Morphology and molecular study of *Pterosiphonia arenosa* sp. nov. (Rhodomelaceae, Rhodophyta) from Jeju Island, Korea

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The genus *Pterosiphonia* is composed of 22 species worldwide and four of these species have been reported in the North-East Asia. In Korea, *P. pennata* originally described from the Mediterranean Sea has been previously recorded from the southern coast as a widespread species. In order to confirm the same species from Korea and Mediterranean, we observed the morphology of Korean *Pterosiphonia* specimen and analyzed *rbcL* sequences for inferring phylogenetic relationships among similar congeners. Korean entity was recognized as a new species, *Pterosiphonia arenosa* sp. nov. The new species is characterized by ecorticate axes with 7-10 pericentral cells, branches formed every two segments, and coalesced with main axis over 1-1.25 axial segments, and tetrasporangia formed in straight series on determinate branches of the upper parts of erect axes. A phylogenetic analysis of *rbcL* sequences demonstrated that *P. arenosa* was distinct from *P. pennata* found in Spain as well as other species. In conclusion, morphological and molecular sequence data indicated that *P. arenosa* sp. nov. has been previously misidentified as *P. pennata* in Korea.

Key Words: morphology; *Pterosiphonia arenosa* sp. nov.; *P. pennata*; *rbcL*; Rhodophyta; taxonomy

INTRODUCTION

The genus *Pterosiphonia* Falkenberg in Schmitz et Falkenberg (1897) was established for a rhodomelacean species based on the type species, *Pterosiphonia cloio-phylla*, having the following characteristics: thallus bilaterally symmetrical differentiated into prostrate and erect systems of indeterminate growth, erect axes with alternately distichous branches of determinate or indeterminate growth, flattened to strongly compressed, and about 1-2 internodes congenital fusions of proximal parts in short laterals with main axes (Maggs and Hommersand 1993). To date, 22 species have been distinguished in *Pterosiphonia*, occurring in temperate to tropical regions

of the Atlantic and Pacific Oceans (Guiry and Guiry 2012). Only four *Pterosiphonia* species have been reported in the North-East Asia: *Pterosiphonia bipinnata* (Postels et Ruprecht) Falkenberg (1901), *P. pennata* (C. Agardh) Sauvageau (1897), *P. pinnulata* (Kützinger) Maggs et Hommersand (1993), and *P. tanakae* S. Uwai et M. Masuda (1999).

Pterosiphonia pennata is widely reported, but taxonomically confusing (Maggs and Hommersand 1993, Díaz Tapia and Bárbara 2004). This species was originally described by Agardh (1824) based on the materials collected from the warmer areas of the Mediterranean to Atlantic Sea. *Pterosiphonia pennata* is characterized by pin-

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nate arrangement of short lateral branchlets, ecorticate, and erect axes with 9-11 periaxial cells (Maggs and Hommersand 1993). The species is generally believed to be ubiquitous outside Mediterranean, i.e., in Europe (Maggs and Hommersand 1993), North and South America (Hoffmann and Santelices 1997, Abbott 1999), Africa (John et al. 2004), Australia and New Zealand (Adams 1994, Womersley 2003), Japan and Korea (Lee et al. 1992). However, Maggs and Hommersand (1993) doubted the species' occurrence, noting that its distribution is difficult to assess at present due to confusion between groups of morphologically similar species.

In Korea, there were reported only one *Pterosiphonia* species that was identified as *P. pennata* (Lee et al. 1992). It was collected at the rocky intertidal zone of a sheltered area from the southern coast of Korea in temperate region. Although erect branches developed connate lateral branches alternately every two segments, the species identification was considered uncertain owing to some extent of differences in dimension (Lee et al. 1992). This might have reflected the possibility of that a different species occurred in Korea and in the Mediterranean, as the specimens inhabited areas far away. Recent collections from Jeju Island, Korea yielded a species apparently belonging to the genus *Pterosiphonia*, but it should be compared with specimens of *P. pennata* from Spain / Mediterranean Sea. Red algal plastid large subunit of ribulose-1,5-bisphosphate carboxylase / oxygenase (*rbcL*) has been used for phylogenetic analysis and for species identification (Kim et al. 2008, 2010, Won et al. 2009). The approach of molecular taxonomy is especially useful for dealing with the species having wide distribution ranges and to investigate the origin of populations on different geographical barriers (Lin et al. 2009). Molecular techniques also allow us to answer questions on determining the species without clear-cut morphological diagnostic criteria. This is the case of the genus *Pterosiphonia* where several synonymies have been suggested due to the species are distinguished solely based on a few vegetative characters, which overlapping in some species (Rull Lluch 2002, Díaz Tapia and Bárbara 2004, 2011, Gabrielson et al. 2004).

In this study, we investigated rhodomelacean specimens identified as *Pterosiphonia pennata* both, from Korea and Spain, near by the type locality. We used detailed morphological information and analysis of gene from the plastid *rbcL*. The several *rbcL* sequences available for *Pterosiphonia* (Kim et al. 2010, 2012) allowed us to compare our results directly with those from other species in the genus and with putative relatives. Here, we describe

Pterosiphonia arenosa sp. nov. from Korea based on materials previously labeled as *P. pennata* and conclude that the name *P. arenosa* must be considered for specimen from the northwestern Pacific marine flora.

MATERIALS AND METHODS

Morphology

Samples were collected from the intertidal zone of Jeju Island and transported live to the laboratory putting into plastic bottle filled with seawater. The specimens were pressed as a voucher or preserved in 4% formalin / seawater for morphological study, with the exception of a small piece of thallus used for molecular analysis. Sections were obtained by hand or with the aid of a freezing microtome NK-101-II (Nippon Optical Works Co., Ltd., Tokyo, Japan). The sectioned preparations were stained with 1% aniline blue acidified with 1% HCl and mounted in 25-30% Karo syrup. Photographs were taken using a Digital Sight DS-Fi1 camera (Nikon, Tokyo, Japan) attached to a microscope (ECLIPSE 80i; Nikon). The voucher specimen was deposited in the herbaria of Jeju National University (JNUB), Jeju and the National Institute of Biological Resources (KB), Incheon, Korea.

Molecular study

Total genomic DNA samples were prepared using the LaboPass Tissue Mini kit (Hokkaido System Science, Hokkaido, Japan) following the manufacturer's instructions. For polymerase chain reaction (PCR) amplification and sequencing reaction of *rbcL* gene, primer pairs were used as follows; *rbcLF145-rbcLR898* and *rbcLF762-rbcLR1442* (Kim et al. 2010). PCR amplification was carried out with a thermal cycler (Techgene Ltd., Duxford, Cambridge, UK) using a TaKaRa Ex Taq reaction kit (Takara Shuzo, Shiga, Japan). PCR was performed with an initial denaturation step at 94°C for 4 min, followed by 35 cycles of 1 min at 94°C, 1 min at 50°C, and 2 min at 72°C, with a final 7 min extension cycle at 72°C. The PCR products were purified with the AccuPrep PCR Purification Kit (Bioneer, Daejeon, Korea) and then sequenced commercially (Macrogen, Seoul, Korea). Both electropherogram outputs from each sample were edited using the program Chromas version 1.45 software (Technelysium Pty Ltd., South Brisbane, Australia).

A total of 26 *rbcL* sequences (17 new) were collated using the multiple-sequence alignment program Se-Al ver-

sion 2.0a11 (Rambaut 2002) and aligned visually. Plastid *rbcL* sequences of *Symphyocladia marchantioides* (Harvey) Falkenberg and *S. latiuscula* (Harvey) Yamada were included as out groups. Collection information and GenBank accession numbers of 17 specimens are listed in Table 1 together with the sequences from our previous study. Phylogenetic analyses were performed using maximum likelihood (ML) by RAxML software (Stamatakis 2006). We used 200 independent tree inferences using -# option with default -I (automatically optimized subtree pruning and regrafting [SPR] rearrangement) and -c (25 distinct rate categories) options of the program to iden-

tify the best tree. To generate bootstrap values for these phylogenies, we used the same program with the same settings and 1,000 replications. In addition, we analyzed all *rbcL* sequences of *Pterosiphonia* registered in GenBank (GQ252571-252573), but GQ252571 (*P. bipinnata*) and GQ252573 (*P. gracilis*) formed a strong clade with *Neosiphonia* group (data not shown). On the other hand, the case of GQ252572 (*P. dendroidea*) sequence would be hinted the possibility of a long branch attraction (data not shown). Finally, we removed three *rbcL* sequence data from GenBank to perform phylogenetic analyses using ML.

Table 1. List of species used in this study for *rbcL* analysis with collection information and GenBank accession numbers

Taxa	Collection data	Voucher	GenBank accession No.	Reference
<i>Pterosiphonia arenosa</i> M. S. Kim et B. Kim	Hyeopjae, Jeju, Korea; Apr 18, 2007	M3_10	JX857412	This study
	Geumneung, Jeju, Korea; Jan 15, 2010	S105	JX857413	This study
	Gimnyeong, Jeju, Korea; Jan 21, 2012	12pte01	JX857414	This study
	Geumneung, Jeju, Korea; Mar 7, 2011	PT01	JX857423	This study
	Geumneung, Jeju, Korea; Apr 4, 2011	PT03	JX857422	This study
	Hyeopjae, Jeju, Korea; May 3, 2011	PT04	JX857421	This study
	Hyeopjae, Jeju, Korea; May 3, 2011	PT05	JX857420	This study
	Gimnyeong, Jeju, Korea; Dec 27, 2011	PT06	JX857419	This study
	Gimnyeong, Jeju, Korea; Jan 21, 2012	PT07	JX857418	This study
	Jongdal, Jeju, Korea; Feb 4, 2012	PT08	JX857417	This study
	Gosung, Jeju, Korea; Mar 23, 2012	PT011	JX857416	This study
	Gimnyeong, Jeju, Korea; Apr 4, 2012	PT012	JX857415	This study
<i>P. ardreana</i> Maggs & Hommersand	Lourido, A Coruña, Spain; Jul 14, 2008	21372	JX857427	This study
	San Román, Lugo, Spain; Jul 16, 2008	21373	JX857428	This study
<i>P. cloiophylla</i> (C. Agardh) Falkenberg	Kommetjie, South Africa; Feb 20, 2006	P2023	GQ867080	Kim et al. (2010)
	Kommetjie, South Africa; Feb 18, 2006	P2024	GQ867081	Kim et al. (2010)
<i>P. complanata</i> (Clemente) Falkenberg	Cambre, Malpica, A Coruña, Spain; Jan 12, 2009	21624	JF967667	Kim et al. (2012)
	San Román, Lugo, Spain; Jan 10, 2009	21625	JF967668	Kim et al. (2012)
	Beganfry, France; Jun 24, 2006	P732	GQ867079	Kim et al. (2010)
<i>P. pennata</i> (C. Agardh) Sauvageau	Ártabra, A Coruña, Spain; Jun 6, 2008	20940	JX857426	This study
	Ensenada de Chanteiro, A Coruña, Spain; Sep 17, 2005	19607	JX857424	This study
	La Franca, Asturias, Spain; Oct 6, 2006	19754	JX857425	This study
<i>Pterosiphonia</i> sp.	Kommetjie, South Africa; Feb 17, 2006	M9	JF967665	Kim et al. (2012)
	Kommetjie, South Africa; Feb 19, 2006	M11	JF967666	Kim et al. (2012)
<i>Symphyocladia latiuscula</i> (Harvey) Yamada	Anin, Gangneung, Korea; Feb19, 2011	CNU884	JF967652	Kim et al. (2012)
<i>S. marchantioides</i> (Harvey) Falkenberg in Schmitz et Falkenberg	Mataikona, Wairarapa, New Zealand; Jan 5, 2010	ASK006	GU731229	Kim et al. (2012)

RESULTS

Pterosiphonia arenosa M. S. Kim et B. Kim, sp. nov. (Fig. 1)

Description. *Thalli epilithius, polysiphonius, ecorticate, ad saxa per rhizoidia unicellularia in intervallis irregularibus affixae, cellulis pericentralibus abscissis, haptero multicellularia terminantibus; 3-7 cm alti, atrobrunneo rubrum, prostratus axes cylindricis, cum cellulis pericentralium 7-9, segmentis 110-160 µm diametro; erecta axes teretes ad leviter compressa, cum cellulis pericentralium 7-10, segmentis 160-210 µm diametro in inferiorem partem, 200-360 µm diametro in medio ad superiorem partem; ramis alternatim distichis cum subacutis apicibus, ferri ad intervalla duo segmentis, leviter incurvatis in superiorem partem, teretes determinatum ramulis formans acuti cellulis apicalibus; rami laterales per segmenta 1-1.25 proximalia axe parentali congenite connate; trichoblasts praesens / absens; trichoblasti vegetative deficientibus; tetrasporangia tetraedrica, singulariter per segmentum fertile in ramis ultimis in seriebus rectis longitudinalibus singularibus facta, 100 µm diametro, a cellulis tegentibus duas presporangialibus sed nee cellulis tegentibus postsporangialibus circumcineta.*

Thalli epilithic, polysiphonous, ecorticate, attached by unicellular rhizoids cut off from pericentral cells, terminating multicellular hapteron; 3-7 cm tall, dark brownish red in color; prostrate axes cylindrical, with 7-9 pericentral cells, segments 110-160 µm in diameter; erect axes terete to slightly compressed, with 7-10 pericentral cells, segments 160-210 µm broad in lower portion, 200-360 µm broad in middle to upper portion; branches alternately distichous with subacute apices, borne at intervals of two segments, slightly incurved in upper portion, terete determinate branchlets forming acute apical cell; lateral branches congenitally fused for proximal 1-1.25 segments with the parental axis; trichoblasts entirely lacking; tetrasporangia tetrahedral, formed one per fertile segment in straight series of ultimate branches, 65-75 µm in diameter, surrounded by two presporangial and one post-sporangial cover cells. Other reproductive structures not observed.

Holotype. JNUB-3PTA1201, tetrasporophyte, collected on Dec 27, 2011 by M. S. Kim and B. Kim (Fig. 1A), deposited in JNUB (the Herbarium of the Department of Biology, Jeju National University, Jeju 690-756, Korea).

Isotypes. JNUB (3PTA1202-3PTA1216), KB (NIBRAL00013308-NIBRAL000013310).

Type locality. Gimnyeong, Jeju Island, Korea (33°33′

28.01″ N, 126°45′29.77″ E).

Etymology. The specific epithet was chosen to represent the natural habitat of the discovery collection, where plants are growing on a stone in sandy place.

Korean name. 나래붉은깃

Other specimens examined. Hyeopjae (M3-10, Apr 18, 2007; 3PTA1217-3PTA1221, Apr 19, 2011; 3PTA1222-3PTA1226, May 3, 2011), Gimnyeong (3PTA1227-3PTA1231, Jan 21, 2012; PT012, Apr 4, 2012), Jongdal (PT08, Feb 4, 2012), Gosung (PT011, Mar 23, 2012), Geumneung (S105, Jan 15, 2010; PT01, Mar 7, 2011; PT03, Apr 4, 2011).

Habitat. *Pterosiphonia arenosa* was collected at the lower intertidal zone of sheltered area. It often grew in dense tufts on a stone in sandy place intermingled with other species such as *Neosiphonia* spp.

Morphology. Thalli are 3-7 cm high, dark brownish red (Fig. 1A). Erect branches are arising irregularly from prostrate axes as dense tufts (Fig. 1B). Plants grow on rocks covered lightly in sand. Thalli have tangled prostrate axes attached by unicellular rhizoids, which are cut off by cross walls from the lower pericentral cells and have discoidal haptera at the terminal portion (Fig. 1C). Prostrate axes are terete, ecorticate, 110-160 µm in diameter and possess segment 80-130 µm long which consist of a central cell and 7-9 pericentral cells (Fig. 1H). Erect axes are mostly terete and slightly compressed in branching portion, ecorticate, 160-210 µm in diameter, with an alternately distichous branching pattern, bearing numerous determinate and indeterminate branches every two segments, becoming tapered upwards, consisting of 7-10 pericentral cells which are 200-360 µm long and 30-50 µm broad (Fig. 1D & I-L). Diameter / length ratio of segments is 0.8-1.3 in prostrate axes and 1.8-2.1 in erect ones. Branches of erect axes are having congenital fusions of proximal parts of 1-1.25 segments with bearing axes (Fig. 1E). Trichoblasts are absent. Lateral branches are determinate, 1.2-2.6 mm long, mainly arranged alternate at lateral portion or spirally on prostrate axes (Fig. 1E & F). The upper portion of erect axes is terete, with alternate branches curved inwards. The apical cells are obtuse, 15-20 µm long, 10-13 µm broad on indeterminate branches, however conic shape, 10-15 µm long, 7-15 µm broad on determinate branches (Fig. 1G). Tetrasporangia are arranged in straight series and formed one per fertile segment in determinate lateral branches of the upper portion of thallus (Fig. 1N). Tetrasporangia are tetrahedral, 65-75 µm in diameter and surrounded by two presporangial and one postsporangial cover cells (Fig. 1M, O & P). Other reproductive structures were not observed.

Fig. 1. *Pterosiphonia arenosa* M. S. Kim & B. Kim, sp. nov. (A) Holotype specimen (JNUB-3PTA1201, tetrasporophyte, 27 December 2011, Gimnyeong) deposited in the Herbarium of Jeju National University (JNUB), Jeju, Korea. (B) Prostrate axes (arrows) with numerous erect branches (arrowheads). (C) Unicellular rhizoids cutting off from pericentral cells of prostrate axes by cross walls (arrows). (D) Erect branches showing alternately distichous branching pattern: determinate branches (arrowheads) and indeterminate branches (arrows). (E) Branches formed every two segments and coalesced with main axis over 1-1.25 axial segments. (F) Prostrate axes bearing spirally numerous young branches. (G) The apex of erect axes with obtuse apical cells (arrowheads) and determinate branches with conic apical cells (arrows). (H) Cross section of prostrate axes having 9 pericentral cells. (I-L) Cross section of erect axes having 7-10 pericentral cells. (M) Cross section of tetrasporangial branches, having tetrasporangia (arrowhead) and two presporangial cover cells (arrows). (N) Erect branches having tetrasporangia in straight series. (O) Postsporogial cover cells (arrows) of tetrasporangia. (P) Tetrasporangia. Scale bars represent: A, 2 cm; B & D, 1 mm; C, 100 µm; E, F & N, 200 µm; G, H, O & P, 50 µm; I, J & K-M, 30 µm.

Fig. 2. Maximum likelihood (ML) phylogenetic tree for *Pterosiphonia* and out group derived from chloroplast *rbcL* sequences. The ML bootstrap values shown above branches are from 1,000 replications.

Molecular analysis. A total of 1,150 bp of *rbcL* were aligned for 26 sequences representing six species of *Pterosiphonia*, and two species of *Symphyocladia* as out-groups. Twelve specimens of “*P. pennata*” from Korea were clearly differed from *P. pennata* collected from Spain that is close by the type locality region of the Mediterranean Sea. The sequences of *P. arenosa* differed 0-4 bp (0-0.35%) within the species, and the specimens from Korea differed by 59-61 bp (5.13-5.30%) from those of *P. pennata* collected from Spain. The sequence divergence within the genus *Pterosiphonia* ranged from 1.22% between *P. complanata* and *P. cloiophylla* to 7.04% between *P. complanata* and *P. pennata*.

In the phylogenetic tree, the genus *Pterosiphonia* was a monophyletic clade with 99% bootstrap supports (Fig. 2). The clade of *Pterosiphonia* consisted of two internodes; one is “*P. pennata*” specimens from Korea with 100% bootstrap supports, and the other is composed of

five *Pterosiphonia* species with moderate bootstrap values (85%) that again diverged in two internodes. The first of these nodes is formed by *P. complanata* from France and Spain, *Pterosiphonia* sp. from South Africa, and the generitype of the genus *Pterosiphonia*, *P. cloiophylla* from South Africa with 100% bootstrap supports, and the second node consisted of *P. pennata* and *P. ardreana* samples from Spain with 100% bootstrap supports.

DISCUSSION

Pterosiphonia arenosa is newly described from Jeju Island, Korea, based on detailed morphological observations and molecular analysis. This new species is epilithic in sand-covered rocks in the sublittoral zone and characterized by the following features: 1) thalli composed of erect and prostrate axes of indeterminate growth, 2)

ecorticate, 3) 7-10 pericentral cells, 4) branches formed every two segments, 5) determinate branches borne in an alternate-distichous pattern, 6) coalesced with main axis over 1-1.25 axial segments, 7) trichoblasts absent, and 8) tetrasporangia formed in straight series in upper branches of determinate growth. Molecular phylogenetic analysis based on *rbcL* sequences demonstrated *P. arenosa*'s distinctness from its congeners, and separated it clearly from the European *P. pennata*, which was collected from Spain close to the type locality, the Mediterranean Sea. Although *P. arenosa* closely resembles *P. pennata* from Spain in appearance, *P. pennata* is characterized by having 9-11 pericentral cells, 1.5-2.5 coalesced segments, trichoblasts sometimes present and prostrate axes bearing a spiral arrangement of simple laterals on every third segment (Maggs and Hommersand 1993). As a result, the specimen from Korea previously labeled under the name *P. pennata* (Lee et al. 1992) should be reassigned to *P. arenosa* because its morphological traits agree well with our description of the new species.

In recognizing the species of *Pterosiphonia*, which currently contains 22 species (Guiry and Guiry 2012), the vegetative characteristics that are viewed as important diagnostic features are 1) number of pericentral cells, 2) the presence or absence of cortication, 3) the degree of coalescence of laterals with their parental axes, and 4) the presence or absence of vegetative trichoblasts (Maggs and Hommersand 1993, Uwai and Masuda 1999a). The diameter / length ratio of segments in prostrate and erect axes is also a valuable characteristic at the species level (Díaz-Tapia and Bárbara 2004). In addition, some morphological traits of reproductive structures could provide additional features, but they are unknown in most species of the genus *Pterosiphonia* (Díaz-Tapia and Bárbara 2011).

In the North-East Asia, four *Pterosiphonia* species have been previously reported: *P. bipinnata* (Postels et Ruprecht) Falkenberg, *P. pennata* (C. Agardh) Sauvageau, *P. pinnulata* (Kützinger) Maggs et Hommersand, and *P. tanakae* S. Uwai et M. Masuda. *Pterosiphonia bipinnata* was originally described from Kamchatka, Russia (Falkenberg 1901). *Pterosiphonia bipinnata* is characterized by large thalli (>10 cm), axes ecorticate with 11-12(18) pericentral cells, and mostly 3 segments between successive branches (Abbott and Hollenberg 1976). *Polysiphonia pinnulata* was collected from Genoa, Italy (Kützinger 1863) and transferred to *Pterosiphonia pinnulata* by Maggs and Hommersand (1993). Although the place of type locality is biogeographically separated from the northeast Asia, *P. pinnulata* with 8-9 pericentral cells has been reported in Japan, for materials previously labeled as *P. pennata*

by Okamura (Yoshida 1998). *Pterosiphonia pinnulata* is characterized by ecorticate axes with 6-7(8) pericentral cells, trichoblasts present, prostrate axes forming a lateral branch every third segments in an apparent spiral, and lateral determinate branches coalesced with main erect axis for 0.5-1.5 axial segments (Maggs and Hommersand 1993). *Pterosiphonia tanakae* Uwai et Masuda (1999b) was established as a new species on the basis of material collected in Hiroshima Bay, Japan. *Pterosiphonia tanakae* is characterized by fan-shaped appearance, with 7-10 pericentral cells, corticated axes, lateral branches congenitally fused for proximal 1.5-2.5 segments with the parental axis, and abundant vegetative trichoblasts (Uwai and Masuda 1999b). They mentioned that the presence or absence of vegetative trichoblasts should be considered as a significant taxonomic feature in *Pterosiphonia*. In *P. arenosa*, vegetative trichoblasts are entirely lacking, which differs from *P. tanakae* and *P. pennata* that sometimes have trichoblasts.

A comparison of *Pterosiphonia arenosa* with the most similar species, *P. pennata*, reported from various localities in the world is given in Table 2. Although *P. arenosa* superficially resembles *P. pennata* from Europe in its basically linear outline of erect axes (Maggs and Hommersand 1993, Díaz-Tapia and Bárbara 2004), it has been shown in our study to differ in several significant ways (Table 2). Our *rbcL* sequences of *P. pennata* from Spain were different from those of Korean specimens, *P. arenosa*. In addition, *P. pennata* was more related to *P. ardreana* from Spain than to *P. arenosa* from Korea. *Pterosiphonia pennata* from Spain grows in a habitat closely similar to that of *P. arenosa* in Korea, and both materials are easily confused considering solely morphological traits. However, *P. pennata* is characterized by 9-11 pericentral cells, sometimes have trichoblasts, and the species usually occurs in extremely wave-sheltered sites, often with moderate to strong current exposure (Maggs and Hommersand 1993). Moreover, other interesting morphological difference is that the tetrasporangia of *P. pennata* from Europe form spiral series on terete extensions of determinate lateral branches from the mid and low parts of the thallus (Díaz-Tapia and Bárbara 2004). While *P. pennata* has been reported from a wide area, including the Atlantic and Pacific Oceans (Guiry and Guiry 2012), our results reveal that these records truly involves more than one species. Therefore, we answered the question on the occurrence of *P. pennata* in the northwest Pacific region, describing *P. arenosa* as a new species endemic from the northwest Pacific region. We demonstrated distinct differences between *P. pennata* from Spain and *P. arenosa* from Korea

Table 2. A comparison of taxonomic characteristics among geographical populations of *Pterosiphonia pennata* and *P. arenosa* sp. nov.

Characteristics	<i>P. arenosa</i> Korea	<i>P. pennata</i> Australia	<i>P. pennata</i> North America	<i>P. pennata</i> Spain	<i>P. pennata</i> Britain
Thalli					
Type	Prostrate and erect	Prostrate and erect	Prostrate and erect	Prostrate and erect	Prostrate and erect
Color	Dark brownish-red	Dark red-brown	-	Dark brownish-red	Brownish-red
Habitat	Epilithic or epiphytic	Epilithic	Epilithic	Epilithic, sandy rocks	Epilithic
Height (cm)	3-7	2-4	2.5	4	1-3
Prostrate axes					
Type, diameter (µm)	Terete, 110-160	Terete, 120-160	Cylindrical, 115	Terete, 120-200	Terete, 190-215
Pericentral cells	7-9	7-8	5	7-9	8-10
Rhizoids					
Cell type	Unicellular	Unicellular	Multicellular	Unicellular	Unicellular
From pericentral cells	Cut off	Cut off	-	Cut off	-
Erect axes					
Type	Terete or slightly compressed	Terete or compressed	Compressed	Terete or slightly compressed	Terete or slightly compressed
Branching pattern	Pinnate or alternate-distichous	Pinnate or alternate	Alternate-distichous	Alternate-distichous	Pinnate or alternate-distichous
Pericentral cells	7-10	8-10	8	7-10(11)	9-11
Diameter (µm)	160-210	180-230	-	100-250	190-360
Lateral branches					
Forming	2 segments	2-4 segments	3 segments	2 segments	3 segments
Branching pattern	Alternately pinnate	Pinnate or alternate	Alternate	Alternate	Pinnate or alternate
Cortical cells	Ecorticate	Ecorticate	-	Ecorticate	Ecorticate
Coalesced with main axis	1-1.25 segments	Over 1-1.5(2) segments	1 segment	1-1.5(2) segments	Over 1.5-2.5 segments
Trichoblasts	Absent	Usually absent	Often present	Absent	Absent or present
Cystocarps					
Shape, diameter (µm)	Unknown	Ovoid, 300-800	Urceolate, 350	Unknown	Unknown
Spermatangia					
Shape	Elongate-ovoid	Elongate-ovoid to clavate, often curved	Elongate heads	Unknown	Unknown
Tetrasprangia					
Arrangement	Straight	Straight	-	Spiral	Unknown
Successive segments	7-12	6-10	Up to 15	Up to 15	
Diameter (µm)	65-75	35-60	60	58-95	
References	This study, Lee et al. (1992)	Womersley (2003)	Abbott (1999)	Díaz Tapia and Bárbara (2004)	Maggs and Hommersand (1993)

based on the morphological and molecular information. The new species was found from winter to spring in the lower intertidal and subtidal zone, often inhabiting sheltered sites. Detailed collections may provide more information on its distribution within the Pacific area. The distant relationships of *P. arenosa* and *P. pennata* from the putative relatives raise questions about divergence pathways, which will be reinvestigated through additional taxon sampling within the genus. Moreover our results also clarify the distinction between *P. cloiophylla* from South Africa and *P. complanata* from Europe (2.2% of sequence divergence), for which the synonymy have been previously suggested (Rull Lluch 2002).

Differences between *Pterosiphonia* and *Symphyocladia* have been said to be slight and insubstantial (Kraft and Wynne 1992). However, Ardré (1973) pointed out that further observations on the species of *Symphyocladia* are necessary before their transfer to *Pterosiphonia*. According to Uwai and Masuda (1999b) these two genera are clearly distinguished by their juvenile plants. All species of *Symphyocladia* have flattened thalli in which there is complete, lateral, congenital fusion between branches and their parental axes during early stages of development, resulting in a leaf-like organization (Uwai and Masuda 1999b). However, in juvenile plants of *Pterosiphonia* apical cells of laterals and the parental axis are completely separate and congenital fusion is found between a few proximal segments of laterals and the axis. This results in a polysiphonous filament-like organization (Uwai and Masuda 1999a).

In conclusion, the analysis of *rbcl* gene in the present study reinforces the taxonomic discrimination of *P. arenosa* from other species in the genus *Pterosiphonia*. It will be very rewarding to investigate other cosmopolitan species of red algae that have phenotypic variation or lack of morphologically clear-cut characters: by comparing type material and molecular analysis. Analyzing more than one gene (e.g., cytochrome c oxidase subunit I [COI] or universal plastid amplicon [UPA] as DNA barcoding marker) will be essential for an accurate assessment of species within large, widespread genera of red algae.

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