The molecular phylogeny and genetic divergence values are sufficient evidence to warrant recognition of the Korean specimens of *Ralfsia* sp. as a new species, *Ralfsia claviformis* sp. nov.

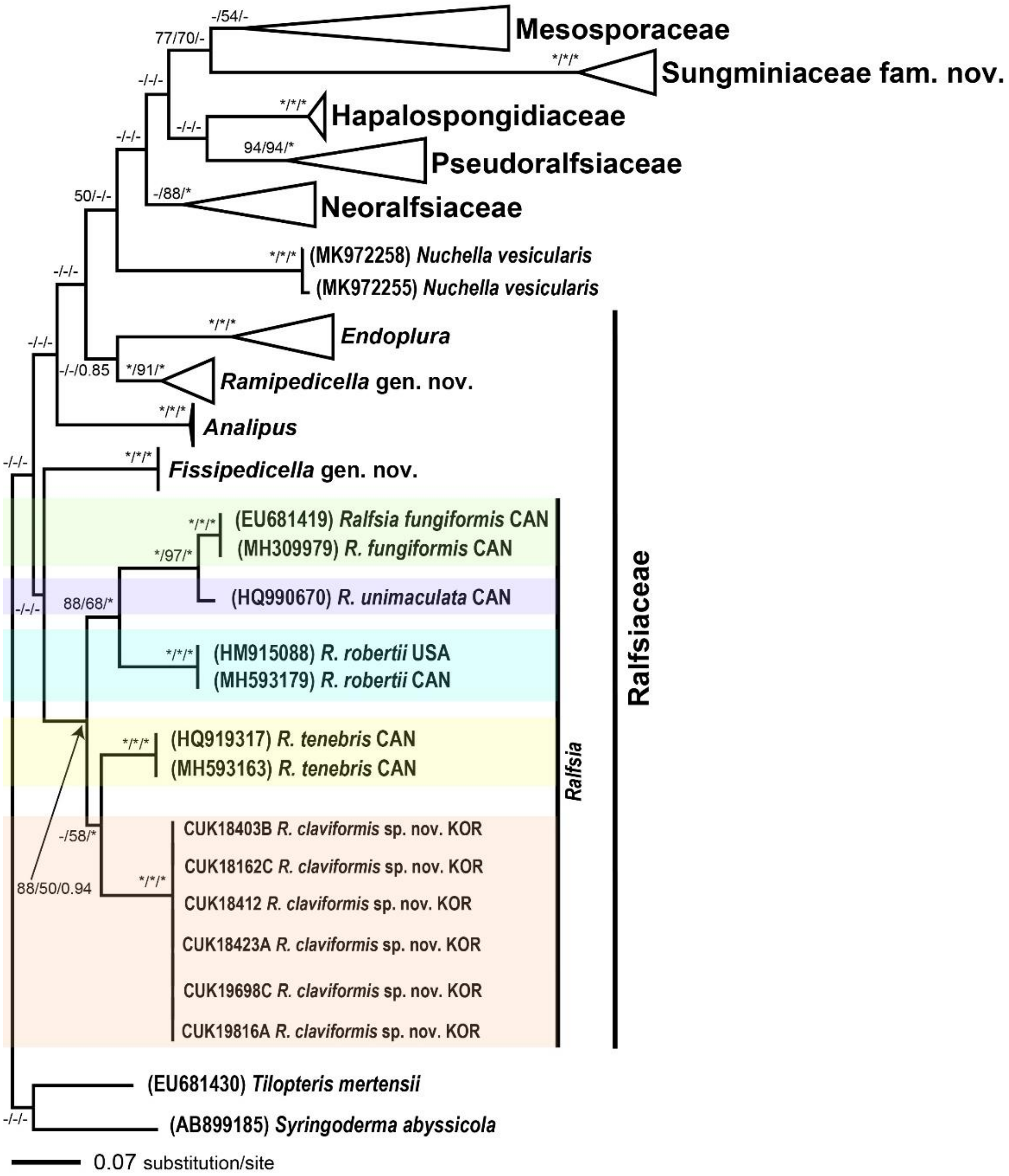


Fig. 39. Phylogenetic tree of COI-5P sequence data from *Ralfsia* and other Ralfsiales species. A phylogenetic analysis was performed using MEGA X, RAxMLGUI v1.5 and MrBayes 3.2.6 methods for Maximum likelihood (ML) analysis (for first two methods) and Bayesian inference. The first two values above branches = ML bootstrap (BS) values in % >50, and the last value = bayesian posterior probability (BPP) >0.75. Values lower than BS 50 or BPP 0.75 are indicated by hyphens (-). Values of bootstrap (BS) 100 or BPP 1.00 are indicated by asterisks (*). Branch lengths are proportional to substitution rate. (CAN, Canada; KOR, Korea; USA, United States of America).

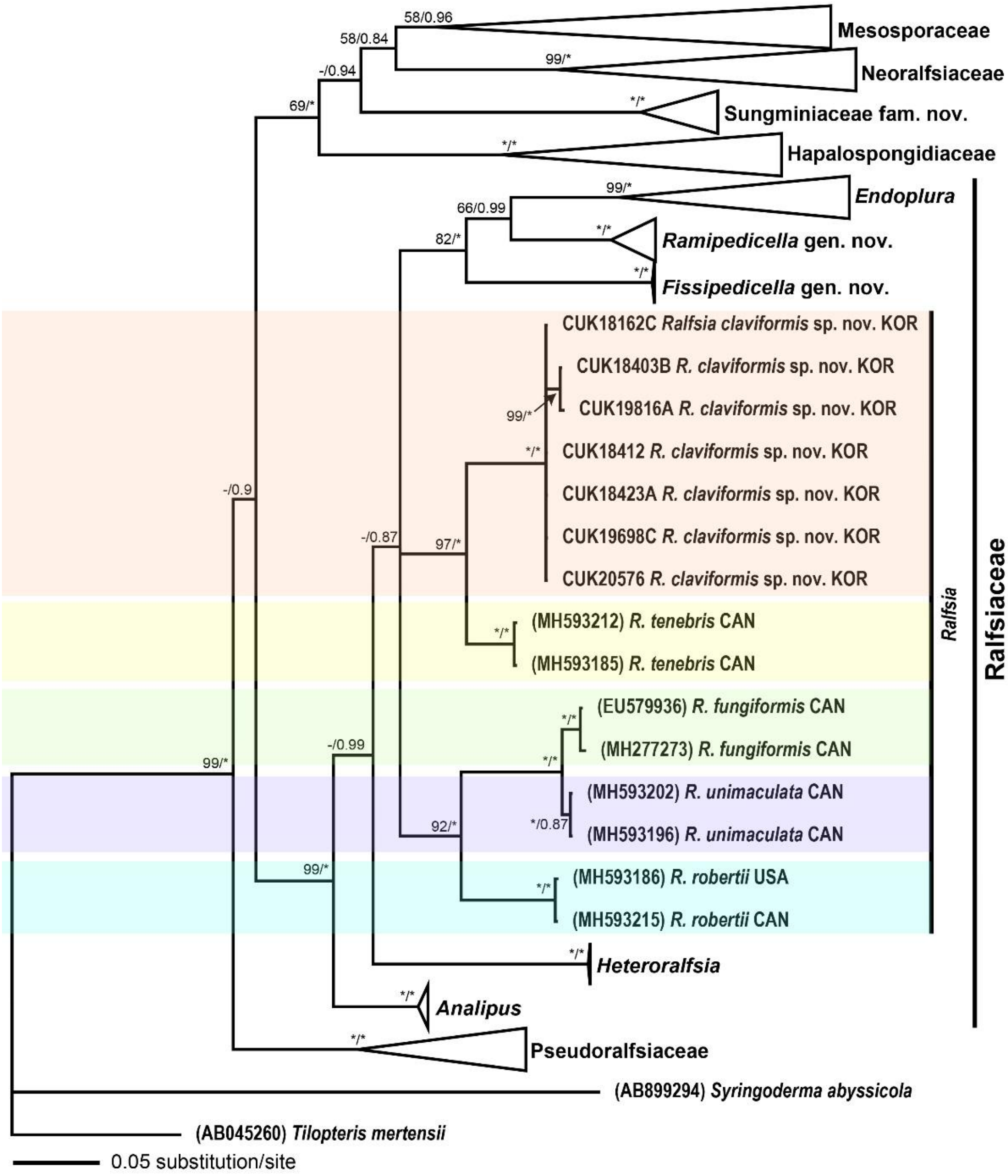


Fig. 40. Phylogenetic tree of *rbc*L gene sequence data from *Ralfsia* and other Ralfsiales species. A phylogenetic analysis was performed using RAxMLGUI v1.5 and MrBayes 3.2.6 methods for Maximum likelihood (ML) analysis and Bayesian inference. The value above branches = maximum likelihood bootstrap (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 bootstrap (BS) 100 or BPP 1.00 are indicated by asterisks (*). Branch lengths are proportional to substitution rate. (CAN, Canada; KOR, Korea; USA, United States of America).

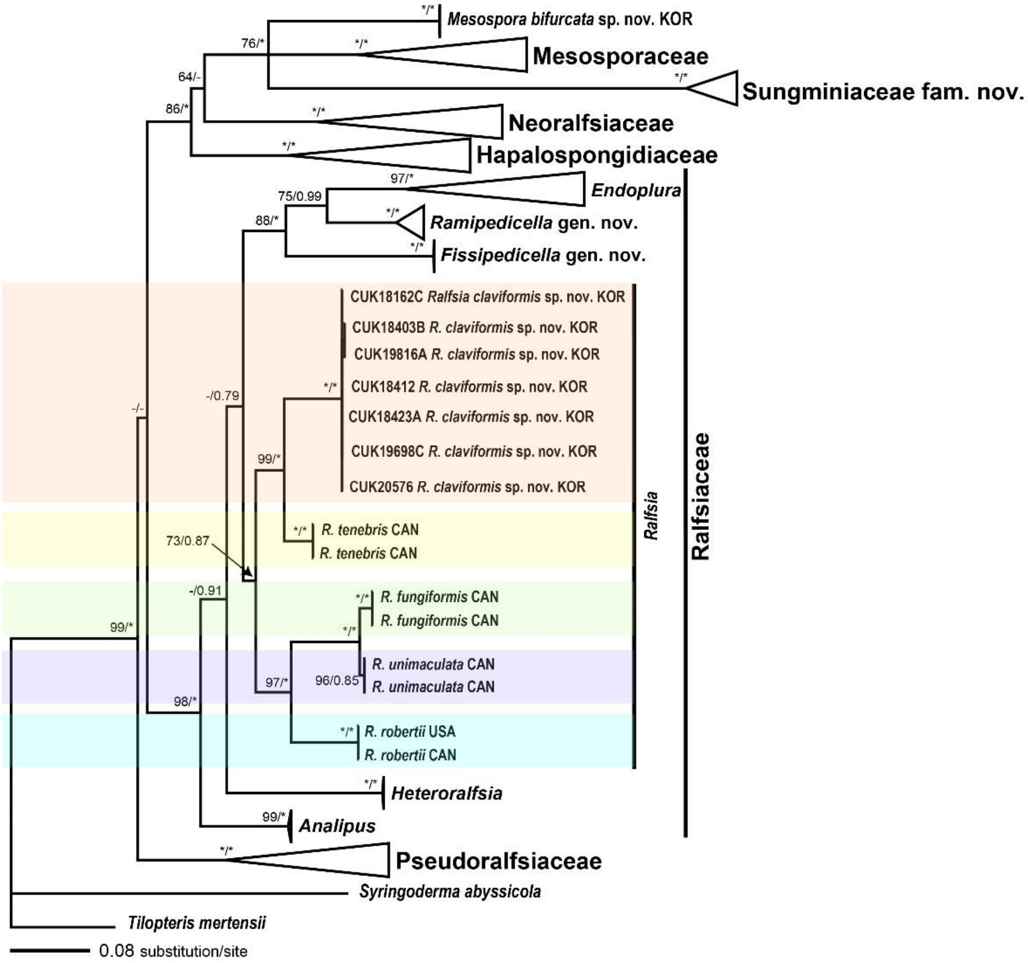


Fig. 41. Phylogenetic tree of concatenated *rbc*L and COI-5P sequence data from *Ralfsia* and other Ralfsiales species. A phylogenetic analysis was performed using RAxMLGUI v1.5 and MrBayes 3.2.6 methods for Maximum likelihood (ML) analysis and Bayesian inference. The value above branches = maximum likelihood 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 bootstrap (BS) 100 or BPP 1.00 are indicated by asterisks (*). Branch lengths are proportional to substitution rate. (CAN, Canada; KOR, Korea; USA, United States of America).

Table 20. Gene sequence divergences (%) between *Ralfsia* species using COI-P sequence data.

	R. claviformis	***R. fungiformis***	***R. robertii***	***R. tenebris***	***R. unimaculata***
R. claviformis	–				
R. fungiformis	16.6	–			
R. robertii	16.1	14.7	–		
R. tenebris	11.3	14.4	13.6	–	
R. unimaculata	15.3	3.8	15.0	13.7	–

Table 21. Gene sequence divergences (%) between *Ralfsia* species using *rbc*L sequence data.

	R. claviformis	***R. fungiformis***	***R. robertii***	***R. tenebris***	***R. unimaculata***
R. claviformis	–				
R. fungiformis	7.8-8.3	–			
R. robertii	7.6-8.1	5.0-5.2	–		
R. tenebris	3.7-4.1	6.9-7.1	6.6-6.7	–	
R. unimaculata	7.8-8.2	0.8-0.9	4.7-4.8	6.9	–

1.3.4. Genus *Ramipedicella* A. O. Oteng'o, B. Y. Won & T. O. Cho gen. nov.

I sequenced a 662–bp portion of COI-5P and a 1315–bp portion of *rbc*L for Ralfsiales species from Korea. In addition, sequences of other recognized species belonging to the order Ralfsiales were downloaded from GenBank (Table 1). Phylogenetic trees based on ML (in MEGA and RAxML) and Bayesian inference (BI) for COI-5P, and ML (in RAxML) and BI for *rbc*L and concatenated alignement of *rbc*L and COI-5P sequence data were obtained. *Syringoderma abyssicola* (Setchell and N.L. Gardner) Levring and *Tilopteris mertensii* (Turner) Kützing were selected as out group taxa. The phylogenetic trees obtained from MEGA ML analyses of COI-5P sequence data resolved a section of Korean specimens of unidentified creeping Ralfsiales species and *Ralfsia longicellularis* forming two lineages in a distinct and well supported clade, sister to *Endoplura*, within the family Ralfsiaceae (Fig. 42). Although COI-5P phylogenetic tree based on BI analysis (not shown) had collapsed branch for genera in family Ralfsiaceae, the section of Korean specimens of unidentified creeping Ralfsiales species and *Ralfsia longicellularis* also formed a distinct clade sister to *Endoplura*. The COI-5P tree based on RAxML (not shown) had a different topology with the unidentified specimens nested in a distinct and well supported clade between *Ralfsia* and *Fissipedicella*. The phylogenetic trees of *rbc*L and concatenated alignments of *rbc*L and COI-5P sequence data for both ML and BI analyses were largely congruent with the section of the unidentified Korean creeping Ralfsiales species and *R. longicellularis* forming two distinct lineages nested in a distinct and well supported clade in family Ralfsiaceae (Figs 43 & 44 respectively). The distinct clade encompassing the two lineages is recognized as a new genus, *Ramipedicella* gen. nov. Genetic distance between *Ramipedicella* gen. nov and other genera in Ralfsiaceae ranged from 15.7% to 20.6% for COI-5P and from 7.6% to 10.4% for *rbc*L gene (Tables 22 & 23 respectively). The gene sequence divergence values between the unidentified Korean creeping Ralfsiales species and *R. longicellularis* ranged from 7.5% to 8.0% for COI-5P and 1.8% for *rbc*L (Tables 24 & 25 respectively). The unidentified Ralfsiales species is recognized as a new species *Ramipedicella microloba* sp. nov. *Ralfsia longicellularis* is transferred to the new genus as *Ramipedicella longicellularis* comb. nov. The molecular phylogeny coupled with genetic divergence values are sufficient to warrant recognition of the new genus *Ramipedicella* and the new species and new combination, *Ra. microloba* and *Ra. longicellularis*, respecitively.

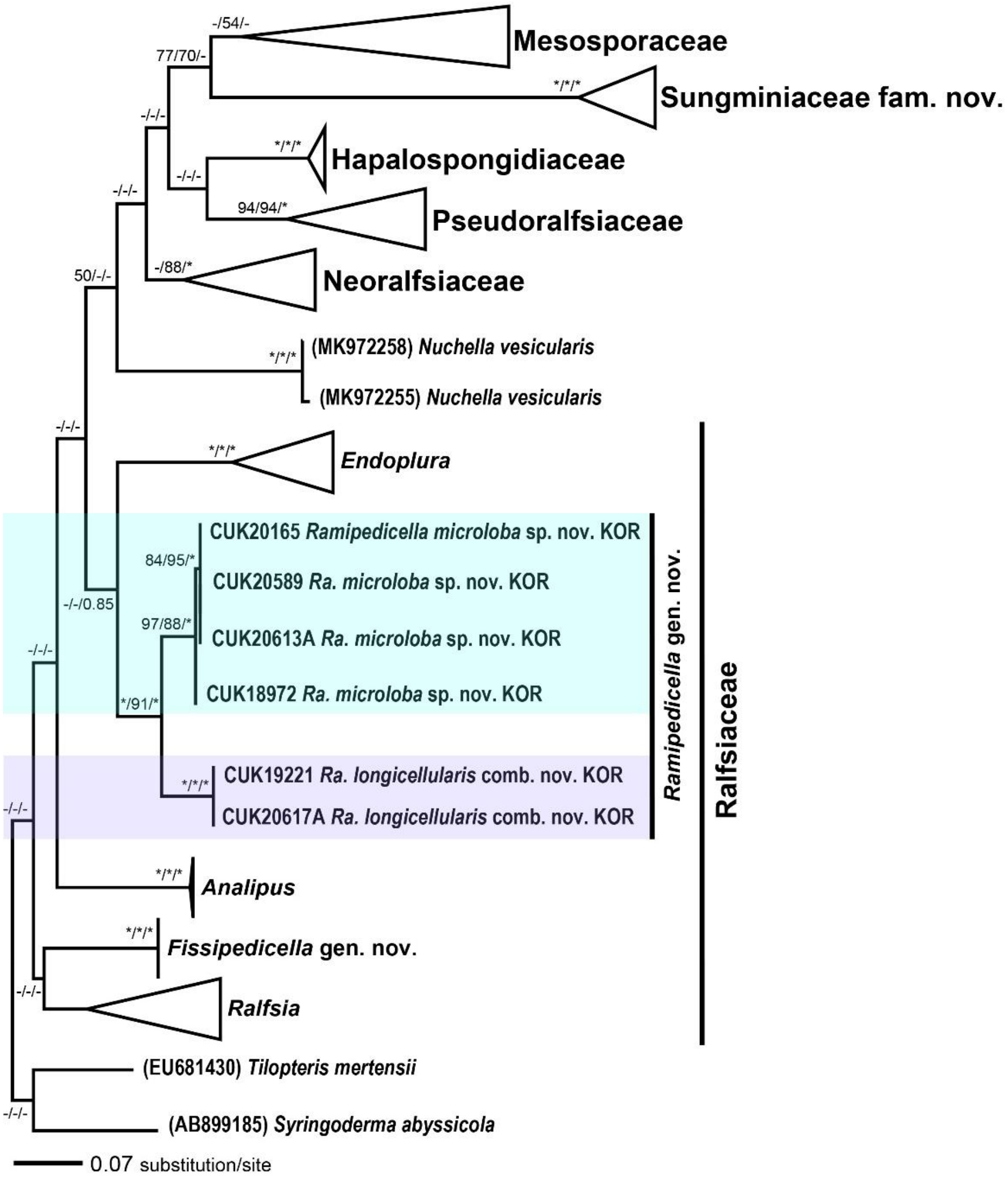


Fig. 42. Phylogenetic tree of COI-5P sequence data from the *Ramipedicella* and other Ralfsiales species. A phylogenetic analysis was performed using MEGA X, RAxMLGUI v1.5 and MrBayes 3.2.6 methods for Maximum likelihood (ML) analysis (for first two methods) and Bayesian inference. The first two values above branches = ML bootstrap (BS) values in % >50, and the last value = bayesian posterior probability (BPP) >0.75. Values lower than BS 50 or BPP 0.75 are indicated by hyphens (-). Values of bootstrap (BS) 100 or BPP 1.00 are indicated by asterisks (*). Branch lengths are proportional to substitution rate. (KOR, Korea).

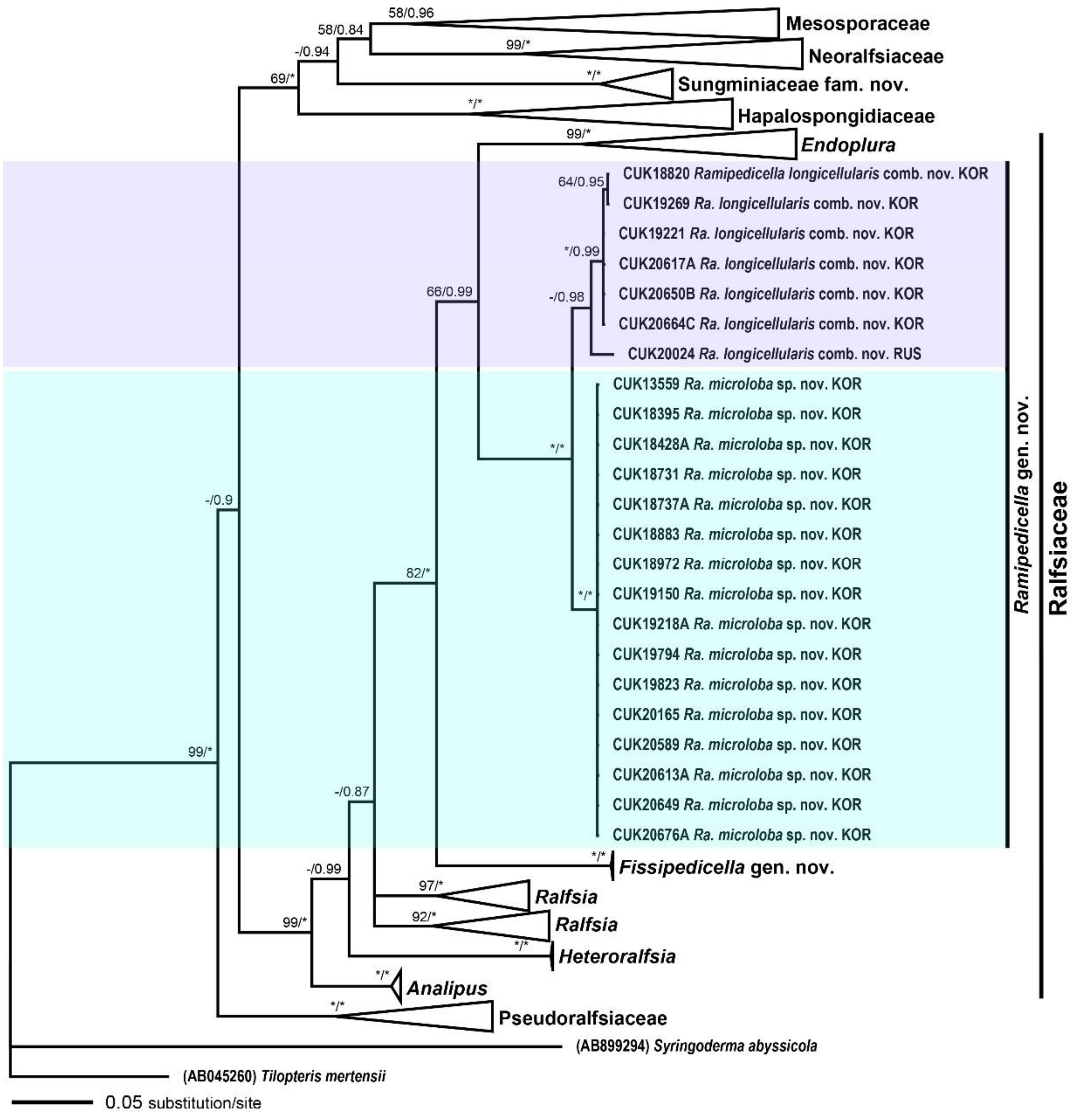


Fig. 43. Phylogenetic tree of *rbc*L gene sequence data from the *Ramipedicella* and other Ralfsiales species. A phylogenetic analysis was performed using RAxMLGUI v1.5 and MrBayes 3.2.6 methods for Maximum likelihood (ML) analysis and Bayesian inference. The value above branches = maximum likelihood 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 bootstrap (BS) 100 or BPP 1.00 are indicated by asterisks (*). Branch lengths are proportional to substitution rate. (KOR, Korea; RUS, Russia).

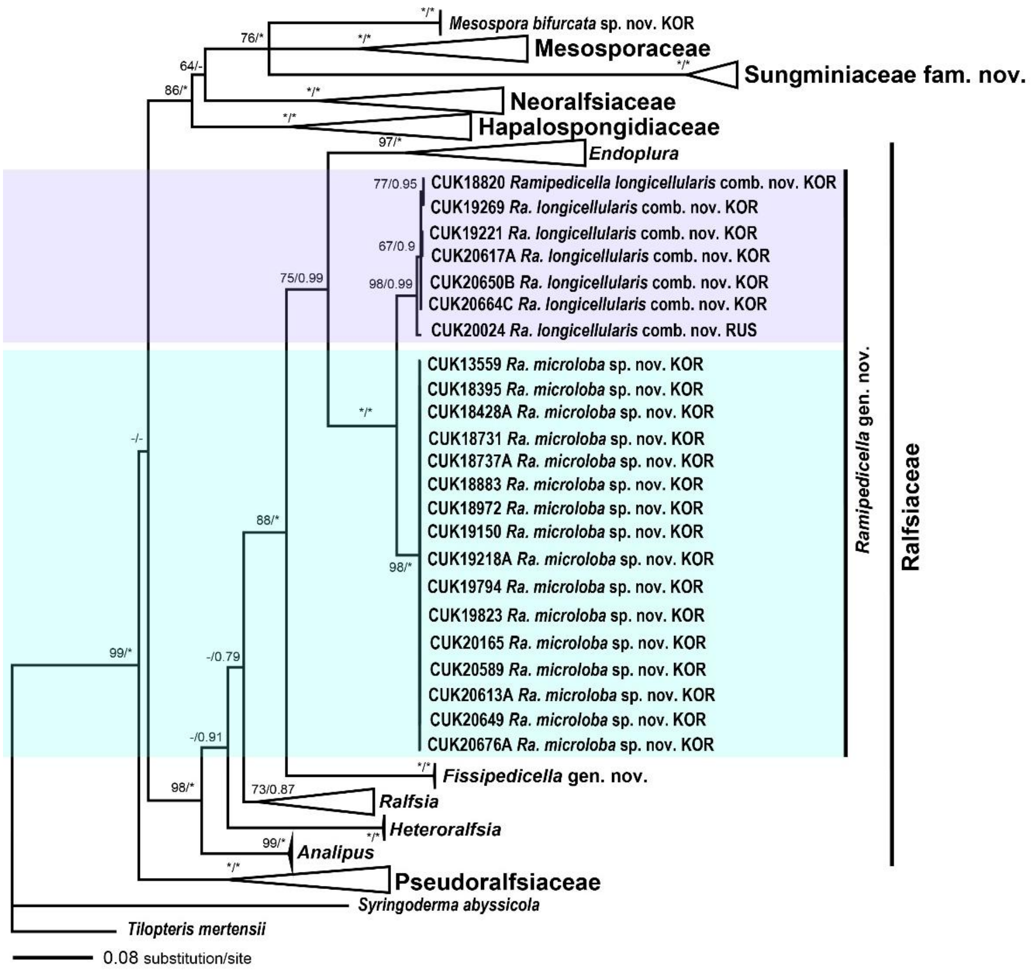


Fig. 44. Phylogenetic tree of concatenated *rbc*L and COI-5P sequence data from *Ramipedicella* and other Ralfsiales species. A phylogenetic analysis was performed using RAxMLGUI v1.5 and MrBayes 3.2.6 methods for Maximum likelihood (ML) analysis and Bayesian inference. The value above branches = maximum likelihood 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 bootstrap (BS) 100 or BPP 1.00 are indicated by asterisks (*). Branch lengths are proportional to substitution rate. (KOR, Korea; RUS, Russia).

Table 22. Gene sequence divergences (%) between *Ramipedicella* and other genera in family Ralfsiaceae using COI-5P sequence data.

	Analipus	***Endoplura***	***Fissipedicella***	***Ralfsia***	***Ramipedicella***
Analipus	–				
Endoplura	19.3-22.5	–			
Fissipedicella	19.5-20.3	20.3-20.9	–		
Ralfsia	17.4-20.4	19.5-23.6	17.4-19.5	–	
Ramipedicella	18.2-19.3	17.9-20.6	17.9-18.8	15.7-20.3	–

Table 23. Gene sequence divergences (%) between *Ramipedicella* and other genera in family Ralfsiaceae using *rbc*L sequence data.

	Analipus	***Endoplura***	***Fissipedicella***	***Heteroralfsia***	***Ralfsia***	***Ramipedicella***
Analipus	–					
Endoplura	9.2-10.6	–				
Fissipedicella	8.5-8.8	9.6-11.9	–			
Heteroralfsia	7.6	11.1-12.2	9.1	–		
Ralfsia	6.3-7.2	9.1-11.6	8.2-9.5	7.7-9.6	–	
Ramipedicella	8.5-9.4	8.6-10.4	7.6-8.0	8.2-8.5	7.6-9.3	–

Table 24. Gene sequence divergences (%) between *Ramipedicella* species using COI-5P sequence data.

	Ramipedicella longicellularis	***Ramipedicella microloba***
Ramipedicella longicellularis	–	
Ramipedicella microloba	7.5-8.0	–

Table 25. Gene sequence divergences (%) between *Ramipedicella* species using *rbc*L sequence data.

	Ramipedicella longicellularis	***Ramipedicella microloba***
Ramipedicella longicellularis	–	
Ramipedicella microloba	1.8	–

1.4. Family Sungminiaceae A. O. Oteng'o, B. Y. Won & T. O. Cho fam. nov.

1.4.1. Genus *Sungminia* A. O. Oteng'o, B. Y. Won & T. O. Cho gen. nov.

A 662–bp portion of COI-5P and a 1315–bp portion of *rbc*L for Ralfsiales species from Korea were sequenced. In addition, sequences of other recognized species belonging to this order were downloaded from GenBank (Table 1). Phylogenetic trees based on ML (in MEGA and RAxML) and Bayesian inference (BI) for COI-5P, and ML (in RAxML) and BI for *rbc*L and concatenated alignement of *rbc*L and COI-5P sequence data were obtained with *Syringoderma abyssicola* (Setchell and N.L. Gardner) Levring and *Tilopteris mertensii* (Turner) Kützing selected as out group taxa. The phylogenetic trees obtained from ML analyses of COI-5P sequence data were largely congruent and both resolved a section of unidentified specimens of creeping brown algae into three disctinct lineages nested in a distinct and well supported clade sister to family Mesosporaceae (Fig. 45). COI-5P tree obtained from BI analysis (not shown) had a slightly different topology with the unidentified specimens of creeping brown algae nested in a clade between *Mesospora bifurcata* and other *Mesospora* species. *Rbc*L phylogenetic trees obtained using both ML and BI analyses were congruent with the unidentified specimens of creeping brown algae nested in a distinct and well supported clade in the order Ralfsiales (Fig. 46). Although the phylogenetic tree obtained from MrBayes analysis of the concatenated alignment of *rbc*L and COI-5P sequence data had collapsed branch between the clade of the unidentified specimens of creeping brown algae, *Mesospora bifurcata* and other Mesosporaceae species (Fig 47), the tree obtained from RAxML (not shown) resovled the unidentified specimens of creeping brown algae into a distinct and well supported clade, separate from the Mesosporaceae. The distinct clade of unidentified specimens of creeping brown algae is recognized as a new family, Sungminiaceae fam. nov. Sequence divergence values between Sungminiaceae fam. nov. and other recognized families in the order

Ralfsiales ranged from 30.7% to 36.3% for COI-5P and 10.2% to 15.0% for *rbc*L gene (Tables 26 & 27 respectively). Sungminiaceae fam. nov. is monogeneric with one new genus, *Sungminia* gen. nov., and three distinct lineages recognized as 3 new species. Gene sequence between *Sungminia* species ranged from 8.5% to 9.4% for COI-5P and from 2.6% to 3.4% for *rbc*L (Tables 28 & 29 respectively). The molecular phylogeny and genetic divergence values are sufficient to warrant recognition of this group of the Korean creeping brown algae as a new family, Sungminiaceae fam. nov. with a new genus, *Sungminia* gen. nov. with three new species, *S. asiatica* sp. nov., *S. gladiata* sp. nov. and *S. pyriformis* sp. nov.

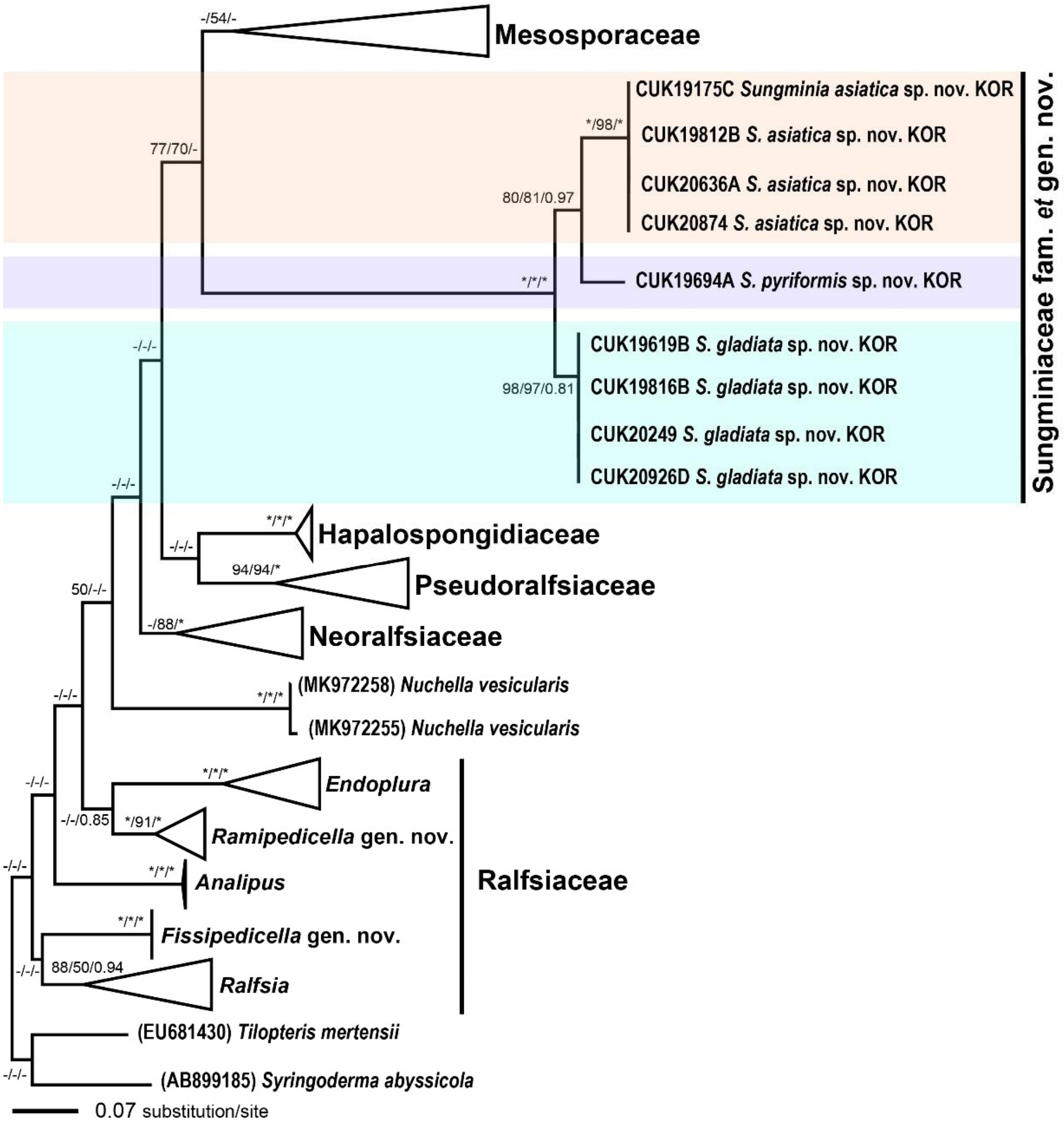


Fig. 45. Phylogenetic tree of COI-5P sequence data from Sungminiaceae and other families in order Ralfsiales. A phylogenetic analysis was performed using MEGA X, RAxMLGUI v1.5 and MrBayes 3.2.6 methods for Maximum likelihood (ML) analysis (for first two methods) and Bayesian inference. The first two values above branches = ML bootstrap (BS) values in % >50, and the last value = bayesian posterior probability (BPP) >0.75. Values lower than BS 50 or BPP 0.75 are indicated by hyphens (-). Values of bootstrap (BS) 100 or BPP 1.00 are indicated by asterisks (*). Branch lengths are proportional to substitution rate. (KOR, Korea).

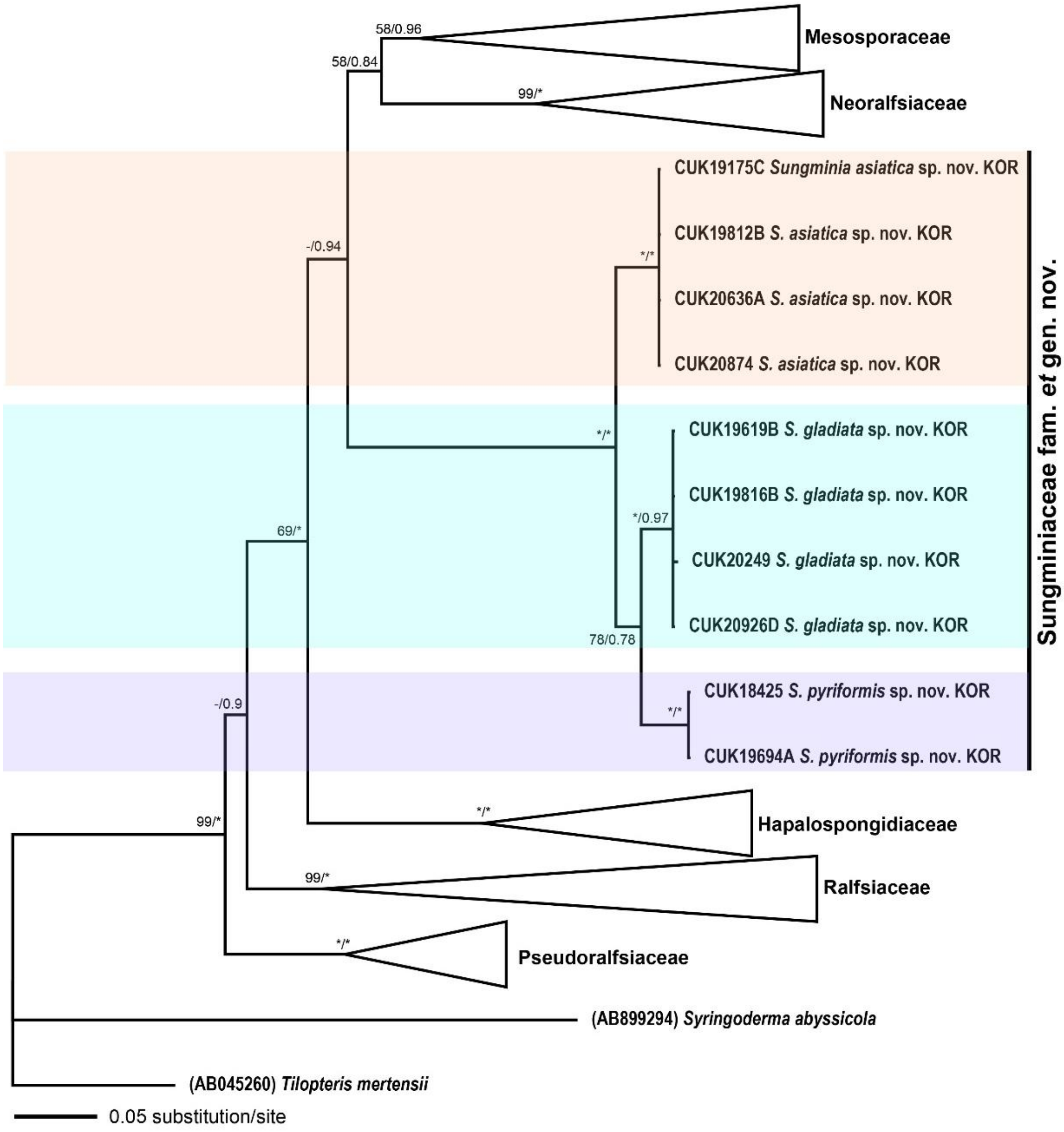


Fig. 46. Phylogenetic tree of *rbc*L gene sequence data from the Sungminiaceae and other families in order Ralfsiales. A phylogenetic analysis was performed using RAxMLGUI v1.5 and MrBayes 3.2.6 methods for Maximum likelihood (ML) analysis and Bayesian inference. The value above branches = maximum likelihood 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 bootstrap (BS) 100 or BPP 1.00 are indicated by asterisks (*). Branch lengths are proportional to substitution rate. (KOR, Korea).

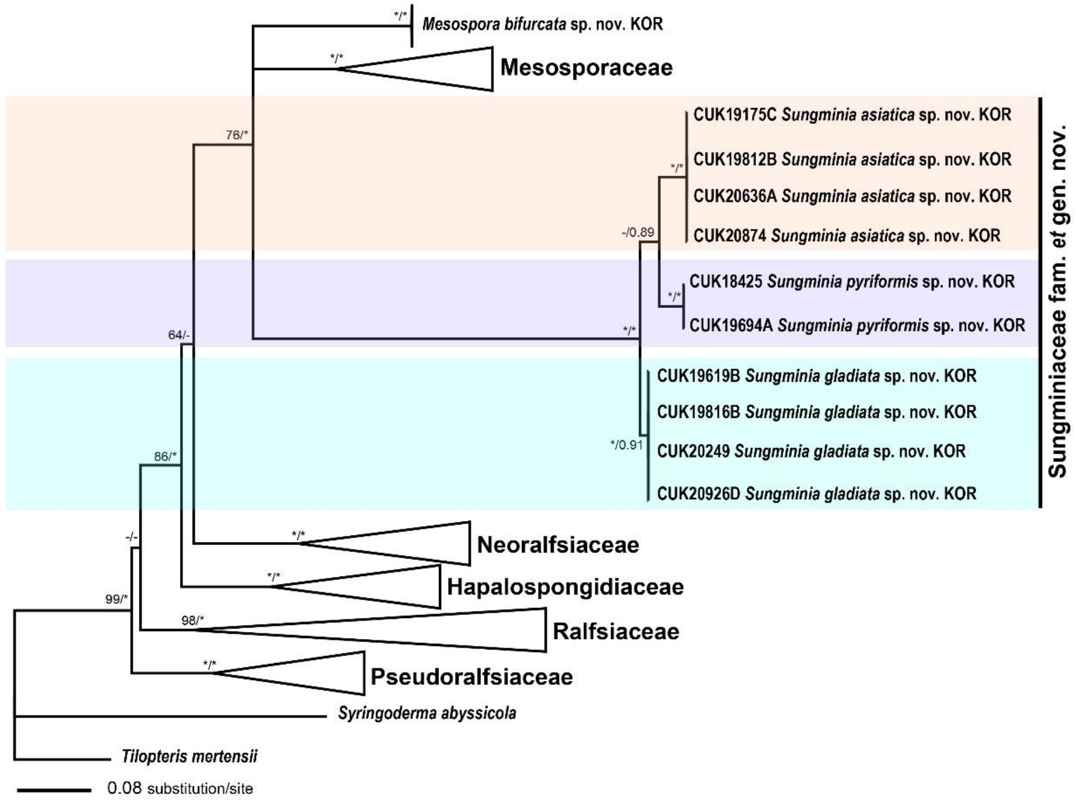


Fig. 47. Phylogenetic tree of concatenated *rbc*L and COI-5P sequence data from Sungminiaceae and other families in order Ralfsiales. A phylogenetic analysis was performed using RAxMLGUI v1.5 and MrBayes 3.2.6 methods for Maximum likelihood (ML) analysis and Bayesian inference. The value above branches = maximum likelihood 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 bootstrap (BS) 100 or BPP 1.00 are indicated by asterisks (*). Branch lengths are proportional to substitution rate. (KOR, Korea).

Table 26. Gene sequence divergences (%) between Sungminiaceae and other families in Ralfsiales using COI-5P sequence data.

	Hapalospongidiaceae	**Mesosporaceae**	**Neoralfsiaceae**	**Pseudoralfsiaceae**	**Ralfsiaceae**	**Sungminiaceae**
Hapalospongidiaceae	–					
Mesosporaceae	22.0-26.8	–				
Neoralfsiaceae	19.7-23.0	22.2-25.9	–			
Pseudoralfsiaceae	22.2-25.3	23.2-28.4	22.4-24.9	–		
Ralfsiaceae	19.9-24.3	21.6-26.5	18.1-25.1	21.7-27.2	–	
Sungminiaceae	32.8-34.3	30.7-35.3	33.2-35.1	33.5-36.3	31.3-34.8	–

Table 27. Gene sequence divergences (%) between Sungminiaceae and other families in Ralfsiales using *rbc*L sequence data.

	Hapalospongidiaceae	**Mesosporaceae**	**Neoralfsiaceae**	**Pseudoralfsiaceae**	**Ralfsiaceae**	**Sungminiaceae**
Hapalospongidiaceae	–					
Mesosporaceae	11.7-14.1	–				
Neoralfsiaceae	12.2-14.3	9.3-12.9	–			
Pseudoralfsiaceae	12.9-14.9	9.6-13.8	11.2-12.8	–		
Ralfsiaceae	11.5-15.4	8.9-15.3	9.9-14.8	7.6-13.5	–	
Sungminiaceae	12.4-15.2	10.2-13.1	11.7-13.1	11.1-14.2	10.4-15.0	–

Table 28. Gene sequence divergences (%) between *Sungminia* species using COI-5P sequence data.

	Sungminia asiatica	***Sungminia gladiata***	***Sungminia pyriformis***
Sungminia asiatica	–		
Sungminia gladiata	9.1-9.4	–	
Sungminia pyriformis	9.1	8.5-8.9	–

Table 29. Gene sequence divergences (%) between *Sungminia* species using *rbc*L sequence data.

	Sungminia asiatica	***Sungminia gladiata***	***Sungminia pyriformis***
Sungminia asiatica	–		
Sungminia gladiata	3.4	–	
Sungminia pyriformis	3.2-3.3	2.6-2.8	–

IV. CONCLUDING REMARKS

The order Ralfsiales is a monophyletic group molecularly based on the plastid *rbc*L gene, and morphologically having creeping form of thallus in at least one of the life history stages, and plurangia terminated by sterile cell(s) (Lim et al. 2007). According to Parente et al. (2021), the Ralfsiales has a hidden diversity especially in *Ralfsia*-like specimens (excluding *Ralfsia*, *Stragularia* spp. and Scytosiphonaceae creeping phases). Based on molecular (using COI-5P and *rbc*L genetic markers) and morpho-anatomical analyses of *Ralfsia*-like samples from the North Atlantic and Europe, they recently established a new family, Pseudoralfsiaceae with two new genera, *Pseudoralfsia* and *Nuchella*. Similarly, our phylogenetic analyses based on mitochondrial-encoded COI-5P, and plastid encoded *rbc*L genetic markers revealed a hiden diversity in *Ralfsia*-like samples collected from the Korean coastal shores. This study uncovers 19 Ralfsiales taxa in Korea including: 1 new candidate family, 4 new candidate genera, 13 new candidate species, and 1 new candidate combination.

The genus *Mesospora* (Mesosporaceae) mainly characterized by creeping thalli with free erect filaments arising from a basal disc of several rows of cells, hairs in tufts arising from basal disc, one chloroplast per cell, unangium on a stalk lateral to parent vegetative filament and without paraphysis, intercalary plurangia terminated by sterile cell(s) (Tanaka and Chihara 1982). There are 6 recognized *Mesospora* species worldwide, and this study adds the following new candidate species: *M. bifurcata.* COI-5P and *rbc*L phylogenies revealed *Mesospora* as a monophyletic genus with strong and well support bootstrap values. This is the first report of *Mesospora* in Korea.

The family Neoralfsiaceae was reported in Korea with only one species, *Neoralfsia expansa*. In this study, a new candidate genus *Neoralfsiopsis* is described with creeping thalli composed of three distinct parts: obvious cortical, medullary and basal layers, with firmly adjoined erect cortical filaments forming unilateral symmetry, with hairs in tufts arising from the basal layer as diagnostic generic level features to include the following new candidate species: *Ne.*

jejuensis. COI-5P and *rbc*L phylogenies revealed *Neoralfsiopsis* as a monophyletic genus with strong and well support bootstrap values.

The family Ralfsiaceae was reported in Korea with species of three genera, *Analipus*, *Heteroralfsia* and *Ralfsia*. In this study, several taxa are reported under Ralfsiaceae in Korea. The genus *Endoplura* is characterized by creeping thalli composed of firmly adjoined straight or assurgent erect filaments arising from an indistinct basal disc, cells with several to many chloroplasts, intercalary plurangia composed of 2–4 reproductive filaments and terminated by a sterile portion consisting of 2–5 cells (Hollenberg 1969). Until recently, *Endoplura* was a monotypic genus with *E. aurea*. But currently there are two new recognized species added to the genus, *E. jejuensis* and *E. koreana* from Korea. This study also includes the following new candidate species: *E. geojensis*, *E. gyeokpoensis* and *E. limpeticola*. COI-5P and *rbc*L phylogenies revealed *Endoplura* as a monophyletic genus with strong and well support bootstrap values. This study gives the first report of *Endoplura* in Korea.

In this study, a new candidate genus *Fissipecicella* (Ralfsiaceae) was described with creeping thalli composed of firmly adjoined straight or assurgent erect filaments arising from a basal disc, with hairs in tufts arising from the basal to mid portion of erect filaments, one parietal chloroplast per cell, intercalary plurangia composed of 1–2 reproductive filaments and terminated by 1–3 sterile cells, unangia on stalks composed of 1–6 cleaved stalk cells, with paraphyses, as diagnostic generic level features to include the following new candidate species: *F. orientalis*. COI-5P and *rbc*L phylogenies revealed *Fissipedicella* as a monophyletic candidate genus with strong and well support bootstrap values.

The genus *Ralfsia* (Ralfsiaceae) mainly identified by creeping thalli with firmly adhering, straight or assurgent or upward and downwardly curving erect filaments arising from a basal disc of several rows of cells, with or without rhizoids on undersurface, one chloroplast per cell, unangia sessile or on a stalk of 1–2 cells, with paraphysis, intercalary plurangia terminated mostly by a sterile cell (Smith and Sowerby 1843, Tanaka and Chihara1980a, 1980b). There are 16 recognized *Ralfsia* species worldwide. Currently, 3 recognized species have been reported in Korea. This study adds the following new candidate species: *R. claviformis.* COI-5P and concatenated alignment of *rbc*L and COI-5P phylogenies revealed *Ralfsia* as a monophyletic genus with moderate and well support bootstrap values.

Also, a new candidate genus *Ramipecicella* (Ralfsiaceae) was described with creeping thalli, multistratose with erect filaments frequently stratified, undersurface frimly attached to the substratum with or without rhizoids, thalli composed of firmly adjoined assurgent erect filaments arising from a basal disc of several layers of cells, with long cells with width to length ratio of 1:1–16 , with or without phaeophycean hairs, with one parietal chloroplast per cell, inter calary plurangia mostly uniseriate, occasionally biseriate, terminated by one to several sterile cells, unangia one to several, sessile or on branched stalks, with paraphyses, as diagnostic generic level features to include the following new candidate species, *Ra. microloba*, and a new candidate combination, *Ra. longicellularis*. COI-5P and *rbc*L phylogenies revealed *Ramipedicella* as a monophyletic genus with strong and well support bootstrap values.

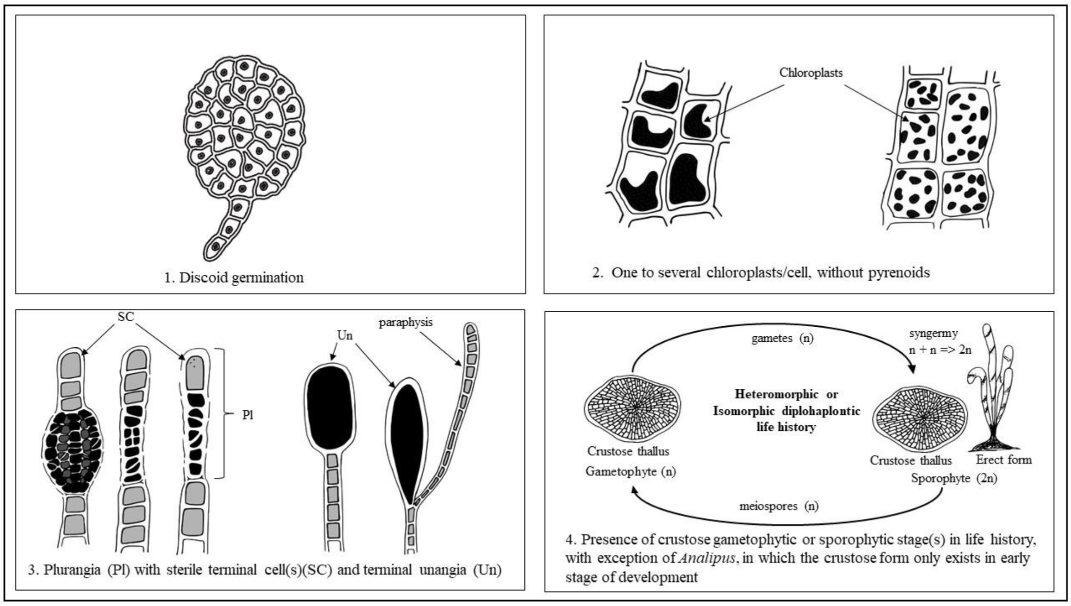
A new candidate family Sungminiaceae (Ralfsiales) was described with gelatinous crusts, firmly attached to substratum without rhizoids, hypothallial layer with one to several cell layers that give rise to erect filaments, erect filaments are moderately adjoined and partially separate with pressure, not tapered, with apical cells larger than other cells, hairs form in tufts, chloroplasts are one per cell, without obvious pyrenoid, life history supposedly diplohaplontic with isomorphic crusts, intercalary plurangia terminated by a sterile cell, unangia on the terminal part of erect filaments, with paraphyses as diagnostic family level features. COI-5P and *rbc*L phylogenies revealed the Sungminiaceae as a monophyletic and monogeneric candidate family with strong and well support bootstrap values.

In this study a new candidate genus *Sungminia* (Sungminiaceae, Ralfsiales) was described with irregularly expanded epilithic crusts, gelatinous and firmly attached to substratum without rhizoids, thalli composed of a basal layer consisting of one to several horizontally elongated cells giving rise to erect filaments, erect filaments straight or slightly curved, simple or sparsely branched, moderately adjoined and partially separates with pressure, not tapered, with apical cells larger than other cells, hairs in tufts, arising from basal layer or basal to mid portions of erect filaments, chloroplasts one per cell, without obvious pyrenoid, intercalary plurangia uniseriate or biseriate, with a terminal sterile cell, unangia sessile or on stalks, on the terminal part of erect filaments, with paraphysis as diagnostic generic level features to include the following new candidate species: *S. asiatica, S. gladiata, S. pyriformis*. COI-5P and *rbc*L phylogenies revealed the *Sungminia* as a monophyletic with strong and well support bootstrap values.

Revision of the systematics of order Ralfsiales

Order Ralfsiales (Figs. 48 & 49)

The order Ralfsiales is mainly distinguished by:



1. Discoid germination

2. One to several chloroplasts/cell, without pyrenoids

3. Plurangia (Pl) with sterile terminal cell(s)(SC) and terminal unangia (Un)

4. Presence of crustose gametophytic or sporophytic stage(s) in life history, with exception of *Analipus*, in which the crustose form only exists in early stage of development

Fig. 48. Morphological characteristics of order Ralfsiales drawn with reference to the description of Lim et al. (2007). This figure is not drawn to scale.

There are six families in this order (Fig. 49). The families can be distinguished based on a combination of characters as follows:

1. Thallus construction (firmly, moderately of loosely adhering filaments)
2. Presense or absence of paraphyses
3. Position of unangia in relation to surrounding filaments
4. Number of sterile cells terminating the plurangial reproductive structures.

Key to members of order Ralfsiales

1. Plants with loosely adhering erect filaments, reproductive structures without paraphyses…. 2

1. Plants with moderate to firmly adhering erect filaments, reproductive structures with paraphyses…... 3

2. Erect filaments with mostly up to 30 cells, one chloroplast per cell, stalked unangia borne laterally on parent filament…………………………………………… **Mesosporaceae**

2. Erect filaments with approximately 40 cells or more, several chloroplasts per cell, unangia borne terminally on parent filament…………………..…… **Hapalospongidiaceae**

3. Plants with intercalary plurangia strictly terminated by one sterile cell ………………..…. 4

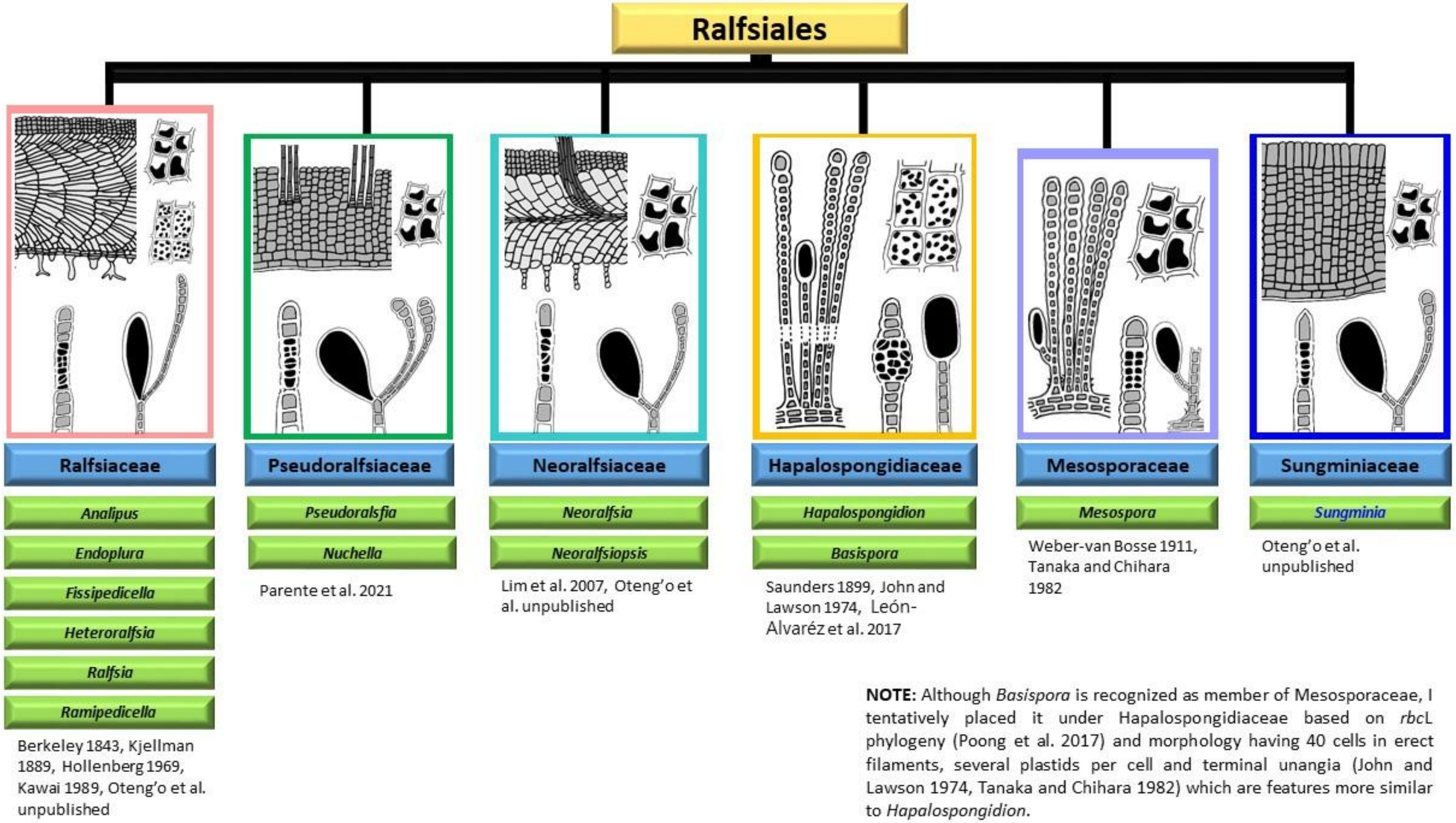
3. Plants with intercalary plurangia terminated by one to several sterile cells ….…………..…. 5

4. Crusts with straight, moderately adhering erect filaments with large apical cells ………….. **Sungminiaceae**

4. Crusts with firmly adhering erect filaments, with obvious cortical and medullary layers ………….. **Neoralfsiaceae**

5. Crusts similar to *Ralfsia* sensu stricto Ralfsiaceae, but with frequent hair pits, typically unsymmetrical, and with distinct molecular data …………………….……… **Pseudoralfsiaceae**

5. Crusts lacking hairs or hairs rarely present, symmetrical to unsymmetrical, and with distinct molecular data ………………………………………………………….……… **Ralfsiaceae**



NOTE: Although *Basispora* is recognized as member of Mesosporaceae, I tentatively placed it under Hapalospongidiaceae based on *rbc*L phylogeny (Poong et al. 2017) and morphology having 40 cells in erect filaments, several plastids per cell and terminal unangia (John and Lawson 1974, Tanaka and Chihara 1982) which are features more similar to *Hapalospongidion*.

Fig. 49. Systematics of the order Ralfsiales.

Family Hapalospongidiaceae (Fig.50)

This family is mainly characterized by: a basal disc that gives rise to free erect filaments which taper downwards, with 17–72 cells per filament, with several plastids per cell, intercalary plurangia terminated by one to several sterile cells, terminal unangia on long stalks composed of 7–31 cells and arising from the basal disc or basal portion of the thallus (Poong et al. 2013, 2017, León-Alvaréz et al. 2017).

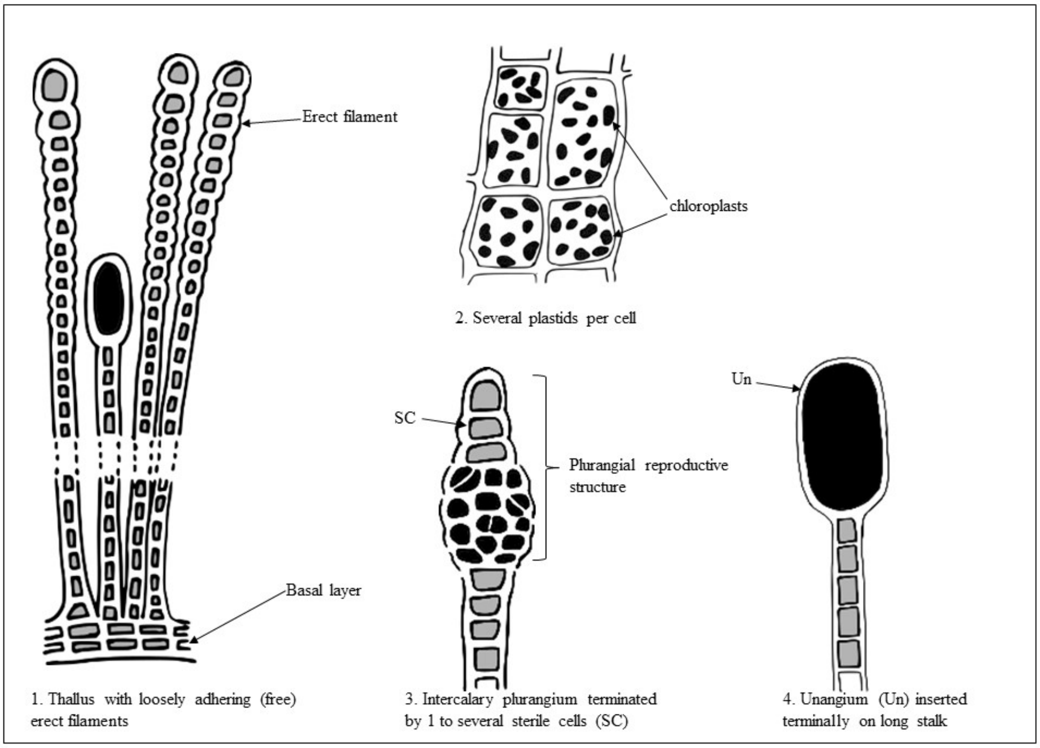


Fig. 50. Morphology of the type genus, *Hapalospongidion* drawn with reference to the description and figures of Saunders (1899). This figure is not drawn to scale.

Although not conclusive, (Poong et al. 2017) indicated that *Basispora*, *Mesospora* and *Hapalospongidion* should not be merged. Their molecular studies based on *rbc*L indicated that *Basispora* is nested in Hapalospongidiaceae. Family Hapalospongidiaceae is currently recognized as monogeneric, but I tentatively add *Basispora* to this family based on the *rbc*L phylogeny of Poong et al. (2017) and the genus having erect filaments longer that those of most species in *Mesospora*, several plastids per cell, and unangia inserted terminally on long stalks that arised from the basal part of the thallus (John and Lawson 1974, Tanaka and Chihara 1982). These morphological charaters makes *Basispora* more similar to *Hapalospongidion* than *Mesospora*.

Hapalospongidion can be distinguished from *Basispora* by having longer, straight erect filaments of upto 85 cells [as in *H. thirumullavaramense* P.Sophiammal Nettar & M.V.N.Panikkar from India (Sophiammal and Panikkar 2009)], and unangial stalks arising from a basal branching postigenous cell while the latter has shorter assurgent erect filaments of about 40 cells and ungial filaments originating from a branching posteginous cell that branches again before forming the unangial stalk and a paraphysis-like filament (León-Alvaréz et al. 2017). Currently, *Basispora* is a monotypic genus with one species, *B. africana*, while *Hapalospongidion* encompasses six recognized species, *H.capitatum, H. gelatinosum, H. macrocarpum, H. saxigenum, H. thirumullavaramense* and *H. vanbosseae* (Guiry and Guiry 2021).

Genus, *Basispora* (Fig.51) is mainly identified by gelatinous crusts with three to several layers of prostrate filaments from which assurgent filaments arise, free and closely packed, consisting up to 40 cells, distinctly clavate towards the upper part, several discoid chloroplasts per cell, terminal unangia on long stalks composed of 4–15 cells from near the base of the simple, often assurgent free vegetative filaments, plurilocular reproductive structures were unknown (John and Lawson 1974). This is a monotypic genus with *B. africana*.

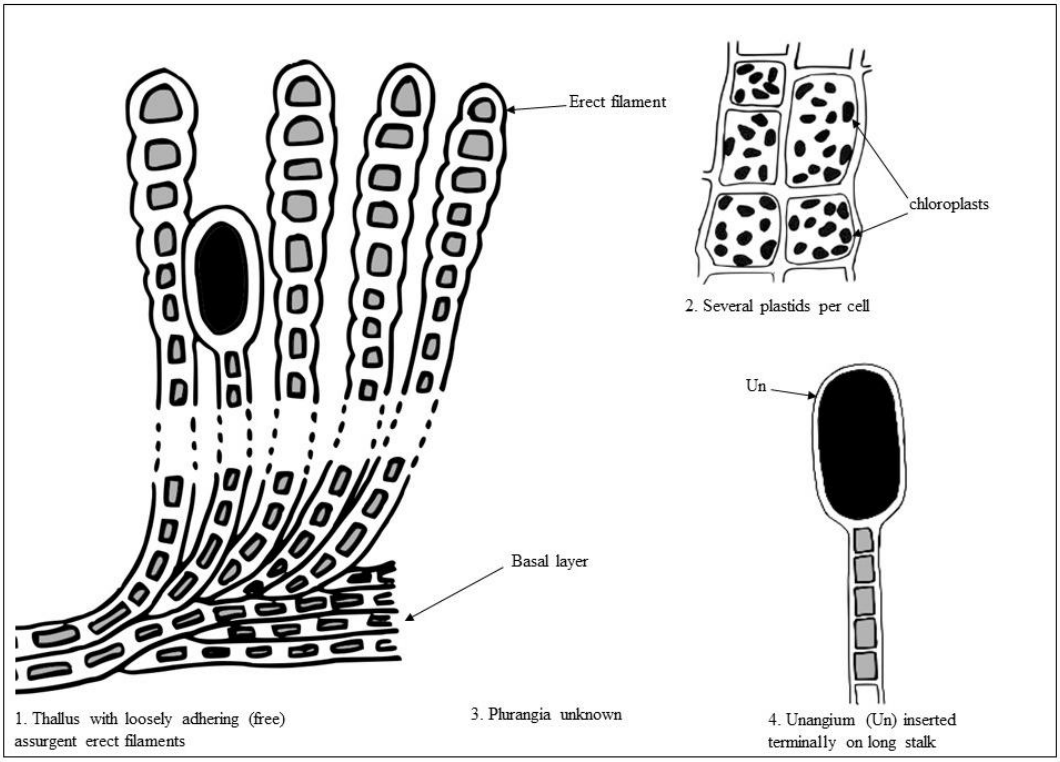


Fig. 51. Morphology of *Basispora* drawn from the description of John and Lawson (1974). This figure is not drawn to scale.

Family Mesosporaceae (Fig. 52)

This family is mainly identified by: a gelatinous crusts with basal layer giving rise to free erect filaments which taper downwards, with 18–32 cells per filament, with one chloroplast per cell, intercalary plurangia terminated by 2–3 sterile cells, unangia on stalks laterally inserted to the parent filament (Weber-van Bosse 1911, 1913, Tanaka and Chihara 1982, Poong et al. 2013, 2017).

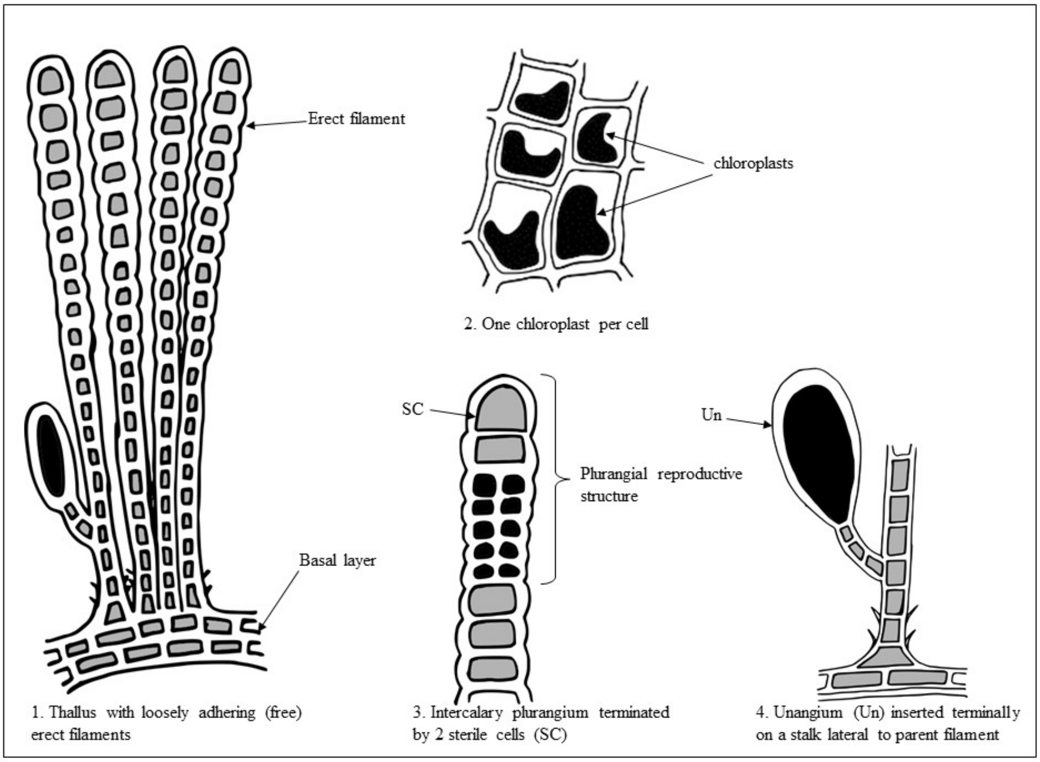
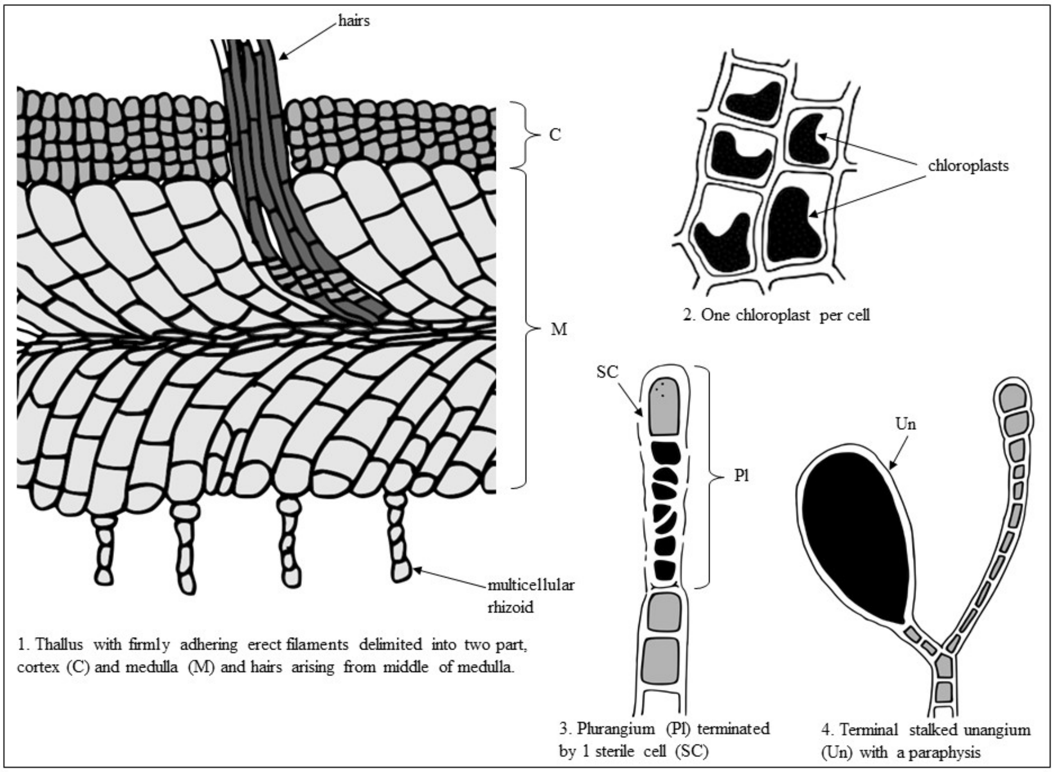


Fig. 52. Morphology of the type genus, *Mesospora* drawn with reference to the descriptions of Weber-van Bosse (1913) and Tanaka and Chihara (1982). This figure is not drawn to scale.

This family is currently recognized as having two genera, *Mesospora* and *Basispora*, but I tentatively transfer *Basispora* to family Hapalospongidiaceae as seen in earlier text. In my opinion, based on the *rbc*L phylogeny and morphological studies by Poong et al (2017), the family Mesosporaceae can tentatively be taken as monogeneric until more authentic specimens of *Hapalospongidion* and *Basispora* are included in analyses to warrant a more conclusive inference. Therefore, there are six recognized species in the monogeneric family Mesosporaceae: *M. elongata*, *M. indopacifica*, *M. lombokensis*, *M. negrosensis*, *M. pangoensis* and *M. schmidtii*. Scpecies in the genus *Mesospora* are morphologically delimited based on the degree and adherence of erect filaments, number of cells in the erect filaments, position of the unangia in relation with the parent filament and number of sterile cells terminating the plurangia. *Mesospora* was not reported in Korea until this study. A new candidate species, *M. bifurcata* was described and delimited based on both molecular and morphological analyses. *Mesospora bifurcata* is generally distinguished from the congeners by having long erect filaments, bifurcated plurangial reproductive structures and sessile unangia vs the congeners generally having shorter erect filaments, unbranched plurangial reproductive structures and stalked unangia (Table 4). Although *M. negrosensis* is sometimes reported to have sessile unangia borne laterally to the middle portion of erect filaments just like *M. bifurcata*, it differ from the latter by having simple erect filaments that are closely packed in the lower half and loosely associated in the upper half portion of thallus as opposed to *M. bifurcata* having sparse dichotomously branched erect filaments that are loosely associated in the whole thallus (Table 4).

Family Neoralfsiaceae (Fig. 53)

Neoralfsiaceae a monogeneric family characterized by: expanded crusts attached to substratum with rhizoids, pseudoparenchymatous tightly adjoined vegetative filaments composed of obvious cortical and medullary layers with hair pits, medullary vegetative filaments are unilateral to bilateral, cells with single plastid, unangia with pedicels and paraphyses, intercalary plurangia with a single sterile terminal cell (Lim et al. 2007).



1. Thallus with firmly adhering erect filaments delimited into two part, cortex (C) and medulla (M) and hairs arising from middle of medulla.

2. One chloroplast per cell

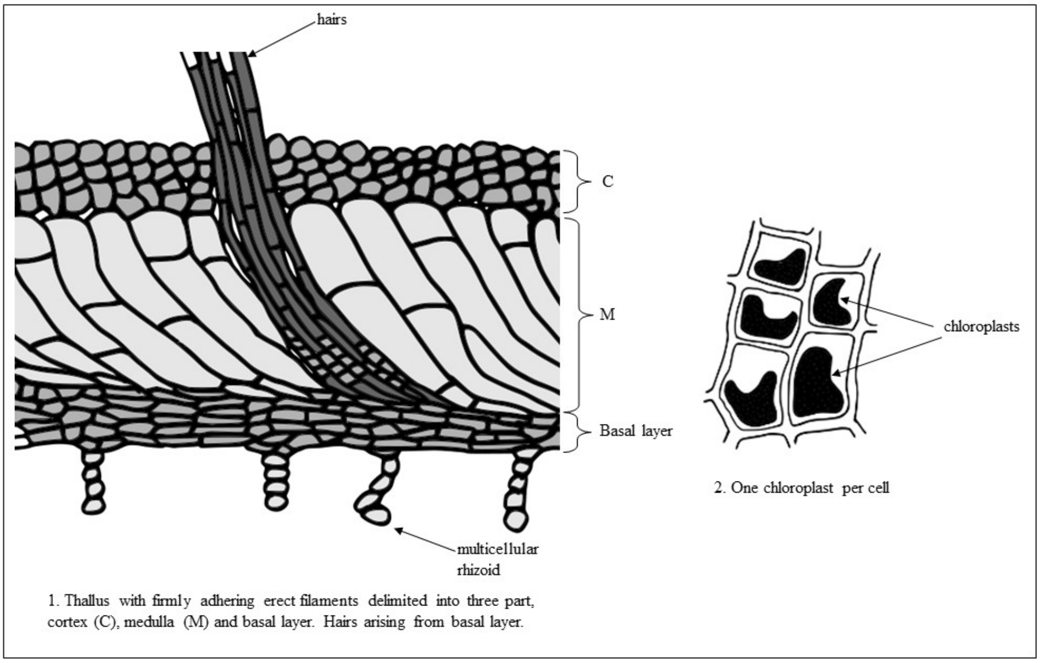
3. Plurangium (Pl) terminated by 1 sterile cell (SC)

4. Terminal stalked unangium (Un) with a paraphysis

Fig. 53. Morphology of the type genus, *Neoralfsia* drawn with reference to the description of Lim et al (2007). This figure is not drawn to scale.

This family was erected to accommodate *Ralfsia expansa* based on molecular and morphology (Lim et al. 2007). Currently there are two recognized species in this family, *N. expansa* and *N. hancockii* (Guiry and Guiry 2021). The species are mainly differentiated by considering the symmetry of vegetative filaments and number of stalk cells with the latter having mostly unilateral symmetry and 2–6 stalk cells while the former has bilaterally symmetrical thallus with 0–1 stalk cell (León-Alvaréz et al. 2014a). Molecular analyses will play an important role in species identification in this genus as the above said characteristics may overlap between the species.

In this study I described a new candidate genus, *Neoralfsiopsis* (Fig. 54) under the family Neoralfsiaceae. It is a monotypic genus with a new candidate species, *Ne. jejuensis.* This genus mainly identified by expanded crusts attached to substratum with rhizoids, pseudoparenchymatous tightly adjoined vegetative filaments composed of three parts (cortex, medulla and basal layers), with single chloroplast per cell, with hair arising from the basal layer, and medullary vegetative filaments upwardly curved to form a unilateral symmetry.



1. Thallus with firmly adhering erect filaments delimited into three part, cortex (C), medulla (M) and basal layer. Hairs arising from basal layer.

2. One chloroplast per cell

Fig. 54. Morphology of *Neoralfsiopsis* drawn from morphology observations in this study. This figure is not drawn to scale.

Neoralfiopsis is nested in a distinct clade in the Neoralfsiceae. *Neoralfsia* is similar to *Neoralfsiopsis* by having obvious delimitation of cortex and medullar, and presence of hairs and rhizoids. Although reproductive structures were not observed, the new candidate genus is molecularly and morphologically distinct. *Neoralfsiopsis* differs from *Neoralfsia* by having thallus divided into 3 parts and hairs arising from the basal part while the latter has thallus forming 2 parts and hairs arising from the middle of the medulla.

Family Pseudoralfsiaceae (Fig.55)

Pseudoralfsiaceae is distingushed by: crusts with or without rhizoids and with or without superimposed thalli, erect filaments are straight or curved upwards, cells with a single chloroplast without pyrenoids, hair pits are frequent, unangia are sessile, or on 1, rarely 2–3 pedicel cells, on the terminal part of erect filaments, with 1–2 paraphyses, intercalary plurangia are uniseriate or biseriate, terminated by 1–2 sterile cells (Parente et al. 2021).

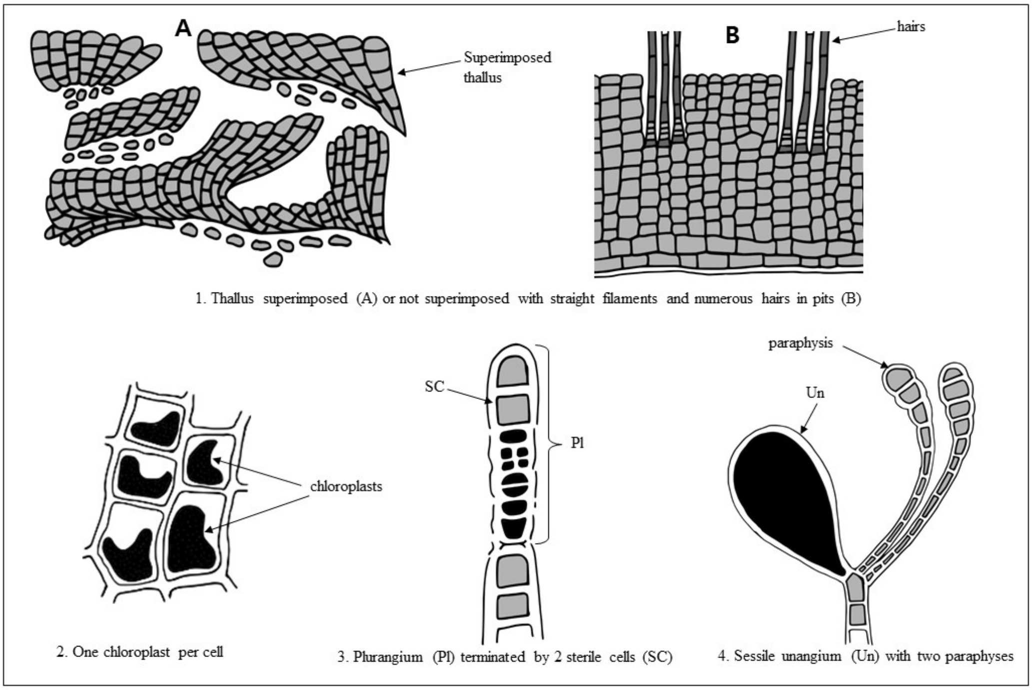


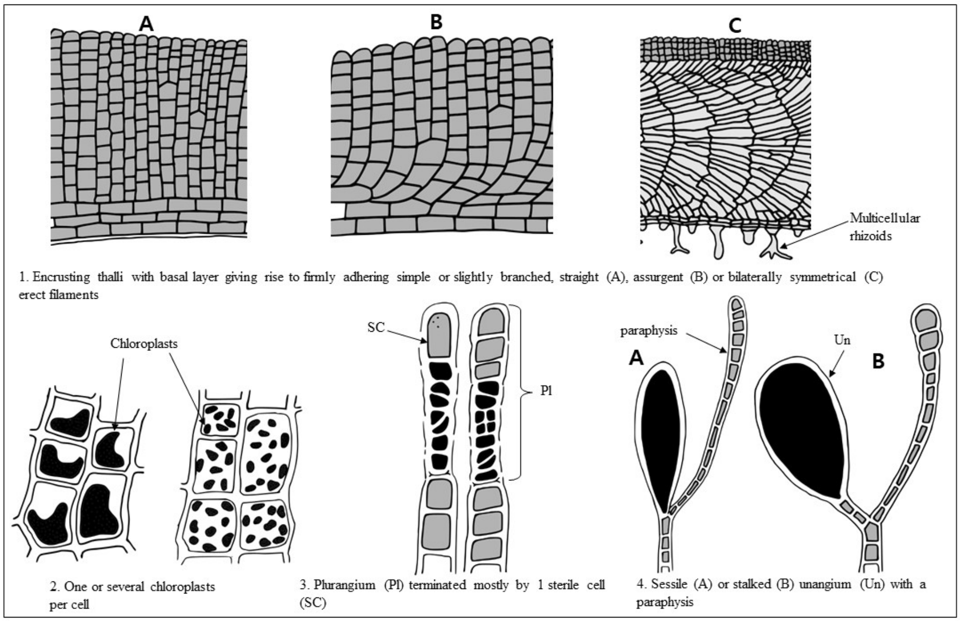
Fig. 55. Morphology of the type genus, *Pseudoralfsia* drawn with reference to the description of Parente et al. (2021). This figure is not drawn to scale.

This family was erected to accommodate *Ralfsia verrucosa*, a new pecies *P. azorica* and new genus and species *Nuchella vesicularis*, based on molecular and morphology (Parente et al.

2021). The thalli construction of species in this family grealty resembles that of *Ralfsia* sensu stricto Ralfsiaceae but can be distingiushed by having frequent hair pits, typically unsymmetrical thalli and in particular they are molecular distinct (Parente et al. 2021). Although *P. verrucosa* has been repoted in Korea (Keum 2010, Ryu and Kim 2021), I could not get any specimens of this species in this study.

Family Ralfsiaceae (Fig. 56)

Family Ralfsiaceae is distinguished by: encrusting thalli with basal layer composed of radiating appressed filaments one to several cells thick, with or without rhizoids, giving rise to firmly adhering simple or slightly branched, straight, assurgent or bilaterally symmetrical erect filaments, chloroplasts are single or few per cell, without pyrenoids, reproductive structures are terminal, or intercalary on vegetative filament and usually associated with surrounding paraphyses. (Farlow 1881, Abbott and Hollenberg 1976, Womersley 1987, Parente et al. 2021).



1. Encrusting thalli with basal layer giving rise to firmly adhering simple or slightly branched, straight (A), assurgent (B) or bilaterally symmetrical (C) erect filaments

2. One or several chloroplasts per cell

3. Plurangium (Pl) terminated mostly by 1 sterile cell (SC)

4. Sessile (A) or stalked (B) unangium (Un) with a paraphysis

Fig. 56. Morphology of the type genus, *Ralfsia* drawn with reference to the descriptions and updated views of (Farlow 1881, Abbott and Hollenberg 1976, Womersley 1987, Parente et al. 2021). This figure is not drawn to scale.

This family was erected based morphology with *Ralfsia* as the type genus (Farlow 1881). Currently there are four recognized genera in family Ralfsiaceae, *Analipus, Endoplura, Heteroralfsia*, and *Ralfsia* (Guiry and Guiry 2021). In this study, 2 new candidate genera, *Fissipedicella* and *Ramipedicella* from Korea are added to the Ralfsiaceae.

Key to the members of Ralfsiaceae

1. Plants with heteromorphic thalli, with creeping (crustose) portion bearing plurangia and erect sporophytic thalli bearing unangia …………….…...……………..…………… ***Heteroralfsia***

1. Plants with isomorphic thalli, with either creeping or erect thalli bearing both plurangia and unangia ………..…………………………………………………………………………. 2

2. Plants with isomorphic erect thalli bearing both plurangia and unangia .... ***Analipus***

2. Plants with isomorphic creeping thalli bearing both plurangia and unangia 3

3. Several chloroplasts per cell, unangia sessile or on soral filaments ……..……… ***Endoplura***

3. Single chloroplast per cell, unangia sessile or on a stalk of 1–2 cells ……………..…***Ralfsia***

4. Single chloroplast per cell, with single unangium on stalk of cleaved cells ………………………………………………………………………….. ***Fissipedicella***

4. Single chloroplast per cell, with one to several unangia on branched stalks …..………………………………………………………………..…. ***Ramipedicella***

Genus, *Analipus* (Fig. 57) is mainly identified by plants having rhizomatous perennial basal disc and annual erect thallus arising from the disc, erect thalli are caespitose, simple or branched, unangia and plurangia are formed on separate thalli, each cell contain a single chloroplast without pyrenoid, except for inner cells which contain several chloroplasts per cell, plurangia are uniseriate or biseriate and terminated by 1–3 sterile cells, unangia are formed from the basal part of assimilatory filaments, among assimilatory filaments, plants are isomorphic with alteration of generation between unangial plants and dioecious plurangial plants. (Kjellman 1889, Nakahara 1984).

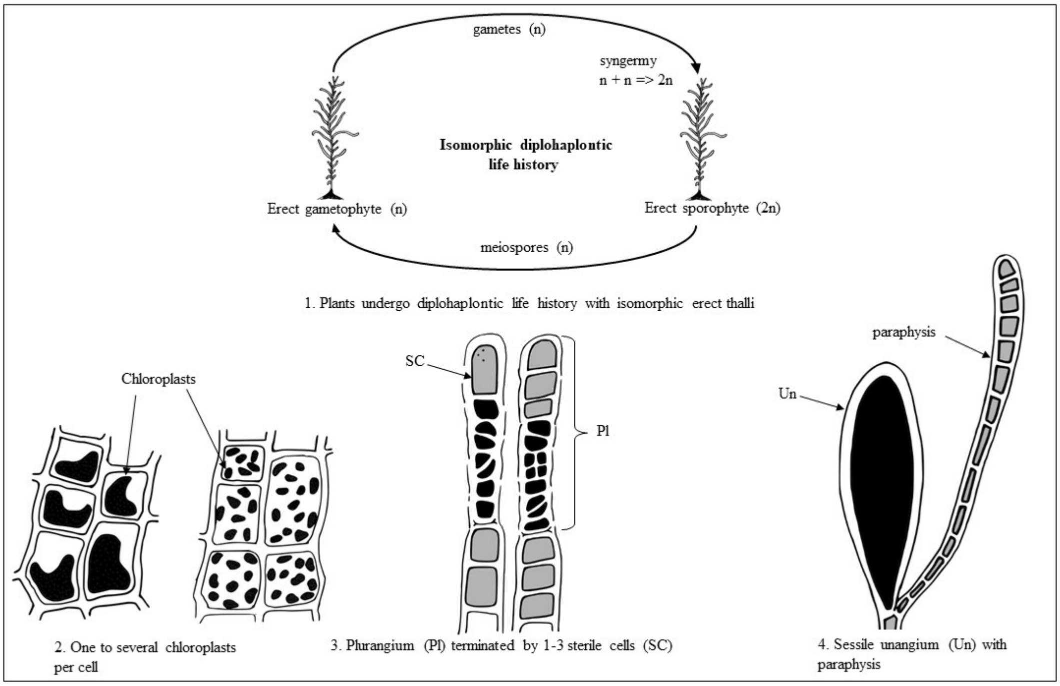


Fig. 57. Morphology of *Analipus* drawn from the description of Kjellman (1889). This figure is not drawn to scale.

Currently there are three recognized species inside the genus *Analipus*: *A. filiformis*, *A. gunjii* and *A. japonicus* (Guiry and Guiry 2021). Although *Analipus japonicus* was previously reported in Korea, there were no samples of this species in the present study.

Genus *Endoplura* (Fig. 58) is distinguished by thin crusts, firmly attached to the substratum with or without rhizoids, with firmly adjoined erect filaments from a hypothallial layer, cells with several to many chloroplasts, with or without hairs, plurangia biseriate to quadriseriate and terminated by a sterile portion consisting of 2–8 cells, unangia are sessile, on the terminal part of vegetative filaments or on soral filaments, with 1–2 paraphyses (Hollenberg 1969, Oteng'o et al. 2021).

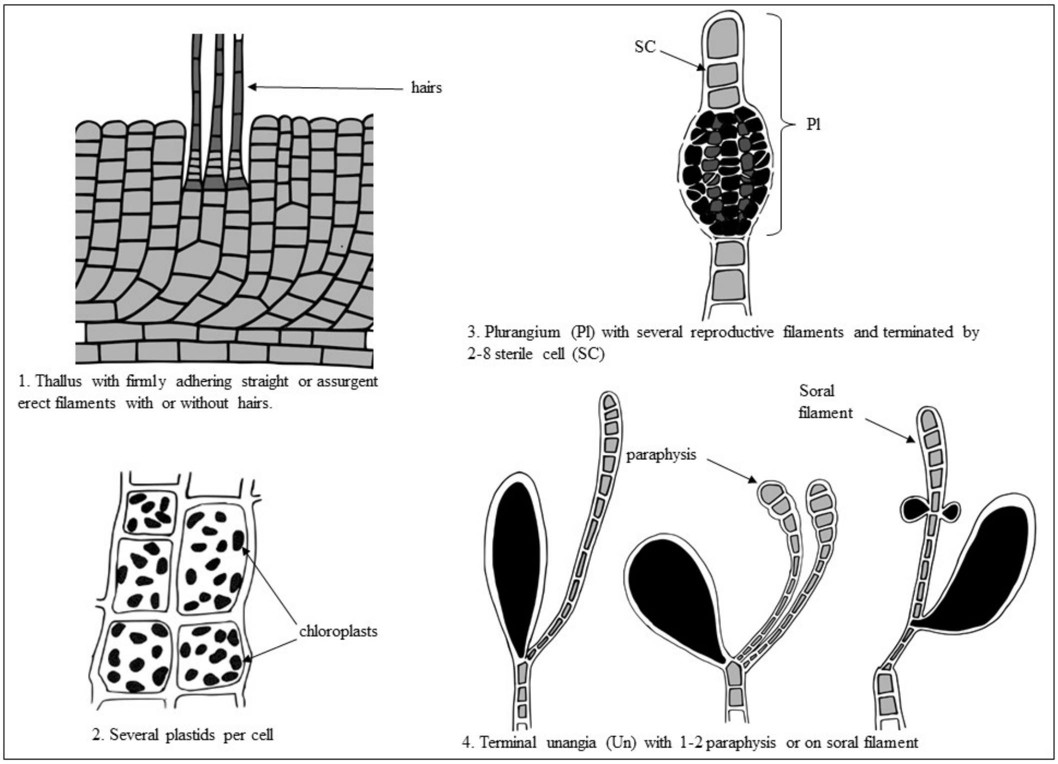


Fig. 58. Morphology of *Endoplura* drawn from the descriptions of Hollenberg (1969), Oteng'o et al. (2021) and other morphological observations in this study. This figure is not drawn to scale.

Endoplura currently consists of 3 recognized species, *E. aurea*, *E. jejuensis* and *E. koreana* (Guiry and Guiry 2021, Oteng'o et al. 2021). The latter two species were recently published from part of the results of this study (Oteng'o et al. 2021). There are yet 3 new candidate species in this genus, *E. geojensis*, *E. gyeokpoensis* and *E. limpeticola*. Below is a key to the species of this genus.

Key to the members of *Endoplura*

1.Crusts up to 10 cm or more broad, without hairs …………………….…………….……… ***E. aurea***

1.Crusts less than 3 cm broad, with hairs ..……………………...…………..……………….2

2.Soral filaments present ……………………………………...……..…… ***E. geojensis***

2.Soral filaments absent ……………………..…………..…………………………..…3

3.Undersuface with multicellular rhizoids ………….……………..………………..…. ***E. limpeticola***

3.Undersuface without multicellular rhizoids …………..…………..…………………..…..……. 4

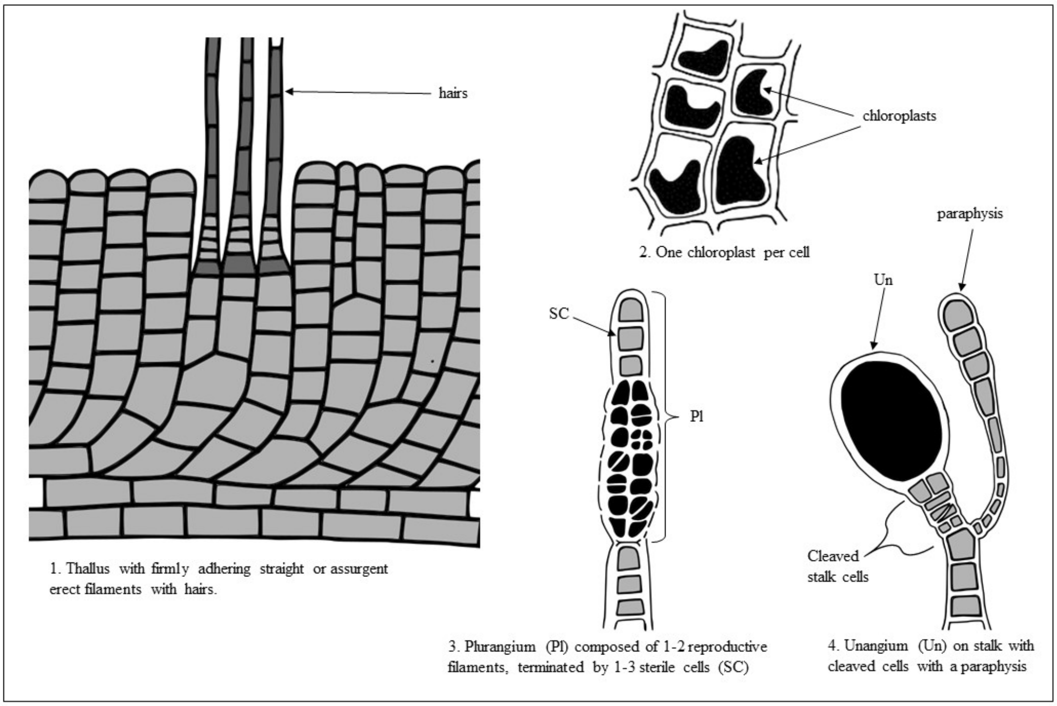
4.Plurangia terminated by 2–8 sterile cells ……………….....…..……..……. ***E. koreana***

4.Plurangia terminated by 2–4 sterile cells …..…………………………………..……5

5.Crusts less than 1 cm across, almost circular in outline, with inconspicuous growth lines and ridges, with distinct molecular data …………………………..………..……..……. ***E. jejuensis***

5.Crusts less than 1 cm across, irregular in outline, and without growth lines and ridges, with distinct molecular data ..……………………………..………..……...…….. ***E. gyeokpoensis***

Fissipedicella (Fig. 59) is a new candidate monotypic genus identified by crusts firmly attached to the substratum without rhizoids, hypothallial layer of one to several cell layers, perithalial layer of firmly adjoined, branched, straight or upwardly curved erect vegetative filaments, chloroplasts one per cell, hairs in hair pits arise from lower to mid cells of erect filaments, intercalary plurangia composed of one to two rows of plurangial reproductive filaments terminated by 1–3 sterile cells, unangia on pedicels of vertically to obliquely cleaved cells, with paraphyses. This genus accommodates one new candidate species, *F. orientalis.*



1. Thallus with firmly adhering straight or assurgent erect filaments with hairs.

2. One chloroplast per cell

3. Plurangium (Pl) composed of 1-2 reproductive filaments, terminated by 1-3 sterile cells (SC)

4. Unangium (Un) on stalk with cleaved cells with a paraphysis

Fig. 59. Morphology of *Fissipedicella* drawn from the morphological observations in this study. This figure is not drawn to scale.

Genus, *Heteroralfsia* (Fig. 60) is a monotypic genus with *H. saxicola* (Guiry and Guiry 2021). This genus is mainly characterized by undergoing a heteromorphic life history with annual erect thalli during summer, and a creeping portion that is probably perennial and produces intercalary plurangia terminated by a sterile cell. Creeping portion is composed of basal cell layer giving rise to closely packed, parallel, erect filaments. The erect portions of thalli bare sessile unangia, on the basal cell of assimilatory filaments. Cells with single chloroplast without pyrenoid, hairs are present (Kawai 1989).

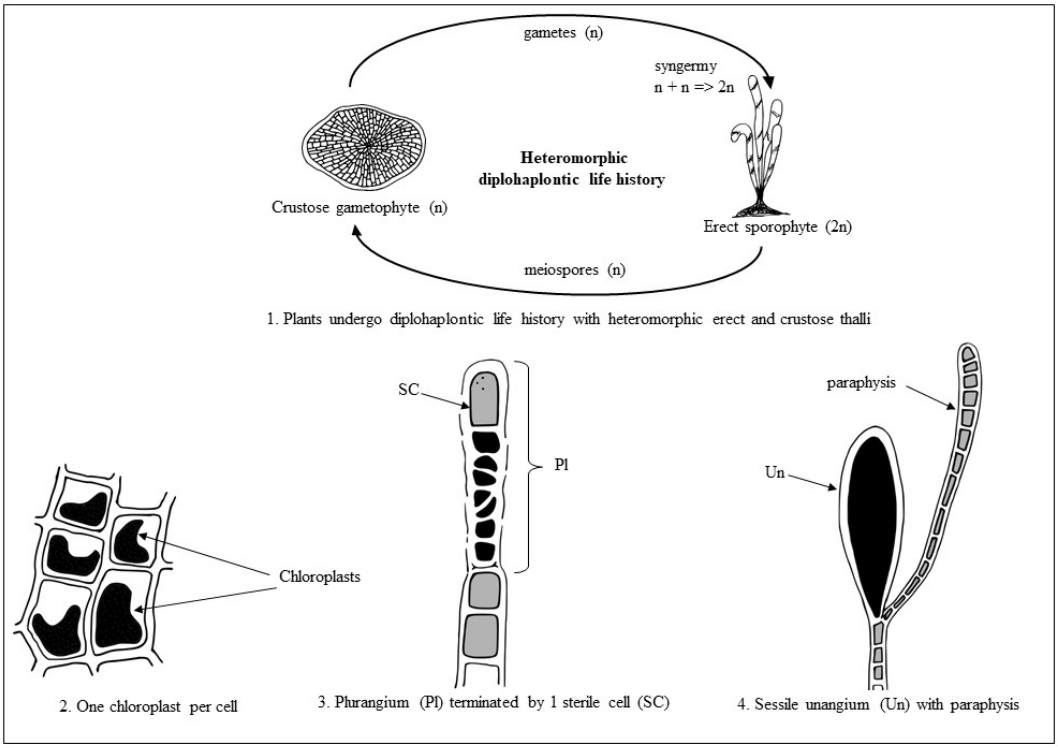


Fig. 60. Morphology of *Heteroralfsia* drawn from the description of Kawai (1989). This figure is not drawn to scale.

Genus, *Ralfsia* (Fig. 56) is the type genus of Ralfsiaceae and mainly identified by having expanded crusts attached to substratum with or without rhizoids, erect vegetative filaments tightly adherent, straight or curved, branched or simple, arising from a hypothallial layer, chloroplasts are one per cell, unangia are sessile or pedicellate, associated with multicellular paraphysis, intercalary plurangia terminated by one or more sterile cells (Setchell and Gardner 1924, Hamel 1931-1939, Hollenberg 1969, Abbott and Hollenberg 1976, Tanaka and Chihara 1980b, Fletcher 1987, Womersley 1987, Parente and Saunders 2019, Oteng'o and Won 2020). Species in this genus are mostly characterized by having sessile or stalked unangia on stalks of 1–2 cell (Tanaka and Chihara 1980b). There are 16 reconized species in this genus, *R. australis*, *R. confusa*, *R. endopluroides*, *R. fungiformis*, *R. hesperia*, *R. huanghaiensis*, *R. integra*, *R. longicellularis*, *R. lucida*, *R. ovata*, *R. pacifica*, *R. pedicellata*, *R. pusilla*, *R. robertii*, *R. tenebris* and *R. unimaculata* (Guiry and Guiry 2021). Although no previously reported *Ralfsia* species in Korea were in this study, a new candidate species, *R. claviformis* was uncovered. *R. claviformis* has a thallus construction similar to those of *R. confusa* and *R. tenebris*, but differs from the latter two by having thicker thalli, with intercalary plurangia terminated by 2–8 sterile cell, while the latter two have thinner thalli with plurangia terminated by a single sterile cell.

Genus, *Ramipedicella* (Fig. 61) is a new candidate genus characterized by crusts firmly attached to the substratum mostly with rhizoids, hypothallial layer give rise to firmly adjoined and usually assurgent erect filaments, cells of basal to apical portions of thalli consistently longer than wide with width to length ratio of 1:1–16, chloroplasts one per cell, hairs present or absent, plurangial reproductive structures uniseriate terminated by one to several sterile cells, unangia one to several on branched stalks or sessile, with paraphysis. Molecular and morphological analyses placed unidentified specimens and specimens of *R. longicellularis* in this genus forming two distinct lineages. Therefore *Ramipedicella* has one new candidate species *Ra. microloba* and a new candidate combination *Ra. longicellularis.* Although both species share the genus characteristics, *Ra. microloba* differs from *Ra. longicellularis* having mirolobes towards the center of the thallus, having hairs and plurangia terminated by 1–4 sterile cells while the later has relatively smooth surface without microlobes, hairs absent and plurangia terminated by 1–2 sterile cells.

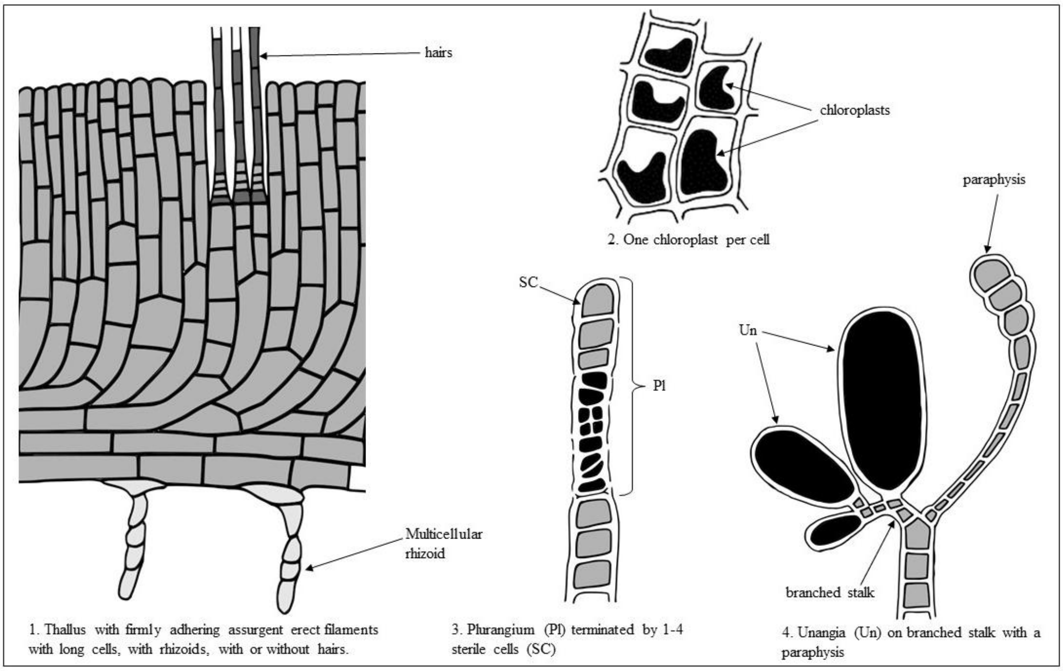


Fig. 61. Morphology of *Ramipedicella* drawn from the morphological observations in this study. This figure is not drawn to scale.

Family Sungmiaceae (Fig. 62)

Sungminiaceae is a monogeneric family distingushed by: small irregular crusts firmly attached to substratum without rhizoids, hypothallial layer of one to several cells, erect filaments moderately adjoined and partially separate with pressure, not tapered and with large apical vegetative cells, chloroplasts one per cell, without pyrenoid, intercalary plurangia terminated by a sterile cell; unangia on the terminal part of erect filaments, with a paraphysis.

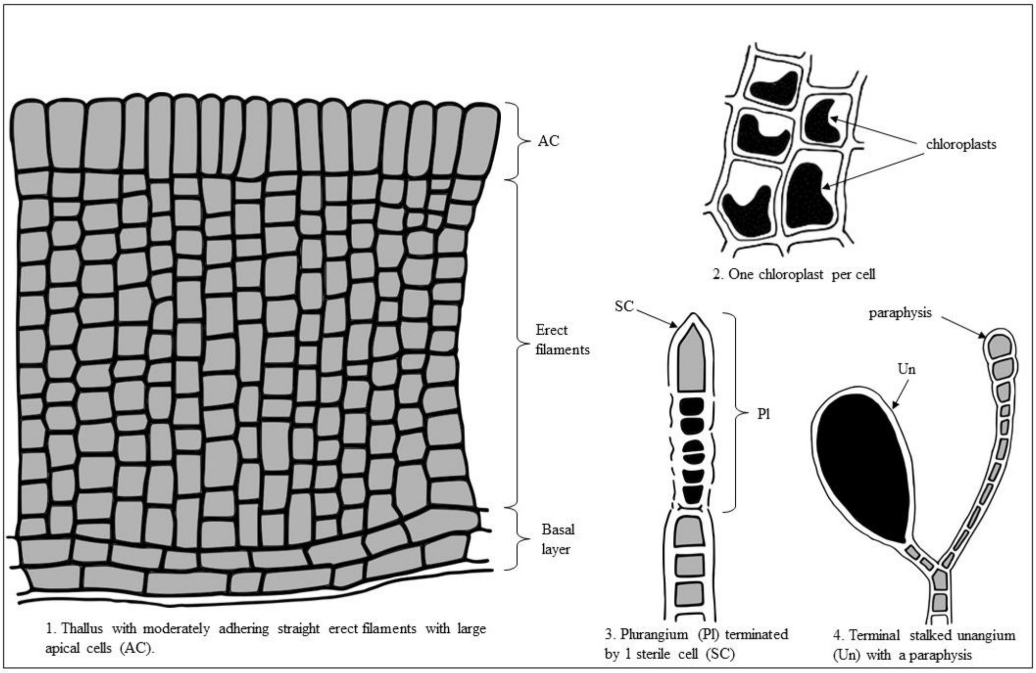


Fig. 62. Morphology of the type genus, *Sungminia* drawn from the morphological observations in this study. This figure is not drawn to scale.

This is a new candidate family erected to accommodate *Ralfsia*-like specimens from Korea studied during my analyses. They were phylogenetically close to Mesosporaceae but morphologically distinct and with high sequence divergence values both for *rbc*L and COI-5P that warranted creating a new family to accommodate the group. The family has three new candidate species, *Sungminia asiatica*, *S. gladiata*, and *S. pyriformis*. The species are identified based on a combination of characters including size of thallus and size and morphology of reproductive structures.

REFERENCES

Abbott I. A. & Hollenberg G. J. 1976. *Marine algae of California*. Stanford University Press, Stanford, CA. 827 pp.

Agardh J. G. 1847. Nya alger från Mexico. *Öfvers af Konglige Vetenskaps-Akademiens Förhandlingar, Stockolm* 4:5-17.

Agardh J. G. 1848. *Species, genera et ordines algarum*. *Algas fucoideas complectens, vol. 1*. C.W.K. Gleerup, Lund, Sweden. 363 pp.

Anderson R. J., Simons R. H. & Bolton J. J. 1988. *Zeacarpa leiomorpha* (Ralfsiaceae), a new genus and species of creeping marine Phaeophyceae from southern Africa. *Phycologia*. 27: 319-326.

Areschoug J. E. 1843. Algarum (Phycearum) minus rite cognitarum pugillus secundus. *Linnaea* 17: 257-269.

Areschoug J. E. 1847. Phycearum, quae in maribus Scandinaviae crescunt, enumeratio: sectio prior Fucuceas continens. *Nova Acta Regiae Sicietatis Scientiarum Upsaliensis* 13: 223-382.

Areschoug J. E. 1876. De algis nonnullis maris Baltici et Bahusiemsis. *Botaniska Notiser* 2: 33-37.

Barton E. S. 1903. List of marine algae collected at the Maldive and Laccadive Islands by J. S. Gardiner, Esq., M. A. *Journal of the Linnean Society of London*, *Botany* 35: 475-482.

Batters E. A. L. 1888. A description of three new marine algae. *Journal of the Linnean Society of London, Botany* 24: 450-453.

Bittner L., Payri C. E., Couloux A., Cruaud C., De Reviers B. & Rousseau F. 2008. Molecular phylogeny of the Dictyotales and their position within the Phaeophyceae, based on nuclear, plastid and mitochondrial DNA sequence data. *Molecular Phylogenetics and Evolution* 49: 211-226.

Boo S. M., Lee W. J., Yoon H. S., Kato A. & Kawai H. 1999. Molecular phylogeny of Laminariales (Phaeophyceae) inferred from small subunit ribosomal DNA sequences. *Phycological Research* 47: 109-114.

Børgesen F. 1912. Two crustaceous brown algae from the Danish West Indies. *Nuova Notarisia, Padova* 23:123-129.

Børgesen F. 1914. The marine algae of the Danish West Indies. Phaeophyceae. *Dansk Bot. Arkiv* 1:157-226.

Buchanan J. 2005. The crustose brown algae of New Zealand: A taxonomic study. MSc. dissertation, Victoria University of Wellington, Wellington, New Zealand. 126 pp.

Cho G. Y., Lee S. H. & Boo S. M. 2004. A new brown algal order, Ishigeales (Phaeophyceae), established on the basis of plastid protein-coding *rbc*L, *psa*A, *psb*A region comparisons. *Journal of Phycology* 40: 921-936.

Cormaci M., Furnari G., Catra M., Alongi G. & Giaccone G. 2012. Erratum. Flora marina bentonica del Mediterraneo: Phaeophyceae. *Bollettino dell'Accademia Gioenia di Scienze Naturali di Catania* 45: 509-510.

Costello M. J., Bouchet P., Boxshall G., Arvanitidis C. & Appeltans W. 2021. European Register of Marine Species. Available at http://www.marbef.org/data/erms.php. Accessed Aug 10, 2021.

Crouan P. L. & Crouan H. M. 1867. *Florule du Finistère: Contenant les descriptions de 360 espèces nouvelles de sporagames, de nombreuses observations et une synonymie des plantes cellulaires et vasculaires qui croissent spontanément dans ce département;*

accompagnées de trente-deux planches où est représentée l'organographie, faite sur l'état vif. des fruits et des tissus de 198 genres d'algues avec la plante grandeur naturelle ou réduite plus une planche supplémentiare ou sont figurés 24 champignons nouveaux. Friedrich Klincksieck, Paris. 262 pp.

Daugbjerg N. & Andersen R. A. 1997. Phylogenetic analyses of the *rbc*L sequences from haptophytes and heterokont algae suggest their chloroplasts are unrelated. *Molecular Biology and Evolution* 14: 1242-1251.

Dawson E. Y. 1944. *The marine algae of the Gulf of California, Allan Hancock Pacific Expeditions Vol. 3 Number 10*. University of Southern California Press, Los Angeles, CA. 189-432 pp.

De Toni G. B. 1895. *Sylloge algarum omnium hucusque cognitarum: Fucoideae Vol. 3*. Sumptibus auctoris, Padua. 638 pp.

Draisma S. G. A., Prud'Homme van Reine W. F., Stam W. T. & Olsen J. L. 2001. A reassessment of phylogenetic relationships within the Phaeophyceae based on Rubisco large subunit and ribosomal DNA sequences. *Journal of Phycology* 37: 586-603.

Farlow W. G. 1881. Marine algae of New England and adjacent coast. *In*: *Report of the U.S. Commissioner of Fish and Fisheries for 1879* (ed.). Government Printing Office, Washington, D.C. 1-210 pp.

Feldmann J. 1931. Contribution à la flore algologique marine de l'Algérie. Les algues de Cherchell. *Bulletin de la Société d'Historie Naturelle de l'Afrique du Nord* 22: 179-254.

Fletcher R. L. 1978. Studies on the family Ralfsiaceae (Phaeophyta) from around the British Isles *In* Irvine, D. E. G. & Price, J. (ed) *Modern approaches to the taxonomy of red and brown algae*. Academic Press, London, New York, 485 pp.

Fletcher R. L. 1987. *Seaweeds of the British Isles. Vol. 3. Part 1. Fucophyceae (Phaeophyceae)*. British Museum (Natural History), London. 359pp.

Foslie, M. H. 1892. Algological notices. *Det Kongelige Norske Videnskabers Selskabs Skrifter* 1892: 1-4 (263-266).

Fries E. M. 1845. *Summa vegetabilium Scandinaviae, seu enumeration, systematica et critica, plantarum tum cotyledonearum, quum nemearum inter mare occidentale et album, inter Eidoram et Nordkap, hactenus lectarum, una cum singulae distribution geographica*. A. Bonnier, Solus Operis Redemtor, Stockholm and Leipzig. 258 pp.

Gobi C. 1878. Die Algenflora des Weissen Meeres und der demselben zunächstliegenden Theile des nördlichen Eismeeres. *Mémoires* de l'Académie Impériale des sciences *de St. Petersburg, VIIé série* 26: 1-92.

Guiry M. D. & Guiry G. M. 2021. *AlgaeBase*. World-wide electronic publication, National University of Ireland, Galway. Available from: http://www.algaebase.org. Accessed Oct 10, 2021.

Gunnerus J. E. 1772. *Flora norvegica, observationibus praesertim oeconomicis panosque norvegici locupletata. Pars posterior, cum iconibus*. Vinding & F. C. Pelt, Trondheim, Copenhagen. 100 pp.

Hamel G. 1931–1939. *Phéophycées de France*. Paris, France. 431 pp.

Hamel G. 1939. Sur la classification des Ectocarpales. *Botaniska Notiser* 1939: 65-70.

Hardy F. G. & Guiry M. D. 2003. *A check-list and atlas of the seaweeds of Britain and Ireland*. British Phycological Society, Galway. 435 pp.

Harvey W. H. 1857. Algae. *In* Gray, A. (ed.) *Account of the Botanical specimens. Narrative of the expedition of an American squadron to the China Seas and Japan, performed in the years 1852, 1853 and 1854, under the command of Commodore M.C. Perry, United States Navy. Volume II - with illustrations*. Senate of the Thirty-third Congress, Second Session, Executive Document. House of Representatives, Washington, D.C. 331-332 pp.

Hollenberg G. J. 1942. Phycological Notes-I. *Bulletin of the Torrey Botanical Club* 69: 528-538.

Hollenberg G. J. 1969. An account of the Ralfsiaceae (Phaeophyta) of California. *Journal of Phycology* 5: 290-301.

Huelsenbeck J. P. & Ronquist F. 2001. MrBayes: Bayesian inference of phylogenetic trees. *Bioinformatics* 17: 754-755.

Hwang I. -K., Kim H. -S. & Lee W. J. 2005. Polymorphism in the brown alga *Dictyota dichotoma* (Dictyotales, Phaeophyceae) from Korea. *Marine Biology* 147: 999-1015.

Inagaki K. 1958. A Systematic Study of the Order Chordariales from Japan and Its Vicinity. *Scientific Papers of the Institute of Algological Research, Hokkaido University* 4: 87-197.

John D. M. & Lawson G. W. 1974. *Basispora*, a new genus of the Ralfsiaceae. *British Phycological Journal* 9: 285-290.

Kawai H. 1989. Life history and systematic position of *Heteroralfsia saxicola* gen. et comb. nov. (Ralfsiaceae, Phaeophyceae). *Phycologia* 28: 243-251.

Kawai H. & Sasaki H. 2004. Morphology, life history, and molecular phylogeny of *Stschapovia flagellaris* (Tilopteridales, Phaeophyceae) and the erection of the Stschapoviaceae. fam. nov. *Journal of Phycology* 40: 1156-1169.

Kawai H., Hanyuda T., Bolton J. & Anderson R. 2016. Molecular phylogeny of *Zeacarpa* (Ralfsiales, Phaeophyceae) proposing a new family Zeacarpaceae and its transfer to Nemodermatales. *Journal of Phycology* 52: 682-686.

Kawai H., Sasaki H., Maeba S. & Henry E. C. 2005. Morphology and molecular phylogeny of *Phaeostrophion irregulare* (Phaeophyceae) with proposal for Phaeostrophiaceae fam. nov., and a review of Ishigeaceae. *Phycologia* 44: 169-182.

Kawai H., Hanyuda T., Draisma S. G., Wilce R. T. & Andersen R. A. 2015. Molecular phylogeny of two unusual brown algae, *Phaeostrophion irregulare* and *Platysiphon glacialis*, proposal of the Stschapoviales ord. nov. and Platysiphonaceae fam. nov., and a re-examination of divergence times for brown algal orders. *Journal of Phycology* 51: 918-928.

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

Kjellman F. R. 1889. Om Beringhafvets algflora. *Kungliga Svenska Vetenskapsakademiens Handlingar* 23: 1-58.

Kogame K., Horiguchi T. & Masuda M. 1999. Phylogeny of the order Scytosiphonales (Phaeophyceae) based on DNA sequences of *rbc*L, partial *rbc*S, and partial LSU nrDNA. *Phycologia* 38: 496-502.

Kraft G. T. 2009. *Algae of Australia: Marine benthic algae of Lord Howe Island and the southern Great Barrier Reef, 2: Brown algae*. ABRS, Canberra; CSIRO Publishing, Melbourne. 364 pp.

Krayesky D. M., Norris J. N., Gabrielson P. W., Gabriel D. & Fredericq S. 2009. A new order of red algae based on the Peyssonneliaceae, with an evaluation of the ordinal classification of the Florideophyceae (Rhodophyta). *Proceedings of the Biological Society of Washington* 122: 364-391.

Kuckuck P. 1894. Bemerkungen zur marinen Algenvegetation von Helgoland. I. *Wissenschaftliche Meeresuntersuchungen. Neue Floge* 1: 223-263.

Kumar S., Stecher G., Li M., Knyaz C. & Tamura K. 2018. MEGA X: Molecular Evolutionary Genetics Analysis across computing platforms. *Molecular Biology and Evolution* 35: 1547-1549.

Kuntze O. 1898. *Revisio generum plantarum. Pars III (3).* Arthur Felix, Dulau & Co., U. Hoepli, Gust. A. Schechert, Charles Klincksierck, Leipzig, London, Milano, New York, Paris. 576 pp.

Kützing F. T. 1859. *Tabulae phycologicae; oder, Abbildungen der Tange. Vol. IX.* Gedruckt auf kosten des Verfassers (in commission bei W. Köhne), Nordhausen. 42 pp.

Kylin H. 1947. Die Phaeophyceen der schwedischen Westkuste. *Acta Universitatis Lundensis* 43: 1-99.

Lane C. E., Lindstrom S. C. & Saunders G. W. 2007. A molecular assessment of northeast Pacific *Alaria* species (Laminariales, Phaeophyceae) with reference to the utility of DNA barcoding. *Molecular Phylogenetics and Evolution* 44: 634-648.

Lanfear R., Frandsen P. B., Wright A. M., Senfeld T. & Calcott B. 2017. PartitionFinder 2: new methods for selecting partitioned models of evolution for molecular and morphological phylogenetic analyses. *Molecular biology and evolution* 34: 772-773.

Lee I. K. & Kang J. W. 1986. A check list of marine algae in Korea. *The Korean Journal of Phycology* 1: 311-325.

Lee Y. P. 2008. *Marine algae of Jeju*. Academy Publication, Seoul. 477 pp.

Lee Y. P. & Kang S. Y. 2001. *A Catalogue of the Seaweeds in Korea*. Jeju National University Press, Jeju. 662 pp.

León-Álvarez D. & González-González J. 1993. Algas Costrosas del Pacifico Tropical. *In* Salazar-Vallejo, S. I. & González, N. E. (ed.) *Biodiversidad marina y costera de México*. Comisión Nacional para el Conocimiento y Aprovechamiento de la Biodiversidad y CIQRO, México. 456-474 pp.

León-Álvarez D. & González-González J. 2003. The morphological distinction of *Ralfsia expansa* and *R. hancockii* (Ralfsiaceae, Phaeophyta) from Mexico. *Phycologia* 42: 613-621.

León-Álvarez D. & Norris J. N. 2005. Terminology and position of reproductive structures in crustose brown algae: misapplication, confusion and clarification. *Cryptogamie Algologie* 26: 91-102.

León-Álvarez D., Núñez-Resendiz M. L. & Pónce-Márquez M. E. 2014a. Morphological and molecular characterization of *Neoralfsia hancockii* comb. nov. (Ralfsiales, Phaeophyceae) from topotype of San José del Cabo, Baja California, Mexico. *Botanica Marina* 57: 139-146.

León-Álvarez D., Núñez-Resendiz M. L. & Wynne M. J. 2014b. Morphological and molecular studies on topotype material of *Neoralfsia expansa* (Phaeophyceae) reveal that Asian specimens assigned to this taxon are genetically distinct. *Botanica Marina* 57: 351-358.

León-Álvarez D., Reyes-Gómez V. P., Wynne M. J., Ponce-Márquez M. E. & Quiróz-González N. 2017. Morphological and molecular characterization of *Hapalospongidion* *gelatinosum*, Hapalospongidiaceae fam. nov. (Ralfsiales, Phaeophyceae) from Mexico. *Botanica Marina* 60: 567-581.

Lim P. -E., Sakaguchi M., Hanyuda T., Kogame K., Phang S. -M. & Kawai H. 2007. Molecular phylogeny of crustose brown algae (Ralfsiales, Phaeophyceae) inferred from *rbc*L sequences resulting in the proposal for Neoralfsiaceae fam. nov. *Phycologia* 46: 456-466.

Lindauer V. W. 1949. Notes on the marine algae of New Zealand. *Pacific Science* 3: 340-352.

Lobban C. S. & Wynne M. J. 1981. *The biology of seaweeds* Vol. 17. University of California Press, Berkeley and Los Angelses, USA. 786 pp.

Loiseaux S. 1968. Recherches sur les cycles de développement des Myrionémacées (Pheophycees), III: Tribu des Ralfsiées, IV: Conclusions générales. *Revue Génerales de Botanica* 75: 295-318.

Lund S. 1959. The marine algae of East Greenland I. Taxonomic part. *Meddelser om Gronland* 156: 1-247.

Lund S. 1967. *Ralfsia lucida*, a new brown alga from Danish waters. *Botaniste* 50: 287-295.

McNeill J., Barrie F. R., Burdet H. M., Demoulin V., Hawksworth D. L., Marhold K., et al. 2006. *International Code of Botanical Nomenclature (Vienna Code) adopted by the Seventeenth International Botanical Congress Vienna, Austria, July 2005. Vol. 146*. Gantner Verlag, Liechtenstein. 568 pp.

Nakahara H. 1984. Alternation of Generations of Some Brown Algae in Unialgal and Axenic Cultures. *Scientific Papers of the Institute of Algological Research, Faculty of Science, Hokkaido University* 7: 77-194.

Nakamura Y. 1972. A proposal on the classification of the Phaeophyta. *In* Abbott, I. A. & Kurogi, M. (ed.) *Contributions to the systematics of the benthic marine algae of the North Pacific*. Japanese Society of Phycology, Kobe. 147-156 pp.

Nelson W. A. 1980. *Analipus japonicus* (Harv.) Wynne (Phaeophyta): studies of its biology and taxonomy. Ph.D. dissertation, University of British Columbia, Vancouver, Canada. 244 pp.

Nelson W. A. 1982. A critical review of the order Ralfsiales, Ralfsiaceae and the taxonomic position of *Analipus japonicus* (Harv.) Wynne (Phaeophyta). *British Phycological Journal* 17: 311-320.

Norris J. N. 2010. *Marine algae of the northern Gulf of California: Chlorophyta and Phaeophyceae. Smithsonian contributions to botany; No. 94*. Smithsonian Institution Scholarly Press, Washington, D.C. 276 pp.

Oteng'o A. O. & Won B. Y. 2020. *Ralfsia longicellularis* (Ralfsiales, Phaeophyceae): a Far East Asian endemic brown alga from Korea. *Korean Journal of Environmental Biolology* 38: 101-105.

Oteng'o A. O., Cho T. O. & Won B. Y. 2021. *Endoplura jejuensis* sp. nov. and *Endoplura koreana* sp. nov. (Ralfsiales, Phaeophyceae) from Korea based on molecular and morphological analyses. *Algae* 36: 155-163.

Papenfuss G. F. 1967. Taxonomic and nomenclatural notes on three species of brown algae. *Botaniste* 50: 319-330.

Parente M. I. & Saunders G. W. 2019. A molecular survey of *Ralfsia* sensu stricto (Ralfsiales, Phaeophyceae) in Canada uncovers three new species: *R. robertii* sp. nov., *R. tenebris* sp. nov., and *R. unimaculata* sp. nov. *Botany* 97: 135-147.

Parente M. I., Fletcher R. L., Costa F. O. & Saunders G. W. 2021. Taxonomic investigation of *Ralfsia*-like (Ralfsiales, Phaeophyceae) taxa in the North Atlantic Ocean based on molecular and morphological data, with descriptions of Pseudoralfsiaceae fam. nov., *Pseudoralfsia azorica* gen. et sp. nov. and *Nuchella vesicularis* gen. et sp. nov. *European Journal of Phycology* 56: 12-23.

Perestenko L. P. 1980. *The Seaweeds of Peter the Great Bay*. Akademia NAUK SSSR, Leningrad, Russia. 231 pp.

Phillips J. A. & Price I. R. 1997. A catalogue of Phaeophyta (brown algae) from Queensland, Australia. *Australian Systematic Botany* 10: 683-721.

Phillips N., Burrowes R., Rousseau F., de Reviers B., & Saunders G. W. 2008. Resolving evolutionary relationships among the brown algae using chloroplast and nuclear genes. *Journal of Phycology* 44: 394-405.

Poong S. W. 2014. Taxonomy and phylogeny of crustose brown algae (Phaeophyceae) from Malaysia and Lombok Island, Indonesia. Ph.D. dissertation, University of Malaya, Kuala Lumpur, Malaysia. 309 pp.

Poong S. -W., Lim P. -E., Phang S. -M., Gerung G. S. & Kawai H. 2013. *Mesospora elongata* sp. nov. (Ralfsiales, Phaeophyceae), a new crustose brown algal species from the Indo-Pacific region. *Phycologia* 52: 74-81.

Poong S. W., Lim P. E., Phang S. M., Sunarpi H., West J. A. & Kawai H. 2014. A molecular-assisted floristic survey of crustose brown algae (Phaeophyceae) from Malaysia and Lombok Island, Indonesia based on *rbc*L and partial *cox*1 genes. *Journal of Applied Phycology* 26: 1231-1242.

Poong S. -W., Lim P. -E., Phang S. -M., Sunarpi H., West J. A., Miller K. A., Nelson W. A. & Kawai H. 2017. Two new species of *Mesospora* (Ralfsiales, Phaeophyceae) from the subtropical Indo-Pacific region. *Phycologia* 56: 487-498.

Rambaut A. 2012. FigTree, tree figure drawing tool v1.4.0. Institute of Evolutionary Biology, University of Edinburgh. Available from: http://tree.bio.ed.ac.uk/software/figtree/. Accessed Oct 2, 2021.

Riisberg I., Orr R. J., Kluge R., Shalchian-Tabrizi K., Bowers H. A., Patil V., Edvardsen B. & Jakobsen K. S. 2009. Seven gene phylogeny of heterokonts. *Protist* 160: 191-204.

Ronquist F. & Huelsenbeck J. P. 2003. MrBayes 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics* 19:1572-1574.

Rosenvinge L. K. 1893. Grønlands Havalger. *Meddelser om Grønland* 3: 763-981.

Rousseau F. & de Reviers B. 1999. Phylogenetic relationships within the Fucales (Phaeophyceae) based on combined partial SSU + LSU rDNA sequence data. *European Journal of Phycology* 34: 53-64.

Ruprecht F. J. 1850. *Algae ochotenses. Die ersten sicheren Nachrichten über die Tange des Ochotskischen Meeres*. Buchdruckerei der Kaiserlichen Akademie der Wissenschaften, St. Petersburg. 243 pp.

Ryu B. D. & Kim Y. S. 2021. Summer Marie Algal flora and vertical distribution in intertidal zone of uninhabited islands around Gageodo, Sinan-gun, west-southern coast of Korea. *Aquatic Nature* 1: 15-23.

Sasaki H., Flores-Moya A., Henry E. C., Müller D. G. & Kawai H. 2001. Molecular phylogeny of Phyllariaceae, Halosiphonaceae and Tilopteridales (Phaeophyceae). *Phycologia* 40: 123-134.

Saunders A. 1899. New or little-known brown algae of the Pacific Coast. *Erythrea* 7: 37-40.

Saunders G. W. 2005. Applying DNA barcoding to red macroalgae: a preliminary appraisal holds promise for future applications. *Philosophical transactions of the Royal Society of London. Series B, Biological sciences* 360: 1879-1888.

Saunders G. W. & McDevit D. C. 2012. Methods for DNA barcoding photosynthetic protists emphasizing the macroalgae and diatoms. *In* Kress, W. J. & Erickson, D. L. (eds.) *DNA Barcodes: methods and protocols*. Humana Press, Totowa, NJ. 207-222 pp.

Scagel R. F., Gabrielson P. W., Garbary D. J., Golden L., Hawkes M. W., Lindstrom S. C., Oliveira J. C. & Widdowson T. B. 1989. *A Synopsis of the Benthic Marine Algae of British Columbia, Southeastern Alaska, Washington and Oregon. Phycological Contribution, No. 3.* Department of Botany, University of British Columbia, Vancouver. 532 pp.

Schneider C. W. & Searles R. B. 1991. *Seaweeds of the Southeastern United States: Cape Hatteras to Cape Canaveral*. Duke University Press, Durham, N.C. 533 pp.

Setchell, W. A. 1924. *American Samoa: Part I. Vegetation of Tutuila Island. Part II. Ethnobotany of the Samoans. Part III. Vegetation of Rose Atoll, Vol. 20*. Department of Marine Biology of the Carnegie Institution of Washington, Washington DC. 275 pp.

Setchell W. A. & Gardner N. L. 1924. Phycological Contributions, VII. *University of California Publications in Botany* 13: 1-13.

Siemer B. L., Stam W. T., Olsen J. L. & Pedersen P. M. 1998. Phylogenetic relationships of the brown algal orders Ectocarpales, Chordiales, Dictyosiphonales, and Tilopteridales (Phaeophyceae) based on RuBisCo large subunit and spacer sequences. *Journal of Phycology* 34: 1038-1048.

Silberfeld T., Leigh J. W., Verbruggen H., Cruaud C., De Reviers B. & Rousseau F. 2010. A multi-locus time-calibrated phylogeny of the brown algae (Heterokonta, Ochrophyta, Phaeophyceae): Investigating the evolutionary nature of the "brown algal crown radiation". *Molecular Phylogenetics and Evolution* 56: 659-674.

Silberfeld T., Rousseau F. & de Reviers B. 2014. An updated classification of brown algae (Ochrophyta, Phaeophyceae). *Cryptogamie Algologie* 35: 117-156.

Silva, P. C. 1996–2018. Index Nominum Algarum, Bibliographia Phycologia Universalis. University Herbarium, University of California, Berkeley. Available from:http://ucjeps.berkeley.edu/CPD/. Accessed Oct 15, 2021.

Silva P. C., Basson P. W. & Moe R. L. 1996. *Catalogue of the benthic marine algae of the Indian Ocean Vol. 79*. University of California Press, Berkeley, CA. 1261 pp.

Silva P. C. & de Reviers B. 2000. Ordinal names in the Phaeophyceae. *Cryptogamie Algologie* 21: 49-58.

Silvestro D. & Michalak I. 2012. raxmlGUI: a graphical front-end for RaxML. *Organisms Diversity & Evolution* 12: 335-337.

Skottsberg C. 1921. Botanische Ergebnisse der Schwedischen Expedition nach Patagonien und dem Feuerlande 1907-1909. VIII. Marine Algae. I. Phaeophyceae. *Kungliga Svenska Vetenskapsakademiens Handlingar* 61: 1-56.

Smith J. E. & Sowerby J. 1843. *Supplement to the English Botany of the late Sir J.E. Smith and Mr. Sowerby. The descriptions, synonyms, and places of growth by William Jackson Hooker, LL.D. Regius Professor of Botany in the University of Glasgow, F.R.S. F.S.A. L.S. &c. &c. and other eminent botanists. The figures by James De Carle Sowerby, F.L.S. &c. Vol. III.*

Sold by the proprietor, J. Sowerby, 3 Mead Place, Lambeth, Longman and Co. and Sherworrd and Co., Patenoster Row, London, UK. 2867 pp.

Smith G. M. 1944. Marine algae of the Monterey peninsula, California. Stanford University Press, Stanford. 622 pp.

Sophiammal Nettar P. & Panikkar M. V. N. 2009. Two new brown algal species from the family Ralfsiaceae (Ectocarpales, Phaeophyceae) from Kerala, India. *Seaweed Research and Utilisation* 31: 7-10.

South G. 1974. *Herpodiscus* gen. nov. and *Herpodiscus durvilleae* (Lindauer) comb. nov., a parasite of *Durvillea antarctica* (Chamisso) Hariot, endemic to New Zealand. *Journal of the Royal Society of New Zealand* 4: 455-461.

Starko S., Bringloe T. T., Gomez M. S., Darby H., Graham S. W. & Martone P. T. 2021. Genomic rearrangements and sequence evolution across brown algal organelles. *Genome Biology and Evolution* 13:evab124.

Svedelius N. 1930. Über die sogenannten Süsswasser-Lithoderman. *Zeitschrift für Botanik* 23: 891-918.

Tan I. H. & Druehl L. D. 1994. A molecular analysis of *Analipus* and *Ralfsia* (Phaeophyceae) suggests the order Ectocarpales is polyphyletic. *Journal of Phycology* 30: 721-729.

Tanaka J. & Chihara M. 1980a. Taxonomic study of the Japanese crustose brown algae (1). General account and the order of Ralfsiales. *Journal of Japanese Botany* 55: 193-201.

Tanaka J. & Chihara M. 1980b. Taxonomic study of the Japanese crustose brown algae (2). Ralfsia (Ralfsiaceae, Ralfsiales), part 1. *Journal of Japanese Botany* 55: 225-236.

Tanaka J. & Chihara M. 1980c. Taxonomic study of the Japanese crustose brown algae (3). *Ralfsia* (Ralfsiaceae, Ralfsiales), part 2. *Journal of Japanese Botany* 55: 337-342.

Tanaka J. & Chihara M. 1981a. Taxonomic study of the Japanese crustose brown algae (4). *Ralfsia* (Ralfsiaceae, Ralfsiales), part 3. *Journal of Japanese Botany* 56: 97-104.

Tanaka J. & Chihara M. 1981b. Taxonomic study of Japanese crustose brown algae (5). *Endoplura* and *Diplura* (Ralfsiaceae, Ralfsiales). *Journal of Japanese Botany* 56: 153-160.

Tanaka J. & Chihara M. 1981c. Taxonomic study of Japanese crustose brown algae (6). *Pseudolithoderma* (Lithodermataceae, Ralfsiales). *Journal of Japanese Botany* 56: 377-381.

Tanaka J. & Chihara M. 1982. Morphology and taxonomic of *Mesospora schmidtii* Weber van Bosse, Mesosporaceae fam. nov. (Ralfsiales, Phaeophyceae). *Phycologia* 21: 382-389.

Taylor W. R. 1945. Pacific marine algae of the Allan Hancock Expeditions to the Galapagos Islands. *Allan Hancock Pacific Expeditions* 12: 1-528.

Taylor W. R. 1957. *Marine algae of the northeastern coast of North America*. The University of Michigan Press, Ann Arbor, Michigan. 509 pp.

Thompson J. D., Higgins D. G. & Gibson T. J. 1994. CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucleic Acids Research* 22: 4673-4680.

Trobajo R., Mann D. G., Clavero E., Evans K. M., Vanormelingen P. & McGregor R. C. 2010. The use of partial *cox*1, *rbc*L and LSU rDNA sequences for phylogenetics and species identification within the *Nitzschia palea* species complex (Bacillariophyceae). *European Journal of Phycology* 45: 413-425.

Vieira C., Camacho O., Wynne M. J., Mattio L., Anderson R. J., Bolton J. J., Sansón M., D'hondt S., Leliaert F., Fredericq S., Payri C. & De Clerck O. 2016. Shedding new light on old algae: matching names and sequences in the brown algal genus *Lobophora* (Dictyotales, Phaeophyceae). *Taxon* 65: 689-707.

Weber-van Bosse A. 1911. Notice sur quelques genres nouveaux d'algues de l'Archipel Malaisien. *Annales du Jardin Botanique de Buitenzorg* 24: 25-33.

Weber-van Bosse A. 1913. Liste des algues du Siboga. I. Myxophyceae, Chlorophyceae, Phaeophyceae. *Siboga Expeditie Monographie* 59a: 1-186.

West J. A. & Calumpong H. P. 1996. *Mesospora negrosensis* sp. nov. (Phaeophyta) from the Philippines. *The Philippine Scientist* 33: 5-15.

Wilce R., Webber E. & Sears J. 1970. *Petroderma* and *Porterinema* in the New World. *Marine Biology* 5: 119-135.

Womersley H.B.S. 1987. *The marine benthic flora of southern Australia*. Part II. South Australian Government Printing Division, Adelaide, Australia. 484 pp.

Wynne M. J. 1969. Life history and systematic studies of some Pacific North American Phaeophyceae (brown algae). *University of California Publications in Botany* 50: 1-88.

Wynne M. J. 1971. Concerning the phaeophycean genera *Analipus* and *Heterochordaria*. *Phycologia* 10: 169-175.

Wynne M. J. 1981. Phaeophyta: Morphology and Classification. *In* Lobban, C. S. & Wynne, M. J. (ed.) *The Biology of Seaweeds*. *Botanical Monographs, No. 17*. University of California Press, Berkeley. 52-85 pp.

Wynne M. J. & Loiseaux S. 1976. Recent advances in life history studies of the Phaeophyta. *Phycologia* 15: 435-452.

Xiyi L. & Junfeng L. 1993. *Ralfsia huanghaiensis*, a new species of *Ralfsia* (Ralfsiales, Phaeophyta). *Oceanologia et Limnologia Sinica* 24: 172-176.

Yamada Y. 1928. Report of the Biological Survey of Mutsu Bay, 9: Marine Algae of Mutsu Bay and Adjacent Waters, II. *Science Reports of the Tohoku Imperial University, 4th Series, Biology* 3: 497-534.

Yang M. Y., Yang E. C. & Kim M. S. 2020. Genetic diversity hotspot of the amphi-Pacific macroalga *Gloiopeltis furcata sensu lato* (Gigartinales, Florideophyceae). *Journal of Applied Phycology* 32: 2515-2522.

Yendo K. 1913. Some new algae from Japan. *Nyt Magazin for Naturvidenskaberne* 51: 275-288.

Yoshida T. 1997. Japanese marine algae: new combinations, new names and new species. *Phycological Research* 45: 163-167.

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