ISSN: 2637-7721

Journal of Plant Biology and Crop Research

Open Access | Research Article

Pyropia yezoensis var. *irifuneiensis* (Bangiales, Rhodophyta), a naturally occurring variety with large ovate shape from Hokkaido, Japan

Yorinari Sunada[1]; Ryunosuke Irie[2]; Masahiro Suda[2]; Hiromi Nakao[3]; Satoshi Shimada[4]; Koji Mikami[5]*

[1]School of Fisheries Sciences, Hokkaido University, Hakodate, Hokkaido, Japan

[2]Graduate School of Fisheries Sciences, Hokkaido University, Hakodate, Hokkaido, Japan

[3]Betsukai Fishery Cooperative, Betsukai-cho, Hokkaido, Japan

[4]Department of Biology, Ochanomizu University, Tokyo, Japan

[5]Faculty of Fisheries Sciences, Hokkaido University, Hakodate, Hokkaido, Japan

***Corresponding Author(s): Koji Mikami**

Faculty of Fisheries Sciences, Hokkaido University, 3-1-1 Minato-cho, Hakodate 041-8611, Hokkaido, Japan

Tel: +81-138-40-8899;

Email: komikami@fish.hokudai.ac.jp

Received: Feb 27, 2020

Accepted: Mar 20, 2020

Published Online: Mar 25, 2020

Journal: Journal of Plant Biology and Crop Research

Publisher: MedDocs Publishers LLC

Online edition: http://meddocsonline.org/

Keywords: Molecular phylogeny; Morphology; *Pyropia yezoensis* var. *irifuneiensis*; *rbc*L gene; Vertical cell division

Abstract

Gametophytic thalli with a large ovate shape were collected at the Irifune fishing port at Hakodate in Hokkaido, Japan. Phylogenetic analysis using *rbc*L sequences identified this alga as the red seaweed *Pyropia yezoensis* (order Bangiales). Since the morphology of the Irifune specimen was different from that of generally recognized *P. yezoensis* specimens, indicating a naturally occurring variety of *P. yezoensis*, the alga was named *Pyropia yezoensis* (Ueda) M. S. Hwang & H. G. Choi var. *irifuneiensis* K. Mikami & H. Nakao. Morphological characteristics of conchocelis filaments and conchosporangia were identical to those of *P. yezoensis* f. *narawaensis*, although the division formula for female gametangia was slightly different. During early development, vertical cell division was frequently observed in the upper parts of thalli, establishing the cuneate and then ovate shape. However, this morphological change was inhibited at 20°C, resulting in production of slender thalli and suggesting a negative influence of mildly high temperature on the frequency of vertical cell division in the upper part of the thalli. Accordingly, the discovery of *P. yezoensis* var. *irifuneiensis* will provide insight into seaweed cell biology and approaches to preserve or improve aquaculture productivity of nori.

Cite this article: Sunada Y, Irie R, Suda M, Nakao H, Shimada S, Mikami K. *Pyropia yezoensis* var. *irifuneiensis* (Bangiales, Rhodophyta), a naturally occurring variety with large ovate shape from Hokkaido, Japan. J Plant Biol Crop Res. 2020; 3(1): 1016.

Pyropia yezoensis is an economically important species of red algae used for production of nori in Asian countries [1,2]. In Japan, current nori cultivars mostly originate from *Pyropia yezoensis* (Ueda) M. S. Hwang & H. G. Choi f. *narawaensis* N. Kikuchi, Niwa & Nakada [3], which grows vigorously to form a large, elongated gametophytic thallus [4]. Genetic heterogeneity is quite low among pure strains of this form due to repetitive self-fertilization [5,6], creating difficulty in establishing high quality, strong growth and stress tolerance traits. Although much effort has been put into breeding *P. yezoensis* by cross-fertilization with other strains and mutagenesis [7-10; for *review*, 11], identifying valuable species such as *P. yezoensis* f. *narawaensis* is indispensable for sustainable development of the nori aquaculture industry.

Taxonomic understanding of the order Bangiales was recently revised by creating 16 genera [12,13], whereas only the foliose *Porphyra* C. Agardh and filamentous *Bangia* Lyngb. had been recognized previously [14]. Of these genera, the genus *Pyropia* is most diverse and contains many unidentified specimens, suggesting the potential for finding valuable species for nori aquaculture among naturally growing *Pyropia* algae. Here, we describe a naturally growing *Pyropia* variety isolated from Hakodate in Hokkaido, Japan; this variety has a large gametophytic thallus and shows vigorous growth in comparison with other naturally grown *Pyropia* species.

Thalli of foliose Bangiales were harvested at slopes in boat lift yards of the Irifune fishing port (41°46′N, 140°42′E), Hakodate, on April 2 and 27, 2018; these specimens were typically found on shellfish with the green alga *Enteromorpha prolifera* (Supplementary Fig. 1). Size and morphology of collected specimens were variable, with elliptical, ovate and round shapes (Supplementary Fig. 1). The type specimen collected on April 2, 2018, is shown in Figure 1A.

We performed molecular phylogenetic analysis to determine the phylogenetic position of the Irifune specimen (HM00003) using sequence information for the plastid *rbc*L gene encoding the large subunit of ribulose 1,5-bisphosphate carboxylase/oxygenase (Rubisco) from various *Pyropia* species. HM00003 clustered in the *P. yezoensis* clade (Supplementary Figure 2), supported by complete identity of the *rbc*L nucleotide sequences with those of *P. yezoensis* specimens in the same clade (data not shown). These findings indicated that the seaweed collected at the Irifune fishing port is a naturally occurring variety of *P. yezoensis* for which we propose the name *P. yezoensis* (Ueda) M. S. Hwang & H. G. Choi var. *irifuneiensis* K. Mikami & H. Nakao; the Japanese name is Irifune susabinori. Morphologies of conchocelis filaments and conchosporangia were indistinguishable from those of *P. yezoensis* f. *narawaensis* (Fig. 1B and 1C). However, most reproductive sori of male gametogonia were parallel to the apical–basal axis of thalli (Supplementary Fig. 1 and Fig. 1A), different from those crossing this axis diagonally in *P. yezoensis* f. *narawaensis*. In addition, representative division formulae in male and female gametogonia were 128 (a/4, b/4, c/8) and 32 (a/2, b/4, c/4), respectively (Figs. 1D–G), the latter being slightly different from 16 (a/2, b/2, c/4) in *P. yezoensis* f. *narawaensis* [15].

Development of unicellular monospores (Figure 2A) released naturally from thalli was monitored in standing culture for 6 weeks and subsequent aeration culture for up to 18 weeks in the laboratory. Monospores first developed into straight and oblanceolate thalli after 2 and 3 weeks of development (Figs. 2B and 2C). Within 6 weeks, thallus morphology changed, with horizontal expansion of the upper part resulting in production of a cuneate-shaped thallus (Figure 2D). Further observations in aerated culture revealed an elliptical shape after a further 3 weeks of culture (total 9 weeks; Figure 2E) and finally a large, ovate shape after 18 weeks of culture (Fig. 2F). The ovate shape of thalli, a representative characteristic of *P. yezoensis* f. *irifuneiensis*, was therefore established in a stepwise fashion during early development via sequential morphological changes from straight to elliptical and then ovate.

To explore growth characteristics, we examined the effects of temperature on growth of asexual monospores at 5, 10, 15, 20 and 25°C under laboratory culture conditions, as previously described [16]. Monospores were prepared by wounding thalli, as established previously for *P. yezoensis* [17]. At 5°C, both height and width of thalli were highly attenuated compared with those at 15°C (Supplementary Figure 3), while incubation of monospores at 25°C promoted the formation of three-dimensional shrink-and-twist morphology with reduction in growth rate (Figure 3D). *P. yezoensis* f. *narawaensis* strain U51 showed the same morphology at 25°C, although frequency of callus production in this form was higher than that in the Irifune specimen (data not shown). These findings indicated that thalli of *P. yezoensis* var. *irifuneiensis* cannot grow normally at 5 or 25°C.

In contrast, thalli grew well at 10 and 15°C, although growth of thalli incubated at 10°C was slightly retarded compared with that at 15°C (Supplementary Fig. 3). We therefore considered the most optimal temperature for growth to be 15°C. As mentioned above, thalli grown at 15°C for 6 weeks (Figures 2D & 3B) as well as at 10°C (Figure 3A) showed a cuneate shape, which was not observed in *P. yezoensis* f. *narawaensis* strain U51 grown under the same conditions (Figures 3E & 3F). However, incubation at 20°C caused repression of expansion in the upper part, with thalli becoming slender in shape (Figures 3C). Therefore, we propose that vertical cell division, which crosses the apical–basal axis of thalli at right angles and is responsible for the expansion of the upper part of thalli, is sensitive to mild heat stress at 20°C in *P. yezoensis* var. *irifuneiensis*.

The large size of *P. yezoensis* var. *irifuneiensis* is similar to that of *P. yezoensis* f. *narawaensis* growing after separation from culture nets in nori aquaculture (See photos by Suzuki M, http://natural-history.main.jp/Tree_of_life/Eukaryote/Plantae/Rhodophyta/Porphyra_yezoensis_narawaensis/Porphyra_narawaensis.html). It is possible that both *P. yezoensis* var. *irifuneiensis* and *P. yezoensis* f. *narawaensis* possess similar mutations in genes responsible for large and vigorous growth through changing the timing and frequency of vertical cell division. How expansion of thalli is regulated via vertical cell division remains an outstanding question. Since heat stress reduced the frequency of vertical cell division in *P. yezoensis* var. *irifuneiensis* (Figure 3C), it is necessary to identify genes whose functions in the regulatory machinery of vertical cell division are repressed by heat stress. In addition, incubation at 25°C resulted in shrink-and-twist morphology by production of callus (Figure 3D), suggesting that *P. yezoensis* var. *irifuneiensis* can be established as a novel experimental material for basic biological research into Bangiales, where regulatory mechanisms of two-dimensional expansion of single or two cell-layered leafy thalli have not been elucidated. In addition, its large and vigorous growth phenotype with adhesion to substrates suggests the potential for application of *P. yezoensis* var. *irifuneiensis* in nori aquaculture, similar to *P. yezoensis* f. *narawaensis*. Therefore, discovery of *P. yezoensis* var. *irifuneiensis* will provide novel insight into basic biological studies of the genus *Pyropia* and *benefit* sustainable aquaculture of nori.

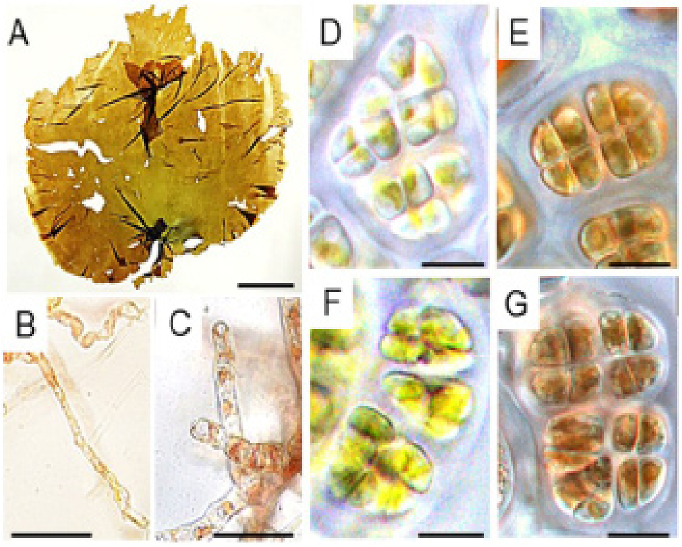

Figure 1: Morphological characteristics of the Irifune specimen. (A) Type specimen collected on April 2, 2018. (B & C) Morphology of conchocelis filaments and conchosporangia. (D & E) Surface views of male and female gametangia. (F and G) Transverse views of male and female gametangia. Bars: 4 cm (A), 50 μm (B, C), 10 μm (D–G).

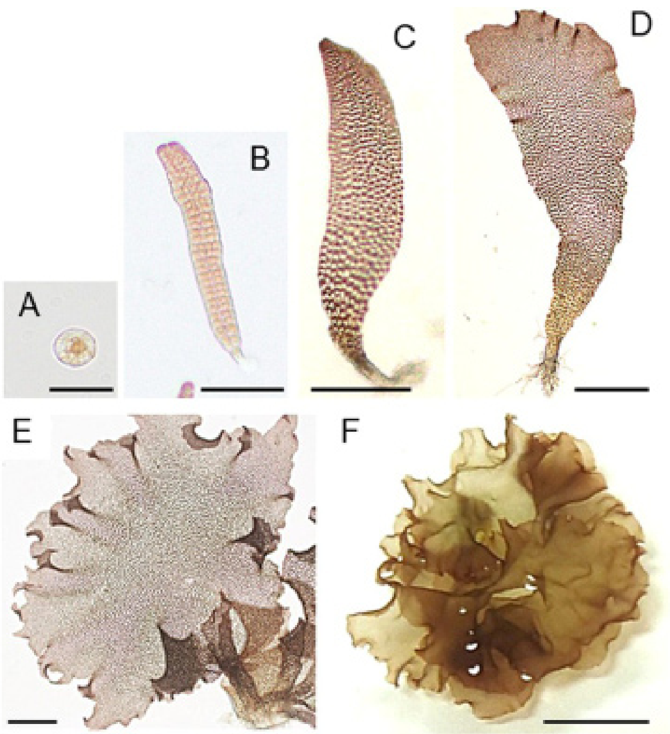

Figure 2: Development of asexual monospores of *Pyropia yezoensis* var. *irifuneiensis*. (A) Monospore. (B) Thallus grown for 1 week. (C) Thallus grown for 2 weeks. (D) Thallus grown for 6 weeks. (E) Thallus grown for 9 weeks. (F) Thallus grown for 18 weeks. Bars: 25 μm(A), 100 μm(B), 250 μm(C), 500 μm (D, E), 1 cm (F).

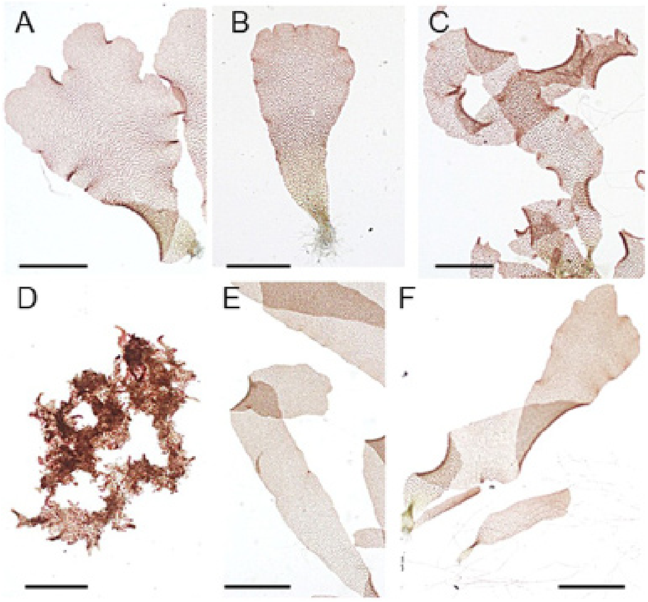

Figure 3: Representative photos of P*yropia yezoensis* var. *irifuneiensis* and *Pyropia yezoensis* f. *narawaensis* U51 cultured at various temperatures for 6 weeks. (A–D) Thalli of *P. yezoensis* var. *irifuneiensis* cultured at 10, 15, 20 and 25°C for 6 weeks. (E & F) Thalli of *P. yezoensis* f. *narawaensis* U51 cultured at 10 and 15°C for 6 weeks. Bars: 500 μm.

Acknowledgements

We are grateful to Kiyohiro Suzuki of the Hakodateshi Cooperative for permission and support in our seaweed collecting at the Irifune fishing port in Hakodate, Japan.

References

1. Sahoo DB, Tang X, Yarish C. *Porphyra* - the economic seaweed as a new experimental system. Current Science. 2002; 11: 1313–1316.

2. Blouin NA, Brodie JA, Grossman AC, Xu P, Brawley SH. *Porphyra*: a marine crop shaped by stress. Trends in Plant Science. 2011; 16: 29-37.

3. Kikuchi N, Nakada T, Niwa K. Proposal of a new combination and a valid name for the two Bangiales taxa (Rhodophyta) used for nori cultivation in Japan. Journal of Japanese Botany. 2015; 90: 380-385.

4. Miura A. A new variety and a new form of *Porphyra* (Bangiales, Rhodophyta) from Japan: *Porphyra tenera* Kjellman var. *tamatsuensis* Miura, var. nov. and *P. yezoensis* Ueda form. *narawaensis* Miura, form. nov. Journal of the Tokyo University of Fisheries. 1984; 71: 1-37.

5. Mizukami Y, Kito H, Kaminishi Y, Murase N, Kunimoto M. Nucleotide sequence variation in the ribosomal internal transcribed spacer regions of cultivated (cultivars) and field-collected thalli of *Porphyra yezoensis*. Fisheries Science. 1999; 65: 788-789.

6. Niwa K, Aruga Y. Identification of currently cultivated *Porphyra* species by PCR-RFLP analysis. Fisheries Science. 2006; 72: 143-148.

7. Ding H, Zhang B, Yan X. Isolation and characterization of a heat-resistant strain with high yield of *Pyropia yezoensis* Ueda (Bangiales, Rhodophyta). Aquaculture and fisheries. 2016; 1: 24-33.

8. Jiang H, Ding H, Yan X. Selection and characterization of an improved strain (A-18) by hybridization recombinant in *Pyropia yezoensis* (Bangiales, Rhodophyta). Haiyang Xuebao. 2018; 40:

95-103.

9. Niwa K, Abe T, Kobiyama A. Possibility of polyploidy breeding using cryptic species in the marine crop *Pyropia yezoensis* (Bangiales, Rhodophyta). Journal of Applied Phycology. 2018; 30: 1197-1205.

10. ShinYJ, Min SR, Kang DY, Lim J-M, Park EJ, Hwang MS, Choi D-W, Ahn JW, Park TI, Jeong W-J. Characterization of high temperature-tolerant strains of *Pyropia yezoensis*. Plant Biotechnology Report. 2018; 12: 365–373.

11. Hwang EK, Yotsukura N, Pang SJ, Su L, Shan TF. Seaweed breeding programs and progress in eastern Asian countries. Phycologia. 2019; 58: 484-495.

12. Sutherland J, Lindstrom S, Nelson W, Brodie J, Lynch M, Hwang MS, Choi HG, Miyata M, Kikuchi N, Oliveira M, Farr T, Neefus C, Mols-Mortensen A, Milstein D, Müller K. A new look at an ancient order: generic revision of the Bangiales. Journal of Phycology. 2011; 47: 1131-1151.

13. Sánchez N, Vergés A, Peteiro C, Sutherland JE, Brodie J. Diversity of bladed Bangiales (Rhodophyta) in western Mediterranean: recognition of the genus Themis and descriptions of *T. ballesterosii* sp. nov., *T. iberica* sp. nov., and *Pyropia parva* sp. nov. Journal of Phycology. 2014; 50: 908-929.

14. Müller KM, Cannone JJ, Sheath RG. A molecular phylogenetic analysis of the Bangiales (Rhodophyta) and description of a new genus and species, *Pseudobangia kaycoleia*. Phycologia. 2005; 44: 146-155.

15. Niwa K, Kikuchi N, Hwang M-S, Choi H-G, Aruga Y. Cryptic species in the *Pyropia yezoensis* complex. (Bangiales, Rhodophyta): Sympatric occurrence of two cryptic species even on same rocks. Phycological Research. 2014; 62: 36-43.

16. Li C, Ariga I, Mikami K. Difference in nitrogen starvation-inducible expression patterns among phylogenetically diverse ammonium transporter genes in the red seaweed *Pyropia yezoensis*. American Journal of Plant Sciences. 2019; 10: 1325-1349.

17. Hafting JT. A novel technique for propagation of *Porphyra yezoensis* Ueda blades in suspension cultures via monospores. Journal of Applied Phycology. 1999; 11: 361-367.