# Four new species of *Padina* (Dictyotales, Phaeophyceae) from the western Pacific Ocean, and reinstatement of *Padina japonica*

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Molecular phylogenetic analyses of *Padina* species collected from the western Pacific Ocean using *rbcL* and mitochondrial *cox3* genes revealed the occurrence of four genetically distinctive clades of unknown *Padina* species: clade A (=*Padina okinawaensis* sp. nov.) from Ryukyu Islands (Japan), Hawaii, Indonesia and Thailand, clade B (=*Padina undulata* sp. nov.), clade C (=*Padina terricolor* sp. nov.) and clade D (=*Padina fasciata* sp. nov.) from Ryukyu Islands (Japan). Morphologically, these new taxa are all bistratose species, and different from any known species in the following morphological features: *P. okinawaensis* sp. nov., reniform or circular thallus of entire margin with inconspicuous hair lines on the superior surface of the thallus, and reproductive organs (oogonia and tetrasporangia) in small groups or discontinuous sori under thin flakes of calcium on the inferior surface; *P. undulata* sp. nov., circular or semicircular thallus with undulate margin, conspicuous hair lines on both surfaces of the thallus, and oogonial and tetrasporangial sori in continuous or discontinuous lines covered with a persistent indusium, and cylindrical oogonia; *P. terricolor* sp. nov., grayish brown on the inferior surface of the thallus, and oogonial and tetrasporangial sori in continuous lines covered with a persistent indusium; *P. fasciata* sp. nov., broad white stripes on both surfaces of the thallus due to unique calcification, and oogonial and tetrasporangial sori in continuous lines, which are entirely embedded in a gelatinous layer, on the inferior surface of the thallus. In addition, the independence of *Padina japonica* from *Padina species* is suggested on the basis of molecular and morphological evidence.

KEY WORDS: cox3, Dictyotales, Padina okinawaensis, Padina undulata, Padina terricolor, Padina fasciata, Phaeophyceae, rbcL, Taxonomy

# INTRODUCTION

The genus *Padina* (Dictyotales, Phaeophyceae) is characterized by fan-shaped thalli with an inrolled margin of meristematic cells. Among Phaeophyceae only *Padina* and *Newhousia imbricata* exhibit calcification of the thallus (Kraft *et al.* 2004). Growth is initiated by the marginal row of apical cells; although, in some species vegetative propagation by so-called *Vaughaniella* stages with a single apical cell is also observed (Taylor 1960; Tanaka & Nozawa 1962; Umezaki & Yoneda 1962; Womersley 1987; Abbott & Huisman 2004; Ni-Ni-Win *et al.* 2008). *Padina* species are widely distributed in tropical and warm temperate coastal areas (Womersley 1987; Prud'homme van Reine & Trono 2002; Abbott & Huisman 2004; Ni-Ni-Win *et al.* 2008), and around 30 species are currently recognized worldwide [Wynne 1998; Guiry & Guiry 2008 (http://www.algaebase. org)]. Eight species have been reported from Japan: Padina arborescens Holmes, Padina australis Hauck, Padina boryana Thivy, Padina crassa Yamada, Padina japonica Yamada, Padina minor Yamada, Padina ryukyuana Y. P. Lee & Kamura, and Padina stipitata Tanaka & Nozawa (Tanaka & Nozawa 1962; Lee & Kamura 1991; Yoshida 1998; Yoshida et al. 2000; Ni-Ni-Win et al. 2008). Recently, Ni-Ni-Win et al. (2008) reported the occurrences of three additional species, i.e. Padina melemele I.A. Abbott & Magruder, Padina moffittiana I.A. Abbott & Huisman and Padina thivyae Doty & Newhouse, from southern Japan.

Taxonomy of *Padina* species has been mainly based on the thallus morphology; however, there has been taxonomic confusion because of their considerable morphological plasticity (e.g. thallus shape, size, color, degree of calcification) and inconsistent usage of taxonomic terminology (Trono 1969). There have been a few taxonomic studies of *Padina* using genetic markers in Japan and worldwide (Lee & Bae 2002; Hoshina *et al.* 2004; De Clerck *et al.* 2006; Bittner *et al.* 2008), but most of the studies were not focused

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on the species-level taxonomy of *Padina*. In this study, we provide taxonomic revision of *Padina* species of the western Pacific Ocean using molecular markers, as well as morphology based on both newly collected specimens and the type specimens of related taxa.

### MATERIAL AND METHODS

### Morphological observations

Padina specimens used in this study were newly collected at a wide range of localities in Japan, Hawaii, Australia, Indonesia, Myanmar and Thailand (Fig. 1, Table 1). Selected voucher specimens (SAP106474-106516) used for morphological study are deposited in the Herbarium of the Graduate School of Science, Hokkaido University (SAP) and the herbarium of the Kobe University Research Center for Inland Seas. Type specimens of Padina distromatica Hauck (L0055592), Padina dubia Hauck (L0055593), Padina haitiensis Thivy, P. japonica (SAP9268) and Padina perindusiata Thivy were also examined. Specimens were hand-sectioned for anatomical observations. Photomicrographs were taken using a VB-7010 digital camera (Keyence, Tokyo, Japan) attached to a BX-51 microscope (Olympus, Tokyo, Japan). For long-term storage, sections were mounted on glass slides in Karo syrup/seawater. Measurement of the sizes of oogonia and tetrasporangia were taken randomly for 20 fertile oogonia and sporangia in each section.

### Molecular phylogenetic analysis

DNA extraction was made from silica gel-dried specimens as well as from herbarium specimens, using a DNeasy Plant Mini Kit (Qiagen, Hilden, Germany), followed by purification using a Geneclean III Kit (BIO 101 Systems, Irvine, CA, USA) according to the manufacturer's protocol. Polymerase chain reaction (PCR) amplifications were carried out using a GeneAmp Thermal Cycler 9700 (Applied Biosystems, Foster City, CA, USA) or a TaKaRa PCR Thermal Cycler Dice (Takara Shuzo, Shiga, Japan) and a TaKaRa Ex Taq Reaction Kit (TaKaRa Shuzo). For both rbcL and cox3, PCR amplifications were done under the following conditions: an initial denaturation step at 94°C for 3 min, followed by 94°C for 0.5 min, annealing at 58°C for 0.5 min, extension at 72°C for 2 min for 28 cycles, and final extension at 72°C for 10 min. The primers used for PCR amplification and cycle sequencing followed Ni-Ni-Win et al. (2008). PCR products were checked by electrophoresis on 1.5% agarose gels dyed with ethidium bromide. Reamplifications were made under the same conditions using 0.5 µl of the first PCR product as a template when the yields were low. PCR products were purified by polyethylene glycol purification (Lis 1980) and used for cycle sequencing. Sequencing was performed using a CE DTCS Quick Start Kit (Beckman Coulter, Fullerton, CA, USA) and a CEQ 8000 Genetic Analysis System (Beckman Coulter).

DNA sequences of chloroplast-encoded large subunit of the ribulose-bisphosphate carboxylase (*rbcL*) and mito-



Fig. 1. Map indicating the collection sites of the specimens used in the present study.

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Canadian	Specimens	Omining Anton Anton Anton Anton Anton	Vouchon no 1	DDBJ code	DDBJ code
Species	code	Urigin (collection date; collector)	Voucher no. <sup>7</sup>	IOT <i>rbc</i> L	10r <i>c0x3</i>
Padina arborescens Holmes	KAG1 NAG1	Shimadomari, Kagoshima Prefecture, Japan (25 May 2005; Ni-Ni-Win) Taira Naøasaki Naøasaki Prefecture Japan (04 July 2006; S. Arai)	SAP105577 SAP105578	AB358904 AB358905	AB358939 AB358940
P. australis Hauck	OK11	Urazoko, Okinawa Island, Okinawa Prefecture, Japan (04 October 2004; T. Hammida)	SAP105580	AB358906	AB358941
	KEP1	L. Hanyuda) Karang Jong E, Kepulauan Seribu, Indonesia (21 September 2005; S.G.A. Draisma)	L0609534	AB489912	AB489953
	MYA1 AUS1	Ngapali Beach, Thandwel (Sandoway), Myanmar (03 May 2006; Ni-Ni-Win) Newcastle: Australia (2005: H. Kawai).		AB489913 AB489914	AB489954 -
P. crassa Yamada	ISK1	Akazumi, Ishikawa Prefecture, Japan (27 July 2005; S. Arai)	SAP105582	AB358909	ı
	NAG2	Taira, Nagasaki, Nagasaki Prefecture, Japan (04 July 2006; S. Arai)	SAP105581	AB358908	AB358943
P. Jasciata sp. nov.	UK12 TAK1	Awase, Okinawa Island, Okinawa Prefecture, Japan (19 November 2006; S. Arai) Taketomi Island, Okinawa Prefecture, Janan (20 November 2006; Ni-Ni-Ni-Nin)	SAP106507	AB489916 A B489916	AB489925
	IRII	Hostizura Beach, Iriomote Island, Okinawa Prefecture, Japan (22 November 7006, NE NURCH	SAP106508	AB489917	AB489956
	IR12	Z000; INI-INI-WIIJ Nakano, Iriomote Island, Okinawa Prefecture, Japan (27 May 2007; M. Uchimura)	SAP106506	AB489918	ı
-	ISHI	Fukido, Ishigaki Island, Okinawa Prefecture, Japan (02 June 2007; M. Uchimura)	SAP106511	AB489919	AB489957
P. <i>japonica</i> Yamada	NAG3 OGA1	Tatra, Nagasaki, Nagasaki Pretecture, Japan (04 July 2006, S. Arai) Futami Bay, Ogasawara Islands, Tokyo Prefecture, Japan (11 May 2007;	SAP10583 -	AB358910 AB489920	AB358942 -
	KOS1	1. Hanyuda) Koshiki Island, Kagoshima Prefecture, Japan (30 September 2005; S. Arai)	ı	AB489921	ı
P. melemele I.A. Abbott & Magruder	HAW1	BISH700753, Hawaii	BISH700753	AB358918	AB358947
	ISH2	Akasaki, Ishigaki Island, Okinawa Prefecture, Japan (20 June 2007; M. Uchimura)	SAP105592	AB358913	AB358945
P. minor Yamada	KOS2 IRI3 Kfp7	Koshiki Island, Kagoshima Prefecture, Japan (30 September 2005; S. Arai) Iriomote Island, Okinawa Prefecture, Japan (21 November 2006; S. Arai) Kotok Resar Kemulanan Serihu Indonesia (16 Sentember 2005; S.G.A. Draisma)	SAP105610 SAP105611 1.0609547	AB358921 AB358920 AB489977	AB358949 AB358948 AB489958
P. moffittiana I.A. Abbott & Huisman	OKI3	Awase, Okinawa Island, Okinawa Prefecture, Japan (15 December 2004; S. Arai)	SAP105613	AB358923	AB358950
	OKI4	Awase, Okinawa Island, Okinawa Prefecture, Japan (19 November 2006; S. Arai)	SAP105618	AB358924	- 1 D 7 5 00 5 1
P. okinawaensis sp. nov.	OKI5	Maro Reet, frawait (21 June 2000, K. MOLIIII) Awase, Okinawa Island, Okinawa Prefecture, Japan (19 November 2006; S. Arai)	SAP1066474	AB330921 AB489923	AB489959
٩	IR14	Hinai, Iriomote Island, Okinawa Prefecture, Japan (25 May 2007; M. Uchimura)	SAP106479	AB490398	AB489960
	ISH3 AMA1	Sekisei, Ishigaki Island, Okinawa Prefecture, Japan (31 May 2007; M. Uchimura) Nakohi Amami Ochima Island, Okinawa Prefecture, Japan (22 June 2007)	SAP106486 SAP106488	AB489924 A R489975	AB489961
		M. Uchimura)	00-001 10/0	C7660402	ı
	HAW3	Hahara Bay, O'ahu Island, Hawaii (11 June 2007; H. Kawaii)	ı	AB489926	AB489962
	HAW4 HAW5	Kaawa Beach Fark, O'ahu Island, Hawaii (11 June 2007; H. Kawaii) Kaawa Beach Park, O'ahu Island, Hawaii (11 June 2007; H. Kawaii)		AB489921 AB489928	AB48990.0
	KEP3	Lancang, Kepulauan Seribu, Indonesia (12 September 2005; S.G.A. Draisma)	L0609551	AB489929	AB489964
	KEP4	Kelapa, Kepulauan Seribu, Indonesia (13 Sept. 2005; S.G.A. Draisma)	L0609514	AB489930	AB489965
	KEP5	Panjang, Kepulauan Seribu, Indonesia (14 September 2005; S.G.A. Draisma)	L0609515	AB489931	AB489966
	KEP0	11 Hourd Rectly, Reputation Serious, Indonesia (18 September 2005; S.G.A. Draisma)	65C60001	AB489952	AB48996/
P. ryukyuana Y.P. Lee & Kamura	OKI6	LIOONG Island, I rang r Tovince, 1 nanang (11 September 2004, A. r rannep) Awase, Okinawa Island, Okinawa Prefecture, Japan (19 November 2006; S. Arai)	- SAP105631	AB409925 AB358929	AB409900 AB358953
P. sanctae-crucis Børgesen	TAK2	Taketomi Island, Okinawa Prefecture, Japan (21 November 2006; Ni-Ni-Win)	ı	AB489934	ı
	OKI7	Haemida Beach, Okinawa Island, Japan (27 May 2007; M. Uchimura) Eutomi Boy, Occessionari Telend, Televo, Desfecture, Tonor, (11 May 2007)	SAP106512	AB489935 AB489935	AB489969
	7000	T utamin Bay, Ogaaawara Isianu, TONYO TIVICCUIC, Japan (11 1914) 2007, T. Hanyuda)	1		01660-00
	ISH4 ISH5	Mikara, Ishigaki Island, Japan (01 June 2007; M. Uchimura) Fukido, Ishigaki Island, Japan (02 June 2007; M. Uchimura)	SAP106513 SAP106516	AB489937 AB489938	

Species	Specimens code	Origin (collection date; collector)	Voucher no.1	DDBJ code for <i>rbc</i> L	DDBJ code for <i>cox</i> 3
	AMA2	Kasari, Amami-Oshima Island, Okinawa Prefecture, Japan (02 Aug. 2007; M Hehimura)		AB489939	
	DQ472037	Ala Moana, Honolulu, O'ahu Island, Hawaii (De Clerck <i>et al.</i> 2006)	I	DQ472037	ı
	HAW6	Hahara Bay, O'ahu Island, Hawaii (11 June 2007; H. Kawaii)	ı	AB489940	AB489971
	HAW7	Kaawa Beach Park, O'ahu Island, Hawaii (11 June 2007, H. Kawaii)	ı	AB489941	AB489972
	HAW8	Kaulou Regional Park, O'ahu Island, Hawaii (11 June 2007; H. Kawaii)	ı	AB489942	
	HAW9	Popukea Beach Park, O'ahu Island, Hawaii (12 June 2007; H. Kawaii)		AB489943	
P. terricolor sp. nov.	OKI8	Awase, Okinawa Island, Okinawa Prefecture, Japan (19 November 2006; S. Arai)	SAP106500	AB489944	AB489973
	OKI9	Awase, Okinawa Island, Okinawa Prefecture, Japan (25 June 2007; M. Uchimura)	SAP106501	AB489945	AB489974
	OKI10	Genka, Okinawa Island, Okinawa Prefecture, Japan (22 June 2007; M. Uchimura)	SAP106499	AB489946	AB489975
	IR15	Hoshizuna Beach, Iriomote Island, Okinawa Prefecture, Japan (22 November 2006; Ni-Ni-Nin)		AB489947	ı
	AMA3	Kasari, Amami-Oshima Island, Okinawa Prefecture, Japan (02 August 2007; M. Uchimura)	SAP106505	AB489948	
P. thivyae Doty & Newhouse	TAN1	Tanega Island, Kagoshima Prefecture, Japan (02 Oct. 2005; S. Arai)	SAP105633	AB358931	AB358954
P. undulata sp. nov.	OKI11	Awase, Okinawa Island, Okinawa Prefecture, Japan (19 November 2006; S. Arai)	SAP106493	AB489949	AB489976
	IR16	Hoshidate, Iriomote Island, Okinawa Prefecture, Japan (27 May 2007; M. Uchimura)	I	AB489950	AB489977
	ISH6	Aragusuku, Ishigaki Island, Okinawa Prefecture, Japan (03 June 2007; M. Uchimura)	SAP106497	AB489951	AB489978
	AMA4	Ishi, Amami-Oshima Island, Okinawa Prefecture, Japan (27 July 2007; M. Uchimura)	,	AB489952	
Dictyota dichotoma (Hudson) Lamouroux		Aburatsubo, Kanagawa Prefecture, Japan (12 September 2004; T. Hanyuda)		AB358934	AB358937
Stypopodium sp		Awase, Okinawa Island, Okinawa Prefecture, Japan (15 December 2004; S. Arai)	I	AB358936	AB358955

Table 1. Continued

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chondrial cox3 genes generated in this study are deposited at GenBank (Table 1). Stypopodium sp. and Dictyota dichotoma were used as outgroups in both rbcL and cox3 analyses (Table 1). Sequences were aligned with Clustal X (Thompson et al. 1997) and then manually adjusted by eye. Maximum parsimony (MP), maximum likelihood (ML), and neighbor joining (NJ) analyses were performed using rbcL, cox3, and concatenated rbcL+cox3 gene sequences of all species in PAUP 4.0b10 (Swofford 2002). MP analysis was accomplished under a heuristic search with 10,000 random sequence addition replicates and a tree bisection reconnection (TBR) branch-swapping algorithm. Modeltest v.3.06 (Posada & Crandall 1998) was used to find the optimal model of sequence evolution to fit data alignment for both ML and NJ analyses, and a general time-reversible model was selected as best fit. ML trees were constructed using the best-fit model with estimated parameters (gamma distribution and proportion of invariable sites) selected by the Akaike information criterion (AIC) for likelihood settings under the options of random additional replicates (100) with TBR branchswapping in the heuristic search. Bootstrap analyses (Felsenstein 1985) were carried out to find support for individual internal branches in a heuristic search option with 1000 replicates (MP) and 100 (ML), random addition (100 for MP, 10 for ML, 1000 for NJ) under TBR branchswapping algorithm.

### RESULTS

### Molecular phylogenetic analyses

**RBCL** GENE SEQUENCES: Forty DNA sequences including 22 new sequences of the examined Padina specimens and two outgroup sequences (Table 1) were aligned and were 1319 base pairs (bp) in length. The ML tree was constructed using a 'general time-reversible model' as the best-fit model derived by the AIC in Modeltest. The selected parameters were as follows: nucleotide frequencies: A = 0.2920, C =0.1502, G = 0.2209, T = 0.3370; substitution rate matrix (A-C = 0.9953, A-G = 2.1642, A-T = 1.9939, C-G =0.3601, C-T = 5.4671); proportion of invariable sites (I) = 0.5787; gamma shape parameter = 0.9748. In the MP analysis, four most-parsimonious trees [length = 701 steps, consistency index (CI) = 0.607, a retention index (RI) = 0.846, and 254 parsimonious informative character] were obtained. MP, ML, and NJ trees showed similar tree topologies except for the position of P. moffittiana: P. moffittiana was basal in the ML (Fig. 2) and NJ trees, but P. melemele was basal in the MP tree.

In all analyses, Hawaiian and Japanese specimens morphologically referable to *Padina sanctae-crucis* (see below) formed a well-supported monophyletic clade. The sequence DQ472037 assigned as *Padina boergesenii* in De Clerck *et al.* (2006) was included in this clade. Japanese specimens morphologically referable to *P. japonica* (see below) formed an independent clade supported by high bootstrap values as a sister to *P. sanctae-crucis* (Fig. 2). Sequence divergences within each clade of *P. sanctae-crucis*  and *P. japonica* were 0-2 bp (0–0.15%) and 1-2 bp (0.07–0.15%), respectively, and those between the two were 11-14 bp (0.83–1.06%).

Four statically well-supported clades were recognized close to the clade of *P. sanctae-crucis* and *P. japonica*, and the specimens included in each clade showed characteristic morphological features different from any known taxa, and are therefore considered to represent independent species: clade A (=*Padina okinawaensis* sp. nov., see below), clade B (=*Padina undulata* sp. nov., see below), clade C (=*Padina terricolor* sp. nov., see below), and clade D (=*Padina fasciata* sp. nov., see below) (Figs 2, 3). The *P. sanctae-crucis/P. japonica* clade was sister to the A/B/C clade, supported by relatively high bootstrap values (100/ 61/56% for ML/MP/NJ), and clade D was basal to these taxa.

COX3 ANALYSIS: Thirty-one sequences including 16 new sequences of the examined *Padina* specimens and two outgroup sequences (Table 1) were aligned and were 734 bp in length. In the ML analysis, a general time-reversible model was used as the best-fit model with the following parameters selected by the AIC in Modeltest: nucleotide frequencies: A = 0.2271, C = 0.1418, G = 0.1982, T = 0.4328; substitution rate matrix (A–C = 2.2426, A–G = 6.8973, A–T = 0.3802, C–G = 0.9293, C–T = 6.8973); proportion of invariable sites (I) = 0.4936; gamma shape parameter = 0.8198. In the MP analysis, two most-parsimonious trees (length = 1064 steps, CI = 0.496, RI = 0.778, and 260 parsimony-informative characters) were produced.

No significant differences in the tree topologies were observed among MP, ML, and NJ trees except for the position of clade B (=*P. undulata* sp. nov.) and clade C (=*P. terricolor* sp. nov.): these two species formed a clade in ML (Fig. 3) but separated in NJ and MP analyses. Clade A formed a clade with that of clade B and clade C, and the clade consisting of these taxa was sister to the clade of *P. sanctae-crucis* and *P. japonica*, supported by moderate to high bootstrap values (98/88/57% for ML/MP/NJ). The clade consisting of these taxa was sister to clade D with low to moderate bootstrap supports (59/81/71% for ML/MP/ NJ). These phylogenetic relationships elucidated by *cox3* agreed with those by *rbcL*.

CONCATENATED RBCL+COX3 ANALYSIS: Thirty DNA sequences including 15 new sequences of the examined Padina specimens and two outgroup sequences were aligned and were 2053 bp in length. The ML tree was constructed using a general time-reversible model as the best-fit model with the following parameters selected by the AIC in Modeltest: nucleotide frequencies: A = 0.2770, C = 0.1398, G = 0.2037, T = 0.3795; substitution rate matrix (A–C = 1.4184, A–G = 3.9490, A–T = 0.9879, C–G = 0.7435, C–T = 6.3983; proportion of invariable sites (I) = 0.5104; gamma shape parameter = 0.5115. The MP analysis produced an MP tree (length = 1754 steps, CI = 0.542, RI = 0.725, and 497 parsimonious informative characters). The resulting ML, MP, and NJ trees were not different from those of the other data set of rbcL and cox3 in tree topologies and thus are not shown here.



-0.1 substitution/site

Fig. 2. Maximum-likelihood (ML) tree based on rbcL gene sequences. Numbers at each node indicate bootstrap values (> 50%) for ML (left), maximum parsimony (MP; middle), and neighbor joining (NJ; right).



## - 0.1 substitution/site

Fig. 3. Maximum-likelihood (ML) tree based on cox3 gene sequences. Numbers at each node indicate bootstrap values (> 50%) for ML (left), maximum parsimony (MP; middle), and neighbor joining (NJ; right).

# Morphological observations

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# Figs 4, 5, 15-20

(representing clade A, see Figs 2, 3)

Thalli semicirculares vel circulares margine integri, latiores quam altiores (usque ad 7 cm lati et 5 cm alti), utraque superficie modice vel dense calcificati, basi stuposa stipite brevi ad 4 mm longo 3 mm lato affixi, omnino distromatici. Lineae pilorum concentricae in utraque superficie alternantes, aequidistantes, in superficie inferiori lineam rubrobrunneam ad marginem distalem zonae latae (0.5–1 mm latae) depressae formantes, in superficie superiori inconspicuae. Thalli dioecii, oogoniis tetrasporangiisque in gregibus parvis vel lineis interruptis e strato calcificato orientibus paulo distalioribus quam lineis pilorum in superficie inferiori dispositis, obovatis, oogoniis 78.25  $\pm$  2.02 µm latis, 106.25  $\pm$  3.58 µm longis, tetrasporangiis 82.25  $\pm$  2.22 µm latis, 116.25  $\pm$  3.93 µm longis.

DESCRIPTION: Thalli semicircular or circular, with entire margin, wider than tall at up to 7 cm wide and 5 cm tall, moderately to heavily calcified on both surfaces, attached by stupose base with short stipe up to 4 mm long and 3 mm wide, two cell layers thick throughout. Concentric hair lines alternating between both surfaces and equidistant, forming a reddish-brown line at the upper end of a broad depressed line (0.5–1 mm wide) on inferior surface but inconspicuous lines on superior surface. Thalli dioecious: Oogonia and tetrasporangia in small groups or in broken lines emerging from the calcium layer, a little more distal than hair lines on inferior surface, obovate, 78.25  $\pm$  2.02 µm wide, 106.25  $\pm$  3.58 µm long, and 82.25  $\pm$  2.22 µm wide, 116.25  $\pm$  3.93 µm long, respectively.

HOLOTYPE: SAP106474 (collected by S. Arai, 19 November 2006), Figs 4, 15, 16, Herbarium of the Graduate School of Science, Hokkaido University, Sapporo 060-0810, Japan.

TYPE LOCALITY: Awase, Okinawa Island, Okinawa Prefecture, Japan.

HABITAT: Subtidal up to 15 m deep.

ETYMOLOGY: Species epithet derives from the name of collection sites.

SPECIMENS EXAMINED: Awase, 19 November 2006 (leg. S. Arai) (SAP106474–6); 28 May 2007 (SAP106480); Nakohi, 22 June 2007 (SAP106488); Yagachikita, 22 June 2007 (SAP106477); Kudakakita, Okinawa Island, 28 June 2007 (SAP106489); Mokutan, 24 May 2007 (SAP106478); Hinai, 25 May 2007 (SAP106479); Amitori, 27 May 2007; Hoshidate, 27 May 2007; Shirahama, Iriomote Island, 28 May 2007; Hatoma Island, 25 May 2007 (SAP106481); Akasaki, 24 May 2007 (SAP106482); Akashioya, 30 May 2007 (SAP106483); Bokajo, 30 May 2007; Mijun, 30 May 2007 (SAP106484); Amparu, 31 May 2007 (SAP106485); Sekisei, 31 May 2007 (SAP106486); Mikara, Ishigaki Island, 01 June 2007(SAP106487); Ishi, 27 July 2007; Akaogi, 28 July 2007 (SAP106490); Ikomo, Amami-Oshima Island, Okinawa Prefecture, Japan, 30 July 2007 (SAP106491) (leg. M. Uchimura); Kaawa Beach Park, O'ahu Island, Hawaii, 11 June 2007 (leg. H. Kawai); Lancang, Kepulauan Seribu, 12 September 2005 (L0609551); Kelapa, Kepulauan Seribu, 13 September 2005 (L0609514); Panjang, Kepulauan Seribu, 14 September 2005 (L0609515); Tidung Kecil, Kepulauan Seribu, 18 September 2005 (L0609539); Sorido resort Lagoon, Kri Island, Raja Ampat Island, 19 November 2007 (SGAD0712009, 0712010), Desa Besir, Raja Ampat Island, 02 December 2007 (SGAD0712397, 0712403, 0712434); Yenweres Bay, Raja Ampat Island, Indonesia, 05 December 2007 (leg. S.G.A. Draisma) (SGAD0712522); Libong Island, Trang Province, Thailand, 11 September 2004 (leg. A. Prathep).

MORPHOLOGY: The erect thalli are semicircular or circular with entire margins (Figs 4, 5), rarely split into segments when young, wider than tall, up to 7 cm wide and 5 cm

long, and attached by a stupose base. The inferior surface of the thallus is moderately calcified except for the hair lines and yellowish brown to yellowish green (Fig. 4); whereas, the superior surface is whitish gray by heavy calcification (Fig. 5). The stipe is short, up to 4 mm in length and 3 mm in width, covered by long fibrous hairs (Fig. 4). The thallus is two cell layers thick throughout (Fig. 19),  $80-85 \mu m$  thick in the basal portion, and  $60-65 \mu m$  thick in the other portions. Cells of the superior side are slightly taller than those of the inferior side. Concentric hair lines alternate between both surfaces of the thallus and are equidistant (3– 4 mm apart on each surface), forming a reddish-brown line at the upper end of a broad depressed line (0.5–1 mm wide) on the inferior surface (Figs 4, 15); whereas, they are inconspicuous on the superior surface (Fig. 5).

The reproductive structures are formed distally on the inferior surface of the thalli, near the hair lines (Fig. 15). The tetrasporangia are arranged in broken lines (Figs 15–17), and the oogonia, formed individually or in small groups (Fig. 18), tend to emerge from the calcified layer. Both oogonia and tetrasporangia are obovate,  $78.25 \pm 2.02 \ \mu\text{m}$  in width,  $106.25 \pm 3.58 \ \mu\text{m}$  in length, and  $82.25 \pm 2.22 \ \mu\text{m}$  in width,  $116.25 \pm 3.93 \ \mu\text{m}$  in length, respectively. Female gametophytes and tetrasporophytes were common, but male gametophytes were not observed.

The thallus structure of this species is similar to that of *P*. *undulata* sp. nov., but they can be distinguished by the structure of indusium covering the reproductive organs: *P*. *okinawaensis* sp. nov. has a indusium-like calcium layer; whereas, *P. undulata* sp. nov. has a transparent indusium.

# Padina undulata sp. nov. Ni-Ni-Win, S. Arai & H. Kawai

## Figs 6, 7, 21-27

### (representing clade B, see Figs 2, 3)

Thalli semicirculares vel circulares margine undulato, latiores quam altiores (usque ad 14 cm lati et 6 cm alti), juniores raro fissi sed seniores in segmenta flabelliformia 2-4 cm lata non profunde fissi, in superficie inferiori flavobrunnei vel brunnei, in superficie superiori albi, in superficie inferiori praeter lineas pilorum modice calcificati, in superficie superiori dense calcificati, basi stuposa affixi, in superficie inferiori a basi usque ad 1 cm supra basin pilis longis fibrosis vestiti, omnino distromatici; cellulis superficiei superioris duplo altioribus quam eis superficiei inferioris; gradibus dictis Vaughaniella adsunt. Lineae pilorum concentricae in utraque superficie alternantes, aequidistantes (utraque superficie 1.5-2.5 mm distantes), in superficie inferiori lineam rubrobrunneam ad marginem distalem zonae latae (1 mm latae) depressae formantes, in superficie superiori angustae. Thalli dioecii, oogoniis tetrasporangiisque in lineis continuis paulo distalioribus quam lineis pilorum in superficie inferiori dispositis, indusio persistenti tectis, oogoniis cylindricis,  $36.15 \pm 3.73 \ \mu m$  latis,  $82.15 \pm 2.28 \ \mu m$  longis, tetrasporangiis obovatis,  $86 \pm 3.84 \ \mu m$  latis,  $112.5 \pm 2.56 \ \mu m$  longis; antheridiis subquadratis, in lineis interruptis vel areis paulo distalioribus quam lineis pilorum in superficie inferiori irregulatim dispositis, sine indusio.

DESCRIPTION: Thalli semicircular or circular, with undulate margin, wider than tall at up to 14 cm wide and 6 cm



Figs 4–14. External morphology.

Figs 4, 5. Padina okinawaensis sp. nov.

Fig. 4. Habit of tetrasporophyte (holotype), showing inferior surface (I.S) with hair lines (arrowhead), tetrasporangial sori (double arrowhead), and fibrous hairs (arrow). Bar = 0.7 cm. Fig. 5. Superior surface (S.S) of the thallus (SAP106485). Bar = 0.7 cm.

Figs 6, 7. *Padina undulata* sp. nov. Fig. 6. Habit of tetrasporophyte (holotype), showing inferior surface with hair lines (arrowhead), tetrasporangial sori (double arrowhead), and fibrous hairs (arrow). Bar = 1 cm.

Fig. 7. Superior surface of the thallus. Bar = 1 cm.

Figs 8, 9. Padina terricolor sp. nov.

Fig. 8. Habit of tetrasporophyte (holotype), showing inferior surface with hair lines (arrowhead), tetrasporangial sori (double

tall, rarely split when young but shallowly split into fanshaped segments (2-4 cm wide) when older, yellowish brown to brown on inferior surface, white on superior surface, moderately calcified on inferior surface except for hair lines, heavily calcified on superior surface, attached by stupose base, covered with long fibrous hairs from the base to 1 cm upward on inferior surface, two cell layers thick throughout; cells of superior side twice as tall as those of inferior side; Vaughaniella stages present. Concentric hair lines alternating between both surfaces and equidistant (1.5-2.5 mm apart on each surface), forming a reddish-brown line at the upper end of a broad depressed line (1 mm wide) on inferior surface, and narrow lines on superior surface. Thalli dioecious; oogonia cylindrical (36.15  $\pm$  3.73  $\mu$ m wide, 82.15  $\pm$ 2.28  $\mu$ m long) and tetrasporangia obovate (86  $\pm$  3.84  $\mu$ m wide, and 112.5  $\pm$  2.56 µm long), both forming continuous lines distally adjacent to the hair lines on inferior surface and covered with a persistent indusium; antheridia squarish, forming broken lines or patches irregularly a little more distal than hair lines on inferior surface, without indusium.

HOLOTYPE: SAP106492 (collected by S. Arai, Nov. 22, 2006), Figs 6, 21, Herbarium of the Graduate School of Science, Hokkaido University, Sapporo 060-0810, Japan.

TYPE LOCALITY: Hoshizuna, Iriomote Island, Okinawa Prefecture, Japan.

HABITAT: Subtidal up to 20 m deep.

ETYMOLOGY: The species epithet refers to the undulated margin of the thallus.

SPECIMENS EXAMINED: Awase, 19 November 2006 (leg. S. Arai) (SAP106493); 28 May 2007 (leg. M. Uchimura); Genka, 22 June 2007 (SAP106494); Nakohi, 22 June 2007; Hamada, 07 July 2007 (SAP106495); Yagachikita, Okinawa Island, 22 July 2007 (SAP106496); Hoshizuna, 22 November 2006 (leg. S. Arai) (SAP106492); Hoshidate, Iriomote Island, 27 May 2007; Aragusuku, Ishigaki Island, 03 June 2007 (SAP106497); Ishi, Amami-Oshima Island, Okinawa Prefecture, Japan, 27 July 2007 (leg. M. Uchimura).

MORPHOLOGY: The erect thalli are semicircular to circular with undulate margins (Figs 6, 7), split into fan-shaped segments 2–4 cm wide when older, wider than tall, up to 14 cm wide and 6 cm tall, yellowish brown to brown on the inferior surface (Fig. 6) and whitish by heavy calcification on the superior surface (Fig. 7), and attached by a stupose

base. The inferior surface has characteristic regular brown stripes because the areas of hair lines lack heavy calcification (Figs 6, 21, 22). The stipe is short, covered with long fibrous hairs, and up to 4 mm long and 3 mm wide (Fig. 6). The entire thallus is composed of two cell layers,  $105-110 \mu m$  thick at the base (Fig. 26) and  $90-95 \mu m$  thick in the other portions (Fig. 27). Cells of the superior surface are twice as tall as those of the inferior surface (Figs 26, 27). Concentric hair lines are conspicuous, equidistant and alternating between both surfaces of the thallus (1.5-2.5 mm apart on each surface), forming reddish-brown lines at the upper end of a broad depressed line on the inferior surface (Figs 21, 22).

The species is dioecious. Oogonial and tetrasporangial sori form distally in continuous or discontinuous lines or patches, near the hair lines on the inferior surface of the thallus (Fig. 21), and covered with an indusium (Figs 23, 24). Reproductive sori are always located distally adjacent to hair lines on the inferior surface of the thallus (Figs 21, 22). Additional discontinuous lines are sometimes formed beside the regular lines (Fig. 23). Oogonia are cylindrical,  $36.15 \pm 3.73 \,\mu\text{m}$  wide,  $82.15 \pm 2.28 \,\mu\text{m}$  long (Fig. 25). Tetrasporangia are obovate,  $86 \pm 3.84 \,\mu\text{m}$  wide and 112.5  $\pm 2.56 \,\mu\text{m}$  long. Antheridial sori are found on the inferior surface of the thallus, forming irregular discontinuous lines or patches on the distal side of the hair lines, and without an indusium (Fig. 22).

# Padina terricolor sp. nov. Ni-Ni-Win, M. Uchimura & H. Kawai

# Figs 8, 9, 28-32

### (representing clade C, see Figs 2, 3)

Thalli semicirculares vel circulares margine integro, latiores quam altiores (usque ad 6 cm lati et 4 cm alti), juniores raro fissi sed seniores in segmenta flabelliformia 2–2.5 cm lata non profunde fissi, rigidi, in superficie inferiori griseo-brunnei, in superficie superiori griseo-albi, utraque superficie praeter lineas pilorum modice vel dense calcificati, haptero discoideo affixi, a basi usque ad 1 vel 2 cm supra basin pilis longis fibrosis vestiti, omnino distromatici; cellulis superficiei superioris paene sesquialtioribus quam eis superficiei inferioris; gradibus dictis Vaughaniella adsunt. Lineae pilorum concentricae in utraque superficie alternantes, inaequidistantes (utraque superficie 2 vel 3 mm distantes), in superficie inferiori lineam rubrobrunneam ad marginem distalem zonae latae (0.1–0.15 mm latae) depressae, in superficie superiori lineam rubram angustam formantes. Thalli dioecii, oogoniis

 $<sup>\</sup>leftarrow$ 

arrowhead), and fibrous hairs (arrow). Bar = 1 cm.

Fig. 9. Superior surface of the thallus. Bar = 0.7 cm.

Figs 10, 11. Padina fasciata sp. nov.

Fig. 10. Habit of tetrasporophyte (holotype), showing inferior surface with hair lines (arrowhead) and fibrous hairs (arrow). Bar = 1 cm. Fig. 11. Superior surface of the thallus with stripes. Bar = 0.6 cm.

Fig. 12. Padina japonica (NNW10), showing inferior surface of the thallus (arrow) with hair lines (arrowhead) and tetrasporangial sori (double arrowhead). Bar = 0.8 cm.

Figs 13, 14. Padina sanctae-crucis.

Fig. 13. Habit of female gametophyte (SAP106514), showing inferior surface of the thallus with hair lines (arrowhead) and oogonial sori (double arrowhead). Bar = 1 cm.

Fig. 14. Habit of inferior surface of tetrasporophyte (SAP106515), with hair lines (arrowhead) and tetrasporangial sori (double arrowhead). Bar = 1 cm.



Figs 15-27. Morphology of P. okinawaensis sp. nov. and P. undulata sp. nov.

Figs 15–20. Padina okinawaensis sp. nov.

Fig. 15. Inferior surface of tetrasporophyte (holotype), showing relationship of hair lines (arrowhead) and tetrasporangial sori (double arrowhead). Bar = 1 mm.

Fig. 16. Tetrasporangial sori emerging from the calcium layer (holotype). Bar = 200  $\mu$ m. Fig. 17. Detail of tetrasporangial sori. Bar = 120  $\mu$ m. Fig. 18. Surface view of small groups of oogonial sori (double arrowhead) emerging from the calcium layer. Bar = 370  $\mu$ m. Fig. 19. Transverse section of the base with fibrous hairs on inferior surface (asterisk) and heavy calcification (c) on superior surface.

 $Bar = 40 \ \mu m.$ 

Fig. 20. Transverse section of tetrasporangial sori, showing obovate tetrasporangia. Bar =  $75 \,\mu m$ .

tetrasporangiisque in lineis continuis paulo distalioribus quam lineis pilorum in superficie inferiori dispositis, indusio persistenti tectis, obovatis, oogoniis  $36.25 \pm 3.58 \mu m$  latis,  $52.5 \pm 2.56 \mu m$  longis, tetrasporangiis  $84.75 \pm 3.76 \mu m$  latis,  $172.75 \pm 2.55 \mu m$  longis. Sori antheridiales in lineis interruptis vel areis dispositis, quam lineis pilorum in superficie inferiori paulo distalioribus, sine indusio.

DESCRIPTION: Thalli circular or semicircular, with entire margin, wider than tall at up to 6 cm wide and 4 cm tall, rarely split when young, becoming shallowly split into fan-shaped segments (2-2.5 cm wide) when older, rigid in texture, grayish brown on inferior surface, grayish white on superior surface, moderately to heavily calcified on both surfaces except for hair lines, covered with long fibrous hairs from the base to 1-2 cm upward, attached by a discoidal holdfast, two cells thick throughout; cells of superior surface nearly 1.5 times as tall as those of inferior surface; Vaughaniella stages present. Concentric hair lines alternating between both surfaces in unequal distance (2-3 mm apart on each surface), forming reddish-brown line at the upper end of a broad depressed line (0.1-0.15 mm wide) on inferior surface, but a red narrow line on superior surface. Thalli dioecious. Oogonia and tetrasporangia obovate,  $36.25 \pm 3.58 \,\mu\text{m}$ wide, 52.5  $\pm$  2.56 µm long, and 84.75  $\pm$  3.76 µm wide,  $172.75 \pm 2.55 \,\mu\text{m}$  long, respectively, in continuous lines adjacent to hair lines distally on inferior surface, and covered with persistent indusium. Antheridial sori in broken lines or patches near hair lines distally on inferior surface, without indusium.

HOLOTYPE: SAP106499 (collected by M. Uchimura, 22 June 2007), Figs 8, 28, Herbarium of the Graduate School of Science, Hokkaido University, Sapporo 060-0810, Japan.

TYPE LOCALITY: Genka, Okinawa Island, Okinawa Pre-fecture, Japan.

HABITAT: Subtidal up to 20 m deep.

ETYMOLOGY: The species epithet refers to the grayishbrown color of the thallus.

SPECIMENS EXAMINED: Awase, Okinawa Island, 19 November 2006 (leg. S. Arai) (SAP106500); 25 June 2007 (leg. M. Uchimura) (SAP106501); Genka, Okinawa Island, 22 June 2007 (SAP106499); Sesoko Island, 26 June 2007 (leg. M. Uchimura) (SAP106502, 106503); Hoshizuna, 22 November 2006 (leg. S. Arai); Hoshidate, 27 May 2007 (SAP106504); Nakano, Iriomote Island, 27 May 2007;

Kasari, Amami-Oshima Island, Okinawa Prefecture, Japan, 02 Aug. 2007 (leg. M. Uchimura) (SAP106505).

MORPHOLOGY: The erect thalli are circular or semicircular with entire margins, wider than tall at up to 6 cm wide and 4 cm tall, rarely split when young, shallowly split into fanshaped segments of 2-2.5 cm wide when older (Figs 8, 9). Both surfaces of the thallus are heavily calcified except for the area of the hair lines, with gravish-brown color on the inferior surface (Fig. 8) and grayish-white color on the superior surface (Fig. 9). The thalli are covered with long fibrous hairs from the base to 1-2 cm upward on the inferior surface (Fig. 8), and attached by a discoidal holdfast. The thallus is rigid in texture when fresh. Creeping Vaughaniella stages are often observed. The stipe is up to 10 mm long and 4 mm wide. The entire thallus is composed of two cell layers, 95-100 µm thick at the base and 80-85 µm thick in other parts of the thallus (Fig. 30). Cells of the superior side are nearly 1.5 times as tall as those of inferior side (Fig. 30). Concentric hair lines are conspicuous and alternate between both surfaces of the thallus in unequal distance (i.e. two alternate hair lines on both surfaces arranged close together resulting in wide and narrow glabrous zones), 2-3 mm apart on each surface, forming a reddish-brown line at the upper end of a concave, slightly broader naked line (0.1-0.15 mm wide) on the inferior surface and a narrower line on the superior surface (Figs 28, 29).

The species is dioecious. Both male and female gametophytes as well as tetrasporophytes were found at Ryukyu Islands, Japan. Reproductive sori are always located distally near the hair lines on the inferior surface of the thallus (Figs 28, 29, 31). Young reproductive sori are embedded under the cuticular layer and the cuticle split as the sori became fertile. The entire mature sori are surrounded by a persistent indusium (Fig. 31). Oogonial and tetrasporangial sori form continuous lines near the hair lines distally on the inferior surface of the thallus (Figs 28, 29, 31) and are covered with a persistent indusium. Both oogonia and tetrasporangia are obovate,  $36.25 \pm 3.58 \ \mu m$ wide,  $52.5 \pm 2.56 \,\mu m$  long and  $84.75 \pm 3.76 \,\mu m$  wide,  $172.75 \pm 2.55 \ \mu m$  long (Fig. 32), respectively. Antheridial sori are borne distally as broken lines or irregular patches near the hair lines on the inferior surface of the thallus and are without an indusium.

This species can be distinguished from other *Padina* species by the grayish-white color of the thallus, rigid texture, heavy calcification on both surfaces of the thallus,

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Figs 21-27. Padina undulata sp. nov.

Fig. 21. Inferior surface of the thallus (holotype), showing relationship of alternating hair lines (on inferior surface–arrowhead; on superior surface–arrow) and tetrasporangial sori (double arrowhead). Bar = 2 mm.

Fig. 22. Inferior surface of the thallus, showing relationship of hair lines (arrowhead) and antheridial sori (double arrowhead). Bar = 2 mm.

Fig. 23. Surface view of tetrasporangial sori (double arrowhead) with indusium. Bar =  $340 \,\mu\text{m}$ .

Fig. 24. Surface view of oogonial sori with indusium (arrowhead). Bar =  $100 \mu m$ .

Fig. 25. Transverse section of oogonial sori, showing cylindrical oogonia. Bar =  $80 \mu m$ .

Fig. 26. Transverse section of the base with fibrous hairs (asterisk) on inferior surface. Bar = 50  $\mu$ m.

Fig. 27. Transverse section of middle portion, showing different thickness of cell layers. Bar =  $45 \,\mu m$ .



Figs 28–41. Morphology of *P. terricolor* sp. nov., *P. fasciata* sp. nov., *P. japonica*, and *P. sanctae-crucis*. Figs 28–32. *Padina terricolor* sp. nov.

Fig. 28. Inferior surface of the thallus with hair lines (arrowhead) and tetrasporangial sori (double arrowhead) (Holotype). Bar = 2.5 mm. Fig. 29. Inferior surface of the thallus, showing relationship of alternating hair lines (on inferior surface–arrowhead; on superior surface–arrow) and tetrasporangial sori (double arrowhead). Bar = 0.8 mm.

Fig. 30. Transverse section of the base with fibrous hairs (asterisk) on inferior surface and heavy calcification (c) on superior surface. Bar =  $55 \ \mu m$ .

Fig. 31. Surface view of tetrasporangial sori (double arrowhead) with indusium (arrow) above hair line (arrowhead). Bar = 0.5 mm. Fig. 32. Transverse section of tetrasporangial sori, showing obovate tetrasporangia. Bar =  $120 \mu m$ . Figs 33–37. *Padina fasciata* sp. nov.

Fig. 33. Inferior surface of the thallus (SAP106508), showing relationship of hair lines (arrowhead), tetrasporangial sori (double arrowhead) with additional sori (arrow). Bar = 4 mm.

Fig. 34. Surface view of tetrasporangial sori in the gelatinous layer. Bar =  $145 \mu m$ .

Fig. 35. Transverse section of oogonial sori with indusium (arrowhead), showing cylindrical oogonia. Bar =  $50 \mu m$ .

and the presence of a conspicuous persistent indusium over reproductive sori.

# Padina fasciata sp. nov. Ni-Ni-Win, M. Uchimura & H. Kawai

### Figs 10, 11, 33-37

#### (representing Clade D, see Figs 2, 3)

Thalli circulares vel semicirculares ad 7 cm diametro margine integri, in segmenta flabelliformia profunde vel non profunde fissi, basi stuposa affixi stipite brevi ad 3 mm longo 2.5 mm lato, in superficie inferiori flavobrunnei vel brunnei, in superficie superiori albi, in utraque superficie praeter lineas pilorum dense calcificati, in superficie superiori dense calcificati, facie fasciata propter ordinationes iterantes zonarum in utraque superficie: in superficie inferiori zonae glabrae calcificatae linea minus calcificata in centro instructae et lineae pilorum nudae, in superficie superiori zonae dense calcificatae linea multo magis calcificata instructae, omnino distromatici; gradibus dictis Vaughaniella vulgaribus. Lineae pilorum concentricae in utraque superficie alternantes, aequidistantes (in utraque superficie 2–3 mm distantes), in superficie inferiori lineam rubrobrunneam ad marginem distalem lineae latae depressae, in superficie superiori lineam rubram angustam formantes. Thalli dioecii, soris oogonialibus tetrasporangialibusque in lineis continuis paulo distalioribus quam lineis pilorum in superficie inferiori dispositis, in strato gelatinoso omnino inclusis, indusio persistenti tectis, oogoniis cylindricis,  $35.71 \pm 3.96 \ \mu m$  latis,  $85.5 \pm 3.94 \ \mu m$ longis, tetrasporangiis obovatis,  $87.55 \pm 2.37$  µm latis, 147.4  $\pm$  2.30 µm longis.

DESCRIPTION: Thalli circular or semicircular, up to 7 cm in diameter, with entire margin, shallowly to deeply split into fan-shaped segments, attached by a stupose base with short stipe up to 3 mm long and 2.5 mm wide, yellowish brown to brown on inferior surface, white on superior surface, heavily calcified on both surfaces except for hair lines, resulting in striped appearance due to a repetitive pattern of calcified glabrous zone with an additional less calcified line at the center and naked hair lines on inferior surface, and substantially calcified zone on with a much more calcified line in equal interval on superior surface, two cells thick throughout; Vaughaniella stages common. Concentric hair lines alternating between both surfaces in equidistant (2-3 mm apart on each surface), forming a reddish-brown line at the upper end of a broad depressed line on inferior surface and a red, narrow line on superior surface. Thalli dioecious; both oogonial and tetrasporangial sori in continuous lines distally adjacent to hair lines on inferior surface, wholly embedded in a gelatinous layer, and covered with a persistent indusium; oogonia cylindrical and tetrasporangia obovate,  $35.71 \pm 3.96 \,\mu\text{m}$  wide,  $85.5 \pm 3.94 \,\mu\text{m}$  long, and  $87.55 \pm 2.37 \,\mu\text{m}$  wide,  $147.4 \pm 2.30 \,\mu\text{m}$  long, respectively; antheridial sori in broken lines or patches distally adjacent to hair lines on inferior surface, without indusium.

HOLOTYPE: SAP106506 (collected by M. Uchimura, 27 May 2007), Fig. 10, Herbarium of the Graduate School of Science, Hokkaido University, Sapporo 060-0810, Japan.

TYPE LOCALITY: Nakano, Iriomote Island, Okinawa Pre-fecture, Japan.

HABITAT: Rock pools in intertidal to subtidal up to 15 m deep.

ETYMOLOGY: The species epithet refers to the striped appearance of the thalli.

SPECIMENS EXAMINED: Urazoko, 15 April 2004; Awase, Okinawa Island, 19 November 2007 (leg. S. Arai) (SAP106507); Taketomi Island, 20 November 2006; Hoshizuna Beach, 22 November 2006 (leg. Ni-Ni-Win) (SAP106508, 106509); Takana, 26 May 2007 (SAP106510); Nakano, Iriomote Island, 27 May 2007 (SAP106506); Fukido, Ishigaki Island, Okinawa Prefecture, Japan, 02 June 2007 (leg. M. Uchimura) (SAP106511).

MORPHOLOGY: The erect thalli are circular or semicircular, up to 7 cm in diameter, with entire margins, shallowly to deeply split into fan-shaped segments, attached by a stupose base, yellowish brown to brown on the inferior surface of the thallus, heavily calcified on both surfaces except for hair lines (Figs 10, 11). They present a striped appearance due to a repetitive pattern of calcified glabrous zones with a less calcified line at the center and naked hair lines on the inferior surface (Fig. 10), and a substantially calcified zone with much more calcified lines on the superior surface of the thallus (Fig. 11). The stipe is short, up to 3 mm long and 2.5 mm wide. Creeping Vaughaniella stages are common. The thallus is composed of two cell layers throughout, 85-95 µm thick at the base and 70-80 µm thick in the other parts (Figs 36, 37). Cells of the superior side are nearly 1.2 times as tall as those of inferior side (Figs 36, 37). Concentric hair lines are conspicuous and alternating between both surfaces equidistantly (2-3 mm apart on each surface), forming a reddish-brown line at the upper end of a broad depressed line (0.6-0.8 mm wide) on the inferior surface (Fig. 33) and narrow line on the superior surface.

The species is dioecious. Reproductive sori are always situated near the hair lines distally on the inferior surface of the thallus (Fig. 33). Oogonial and tetrasporangial sori

Fig. 36. Transverse section of the base with fibrous hairs (asterisk) and heavy calcification (c). Bar =  $60 \mu m$ .

Fig. 37. Longitudinal section of middle portion. Bar =  $55 \,\mu m$ .

Fig. 38. *Padina japonica* (no. 10), showing hair lines (arrowhead) and tetrasporangial sori (double arrowhead) on inferior surface of the thallus. Bar = 2 mm.

Figs 39–41. Padina sanctae-crucis.

Fig. 39. Surface view of inferior surface (SAP106514), showing hair lines (arrowhead) and tetrasporangial sori (double arrowhead). Bar = 3 mm.

Fig. 40. Detail of oogonial sori with indusium (arrowhead). Bar =  $140 \ \mu m$ .

Fig. 41. Transverse section of middle portion. Bar =  $35 \ \mu m$ .

Table 2.	Comparison	of morpholog	ical features ar	nong bistratose	species of <i>Padina</i> .

Characters	Clade A (=P. okinawaensis sp. nov.)	Clade B (=P. undulata sp. nov.)	Clade C (= <i>P. terricolor</i> sp. nov.)	Clade D (= <i>P. fasciata</i> sp. nov.)	P. distromatica Hauck	P. elegans Koh ex Womersley
Thallus						
Shape of margin	entire	undulate	entire	entire	entire	split
Calcification on IF/SP surfaces	moderate/heavy	moderate/ heavy	heavy/heavy	moderate/ heavy	light/moderate	no/light
Vaughaniella stages	present	present	present	present	absent	absent
Distance between alternate HLs	equal	equal	unequal	equal	-	equal
Hairlines (IF/SP surfaces)	conspicuous/ inconspicuous	conspicuous/ conspicuous	conspicuous/ conspicuous	conspicuous/ moderate	conspicuous/ inconspicuous	conspicuous/ conspicuous
Sporangial sori		*			*	*
Position (surface)	$IF^1$	IF	IF	IF	IF	SP
Number in row between HLs	one	one	one	one <sup>2</sup>	two	one
Arrangement	continuous lines; just above HLs	continuous lines; just above HLs	continuous lines; just above HLs	continuous lines; just above HLs	abutting HLs	continuous or broken lines; above HLs
Oogonia						
Shape	obovate	cylindrical	obovate	cylindrical	-	-
Size – wide $\times$ long ( $\mu$ m)	$78.25 \pm 2.02 \times 106.25 \pm 3.58$	36.15±3.73 x 82.15±2.28	$36.25 \pm 3.58 \times 52.5 \pm 2.56$	$32.71 \pm 3.96 \times 85.5 \pm 3.94$	-	-
Tetrasporangia						
Shape	obovate	obovate	obovate	obovate	-	ovoid
Size – wide $\times$ long ( $\mu$ m)	$\begin{array}{c} 82.25 \pm 2.22 \times \\ 116.25 \pm 3.93 \end{array}$	86±3.24 x 112.5±2.56	$\begin{array}{c} 84.75 \pm 3.76 \times \\ 172.75 \pm 2.55 \end{array}$	$87.55 \pm 2.37 \times 147.4 \pm 2.30$	-	50–100 × 80–120
Fertile zone	alternate <sup>3</sup>	alternate <sup>3</sup>	alternate <sup>3</sup>	alternate <sup>3</sup>	successive4	alternate <sup>3</sup>
Indusium	present (calcium layer)	present	present	present	absent	present
Reference	herein	herein	herein	herein	Hauck 1887	Womersley 1987

<sup>1</sup> IF, inferior; SP, superior; HLs, hair lines.

<sup>2</sup> Sometimes with additional broken line.

<sup>3</sup> Fertile zones are separated by sterile zones when both surfaces are viewed together.

<sup>4</sup> Sterile zones are absent.

form continuous distal lines near the hair lines on the inferior surface of the thallus, and additional broken lines or small patches are sometimes found beside the regular sori (Fig. 33). The sori are entirely embedded in a gelatinous layer and covered with a persistent indusium (Fig. 34). Oogonia are cylindrical,  $35.71 \pm 3.96 \mu$ m wide and  $85.5 \pm 3.94 \mu$ m long (Fig. 35); whereas, tetrasporangia are obovate,  $87.55 \pm 2.37 \mu$ m wide and  $147.4 \pm 2.30 \mu$ m long. Antheridial sori are formed as patches near the hair lines distally on the inferior surface of the thallus and without an indusium.

Young thalli resemble *P. sanctae-crucis* in appearance. However, this species is unique in appearing striped because of the alternating pattern of the glabrous zones of uncalcified lines at the center with naked hair lines on the inferior surface and heavily calcified lines on the superior surface.

### Padina sanctae-crucis Børgesen 1914

### Figs 13, 14, 39-41

SYNONYM: Padina jamaicensis (F. S. Collins) Papenfuss

TYPE LOCALITY: St. Croix, Virgin Islands.

DISTRIBUTION: Belize (Taylor 1960 as *P. sanctae-crucis*; Littler & Littler 1997 as *P. jamaicensis*), Venezuela (Ganesan 1990 as *P. jamaicensis*), Bermuda (Taylor 1960), Florida (Taylor 1960), Caribbean Islands (Taylor 1960, 1969; Littler & Littler 2000; Cabrera *et al.* 2004; Suárez 2005; Duncan & Lee Lum 2006), Brazil (Taylor 1930), Bangladesh (Silva *et al.* 1996), Pakistan (Silva *et al.* 1996), Philippines (Silva *et al.* 1987), Indonesia (Verheij & Prud'homme van Reine 1993), Australia and New Zealand (Womersley 1987; Huisman & Walker 1990), Micronesia (Lobban & Tsuda 2003), Fuji (South & Skelton 2003; Littler & Littler 2003), Hawaii (Abbott & Huisman 2004), southern Japan (this study).

SPECIMENS EXAMINED: Haemida Beach, Okinawa Island, 27 May 2007 (SAP106512); Mikara, 01 June 2007 (SAP106513, 106514); Miyahama, 01 June 2007 (SAP106515); Fukido, 02 June 2007 (SAP106516); Tsukuji Beach, 02 June 2007; Aragusuku, Ishigaki Island, 03 June 2007; Kasari, Amami-Oshima Island, Okinawa Prefecture, Japan, 02 August 2007 (leg. M. Uchimura); O'ahu Island, Hawaii, 11 June 2007 (leg. H. Kawai).

HABITAT: Rock pools in intertidal to subtidal zones up to 10 m deep.

MORPHOLOGY: The erect thalli are flabelliform with entire margins, wider than tall at up to 10 cm wide and 7 cm tall, semicircular and rarely split when young, becoming shallowly split into fan-shaped segments 2–3 cm wide when older, and attached by a stupose base (Figs 13, 14). The thalli grow in clusters, yellowish brown and moderately calcified on the inferior surface except for hair lines

P. fernandeziana Skottsberg et Levring	P. haitiensis Thivy	P. <i>japonica</i> Yamada	P. jonesii Tsuda	P. perindusiata Thivy	P. plumbea (Areschoug) Levring	P. sanctae-crucis Børgesen
split no/light	split heavy/heavy	entire light/moderate	- heavy/heavy	- light/light	- light/ moderate	entire moderate/ heavy
absent equal	absent equal	present equal	absent equal	absent irregular	absent irregular	present equal
conspicuous/ inconspicuous	conspicuous/ conspicuous	conspicuous/ conspicuous	conspicuous/ inconspicuous	conspicuous / conspicuous	inconspicuous / inconspicuous	conspicuous/ conspicuous
IF two	IF one	IF 2–3	IF one	IF 2–3	SP -	IF one 1
continuous or broken lines; abutting HLs	broken lines; in the middle of HLs	continuous lines; above HLs or spreading between HLs	continuous lines; above HLs	continuous or broken lines; between HLs	broken lines or patches; between HLs	continuous lines; just above HLs
-	-	obovate 32.85 ± 2.13 × 42.75 ± 1.83	-	-	-	spherical 112.25±6.58 x 112.25±6.58
$60-75 \times 90-120$ successive <sup>4</sup> present	$\frac{1}{10}$ alternate <sup>3</sup> present	obovate $45 \pm 3.97 \times 77.5$ $\pm 2.56$ alternate 2)/successive 3) present	- 75 × 75 alternate 2) absent	- 170 x 170 successive present	- 30–35 x 45–55 successive present	spherical 117±5.48 x 117±5.48 alternate present
Levring 1941	Taylor 1960	herein	Tsuda 1972	Taylor 1960	Levring 1940	herein

### Table 2. Extended

(Figs 13, 14), and are heavily calcified on the superior surface. The stipe is short, up to 5 mm long and 2 mm wide (Figs 13, 14). Creeping *Vaughaniella* stages are present. The thallus is composed of two cell layers throughout, 75–80  $\mu$ m thick at the base and 65–70  $\mu$ m thick in the other parts (Fig. 41). Cells of the superior side of the thallus are nearly 1.5 times as tall as those of the inferior side (Fig. 41). Concentric hair lines are conspicuous and alternating between both surfaces of the thallus and equidistant (1.5–2.5 mm apart on each surface), forming a reddish-brown line at the upper end of a broad depressed line (0.3–0.7 mm wide) on the inferior surface and narrow lines on the superior surface of the thallus.

The species is dioecious. Both male and female gametophytes as well as tetrasporophytes were found at Ryukyu Islands, Japan. In contrast, only tetrasporophytes were found from Hawaii. Oogonial and tetrasporangial sori are borne only on the inferior surface, forming continuous or discontinuous lines or sometimes patches distally near the hair lines (Figs 13, 39), covered with an indusium (Fig. 40). Additional broken lines or small patches, irregularly spreading between hair lines in the lower part of the thallus are sometimes found. Both oogonia and tetrasporangia are more or less spherical,  $112.25 \pm 6.58 \,\mu\text{m}$  and  $117 \pm 5.48 \,\mu\text{m}$  in diameter, respectively. Antheridial sori occur only on the inferior surface of the thallus, forming discontinuous lines or patches near hair lines and without an indusium. The antheridia are squarish.

Thalli growing in the intertidal zone are generally smaller and the distances between the hair lines are narrower than those of the subtidal zone. Specimens from the intertidal zone resemble *P. minor* in appearance, but they are distinguishable by the presence of a persistent indusium covering the sporangial sori, absent in *P. minor*.

### DISCUSSION

Molecular phylogenetic analyses of Padina species collected from the western Pacific Ocean and Hawaii using rbcL and cox3 gene sequences revealed the occurrence of four unknown taxa, which are genetically closely related to P. sanctae-crucis and P. japonica. They represent independent clades supported by moderate to high bootstrap values in rbcL, cox3 (Figs 2, 3), and concatenated rbcL+cox3 (data not shown). DNA sequence divergences within the clades ranged from 0 to 0.39% in rbcL and 0 to 2.6% in cox3; whereas, divergences between the clades were 1.48-3.64% in rbcL and 7.08-13.14% in cox3. These values are comparable with those between different Padina species (Ni-Ni-Win et al. 2008), supporting their genetic separation. The specimens comprising the clades shared the same morphological characters as follows, and are distinguishable from each other and from any known taxa (Table 2): clade A (=P. okinawaensis sp. nov.), reniform or circular thallus of entire margin with inconspicuous hair lines on the superior surface of the thallus, and reproductive organs (both oogonia and tetrasporangia) in small groups or discontinuous sori under thin flakes of calcium on the inferior surface; clade B (=P. undulata sp. nov.), circular or semicircular thallus of undulate margin with conspicuous

hair lines on both surfaces of the thallus, oogonial and tetrasporangial sori in continuous or discontinuous lines covered with a persistent indusium, and cylindrical oogonia; clade C (=*P. terricolor* sp. nov.), grayish brown on the inferior surface of the thallus, and oogonial and tetrasporangial sori in continuous lines, covered with a persistent indusium; clade D (=*P. fasciata* sp. nov.), presence of broad white stripes on both surfaces of the thallus due to unique calcification, and oogonial and tetrasporangial sori in continuous lines, entirely embedded in a gelatinous layer, on the inferior surface of the thallus.

All four new taxa are bistratose throughout. Of the 32 commonly recognized Padina species, 14 species (including P. japonica) are reported to be bistratose throughout (Taylor 1960; Abbott & Huisman 2003, 2004). Seven of these species were included in the molecular analyses of this study and were revealed to be genetically distant from the four new species (Figs 2, 3). However, for the rest of the species, molecular analysis was not possible because of the difficulties in collecting new specimens from their type localities; therefore, their morphology was compared in detail with the new taxa. Among the remaining seven species together with P. japonica and P. sanctae-crucis (Table 2), Padina fernandeziana Skottsberg & Levring, P. haitiensis Thivy, P. perindusiata Thivy and Padina plumbea (Areschoug) Levring are relatively close to our new species. However, P. fernandeziana and P. plumbea contrast with each of four new species mainly by the presence of successive fertile zones (Levring 1940; Abbott & Huisman 2003); whereas, alternation of fertile and sterile zones was observed in our materials (i.e. fertile zones were separated by sterile zones when both surfaces are viewed together). Padina haitiensis differs from other species in the presence of tetrasporangial sori in the middle between two hair lines (Taylor 1960; Abbott & Huisman 2003); whereas, tetrasporangial sori were always located distally near the hairlines in the four new species. Re-examination of type specimen of P. haitiensis also showed this morphological difference. Likewise, P. perindusiata differs from other species especially in its weaker calcification on both surfaces of the thallus, in the irregular arrangement of alternating hair lines, and in the arrangement of sporangial sori in two to three rows in fertile zone (Taylor 1960; Littler & Littler 2000). Re-examination of the type specimen of P. perindusiata also confirmed the above-mentioned morphological differences. Considering the above-mentioned morphological differences, we conclude that the four new taxa are distinctive from known Padina species.

Taylor (1960) reported that the monotypic species *Dictyerpa jamaicensis* Collins represented a growth form of a *Padina* species, and stated that it was synonymous with *P. sanctae-crucis* Børgesen. Subsequently, Gaillard (1975) pointed out the morphological similarities between *P. sanctae-crucis* and *P. japonica* Yamada, and suggested their synonymy. Papenfuss (1977) proposed a new combination of *P. jamaicensis* (= *D. jamaicensis*) for *P. sanctae-crucis*. Later, Abbott and Huisman (2003, 2004) re-examined the morphology of the type specimen of *P. japonica*, compared with several specimens of *P. sanctae-crucis* from Hawaii and St. Croix, Virgin Islands, Caribbean Sea (type locality),

and concluded that those two species were synonymous. However, the present study highlights the genetic distance separating P. sanctae-crucis collected from Hawaii and southern Japan from P. japonica collected from Japan, in both rbcL and cox3 sequences. The DNA sequence (DQ472037) based on the specimen collected from Hawaii and assigned as P. boergesenii by De Clerck et al. (2006) we consider to be *P. sanctae-crucis*; although, the morphology of the specimen is unclear since the authors did not mention its morphology. This notion is supported by the following morphological features: P. boergesenii differs from P. sanctae-crucis in the number of cell layers of the thallus (i.e. two to three layers in the former vs two layers in the latter) and in the presence or absence of an indusium (absent in the former vs present in the latter) (Dawson 1944; Allender & Kraft 1983; Littler and Littler 2000, 2003; Abbott & Huisman 2003, 2004).

Morphologically P. sanctae-crucis and P. japonica are shown to differ in the following features: the inferior surface of the thallus is much more calcified in P. sanctaecrucis than in P. japonica; both oogonia and tetrasporangia are more or less spherical and large in P. sanctae-crucis; whereas, both are obovate and small in P. japonica; the regular arrangement of a single row of tetrasporangial sori was observed in P. sanctae-crucis and additional broken lines or small patches were occasionally found outside the regular one; whereas, in P. japonica an irregular arrangement of two to three rows of tetrasporangial sori, as reported by Yamada (1931) and confirmed by ourselves by examining the type (SAP9268), were commonly observed between two hair lines besides the regular one (Table 2). We therefore conclude that P. sanctae-crucis and P. japonica should be distinguished at the species level.

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