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A reassessment of the taxonomic status of *Porphyra suborbiculata*, *Porphyra carolinensis* and *Porphyra lilliputiana* (Bangiales, Rhodophyta) based on molecular and morphological data

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We examined three species of diminutive *Porphyra*, *Porphyra suborbiculata* Kjellman from the North Pacific, *Porphyra lilliputiana* W. A. Nelson, G. A. Knight *et* M. W. Hawkes from the South Pacific, and *Porphyra carolinensis* Coll *et* J. Cox from the western North Atlantic. These taxa were compared in terms of morphology, habitat data and sequence haplotypes of nuclear small subunit rDNA (SSU) and internal transcribed spacers of the nuclear rDNA cistron (ITS). These three species have similar morphologies and growth habits, and share very similar type descriptions and habitat records. Haplotype variation was found within the 11 samples of *P. lilliputiana* we examined and within *P. suborbiculata* samples from two locations, but the single *P. carolinensis* haplotype (from collections from two separate locations) was identical to one found in several widespread *P. lilliputiana* samples. Unrooted phylogenetic trees based on sequence data do not support any of the three species as being a monophyletic group. We conclude that these three taxa represent a single species with the oldest name *P. suborbiculata* having nomenclatural priority. It is likely that *P. suborbiculata* has recently been introduced to the western Atlantic from the Pacific region.

Key words: 18S rDNA, Bangiaceae, Group I intron, ITS, New Zealand, *Porphyra carolinensis*, *P. lilliputiana*, *P. suborbiculata*, red algae, Rhodophyta, nuclear SSU, systematics

Introduction

The intertidal red algal genus *Porphyra* C. Agardh is found on rocky shores from polar to tropical seas (Bold & Wynne, 1978). Although the genus itself is cosmopolitan, individual species are not so widespread. Current understanding of the genus suggests that most species have distributions that are regionally confined (Yoshida, 1997; Guiry & Nic Dhonncha, 2001). For instance, there are very few species common both to Japan and to the Pacific coast of North America, two areas where the genus is particularly well studied. To our knowledge *Porphyra suborbiculata* Kjellman is the only species reported to be common to both hemispheres and more than one ocean basin, having been recorded from the North Pacific, South Pacific and Indian Oceans (Kjellman, 1897; Silva *et al.*, 1987; Silva *et al.*, 1996). *P. spiralis* var. *amplifolia* Oliveira Filho *et* Coll has been reported to occur in both the northern and southern hemispheres, extending from the United States into the southern Atlantic in Brazil (Oliveira & Coll, 1975; Kapraun & Lemus, 1987). Although *P. leucosticta* Thuret in Le Jolis has been reported from a number of areas (Le Jolis, 1863; Coll & Oliveira, 1976), recent critical examination of this species (J. Brodie & L. M. Irvine, pers. comm.) strongly suggests that this species is restricted to the northern Atlantic and the Mediterranean Sea.

Species identification in *Porphyra* is hampered by the simple morphology of members of this genus

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and a concomitant relative paucity of characters for species recognition. Nevertheless, Yoshida *et al.* (1997) list more than 130 species of *Porphyra*. Recent studies applying molecular techniques to the problem of species identification in *Porphyra* (Stiller & Waaland, 1993; Oliveira *et al.*, 1995; Kunimoto *et al.*, 1999*a*; Broom *et al.*, 1999) have revealed considerable interspecies diversity of the nuclear small subunit ribosomal DNA (SSU) gene within this genus and suggest that nuclear SSU sequences provide useful characters for species identification.

As part of an ongoing study of the New Zealand *Porphyra* flora (Broom *et al.*, 1999), we recently sequenced the nuclear SSU gene of *Porphyra lilliputiana* W. A. Nelson, G. A. Knight *et* M. W. Hawkes, a New Zealand endemic species described in 1998 (Nelson *et al.*, 1998). We noted that the exon portion of the nuclear SSU sequence from this species was identical to that attributed to the Japanese species *Porphyra suborbiculata* by Kunimoto *et al.* (1999*b*). Also present in GenBank was a partial nuclear SSU sequence (AF133792, Klein *et al.*, 1999) attributed to *Porphyra carolinensis* Coll *et* J. Cox, a western Atlantic species described from the eastern seaboard of the United States in 1977 (Coll & Cox, 1977). This sequence included a Group I intron as well as a portion of the nuclear SSU exon, and was identical to sequence data we had obtained from *Porphyra lilliputiana*. This, combined with the very similar type descriptions of these three species, led us to speculate that these three entities might be conspecific.

The internal transcribed spacer (ITS) region of the nuclear rDNA cistron is a variable region that has been widely used in intrageneric studies in angiosperms (Baldwin, 1992), Chlorophyta (Famá *et al.*, 2000) and also in members of Rhodophyta (van Oppen *et al.*, 1995, Vis & Sheath, 1997, Kunimoto *et al.*, 1999*a*). ITS sequences are under considerably less sequence constraint than are the nuclear SSU exons, and accumulate sequence changes much more rapidly. Kunimoto *et al.* (1999*a*) used the ITS1 region to discriminate among populations of *Porphyra yezoensis*, sampling both cultured strains and wild populations, and between *P. yezoensis* and the related species *P. tenera*. Group I introns present in the nuclear SSU rDNA of many *Porphyra* species, including *P. suborbiculata*, *P. carolinensis* and *P. lilliputiana*, also show significant interspecific and intraspecific sequence variation (Oliveira & Ragan, 1994; Kunimoto *et al.*, 1999*a*). In this study we compare nuclear SSU, Group I intron and ITS sequences of *P. suborbiculata*, *P. carolinensis* and *P. lilliputiana* in order to determine whether these three entities are in fact three separate species, or whether they represent widely distributed populations of a single cosmopolitan *Porphyra* species.

Methods

Samples for morphological assessment and molecular analysis

Specimens examined in this study are listed in Table 1, which gives the haplotype nomenclature, details of voucher specimens and the accession numbers of sequences lodged in GenBank. Herbarium abbreviations are according to Holmgren *et al.* (1990). Eleven samples identified as *P. lilliputiana* were collected from Australia and New Zealand, including one from the type locality of this species, Frank Kitts Park, Wellington. Three diminutive north-eastern Pacific *Porphyra* samples collected from Baja California, Mexico, were included in the analysis. Two samples of *P. carolinensis* were examined, one from the type locality of this species at Fort Macon, North Carolina and another from Waterford, Connecticut, near the mouth of Long Island Sound. The locations of all collections are shown in Fig. 1.

Three sequences attributed to *P. suborbiculata* were included in this study. Sequences of the *P. suborbiculata* nuclear SSU rDNA, including the 516 intron (AB013180), and ITS1 (AB017089) were deposited in GenBank by Kunimoto *et al.* (Kunimoto *et al.*, 1999*a*,*b*). To simplify analysis we have assumed these sequences are from a single individual. A further *P. suborbiculata* sample collected at Weihai, Shandong Peninsula, China was sequenced by us. Our sequence data were also compared with a partial nuclear SSU sequence (AF133792.2) which includes the 516 intron, deposited in GenBank by Klein *et al.* (1999).

The type collection of *P. suborbiculata* was examined, and its morphology compared with the three samples of diminutive *Porphyra* from Mexico, *P. lilliputiana* and *P. carolinensis*. A summary of the characters used in the descriptions of *Porphyra carolinensis*, *P. lilliputiana*, and *P. suborbiculata* is presented in Table 2.

DNA extraction, amplification and sequencing

DNA was extracted according to Goff & Moon (1993). PCR amplification and sequencing of the nuclear SSU rDNA region were performed as in Broom *et al.* (1999). The ITS region was amplified using primers TW81 and AB28 (Steane *et al.*, 1991) and sequenced using these primers and primer ITS2 (White *et al.*, 1990). For some samples primer TW81 was replaced by primer JBITS7 (GTAGGTGAACCTGCGGAAGG) which consistently gave better amplification. Some samples were also amplified using primer pair G02 and ITS2 (Saunders & Kraft, 1994; White *et al.*, 1990) to give a product spanning the nuclear SSU/ITS1 boundary. Amplification products were purified by PEG precipitation and sequenced on an ABI 377 automated sequencer according to standard methods.

Sequence alignment and phylogenetic analysis

Sequences were aligned using HOMED (Stockwell & Petersen, 1987). Two Group I introns were present in the nuclear SSU sequences of some samples. The positions of these introns are given with respect to the *Escherichia coli*

Table 1. Locations and collection information of samples used in this study

Location	Collection date	Collector	Voucher no/ herbarium number	Identification	GenBank accession no.	Haplotype
New Zealand						
Frank Kitts Park, Wellington	2 Jul 1997	G. A. Knight	WELT A22855	*P. lilliputiana*	AF136424 (nuclear SSU rDNA), AF378649	NZFKitts
Palmer Head, Wellington	1 Mar 2000	W. A. Nelson	WELT A22980	*P. lilliputiana*	AF378665	NZ/Aus/USA
Mount Maunganui	9 Aug 1998	W. A. Nelson	WELT A22978	*P. lilliputiana*	AF378659	NZMtM/Mu
Muriwai Beach	4 Apr 2000	G. C. Williams	WELT A22979	*P. lilliputiana*	AF378658	NZMtM/Mu
Okurei Point, Maketu	9 Aug 1998	W. A. Nelson & G. A. Knight	WELT A22853	*P. lilliputiana*	AF378650	NZMaketu
Opunake	6 Sep 1998	G. A. Knight	WELT A22852	*P. lilliputiana*	AF378664	NZ/Aus/USA
Kaupokonui	6 Sep 1998	W. A. Nelson & G. A. Knight	WELT A22854	*P. lilliputiana*	AF378663	NZ/Aus/USA
Punakaiki	12 Nov 2000	W. A. Nelson & T. J. Farr	WELT A23002	*P. lilliputiana*	AF378651	NZPunakaiki
Australia						
Gosford, NSW	28 Aug 1996	G. A. Knight	WELT A22987	*P. lilliputiana*	AF378660	NZ/Aus/USA
Crescent Head, NSW	15 Dec 1998	L. E. Phillips	WELT A22981	*P. lilliputiana*	AF378662	NZ/Aus/USA
Trigg Bay, Western Australia	17 Apr 1999	G. A. Knight	WELT A22984	*P. lilliputiana*	AF378661	NZ/Aus/USA
USA						
Fort Macon, NC	25 Nov 2000	D. W. Freshwater	WELT A23000	*P. carolinensis*	AF378654	NZ/Aus/USA
Millstone Point, Waterford, CT	2 Nov 1999	B. Kerin	WELT A22971	*P. carolinensis*	AF378653	NZ/Aus/USA
Mexico						
Punta Popotla, Baja California	24 Feb 1999	R. Aguilar Rosas & L. E. Aguilar Rosas	WELT A22973	–	AF378655	Mexico
Bajamar, Baja California	1 Mar 1999	R. Aguilar Rosas	WELT A22976	–	AF378656	Mexico
Villa de Las Rosas, Baja California	21 Apr 1999	R. Aguilar Rosas	WELT A22977	–	AF378657	Mexico
Japan						
Goto Islands	Jul 1881	J. Petersen	UPS-type collection	*P. suborbiculata*	–	–
Goto Islands	Jul 1881	J. Petersen	L-type collection	*P. suborbiculata*	–	–
Kawatana Yamaguchi* (nuclear SSU rDNA)	–		–	*P. suborbiculata*	AB013180	PsubJapan
Kawatana Yamaguchi* (ITS1)	–		–	*P. suborbiculata*	AB017089	PsubJapan
China						
Weihai, Shandong Peninsula	12 Jan 2001	J. X. Mei	WELT A22999	*P. suborbiculata*	AF378652	PsubWeihai

*Sequenced by Kunimoto *et al*., 1999.

Table 2. Original descriptions of the three species of *Porphyra* studied

	P. carolinensis	*P. lilliputiana*	*P. suborbiculata*
Size and shape	2·5 × 4 cm; oval-lanceolate, irregular	0·5–2·0 (3·5) cm; ovate to cordate to reniform	1–2 (3) cm; round to kidney-shaped, becoming rolled into a cornet shape
Thallus	monostromatic; marginal microscopic teeth	monostromatic; margin microscopically dentate	monostromatic; margin has distinct dentation
Colour	brownish red to pale violaceous	pink to bronze	violet with a tinge of dirty yellow
Habitat	upper littoral fringe on other red algae, barnacles, mussels and rocks	upper to mid intertidal zone on rock, algae, limpets and barnacles	attached to barnacles, molluscs, and on other algae
Seasonality	year round although primarily in spring and summer	autumn to spring	summer
Reproduction	monoecious; archeospores; 32 spermatangia in 4 tiers; 16 zygotosporangia in 2 tiers	monoecious; reproductively mature at a very small blade size; archeospores; spermatangia 2 × 2(4) × 4; zygotosporangia 2 × 2 × 2;	monoecious; impossible to find in solely vegetative state; many spermatia per spermatangium; 8 spores per zygotosporangium
Type locality	North Atlantic – USA, North Carolina, Fort Macon	South Pacific – New Zealand, Wellington	North Pacific – Japan, Goto Islands
Source	Coll & Cox, 1977	Nelson *et al.*, 1998	Kjellman, 1887

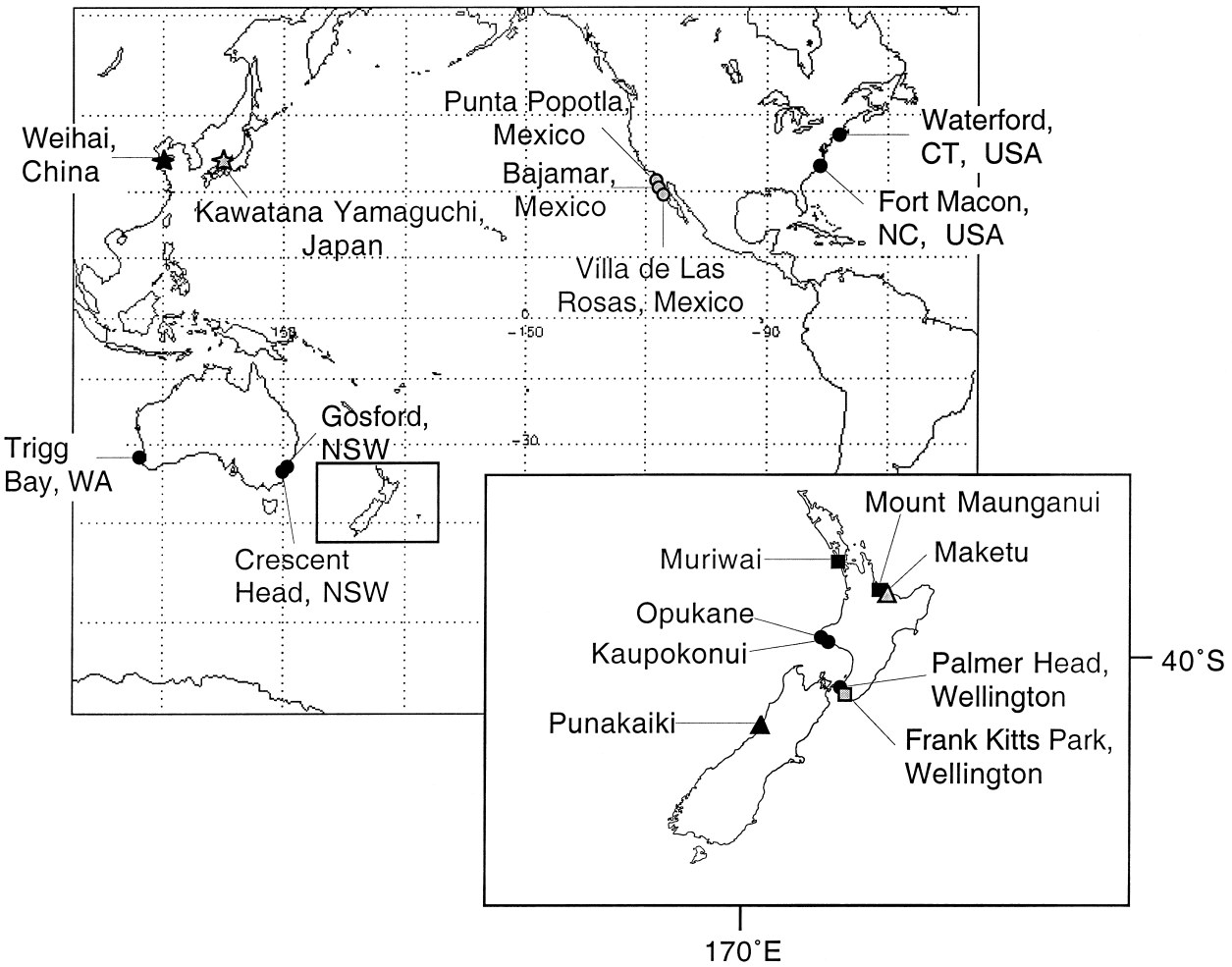

Fig. 1. Locations of samples used in this study. Each haplotype is represented by a unique symbol as follows: NZ/Aus/USA ●; NZFKitts ▣; NZMtM/Mu ■; Mexico ◎; PsubJapan ☆; NZMaketu △; NZPunakaiki ▲; PsubWeihai ★.

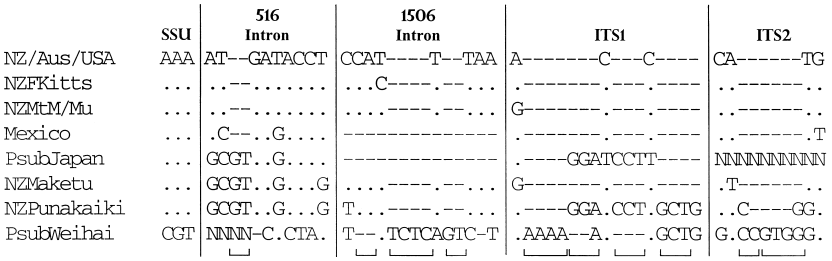

Fig. 2. Condensed haplotypes of *P. suborbiculata*, *P. carolinensis* and *P. lilliputiana* samples from the dataset. Haplotype names are as in Table 1; the reference haplotype is NZ/Aus/USA. Dots represent nucleotides which are identical to the reference sequence; a dash indicates a gap. The unalignable portion of the PsubWeihai 516 intron has been replaced by Ns. Brackets indicate nucleotides that are inserted or deleted consecutively, and so probably represent a single mutation event. Note that the 1506 intron is absent from both the Mexico and the PsubJapan sequences.

gene (516 and 1506) according to the usual convention. Bases in the 516 intron of PsubWeihai which could not be confidently aligned were scored as missing (Hershkovitz & Leipe, 1998). In order to maximize the number of informative sites under parsimony analyses, gaps of more than one nucleotide were recoded, scoring one of the gap positions a gap and the remainder as missing (Hershkovitz & Leipe, 1998). Three insertions in ITS1 and ITS2 were shared in part by haplotypes PsubWeihai and NZPunakaiki (Fig. 2). This recoding scheme would therefore tend to shorten the branch leading to PsubWeihai, as the gap characters, which distinguish these two taxa, were recoded to unknown bases. The absence of the 1506 intron in samples from Mexico and from Japan was indicated in our alignment by one gap character followed by unknown base characters, so that the phylogenetic information of the presence/absence polymorphism was retained.

Phylogenetic analysis of the dataset was performed using PAUP*4.04b8 (Swofford, 2001). In order to avoid the possibility of confounding two independent phylogenies, trees were constructed from 516 intron sequences and ITS sequences separately, as well as from the entire dataset as a single matrix. Since the 1506 intron contributes only two parsimony informative characters (Fig. 2), construction of a phylogenetic tree from this intron was not performed. Maximum parsimony trees were obtained by an exhaustive search considering gaps as a fifth base. Bootstrap support was assessed (1000 replicates) to check that support for any particular branch was not excessively localized in the phylogenetic matrix. For the maximum likelihood analysis, an exhaustive search was performed using parameters (R matrix, proportion of invariant sites and gamma shape parameter) estimated from the single most parsimonious tree. Bootstrap support was assessed (100 replicates) by a heuristic search using stepwise addition (10 random-order replicates) followed by TBR branch swapping. Trees constructed with PsubJapan and PsubWeihai constrained to be monophyletic were compared to unconstrained trees using the Kishino-Hasegawa test in PAUP*4.04b8.

Attempts to identify appropriate outgroups for these analyses were thwarted by the large amounts of sequence variation between the sequences obtained from our study samples and all available ITS sequences from *Porphyra*, which rendered possible outgroup sequences unalignable over virtually all informative regions of the phylogenetic matrix. For this reason, phylogenetic analyses are presented as unrooted trees.

Results

Morphology

All fresh samples examined consisted of diminutive blades with microscopically dentate thallus margins. The samples were all collected from the upper to mid intertidal region and were found to be growing epiphytically, epilithically and epizoically on molluscs or barnacles. The comparison of original descriptions of the three species (Table 2) – *Porphyra carolinensis*, *P. lilliputiana*, and *P. suborbiculata* – emphasizes the similarities among these species in terms of size, shape, thallus margin, early maturity and arrangement of reproductive cells.

Porphyra suborbiculata was described from material collected in Goto, Japan, in July 1881 by J. Petersen, and predates the description of both *Porphyra carolinensis* and *P. lilliputiana*. The name *P. suborbiculata* has been applied to material collected from various parts of Asia. Although it appears that the species concept used by different authors for this taxon has varied (Masuda *et al.*, 1991), all refer to this species being monostromatic, monoecious, with a microscopically dentate margin, and small, rounded to funnelled blades (Miyata & Kikuchi, 1997). Kjellman (1897) included a photograph of the dentation of the thallus margin when describing this species.

Examination of material from the type collection of *Porphyra suborbiculata* held at UPS and at L (Holmgren *et al.*, 1990) and communication with herbarium staff at UPS revealed that a type specimen had not been selected by Kjellman and thus that this species requires lectotypification.

Sequence data

For each sample, sequence data totalling up to 4369 bp were obtained, extending from the 5′ end of the nuclear SSU through the ITS1, 5.8S rDNA and ITS2 regions to the beginning of the large subunit

rDNA gene. All samples contained a Group I intron inserted at the SSU position equivalent to base 516 in *Escherichia coli*. A second Group I intron was inserted at *E. coli* equivalent position 1506 in all samples except that from Japan and the three from Mexico.

Sequence variation and haplotypes

The complete dataset included five haplotypes unique to a single individual – those of *P. suborbiculata* from Japan (PsubJapan), *P. suborbiculata* from China (PsubWeihai), *P. lilliputiana* from Maketu, NZ (NZMaketu), *P. lilliputiana* from Punakaiki, NZ (NZPunakaiki), and *P. lilliputiana* from Frank Kitts Park, Wellington, NZ (NZFKitts) – and three haplotypes common to more than one individual (Table 1 and Fig. 1). Sequences obtained from the three samples from Baja California, Mexico, were identical in all respects. Two samples from Mount Maunganui and from Muriwai Beach, both located in the northern part of the North Island of New Zealand, also had an identical haplotype. Interestingly, sequences obtained from three New Zealand samples (Palmer Head, Opunaki and Kaupokonui) and from all three Australian samples were absolutely identical to the two complete and one partial *P. carolinensis* sequences, despite the wide geographic distribution of these individuals. A single representative sequence from each of these three haplotypes (Mexico, NZMtM/Mu and NZ/Aus/USA) was used in subsequent analyses. NZFKitts haplotype differed from the NZ/Aus/USA haplotype by only a single nucleotide substitution within the 1506 intron. The NZMtM/Mu haplotype also differed from the NZ/Aus/USA haplotype by a single nucleotide substitution, in the ITS1 region. For convenience, we designate the NZ/Aus/USA haplotype as the reference sequence for the present analysis. Fig. 2 presents the haplotypes as a condensed alignment, with all invariant characters removed. Each section of the alignment is discussed in turn below.

Exon sequences of the nuclear SSU and 5.8S rDNA genes were identical in all samples with the exception of PsubWeihai, which differed from the others by one nucleotide substitution in helix 8 and two in helix E10-1 of the nuclear SSU. All three substitutions fall within variable region V2 of the nuclear SSU (Neefs *et al*., 1993).

PsubWeihai contained a unique 615 bp insertion beginning 6 bp from the start of the 516 intron, replacing bases 7-139 of the reference intron sequence (NZ/Aus/USA). This insertion contains a 576 bp open reading frame (ORF). Size polymorphism of Group I introns within a single species has previously been reported for *P. spiralis* (Oliveira & Ragan, 1994). The gain and loss of ORFs within Group I introns has been reported previously, and is a source of genetic variation within these mobile genetic elements (reviewed in Gimble, 2000). The PsubWeihai 516 intron sequence also contains four single nucleotide substitutions in regions that are alignable to the reference sequence. Of the remaining samples, sequence data from the 516 intron showed little variation, with only four point substitutions and a single 2 bp insertion over a total of 492 bp (Fig. 2). The 2 bp insertion was common to haplotypes PsubJapan, NZPunakaiki and NZMaketu. These three sequences also shared a unique A to G transversion at position 13 of the 516 intron.

Over the 1037 bp ITS1-5.8S-ITS2 region, PsubWeihai contained six small (1–4 bp) insertions, 5 of which were shared at least in part with NZPunakaiki. Other sequence variation was limited to seven single nucleotide substitutions, five of which are unique to a single haplotype and therefore phylogenetically uninformative, and a 3 bp insertion shared by PsubJapan and NZPunakaiki. No ITS2 sequence data were available for *P. suborbiculata* from Japan.

Trees

The final alignment contained 4415 characters, of which 41 were variable, and 18 were parsimony-informative. The single most parsimonious tree calculated over the entire dataset (length = 43, CI = 0·884) is presented in Fig. 3. All branches received moderate to strong bootstrap support. Trees constructed using the ITS region alone grouped PsubJapan, PsubWeihai and NZPunakaiki together with even higher bootstrap support (97%), while trees based on the 516 intron alone grouped PsubWeihai with the NZ/Aus/USA, NZFKitts and NZMtM/Mu clade (bootstrap support 73%). Under the topological constraint of monophyly of PsubWeihai and PsubJapan, a single most parsimonious tree of length 45 was found over the entire dataset. This tree was not significantly less likely than the unconstrained maximum parsimony tree under a Kishino-Hasegawa test (P = 0·1573); however all 15 trees within 2 steps of the MP tree supported monophyly of the (Weihai, Japan, Punakaiki) clade. Over the ITS region alone, the constrained tree was again not significantly worse than the unconstrained tree (P = 0·1574), but all 19 trees within 2 steps of the maximum parsimony tree supported the (Weihai, Japan, Punakaiki) clade as a monophyletic group.

Under maximum likelihood, gaps must be considered as unknown bases, which significantly reduces the phylogenetic information in the data matrix, much of which depends on shared small insertions or deletions. An exhaustive search under the optimality criterion of maximum likelihood

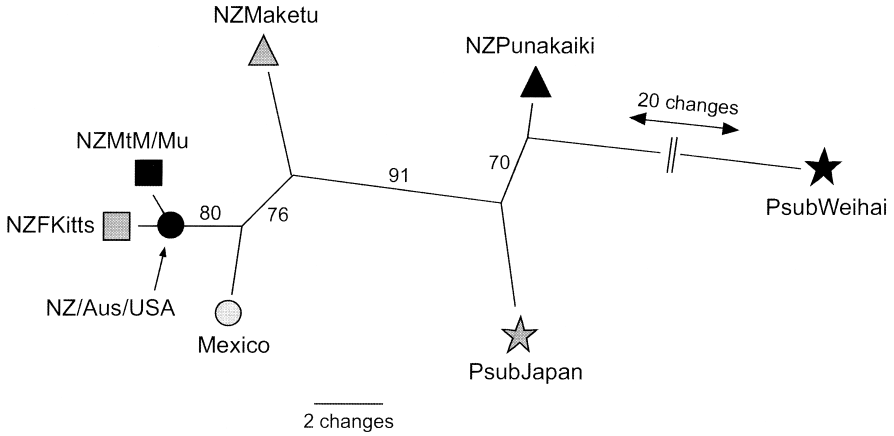

Fig. 3. The single most parsimonious tree derived from the entire dataset. Percentage MP bootstrap support (1000 replicates) is shown for each branch. Haplotype names are as in Table 1, symbols correspond to those from Fig. 1.

over the whole dataset generates a tree grouping PsubWeihai with 3 NZ haplotypes (NZ/Aus/USA, NZFKitts and NZMtM/Mu) while an analysis over the ITS region alone produces a tree grouping PsubWeihai, PsubJapan and NZPunakaiki (bootstrap support 66 % over 100 replicates). Neither tree therefore supports monophyly of *P. suborbiculata* with respect to *P. lilliputiana* and *P. carolinensis*.

Discussion

The type descriptions of *Porphyra suborbiculata*, *P. carolinensis* and *P. lilliputiana* are all remarkably similar. The examination of fresh and herbarium material including the type of *P. suborbiculata* has reinforced this conclusion. These three taxa were all established as being distinctly different from other local *Porphyra* species by the authors of the original papers (Kjellman, 1897; Coll & Cox, 1977; Nelson *et al*., 1997).

The molecular dataset was remarkable for the very high level of similarity between all samples. A single haplotype was common to samples of *P. carolinensis* from Connecticut and North Carolina and to samples of *P. lilliputiana* from the east and west coasts of Australia and from the North Island of New Zealand. The conspecificity of *P. carolinensis* and *P. lilliputiana* seems indisputable. In contrast, within *P. lilliputiana* from New Zealand, five distinct haplotypes were found, and the two *P. suborbiculata* sequences from Asia both had unique haplotypes.

Our sequence data include the ITS region and also two Group I introns inserted in the nuclear SSU. How much variation is present at these loci within currently well accepted *Porphyra* species? The 1999 study of *Porphyra yezoensis* by Kunimoto *et al*. (Kunimoto *et al*., 1999b) provides an excellent benchmark for comparison. Kunimoto *et al*. obtained sequence data from twelve samples of *P. yezoensis*, both wild collected and cultured strains, and from samples of the closely related Japanese taxon *P. tenera*. Over the 346 bp ITS1 region, intraspecific sequence similarity between samples of *P. yezoensis* was 96–100 %. Both point substitutions and small insertions/deletions (indels) were present in the dataset. Sequence similarities between samples of *P. yezoensis* and the closely related species *P. tenera* over the ITS1 region were considerably lower, at 88–90 %. Raw sequence similarity among samples of *P. suborbiculata*, *P. carolinensis* and *P. lilliputiana* at ITS1 was 94·6–100 % (over 279 bp). The lowest similarity score of 94·6 % was between the two *P. suborbiculata* haplotypes from Yamaguchi, Japan and Weihai, China (PsubWeihai and PsubJapan). Similarity scores between the *P. suborbiculata* haplotypes and all *P. lilliputiana*/*P. carolinensis* ITS1 haplotypes were 96·0 % or greater. These ITS1 similarity scores support the recognition of all these samples as conspecific.

Kunimoto *et al*. (1999*b*) also examined sequence variation of the 1506 rDNA intron for samples of *P. yezoensis* and *P. tenera*. Here, they observed more sequence variation within *P. yezoensis* than is present in our dataset. The *P. yezoensis* samples showed presence/absence polymorphism for this intron, as has been observed in other *Porphyra* species (Oliveira & Ragan, 1994). Intron sequence within *P. yezoensis* was variable, with up to eight substitutions and 16 gaps distinguishing samples of *P. yezoensis*, which were all collected from locations within Japan. This contrasts with the four indels of 1–4 bp (a total of nine gaps) and five nucleotide substitutions in our dataset. We also observed presence/absence polymorphism in our dataset: this intron was not present in PsubJapan, nor in any of the three Mexican samples.

The structure observed in the phylogenetic tree clearly does not support the notion that *P. suborbiculata* and *P. carolinensis*/*P. lilliputiana* are genetically distinct species. The *P. suborbiculata* sequences are not supported as a monophyletic group

over the dataset as a whole, or over either the 516 intron or the ITS region alone. Instead, they are grouped with significant bootstrap support with either the *P. lilliputiana* haplotype NZMaketu (516 intron) or with the *P. lilliputiana* haplotype NZPunakaiki (ITS1 and ITS2). Although a tree constraining the *P. suborbiculata* sequences to monophyly was not significantly less likely than the most likely trees by the Kishino-Hasegawa test, this is likely to reflect the relatively few informative sites in the dataset, which is itself a probable indication of relatively recent gene flow between the populations. The relationship between the haplotypes PsubWeihai, PsubJapan and NZPunakaiki over the ITS region is particularly convincing because it rests on shared insertions and deletions, which are unlikely to represent independent mutations.

We conclude that neither morphological descriptions nor molecular data support the retention of *P. carolinensis*, *P. lilliputiana* and *P. suborbiculata* as separate taxa. As *P. suborbiculata* is the oldest name, it has priority and *P. carolinensis* and *P. lilliputiana* become synonyms of *P. suborbiculata*.

Synonymy: *Porphyra suborbiculata* Kjellman 1887: 11; *Porphyra carolinensis* Coll *et* J. Cox 1977: 155–156; *Porphyra lilliputiana* W. A. Nelson, G. A. Knight *et* M. W. Hawkes 1998: 57–58.

Lectotype: UPS. Type material of *P. suborbiculata* is housed at both UPS and L. The lectotype is a sheet with 16 pressed thalli. Duplicate sheets and packets of unmounted thalli are also housed at UPS.

This species is reported to have a widespread distribution, and is recorded from Japan (Yoshida *et al*., 1990), China (Tseng, 1984), Sri Lanka (Silva *et al*., 1996), both the west and east coasts of Australia, New Zealand, Mexico, and the Atlantic coast of North America from Waterford, Connecticut to Florida (Freshwater & Kapraun, 1986). Such a cosmopolitan distribution has not previously been reported for a species within *Porphyra*, members of which are generally presumed to have a localized distribution. This is the first confirmed record of a *Porphyra* species being present in both the Atlantic and Pacific Oceans and also in both the Northern and Southern hemispheres.

The haplotype variation found in samples from New Zealand, Mexico, Japan and China suggests a longstanding distribution of this species in the Pacific. This is consistent with reports of *P. suborbiculata* from the Philippines (Silva *et al*., 1987). This natural distribution may extend into the Indian Ocean (Silva *et al*., 1996). Both Maketu and Punakaiki, the two locations within New Zealand from which the two most divergent Australasian haplotypes were collected, are relatively unmodified habitats which are not adjacent to any major ports. The locations within New Zealand from which the NZ/Aus/USA haplotype has been recovered are associated with substantial shipping activity. Wellington is a major port, while the Taranaki region (Opunake and Kaupokonui) has been subject to a great deal of offshore oil and gas prospecting in recent years, with concomitant movement of vessels in the area.

The identical haplotypes found in samples from the Western Atlantic and the Western Pacific suggests very recent extensive dispersal of this entity. We suggest that *P. suborbiculata* is particularly well suited to distribution by shipping. *P. suborbiculata* is able to tolerate warm water temperatures (up to 30 °C, Freshwater & Kapraun, 1986) which would enable it to withstand passages across the equator. This is also supported by its presence in the Philippines. Although to our knowledge this species has not been reported as a fouling organism on vessels, its small size would make it easy to overlook. *P. suborbiculata* is known to grow readily on a variety of substrata including barnacles, which are often present as fouling organisms on the hulls of seagoing vessels. It is easy to imagine that these very small thalli might remain within the boundary layer around a ship's hull and thus experience minimal shear forces such as would tend to disrupt larger *Porphyra* thalli. *P. suborbiculata* is also known to reproduce freely via copious production of archeospores (Freshwater & Kapraun, 1986; Nelson *et al*., 1998; Notoya, 1999) providing a means of rapid clonal reproduction in a new location. If vessels are in fact an important vector in the distribution of this taxon, then it is also likely to be present in Europe, on the Eastern Atlantic coast and in the Mediterranean Sea. We suggest that careful observation of the upper intertidal reaches of warm water shorelines may reveal its presence in these regions, now or in the near future.

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