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Four newly recorded species of the calcified marine brown macroalgal genus *Padina* (Dictyotales, Phaeophyceae) for Australia

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Abstract. Molecular phylogenetic analyses based on plastid-encoded *rbc*L and mitochondrial *cox*3 gene sequences, in combination with morphological observations, revealed the existence of the following four bistratose *Padina* species previously unreported from Australian coasts: *Padina calcarea* Ni-Ni-Win, S.G.A.Draisma, W.F.Prud'homme van Reine & H.Kawai, characterised by its bright yellow–orange inferior thallus surface and chalky white, heavily calcified superior surface, and the presence of hairlines only on the inferior surface; *P. macrophylla* Ni-Ni-Win, M.Uchimura & H.Kawai, characterised by a moderately calcified thallus with broad, depressed hairlines on the inferior surface and narrow, not depressed hairlines on the superior surface, those hairlines that are largely spaced on each surface; *P. moffittiana* I.A.Abbott & Huisman, characterised by lightly calcified thalli with narrow, slightly depressed hairlines that are distributed in alternate sequence between the two surfaces at unequal distances, and broad reproductive sori in one or two rows in the fertile zone; and *P. okinawaensis* Ni-Ni-Win, S.Arai, M.Uchimura & H.Kawai, characterised by heavily calcified thalli, except at the hairlines, which form an alternation of uncalcified furrows and calcified glabrous zones on the inferior surface. With the addition of these four species, 13 *Padina* species are known from Australia.

Additional keywords: *cox*3, molecular phylogeny, morphology, *Padina calcarea*, *P. macrophylla*, *P. moffittiana*, *P. okinawaensis*, *rbc*L, taxonomy.

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Introduction

The genus *Padina*, a common and widely distributed group of marine brown algae from warm-temperate to tropical seas worldwide, is generally characterised by its fan-shaped calcified thalli with inrolled marginal meristems. Some species, however, have a '*Vaughaniella*' stage of creeping rhizomes with a single apical cell from which the fan-shaped thalli develop (Børgesen 1951; Cribb 1951; De Clerck and Coppejans 1997). Calcification occurs to varying degrees, particularly on the superior surface (which is facing to the inrolled margin). Thalli may be upright or decumbent and are composed of two to many (up to 20) cell layers, depending on the species. Phaeophycean hairs form in concentric rows termed hairlines and may occur on one or both surfaces, except in *P. glabra* Gaillard whose thalli are reported to lack hairlines (Gaillard 1966; Wynne and De Clerck 1999). The position and arrangement of reproductive sori are generally related to hairlines, both being important for species identification. *Padina* exhibits an alternation of diplohaplontic isomorphic generations.

Currently, 43 species are recognised in the genus *Padina* worldwide (Ni-Ni-Win *et al*. 2011*a*, 2011*b*; see also Algaebase, http://www.algaebase.org/, accessed January 2012); nine of these have been reported in Australia, namely, *P. australis* Hauck, *P. boergesenii* Allender & Kraft, *P. boryana* Thivy, *P. condominium* Kraft, *P. elegans* Koh & Womersley, *P. fraseri* (Greville) Greville, *P. gymnospora* (Kützing) Sonder, *P. melemele* I.A.Abbott & Magruder and *P. sanctae-crucis* Børgesen (Womersley 1987; Huisman 2000; Kraft 2009). There have been no studies of Australian *Padina* using a molecular-based taxonomic approach supplemented by morphological observations; all previous studies were based only on morphology. In the present study, we aimed to produce a detailed taxonomic assessment of Australian *Padina* species on the basis of molecular phylogenetic analyses using *rbc*L and *cox*3 genes, in combination with morphological observations.

Materials and methods

Morphological observations

Padina specimens used in the present study were newly collected from a wide range of sites on the eastern and western coasts of Australia, such as Lizard Island, Heron Island (Great Barrier Reef), Newcastle, Sydney (New South Wales), Rottnest Island, Cottesloe and Ningaloo Reef (Western Australia). Selected voucher specimens used for morphological and molecular studies are deposited in the herbarium of Kobe University Research Center for Inland Seas and the State Herbarium of South Australia (AD). Type specimens of *P. australis* Hauck (L0055591), *P. distromatica* Hauck (L0055592), *P. dubia* Hauck (L0055593), *P. somalensis* Hauck (L0055595), *P. tetrastromatica* Hauck (L0055597; *Hauck No. 68*), *P. haitiensis* Thivy (MICH, *Taylor 20987*), *P. perindusiata* Thivy (MICH, *Taylor 1356*) and *P. japonica* (SAP 9268) were loaned from the National Herbarium of the Netherlands, Leiden (L), the Herbarium of the University of Michigan (MICH), and the Herbarium of the Graduate School of Science, Hokkaido University (SAP). Type specimens of three recently described species, namely *P. calcarea* (SAP111112), *P. macrophylla* (SAP107787) and *P. okinawaensis* (SAP106474), were also examined. Specimens were hand-sectioned for anatomical observation and the sections were mounted on glass slides in 50% Karo corn syrup–seawater. Photomicrographs were taken using a VB-7010 digital camera (Keyence, Tokyo, Japan) attached to a BX-51 microscope (Olympus, Tokyo, Japan). Herbarium abbreviations follow Index Herbariorum (http://sciweb.nybg.org/science2/IndexHerbariorum.asp, accessed December 2011).

Molecular phylogenetic analyses

DNA extraction, polymerase chain reaction (PCR) amplifications and sequencing were carried out as specified in Ni-Ni-Win *et al.* (2008). In all, 19 new *rbc*L and 16 new *cox*3 sequences were generated in the present study and analysed in combination with previously published sequences (Table 1). Seven species, including close and distant relatives to *Padina*, namely *Distromium didymothrix* EU579948 and *Homoestrichus* sp. EU579951 (Dictyotales), both putative sister groups to *Padina* (Bittner *et al*. 2008), *Dictyota dichotoma* AB358934, *Dictyopteris australis* EU579940, *Lobophora* sp. AB548390, *Stypopodium* sp. AB358936 and *Zonaria stipitata* EU579965, were used as outgroup taxa in *rbc*L and concatenated *rbc*L + *cox*3 analyses (Table 1). *Stypopodium* sp. AB358955 was used as the outgroup taxon in *cox*3 analyses because this marker was unavailable for the other above-mentioned species. Sequences were aligned with Clustal X (Thompson *et al*. 1997). Three alignments were created, namely *rbc*L, *cox*3 and the combined *rbc*L+*cox*3. Phylogenetic trees were obtained using maximum likelihood (ML) and Bayesian inference (BI) analyses. The best-fit evolutionary model for each codon position of each gene (six partitions) were performed by comparing different evolutionary models via the corrected Akaike information criterion (Akaike 1974) for ML analysis, and the Bayesian information criterion (Schwarz 1978) for the BI analysis implemented in KAKUSAN3 (Tanabe 2011). The selected models for each partition for ML and BI are described in Table 2. The ML analyses were performed by the likelihood ratchet method (Vos 2003). For the ML tree search, 1000 sets of 25% site-upweighted data were created by using the pgresampleseq command in Phylogears 2.0.2010.08.31 (Tanabe 2010), and the ML trees with the upweighted data were estimated by using Treefinder (Jobb *et al*. 2004) with application of the best-fit substitution model. The robustness of the resulting phylogenies was tested by bootstrap resampling (Felsenstein 1985), using Treefinder (1000 replicates). Bayesian analyses with the selected evolutionary models were conducted using MrBayes v.3.2 (Ronquist and Huelsenbeck 2003). The Bayesian analyses were initiated with a random starting tree, using four Markov chain Monte Carlo chains simultaneously for 10 000 000 generations, sampling one tree every 100 generations. The first 10 000 trees sampled were discarded as 'burn-in' on the basis of the stationarity of -ln L, as assessed using Tracer version 1.5 (Rambaut and Drummond 2009). Consensus-tree topology and posterior probability were calculated with the remaining trees by MrBayes v.3.2.

Results

Molecular analyses

The *rbc*L alignment consisted of seven outgroup taxa and 60 ingroup sequences, with a total of 1319 bp, these representing 30 *Padina* species, including 11 new sequences of Australian specimens and 9 of the 13 species now reported from Australia. The *cox*3 alignment consisted of 748 characters of 52 sequences, representing 29 *Padina* species (9 Australian), including 11 new sequences of Australian specimens plus one outgroup taxon (Table 1). The *rbc*L + *cox*3 alignment comprised 57 sequences representing 30 *Padina* species and seven outgroup taxa, and was 2067 bp in length. All sequences were unambiguously aligned and no gaps were present. ML and BI analyses using these three alignments (separate and combined loci) showed almost identical tree topologies, except for some nodes that exhibited low support. The ML trees inferred from separate *rbc*L and *cox*3 analyses are respectively shown in Figs 1 and 2 (similar results from the combined analysis are not shown). Sequences of Australian specimens were placed in the lineages of the following nine known species: *P. australis*, *P. boergesenii*, *P. calcarea*, *P. elegans*, *P. fraseri*, *P. gymnospora*, *P. macrophylla*, *P. moffittiana* and *P. okinawaensis*. All species were supported by high bootstrap values (95–100%) and posterior probabilities (1.0). All newly collected Australian specimens showed sequences identical or very similar to those of known taxa both in *rbc*L and *cox*3 (Figs 1, 2). Intra-species variation in *rbc*L ranged from 0% (*P. macrophylla*) up to a maximum of 1.2% (1.2%

Table 1. Origin of specimens used in the study and their DNA Data Bank of Japan (DDBJ) accession numbers

For voucher numbers: IA, the herbarium of I. A. Abbott; BISH, the Herbarium of Bishop Museum; SAP, the Herbarium of the Graduate School of Science, Hokkaido University; and AD, the State Herbarium of South Australia. KURCIS, KU and KU-d serial numbers refer to specimen numbers of the voucher specimens, culture specimens and silica-gel specimens respectively, housed in the herbarium of the Kobe University Research Centre for Inland Seas. L, Nationaal Herbarium Nederland, Universiteit Leiden branch. Bold lettering for DDBJ code for *rbc*L and DDBJ code for *cox*3 indicates new sequences generated in the present study

Species	Specimen code	Origin, collection date, collector	Voucher number	DDBJ code for *rbc*L	DDBJ code for *cox*3
Padina arborescens Holmes	NAG1	Taira, Nagasaki, Japan, 4 July 2006, *S. Arai*	SAP105578	AB358905	AB358940
P. australis Hauck	OKI1	Urazoko, Okinawa Island, Japan, 4 October 2004, *T. Hanyuda*	SAP105580	AB358906	AB358941
	MAL1	Johor, Malaysia, 2005, *H. Kawai*	–	AB512524	AB512564
	AUS1	Newcastle, NSW, Australia, 2005, *H. Kawai*	–	AB489914	AB512565
	MIY1	Toguchinohama, Miyako Island, Okinawa Pref., Japan, 22 November 2010, *Z.M. Sun*	KU-d9503	**AB690271**	**AB690280**
	CHI1	Chichi-jima, Ogasawara Island, Tokyo Pref., Japan, 12 December 2006, *H. Kawai*	KU-d3208	**AB690272**	–
P. boergesenii Allender & Kraft	ROT2	Rottnest Island, Australia, 11 March 2011, *C.F.D. Gurgel*	No. 9907 in KURCIS	**AB820943**	**AB844683**
P. boryana Thivy	MYA4	Setsei, Kyaikkhami, Mon State, Myanmar, 20 April 2005, *Mya Kyawt Wai*	No. 65542 in KURCIS	AB512527	AB512568
P. calcarea Ni-Ni-Win, S.G.A. Draisma, W. F. Prud'homme van Reine & H.Kawai	BAB1	Back Reef (07°57.271′N, 134°37.661′E), north of Babeldaob Island, Palau, 31 March 2009, *E. Verheij and W. F. Prud'homme van Reine*	SAP111112	AB671201	AB671210
	GAM1	Cape Besir, Besir Bay, West-Papua, Gam Island, Indonesia, 27 November 2007, *S.G.A. Draisma*	L SGAD 0712219	AB671198	AB671208
	LIZ1	12-m depth, scuba dive, Yong Reef (14°37′12.5″S, 145°37′11.1″E), Lizard Island Group, Great Barrier Reef, Australia, 11 February 2009, *C.F.D. Gurgel and R. R. M. Dixon*	KU-d6826 and AD-A89444a	**AB690273**	**AB690281**
P. crassa Yamada	NAG2	Taira, Nagasaki, Japan, 4 July 2006, *S. Arai*	SAP105581	AB358908	AB358943
P. ditristromatica Ni-Ni-Win & H.Kawai	BRU1	Brucoli, Augusta SR, Italy, 11 September 2009, *G. Furnari*	SAP108068	AB548382	–
P. elegans Koh & Womersley	COT1	Cottesloe, Western Australia, Australia, 26 February 2011, *C.F.D. Gurgel*	No. 9807 in KURCIS	**AB820944**	**AB820953**
P. fasciata Ni-Ni-Win, M. Uchimura & H. Kawai	OKI2	Awase, Okinawa Island, Japan,19 November 2006, *S. Arai*	SAP106507	AB489915	AB489955
P. fraseri (Greville) Greville	AUS2	Flat Rock, northern New South Wales, Australia, 16 December 2006, *J. Phillips*	No. 65550 in KURCIS	AB548389	AB548397
	AUS3	Sydney, New South Wales, Australia, 11 March 2010, *H. Kawai*	KU-d7086	**AB690274**	**AB690282**
P. gymnospora (Kützing) Sonder	SCA1	Scarborough, Queensland, Australia, 3 September 2006, *J. Phillips*	KU-d3201	**AB820945**	**AB820954**
	NEW1	Newcastle, New South Wales, Australia, 12 March 2010, *H. Kawai*	No. 7120 in KURCIS	**AB820947**	**AB820955**
	COT2	Cottesloe, Western Australia, Australia, 26 February 2011, *C.F.D. Gurgel*	No. 9816 in KURCIS	**AB820948**	**AB820956**
	ROT3	Rottnest Island, Western Australia, Australia, 26 February 2011, *C.F.D. Gurgel*	No. 9741 in KURCIS	**AB820949**	**AB820957**
	CUR3	Playa Piskado (a.k.a. Playa Grandi), Curaçao, 9 May 2009, *S.G.A. Draisma*	L SGAD 0905018	**AB820946**	**AB820958**
	KEN1	Kenting, Taiwan, 31 May 2007, *H. Kawai*	KU-d3601	**AB820950**	**AB820959**
	crassaDQ472038	De Clerck *et al.* (2006)	–	DQ472038	–
	sanctae-crucisDQ472036	De Clerck *et al.* (2006)	–	DQ472036	–
	EU579933	Bittner *et al.* (2008)	FRA0311 (IRD162)	EU579933	–

Table 1. (*continued*)

Species	Specimen code	Origin, collection date, collector	Voucher number	DDBJ code for *rbc*L	DDBJ code for *cox*3
P. ishigakiensis Ni-Ni-Win, S.Arai, M. Uchimura & H. Kawai	ISH7	Hunakoshi, Ishigaki Island, Okinawa Pref., Japan, 29 May 2007, *M. Uchimura*	SAP107778	AB512534	AB512575
P. japonica Yamada	NAG3	Taira, Nagasaki, Nagasaki Pref., Japan, 4 July 2006, *S. Arai*	SAP105583	AB358910	AB358942
P. macrophylla Ni-Ni-Win, M.Uchimura & H.Kawai	ISH9	Akasaki, Ishigaki Island, Okinawa Pref., Japan,30 May 2007, *M. Uchimura*	SAP107787	AB512539	AB512580
	HER2	Shallow subtidal, 0.2-m depth, reef pool on Heron Island (23°26′38.99″S, 151°54′39.26″E), Great Barrier Reef, Australia, 26 August 2008, *C.F.D. Gurgel*	KU-d6980 and AD-A88777	**AB690275**	**AB690283**
P. maroensis Ni-Ni-Win & H.Kawai	HAW11	23.6°N, 164.6°W, Maro Reef, Hawaii, 21 June 2006, *R. Moffit*	SAP108066	AB512541	AB512582
P. melemele I.A. Abbott & Magruder	HAW1	BISH700753, Hawaii	BISH700753	AB358918	AB358947
	ISH10	Akasaki, Ishigaki Island, Okinawa Pref., Japan, 20 June 2007, *M. Uchimura*	SAP105592	AB358913	AB358945
P. minor Yamada	IRI3	Iriomote Island, Okinawa Pref., Japan, 21 November 2006, *S. Arai*	SAP105611	AB358920	AB358948
P. moffittiana I.A. Abbott & Huisman	OKI3	Awase, Okinawa Island, Okinawa Pref., Japan, 15 December 2004, *S. Arai*	SAP105613	AB358923	AB358950
	HAW2	Maro Reef, Hawaii, 21 June 2006, *R. Moffitt*	IA31668	AB358927	AB358951
	HER1	28-m depth, scuba dive, Wistari channel (23°27.203′S, 151°54.988′E), Heron Island, Great Barrier Reef, Australia, 3 September 2008, *C.F.D. Gurgel*, *J.M. Huisman, R.R.M. Dixon*	KU-d7007 and AD-A88468k	**AB690276**	**AB690284**
	ROT1	Rottnest Island, Western Australia, Australia, 26 February 2011, *H. Kawai*	No. 9753 in KURCIS	**AB690277**	**AB690285**
P. okinawaensis Ni-Ni-Win, S.Arai & H. Kawai	OKI5	Awase, Okinawa Island, Okinawa Pref., Japan, 19 November 2006, *S. Arai*	SAP106474	AB489923	AB489959
	HAW3	Kahara Bay, O'ahu Island, Hawaii, 11 June 2007, *H. Kawai*	–	AB489926	AB489962
	HAW14	Kaaawa Beach Park, O'ahu Island, Hawaii, 11 June 2007, *H. Kawai*	KU-d3673	**AB820951**	–
	KEP3	Lancang, Kepulauan Seribu, Indonesia, 12 September 2005, *S.G.A. Draisma*	L0609511	Ab489929	AB489964
	KEP4	Kelapa, Kepulauan Seribu, Indonesia, 13 September 2005, *S.G.A. Draisma*	L0609514	AB489930	AB489965
	KEP5	Panjang, Kepulauan Seribu, Indonesia, 14 September 2005, *S.G.A. Draisma*	L0609515	AB489931	
	KEP6	Tidung Kecil, Kepulauan Seribu, Indonesia, 18 September 2005, *S.G.A. Draisma*	L0609539	AB489932	AB489967
	KRI1	Sorido resort lagoon, Kri Island, Indonesia, 19 November 2007, *S.G.A. Draima*	L SGAD 0712009	**AB820952**	–
	LIB1	Libong Island, Trang Prov., Thailand, 11 September 2004, *A. Prathep*	–	AB489933	AB489968
	NIN1	3–4-m depth, scuba dive, Inner Lagoon (21°52.926′S, 113°58.589′E), Ningaloo Reef, West Australia,14 June 2008, *C.F.D. Gurgel*, *R.R.M. Dixon*	KU-d6956 and AD-A89914a	**AB690278**	**AB691762**
P. pavonica (Linnaeus) Thivy	SPA2	Port des Canonges, Mallorca, Spain, 22 June 2006, *S.G.A. Draisma*	SGAD0606012 (housed in KURCIS)	AB512545	AB512586

(*continued next page*)

Table 1. (*continued*)

Species	Specimen code	Origin, collection date, collector	Voucher number	DDBJ code for *rbc*L	DDBJ code for *cox*3
P. pavonicoides Ni-Ni-Win & H.Kawai	SPA3	La Llosa d'en Patro Pere, Menorca Island, Spain, 24 June 2006, *S.G.A. Draisma*	SGAD0606151 (housed in KURCIS)	AB602783	AB602786
P. ryukyuana Y.P.Lee & Kamura	OKI6	Awase, Okinawa Island, Okinawa Pref., Japan,19 November 2006, *S. Arai*	SAP105631	AB358929	AB358953
P. sanctae-crucis Børgesen	OKI7	Haemida Beach, Okinawa Island, Japan, 27 May 2007, *M. Uchimura*	SAP106512	AB489935	AB489969
P. sulcata Ni-Ni-Win, S.G.A.Draisma & H.Kawai	MAL7	Pulau Rusukan Kecil, Pulau Labuan, Malaysia, June 1998, *M. Masuda* and *S-M. Phang*	MAL7	AB671206	AB671214
	KEP12	Semak Daung, Kepulauan Seribu, Indonesia, 17 September 2005, *S.G.A. Draisma*	L0609544	AB671205	AB671213
P. terricolor Ni-Ni-Win, M.Uchimura & H.Kawai	OKI8	Awase, Okinawa Island, Okinawa Pref., Japan,19 November 2006, *S. Arai*	SAP106500	AB489944	AB489973
P. tetrastromatica Hauck	MAL2	Desaru, Johor, Malaysia, 22 May 2005, *P-E. Lim*	–	AB512554	AB512595
P. thivyae Doty & Newshouse	TAN1	Tanega Island, Kagoshima Pref., Japan, 2 October 2005, *S. Arai*	SAP105633	AB358931	AB358954
P. undulata Ni-Ni-Win, S.Arai & H.Kawai	OKI11	Awase, Okinawa Island, Okinawa Pref., Japan,19 November 2006, *S. Arai*	SAP106493	AB489949	AB489976
P. usoehtunii Ni-Ni-Win & H.Kawai	MYA2	Chaung Thar beach, Pathein (Bassein), Myanmar, 2 April 2005, *Ni-Ni-Win*	SAP107801	AB512559	AB512597
Padina sp.	FER1	Puerto Franes, Robison Crusoe, Jaun Fernandez, Chile, 2004, *D.G. Mueller*	KU-1175	**AB690279**	**AB690286**
Dictyota dichotoma (Hudson) Lamouroux	–	Aburatsubo, Kanagawa Pref., Japan, 12 September 2004, *T. Hanyuda*	–	AB358934	–
Dictyopteris australis (Sonder) Askenasy		Bittner *et al.* (2008)	FRA0359 (IRD302)	EU579940	–
Distromium didymothrix Allender & Kraft	–	Bittner *et al.* (2008)	FRA0361 (IRD320)	EU579948	–
Homoeostrichus sp.	–	Bittner *et al.* (2008)	FRA0425	EU579951	–
Lobophora sp.	–	Awase, Okinawa Island, Okinawa Pref., Japan, 15 December 2004, *S. Arai*	No. 65551 in KURCIS	AB548390	–
Stypopodium sp.	–	Awase, Okinawa Island, Okinawa Pref., Japan, 15 December 2004, *S. Arai*	–	AB358936	AB358955
Zonaria stipitata Tanaka & Nozawa	–	Bittner *et al.* (2008)	FRA0434 (IRD267)	EU579965	–

Table 2. Selected models for each codon position of each gene for maximum likelihood (ML) and Bayesian inference (BI) analyses

Analysis	Dataset	1st codon position	2nd codon position	3rd codon position
Maximum likelihood (ML)	*rbc*L	GTR+G	GTR+G	GTR+G
	*cox*3	GTR+G	HKY85	GTR+G
	*rbc*L+*cox*3	Same model as in separate datasets	Same model as in separate datasets	Same model as in separate datasets
Bayesian inference (BI)	*rbc*L	GTR+G	JC+G	GTR+G
	*cox*3	GTR+G	F81	GTR+G
	*rbc*L+*cox*3	Same model as in separate datasets	Same model as in separate datasets	Same model as in separate datasets

within *P. calcarea*, and 0–1.2% within *P. gymnospora* and *P. okinawaensis*); some of these differences are slightly greater than that between the sibling species *P. sanctae-crucis* and *P. japonica*, 0.83–1.06% (see Ni-Ni-Win *et al.* 2010 for comparisons based on a larger set of sequences for *P. sanctae-crucis* and *P. japonica* than reported here). Australian specimens

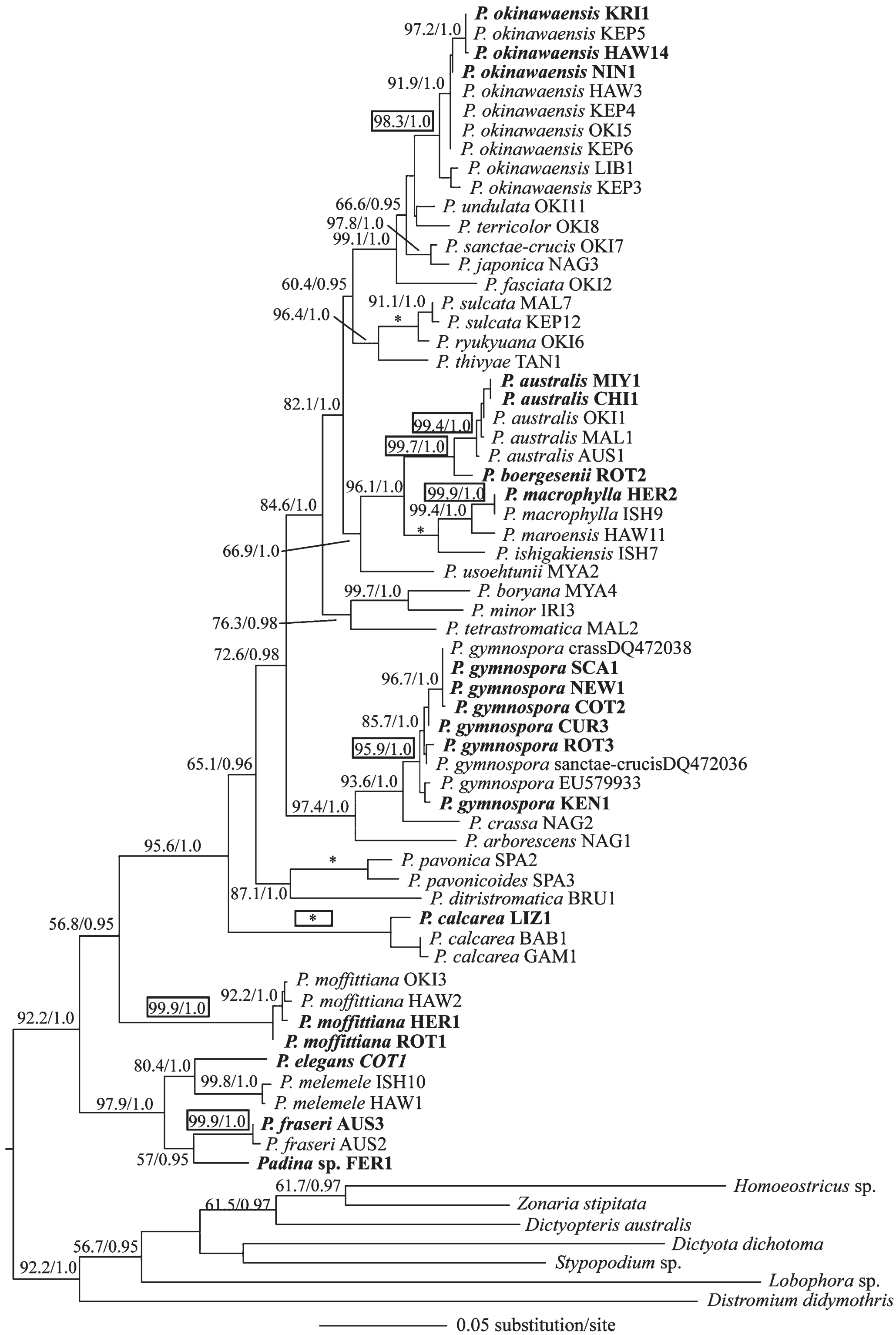


Fig. 1. Maximum-likelihood (ML) tree for selected species of the genus *Padina*, with emphasis on Australian species on the basis of *rbc*L DNA sequences. Numbers at each node indicate bootstrap values (>50%) for ML (left) and posterior probabilities (>0.90) for Bayesian inference (BI; right). Asterisks indicate 100% bootstrap values and 1.0 posterior probabilities. Bold letters indicate the specimens newly collected from Australia and other countries. Boxes with support values indicate the clades where Australian samples are included.

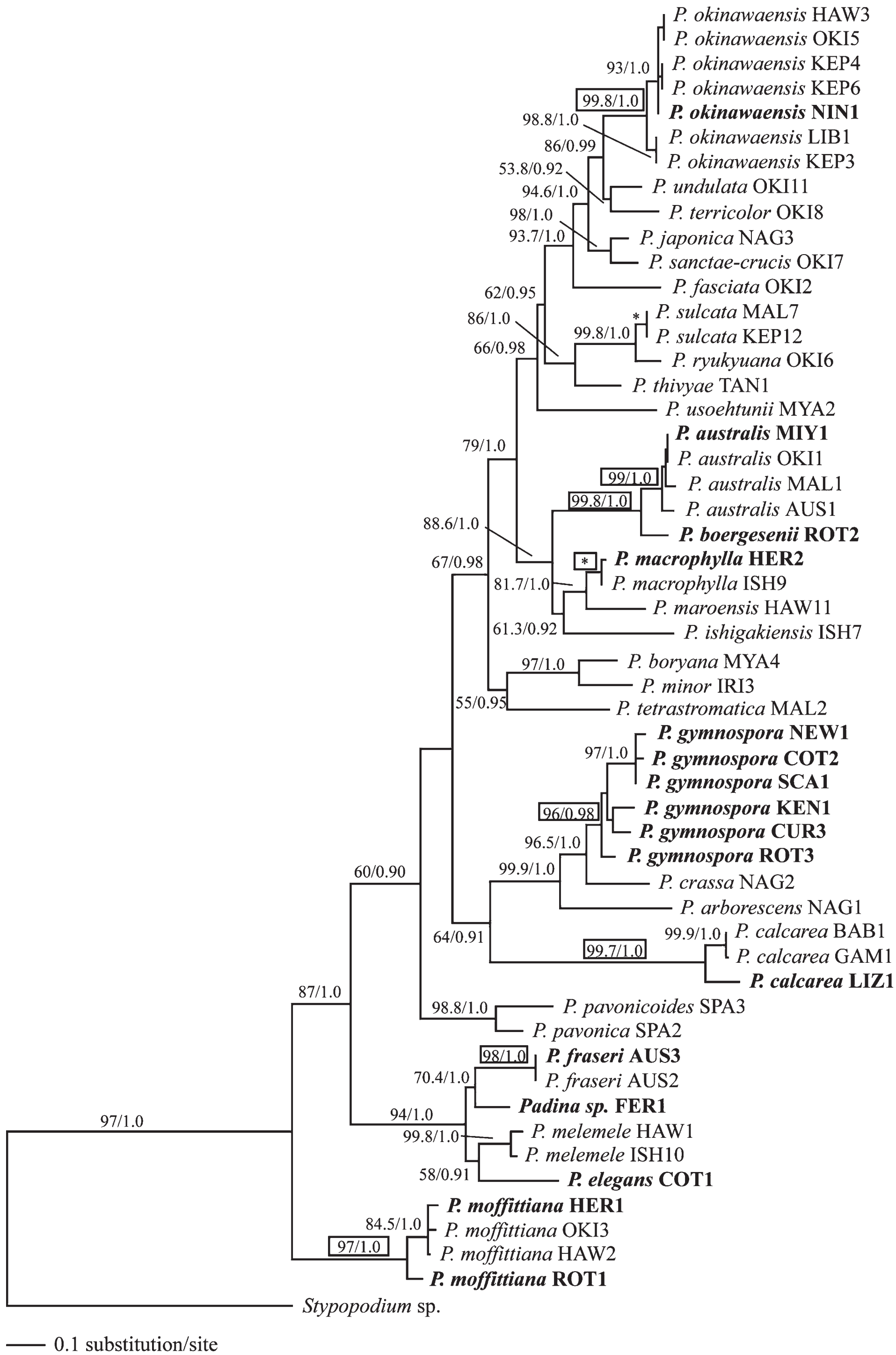


Fig. 2. Maximum-likelihood (ML) tree for selected species of the genus *Padina*, with emphasis on Australian species on the basis of *cox*3 DNA sequences. Numbers at each node indicate bootstrap values (>50%) for ML (left) and posterior probabilities (>0.90) for Bayesian inference (BI; right). Bold letters indicate the specimens newly collected from Australia and other countries. Boxes with support values indicate the clades where Australian samples are included.

of *P. okinawaensis* exhibited a sequence identical to those from Japan, Hawaii and Indonesia. Within-species variation in *cox*3 ranged from 0.4% in *P. macrophylla*, to a maximum of 4.8% in *P. calcarea* (0.13–4.8% between different accessions of *P. calcarea*). Newly collected Australian specimens from Sydney that were morphologically referable to *P. fraseri* (and near its type locality) formed a strongly supported clade with the previously collected Australian specimen from northern New South Wales, and exhibited little sequence variation in *rbc*L (0.23%), but no variation among *cox*3 DNA sequences. Three sequences downloaded from GenBank that were assigned as *P. crassa* (DQ472038, from Lord Howe Island) and *P. sanctae-crucis* (DQ472036, from Bermuda) by De Clerck *et al*. (2006), and *P. gymnospora* (EU579933, from New Caledonia) by Bittner *et al*. (2008), grouped with specimens from Curaçao, Taiwan and Australia in the *P. gymnospora* clade. The specimens from Curacao (near the type locality, St Thomas, Virgin Islands), Taiwan and Australia all morphologically agreed well with the original description as well as the description of *P. gymnospora* by Womersley (1987), and thus the specimens described by De Clerck *et al*. (2006) should undergo further morphological examination. A total of 11 Australian specimens morphologically assigned as *P. elegans* exhibited identical sequence to those collected from its type locality, Cottesloe, Western Australia, and thus only one representative sequence for both *rbc*L and *cox*3 was used (Table 1). The species forms a sister relationship to *P. melemele* in all analyses of both *rbc*L and *cox*3, with interspecies sequence variation of 3.79–4.02% in *rbc*L and 8.02–8.82% in *cox*3. Likewise, four specimens morphologically referable to *P. boergesenii*, collected from Mary Cove, Rottnest Island, Western Australia, showed identical sequence in both *rbc*L and *cox*3 and thus, only one representative sequence for each gene was used. It was sister to *P. australis* in all analyses, with interspecies sequence divergence of 1.4–1.7% in *rbc*L and 5.9–6.7% in *cox*3.

Morphological observations

Key to species of Australian *Padina*

1. Thallus mostly 3 or more layers thick 2
   Thallus 2 layers thick throughout or sometimes 3 layers thick at the base 4
2. Thallus 4–8 layers thick; hairlines irregularly on both surfaces .... *P. gymnospora*
   Thallus mostly 3 layers thick; hairlines alternating between both surfaces in equal distance 3
3. '*Vaughaniella*' stage absent; central cell layer tallest; sporangial sori broad, located only on the superior surface, arranged in 1–3 continuous or separated lines between the hairlines .... *P. fraseri*
   '*Vaughaniella*' stage present; cells of the inferior layer taller than those of the superior layer and central cell layer shortest; sporangial sori narrow, located on the inferior surface, arranged in 1 continuous line just above the hairline .... *P. boergesenii*
4. Thallus mostly 2 layers thick, sometimes 3 layers thick at the base .... *P. boryana*
   Thallus 2 layers thick throughout. 5
5. Inferior thallus surface golden brown to bright yellow or orange and superior surface brilliant white due to heavy calcification (see Abbott (1996), fig. 1; Littler and Littler (2003), p. 175, Ni-Ni-Win *et al*. (2008), fig. 3) 6
   Inferior thallus surface pale to dark brown or yellowish-brown and superior surface whitish or pale brown 8
6. Hairlines alternating on both surfaces of the thallus; sporangial sori mostly in concentric lines, located on the superior surfaces, just below of the hairlines of the same surface (i.e. in the middle of two alternating hairlines when the hairlines on both surfaces viewed together) .... *P. elegans*
   Hairlines only on the inferior surface and inconspicuous; sporangial sori mostly in broken lines 7
7. Sporangial sori on the inferior surface of the thallus, just above the hairlines, not embedded .... *P. calcarea*
   Sporangial sori on the superior surface of the thallus, in 1–3 broken lines between the hairlines of the opposite surface, partially embedded in the cuticle .... *P. melemele*
8. Alternating hairlines unequally distanced on both surfaces 9
   Alternating hairlines equally distanced on both surfaces 10
9. Hairlines of the inferior surface broad and depressed, those of the superior surface inconspicuous; sporangial sori placed on thallus surface .... *P. macrophylla*
   Hairlines of both surfaces narrow, depressed and conspicuous; sporangial sori partially embedded in the cuticle .... *P. moffittiana*
10. '*Vaughaniella*' stage absent; gametophytes monoecious 11
    '*Vaughaniella*' stage present; gametophyte dioecious 12
11. Oogonial sorus covered with indusium but antheridial sorus without indusium .... *P. australis*
    Both oogonia and patches of antheridia covered with the common indusium .... *P. condominium*
12. Thallus moderately to heavily calcified, forming strips because of the presence of calcified glabrous zones and uncalcified hairlines in alternate sequences on the inferior surface; hairlines of the superior surface inconspicuous; sporangial sori in broken lines or small groups, tending to emerge from the calcified layer, covered with an indusium-like calcium layer .... *P. okinawaensis*
    Thallus slightly to moderately calcified on the inferior surface; hairlines of the superior surface moderate to conspicuous; sporangial sori mainly in continuous lines, covered with transparent indusium .... *P. sanctae-crucis*

Padina calcarea Ni-Ni-Win, S.G.A.Draisma, W.F. Prud'homme van Reine & H.Kawai *in* Ni-Ni-Win *et al.* (2011*a*)

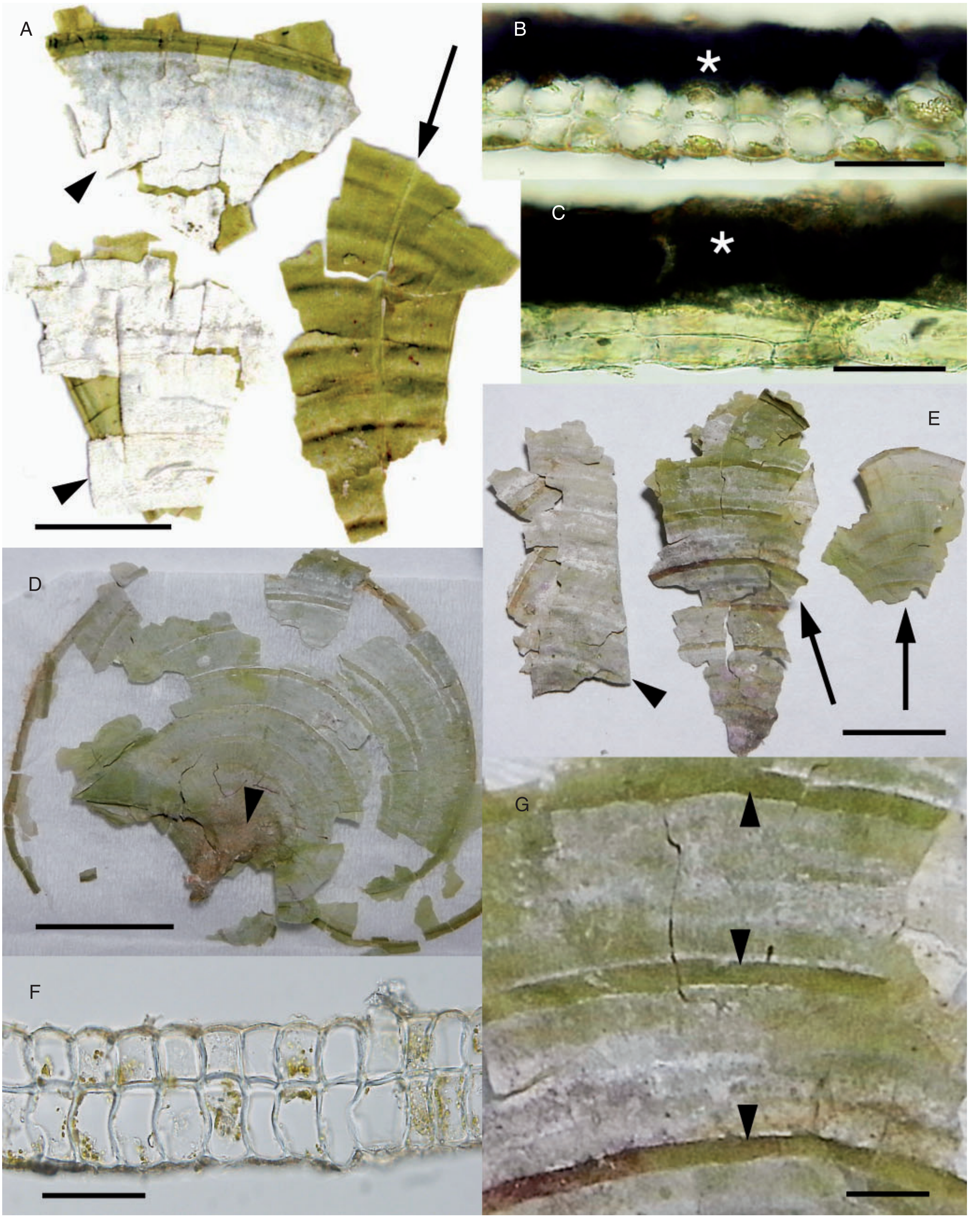
Fig. 3A–C

Type locality: Back Reef, north of Babeldaob Island, Palau (07°57.271′N, 134°37.661′E).

Geographical distribution: Indonesia (Ni-Ni-Win *et al*. 2012), Palau (Ni-Ni-Win *et al*. 2012), and Australia (the present study).

Habitat: in Australia, *P. calcarea* is a common coral-reef species growing on bommies located inside reef lagoons, back reef and protected tropical reef habitats. Observed at depths of 2–15 m.

Specimens examined: AUSTRALIA. Lizard I.: (*C.F.D. Gurgel*, 16 Apr. 2008, KU-d6874–6890/AD-A88206*, KU-d6900–6933/AD-A882206A*), (*C.F.D. Gurgel*, 10 Feb. 2009, KU-d6831–6834/AD-A89441a-d), (*C.F.D. Gurgel*, 11 Feb. 2009, KU-d6826–6830/AD-A89444a-e, 6835/AD-A89442), (*C.F.D. Gurgel*, 17 Feb. 2009, KU-d6847–6852/AD-A89445a-f); Heron I.: (*C.F.D. Gurgel*, 27 Aug. 2008, KU-d6983/AD-A88391*). INDONESIA: Gam I.: West-Papua, Besir Bay, Cape Besir (*S.G.A. Draisma*, 27 Nov. 2007, L SGAD 0712219); Batanta I.: Gegenlol Bay (*S.G.A. Draisma*, 27 Nov. 2007, L SGAD 0712254); Wai I.: Yenweres Bay (a.k.a. Jerief I.; *S.G.A. Draisma*, 5 Dec. 2007, L SGAD 0712521); lagoon (P. Jerief; *S.G.A. Draisma*, 11 Dec. 2007, L SGAD 0712684). PALAU. N of Babeldaob I.: Back Reef (*E. Verheij and W.F. Prud'-homme van Reine*, 31 Mar. 2009, SAP111112 (holotype), L0821280 (isotype)). Note: for Australian samples, asterisks indicate that pressed voucher specimens are available in AD; other samples are represented only by materials preserved in silica gel.


A
B
C
D
E
F
G

Morphology

Thalli are erect, semicircular or circular, with entire margins, up to 2–5 cm wide and 1–4 cm tall, bright yellow or orange on the uncalcified or lightly calcified inferior surface and brilliant white on the superior surface because of heavy calcification (Fig. 3A), composed of two cell layers throughout (~45–60 µm), and attached by a discoidal holdfast with a short stipe. The cell layers are of almost equal height, and the calcium carbonate layer on the superior surface is as thick as or slightly thicker than both cell layers (~50–60 µm) (Fig. 3B, C). Concentric hairlines are inconspicuous and confined only on the inferior surface, measuring ~2–3 mm apart from each other. Reproductive organs were not observed in Australian samples.

Padina macrophylla Ni-Ni-Win, M.Uchimura & H.Kawai *in* Ni-Ni-Win *et al*. (2011*a*)

Fig. 3D–G

Type locality: Akasaki, Ishigaki Island, Okinawa, Japan.

Geographical distribution: southern Japan (Ni-Ni-Win *et al*. 2011*a*), Australia (the present study).

Habitat: same as for *P. calcarea*.

Specimens examined: AUSTRALIA. Heron I.: (*C.F.D. Gurgel*, 26 Aug. 2008, KU-d6980/AD-A88777), (*C.F.D. Gurgel*, 3 Sep. 2008, KU-d6997–6999/AD-A88468a-c*, KU-d7001/AD-A88468e*, KU-d7004/AD-A88468h). JAPAN. Iriomote I.: Hinai (*M. Uchimura*, 27 May 2007, SAP107793); Ishigaki I.: Aashioya (*M. Uchimura*, 30 May 2007, SAP107785), Akahashi (*M. Uchimura*, 30 May 2007, SAP107786), Akasaki (*M. Uchimura*, 30 May 2007, SAP107787 (holotype), –107788); Sesoko I.: (*M. Uchimura*, 26 June 2007, SAP107797–9); Oura Wan: Shuwabo (*M. Uchimura*, 21 June 2007, SAP107800); Okinawa I.: Genka (*M. Uchimura*, 22 June 2007, SAP107791), Awase (*M. Uchimura*, 25 June 2007, SAP107789), Agonoura (27 June 2007), Kudakakita (*M. Uchimura*, 28 June 2007), Miyagi (*M. Uchimura*, 29 June 2007, SAP107795), Hamada (7 July 2007, SAP107792); Amami-Oshima I.: Ikomo (*M. Uchimura*, 30 July 2007), Saneku (30 July 2007, SAP107796), Doran (31 July 2007, SAP107790), Ikema (31 July 2007, SAP107794). Note: for Australian samples, asterisks indicate that pressed voucher specimens are available in AD; other samples are represented only by materials preserved in silica gel.

Morphology

Thalli are erect, semicircular or circular, with entire margins, up to 4–6 cm wide and 3–5 cm tall, lightly calcified on the inferior surface and moderately calcified on the superior surface, yellowish-brown or pale brown, composed of two cell layers throughout (85–105 µm thick) and attached by a stupose base with a short stipe (Fig. 3D–F). Cells of the inferior layer are slightly shorter than those of the superior layer (Fig. 3F). *Vaughaniella* stages were not observed. Concentric hairlines are found on both surfaces in alternate sequence at unequal distances from one another and measure ~5–7 mm apart on each surface (Fig. 3D, E, G). They are broad and depressed on the inferior surface (Fig. 3G, arrowheads), but narrow and not depressed on the superior surface of the thallus (Fig. 3E, arrowhead). Reproductive organs were not observed in Australian samples.

Padina moffittiana I.A. Abbott & Huisman 2003

Fig. 4A–D

Type locality: Maro Reef, north-western Hawaiian Islands.

Geographical distribution: Hawaii (Abbott and Huisman 2003; 2004), southern Japan (Ni-Ni-Win *et al*. 2008), Australia (the present study).

Habitat: same as for *P. calcarea*.

Specimens examined: AUSTRALIA. Heron I.: (*C.F.D. Gurgel*, 26 Aug. 2008, KU-d6982/AD-A88371*), (*C.F.D. Gurgel*, 1 Sep. 2008, KU-d6988/AD-A88429*), (*C.F.D. Gurgel*, 3 Sep. 2008, KU-d7005–7007/AD-A88468i-k; KU-d7009//AD-A88468m*, KU-d7012/AD-A88468p, KU-d7016/AD-A88468s, KU-d7017/AD-A88468t*), (*C.F.D. Gurgel*, 4 Sep. 2008, KU-d7020/AD-A88488*); Rottnest I.: (*H. Kawai*, 26 Feb. 2010, KURCIS No. 9725–9727, 9743–9745, 9750, 9753, 9755, 9889). JAPAN. Okinawa I.: Awase (*S. Arai*, 15 Dec. 2004, SAP105613–105617), (*S. Arai*, 19 Nov. 2006, SAP105618–21), (*S. Arai*, 5 Apr. 2007, SAP105622–105627); Iriomote I.: Akahashi (*M. Uchimura*, 30 May 2007, SAP105628–30). HAWAII. Necker I.: (*R. Moffitt*, 30 June 2001, IA20659); Maro Reef: (*R. Moffitt*, 21 June 2006, IA31668, –31669). Note: for Australian samples, asterisks indicate that pressed voucher specimens are available in AD; other samples are represented only by materials preserved in silica gel.

Morphology

Thalli are erect, semicircular or circular to flabelliform, with entire margins, sometimes shallowly split into fan-shaped segments, up to 4–9 cm wide and 3–7 cm tall, lightly calcified on both surfaces except at the hairlines, yellowish-green or yellowish-brown, composed of two cell layers throughout (75–100 µm), and attached by a stupose base with a short stipe (Fig. 4A). The holdfast and 1 cm of the basal portion of the frond are covered by long fibrous hairs that are confined to the inferior surface (Fig. 4A, arrowhead). Cells of the superior surface layer are nearly 1.5 times thicker than those of the inferior surface layer. *Vaughaniella* stages were not observed. Concentric hairlines are found on both surfaces in an alternate sequence at unequal distances. The hairline of the superior surface is distally ~3–4 mm from that of the inferior surface, which is then, in turn, followed by the hairline of the inferior surface at ~1.5–2 mm (Fig. 4A, C). Both of the alternating hairlines are narrow and depressed, but the hairlines of the inferior surface (Fig. 4C, arrowheads) are slightly broader than those of the superior surface (Fig. 4B, arrowheads, Fig. 4C, double arrowhead).

Gametophytes dioecious. Reproductive sori (oogonial, antheridial and tetrasporangial sori) primarily observed on the inferior surface of the thallus. Both oogonial and tetrasporangial

Fig. 3. Morphology of *Padina calcarea* and *P. macrophylla*. A–C. *Padina calcarea*. A. Habit of sterile plant, showing bright yellow inferior (arrow) and heavily calcified superior (arrowheads) surfaces. B. Transverse section of middle portion of the thallus with thick calcium layer (asterisk) on the superior thallus surface. C. Longitudinal section of middle portion of the thallus, with thick calcium layer (asterisk) on the superior thallus surface. D–G. *Padina macrophylla*. D. Habit, showing the inferior surface of a sterile plant with fibrous hairs (arrowhead) at the base. E. Broken sterile plant, showing superior (arrowhead) and inferior (arrows) surfaces. F. Transverse section of the marginal portion of the thallus. G. Detail of broad-depressed hairlines (arrowheads) on the inferior thallus surface. Scale bars: 1 cm (A), 100 µm (B, C), 1 cm (D), 2 cm (E), 50 µm (F) and 2 mm (G).

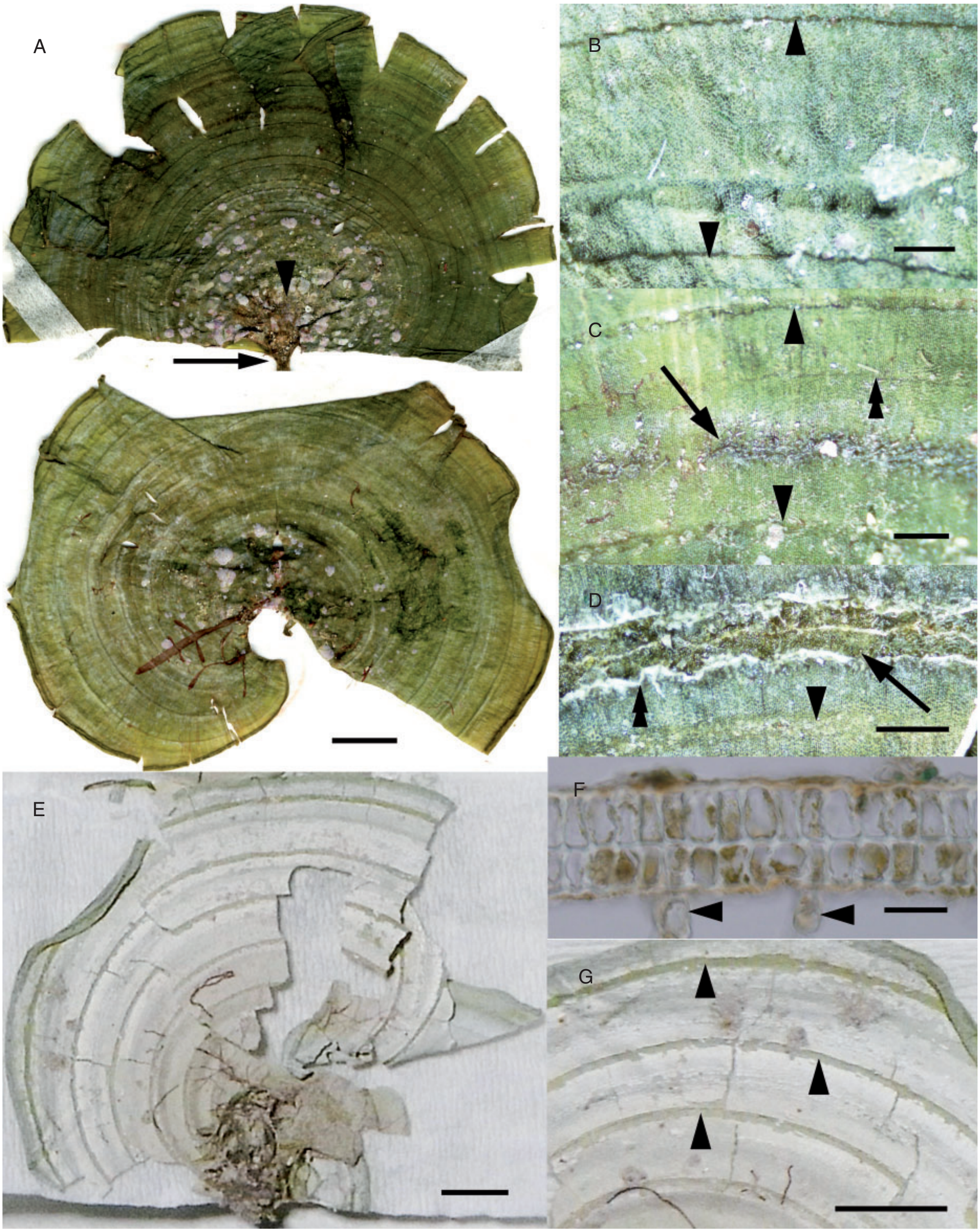


Fig. 4. Morphology of *Padina moffittiana* and *P. okinawaensis*. A–D. *Padina moffittiana*. A. Habit of tetrasporophytes, showing inferior (upper) and superior (lower) surfaces with fibrous hairs (arrowhead) at the base (arrow). B. Hairlines (arrowheads) on the superior thallus surface. C. Inferior thallus surface, showing relationship of the alternating hairlines (arrowheads on inferior surface; double arrowhead on superior surface) and immature tetrasporangial sori (arrow). D. Detail of mature tetrasporangial sori (arrow), with indusium (double arrowhead) located just above the hairline (arrowhead). E–G. *Padina okinawaensis*. E. Habit of sterile plant. F. Transverse section of near-basal portion of the thallus, with fibrous hairs (arrowheads) on the inferior surface. G. Detail of the inferior surface, with broad-depressed hairlines (arrowheads). Scale bars: 1 cm (A), 1 mm (B–D), 1 cm (E), 50 μm (F) and 5 mm (G).

sori are rather broad, forming broken lines or patches and arranged in concentric rows. Sori are located in the middle of fertile zones (~1–1.5 mm distal to the hairlines of the inferior surface) that are bordered by sterile zones on both sides (Fig. 4C). Oogonial and tetrasporangial sori are partially embedded in the epidermal layer and surrounded by a persistent indusium (Fig. 4D). Antheridial sori are non-indusiate and form brown patches or broken lines just distal to the hairlines of the inferior surface.

Padina okinawaensis Ni-Ni-Win, S.Arai, M.Uchimura & H.Kawai in Ni-Ni-Win *et al*. 2010

Fig. 4E–G

Type locality: Awase, Okinawa Island, Okinawa, Japan.

Geographical distribution: southern Japan (Ni-Ni-Win *et al*. 2010), Hawaii (Ni-Ni-Win *et al*. 2010), Thailand (Ni-Ni-Win *et al*. 2010), Indonesia (Ni-Ni-Win *et al*. 2010), Australia (the present study).

Habitat: same as for *P. calcarea*.

Specimens examined: AUSTRALIA. Ningaloo reef: (*C.F.D. Gurgel*, 8 June 2008, KU-d6935/AD-A87672*, KU-d6938/AD-A89507), (*C.F.D. Gurgel*, 10 June 2008, KU-d6944/AD-A89507), (*C.F.D. Gurgel*, 14 June 2008, KU-d6956/AD-A89914a, KUd6961–6965/AD-A89914f-j, KU-d6967–6975/AD-A89914m-s, -6978/AD-A89914x); Heron I.: (*C.F.D. Gurgel*, 3 Sep. 2008, KU-d7000/AD-A88468d, KU-d7011/AD-A88468o); Lizard I.: (*C.F.D. Gurgel*, 20 Feb. 2009, KU-d6843–6845AD-A94632b-d). JAPAN. Okinawa I.: Awase (*S. Arai*, 19 Nov. 2006, SAP106474–6), (*S. Arai*, 28 May 2007, SAP106480); Nakohi (*M. Uchimura*, 22 June 2007, SAP106488), Yagachikita (*M. Uchimura*, 22 June 2007, SAP106477); Kudakakita (*M. Uchimura*, 28 June 2007, SAP106489); Iriomote I.: Mokutan (*M. Uchimura*, 24 May 2007, SAP106478); Hinai (*M. Uchimura*, 25 May 2007, SAP106479); Amitori (*M. Uchimura*, 27 May 2007); Hoshidate (*M. Uchimura*, 27 May 2007); Shirahama (*M. Uchimura*, 28 May 2007); Hatoma I.: (*M. Uchimura*, 25 May 2007, SAP106481); Ishigaki I.: Akasaki (*M. Uchimura*, 24 May 2007, SAP106482); Akashioya (*M. Uchimura*, 30 May 2007, SAP106483); Bokajo (*M. Uchimura*, 30 May 2007); Mijun (*M. Uchimura*, 30 May 2007, SAP106484); Amparu (*M. Uchimura*, 31 May 2007, SAP106485); Sekisei (*M. Uchimura*, 31 May 2007, SAP106486); Mikara (*M. Uchimura*, 1 June 2007, SAP106487); Amami-Oshima I.: Ishi: (*M. Uchimura*, 27 July 2007); Akaogi (*M. Uchimura*, 28 July 2007, SAP106490); Ikomo (*M. Uchimura*, 30 July 2007, SAP106491). HAWAII. O'ahu I.: Kaawa Beach Park (*H. Kawai*, 11 June 2007). INDONESIA. Kepulauan Seribu: Lancang (*S.G.A. Draisma*, 12 Sep. 2005, L0609551); Kelapa (*S.G.A. Draisma*, 13 Sep. 2005, L0609514); Panjang (*S.G.A. Draisma*, 14 Sep. 2005, L0609515); Tidung Kecil (*S.G.A. Draisma*, 18 Sep. 2005, L0609539); Raja Ampat I.: Kri Island, Sorido resort Lagoon (*S.G.A. Draisma*, 19 Nov. 2007, L SGAD 0712009, 0712010); Desa Besir (*S.G.A. Draisma*, 2 Dec. 2007, L SGAD 0712397, 0712403, 0712434); Yenweres Bay (*S.G.A. Draisma*, 5 Dec. 2007, L SGAD 0712522). THAILAND. Trang Province: Libong I. (*A. Prathep*, 11 Sep. 2004). Note: for Australian samples, asterisks indicate that pressed voucher specimens are available in AD; other samples are represented only by materials preserved in silica gel.

Morphology

Thalli are erect, circular or semicircular, with entire margins, moderately to heavily calcified on the inferior surface, except at the hairlines, and heavily calcified on the superior surface, yellowish-brown or pale brown or whitish-brown (Fig. 4E), composed of two cell layers throughout (60–90 µm thick; Fig. 4F), and attached by a stupose base with a short stipe (Fig. 4E). Calcified glabrous zones and uncalcified hairlines are formed in alternate sequence on the inferior surface of the thallus, forming brown stripes or furrows on the whitish-brown calcified thallus surface (Fig. 4E, G). The superior thallus surface exhibits continuous calcification. Cells of the superior surface layer are slightly thicker than those of the inferior surface layer (Fig. 4F). *Vaughaniella* stages are found at the base of the specimens collected from Lizard Island. Concentric hairlines are present on both surfaces and placed in alternate sequence at equal distance between the two surfaces. Hairlines on the inferior surface are broad and depressed (Fig. 4E, G), whereas those on the superior surface are narrow, not depressed and sometimes inconspicuous or rudimentary.

Most of the specimens observed were sterile or without reproductive organs, although one specimen from Lizard Island displayed a mixture of antheridial sori and immature oogonial or tetrasporangial sori in a single row located just distally to the hairlines of the inferior surface. Determination of oogonia or tetrasporangia was difficult because of the early developmental stages observed.

Discussion

Molecular phylogenetic analyses using chloroplast *rbc*L and mitochondrial *cox*3 sequences, in combination with morphological observations, confirmed the presence of four newly recorded species of *Padina* in Australia (*P. calcarea*, *P. macrophylla*, *P. okinawaensis* and *P. moffittiana*); all but one of these have also been newly reported in the southern hemisphere. Three of the newly reported species (*P. calcarea*, *P. macrophylla*, *P. okinawaensis*) were recently described from the central Indo-Pacific and southern Japan (Ni-Ni-Win *et al*. 2010, 2011*a*, 2012), and one (*P. moffittiana*) was first described from Hawaii (Abbott and Huisman 2003, 2004).

Anatomically, Australian specimens of *P. calcarea*, *P. macrophylla*, *P. okinawaensis* and *P. moffittiana* agree well with the original descriptions and the accounts of Abbott and Huisman (2003, 2004) for *P. moffittiana* and those of Ni-Ni-Win and co-workers for *P. okinawaensis*, *P. macrophylla* and *P. calcarea* (Ni-Ni-Win *et al*. 2010, 2011*a*, 2012 respectively). In the case of *P. moffittiana*, however, Australian specimens were more similar to Japanese specimens than Hawaiian specimens in thallus appearance. For example, Japanese and Australian specimens had dark green or yellowish-green thalli, whereas Hawaiian specimens exhibit a reddish-brown or dark brown colour. In Japanese and Australian specimens, the thallus margins are always entire rather than fimbriate, as is commonly found in the Hawaiian specimens. Also, fibrous hairs at the base of the thallus are less pronounced in the Japanese and Australian populations than in the Hawaiian specimens in which long fibrous hairs thickly cover the base to 1–2 cm up the frond. In addition, Hawaiian specimens showed many small pores or tears on the thallus that were not found in the Australian and Japanese specimens. These morphological differences do not correlate with DNA sequence variation (Figs 1, 2) and, as previously suggested by Ni-Ni-Win *et al*.

(2008), it is likely that these features are influenced by the environmental conditions because Japanese and Australian specimens were both collected from the shallow habitats (~1–15-m depths) whereas Hawaiian specimens were collected from the deep-water habitats, ~30-m depth (Abbott and Huisman 2003, 2004; Ni-Ni-Win *et al*. 2008).

Although reproductive organs have not been observed in Australian specimens of *P. calcarea*, *P. okinawaensis* (except for one specimen from Lizard Island having a mixture of antheridial and oogonial or tetrasporangial sori) and *P. macrophylla*, the combination of several distinctive characters allowed the determination of each species. For example, the bright yellow colour of the inferior thallus surface, heavy calcification on the superior thallus surface where the calcium carbonate layer is as thick as the thallus, and the structure and position of the inconspicuous hairlines that are confined to the inferior thallus surface, were seen in all Australian specimens of *P. calcarea*. Similarly, in Australian *P. okinawaensis*, continuous calcification on both surfaces of the thallus except at the hairlines, resulting in an alternation of calcified glabrous zones and uncalcified hairlines on the inferior thallus surface (forming brown stripes on white calcified thalli), and the broad and depressed nature of the hairlines of the inferior thallus surface, distinguish this species from all other Australian *Padina* (see in Table 3 where comprehensive morphological comparisons of all Australian *Padina* species are described). Likewise, some distinguishing morphological characters of *P. macrophylla*, such as its less calcified thallus, and the structure and arrangement of inferior thallus-surface hairlines, which are broad and depressed and are separated by a large distance (~5–10 mm apart), separate the Australian specimens of *P. macrophylla* from all other *Padina* species found in Australia (Table 3). All molecular phylogenetic analyses using *rbc*L and *cox*3 gene sequences were consistent with morphology-based identifications of these four species using the above-mentioned characters, supporting their taxonomic utility.

Several recent molecular-based studies on the genus *Padina*, as well as on other algae from European, Mediterranean and Indo-West Pacific regions have reported the existence of many new and cryptic species among specimens previously attributed to a single species (De Clerck *et al*. 2005; Saunders and Lehmkuhl 2005; Brodie *et al*. 2007; Rodríguez-Prieto and De Clerck 2009; Tronholm *et al*. 2010; Coyer *et al*. 2011; Ni-Ni-Win *et al*. 2011*a*, 2011*b*; Payo *et al*. 2013). These studies also suggest that morphology-based taxonomy alone may have contributed to an erroneous taxonomy by attributing morphologically different specimens of one species to numerous species or by underestimating species diversity because of anatomical similarity, indicating the inadequacy of traditional morphological data alone for estimates of algal diversity and knowledge of species boundaries. Accordingly, we assume that a similar case might have occurred in Australian *Padina* taxonomy, underestimating species diversity because of morphological similarities.

Padina okinawaensis can be distinguished from all other members of the genus by its yellowish-brown to pale or whitish-brown thallus with continuous calcification on both surfaces except at the hairlines, forming calcified glabrous zones and uncalcified, broad and depressed hairlines (0.5–0.9 mm wide) in alternate sequence on the inferior thallus surface, and narrow and not depressed or sometimes inconspicuous hairlines on the superior surface. As in Ni-Ni-Win *et al*. (2010), most *P. okinawaensis* specimens from western and eastern Australian coasts lacked reproductive organs, except for one specimen from Ningaloo Reef that exhibited antheridia and oogonia or tetrasporangia (the identity of which was difficult to determine because of their very young stage) mixed in a single row. However, Ni-Ni-Win *et al*. (2010) reported dioecious gametophytes of this species on the basis of the materials collected from southern Japan, Indonesia, Hawaii and Thailand. A mixture of antheridia and mature tetrasporangia in a single concentric or broken row on the same thallus surface was also observed among the specimens of *P. japonica* collected from Japan (Ni-Ni-Win, pers. obs.), suggesting the possibility that a simultaneous existence of antheridial and tetrasporangial sori is more widespread in the genus than is observed. A careful examination of the identity of oogonia or tetrasporangia is critical in the genus *Padina*, so as to avoid misinterpretation of dioecy or monoecy, one useful character for species delimitation in the genus.

Padina melemele and *P. elegans*, the latter being originally reported from Western Australia, are morphologically very similar to one another as well as to the recently described central Indo-Pacific *P. calcarea* in thallus appearance, with all sharing orange or bright yellow inferior thallus surface and heavy calcification of the superior surface. It is, therefore, possible that Australian *P. calcarea* specimens have been erroneously assigned to *P. melemele* or *P. elegans* before. However, these three similar species can be distinguished from each other by the position and arrangement of hairlines and reproductive sori. Hairlines are found only on the inferior thallus surface in *P. calcarea* and *P. melemele*, whereas they are present on both surfaces in *P. elegans.* Although not always obvious, hairlines of the superior thallus surface in *P. elegans* can be seen as fissures on the continuous calcified thallus surface. Reproductive sori are found on the superior thallus surface in *P. melemele* and *P. elegans*, whereas they are found on the inferior surface in the type specimen of *P. calcarea* (no reproductive organs were found in Australian specimens). Sori form in more or less continuous lines and are situated distal to the hairlines or between the hairlines of the superior surface in *P. elegans* (the present study), but those in broken lines or patches are irregularly placed between the hairlines of the opposite surface (inferior surface) in *P. melemele* when the hairlines on both surfaces are viewed together (Abbott 1996; Abbott and Huisman 2003, 2004; Ni-Ni-Win *et al*. 2008, 2012). In *P. calcarea*, sori are located just distal to the hairlines (Ni-Ni-Win *et al*. 2012). Without careful examination of the disposition of hairlines and reproductive organs, species identification is often impossible and the three species might easily be confused or misidentified.

In the present study, extensive sample collections were made on the eastern (Lizard Island, Heron Island, Newcastle and Sydney) and western (Ningaloo Reef, Cottesloe and Rottnest Island) coasts of Australia. *P. moffittiana* was found in the Capricorn group (Heron Island) and Rottnest Island, whereas *P. okinawaensis* was found in Ningaloo Reef, Lizard Island and Heron Island, showing their wide distribution range in

Table 3. Comparison of morphological features of Australian *Padina* species, including the four species that are newly recorded here

All alternate fertile zones are separated by a sterile zone when both surfaces are viewed together. For successive zones, the sterile zone was absent. IF, inferior surface; SP, superior surface

Character	*P. calcarea*	*P. macrophylla*	*P. moffittiana*	*P. okinawaensis*	*P. australis*	*P. boergesenii*	*P. boryana*	*P. condominium*	*P. elegans*	*P. fraseri*	*P. gymnospora*	*P. melemele*	*P. sanctae-crucis*
Vegetative characters													
Thallus													
Shape	Semicircular or circular	Semicircular or circular	Flabelliform or circular	Semicircular or circular	Flabelliform	Flabelliform	Flabelliform	Flabelliform	Flabelliform	Flabelliform	Flabelliform	Flabelliform	Flabelliform
Colour (IF surface; SP surface)	Orange or bright yellow; white	Pale or yellowish-brown; pale or whitish-brown	Yellowish-green or yellowish-brown; yellowish-green or yellowish-brown	Yellowish-brown or pale brown; whitish-brown	Yellowish-brown or pale brown; pale brown	Light brown to tan; pale brown	Pale or medium brown; whitish-brown or pale brown	–	Bright yellow to yellowish-brown; brownish-white	Medium to dark olive brown; pale brown	Yellowish-green or greenish-brown or dark brown; pale brown or whitish brown	Golden brown to bright yellow-orange; brilliant white	Medium brown; whitish-brown
Calcification on IF; SP surfaces	No or light; heavy	Light; moderate	Light; light to moderate	Moderate to heavy; heavy	Light to moderate; moderate to heavy	Moderate; moderate	Light; moderate	–	No or light; heavy	Light; light to moderate	Light; light to moderate	No; heavy	Light; moderate
Number of cell layers													
Marginal portion (inrolled margin)	2 (2)	2 (2)	2 (2)	2 (2)	2 (2)	3 (2)	2 (2)	2 (2)	2 (2)	3 (2)	4 (2)	2 (2)	2 (2)
Middle portion	2	2	2	2	2	Mostly 3, sometimes 2 layers where second transverse divisions fail to take place	2	2	2	3	4–6	2	2
Basal portion	2	2	2	2	2	3	2 or 3	2	2	3	6–8	2	2
Other characters													
Thickness of cell layers	Both cell layers in same height	Cells of superior layer 1.2 times taller than those of inferior layer	Cells of superior layer 1.2 to 1.5 times taller than those of inferior layer	Cells of inferior layer slightly shorter than those of superior layer	Cells of inferior layer slightly shorter than those of superior layer	Cells of inferior layer taller than those of superior layer and central cell layer shorter than the surface layers	Cells of inferior layer slightly shorter than those of superior layer	Cells of inferior layer smaller and shorter than those of superior layer	Both cell layers in similar size at the basal portion; cells of superior layer 1.2–1.4 times taller than those of inferior layer in middle and marginal portions	Cells of inferior layer shortest; central cell layer tallest	All cell layers in same height	Cells of superior layer shorter than those of inferior layer	Cells of superior layer 1.2 to ~1.8 times taller than those of inferior layer
'*Vaughaniella*' *stage*	Absent	Absent	Absent	Present	Absent	Present	Present	–	Absent	Absent	Absent	Present (in Hawaiian samples (Abbott and Huisman 2004), but unknown in Australian samples (Kraft 2009)	Present

(*continued next page*)

Table 3. (*continued*)

Character	*P. calcarea*	*P. macrophylla*	*P. moffittiana*	*P. okinawaensis*	*P. australis*	*P. boergesenii*	*P. boryana*	*P. condominium*	*P. elegans*	*P. fraseri*	*P. gymnospora*	*P. melemele*	*P. sanctae-crucis*
Hairlines													
Position	Only on IF surface	Alternating on both surfaces	Alternating on both surfaces	Alternating on both surfaces	Alternating on both surfaces	Alternating on both surfaces	Only on IF surface (Franscesca Thivy in Taylor 1966; Allender and Kraft 1983, as *P. tenuis*; Ni-Ni-Win *et al*. 2011*a*)	Alternating on both surfaces	alternating on both surfaces	Alternating on both surfaces	On both surfaces	Only on IF surface	Alternating on both surfaces
Hairlines (IF surface; SP surface)	Inconspicuous; –	Conspicuous; inconspicuous	Conspicuous; conspicuous	Conspicuous; inconspicuous	Conspicuous; conspicuous	Conspicuous; conspicuous	Inconspicuous; – (Ni-Ni-Win, pers. observ.)	Conspicuous; conspicuous	Conspicuous; conspicuous	Conspicuous; conspicuous	Conspicuous; conspicuous	Inconspicuous; –	Conspicuous; moderate
Structures (IF surface; SP surface)	Narrow–undepressed; –	Broad–depressed; narrow–undepressed	Slightly broad–depressed; narrow–depressed	Broad–depressed; narrow–undepressed	Narrow–undepressed (sometimes slightly broad and depressed); narrow–undepressed)	Narrow–undepressed; narrow–undepressed	Narrow (Ni-Ni-Win, pers. obs.)	Narrow; narrow	Narrow–undepressed; narrow, slightly depressed	Narrow, slightly depressed; narrow–undepressed	Narrow–undepressed; narrow	Narrow–undepressed; –	Slightly broad and depressed; narrow–undepressed
Arrangement of alternating hairlines between both surfaces	–	Unequal distance	Unequal distance	Equal distance	Equal distance	Equal distance	–	Equal distance	Equal distance	Equal distance	Equal distance	–	Equal distance
Reproductive structures													
Reproductive system	Dioecious (Ni-Ni-Win *et al*. 2012) but no reproductive organs found in Australian specimens	Dioecious (Ni-Ni-Win *et al*. 2011*a*), but no reproductive organs in Australian specimens	Dioecious	Dioecious in the specimens of Japan, Hawaii, Indonesia and Thailand (Ni-Ni-Win *et al*. 2010), but a mixture of antheridia and young oogonia or tetrasporangia found in Australian specimen	Monoecious	Dioecious	Dioecious	Monoecious	Sexual plant unknown	Dioecious	Dioecious	Dioecious	Dioecious (Gaillard 1975, Ni-Ni-Win *et al*. 2010), but sexual plants unknown on Australian coasts (Womersley 1987)
Sporangial sori													
Position (surface)	On IF in Type specimen (Ni-Ni-Win *et al*. 2012)	On both surfaces but mainly on IF	On IF	On IF	On IF	On IF	On IF by Fransesca Thivy in Taylor 1966 and Ni-Ni-Win *et al*. (2011*a*)	Only on IF	Only on SP	Only on SP	On both surfaces but mainly on IF	Only on SP	Only on IF
Structure	Narrow	Broad	Broad	Narrow	Narrow	Narrow	Narrow (Fransesca Thivy in Taylor 1966; Ni-Ni-Win, pers. obs.)	–	Broad	Broad	Moderate	Broad	Narrow to moderate

Table 3. (*continued*)

Character	*P. calcarea*	*P. macrophylla*	*P. moffittiana*	*P. okinawaensis*	*P. australis*	*P. boergesenii*	*P. boryana*	*P. condominium*	*P. elegans*	*P. fraseri*	*P. gymnospora*	*P. melemele*	*P. sanctae-crucis*
Arrangement	In continuous line; just above the hairlines	Mainly in continuous line; nearly in the middle of two alternating hairlines when the hairlines on both surfaces are viewed together	In continuous or broken lines or patches; between two alternating hairlines when the hairlines on both surfaces are viewed together	Broken lines or small group; just above the hairlines	Tetrasporangial sori in concentric, continuous line; oogonial and antheridial sorus mixing at the same row; located just above the hairlines of the IF (i.e. in the middle of two alternating hairlines when the hairlines on both surfaces are viewed together)	In continuous lines; above the hairlines	In concentric, continuous line (Fransesca Thivy in Taylor 1966; Ni-Ni-Win pers. obs.),	Oogonial and antheridial sori mixing at the same row; located just above the hairlines of the IF (i.e. in the middle of two alternating hairlines when the hairlines on both surfaces are viewed together)	In concentric or broken lines; below the hairlines of the SP (i.e. in the middle of two alternating hairlines when the hairlines on both surfaces are viewed together)	In continuous or broken lines; between the hairlines	In concentric lines; above or between the hairline	In discontinuous lines or patches; between the hairlines of the opposite surface	In concentric, continuous or broken lines; above the hairline
Number in row between hairlines	1	1 or 2	1–3	1	1	1	1 (Ni-Ni-Win pers. obs.)	–	1	1–3	1 or 2	1–3	1
Location	On thallus surface	On thallus surface	Partially immersed in the cuticle layer	Likely to emerge from the calcified layer	On thallus surface	On thallus surface	On thallus surface (Ni-Ni-Win, pers. obs.)	On thallus surface	Partially immersed in the cuticle layer	Partially immersed in the cuticle layer	On thallus surface	Partially immersed in the cuticle layer	On thallus surface
Fertile zone	Successive	Alternate	Alternate	Alternate	Alternate	Alternate	Successive	Alternate	Alternate	Successive	Successive	Successive	Alternate
Indusium	Present[A]	Present[A]	Present[A]	Present[A] (indusium-like calcium layer)	Present (oogonial sori) but absent (antheridial sorus and tetrasporophyte)	Absent	Absent (Fransesca Thivy in Taylor 1966; Ni-Ni-Win, pers. obs.)	Present (both oogonia and antheridia); tetrasporangia unknown	Present[A]	Present[A]	Present[A]	Present[A]	Present[A]
References	Ni-Ni-Win *et al*. (2012); herein (Note: description for reproductive organs based on Type specimen reported by Ni-Ni-Win *et al*. 2012)	Ni-Ni-Win *et al*. 2011*a*); herein	Abbott and Huisman 2003, 2004; Ni-Ni-Win *et al*. 2008; herein	Ni-Ni-Win *et al*. 2010; herein	Ni-Ni-Win *et al*. 2011*a*; herein	Allender and Kraft 1983; Kraft 2009; here in	Taylor 1966; Ni-Ni-Win *et al*. 2011*a*; Ni-Ni-Win, pers. obs.	Kraft 2009	Womersley 1987; herein	Womersley 1987; herein	Womersley 1987; Kraft 2009; herein	Kraft 2009	Womersley 1987; Ni-Ni-Win *et al*. 2010

[A]Indusium present in female gametophyte and tetrasporophyte but absent in male gametophyte.

both eastern and western Australia. However, *P. calcarea* was found only around Lizard Island and Heron Island on the Great Barrier Reef and *P. macrophylla* was observed only around Heron Island. *P. elegans* and *P. gymnospora* appear to be widely distributed along Australian coasts because they were found in almost all collection areas. We extend the known distribution of *P. elegans* to the eastern coast, with new records from Heron Island and Newcastle from the previously recorded locales in Western Australia and South Australia by Womersley (1987). In the present study, *P. fraseri* and *P. boergesenii* were confirmed as present only in New South Wales and around Rottnest Island respectively, although broader Australian distributions are likely to be based on morphological identifications from areas not surveyed in the present study (Womersley 1987). Following the present study, 13 species of *Padina* should be recognised in Australia instead of nine, this genus being composed of species with wide geographic distributions across the eastern Indian Ocean and warm-temperate to tropical regions of the Pacific Ocean, most likely including the entire coral triangle.

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