Polysiphonia ulleungensis sp. nov. (Rhodomelaceae, Rhodophyta): a new diminutive species from Korea belonging to Polysiphonia sensu stricto

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Polysiphonia sensu stricto is characterized by having 4 ecorticate pericentral cells, rhizoids in open connection with the pericentral cells, four-celled carpogonial branches, spermatangial branches replacing the whole trichoblast, and tetrasporangia arranged in straight series. Polysiphonia ulleungensis sp. nov. is newly described from Sadongri, Ulleung Island, Korea, based on morphological and molecular evidence. It is mainly characterized by having ecorticate axes with 4 pericentral cells, apical cells transversely or obliquely divided, unicellular rhizoids in open connection with pericentral cells, very scarce trichoblasts and scar cells, procarps with a four-celled carpogonial branch, and spermatangial branches replacing the whole trichoblast. Polysiphonia ulleungensis is closely similar in morphology to P. atlantica sensu lato. We concluded that materials of P. atlantica sensu Nam and Kang from Korea correspond to P. ulleungensis. By contrast, the new species differs morphologically from the Atlantic specimens of P. atlantica as well as from P. atlantica sensu Kim and Lee from Korea. Morphological characteristics and rbcL sequence analyses support the taxonomic placement of P. ulleungensis within Polysiphonia sensu stricto.

Key Words: Polysiphonia atlantica; Polysiphonia ulleungensis sp. nov.; rbcL; Rhodomelaceae; taxonomy

INTRODUCTION

Polysiphonia Greville is a large, morphologically heterogeneous group of red algae. Kim and Lee (1999) attempted to resolve some of this heterogeneity by segregating some of the species that are not considered to represent Polysiphonia sensu stricto into the new genus Neosiphonia Kim et Lee. Subsequently, it was established the Polysiphonia sensu stricto group, which has been confirmed by analyses of nuclear-encoded 18S rDNA (small subunit) sequences (Choi et al. 2001, Mamoozadeh and Freshwater 2011).

The type species of Polysiphonia is P. urceolata (Lightfoot ex Dillwyn) Greville, a synonym of P. stricta (Dillwyn) Greville. Polysiphonia sensu stricto is characterized by having ecorticate axes with 4 pericentral cells, rhizoids in open connection with pericentral cells, four-celled carpogonial branches, spermatangial branches replacing the whole trichoblasts, and tetrasporangia arranged in straight series (Magg and Hommersand 1993, Kim et al. 2000, Choi et al. 2001). Currently, the combination of morphological and molecular analyses have confirmed the following Polysiphonia sensu stricto species from

Yoon (1986) published a study on the morphology of 15 *Polysiphonia sensu lato* species from Korea, but only 5 of these species are currently recognized as members of the *Polysiphonia sensu stricto* group: *P. atlantica*, *P. morrowii*, *P. scopulorum*, *P. senticulosa*, and *P. subtilissima* (Lee and Kang 2002). The vegetative and reproductive morphology of most of these species has been well characterized in Korea (Kim et al. 1994, Kim and Lee 1996, Nam and Kang 2012), but none has been included in molecular analyses.

We collected unidentified samples of *Polysiphonia sensu stricto* from Sadongri, Ulleung Island, Korea in the spring of 2013. In this study, we characterize these samples morphologically and examine their phylogenetic relationships by analyses of *rbcL* sequences.

**MATERIALS AND METHODS**

**Morphology**

Samples were collected from Ulleung Island, Korea, during April 2013. They were preserved in 4-5% formalin / seawater for morphological study and in silica gel for molecular study. Microscope observations were made on materials stained with 1% aqueous aniline blue acidified with 0.1% diluted HCl. Photomicrographs were taken under an Olympus microscope (BX51TRF; Olympus, Tokyo, Japan) with an Olympus DP71 camera. A total of 25 individuals from 5 tufts were selected for the determination of quantitative characters and their means and standard deviations were calculated. Voucher specimens were deposited in the herbarium of Chosun University (CUK), Korea.

**Molecular study**

Genomic DNA was extracted from silica gel-dried samples using the NucleoSpin Plant II kit (Macherey-Nagel, Düren, Germany), following the manufacturer’s instructions. Polymerase chain reactions (PCR) were set up with a final volume of 20 μL using 2.5 μL of genomic DNA, 2 μL of 10 mM dNTP mix, 2 μL of 10× reaction buffer, 1 μL of 5-10 μM forward and reverse primers and 0.2 μL of TOP DNA polymerase (Bioneer, Daejeon, Korea). The *rbcL* locus was amplified using the primer combination F7-R753 and F645-Rrbcst (Lin et al. 2001, Bustamante et al. 2013) and purified with PCR quick-spin PCR product purification kit (iNtRON Biotechnology Inc., Seongnam, Korea). Cycle sequencing was performed with the primers F7, F645, F993, R376, R753, R1150, and RrbcStart (Freshwater and Rueness 1994, Cho et al. 2003, Bustamante et al. 2012). Sequences were determined for both forward and reverse strands using an ABI Prism 3100 Genetic Analyzer (Life Technologies, Seoul, Korea). A *rbcL* sequence was obtained from *Polysiphonia ulleungensis* sp. nov. and was deposited in EMBL / GenBank under the accession number KJ028026. This sequence and others obtained from GenBank were initially aligned with ClustalW (Thompson et al. 1994) and adjusted manually using the MEGA5 software (Tamura et al. 2011). Maximum likelihood analyses were conducted with 1,000 bootstrap replications in MEGA5 using the GTR + I model. A Bayesian inference was performed using MrBayes 3.1.2 (Huelsenbeck and Ronquist 2001, Ronquist and Huelsenbeck 2003). The Markov chain Monte Carlo runs were carried out for 2,000,000 generations each with 1 cold chain and 3 heated chains employing the GTR + I + I evolutionary model, sampling and printing every 1,000 generations. Summary trees were generated using a burn-in value of 200.

**RESULTS**

*Polysiphonia ulleungensis* D. E. Bustamante, B. Y. Won et T. O. Cho sp. nov.

**Diagnosis.** Thalli 0.9-1.8 cm tall, saxicolous or epiphytic, and composed of prostrate and erect systems. Axes with 4 pericentral cells, ecorticate throughout, and alternate branches which are independent from trichoblasts. Trichoblasts small and scarce and their scar cells inconspicuous and scarce. Rhizoids unicellular, in open connection with and produced from the center or the proximal end of pericentral cells. Procarps bearing a four-celled carpogonial branch. Spermatangial branches replacing trichoblast.

**Holotype.** CUK9483 (Fig. 1A).

**Type locality.** Sadongri, Ulleung-eup, Ulleung-gun (Ulleung Island), Gyeongsangbuk-do, Korea; 37°28’11.04” N,
Fig. 1. Vegetative structures of *Polysiphonia ulleungensis* sp. nov. (A) Holotype specimen (CUK9483) from Sado, Dokdo-ri, Ulleung Island, Korea. (B) Habit of vegetative plant showing the extended prostrate axes and regularly branched erect axes. (C) Thallus with an alternate branching pattern. (D) Young erect axes showing short segments with alternate laterals and without trichoblasts. (E) Lower part of erect axes showing long segments without scar cells. (F) Apices showing obliquely (arrow) and transversely (arrowhead) divided apical cells. (G & H) Cross section views of erect axes (G) and prostrate axes (H). ax, axial cell; p, pericentral cell. (I & J) Rhizoids (r) showing open connection to pericentral cells (p). Scale bars represent: A, 5 mm; B, 1 mm; C, 200 μm; D, 50 μm; E & I, 100 μm; F & G, 10 μm; H & J, 20 μm.
Fig. 2. Reproductive structures of *Polysiphonia ulleungensis* sp. nov. (A) Female gametophyte. (B) Upper part of female thallus showing subapical cystocarps (cy) in densely branched axes with scarce trichoblast (arrowhead). (C) Procarp with a four-celled carpogonial branch. cb1-cb3, sequence of carpogonial branch cells. cp, carpogonium; su, supporting cell. (D) Young cystocarps. (E) Mature cystocarp showing slightly urceolate shape. (F) Young spermatangial branch (arrowhead) replacing trichoblast. (G) Mature spermatangial branch having spermatangia. Scale bar represents: A, 2 mm; B, 100 μm; C & F, 20 μm; D, 10 μm; E & G, 50 μm.
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*Polysiphonia ulleungensis* sp. nov. from Korea

**Isotype.** CUK9583.

**Etymology.** The specific epithet ‘ulleungensis’ is derived from the collection locality, the island of Ulleung.

**Description.** Plants are diminutive, 0.9-1.8 cm high (Fig. 1A), blackish red in color, and associated with other filamentous species. They form small tufts and are predominantly attached to rock surfaces of tide pools. Thalli are composed of prostrate and erect system (Fig. 1B). Prostrate system is extensive, entangled, and decumbent with rigid texture. Segments of prostrate axes are 85.45 ± 7.56 μm long and 61.36 ± 5.45 μm in diameter, being 0.7 times broader than long (1.4 ± 0.18 in L / D). The erect system is composed of interwoven indeterminate axes. Erect axes are slender (Fig. 1E), delicate, and arise endogenously from prostrate axes at intervals of 1-4 (3.24 ± 1.39) axial cells (Fig. 1B). They are composed of 4 pericentral cells (Fig. 1G & H), ecorticate throughout and radially branched in an alternate pattern every 2-3 axial cells (Fig. 1C). Adventitious branches are absent. Young erect axes are slightly curved in the direction of the apices of prostrate axes (Fig. 1B) and have short segments (Fig. 1D). Older segments of erect axes are 70.15 ± 43.67 μm long and 35.76 ± 10.71 μm in diameter, being 0.5 times broader than long (1.98 ± 1.02 in L/D). Apical cells are prominent, 6.78 ± 1.85 μm long and 6.32 ± 1.04 μm wide, and obliquely or transversely divided (Fig. 1F). Trichoblasts are absent in vegetative thalli. Rhizoids are ventrally produced from the center or proximal end of pericentral cells of prostrate axes. They are in open connection with pericentral cells, unicellular with multilobed terminations, and 33.33 ± 12.8 μm in diameter and 180.68 ± 87.50 μm long (Fig. 1I & J).

Erect axes of female gametophytes are densely branched distally and bear small, scarce trichoblasts (Fig. 2A & B). Procarps are positioned laterally and subapically on erect axes and are composed of a supporting cell bearing a four-celled carpogonial branch and a basal sterile cell (Fig. 2C). Cystocarps are elongate, slightly urceolate (Fig. 3D & E), 270.01 ± 72.73 μm high, and 200.79 ± 42.43 μm in diameter. Spermatangial branches of male gametophytes are clustered at the apices of erect axes, replace the whole trichoblast, develop one to several segments apart (Fig. 2F) and they have 2-4 sterile tip cells when mature (Fig. 2G). Tetrasporangial plants were not found.

**Habitat and distribution.** Plants grow from the intertidal to subtidal zones. They were found in sheltered to wave-exposed areas, and attached to rocks inside tide pools. Tufts of *P. ulleungensis* were entangled with filamentous algae like *Antithamnion*, *Ceramium*, and other *Polysiphonia* species.

**Phylogenetic analyses.** A 1,245-bp portion of the 1,467-bp *rbcL* (84.8%) was sequenced for *Polysiphonia ulleungensis* sp. nov. This sequence was aligned with 31 other *Polysiphonia sensu lato* sequences downloaded from GenBank, and *Lophosiphonia* sp. (GU385835) and *Herposiphonia* sp. (GU385834) were included as outgroups. Phylogenetic analyses placed *Polysiphonia ulleungensis* sp. nov. sister to *Polysiphonia* sp. (EU492920) within a clade of *Polysiphonia sensu stricto* (Fig. 3). The sequence divergence between *P. ulleungensis* and *Polysiphonia* sp. (EU492920) was 3.2%. *Polysiphonia ulleungensis* diverged from eastern Atlantic and western Atlantic specimens of *P. atlantica* by 9.6% and 8.4%, respectively.

**DISCUSSION**

*Polysiphonia ulleungensis* sp. nov. is newly described from Sadongri, Ulleung Island, Korea, based on morphological and molecular evidence. *P. ulleungensis* is mainly characterized by having 4 pericentral cells, ecorcicate thallus, unicellular rhizoids in open connection with pericentral cells, very scarce trichoblasts and scar cells, branches independent from trichoblasts, procarps with a four-celled carpogonial branch, and spermatangia replacing the whole trichoblast. These character states are consistent with its classification in *Polysiphonia sensu stricto*, and *rbcL* sequence analyses also support this taxonomic placement.

*Polysiphonia ulleungensis* sp. nov. is morphologically most similar to *P. atlantica*. *P. atlantica* was originally described by Harvey (1836) from Ireland as *P. macrocarpa* Harvey nom. Illeg. and later renamed as *P. atlantica* by Kapraun and Norris (1982). Maggs and Hommersand (1993) described in detail the morphology of *P. atlantica* from the British Isles, and European specimens were sequenced by Bárbara et al. (2013) and Díaz-Tapia and Bárbara (2013). *P. atlantica* has been reported from the northeastern Atlantic, western Atlantic, and Korea (Kapraun 1977, Yoon 1986, Maggs and Hommersand 1993, Kim and Lee 1996, Stuercke and Freshwater 2008, Mamoozadeh and Freshwater 2011, Nam and Kang 2012, Bárbara et al. 2013, Díaz-Tapia and Bárbara 2013). However, these records apparently involve four species, as it is explained below. Although our *P. ulleungensis* is similar to the European *P. atlantica* in having a diminutive habit and the typical features for the *Polysiphonia sensu stricto* members, the former is morphologically distinguished from the latter by the scarce trichoblasts and 2-4 sterile...
Polysiphonia atlantica sensu Polysiphonia atlantica sensu stricto has not been included in molecular analyses, and it may represent a different species of Polysiphonia sensu stricto. Polysiphonia atlantica sensu Nam and Kang (2012) was collected from the eastern and southern coasts of Korea. It is distinguished from European P. atlantica by the scarce trichoblasts and 2-4 sterile tip cells in the spermatangia. It also differs from P. atlantica sensu Kim and Lee (1996) in tip cells in the spermatangia (Table 1).

P. atlantica was initially reported and described from Korea by Yoon (1986), and additional detailed morphological descriptions were provided by Kim and Lee (1996) and Nam and Kang (2012). Kim and Lee (1996) reported the plants collected from Daesambudo as “P. atlantica” on the basis of field and laboratory cultured materials. However, these plants are distinguished from P. atlantica described from Europe by paired branches being borne on one side of the axis and then a pair on the other side.

Fig. 3. Phylogenetic tree based on maximum likelihood analysis of rbcL sequences. Value above branches = maximum likelihood bootstrap values (BS) >50% / Bayesian posterior probabilities (BPP) >0.75. Values lower than BS 50 or BPP 0.75 are indicated by hyphens (-). Values of BPP 1.00 or BS 100 are indicated by asterisks (*).

Polysiphonia atlantica sensu stricto

Polysiphonia atlantica sensu Kim and Lee (1996) has not been included in molecular analyses, and it may represent a different species of Polysiphonia sensu stricto.

Polysiphonia atlantica sensu Nam and Kang (2012) was collected from the eastern and southern coasts of Korea. It is distinguished from European P. atlantica by the scarce trichoblasts and 2-4 sterile tip cells in the spermatangia. It also differs from P. atlantica sensu Kim and Lee (1996) in...
Table 1. Comparisons between members of *Polysiphonia sensu stricto* group

<table>
<thead>
<tr>
<th></th>
<th><em>P. ulleungensis</em> sp. nov.</th>
<th><em>P. atlantica sensu Kim and Lee</em></th>
<th><em>P. atlantica sensu Maggs and Hommersand</em></th>
<th><em>P. caespitosa</em></th>
<th><em>P. decussata</em></th>
<th><em>P. devoniensis</em></th>
<th><em>P. funebris</em></th>
<th><em>P. kapraunii</em></th>
<th><em>P. macrocarpa</em></th>
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<td>0.9-1.8</td>
<td>0.5-1.5</td>
<td>0.5-3</td>
<td>Up to 2</td>
<td>1-2</td>
<td>1.2-5</td>
<td>Up to 0.5</td>
<td>Up to 10</td>
<td>1-3</td>
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<td>Extremely soft and flaccid</td>
<td>Extremely soft and flaccid</td>
<td>Robust to slender</td>
<td>-</td>
<td>Flaccid</td>
<td>-</td>
<td>-</td>
<td>Delicate</td>
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<td>Each pair of branches laterally on each side</td>
<td>Alternate to irregular</td>
<td>Distichous</td>
<td>Irregular</td>
<td>Alternate-subdistichous</td>
<td>Alternate</td>
<td>Subdichotomous to alternate</td>
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<td>0.5-2.8</td>
<td>0.5-1.5</td>
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<td>-</td>
<td>2-3</td>
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<td>Dome shape</td>
<td>Dome shape</td>
<td>Dome shape</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>Dome shape</td>
<td>-</td>
<td></td>
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<td>Transverse</td>
<td>Transverse</td>
<td>-</td>
<td>-</td>
<td>Transverse</td>
<td>-</td>
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<td>Occasional to common</td>
<td>Occasional to common</td>
<td>Scarce to numerous</td>
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<td>Numerous</td>
<td>Scarce</td>
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<td>30-50</td>
<td>20-30</td>
<td>20-80</td>
<td>-</td>
<td>20-80</td>
<td>-</td>
<td>-</td>
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<td>Straight</td>
<td>Straight and slightly spiral</td>
<td>Slight spiral</td>
<td>Slight spiral</td>
<td>Straight</td>
<td>Straight</td>
<td>Straight</td>
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<td>Tetraspore diameter (μm)</td>
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<td>50-60</td>
<td>35-70</td>
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<td>25-40</td>
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<td>Spermatangia</td>
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<td>Replacing trichoblast</td>
<td>Replacing trichoblast</td>
<td>Replacing trichoblast</td>
<td>Primary branch of trichoblast</td>
<td>Primary branch of trichoblast</td>
<td>-</td>
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<td>Sterile tip cell</td>
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<td>1-2</td>
<td>Absent</td>
<td>Absent</td>
<td>-</td>
<td>0-2</td>
<td>Absent</td>
<td>-</td>
<td></td>
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<td>Urceolate</td>
<td>Slightly urceolate</td>
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<td>Ovoid</td>
<td>Globose to ovoid</td>
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<td>Cystocarp diameter (μm)</td>
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<td>260-330</td>
<td>190-450</td>
<td>250-320</td>
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<td>270-300</td>
<td>175-315</td>
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<td>British Isles</td>
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<td>Los Angeles, CA, USA</td>
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<td>Port-au-Prince, Haiti</td>
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Table 1. Continued

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<th><em>P. morrowii</em></th>
<th><em>P. pacifica</em></th>
<th><em>P. pernacola</em></th>
<th><em>P. rudis</em></th>
<th><em>P. scopulorum</em></th>
<th><em>P. senticulosa</em></th>
<th><em>P. stricta</em></th>
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<td>-</td>
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<td>Irregular</td>
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<td>-</td>
<td>4-6</td>
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<td>Dome shape</td>
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<td>Dome shape</td>
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<td>Transverse</td>
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<td>-</td>
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<td>Trichoblast and scar cells</td>
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<td>Scarcely</td>
<td>Absent</td>
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<td>Scarce</td>
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<td>Scarcely</td>
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<td>Tetradsporangia arrange-</td>
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<td>Straight</td>
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<td>1 to several</td>
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<td>5-7</td>
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<td>Urceolate</td>
<td>Conspicuously urceolate</td>
<td>Urceolate</td>
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<td>Ovoid to urceolate</td>
<td>Urceolate</td>
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</table>
having an alternate branching pattern. *P. atlantica sensu* Nam and Kang (2012) resembles *P. ulleungensis* sp. nov. based on the morphology of vegetative, male, and female thalli, although it has been reported with a dichotomous branching pattern that seems to be alternate in the tetrasporophyte (see Fig. 18I in Nam and Kang 2012). We recognize the *P. atlantica* from Korea reported by Nam and Kang (2012) as *P. ulleungensis* sp. nov.

The following seven *Polysiphonia sensu stricto* species are also similar to our new species: *P. funebris*, *P. morrowii*, *P. pacifica*, *P. pernacola*, *P. scopulum*, *P. senticulosa*, and *P. subtilissima* by having the combined features of scarce trichoblasts and scar cells and spermatangial branches replacing trichoblasts (Table 1). However, *P. funebris* differs from *P. ulleungensis* sp. nov. by the scattered disposition of the ovoid cystocarps throughout the female gametophyte and an alternate-distichous branching pattern (Afonso-Carrillo and Rojas-González 2004). *Polysiphonia morrowii* is distinguished by the sharply pointed vegetative tips and 5 to 8 sterile tip cells in spermatangial branches (Kim et al. 1994, Geoffroy et al. 2012). *P. pacifica* is distinguished by the lax texture and distichous branching pattern (Hollenberg 1942). *P. pernacola* is distinguished by the absence of trichoblasts and conspicuous urceolate cystocarps (Adams 1991). *P. scopulum* differs by the rigid texture of filaments, presence of adventitious erect branches and cicatrigenous branching, absence of sterile tip cells in spermatangial branches, and slightly spiral tetrasporangia arrangement (Womersley 1979, see Fig. 18I in Nam and Kang 2012). *P. senticulosa* differs by the laterals hooked in a proximal direction and 5 to 7 sterile tip cells (Nam and Kang 2012). *P. subtilissima* is distinguished by its lax texture, irregular branching, and both straight and spiral arrangement of tetrasporangia (Womersley 2003).

Molecular-assisted identification with plastid-encoded rbcL has proven useful for discriminating species of *Polysiphonia sensu lato* (Mamoozadeh and Freshwater 2011). Our molecular phylogenetic analyses using rbcL sequences reveal that *P. ulleungensis* sp. nov. is firmly embedded within *Polysiphonia sensu stricto* (Fig. 3). *P. ulleungensis* is situated in a well-supported clade with the generitype, *P. stricta*, and other species that have in common the rhizoids in open connection to pericentral cells, 4 pericentral cells, and branches radially emerged. *P. ulleungensis* shows sufficient sequence divergence from morphologically similar *P. atlantica* from Europe, “*P. atlantica*” from the USA, and other *Polysiphonia* species to warrant recognition as a new species. *P. ulleungensis* sp. nov. has been collected Ulleung Island (~135 km off the eastern coast of the Korean Peninsula) and it was also reported from the western and eastern coasts of Korea as *P. atlantica sensu* Nam and Kang (2012).

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