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Miuraea migitae, a new record of the order Bangiales (Bangiophyceae, Rhodophyta) from Korea

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Abstract

We found specimens of foliose Bangiales from the subtidal zone of Udo, Jeju Island, Korea. In molecular analyses of *rbcl* sequences, these Korean specimens were almost identical to *Miuraea migitae* from Osaka, Japan. In the morphological comparison, Korean specimens were consistent with habitat, color, and vegetative characteristics with the description of *M. migitae*. This is the first record of *M. migitae* outside the type locality and Nagasaki in Japan. This study confirms that new or unrecorded species of the order Bangiales may be discovered from subtidal habitats.

Keywords: Bangiales, Korea, *Miuraea migitae*, New record, *rbcl*

Background

The order Bangiales (Nägeli 1874) is an order of distinctive and morphologically simple red algae that represents an ancient lineage (Butterfield 2000). The foliose Bangiales includes the most highly valued seaweeds grown in aquaculture from Korea, Japan, China, and Southeast Asia for the past several hundred years (Mumford and Miura 1988). This order has been considered monophyletic with the single family Bangiaceae. Traditionally, *Bangia* Lyngbye and *Porphyra* C. Agardh have been recognized on the basis of gametophyte morphology: unbranched uni- to multiseriate filaments in the genus *Bangia* and blade in the genus *Porphyra* (Oliveira et al. 1995). Recently, the revision of the order Bangiales, based on the phylogeny using nrSSU and *rbcl* genes, has greatly improved the taxonomic understanding of this group (Sutherland et al. 2011). According to Sutherland et al. (2011), the order Bangiales was split into eight of foliose (*Boreophyllum*, *Clymene*, *Lysithea*, *Miuraea*, *Porphyra*, *Pyropia*, and *Wildemanina*) and seven of filamentous genera.

The genus *Miuraea* was established by Sutherland et al. (2011) based on molecular analyses and composed of only one species, *M. migitae* (N.Kikuchi, S.Arai, G.Yoshida & J.A.Shin) N.Kikuchi, S.Arai, G.Yoshida, J.A.Shin & M.Miyata. This species was originally described as *Porphyra migitae*, based on the specimen

collected from Misaki town in Osaka Prefecture, Japan (Kikuchi et al. 2010). *M. migitae* was distinguished from other foliose Bangiales species by growing on the subtidal habitat and having fire red to pink color (Kikuchi et al. 2010). The distribution of *M. migitae* was reported only from Japan (Osaka and Nagasaki), attached to the dead bivalve shell and rope (Sutherland et al. 2011).

In Korea, the study of *Porphyra* has been carried out on morphology, physiology, flora, and culture by several authors (Hwang and Lee 2001; Kim and Kim 2011). To date, seven species of *Porphyra* and 13 species of *Pyropia* J.Agardh (previously known *Porphyra* from Korea) have been reported from Korea, mostly collected in the intertidal zone (Kim et al. 2013). Two species, *Pyropia koreana* (M.S. Hwang & I.K. Lee) M.S. Hwang, H.G. Choi, Y.S. Oh & I.K. Lee and *Pyropia yezoensis* (Ueda) M.S. Hwang & H.G. Choi, are known from subtidal habitats (Hwang and Lee 2001; Kim and Kim 2011). However, the specimens of the order Bangiales from subtidal habitats have been scarcely explored because of the difficulty of sampling in this environment, despite the possibility of discovering new or unrecorded species.

In this study, we collected foliose Bangiales specimens from a depth of 15 m in Udo, Jeju Island, Korea. We conducted morphological observations and *rbcl* sequence analysis in order to confirm the taxonomic position of these subtidal specimens. This is the first record

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of the occurrence of the order Bangiales species from under 15 m depth of subtidal habitats in Korean coasts.

Methods

Four samples were collected by SCUBA diving from a depth of 15 m of Udo (33° 30' N, 126° 55' E), Jeju Island, Korea, in 9 May 2015 (E15019) and 17 May 2016 (E16064, E16065, E16066). Thalli were mounted on herbarium sheets as voucher specimens. Some parts of thallus were cutoff to stain with 1% aqueous aniline blue acidified with a drop of 1% HCL and to make permanent slide in a solution of 50% Karo syrup for microscopic observations. Photomicrographs were taken with a Canon EOS 600D digital camera mounted on the Olympus BX43

microscope. Voucher specimens were deposited in the herbarium of Jeju National University, Korea (JNUB).

Genomic DNA was extracted from the specimens using the DNeasy Plant Mini Kit (Qiagen, Hilden, Germany) following the manufacturer's instruction. The primer pairs for *rbcL* gene were *rbcLJNF1-rbcLJNR1* and *rbcLJNF2-rbcLJNR2* (Kang and Kim 2013). Amplification condition for *rbcL* consisted of 7 min at 97 °C for pre-denaturation, followed by 45 cycles of 1 min at 97 °C, 1 min 47 °C, and 2 min at 72 °C, with a final 7 min extension cycle at 72 °C, and a soak cycle at 4 °C. PCR products were purified using the AccuPrep® PCR Purification Kit (Bioneer, Daejeon, Korea) following the manufacturer's instructions. Nucleotide sequences of *rbcL* were determined on strands of PCR

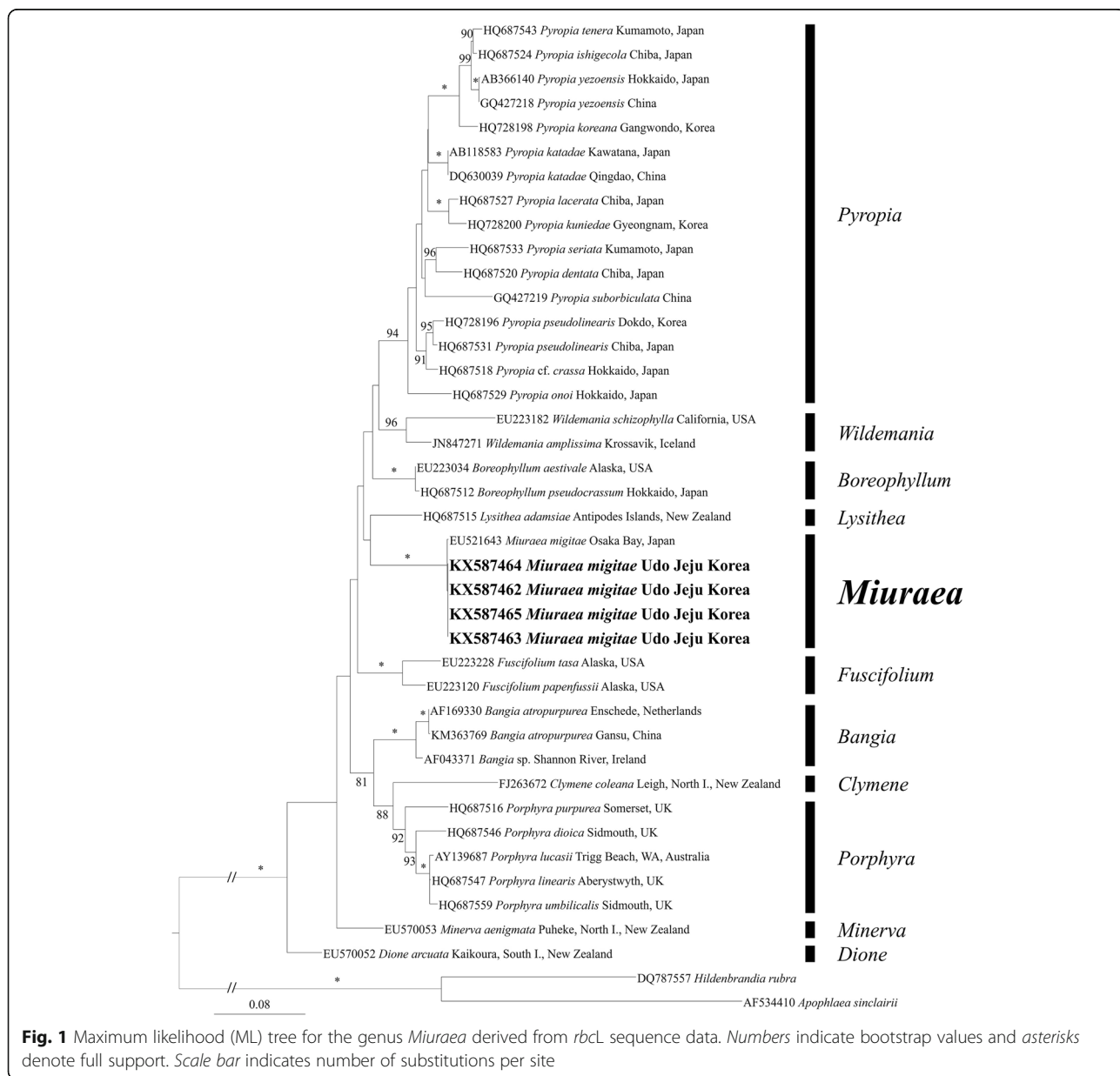


Fig. 1 Maximum likelihood (ML) tree for the genus *Miuraea* derived from *rbcL* sequence data. Numbers indicate bootstrap values and asterisks denote full support. Scale bar indicates number of substitutions per site

amplification products at the MacroGen sequencing facility (MacroGen Inc., Seoul, Korea). The sequences were edited using Mega ver. 7.0.14 (Tamura et al. 2013), and multiple sequence alignments were constructed with 39 taxa of the order Bangiales from GenBank with two taxa of Hildenbrandiales as out-group.

Maximum likelihood (ML) analysis was conducted using RAxML software (Stamatakis 2006) with the GTR + I + Γ evolutionary model to confirm the taxonomic position of Korean specimens. We used 1000 independent tree inferences using the “-#” option to generate bootstrap values for phylogenetic analysis with the algorithm “a” of “-f” option to search the best-scoring tree in one program run. The other options were used as a default set of the software.

Results

Molecular analyses

In total, 1174 base pairs of *rbcL* were aligned with 444 variable positions (37.8%) and 353 phylogenetically informative positions (30.0%). The Korean specimens (GenBank accession number KX587462-KX587465) were almost identical with *M. migitae* from Japan (EU521643), showed 0.2% sequence divergence (Fig. 1). In addition, *M. migitae* was clearly separated from other Korean specimens, *Pyropia koreana* (HQ728198), *Py. kuniedae* (HQ728200), *Py. pseudolinearis* (HQ718196), and *Py. yezoensis* (AB366140 and GQ427218), by 10.2–10.5%, 10.1–10.3%, 9.4–9.7% and 11–11.1% sequence divergences, respectively.

The clade of the genus *Miuraea* formed a monophyletic group with strong support, whereas there was a sister genus *Lysithea* without support. The monophyly of each genus was supported by strong bootstrap values (100% for *Bangia*, *Boreophyllum*, *Fuscifolium*, and *Miuraea*; 96% for *Wildemanina*; 94% for *Pyropia*; and 92% for *Porphyra*). *Pyropia* formed a clade with *Wildemanina* and *Boreophyllum*, but not supported. *Porphyra* was related to *Clymene* (a bladed species) and *Bangia* with filamentous morphology by moderate support values.

***Miuraea migitae* (N.Kikuchi, S.Arai, G.Yoshida & J.A.Shin) N.Kikuchi, S.Arai, G.Yoshida, J.A.Shin & M.Miyata**

Basionym: *Porphyra migitae* N.Kikuchi, S.Arai, G.Yoshida & J.A. Shin

Type locality: Misaki town, Osaka Prefecture, Japan.

Holotype: SAP105477 (Kikuchi et al. 2010).

Korean name: Chambunhonggim (참분홍김).

Thalli are membranaceous, monostromatic, elliptic, obovate, and circular shape with cordate and rotund base, fire red to pink in color, and undulated entire margin (Fig. 2a, b). Thalli are up to 13 cm long, 6 cm wide, and 25–40 μ m thick in the central portion (Fig. 2a, e).

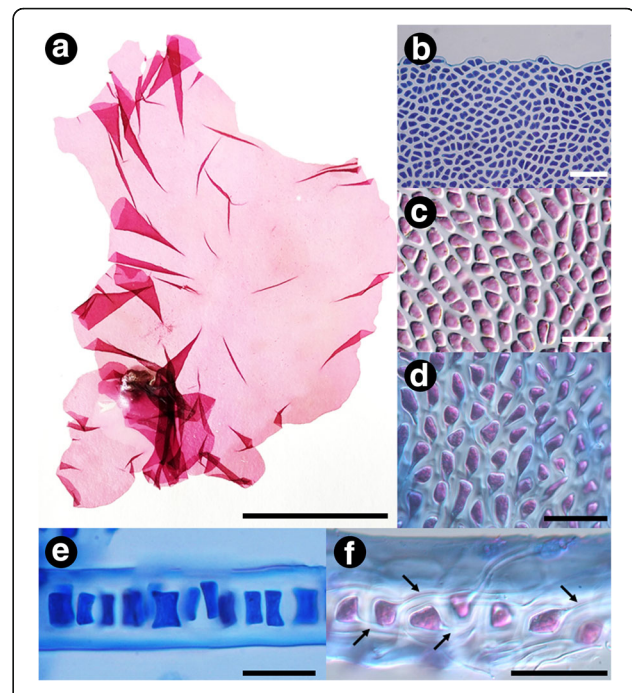


Fig. 2 *Miuraea migitae* (N.Kikuchi, S.Arai, G.Yoshida & J.A.Shin) N.Kikuchi, S.Arai, G.Yoshida, J.A.Shin & M.Miyata. **a** Vegetative thallus of *M. migitae* (E15019) collected from Udo, Jeju Island, Korea, 9 May 2015. **b** Surface view of thallus with entire margin. **c** Surface view of the central portion of thallus. **d** Surface view of the basal portion with rhizoidal cells. **e** Sectional view of vegetative cells in the central portion. **f** Sectional view of rhizoidal filaments toward both sides of thallus (arrows). Scale bars represent **a** 4 cm; **b, d, f**, 50 μ m; **c, e**, 25 μ m

Vegetative cells are oblong, triangular, polygonal with rounded angles in the surface view, and 10–17 μ m long \times 7–10 μ m broad in size (Fig. 2b, c). In the sectional view, cells are oblong and 15–20 μ m high \times 7–15 μ m broad in size (Fig. 2e). Basal cells are 15–35 μ m long \times 6–20 μ m broad in the surface view (Fig. 2d) and having rhizoidal filaments in both sides of thallus in the sectional view (Fig. 2f).

To date, *M. migitae* has been only collected from the subtidal zone in Udo, Jeju Island, Korea. The habitat is an area in a strong current and is covered with sand and a rhodolith bed.

Discussion

Miuraea migitae has been known as an endemic species in the subtidal zone of Japan. It is characterized by monostromatic thallus elliptic and obovate in shape, with fire red to pink color and 25 cm length \times 13 cm width in size (Kikuchi et al. 2010). According to the culture study by Kikuchi et al. (2010), *M. migitae* can be distinguished from other subtidal species of Bangiales in Japan by the presence of asexual reproductive subcycles involving archeospores and neutral spores on the foliose thallus. Although our specimens were not observed any

archeospores and neutral spores on thallus, Korean specimens were consistent with habitat, color, and vegetative characteristics in the description of *M. migiatae*.

In recognizing the species of *Pyropia*, the vegetative features are viewed as important diagnostic characters, such as color, length, thickness, and projection of rhizoidal filaments including habitat (Table 1). *M. migiatae* was found in the subtidal habitat like *Py. koreana* and *Py. yezoensis* with monostromatic thallus, but they can be distinguished from *M. migiatae* by color, basal cell size, and the projection of rhizoidal filaments (both sides in *M. migiatae* and one side in *Py. koreana*) (Table 1). *Porphyra oligospermatangia*, *Pyropia katadae*, and *Py. tenera* are having elliptic to obovate thallus with cordate base (Kim and Kim 2011), but they are different from *M. migiatae* in length (24–47 cm in *M. migiatae* and 30–56 cm in *P. oligospermatangia*), the thickness of thallus (25–40 μm in *M. migiatae*, 40–50 μm in *Py. katadae* and 35–60 μm in *Py. yezoensis*), the habitats (subtidal zone in

M. migiatae and intertidal zone in *P. oligospermatangia*, *Py. katadae*, and *Py. tenera*), and the color (fire red to pink in *M. migiatae*, yellowish to greenish in *P. oligospermatangia*, *Py. tenera*, and *Py. yezoensis*, and purple in *Py. katadae*) (Hwang and Lee 1994; Kim and Kim 2011).

The molecular analyses demonstrated that the *rbcL* sequences from Korean specimens and *M. migiatae* from Japan (EU521643) were almost identical with 0.2% genetic distances. Lindstrom and Fredericq (2003) mentioned that the sequence divergences of *rbcL* within species of *Porphyra* from different populations differed by 0.1% in *Porphyra fallax* from British Columbia, Canada, to 0.9% in *Porphyra pseudolanceolata* and *P. pseudolinearis* from Alaska, USA. The sequence divergence between Korean and Japanese specimens fell into the intraspecific variation, showing the specimens to be conspecific. In the phylogenetic tree, *Miuraea* formed a clade with four genera, *Pyropia*, *Wildemania*, *Boreophyllum*, and *Lysithea*, without supporting value. These

Table 1 Morphological features of *Miuraea migiatae* and five foliose Bangiales species having similar morphology or habitat from Korea

	<i>Miuraea migiatae</i>	<i>Porphyra oligospermatangia</i>	<i>Pyropia kuniedae</i>	<i>Py. koreana</i>	<i>Py. tenera</i>	<i>Py. yezoensis</i>
Habitat	6–15-m depth of subtidal zone	Intertidal zone	Low intertidal zone	Low intertidal to 5-m depth of subtidal zone	Upper to middle intertidal zone	Middle intertidal to 5-m depth of subtidal zone
Color	Fire red, pink	Yellowish brown, pale-green toward the base	Purple, greenish red, brownish red	Bright red, brownish red	Yellowish red, brownish red, greenish red	Brownish red, greenish red
Shape	Narrow elliptic, elliptic, obovate, circular	Elliptical, linear, lanceolate	Round, ovate	Elliptical, obovate	Elliptical, round, ovate, obovate	Round, ovate, obovate, oblanceolate, reniform
Basal shape	Cordate, rotund, navel	Round, cordate	Cordate	Cuneate, round	Cuneate, round, cordate	Cuneate, round, cordate, umbilicate
Dentation	Absent	Absent	Absent	Absent	Absent	Absent
Undulation	Present	Present	Present	Present	Present	Present
Length (cm)	13–25	20–40 (–55)	7–9.5	6–10	4.5–20 (–40)	3.5–13
Width (cm)	6–13	5–15	6.5–9	4–8	1.5–9	1–9
Thickness (μm)	24–47	30–56	40–50	20–27	20–40	35–60
Vegetative cell shape	Oblong, triangular, polygonal	Circle, rectangle	Circle, rectangle, triangle	Rectangle	Rectangle	Rectangle
Vegetative cell size (μm)	8–17 \times 6–10	7–11 \times 6–9	12–15 \times 7–10	13–17 \times 9–10	15–21 \times 9–12	13–19 \times 7–14
Basal cell shape	Capitate	Elliptic	Elliptic, capitate	Elliptical	Circle, elliptic	Circle, elliptical
Basal cell size (μm)	15–35 \times 6–20	18–42 \times 13–21	22–27 \times 13–16	22–25 \times 9–12	22–33 \times 13–21	22–33 \times 10–23
Projection of rhizoidal filaments	Both sides	Both sides	Both sides	One side	Both sides	Both sides
Division formula of spermatangia	4 \times 4 \times 8	4 \times 2 \times 8	4 \times 2–4 \times 4–8	4 \times 2–4 \times 4	4 \times 2–4 \times 4–8	4 \times 2–4 \times 8–16
Division formula of zygotosporangia	2 \times 2 \times 4	2 \times 2 \times 2–4	2 \times 2 \times 4	2 \times 1 \times 2	2–4 \times 1–2 \times 2–4	2 \times 2 \times 4
Reference	This study, Kikuchi et al. 2010	Kim and Kim 2011	Hwang and Lee 2001, Kim and Kim 2011	Hwang and Lee 2001, Kim and Kim 2011	Hwang and Lee 2001, Kim and Kim 2011	Hwang and Lee 2001, Kim and Kim 2011

phylogenetic relationships were different from two previous studies: one is that the genus *Miuraea* formed a basal node of the order Bangiales except for two filamentous genera *Dione* and *Minerva* (Sutherland et al. 2011), in the other is that the genus *Miuraea* formed a sister clade composed of *Boreophyllum*, *Fuscifolium*, *Pyropia*, *Wildemenia*, and an undescribed filamentous genus (Sánchez et al. 2014). In addition, the taxonomic confusion still remained on the phylogenetic relationships of three genera, *Porphyra*, *Bangia*, and *Clymene*, even though they had moderate support value in the *rbcL* tree (Sutherland et al. 2011; Sánchez et al. 2014).

The new record of *M. migitae* from Korea extends the distribution range of this species previously restricted to only two localities, Osaka and Nagasaki in Japan (Guiry and Guiry 2016). Udo of Jeju Island is distant from the type locality of *M. migitae*, Osaka, Japan. Hwang (2008) mentioned that Udo had been connected with Osaka by shipping previously (before 1945). Although the origin of this species is unclear, this report indicated that this species might be occurred in Korea prior to that time. Lack of the records on the distribution of *M. migitae* from Udo before our finding might be due to the difficulty of collecting in its subtidal habitat where there are swift currents. Since the foliose thallus of *M. migitae* is growing up to 28 cm as the maximum length and producing asexual reproductive cells during summer season (Kikuchi et al. 2010), it can be a potential genetic resource for plant breeding to increase the production in seaweed aquaculture.

Conclusions

In conclusion, we collected the foliose Bangiales specimens from the subtidal zone of Udo, Jeju Island. Based on molecular and morphological analyses, these specimens were identified as *M. migitae*, previously considered endemic in Japan. This is a new record for Korea and indicates that collections from the subtidal zone are necessary to increase understanding of the biodiversity of seaweeds including potential genetic resources in Korea. In further studies, we are performing morphological and molecular investigations of the order Bangiales to understand the accurate phylogenetic relationships.

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Availability of data and materials

The sequences are available in the GenBank (<http://www.ncbi.nlm.nih.gov/>).

Authors' contributions

YHK carried out the research and drafted the manuscript. HWL collected the samples from the subtidal zone by SCUBA diving. MSK designed this study

and revised the manuscript. All authors read and approved the final manuscript.

Competing interests

The authors declare that they have no competing interests.

Consent for publication

Not applicable

Ethics approval

Not applicable

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