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Confirmation of *Neoporphyra* cf. *dentata* on Shikinejima, Izu Islands, southcentral Japan, and comparison with co-occurring *Neoporphyra haitanensis*

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SUMMARY

The Izu Islands of southcentral Japan are thought to fall within the distribution range of *Neoporphyra dentata*. However, the gametophytic blades of Bangiales collected from Shikinejima and Hachijojima, Izu Islands, were identified as *Neoporphyra haitanensis* in our previous study. Thus, it became uncertain whether *N. dentata* is distributed in Izu Islands, including Shikinejima. To clarify whether *N. dentata* grows on Shikinejima, we conducted a further distribution survey of *N. dentata* on the island. The morphological features of the blade samples collected from an additional sampling site on Shikinejima were more similar to those of *N. dentata* than to those of *N. haitanensis*: the blade thickness and the division formula of spermatangia resembled those of the former species rather than the latter species. However, the division formula of zygotosporangia was different from those of either species. The phylogenetic analyses of the *rbc*L gene indicated that the samples were resolved in a clade including *N. dentata* collected from Shirahama, Chiba Prefecture, and Enoshima, Kanagawa Prefecture, Honshu, Japan. The *p*-distances of the chloroplast *rbc*L gene and nuclear 18S rRNA also supported identification of the samples as *N. dentata*. The results demonstrated that *N. dentata* is also distributed on Shikinejima with co-occurring *N. haitanensis*, and that the island materials of the two species were genetically different from other materials of the two species, respectively.

Key words: bladed Bangiales, molecular phylogeny, *Neoporphyra dentata*, *rbc*L, taxonomy

INTRODUCTION

Until 2011, the bladed Bangiales were composed of a single genus, *Porphyra*. However, Sutherland et al. (2011) divided the genus into eight genera based on molecular phylogenetic analysis, and then Sánchez et al. (2014) added a new genus. Furthermore, Yang et al. (2020) proposed a revision of the genus *Pyropia* in bladed Bangiales to six genera, including four new genera and a resurrected genus. Consequently, the bladed Bangiales is now divided into 14 genera, and molecular phylogenetic studies have revealed surprisingly high biodiversity in the bladed Bangiales. Recently, Zuccarello et al. (2022) proposed that newly established genera should be restored to *Pyropia* because of the low confidence of branches in phylogenetic analyses. However, the scientific names in the present study follow Yang et al. (2020).

In Japan, 29 species of bladed Bangiales are distributed as reported in Yoshida et al. (2015). However, new species, cryptic species and species newly recorded from Japan have been discovered in bladed Bangiales based on detailed morphological observations and molecular analysis, and further genetic diversity in Japan has also been revealed (Niwa et al. 2014, Abe et al. 2021, Sano et al. 2021). Although previously reported from Japan by Sutherland et al. (2011), *Neoporphyra haitanensis* was recently recorded from Shikinejima and Hachijojima, Izu Islands, Japan (Sano et al. 2021), islands thought to be inhabited by *N. dentata*, a morphologically similar species (Okamura 1930, Segawa 1936, Takahashi et al. 2015). Thus, Sano et al. (2021) questioned whether *N. dentata* even occurred on these islands.

Although the type locality of *N. dentata* is Amakusa, western Kyushu (Kjellman 1897), there are no papers using the samples based on detailed morphological observations and molecular analyses. On the other hand, Sutherland et al. (2011) carried out molecular

phylogenetic analyses using *N. dentata* collected from Shirahama, Chiba Prefecture, on the Pacific coast of Honshu. In the present study, we investigated whether *N. dentata* (sensu Sutherland et al. 2011) is distributed on Shikinejima, Izu Islands, based on detailed morphological observations and molecular analyses using the sequence data of Sutherland et al (2011). However, since the molecular data for *N. dentata* were not obtained from samples collected from the type locality, it is uncertain that the species is authentic *N. dentata*. Thus, the species identified by Sutherland et al. (2011) and the present study is called *Neoporphyra* cf. *dentata*.

MATERIALS AND METHODS

Morphological observation and isolation of the conchocelis strain

Gametophytic blades of Bangiales were collected from site 1 on Shikinejima in February 2020 (Fig. 1a and b). They grew in the high intertidal zone of the rocky shore (Fig. 1c, d). Site 1 was different from a site of the *Neoporphyra haitanensis* habitat (site 2 in Fig. 1b) reported by Sano et al. (2021). The collected blades were transported in a cool box to our laboratory. The collections were wiped with paper towels and stored at -30 °C. After thawing, some of the blades were spread on paper and immediately photographed. For molecular identification, a blade piece (approximately 2-3 cm^2) was cut from each blade and used for DNA extraction. Morphological observation was conducted using the blade samples identified by 77 bp of the RUBISCO spacer region (see below). For anatomical observation, blade sections were made by hand using a razor blade. In addition, some of the blades after defrosting were used to isolate the conchocelis (filamentous thalli) strain, according to the procedures of Niwa et al. (2005).

Molecular analyses and phylogenetic analyses

Total DNA was extracted using the ISOPLANT II kit (Nippon Gene, Tokyo, Japan) following the method of Niwa et al. (2005). The chloroplast RUBISCO spacer region, the *rbc*L gene, and the nuclear V9 region of 18S rRNA were amplified with primer pairs from Sano et al. (2020), Hanyuda et al. (2004), and Yang et al. (2020), respectively. For sequence analysis of the *rbc*L gene, two additional primers, Rh1-2 and LS1-2, were also used, as previously described (Niwa et al. 2008). The nucleotide sequences were determined by a DNA sequencing service (FASMAC, Atsugi, Japan). In addition, we determined the *rbc*L and V9 sequences of a blade (SND-M2) collected from site 2 in a previous study (Sano et al. 2021), because the RUBISCO spacer sequences of the blade were different from those of the *N. haitanensis* blades in the previous study. In each region, highly homologous sequences were investigated by BLAST search. The determined sequences have been deposited in GenBank/EMBL/DDBJ. The accession numbers of the RUBISCO spacer region, the *rbc*L gene, and the V9 region are described as follows.

RUBISCO spacer: the blade from site 1, LC616360; the blade from site 2, LC616361. *rbc*L gene: the blade from site 1, LC616364; the blade from site 2, LC616365. V9: the blade from site 1, LC616368; the blade from site 2, LC616369.

Phylogenetic analyses of the *rbc*L gene (1,230 bp) were carried out using maximum-likelihood (ML) and Bayesian inference (BI) methods. The determined sequences and published sequence data of bladed Bangiales were used for the phylogenetic analyses (Table S1). The ML tree was constructed using MEGA XI (Kumar et al. 2018) with the Tamura-Nei model. Bootstrap analysis was performed using 1,000 pseudoreplicates. BI analyses were run using MrBayes v. 3.2.7 (Ronquist et al. 2012) with the GTR+I+G model selected for each gene, and data were partitioned by codon position. Analyses were

started from random trees and were run with four chains of Markov chain Monte Carlo (MCMC) iterations. Trees were sampled every 100 generations for 100 000 generations, and the results verified that the average standard deviation of split frequencies (ASDSF) was <0.01 and that potential scale reduction factor (PSRF) values were stable at approximately 1.00. The first 25% of trees sampled were discarded as 'burn-in', and posterior probability values were calculated from the remaining trees. The *p*-distances of *rbc*L and V9 were also calculated from the determined and published sequences using MEGA XI.

RESULTS

Morphological observations

Some of the gametophytic blades from site 1 and site 2 are shown in Fig. 2. The blade shape was lanceolate or linear-lanceolate. The conchocelis SND-2 strain was established from the third blade from the left in Fig. 2. The shape of the basal portion was cordate or rounded. The blade color was light brownish purple, light reddish brown or dark purplish red. Herbarium specimens were deposited in the Museum of Marine Science, Tokyo University of Marine Science and Technology: the specimen numbers are MTUF-43303 and MTUF-43304.

Microscopic observations were conducted using blades from site 1. Microscopic denticulae were observed along the blade margin (Fig. 3a). Each vegetative cell possessed a single chloroplast (Fig. 3b). In sectional view, the vegetative portions were monostromatic, and the shape of the vegetative cells was quadrate with rounded corners (Fig. 3c). The blade thickness in the central vegetative portions ranged from approximately 41 to 57 μm. The blades were dioecious. The division formula of spermatangia was presumed to be 128 (a/4, b/4, c/8) (Fig. 3d–g), although the division in the sectional view was not always complete.

142 The surface view in the division formula of zygotosporangia was presumed to be a/4 and
143 b/2 (Fig. 3h) or a/4 and b/4, although the division (a/4, b/4) was not always complete (Fig.
144 3i and j). The sectional cell number c in the division formula for most samples was 2 (Fig.
145 3k); however, c values up to 4 were observed (Fig. 3l and m). Thus, the division formula of
146 zygotosporangia was 16–64 (a/4, b/2–4, c/2–4) (Fig. 3h–m).
147
148 Molecular analyses
149 The nucleotide sequences (77 bp) of the RUBISCO spacer regions were determined from
150 approximately ten samples, including the conchocelis SND-2 strains, all of which originated
151 from site 1. Their sequences were identical to each other, and the sequence haplotype was
152 first detected in the present study. The RUBISCO spacer sequences of SND-M2 that were
153 collected from site 2 were different from those of site 1 samples by 1 bp. In addition, the
154 difference between the sequences of SND-2 and *Porphyra* sp. DE001 (AB287971) that was
155 collected from Enoshima (Fig. 1a) in Kanagawa Prefecture was 2 bp, whereas the difference
156 between the sequences of SND-M2 and *Porphyra* sp. DE001 was 1 bp.
157 The nucleotide sequences of the *rbc*L gene and the V9 region were determined from
158 SND-2 (the sample from site 1) and SND-M2 (the sample from site 2). The number of
159 differences and *p*-distances between the *rbc*L sequences of the two samples and the related
160 species are shown in Table 1. The *rbc*L sequences of SND-2 and SND-M2 differed by 11 bp.
161 The difference between the *rbc*L sequences of SND-2 and *N.* cf. *dentata* (Sutherland et al.
162 2011, HQ687520) that was collected from Shirahama (Fig. 1a) in Chiba Prefecture was 11
163 bp, whereas the difference between SND-M2 and *N.* cf. *dentata* was 8 bp. Thus, the
164 *p*-distances between all samples were < 1.0% (Table 1). In addition, the difference between
165 the *rbc*L sequences of *N.* cf. *dentata* from Shirahama and *Porphyra* sp. DE001 (AB287971)

166 from Enoshima was 1 bp. The phylogenetic tree of the *rbc*L gene using the maximum
167 likelihood (ML) method and Bayesian inference (BI) method is presented in Fig. 4. The two
168 samples, SND-2 from site 1 and SND-M2 from site 2, were resolved in a clade including *N.*
169 cf. *dentata* and *Porphyra* sp. DE001 with high bootstrap.
170 The number of differences and *p*-distances between the V9 sequences of the two
171 samples (SND-2 and SND-M2) and the related species are shown in Table 2. The V9
172 sequences of SND-2 and SND-M2 differed by 1 bp. The difference between the sequences
173 of SND-2 and *N.* cf. *dentata* was 1 bp, whereas the sequences of SND-M2 were identical to
174 those of *N.* cf. *dentata* (Sutherland et al. 2011, HQ687588) and *Porphyra* sp. DN002
175 (AB293534), which were collected from Shirahama and Enoshima, respectively (Table 2).
176
177 **DISCUSSION**
178 Since the RUBISCO spacer sequences of the examined blades that were collected from site
179 1 on Shikinejima were identical to each other, it was inferred that the population of the
180 bladed Bangiales was composed of the same species. External and anatomical features of
181 the blades were similar to those of *N. dentata* and *N. haitanensis* (Kjellman 1897; Ueda
182 1932; Okamura 1936; Tanaka 1952; Chang & Zheng 1960; Fukuhara 1968; Kim 1999; Sano
183 et al. 2021). We compared these features in Table 3. The features of *N. dentata* and *N.*
184 *haitanensis* were quite similar: both species had microscopic denticulae, and both were
185 dioecious, although some monoecious blades consisting of male and female sectors were
186 also observed in both species (Chang & Zheng 1960; Kurogi & Yamada 1986). Moreover,
187 the division formula of the reproductive cells of *N. dentata* was also similar to that of *N.*
188 *haitanensis*. However, blade thickness in the vegetative portion of *N. dentata* was thinner
189 than that of *N. haitanensis*: the former was 30-58 μm (Ueda 1932; Okamura 1936; Tanaka

1952), whereas the latter was 55-95 μm. The thickness of the blades collected from site 1 on Shikinejima was 41-57 μm. These results indicated that the samples from site 1 were more similar to *N. dentata* than *N. haitanensis*.

The division formula of spermatangia of *N. dentata* and *N. haitanensis* was mostly the same number (Ueda 1932; Okamura 1936; Tanaka 1952; Chang & Zheng 1960; Fukuhara 1968), and in *N. haitanensis,* a different cell number was also observed (Chang & Zheng 1960), as previously reported in other species of the bladed Bangiales (Lindstrom & Cole 1992). In the present study, c/16 of the sectional view, which was reported in *N. haitanensis* (Chang & Zheng 1960), was not observed in the examined samples, indicating that their division formula of spermatangia was identical to that of *N. dentata*. On the other hand, the division formula of zygotosporangia of *N. dentata* was 16 (a/2, b/2, c/4) (Ueda 1932; Okamura 1936; Tanaka 1952; Fukuhara 1968), whereas that of *N. haitanensis* was 8–32 (a/2–4, b/2, C/2–4) (Chang & Zheng 1960; Sano et al. 2021). In the examined blades, the surface view of the division formula was mostly a/4 and b/2 and rarely a/4 and b/4. Additionally, the sectional view of the division formula was mostly c/2 and rarely c/4, although the sectional view of the division formula of *N. dentata* was c/4. These results indicated that the division formula of zygotosporangia of the examined blades was different from those of *N. dentata* and N. *haitanensis*. Therefore, although the division formula of zygotosporangia was different between the examined samples, morphological and anatomical features of the blades that were collected from site 1 in Shikinejima were more similar to *N. dentata* than to *N. haitanensis*.

The phylogenetic analysis demonstrated that SND-2 and SND-M2 were resolved in a clade including *N.* cf. *dentata* (HQ687520) from Shirahama. *Porphyra* sp. DE001 (thus, it was identified as *N.* cf. *dentata* DE001) from Enoshima was also included in the clade,

214 although the RUBISCO spacer sequences of SND-2, SND-M2 and *Porphyra* sp. DE001
215 were different from each other. All *p*-distances between SND-2, SND-M2, and *N.* cf.
216 *dentata* were < 0.9% (8-11 bp). In northwestern Atlantic *Porphyra* species, very little
217 intraspecific variations in *rbc*L sequences were confirmed to be < 0.1% (Klein et al. 2003).
218 Lindstrom and Fredericq (2003) showed that the intraspecific sequence divergences for
219 approximately half of the species in the bladed Bangiales, which were examined by them,
220 ranged from 0.0 to 0.5%. On the other hand, Milstein et al. (2015) reported that interspecific
221 divergences in the bladed Bangiales were > 1.9% for the *rbc*L gene, despite exhibiting some
222 exceptions. In *N. haitanensis*, which is a member of the same genus of *N. dentata*, the
223 intraspecific divergences were confirmed to be < 1.3% (Sano et al. 2021). Therefore,
224 although the sequence variations between SND-2, SND-M2, and *N.* cf. *dentata* were
225 slightly higher than those expected for conspecies compared to the former two studies
226 (Klein et al. 2003; Lindstrom and Fredericq 2003), SND-2 and SND-M2 from Shikinejima
227 were presumed to be *N.* cf. *dentata* based on phylogenetic analysis and the *p*-distance of the
228 *rbc*L gene. Additionally, it was also presumed that *N.* cf. *dentata* possessed 3 haplotypes of
229 RUBISCO spacer sequences from the above-mentioned results.
230 In the V9 region of the 18S rDNA, the *p*-distance between SND-2 and *N.* cf. *dentata*
231 was 0.3% (1 bp), whereas the distance between SND-M2 and *N.* cf. *dentata* was 0%
232 because the sequences were identical. According to a previous study, interspecific
233 divergences of 18S rRNA in bladed Bangiales were > 1.1% (Milstein et al. 2015). On the
234 other hand, the intraspecific divergences of 18S rRNA in *N. haitanensis* were confirmed to
235 be < 0.4% (Sano et al. 2021). The results of the nuclear DNA region also identified SND-2
236 from site 1 and SND-M2 from site 2 as *N.* cf. *dentata*. Although *Porphyra* sp. DN002 in
237 Table 2 was different from the specimen *Porphyra* sp. DE001, the *rbc*L sequences of which

were determined, *Porphyra* sp. DN002 was also collected from Enoshima. Because the V9 sequences of the specimen were identical to those of *N.* cf. *dentata*, *Porphyra* sp. DN002 could also be identified as *N.* cf. *dentata*.

As mentioned above, the results of detailed morphological observations and molecular analyses of chloroplast and nuclear DNA regions identified the blades and conchocelis of strain SND-2 from site 1 and the blade of SND-M2 from site 2 as *N.* cf. *dentata*; these samples were genetically different from those from Shirahama and Enoshima in Honshu, Japan. Thus, in addition to *N. haitanensis*, it was shown that *N.* cf. *dentata* is also distributed on Shikinejima, Izu Islands, southcentral Japan. However, to clarify that these samples are *N. dentata* (see Introduction), further morphological and molecular studies using the samples collected from the type locality of *N. dentata* are needed.

In Japan, most cultivated strains of nori are *Neopyropia yezoensis* f. *narawaensis* (Miura 1984; Niwa & Aruga 2006), and the species is considered a northern species in Japan (Miura 1988). However, since nori cultivation has been damaged due to the influence of high seawater temperatures caused by global warming (Niwa 2020), it has become important to establish and collect conchocelis strains of southern species as potential breeding materials. Recently, the strains of the southern species, *N. haitanensis* and *N. kitoi*, have been isolated (Sano et al. 2021; Niwa et al. 2022). Despite faster maturity, the later strain has potential as a new cultivated species with high growth and high temperature tolerance as determined from the results of laboratory culture and experimental cultivation on a nori farm (Niwa et al. 2022, 2023). Thus, it is expected that the isolated conchocelis strain SND-2 of *N.* cf. *dentata* also has the potential to be used as breeding material for the development of new cultivars with high temperature tolerance.

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Figure legends

Fig. 1. Map showing the sampling localities: Shikinejima, Izu Islands, Tokyo Metropolitan Area, with the related localities, Shirahama, Chiba Prefecture, and Enoshima, Kanagawa Prefecture, Honshu, Japan (a, b); a natural habitat of the bladed Bangiales at site 1 on Shikinejima Island (c); and gametophytic blades of the bladed Bangiales at site 1 (d). Black circles in b indicate the sampling sites. Arrows in c indicate the zonation of the bladed Bangiales in the high intertidal zone on the rocky shore.

Fig. 2. Gametophytic blades of *Neoporphyra* cf. *dentata* collected from Shikinejima, Tokyo Metropolitan Area, Japan. Three blades collected from site 1 and the SND-M2 blade collected from site 2 are shown from left to right. Scale bar = 10 cm. The conchocelis strain SND-2 was isolated from the third blade from the left.

Fig. 3. Morphology of gametophytic blades of *Neoporphyra* cf. *dentata* collected from Shikinejima, Tokyo Metropolitan Area, Japan. (a) Marginal portion with microscopic denticulae. (b) Surface view of vegetative cells. (c) Section of vegetative cells in the central portion. (d) Surface view of spermatangia composed of 16 cells (a/4, b/4) (arrow). (e) Line drawing of Fig. 3c. (f) Section of spermatangia containing 8 cell layers (c/8) (arrow). (g) Line drawing of Fig. 3g. (h) Surface view of zygotosporangia composed of 8 cells (a/4, b/2) (arrow). (i) Surface view of zygotosporangia composed of 16 cells (a/4, b/4). (j) Line drawing of Fig. 3i. (k) Section of zygotosporangia composed of 2 cell layers (c/2) (arrow). (l) Section of zygotosporangia composed of 4 cell layers (c/4) (arrow). (m) Line drawing of Fig. 3l. Scale bars = 20 μm.

Fig. 4. Maximum likelihood (ML) tree of the bladed Bangiales based on the *rbc*L gene (total 1230 bp). Numbers below the branches indicate the bootstrap values (BP, left) and Bayesian posterior probability (PP, right). Only BP ≥ 50% and PP ≥ 0.85% are

401 shown.

Table 1. The number of differences (below diagonal) and *p*-distance (above diagonal) between the *rbc*L sequences (1230 bp) of the two samples (SND-2, SND-M2), *Neoporphyra* cf. *dentata* and *Neoporphyra haitanensis*, and the related species.

	Sample	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
1	SND-2	—	0.0089	0.0089	0.0081	0.0358	0.0366	0.0374	0.0366	0.0301	0.0309	0.0390	0.0382	0.0439	0.0285	0.0325
2	SND-M2	11	—	0.0065	0.0057	0.0382	0.0390	0.0398	0.0390	0.0325	0.0333	0.0398	0.0390	0.0463	0.0309	0.0350
3	*Neoporphyra* cf. *dentata*	11	8	—	0.0008	0.0366	0.0374	0.0382	0.0374	0.0309	0.0317	0.0398	0.0374	0.0431	0.0309	0.0350
4	*Porphyra* sp. DE001	10	7	1	—	0.0358	0.0366	0.0374	0.0366	0.0301	0.0309	0.0390	0.0366	0.0439	0.0301	0.0341
5	*Neoporphyra haitanensis* PH-38, Yuge	44	47	45	44	—	0.0008	0.0016	0.0008	0.0114	0.0106	0.0398	0.0398	0.0496	0.0350	0.0390
6	*Neoporphyra haitanensis* LYCN162	45	48	46	45	1	—	0.0008	0.0008	0.0122	0.0114	0.0407	0.0407	0.0504	0.0358	0.0398
7	*Neoporphyra haitanensis* LYCN192	46	49	47	46	2	1	—	0.0016	0.0130	0.0122	0.0415	0.0415	0.0512	0.0366	0.0407
8	*Neoporphyra haitanensis* LYCN202	45	48	46	45	1	1	2	—	0.0122	0.0114	0.0407	0.0407	0.0504	0.0358	0.0398
9	*Neoporphyra haitanensis* SNH-1, SNH-2	37	40	38	37	14	15	16	15	—	0.0008	0.0390	0.0390	0.0463	0.0309	0.0366
10	*Neoporphyra haitanensis* HJH-1	38	41	39	38	13	14	15	14	1	—	0.0382	0.0382	0.0455	0.0301	0.0358
11	*Neoporphyra kitoi* Psp1	48	49	49	48	49	50	51	50	48	47	—	0.0024	0.0472	0.0252	0.0293
12	*Neoporphyra kitoi* UM-KT6, OHN-1	47	48	46	45	49	50	51	50	48	47	3	—	0.0455	0.0244	0.0285
13	*Neoporphyra seriata*	54	57	53	54	61	62	63	62	57	56	58	56	—	0.0415	0.0463
14	*Porphyra* sp. 6POR	35	38	38	37	43	44	45	44	38	37	31	30	51	—	0.0073
15	*Porphyra* sp. ZLI1045	40	43	43	42	48	49	50	49	45	44	36	35	57	9	—

414 **Table 2.** The number of differences (below diagonal) and the *p*-distance (above diagonal) between the V9 sequences (333 bp) of the two
415 samples (SND-2, SND-M2), *Neoporphyra* cf. *dentata* and *Neoporphyra haitanensis*, and the related species.

	Sample	1	2	3	4	5	6	7	8	9
1	SND-2	—	0.0030	0.0030	0.0030	0.0120	0.0150	0.0120	0.0240	0.0270
2	SND-M2	1	—	0.0000	0.0000	0.0150	0.0180	0.0150	0.0210	0.0300
3	*Neoporphyra* cf. *dentata*	1	0	—	0.0000	0.0150	0.0180	0.0150	0.0210	0.0300
4	*Porphyra* sp. DN002	1	0	0	—	0.0150	0.0180	0.0150	0.0210	0.0300
5	*Neoporphyra haitanensis* LYCN064, 106, 123, Yuge	4	5	5	5	—	0.0030	0.0120	0.0240	0.0270
6	*Neoporphyra haitanensis* LYCN108	5	6	6	6	1	—	0.0150	0.0270	0.0300
7	*Neoporphyra kitoi* Psp1, UM-KT6, OHN-1	4	5	5	5	4	5	—	0.0240	0.0150
8	*Neoporphyra seriata*	8	7	7	7	8	9	8	—	0.0330
9	*Porphyra* sp. ZLI1045	9	10	10	10	9	10	5	11	—

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Table 3. Morphological features of the bladed Bangiales collected from site 1 on Shikinejima (present study), *Neoporphyra haitanensis* and *Neoporphyra dentata*.

Features	Present study	*Neoporphyra dentata*	*Neoporphyra haitanensis*
Blade shape	Lanceolate, linear–lanceolate	Lanceolate, linear-lanceolate, linear, bamboo leaf shape, long ovate, oblong ovate, ovate, obovate	Lanceolate, linear-lanceolate, elongated subovate, subovate
Shape of basal portion	Cordate or rounded	Cordate	Cordate, sometimes rounded or cuneate
Blade color	Dark purplish red, light brownish purple, light reddish brown	Dark mauve, mixture of violet and purple, light purple, faded purple, light purplish red, pale brownish red	Dark greenish purple with brownish tinge, dark purplish red, light brownish purple
Blade margin	Microscopic denticulae	Microscopic denticulae	One– to three–celled denticulae
Chloroplast	Single stellate	Single stellate	Single stellate, sometimes two stellates
Blade section	Monostromatic	Monostromatic	Monostromatic but distromatic portions present
Shape of vegetative cells in sectional view	Quadrate with rounded	Square, quadrate with rounded angles	Rectangular
Thickness of vegetative portion (μm)	41–57 μm	30–58 μm	55–95 μm
Sex type	Dioecious	Dioecious[1)], rarely monoecious	Mostly dioecious, sometimes monoecious, or monoecious[2)]
Division formula of spermatangia	128 (a/4, b/4, c/8)	128 (a/4, b/4, c/8)	128 (a/4, b/4, c/8), 256 (a/4, b/4, c/16)
Division formula of zygotosporangia	16–32 (a/4, b/2–4, c/2–4)	16 (a/2, b/2, c/4)	8–32 (a/2–4, b/2, c/2–4)[3)]
Habitat	Upper intertidal zone	Upper intertidal zone	Upper intertidal zone
Reference		Kjellman (1897), Ueda (1932), Okamura (1936), Tanaka (1952), Fukuhara (1968), Kim (1999)	Chang and Zheng (1960), Zhang *et al*. (2013), Sano et al. (2021)

[1)] Kurogi and Yamada (1986) observed the specimens of Kjellman (1897). They suggested that the specimen is monoecious, although Kjellman reported that *N. dentata* is dioecious.

[2)] Zhang *et al*. (2013) reported that *N. haitanensis* is monoecious.

[3)] Although Chang and Zheng (1960) reported that the formula of zygotosporangia was 32 (a/2, b/4, c/4), we described it as 32 (a/4, b/2, c/4).

441 **Supplemental Table S1:** List of accession numbers acquired from GenBank for the *rbc*L
442 and 18S genes for molecular analyses and phylogenetic analyses.

Species	***rbc*L**	**18S**	**Source**
Neoporphyra cf. *dentata* SND-2, Japan	LC616364	LC616368	Present study
Neoporphyra cf. *dentata* SND-M2, Japan	LC616365	LC616369	Present study
Neoporphyra cf. *dentata*, Japan	HQ687520	HQ687588	Sutherland et al. (2011)
Neoporphyra haitanensis LYCN162, China	MG604540	—	Yang et al. (2018)
Neoporphyra haitanensis LYCN192, China	MG604567	—	Yang et al. (2018)
Neoporphyra haitanensis LYCN202, China	MG604576	—	Yang et al. (2018)
Neoporphyra haitanensis PH-38	KC464603	—	Wang et al. (2013)
Neoporphyra haitanensis SNH-1, Japan	LC599648	—	Sano et al. (2021)
Neoporphyra haitanensis SNH-2, Japan	LC599649	—	Sano et al. (2021)
Neoporphyra haitanensis HJH-2, Japan	LC599650	—	Sano et al. (2021)
Neoporphyra haitanensis Yuge, Ehime Japan	AB118585	AB013181	Sutherland et al. (2011)
Neoporphyra haitanensis LYCN064, China	—	MG647683	Yang et al. (2018)
Neoporphyra haitanensis LYCN106, China	—	MG647723	Yang et al. (2018)
Neoporphyra haitanensis LYCN108, China	—	MG647725	Yang et al. (2018)
Neoporphyra haitanensis LYCN123, China	—	MG647739	Yang et al. (2018)
Neoporphyra kitoi UM-KT6, Chiba Japan	LC634521	LC634518	Abe et al. (2021)
Neoporphyra kitoi Psp1, Yamaguchi Japan	LC328550	LC328547	Abe et al. (2021)
Neoporphyra kitoi OHN-1, Chiba Japan	LC626356	LC626357	Niwa et al. (2022)
Neoporphyra seriata, Kumamoto Japan	HQ687533	HQ687576	Sutherland et al. (2011)
Neoporphyra seriata E16047, Korea	MG845442	—	Koh and Kim (2018)
Porphyra sp. DE001, Kanagawa Japan	AB287971	—	
Porphyra sp. DN002, Kanagawa Japan	—	AB293534	
Porphyra sp. 6POR, Texas America	JN029003	—	Kucera and Saunders (2012)
Porphyra sp. ZLI1045, South Africa	GU165839	AY292635	Sutherland et al. (2011)
Wildemania amplissima, Hokkaido Japan	HQ687560	—	Sutherland et al. (2011)

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