



Desmarestia japonica subsp. *angustifolia* (Desmarestiales, Phaeophyceae), a new subspecies from Korea

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Abstract

Desmarestia japonica H. Kawai, T. Hanyuda, D. G. Müller, E. C. Yang, A. F. Peters, & F.C. Küpper (2014: 161) was recently reported in Japan, Korea, and China. In this study, we found intraspecific morphogenetic variation in *D. japonica*. Morphologically, the new taxonomic entity was characterized by narrow filamentous primary and secondary branches (morphotype 2), which was distinct from the broad (ligulate) branches in the original description of *D. japonica* (morphotype 1). Korean individuals with morphotype 2 were clearly separated from *D. japonica* with morphotype 1 in the molecular phylogenetic tree using the mitochondrial cytochrome c oxidase 1 (*cox1*) gene. Therefore, we propose that this new taxonomic entity be established as *Desmarestia japonica* subsp. *angustifolia* subsp. nov. (Phaeophyceae, Desmarestiales) based on its morphological and molecular characteristics.

Keywords: *cox1*, *Desmarestia japonica*, intraspecific variation, Korea, new subspecies

Introduction

Desmarestia are brown algae that are distributed mainly in the cold seawaters of the Northern and Southern Hemispheres, which have branched or foliose macroscopic thalli with free sulfuric acid (Graham *et al.* 2009; Yang *et al.* 2014). Yang *et al.* (2014) examined the molecular phylogenetic relationships of species in the genus *Desmarestia* and revised their taxonomic status at intra/interspecific levels. In addition, the new species *D. japonica* H. Kawai, T. Hanyuda, D.G. Müller, E.C. Yang, A.F. Peters, & F.C. Küpper was established for Japanese individuals. In Korea, three *Desmarestia* species have been reported based on morphological analyses: *D. ligulata*, *D. tabacoides*, and *D. viridis* (Lee & Hwang 2010). Recently, we examined the distribution of *D. japonica* in Korea and China using *cox1* DNA sequences (Lee & Lee 2018).

Yang *et al.* (2014) established new subspecies of *D. ligulata*, *D. dudresnayi*, and *D. herbacea* based on genetic variation at the intra/interspecific level. Specifically, *D. ligulata* was subdivided into subsp. *ligulata* f. *distans* (C. Agardh) A.F. Peters, E.C. Yang, F.C. Küpper, & Prud'Homme van Reine comb. nov., subsp. *muelleri* (M.E. Ramirez et A.F. Peters) A.F. Peters, E.C. Yang, F.C. Küpper, & Prud'Homme van Reine comb. nov., and subsp. *gayana* (Montagne) A.F. Peters, E.C. Yang, & F.C. Küpper comb. nov. (Yang *et al.* 2014). These *D. ligulata* subspecies formed different groups in the molecular phylogenetic tree at the intraspecific level (Yang *et al.* 2014).

However, no morphological or genetic variation in *D. japonica* at the intraspecific level has been reported. Therefore, in this study, we performed morphological and molecular phylogenetic analyses of Korean *D. japonica* and recognized intraspecific differences. Here, we discuss a new taxonomic entity.

Materials and Methods

We collected field samples and examined the morphology of herbarium specimens of *D. japonica* deposited in the National Institute of Biological Resources, Korea (Figs. 1 & 2; Table 1). We conducted a molecular analysis of herbarium specimens previously identified as *D. ligulata* morphologically, but subsequently annotated as *D. japonica*. The field samples were deposited on a silica gel for DNA analysis. The morphological analysis was conducted using light microscopy (BX50, Olympus, Tokyo, Japan) and photographs were obtained with a digital camera (C-4040 Zoom, Olympus). To analyze images of herbarium specimens, we used a scanner (Epson, Seiko Epson, Nagano, Japan).

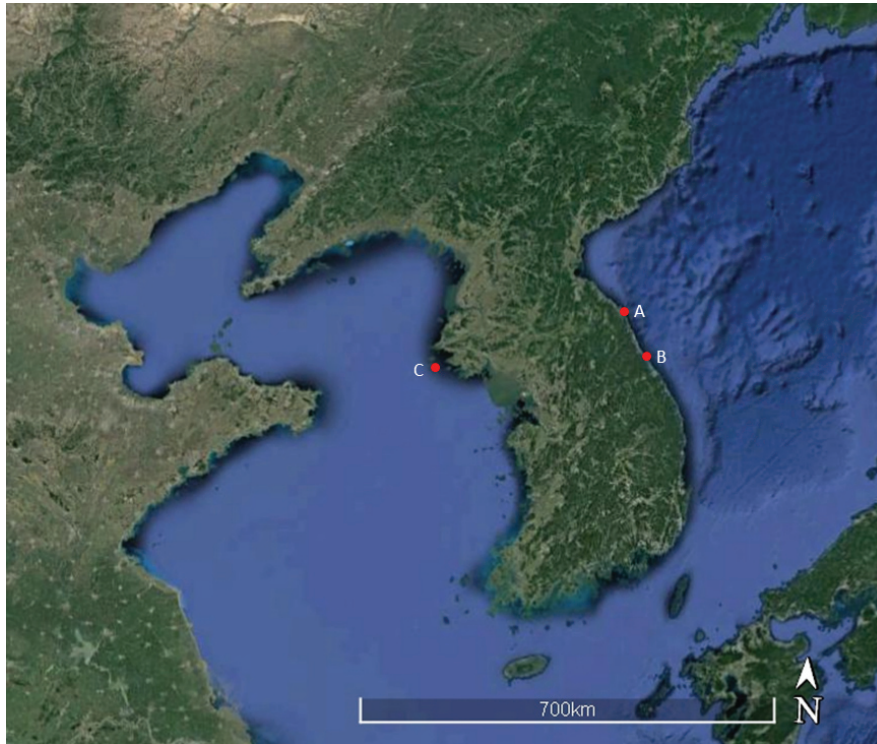


FIGURE 1. Korean *Desmarestia japonica* collection sites: A, Goseong; B, Gangneung; C, Socheong Island.

TABLE 1. Sampling information of *Desmarestia japonica*

Specimen no.	Collection site	Collection date		
NIBRAL0000000705	Gangwon-do Goseong-gun Toseong-myeon	2005-07-23	38°17'12.6"N	128°33'0.6"E
NIBRAL0000000724	Gangwon-do Gangneung-si Gangdong-myeon	2006-03-07	37°43'55"N	128°59'32"E
NIBRAL0000122788	Gangwon-do Gangneung-si Gangdong-myeon Aninjin-ri	2009-05-08	37°43'55"N	128°59'32"E
NIBRAL0000122790	Gangwon-do Gangneung-si Gangdong-myeon Aninjin-ri	2009-05-08	37°43'55"N	128°59'32"E
NIBRAL0000132118	Gangwon-do Gangneung-si Gangdong-myeon Aninjin-ri	2011-05-14	37°43'55"N	128°59'32"E
NIBRAL0000132117	Gangwon-do Gangneung-si Sacheon-myeon Sacheonjin-ri	2011-06-15	37°50'16.2"N	128°52'44"E
NIBRAL0000132116	Gangwon-do Gangneung-si Sacheon-myeon Sacheonjin-ri	2011-06-15	37°50'16.2"N	128°52'44"E
NIBRAL0000123497	Gangwon-do Gangneung-si Gangdong-myeon Anin-ri	2010-05-21	37°44'20.67"N	128°59'7.79"E
NIBRAL0000122789	Gangwon-do Gangneung-si Gangdong-myeon Aninjin-ri	2009-05-08	37°43'55"N	128°59'32"E
NIBRAL0000142337	Gangwon-do Gangneung-si Gangmun-dong	2013-04-25	37°47'50.65"N	128°55'3.14"E
NIBRAL0000142338	Gangwon-do Gangneung-si Gangmun-dong	2013-04-25	37°47'50.65"N	128°55'3.14"E
NIBRAL0000142339	Gangwon-do Gangneung-si Gangmun-dong	2013-04-25	37°47'50.65"N	128°55'3.14"E
NIBROR0000001248	Incheon Ongjin-gun Daecheong-myeon Socheong-ri	2017-05-14	37°46'5.00"N	124°45'43.00"E



FIGURE 2. Specimens of *D. japonica* from Korea. (A) Morphotype 1, *D. japonica* subsp. *japonica* (B) Morphotype 2, *D. japonica* subsp. *angustifolia* subsp. nov.

After morphological observation, we removed a small piece ($< 0.5 \text{ cm}^2$) from each of the dried samples for molecular phylogenetic analysis. DNA extraction, polymerase chain reaction (PCR), and sequencing were conducted according to Lee & Lee (2018). We selected the *cox1* region for molecular phylogenetic analysis and used primer pairs suggested by Lee & Lee (2018) (*cox1*-desm-193F/*cox1*-desm-504R). The PCR conditions were as follows: 3 min at 95°C , 40 cycles of 30 s at 94°C , 30 s at 50°C , and 30 s at 72°C , and a final 7 min extension at 72°C . We used a commercial sequencing service (Genotech, Daejeon, Korea) and the program Sequencher 5.4.6 (Gene Codes, Ann Arbor, MI, USA) for assembling chromatograms. MEGA ver. 6 (Tamura *et al.* 2013) was used for phylogenetic analysis with the neighbor-joining method with 2000 bootstrap replicates and the pairwise distance calculation.

Results

The overall morphology of the *D. japonica* specimens varied in terms of branching and branch width (Figs. 2 & 3). Prominent morphological features to differentiate among subsp. *angustifolia* and the typical subspecies are supplied in Table 2. The calculation was performed basing on four herbarium specimens with morphotype 2 and nine of morphotype 1 (Table 1).

Two morphotypes showed significantly the different values (e.g. primary blade width and secondary blade width) (Table 2). The narrow filamentous type (morphotype 2) had narrow primary and secondary branches. The difference in branch width was greater for the secondary branch than the primary branch. The secondary branch of the narrow type was longer than that of the broad type (Figs. 2 & 3; Table 2). The morphotype 2 specimens were collected at two nearby sites in Korea (Gangmun-dong, Gangneung in 2009 and Aninjin-ri, Gangneung in 2013) and had a restricted distribution (Table 2).

Six *cox1* sequences (272 bases) were newly determined from herbarium specimens and field samples from the Korean coast. *D. japonica* showed 2.6–17.6% pairwise distances with other *Desmarestia* species.

At the intra/intersubspecific level, the *Desmarestia* species showed marked genetic variation (Table 3). The *cox1* sequences of *D. japonica* reported from Korea and Japan consisted of two *cox1* haplotypes. The *cox1* sequences of two samples (herbarium specimen NIBRAL0000122788 and a field sample collected from Socheong Island, Korea on 14 May 2017) were identical to the sequence of specimens from Japan [HE866773 in Yang *et al.* (2014)] and Korea

[MF363010; NIBRAL0000000724, NIBRAL0000122790, and NIBRAL0000000705 in Lee & Lee (2018)]. Another *cox1* haplotype (GenBank accession MH423500 (NCBI, National Center for Biotechnology Information)) found in four Korean samples (NIBRAL0000122789, NIBRAL0000142337, NIBRAL0000142338, and NIBRAL0000142339) differed from MF363010 by two bases (99.3% similarity). In the phylogenetic tree, the two *D. japonica* haplotypes formed a single clade that was distantly related to other *Desmarestia* species (Fig. 4).

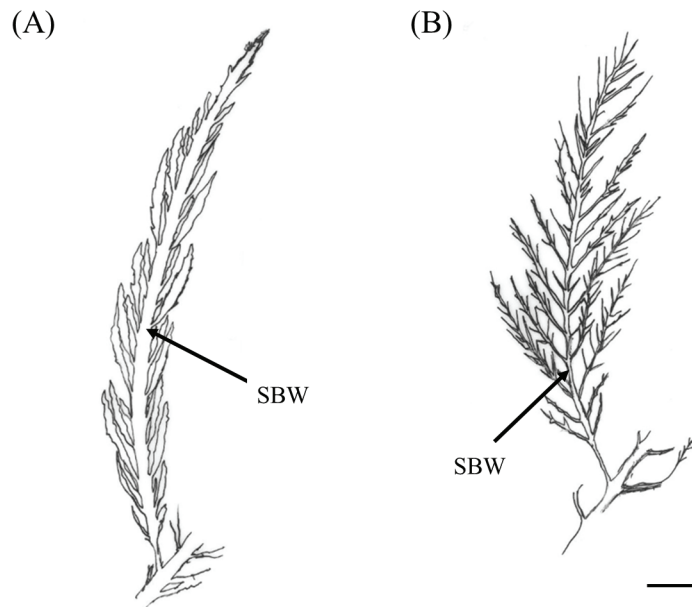


FIGURE 3. Comparison of the morphological characteristics of the branching patterns of the *Desmarestia japonica* subspecies. (A) Morphotype 1, *D. japonica* subsp. *japonica* (broad); (B) Morphotype 2, *D. japonica* subsp. *angustifolia* subsp. nov. (narrow). SBW, secondary blade width. Scale bar indicates 1 cm.

TABLE 2. Prominent morphological differences between *D. japonica* subsp. *angustifolia* and *D. japonica* subsp. *japonica*. Parentheses are average values of quantitative characteristics of blade morphology

Characters	<i>D. japonica</i> subsp. <i>angustifolia</i>	<i>D. japonica</i> subsp. <i>japonica</i>
Overall length (cm)	27–64(44)	23–48(36)
Primary blade length (cm)	27–64(44)	17–48(34)
Primary blade width (mm)	0.6–2(1.5)	1.7–3.5(2.5)
Secondary blade length (cm)	7–16(11)	9–25(15)
Secondary blade width (mm)	0.3–1(0.6)	1.6–2.9(1.9)
Stipe length (cm)	1–4(2)	0.4–3(1)

TABLE 3. Intraspecific *cox1* sequence variation (%) of *Desmarestia* species in the phylogenetic tree (Fig. 4). Pairwise distances were calculated by Kimura-2-parameter method

Species	Intraspecies	Interspecies
<i>D. japonica</i>	0–0.7	0.7
<i>D. aculeata</i>	0–2.6	-
<i>D. dudresnayi</i>	1.5	1.5
<i>D. herbacea</i>	0–1.1	0–0.7
<i>D. ligulata</i>	0–3.4	0–3.4

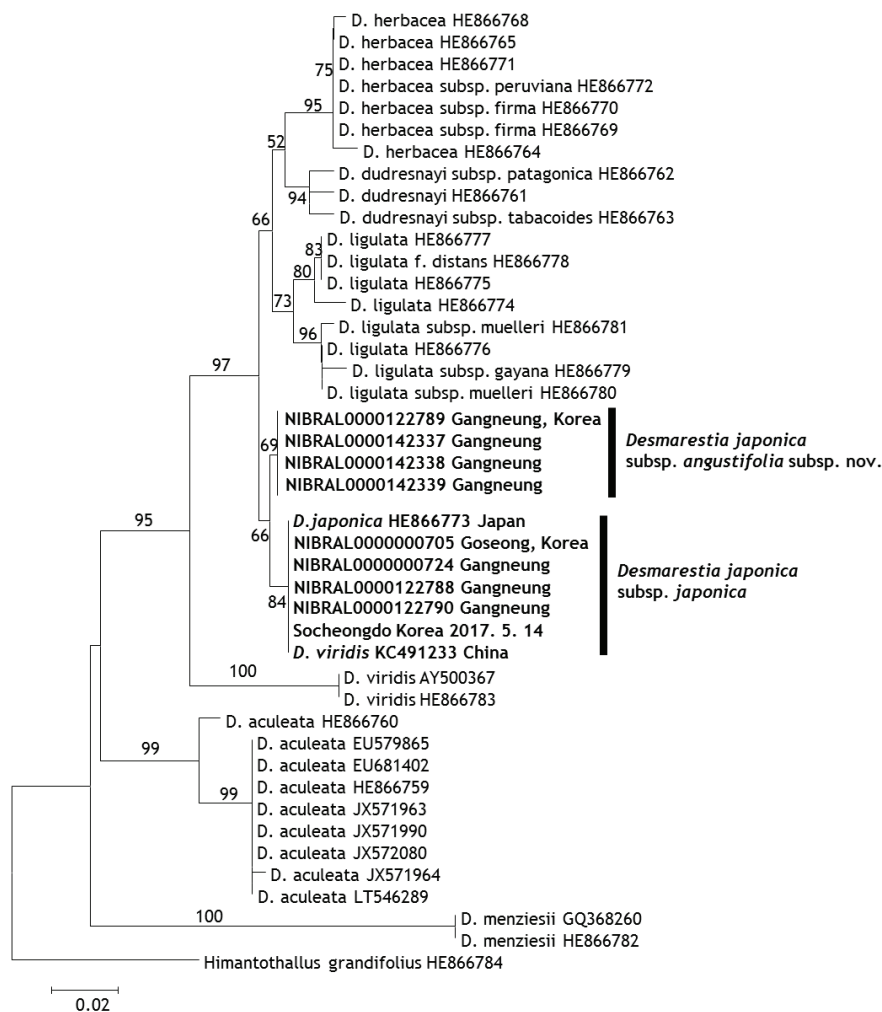


FIGURE 4. Phylogenetic relationships among the *Desmarestia* species. A. The neighbor-joining tree was constructed using 2,000 bootstrap replicates.

Discussion

Yang *et al.* (2014) reported genetic variation in the *cox1* sequences of *Desmarestia* species and made new intraspecific combinations under *D. ligulata*, *D. dudresnayi*, and *D. herbacea*. In their study, *cox1* sequence variation provided evidence for new taxonomic treatment at intra/interspecific levels. Saunders & McDevit (2013) also reported genetic variation in the *cox1* region of *D. aculeata* and proposed the possibility of a new species. And, 1.2% of pairwise distance (PWD) was suggested as the species-level cut-off (Yang *et al.* 2014). Therefore, PWD between two groups of Korean *D. japonica* showed an intraspecific level (0.7%) (Table 3).

We first reported the distribution in Korea and China, but not the Japanese type locality. Moreover, no report has examined the existence of intraspecific variation. After the first report on Korean *D. japonica*, we conducted an intensive taxonomic study of Korean *D. japonica* using herbarium specimens and field samples. Our morphological and molecular phylogenetic examination identified two morpho-genotypes of *D. japonica*.

One morphotype (morphotype 1, broad branchlets) had the same morphological characteristics as originally described for *D. japonica* (Figs. 2 & 3). The other morphotype (morphotype 2) had distinct narrow filamentous branches (Figs. 2 & 3). Individuals with morphotype 2 were found only on the east coast of Korea near Gangneung. Anderson (1985) used the branching patterns as a key characteristic in distinguishing *Desmarestia* species. Moreover, the branch morphology of *Desmarestia* species was used to typify *D. firma* (Anderson 1985; overall length, primary blade length and maximum primary blade width, maximum secondary blade length, and maximum secondary blade width).

The Korean individuals with morphotype 2 were distantly related to morphotype 1 of *D. japonica* reported from Japan and Korea (Table 3) and were separated in the *cox1* gene tree and formed a sister group (Fig. 4). Using the morphogenetic variation, we determined that Korean *D. japonica* was a distinct taxonomic group. Examining the intraspecific taxonomic treatment of *D. ligulata*, we proposed that the Korean individuals were a new subspecies of *D. japonica*. The sympatric distribution of the two *D. japonica* subspecies needs further study from an eco-physiological perspective.

Taxonomic treatment

Desmarestia japonica subsp. *japonica*

The thallus is light olive brown in color and becomes greenish brown when exposed to air. The Korean specimens are up to 67 cm in height and have mostly three orders of branching. The main primary blades were 3.5 mm wide, although they were up to 4 mm wide in tall specimens. The secondary blades had a maximum length of 7–25 cm and maximum width of 0.3–2.9 mm. The gross morphology, with feather-like pinnate branching, was similar to that of Japanese ligulate *Desmarestia* species.

Examined specimens: NIBRAL0000000724 (a typical morphology of *D. japonica* subsp. *japonica*), NIBRAL0000000705, NIBRAL0000122788, NIBRAL0000122790, NIBRAL0000132118, NIBRAL0000132117, NIBRAL0000132116, NIBRAL0000123497, NIBROR0000001248. NIBRAL0000000724

Molecular sequences of typical material (NIBRAL0000000724): *cox1* (GenBank accession MF363010), 18S rDNA (GenBank accession MF363011)

Korean name: Git-san-mal (git means feather-like morphology and san-mal is Korean name of *Desmarestia* genus)

Desmarestia japonica subsp. *angustifolia* S.R. Lee et E.-Y. Lee subsp. nov. (Figs. 1 & 2)

Differs from the typical *D. japonica* subspecies in that the maximum primary blade is more slender (width 0.6–2 mm, average 1.5 mm) and the maximum secondary blade is shorter (average 11 cm) and narrower (average width 0.7 cm); the stipe length averages 1 cm.

Holotype: NIBRAL 0000142337, Gangwon-do, Gangneung-si, Gangmun-dong. Korea. 25 April 2013 by Hyung-Seop Kim in “Survey of indigenous biological resources of Korea” project under MOE.

Molecular sequences of type material: *cox1* (GenBank accession MH423500)

Phenology: Occurrence was recorded for subsp. *angustifolia* from April to May

Distribution and habitat (Fig. 1): Korean *D. japonica* had a restricted distribution, mainly on the northeastern coast, mostly in subtidal habits (Lee and Hwang 2010). Occurs sympatrically with *D. japonica* subsp. *angustifolia* and is restricted to Gangmun-dong, Gangneung-si, and Gangwon-do under seawater on a beach of the East Sea.

Etymology: The specific epithet refers to the slender blade morphology not to the ligulate blade.

Korean name: Jop-eun-git-san-mal (Jop-eun means narrow morphology)

Additional specimens examined (paratypes): NIBRAL 0000142338, Gangwon-do, Gangneung-si, and Gangmun-dong. Korea 25 April 2013; NIBRAL 0000142339, Gangwon-do, Gangneung-si, and Gangmun-dong. Korea 25 April 2013; NIBRAL 0000122789, Gangwon-do, Gangdong-myeon, Aninjin-ri, and Anin Beach. Korea 8 May 2009.

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