

Making the links: towards a global taxonomy for the red algal genus *Porphyra* (Bangiales, Rhodophyta)

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Abstract Species assigned to *Porphyra* sensu lato (Bangiales) make up one of the largest groups of red algae in the world. While the Bangiales are monophyletic, *Porphyra* is not, but the number of genera remains unresolved and a consensus needs to be reached on how to proceed. Here, it is proposed that a global taxonomy for the group would enable resolution and consensus. Using examples from our work on *Porphyra* in both the northern and southern hemispheres, including the North Atlantic, Mediterranean and Pacific, notably Chile, we have addressed the following questions: i) how many species are there, ii) how many species are there in different parts of the world, and iii) how do these species relate to those in different parts of the world? A cumulative total of species described in the North Atlantic and Mediterranean since 1800 indicates that there are still species to be added to the flora for these regions. Determination of intraspecific diversity or diversity within species complexes was examined using the mitochondrial *cox1*. The results split the species into two distinct groups, revealed differences between the British and Faroese floras and indicated a new species in the Faroes. Detection of species diversity in different geographical areas of the

world and their relationship with the North Atlantic and southeast Pacific (Chile) was studied using the plastid Rubisco spacer and partial rDNA SSU data. While the Rubisco spacer was limited for global comparisons, it was useful for species identification adding five more species to the Atlantic flora. The partial rDNA SSU data, while its shortness made it unsuitable for generic interpretation, revealed the spread of taxa from different geographical areas throughout the tree. We have concluded that to achieve a global taxonomy standard molecular markers should be used and we propose that the *rbcL* and complete rDNA SSU would be suitable. We have also concluded that while a few regions of the world have been well studied, before a consensus on generic boundaries can be achieved detailed taxonomic studies are needed in many other parts of the world including circumpolar Arctic, mid and southwest Atlantic and southeast Asia.

Keywords Chile · *cox1* · Mediterranean · North Atlantic · *Porphyra* · rDNA SSU · Rhodophyta · Rubisco spacer · Taxonomy

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Introduction

The genus *Porphyra* sensu lato (*Porphyra* and *Bangia*) is one of the largest genera of red algae (Brodie and Zuccarello 2007), and although the exact number of species is unknown, there are almost certainly well over 150. While the Bangiales is monophyletic (Saunders and Hommersand 2004; Milstein and de Oliveira 2005), the genus is polyphyletic (Oliveira et al. 1995; Müller et al. 1998; Nelson et al. 2006) although the exact number of genera is currently unresolved. Five genera are currently recognised (*Porphyra*, *Bangia*, *Dione*, *Pseudobangia*, *Minerva*) although

Nelson et al. (2006) speculated, based on the analysis of the 18S rDNA for 127 samples, that there would be at least ten genera. Species occur in the intertidal and shallow subtidal throughout the world. Although they are particularly abundant in boreal and cold temperate waters, they are also found in the Arctic and Antarctic and warm temperate and tropical regions (Kapraun and Lemus 1987), with evidence that some species in the tropics occur during the rainy season (Sahoo et al. 2006; Fujiyoshi et al. 2007). Fossil evidence based on diagnostic cell division suggests that bangiacean red algae occurred at least 1.2 billion years ago, with *Bangiomorpha pubescens* Butterfield representing the oldest taxonomically resolved eukaryote (Butterfield 2000). The remarkable diversity of life histories exhibited by the species (e.g. Notoya 1997; Brodie and Irvine 2003) and an array of life history strategies (Holmes and Brodie 2004) may reflect this long evolutionary history. Species of *Porphyra* are economically important, and have traditionally been eaten in many parts of the world, notably as nori in Japan, laver in Wales and luche in Chile. This has also led to cultural differences in different parts of the world in the way that the species are prepared and consumed, ranging from subsistence foods to haute cuisine. The success of the nori industry, owes much to the discovery by Kathleen Drew of the *Porphyra* life cycle (Drew 1949, 1954) where the blade phase, which is conspicuous on the shore, produces spores which grow into shells to produce a shell-boring filamentous or conchocelis phase which is sometimes visible as pink staining on shells. The diversity of species, global spread and antiquity of the genus suggest a remarkably successful group of organisms with the ability of both the blade and conchocelis phases to survive environmental changes over millions of years.

Species of *Porphyra* are often notoriously difficult to identify, so despite at least 250 years of study, the complete number of species remains unknown. However, major advances in recent years with the application of molecular techniques, the ability to use these methods on type or historic material plus the sharing of knowledge from different parts of the world, means that the goal of achieving a complete taxonomy for *Porphyra* is timely and a real possibility.

This paper focuses on the species of *Porphyra* in the North Atlantic and their possible relationships with other parts of the world. *Porphyra* is very common in the North Atlantic and while there is evidence of speciation in the region (Robba et al. 2006), and at least one species, *P. dioica*, appears to be endemic to the northeastern part, the genus almost certainly originally evolved in the Pacific, and it has also been proposed that the origin for the modern day Bangiales is centred upon New Zealand (Broom et al. 2004). Given the apparent antiquity of the genus, there are different routes and different times by which species could

potentially have arrived since the formation of the North Atlantic, e.g. via the Tethys Sea, the Panama Isthmus, the Arctic Seaways, or from the South Atlantic (see Brodie and Nielsen 2005).

To achieve a global taxonomy, we need to resolve the following questions:

1. How many species are there?
2. How many species are there in different parts of the world?
3. How do these species relate to those in different parts of the world?
4. How many genera are there?

The aim of this paper is to use examples, notably from the North Atlantic but also from the Pacific coast of Chile to put forward a possible approach to achieve a global taxonomy, concentrating on highlighting some of the major problems which require resolution.

Materials and methods

Cumulative total of species in the North Atlantic and Mediterranean

In order to predict whether there are still species to be discovered in the North Atlantic and Mediterranean, a cumulative total of species was derived by adding up the number of species described before 1800, and within 50 year time intervals since that time until 2000 and then after that date. Species were only included if there was enough evidence that they were considered distinct.

Molecular analysis

Sequence data were analysed for mitochondrial (*cox1*), plastid (Rubisco spacer), and nuclear (partial rDNA SSU) regions. The *cox1* sequence data were used to compare differences within species in Great Britain and the Faroes. Rubisco spacer sequences were mainly generated from specimens collected in the North Atlantic and were used for species identification primarily in this region. The partial rDNA SSU was used to compare specimens from the North Atlantic with those from the Pacific, including Japan, New Zealand, Australia and South Africa, based on the phylogenetic tree for the Bangiales (see Nelson et al. 2006) and from our collections from Chile. In addition to our own data, sequences were selected based on the genera and clades recognised by Nelson et al. (2006).

Specimens used for molecular analysis are given in Table 1. DNA extraction, PCR amplification and sequencing for *cox1* and Rubisco spacer sequences generated in this study followed methods given in Robba et al. (2006).

Table 1 Taxa from which sequence data were obtained with their location and GenBank accession numbers

Taxa	Code	Location	GenBank accession no.			Reference
			<i>rbcL-rbcS</i> spacer	partial rDNA SSU	<i>cox1</i>	
<i>Bangia atropurpurea</i>	NH	Atlantic US	-	AF043353	-	Müller et al. (1998)
<i>B. atropurpurea</i>	CA-At	Atlantic Canada	-	AF043353	-	Müller et al. (1998)
<i>B. fuscopurpurea</i>	JB72	Wales	AJ010785	-	-	Brodie et al. (1998)
<i>B. fuscopurpurea</i>	IR	Ireland	-	AF175534	-	Müller et al. (2005)
<i>B. fuscopurpurea</i>	JB212	England	-	-	DQ442886	Robba et al. (2006)
<i>B. fuscopurpurea</i>	JB238	England	-	-	DQ442886	Robba et al. (2006)
<i>B. fuscopurpurea</i>	225	England	-	-	DQ442887	Robba et al. (2006)
<i>Bangia</i> sp.	MA	Atlantic US	-	AB013179	-	Müller et al. (2005)
<i>Bangia</i> sp.	BOHNZ	New Zealand	-	AY909600	-	Nelson and Broom (2005)
<i>Bangia</i> sp.	BMWNZ	New Zealand	-	AY184344	-	Broom et al. (2004)
' <i>Dione arcuata</i> '	NZ	New Zealand	-	AY184338	-	Broom et al. (2004)
<i>Minerva aenigmata</i>	NZ	New Zealand	-	AY184347	-	Broom et al. (2004)
<i>P. amplissima</i>	JB91	Scotland	AJ010780	-	-	Brodie et al. (1998)
<i>P. 'amplissima'</i>	CA-At	Atlantic Canada	-	L36048	-	Oliveira et al. (1995)
<i>P. birdiae</i>	10A	Faroese	AY458657	-	-	Brodie and Nielsen (2005)
<i>P. cinnamomea</i>	NZ	New Zealand	-	AH008010	-	Broom et al. (1999)
<i>P. coleana</i>	NZ	New Zealand	-	AY136423	-	Broom et al. (1999) (as PAP52)
<i>P. columbina?</i>	CH7	Chile	-	AM943385	-	This paper
<i>P. cuneiformis</i>	CH3	Chile	-	AM943386	-	This paper
<i>P. dentata</i>	JP	Japan	-	AB013183	-	Kunimoto et al. (1999)
<i>P. dioica</i>	P163	Scotland	AJ010779	-	-	Brodie et al. (1998)
<i>P. dioica</i>	JB261	England	-	AM943387	-	This paper
<i>P. dioica</i>	JB347	England	-	-	DQ191340	Robba et al. (2006)
<i>P. dioica</i>	JB137	Wales	-	-	DQ442888	Robba et al. (2006)
<i>P. dioica</i>	JB246	Scotland	-	-	DQ442889	Robba et al. (2006)
<i>P. dioica</i>	JB314	Scotland	-	-	DQ442889	Robba et al. (2006)
<i>P. drachii</i>	Pdr	Wales	AJ010788	-	-	Brodie et al. (1998)
<i>P. gardneri</i>	MX	Mexico	-	DQ084423	-	Nelson et al. (2006)
<i>P. haitanensis</i>	JP	Japan	-	AB013181	-	Kunimoto et al. (1999)
<i>P. katadae</i>	JP	Japan	-	AB013184	-	Kunimoto et al. (1999)
<i>P. kunthiana?</i>	CH49	Chile	AM943010	AM943388	-	This paper
<i>P. kunthiana?</i>	CH54	Chile	-	AM943389	-	This paper
<i>P. lanceolata</i>	MX	Mexico	-	AY909594	-	Nelson and Broom (2005)
<i>P. 'leucosticta'</i>	JB402	England	AM943011	AM943390	-	This paper
<i>P. leucosticta</i>	Pgu	Channel Islands	AJ010789	-	-	Brodie et al. (1998)
<i>P. leucosticta</i>	AF175557	Atlantic Canada	-	AF175557	-	Müller et al. (2001a)
<i>P. leucosticta</i>	GE	Helgoland	-	AF342746	-	Müller et al. (2001b)
<i>P. leucosticta</i>	Ca-At	Atlantic Canada	-	L26199	-	Ragan et al. (1994)
<i>P. leucosticta</i>	AMM06SF1240	Faroese	-	-	AM943398	This paper
<i>P. leucosticta</i>	JB322	England	-	-	DQ191338	Robba et al. (2006)
<i>P. leucosticta</i>	JBLR21	England	-	-	DQ191337	Robba et al. (2006)
<i>P. leucosticta</i>	JB340	England	-	-	DQ442890	Robba et al. (2006)
<i>P. linearis</i>	125	Wales	AJ010781	AM943391	-	Brodie et al. (1998); this paper
<i>P. lucasii</i>	AU-ln	Australia	-	AY139685	-	Farr et al. (2003)
<i>P. miniata</i>	Psam	Faroese	AJ010786	-	-	Brodie et al. (1998)
<i>P. miniata</i>	CA-At	Atlantic Canada	-	AF175540	-	Müller et al. (2005)
<i>P. olivii</i>	TES23	Greece	DQ834623	-	-	Brodie et al. (2007)
<i>P. olivii</i>	TES19	Greece	-	DQ834632	-	This paper
<i>P. perforata</i>	MX	Mexico	-	AY909592	-	Nelson and Broom (2005)
<i>P. pseudolinearis</i>	P2	Japan	AJ010787	-	-	Brodie et al. (1998)
<i>P. pseudolinearis</i>	JP	Japan	-	AB013185	-	Kunimoto et al. (1999)
<i>P. purpurea</i>	P127	England	AJ010776	-	-	Brodie et al. (1998)
<i>P. purpurea</i>	JB257	England	-	AM943392	-	This paper

Table 1 (continued)

Taxa	Code	Location	GenBank accession no.			Reference
			<i>rbcL-rbcS</i> spacer	partial rDNA SSU	<i>cox1</i>	
<i>P. purpurea</i>	CA-At	Atlantic Canada	-	L26201	-	Ragan et al. (1994)
<i>P. purpurea</i>	(GenBank)		-	-	NC0020007	GenBank
<i>P. rosenfurtii</i>	MAL24	Spain	DQ834627	-	-	Brodie et al. (2007)
<i>P. rosenfurtii</i>	MAL26	Spain	-	DQ834638	-	This paper
<i>P. rosenfurtii</i>	AMM06SF1308	Faroes	-	-	AM943399	This paper
<i>P. rosenfurtii</i>	JB286	England	-	-	DQ191335	Robba et al. (2006)
<i>P. rakiura</i>	NZ	New Zealand	-	AF136425	-	Broom et al. (1999) (as RAK49)
<i>P. spiralis</i>	BR	Brazil	-	L26177	-	Ragan et al. (1994)
<i>P. suborbiculata</i>	NZ	New Zealand	-	AF136424	-	Broom et al. (1999)
<i>P. thulaea</i> Holotype	PthH	Greenland	AM943012	-	-	This paper
<i>P. thulaea</i>	564557	Iceland	AM943013* ¹	AM943393	-	This paper
<i>P. umbilicalis</i>	P126	England	AJ010782	-	-	Brodie et al. (1998)
<i>P. umbilicalis</i>	JB258	England	-	AM943394	-	This paper
<i>P. umbilicalis</i>	AMM06SF1246	Faroes	-	-	AM943400	This paper
<i>P. umbilicalis</i>	AMM06SF1144	Faroes	-	-	AM943401	This paper
<i>P. umbilicalis</i> * ²	JB178	Scotland	-	-	DQ442891	Robba et al. (2006)
<i>P. umbilicalis</i>	JB329	Northern Ireland	-	-	DQ191334	Robba et al. (2006)
<i>P. umbilicalis</i>	AMM06SF1351	Faroes	-	-	AM943402	This paper
<i>P. umbilicalis</i>	JB342	England	-	-	DQ191336	Robba et al. (2006)
<i>P. umbilicalis</i>	JB334	England	-	-	DQ191333	Robba et al. (2006)
<i>P. umbilicalis</i>	JB324	England	-	-	DQ442892	Robba et al. (2006)
<i>P. umbilicalis</i>	JB357	England	-	-	DQ191332	Robba et al. (2006)
<i>P. umbilicalis</i>	JB352	England	-	-	DQ191332	Robba et al. (2006)
<i>P. umbilicalis</i>	AMM06SF4241	Faroes	-	-	AM943403	This paper
<i>P. virididentata</i>	NZ	New Zealand	-	AF136421	-	Broom et al. (1999)
<i>P. yezoensis</i>	Pye	Japan	AJ010783	-	-	Brodie et al. (1998)
<i>P. yezoensis</i>	JP	Japan	-	AB013177	-	Kunimoto et al. (1999)
<i>P. yezoensis</i> sensu Kormmann	Pmu	Helgoland	AJ010778	-	-	Brodie et al. (1998)
<i>Porphyra</i> sp.	PO2	Faroes	AJ634466	-	-	Brodie and Nielsen (2005)
<i>Porphyra</i> sp.	AZ23	Azores	AM943014	-	-	This paper
<i>Porphyra</i> sp.	RNO2	Denmark	AM943016	-	-	This paper
<i>Porphyra</i> sp.	AZ21	Azores	AM943015	-	-	This paper
<i>Porphyra</i> sp.	JB403	England	-	AM943395	-	This paper
<i>Porphyra</i> sp.	SYJP	Japan	-	AB013182	-	Kunimoto et al. (1999)
<i>Porphyra</i> sp.	CH5	Chile	-	AM943396	-	This paper
<i>Porphyra</i> sp.	CH4	Chile	-	AM943397	-	This paper
<i>Porphyra</i> sp.	ZGR SA	South Africa	-	AY292631	-	Jones et al. (2004)
<i>Porphyra</i> sp.	ZEKSA	South Africa	-	AY292630	-	Jones et al. (2004)
<i>Porphyra</i> sp.	GRB368	New Zealand	-	AF136420	-	Broom et al. (1999)
<i>Porphyra</i> sp.	CHLCH	Chile	-	AY913952	-	Nelson and Broom (2005)
<i>Porphyra</i> sp.	Wal	Wales	-	AF175554	-	Müller et al. (2005)
<i>Porphyra</i> sp.	AMM06SF2347	Faroes	-	-	AM943404	This paper
<i>Pseudobangia</i> <i>kaycoleia</i>	-	Virgin Islands	-	AF043364	-	Müller et al. (1998)
<i>Chondrus crispus</i>	-	England	-	-	DQ191341	Robba et al. (2006)
<i>Corallina officinalis</i>	-	England	-	-	DQ191342	Robba et al. (2006)
<i>Erythrocladia</i> sp.	-		-	L26188	-	Ragan et al. (1994)
<i>Erythrotrichia carnea</i>	-		-	L26189	-	Ragan et al. (1994)
<i>Gracilaria gracilis</i>	-	England	-	-	DQ191347	Robba et al. (2006)
<i>Cyanidium caldarium</i>	(GenBank)		-	-	Z48930	GenBank

*¹ sequence not used in analysis*² proposed neotype of *Porphyra umbilicalis* (Brodie et al. in press)

Sequence data obtained for the Xs region of rDNA SSU followed the methods given in Broom et al. (1999) as described in Brodie et al. (2007). All other sequences used in this paper were obtained from GenBank.

Data analysis

Sequence data obtained were assembled in Lasergene (DNA*Star Inc., 2005) and aligned with BioEdit Sequence Alignment Editor v6.0.5 (Hall 2004), with a final alignment by eye. GenBank sequences (Table 1) were then included in the analysis. The sequences were analysed using PAUP* 4.0 (Swofford 2002). Thirty taxa of 533 bp for *cox1*, 22 taxa consisting of 99 bp (22 bp of the 3' end of *rbcL* and the spacer which is 77 bp) were used for the Rubisco spacer analysis and 54 taxa of 501 base pairs (bp) for the rDNA SSU analysis. No exclusions were made within the sequences. Parsimony analysis was undertaken using a full heuristic search with tree bisection reconnection branch-swapping (TBR), MULTrees option on, collapse branching on if maximum length equalled zero for 1000 random-additional sequence replicates. For the *cox1* data, which generated 1 tree, a phylogram was generated, rooted against the outgroups *Chondrus crispus*, *Corallina officinalis*, *Gracilaria gracilis* and *Cyanidium caldarium* and bootstrap analysis undertaken using 20 repetitions with 1000 additional repetitions. For the Rubisco spacer data, where parsimony analysis generated 62 trees, an unrooted strict consensus tree was produced. Bootstrap analysis was then undertaken for clade support using 100 repetitions with 1000 additional repetitions. For partial rDNA SSU parsimony analysis generated 200 trees. The strict consensus tree was rooted against the outgroups *Erythrocladia* sp. and *Erythrotrichia carnea*. Bootstrap analysis undertaken for clade support used 10 repetitions with 1000 additional repetitions. AS-IS maximum likelihood analysis was completed for the three datasets using default settings for the HKY85 model. On completion of the analysis, bootstrap analysis was undertaken for 100 repetitions with 1000 additional repetitions for both the *cox1* and Rubisco datasets and 10 repetitions with 1000 additional repetitions for the rDNA SSU. Bayesian analysis was run in MrBayes v3.1.2. The model parameters (1set + prset) were selected using MrModelTest2.2 (Nylander 2005).

Results and discussion

Determination of species diversity: Example: North Atlantic and Mediterranean species

The results of the number of species reported from the North Atlantic and Mediterranean (Table 2) show that the

first half of the 18th century and the second half of the 20th century were periods of time when more species were described than at others. The evidence from the first seven years of the 21st century also suggests that there are still more species to be added to the list. In addition to those listed in Table 2, we know that there are other species that will be added to the list (Neefus 2007; Brodie, pers. obs.) However, not all these species will necessarily be new to science. *Porphyra carolinensis* is a synonym of *P. suborbiculata* Kjellman, a cosmopolitan species (Milstein and de Oliveira 2005) and is possibly an introduced species in the North Atlantic (Brodie et al. 2007). *Porphyra yezoensis* which was being grown by an aquaculture company in Maine, USA as a source of nori (Levine 1998), escaped and was confirmed on the New Hampshire coast by West et al. (2005). It is probable that further species will be introduced by direct anthropogenic activities but with the warming of the Arctic waters, we might predict that more species will find their way into the North Atlantic and some species will possibly move in the opposite direction. Some species listed may represent species complexes, notably *P. leucosticta*, a name which has been misapplied to a number of different species in the North Atlantic (Neefus 2007).

Determination of intraspecific diversity or diversity within species-complexes: Example: *cox1* as a tool to resolve intraspecific diversity in Great Britain and Faroes specimens

The *cox1* data (Fig. 1) are useful for species identification whilst suggesting differences within species. As previously reported (Robba et al. 2006), variation within species is between 0 and 4 bp. While the number of species included and the geographical range is more restricted in the dataset than for the other two molecular markers used in this study, the results provide an excellent preliminary comparison between Great Britain and the Faroes which have similar *Porphyra* floras but with southerly and northerly elements respectively (see Brodie and Nielsen 2005). Here, *Porphyra* sensu lato is split into two groupings separated by strong bootstrap support. One of these contains species which have been considered as the 'Atlantic Group' (Brodie et al. 1998): *P. dioica*, *P. purpurea*, *P. umbilicalis* and *Bangia fuscopurpurea*. *Porphyra dioica* and *P. umbilicalis* both show slight variation within the species and there is a suggestion of geographical variation. In both species, there are northerly and southerly groupings, but whether this will hold true with the inclusion of more samples remains to be tested. The sample of *P. umbilicalis* from Scotland which groups with specimens from Northern Ireland and the Faroes has been selected as the neotype for the species (Brodie et al. in press). It is well established that *Porphyra*

Table 2 Cumulative total with time for species described or reported in the North Atlantic and Mediterranean

Species	Time period					
	<1800	1800–1849	1850–1899	1900–1949	1950–1999	>2000
<i>P. atropurpurea</i> (Olivi) De Toni	+					
<i>P. purpurea</i> (Roth) C. Agardh		+				
<i>P. linearis</i> Greville		+				
<i>P. umbilicalis</i> (Linnaeus) Kützing		+				
<i>P. miniata</i> (C. Agardh) C. Agardh		+				
<i>P. leucosticta</i> Thuret in Le Jolis			+			
<i>P. amplissima</i> (Kjellman) Setchell & Hus ex Hus				+		
<i>P. suborbiculata</i> (as <i>carolinensis</i>) J. Coll & J. Cox					+	
<i>P. rosenfurtii</i> J. Coll & J. Cox					+	
<i>P. drachii</i> J. Feldmann					+	
<i>P. thulaea</i> I.M. Munda & P.M. Pedersen					+	
<i>P. yezoensis</i> sensu Kornmann					+	
<i>P. dioica</i> Brodie & L. Irvine					+	
<i>P. yezoensis</i> Ueda						+
<i>P. birdiae</i> Neefus & Mathieson						+
<i>P. olivii</i> Orfanidis, Neefus & Bray						+
Total	1	4	1	1	6	3
Cumulative total	1	5	6	7	13	16

and *Bangia* are not separate taxonomic entities (Müller et al. 2005) and the diversity observed in *Bangia* was already noted in Robba et al. (2006). The other group contains the ‘*P. leucosticta*’ complex (Brodie et al. 2007), including *P. rosenfurtii* which is recognised for the first time in the Faroes flora. The data also reveal an unidentified species which groups with the *P. leucosticta* complex. That there were almost certainly unidentified species in the Faroese flora was predicted by Brodie and Nielsen (2005), who found several species that they were unable to name based on morphological observations. The presence of *P. rosenfurtii* in the Faroese flora extends the known distribution of this species in the North Atlantic and Mediterranean.

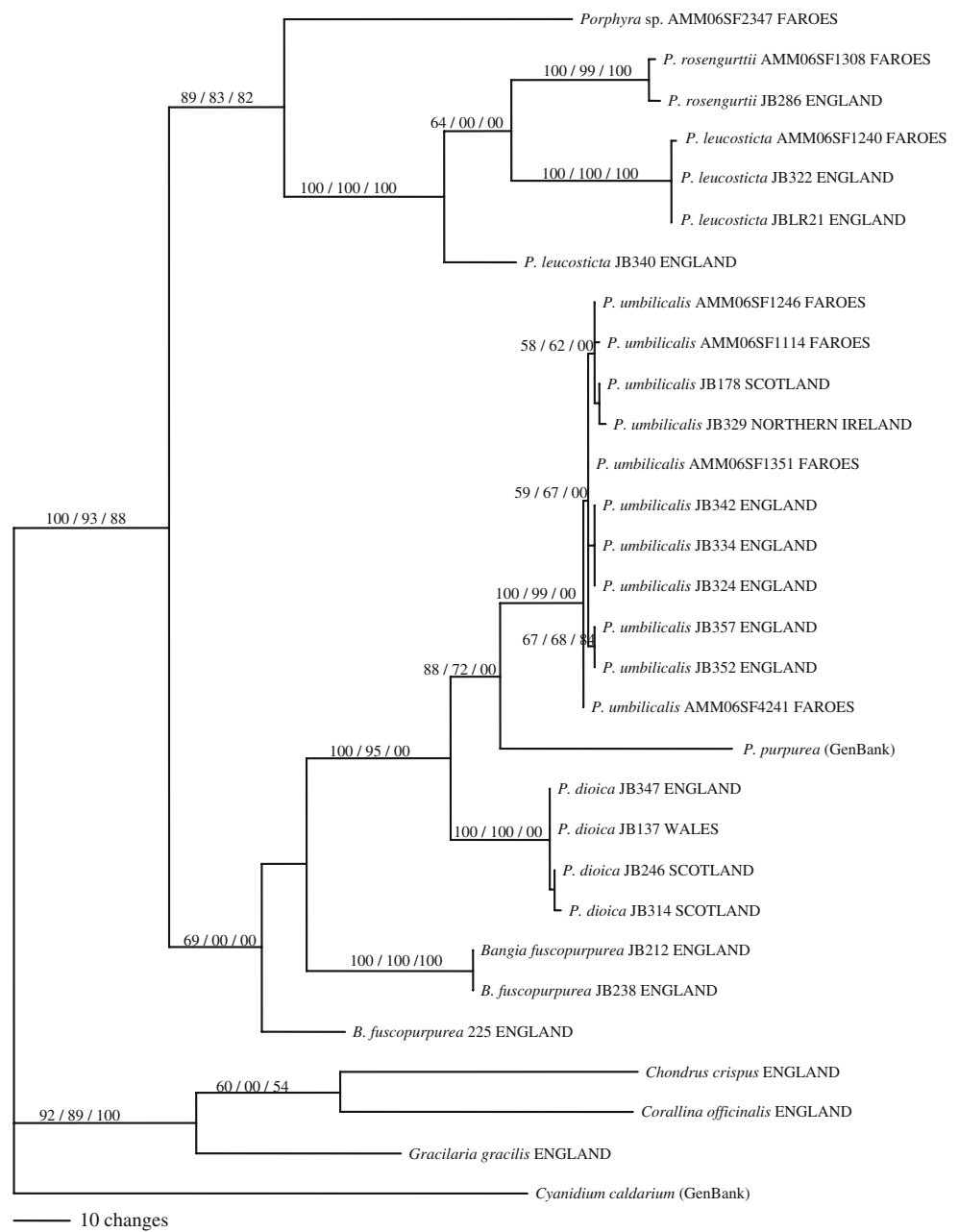
These results indicate the value of such comparisons between floras from different regions and are vital to resolve the global taxonomy of the genus. This was demonstrated in Brodie et al. (2007) where two species thought to be confined to the Mediterranean were much more widespread in the North Atlantic following comparison of data between research groups in the region. One species of the species which was about to be described as new from Spain (Mediterranean) was established as *P. rosenfurtii* (described from North Carolina). The other, *P. olivii*, while considered new in Greece, had concurrently been recognised as undescribed in the northeastern US. By joining up research groups and sharing data, unnecessary descriptions of species were avoided and a much better understanding of the taxonomy, ecology and distribution were able to be made.

All the Faroese samples were collected from one fjord which was sampled at several different times during a year (Mortensen et al. 2007) and will be the subject of a separate paper. This work demonstrates the value of such detailed studies and comprehensive collecting over a small area at different times of the year in really understanding the *Porphyra* flora of a region, as species can easily be overlooked or misidentified. Furthermore, species may be ephemeral or have restricted times of the year when they occur.

Detection of species diversity in different geographical areas of the world and their relationship: Example: North Atlantic and SE-Pacific (Chilean) Porphyra flora

While the Rubisco spacer data are unsuitable for phylogenetic analysis, the unrooted tree (Fig. 2) suggests three groupings. The first (top group in Fig. 2), separated by strong bootstrap support (98), has species from the Azores (mid Atlantic) to the Faroes (northeastern Atlantic) and contains members of the ‘Atlantic Group’ (Brodie et al. 1998), notably *P. dioica*, *P. linearis*, *P. purpurea* and *P. umbilicalis*. *Porphyra dioica* appears to be confined to the northeastern Atlantic (Brodie and Irvine 2003) and has not been reported from the eastern US although we have looked for it. *Porphyra linearis* and *P. umbilicalis* occur in the northeastern and northwestern Atlantic although the names have been misapplied both in this region and elsewhere (Brodie pers obs.). *Porphyra purpurea* is common in the North Atlantic and occurs in the northeast-

Fig. 1 Phylogram of *cox1* for *Porphyra* species from Great Britain and the Faroes. Numbers from left to right represent: i) parsimony bootstrap values, ii) maximum likelihood bootstrap values and iii) Bayesian posterior probabilities; zero values are where statistical support was ≤ 50

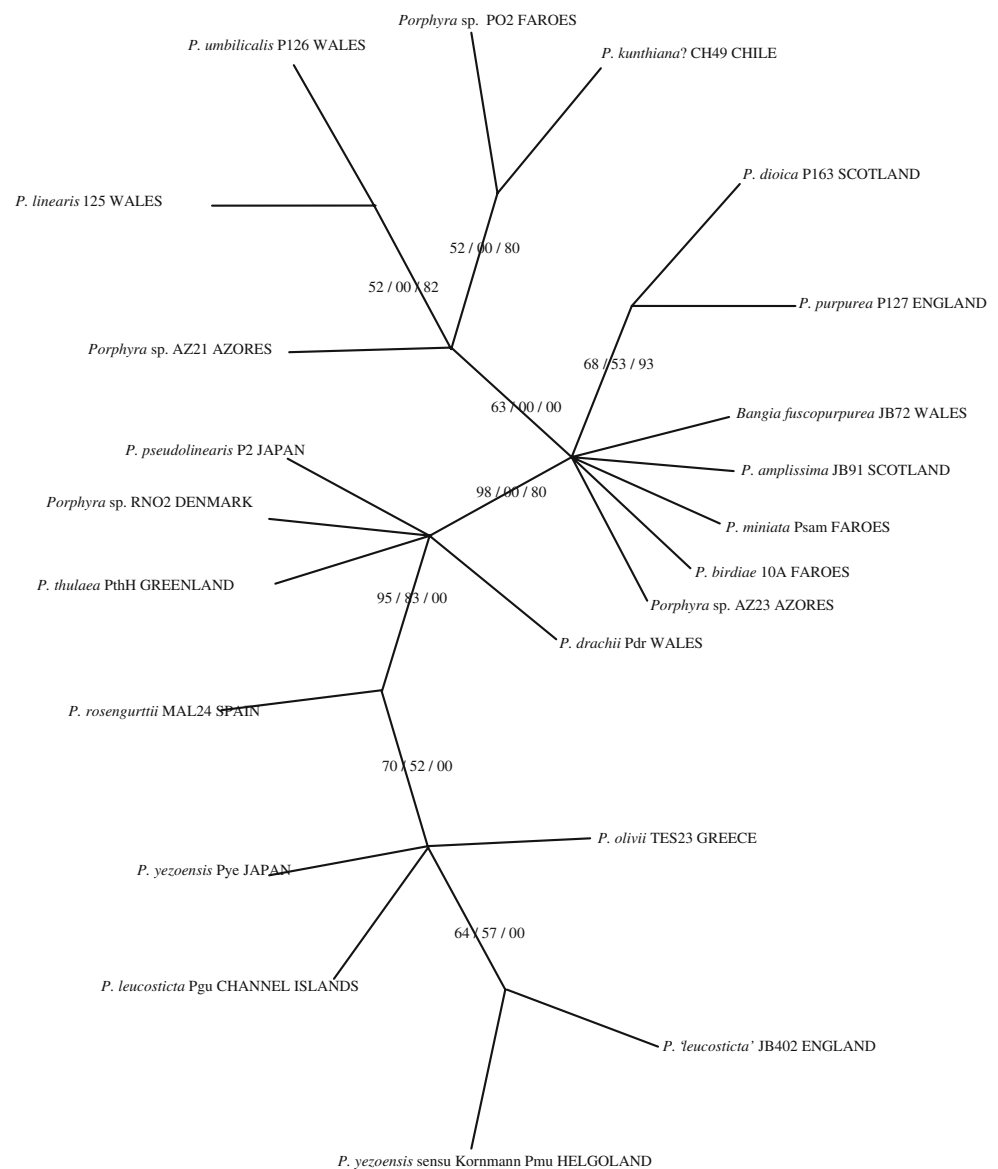


ern Pacific coast of the US (as *P. rediviva* Stiller and Waaland; Bray et al. 2006; see also Lindstrom and Fredericq 2003; Lindstrom 2008), although we need to know more about its distribution in this region.

The inclusion of one sample of *Porphyra* from Chile, and the closeness to species in the group points to the need for work to continue to establish the relationships between floras in different parts of the world. Further evidence of a possible link between the Chilean flora and those in the North Atlantic can be found between *P. amplissima* (North Atlantic) and *P. cuneiformis* (southern Chile) where the Rubisco spacer region (not shown in this paper) is identical (Brodie, unpubl. data).

The second group (middle of the unrooted tree) contains the holotype of *P. thulaea* from Greenland. The sequence for *P. thulaea* from Iceland (564557) is identical to that of the holotype. The species has only been confirmed from Greenland and Iceland and appears to be confined to these more northerly regions, although the sequence from *Porphyra* sp. RNO2 from Denmark only differs by an extra bp and all three are close to *P. pseudolinearis* from Japan. However, a single bp in the Rubisco spacer suggests that these are distinct. *Porphyra dioica* was separated from *P. purpurea* by 1 bp (in conjunction with morphological and ecological characters; Brodie et al. 1998) and this is borne out for at least *P. thulaea* and *P. pseudolinearis* from the

Fig. 2 Unrooted strict consensus tree of the Rubisco spacer (including the 3' end of *rbcL*) for *Porphyra* species. Numbers from left to right represent: i) parsimony bootstrap values, ii) maximum likelihood bootstrap values and iii) Bayesian posterior probabilities; zero values are where statistical support was ≤ 50



partial rDNA SSU data (Fig. 3) where they are clearly separated.

Porphyra drachii also occurs with the second group, although given the distinctive, *P. leucosticta*-like male sori, might have been predicted to come out with the third group, separated by high bootstrap support (95), which contains species of the *P. leucosticta* complex (Brodie et al. 2007). It includes *P. rosenfurtii* and *P. olivii* which occur in the North Atlantic and Mediterranean (Brodie et al. 2007), other species under the name *P. leucosticta* (Neefus 2007) and closely related *P. yezoensis* from Japan. *Porphyra yezoensis sensu Kornmann*, recorded from Helgoland, may represent another species in the '*leucosticta*' complex.

There are five species to add to the North Atlantic flora based on these results alone. Preliminary data indicate that in addition to the two unidentified species from the Azores, there are probably at least three more unidentified species

from this region (Brodie, pers. obs.). There are still several species in the *P. leucosticta* complex that remain unresolved.

The discovery of a possible Atlantic group within the *cox1* and Rubisco spacer data can be compared with Milstein and de Oliveira (2005), who studied the molecular phylogeny of the Bangiales based on the complete rDNA SSU with the emphasis on Brazilian *Porphyra* species. Here they recovered two monophyletic sister groups one of which (G1) contained *P. umbilicalis*, *P. purpurea* and *B. atropurpurea*, species of our Atlantic group, but also species from New Zealand, South Africa and Australia. Their second group (G2) contained *P. leucosticta* and all the species they included in their analysis from Brazil as well as Pacific representatives from Japan and New Zealand.

While the Rubisco spacer has proved valuable as an initial identification tool for *Porphyra* species and as a pointer for further investigation, at only 77 bp long, its use

is almost certainly limited for global comparisons. A more useful plastid marker that would give much the same information as well as being informative phylogenetically would be the *rbcL* (e.g. Klein et al. 2003; Lindstrom and Fredericq 2003; Lindstrom 2008) and it is proposed that this should be one of the molecular markers used in determining a global taxonomy.

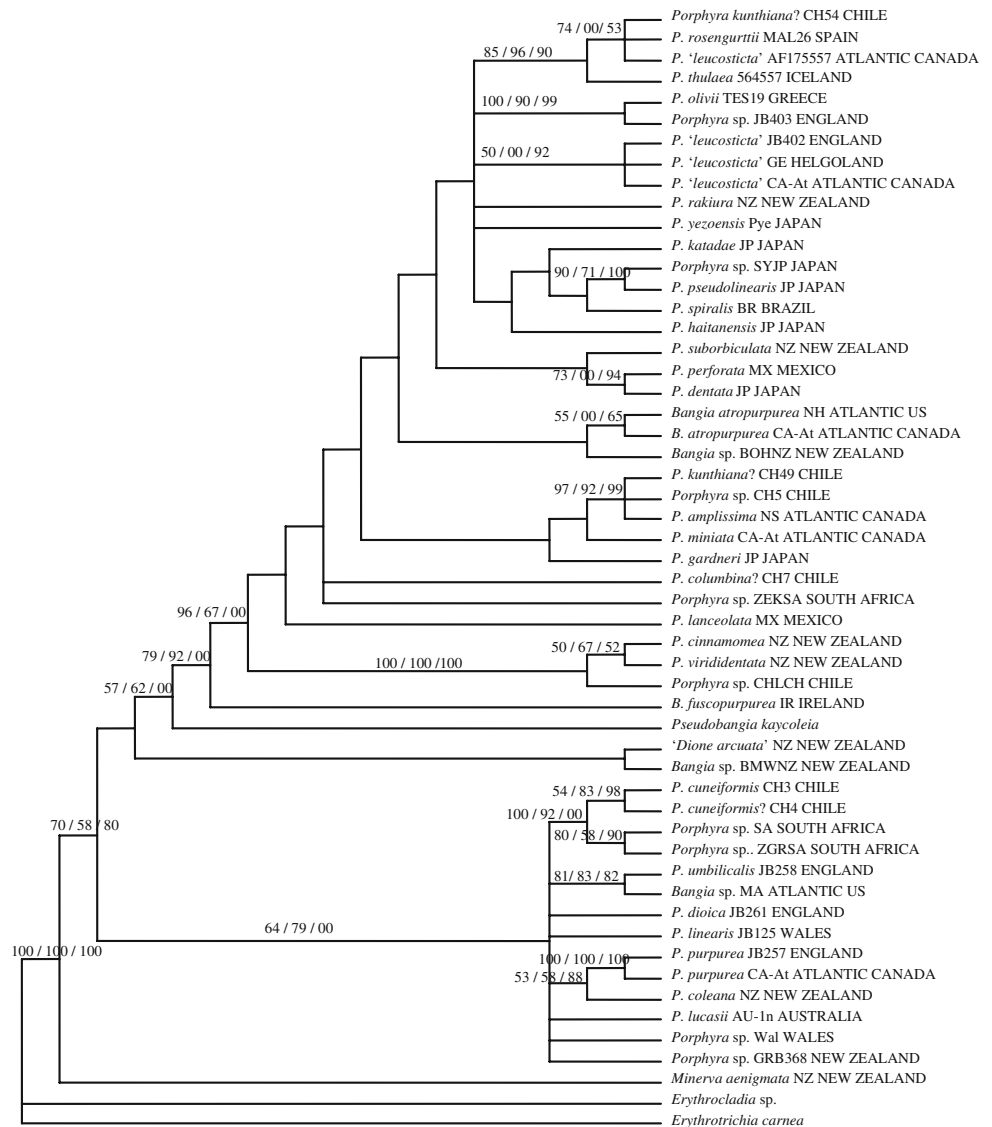
For a much more comprehensive analysis of a Bangiales rDNA SSU dataset and discussion of generic concepts, refer to Nelson et al. (2006). In our study the partial rDNA SSU dataset represents just under a third of the gene in a region that is useful for species identification in *Porphyra*. The results (Fig. 3) show several groupings, although these are not clearly resolved, and the relatively short length of the sequences for this gene and the limited number of taxa included in the tree make the data unsuitable for any generic interpretation. However, notable in the data, is the spread of

Table 3 Details of parsimony analysis for the three molecular markers

Marker	Rubisco spacer	Partial rDNA SSU	<i>cox1</i>
No. of taxa	22	54	30
No. of bp included in analysis	99	492	533
No. of trees generated by parsimony analysis	62	54*	1
No. of parsimony informative characters	20	142	194
Tree length	46	630	698
CI	0.826	0.529	0.593
RI	0.924	0.710	0.762

*saved no more than one tree \geq a score of 10 each replication.

Fig. 3 Strict consensus tree of partial rDNA SSU for *Porphyra* species. Numbers from left to right represent: i) parsimony bootstrap values, ii) maximum likelihood bootstrap values and iii) Bayesian posterior probabilities; zero values are where statistical support was ≤ 50



taxa from different geographical areas throughout the tree, which highlights the need for more comprehensive sampling in targeted areas of the world where the taxonomy of *Porphyra* has been under worked to be certain of determining reliable generic concepts. The taxa included in the data set from Chile indicate that the *Porphyra* flora of this region is rich and that there are a considerable number of unidentified species. Species from this area occur throughout the tree, notably one of the taxa identified as *P. kunthiana?* (CH54) grouping with *P. rosengurttii* (MAL26) from the Mediterranean and a species identified as *P. leucosticta* (AF175557) from Eastern Canada, and another species named *P. kunthiana?* (CH49) close to *Porphyra* sp. (CH5) from Chile and a sample identified as *P. amplissima* from Nova Scotia. Specimens identified as *P. cuneiformis* appear to be closely related to an unidentified South African species. Additional data (not shown here) also suggest a close link between the Chilean and South African *Porphyra* flora. Names have almost certainly been misapplied in the region, notably *P. columbina* whose presence in the Chilean flora remains to be confirmed. Further detailed work is required on the *Porphyra* flora of Chile to sort out the names and study the relationships of these species, notably with South Africa, Japan and the North Atlantic.

The sequence '*Dione arcuata*' AY184338 from Nelson et al. (2006) does not match the type sequence (Nelson et al. 2005) but groups with a species of *Bangia* (BMWNZ).

Parsimony analysis of the three molecular markers showed that *cox1* (Table 3) had the highest number of parsimoniously informative characters and just one tree. The large number of trees and lowest number of parsimony informative characters for the Rubisco spacer reflects the short length of the sequences. The partial rDNA SSU represents approximately a third of the gene, excluding introns, and for a more complete analysis see Nelson et al. (2006). Nevertheless, all three markers provide useful information in our understanding of the number of species of *Porphyra*, relationships both between and within species in different parts of the world.

Resolving the genera involved in worldwide Porphyra sensu lato

Before determining the taxonomy at the generic level, it is crucial to include as many species as possible in the analysis. It is also necessary to decide which molecular markers to use for this work. The Rubisco spacer has been shown as unsuitable for this work, but the *rbcL* is suitable along with the whole rDNA SSU. While the *cox1* data may be unsuitable for generic determination, they are valuable for species discrimination and intra-specific variation.

It is evident that we do not yet know the number of species of *Porphyra* in the world, or how many species

occur in different parts of the world. While there is evidence that different regions have distinct floras, it is important to consider that species may be much more widely distributed than recognised and may be going under different names in different regions. Conversely species may be going under the same name but be different species. A lot more work needs to be done to establish how species in different parts of the world relate to each other. While detailed molecular studies of the Bangiales have been undertaken in some parts of the world, notably parts of the northeastern Atlantic (Brodie et al. 1998; Klein et al. 2003), the northeastern Pacific (Lindstrom and Fredericq 2003; Lindstrom 2008), South Africa (Jones et al. 2004) and New Zealand (Nelson et al. 2006), many other regions of the world remain to be studied. It has also been noted previously (Brodie and Nielsen 2005) that northern areas of the North Atlantic and Arctic require investigation and there is evidence (Brodie, pers. obs.) of additional unidentified species in these regions.

Here, the results indicate that more detailed taxonomic studies are required for the Azores and Chile but many other regions need such study, e.g. circumpolar Arctic, southwest Atlantic and southeast Asia. While not the focus of this paper, in addition to the molecular work, detailed morphological descriptions need to be made of all the species along with consideration of ecological data.

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