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A REAPPRAISAL OF *PORPHYRA* AND *BANGIA* (BANGIOPHYCIDAE, RHODOPHYTA) IN THE NORTHEAST ATLANTIC BASED ON THE *rbc*L–*rbc*S INTERGENIC SPACER[1]

Juliet Brodie[2]

Faculty of Applied Sciences, Bath Spa University College, Newton Park, Newton St. Loe, Bath BA2 9BN, United Kingdom

Paul K. Hayes, Gary L. Barker

School of Biological Sciences, University of Bristol, Woodland Road, Bristol BS8 1UG, United Kingdom

Linda M. Irvine

Botany Department, The Natural History Museum, Cromwell Road, London SW7 5BD, United Kingdom

and

Inka Bartsch

Biologische Anstalt Helgoland, Zentrale Hamburg, Notkestrasse 31, D22607 Hamburg, Germany

ABSTRACT

Sequence data of the rbc*L*–rbc*S noncoding intergenic spacer of the plastid genome for 47 specimens of* Porphyra *and* Bangia *from the northeast Atlantic reveal that they fall into 11 distinct sequences:* P. purpurea, P. dioica *(includes a sample of* P. ''ochotensis'' *from Helgoland),* P. amplissima *(includes* P. thulaea *and British records of* P. ''miniata''*),* P. linearis, P. umbilicalis, P. ''miniata'', B. atropurpurea s.l. *from Denmark and* B. atropurpurea s.l. *from Wales,* P. drachii, P. leucosticta *(includes a British record of* P. ''miniata *var.* abyssicola''*), and* P. ''insolita'' *(includes* P. ''yezoensis'' *from Helgoland). Of these, data obtained for* P. purpurea, P. dioica, P. amplissima, P. linearis, P. umbilicalis, P. drachii, *and* P. leucosticta *were based on type specimens or material compared with types. Comparison of sequence data for* Porphyra *spp. and* Bangia atropurpurea s.l. *(including* B. fuscopurpurea, *the type species of* Bangia*) confirms that the species are congeneric. The data also confirm that the number of layers that make up the* Porphyra *thallus are not taxonomically significant. Comparison of sequence data for species from the northeast Atlantic with those for material of two species from the Pacific reveals that the species fall into two distinct groupings: an* Atlantic group, *containing* P. purpurea, P. dioica, P. amplissima, P. linearis, P. umbilicalis, P. ''miniata'', *and* B. atropurpurea, *and a* Pacific group, *containing* P. ''pseudolinearis'', P. drachii, P. leucosticta, P. ''yezoensis'' *(including a sample of* P. ''tenera''*), and* P. ''insolita'' *(including* P. ''yezoensis'' *from Helgoland). The possibility of alien species in the northeast Atlantic is discussed.*

Key index words: Atlantic; Bangia; *introduced species; Pacific;* Porphyra; *Rhodophyta; RUBISCO spacer; taxonomy*

Abbreviation: s.l., sensu lato

The red algal family Bangiaceae currently has two genera assigned to it, *Porphyra* and *Bangia,* but in this paper we now have good evidence that the type species are congeneric. Species of *Porphyra* occur in the intertidal and shallow subtidal zones in cool- to warm-temperate regions of the world and at certain times of the year can be the dominant algae in some shore regions. Some species are economically important, being harvested from the wild or grown commercially as food; for example, laver and nori. *Bangia* occurs mainly in the intertidal zone in cool to tropical regions.

Despite being the subject of much study over at least the last 100 years, the taxonomy within the family remains problematic, mainly because of the highly variable morphology and lack of easily recognizable characters. Molecular techniques have been used to assist in the discrimination of species of *Porphyra* (Lindstrom and Cole 1992a, b, Stiller and Waaland 1993, 1996, Oliveira et al. 1995), and an analysis of nucleotide sequence data of the plastid-borne *rbc*L–*rbc*S intergenic spacer (RUBISCO spacer) has proved useful in differentiating between two often confused species of *Porphyra* (Brodie et al. 1996): *Porphyra dioica* Brodie et L. Irvine (as *P. laciniata*) was distinguished from *P. purpurea* using this technique, and sound morphological characters confirmed it as a distinct species (Brodie and Irvine 1997). In this paper, species-level taxonomy based on analysis of the RUBISCO spacer data is explored for specimens of *Porphyra* and *Bangia* from the northeast Atlantic. We also compare sequence data for three Pacific samples of *Porphyra.*

Six species of *Porphyra,* as well as *Bangia atropurpurea,* were recognized for the coasts of Britain, Ireland, and adjacent waters by Parke and Dixon (1976). A further five species were reported for the northeast Atlantic by South and Tittley (1986) and five more by Guiry (1997) (Table 1). We have tested specimens purporting to belong to all these species except for *P. helenae*; although we have been able to

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[2] Corresponding author; e-mail j.brodie@bathspa.ac.uk

TABLE 1. *Species of* Porphyra *and* Bangia *listed for Great Britain, Ireland, and the North Atlantic. nd = not determined.*

Parke and Dixon (1976)	South and Tittley (1986)	Guiry (1997)	This study
Bangia atropurpurea (Roth) C. Agardh	*B. atropurpurea*	*B. atropurpurea*	*B. atropurpurea*
	P. abyssicola Kjellman		nd
	P. abyssicola Kjellman		nd
P. amethystea Kützing	*P. amethystea*	*P. amethystea*	nd
	P. amplissima (Kjellman) Setchell et Hus		*P. amplissima*
	P. drachii J. Feldmann	*P. drachii*	*P. drachii*
	P. helenaea A. Zinova		nd
		P. insolita Kornmann & Sahling	*P.* ''*insolita*''
		P. laciniata (Lightfoot) C. Agardh	*P. dioica* Brodie & L. Irvine
P. leucosticta Thuret in Le Jolis	*P. leucosticta*	*P. leucosticta*	*P. leucosticta*
P. linearis Greville	*P. linearis*	*P. linearis*	*P. linearis*
P. miniata (C. Agardh) C. Agardh	*P. miniata*	*P. miniata*	*P. amplissima* (most samples)
		P. ochotensis Nagai[a]	*P. dioica*[a]
P. purpurea (Roth) C. Agardh	*P. purpurea*	*P. purpurea*	*P. purpurea*
		P. purpureo-violacea (Roth) Krishnamurthy	*P. purpurea*[b]
	P. thulaea Munda & Pedersen		*P. amplissima*
P. umbilicalis (Linnaeus) J. Agardh	*P. umbilicalis*	*P. umbilicalis*	*P. umbilicalis*
		P. yezoensis Ueda	*P.* ''*insolita*''

[a] Abstract includes *P.* ''*ochotensis*'' in *P. dioica*; see also Results and Discussion.
[b] See Brodie and Irvine (1997).

extract DNA, we have not been able to obtain a RUBISCO spacer sequence for type material of *P. amethystea,* and we have no sequences for verified *P. abyssicola* or *P. miniata.*

A major problem exists in relating our data to those published elsewhere, as specimens given the same names are not necessarily conspecific. Thus, whenever possible, our sequence data are based only on types or authentic or similar material; nonauthenticated names are given in quotation marks (Table 1).

MATERIALS AND METHODS

Material. Specimens used for molecular analysis were from Great Britain, Germany (Helgoland), Ireland, Norway, Faroes, Denmark, Greenland, and Japan (Table 2). DNA was extracted from one of the following: freshly collected material, herbarium specimens, silica gel- preserved plants, *Conchocelis,* or blade-phase cultures. *Conchocelis* cultures were initiated from zygotospores (sensu Guiry 1990) released from fertile sections of the blade phase as described by Brodie et al. (1996). Blades developed in culture either directly (*P. umbilicalis* from Brighton) or from spores released by the *Conchocelis*-phase. Cultures were grown in nutrient-enriched seawater as described by Brodie and Guiry (1988) and incubated at 15° C, 16:8 h LD cycle, incident irradiance of 20 μmol photons·m^{-2}·s^{-1}. Some *Conchocelis* cultures arrived by post, having been initiated elsewhere.

DNA extraction and PCR amplification. Procedures for DNA extraction and PCR amplification were as descibed by Brodie et al. (1996). Primers used were complementary to the 3′ end of *rbc*L (5′TGTGGACCTCTACAAACAGC3′) and the 5′ end of *rbc*S (5′CCCCTAGTTCCCAAT3′) (Maggs et al. 1992).

Sequencing and analysis. Manual sequencing was carried out according to the method described by Brodie et al. (1996). Automated sequencing was performed commercially on an ABI prism 377 sequencer. Sequences were visually aligned, and a Jukes-Cantor–corrected distance matrix was calculated using DNADIST in the PHYLIP package (Felsenstein 1989).

RESULTS AND DISCUSSION

PCR amplification of the *rbc*L–*rbc*S spacer and flanking coding regions for *Porphyra* species and *Bangia atropurpurea* yielded a product of about 320 bp for all isolates. A common region including the 3′ end of *rbc*L, the 77-bp spacer, and the rbc*S* start codon was sequenced for all isolates (Fig. 1); from a total of 50 specimens, we found 13 distinct sequences represented by (with EMBL accession number): *P. purpurea* (AJ010776), *P. dioica* (includes *P. "ochotensis"*) (AJ010779), *P. amplissima* (includes *P. thulaea*) (AJ010780), *P. linearis* (AJ010781), *P. umbilicalis* (AJ010782), *P.* ''*miniata*'' (AJ010786), *B. atropurpurea s.l.* from Denmark (AJ010784), *B. atropurpurea s.l.* from Wales (AJ010785), *P. "pseudolinearis"* (AJ010787), *P. drachii* (AJ010788), *P. leucosticta* (AJ010789), *P."yezoensis"* (AJ010783), and *P. "insolita"* (AJ010778) (Table 2).

These data have thus helped clarify the circumscriptions of the species of *Porphyra* in the northeast Atlantic. Samples of type specimens or material carefully compared with types (*P. purpurea, P. dioica, P. amplissima, P. linearis, P. thulaea, P. umbilicalis, P. drachii,* and *P. leucosticta*) can, with the exception of *P. thulaea,* be distinguished morphologically and have different *rbc*L–*rbc*S spacer sequences. The sequence for *P. amplissima* is the same as that for type material of *P. thulaea.*

No sequence variation was found within species, with the exception of one specimen of *P. purpurea* from Hayling Island. This sample differed from the other *P. purpurea* isolates by a single base transition, T to C, in the third position of an alanine codon seven base pairs before the stop codon of *rbc*L (Fig.

TABLE 2. *Specimens used for molecular analysis. C = culture, Cc = culture,* Conchocelis *phase, Cb = culture, blade phase, D = dried blade, H = herbarium specimen, Sg = blade dried in silica gel, BM = The Natural History Museum, London.*

Specimens	Code	Location	Date	Collector	Material	Species according to *rbc*L–*rbc*S sequence
Northeast Atlantic						
P. purpurea	Phy	Seabrook, nr Hythe, Kent, England	9.viii.1956	K. Drew (Drew no. 2885 BM)	H	*P. purpurea*
	P121	Lilstock, Somerset, England	28.ii.1994	J. Brodie (JB culture no. 121)	Cc	*P. purpurea*
	PS	Lilstock, Somerset, England	9.vi.1994	J. Brodie (on pebble in sand)	D	*P. purpurea*
	P127	Lilstock, Somerset, England	9.vi.1994	J. Brodie (on boulder, JB culture no. 127)	Cc	*P. purpurea*
	P161	Langstone Harbour, Hayling Is., England	13.xii.1994	J. Brodie (on boulder, JB culture no. 161)	Cc	*P. purpurea* (Hayling Is.)
	Psm	Portaferry, N. Ireland	?.i.1995	D. Birkett	D	*P. purpurea*
	Pfe	Lyme Regis, Dorset, England	16.v.1995	J. Brodie	Sg	*P. purpurea*
	JB28	Helgoland, Germany	23.viii.1995	J. Brodie	Sg	*P. purpurea*
P. dioica	063	Helgoland, Germany	26.ii.1991	I. Bartsch (Bartsch culture no. 063)	Cc	*P. dioica*
	P156	Sidmouth, Devon, England	3.xi.1994	J. Brodie (JB culture no. 156)	Cc	*P. dioica*
	163	Cruden Bay, Aberdeenshire, Scotland	26.i.1995	L. Terry (JB culture no. 163)	Cc	*P. dioica*
	167	Cruden Bay, Aberdeenshire, Scotland	26.i.1995	L. Terry (JB culture no. 167)	Cc	*P. dioica*
	JB48	Sidmouth, Devon, England	9.ix.1995	J. Brodie (amongst *Enteromorpha*)	Sg	*P. dioica*
P. amplissima	Pstr	Strangford Lough, N. Ireland	1.iv.1995	C. A. Maggs (subtidal, epiphytic)	Sg	*P. amplissima*
	Pmvi	Vatlestraumen, Norway	11.vi.1996	J. Rueness	Sg	*P. amplissima*
	JB89	Dale, Pembrokeshire, Wales	16.vi.1996	J. Brodie (drift on mudflats)	Sg	*P. amplissima*
	JB91	Clyde Estuary, Scotland	2.vii.1996	J. Hall-Spencer (subtidal)	Sg	*P. amplissima*
P. linearis	P050	Helgoland, Germany	25.ii.1991	I. Bartsch (herbarium ref: Pli2/12)	Cc	*P. linearis*
	P052	Helgoland, Germany	25.ii.1991	I. Bartsch (herbarium ref: Pli1/13)	Cc	*P. linearis*
	Plinh	Hayling Island, England	28.ii.1995	W. F. Farnham	Sg	*P. linearis*
	125	South Beach, Aberystwyth, Wales	10.iv.1994	J. Plumb	Cc	*P. linearis*
P. umbilicalis	P143	The Smalls, Pembrokeshire, Wales	30.viii.1994	S. Scott (on lighthouse wood)	Cb	*P. umbilicalis*
	Pum	Margate, Kent, England	?.viii.1995	I. Tittley	D	*P. umbilicalis*
	P126	Brighton, Sussex, England	16.iv.1994	J. Brodie (near working pier)	Cb	*P. umbilicalis*
	2108	Helgoland, Germany	No date	K. Lüning	Cc	*P. umbilicalis*
	P170	Cook Street Pier, N. Ireland	8.ii.1995	D. Birkett	D	*P. umbilicalis*
	N1-3	Velbastadur, Streymoy, Faroes	2.v.1996	K. Gunnarsson & R. Nielson (herbarium ref: F961352 10, 3 individuals)	Sg	*P. umbilicalis*
P. "miniata"	Psam	Lirberg, Sandoy, Faroes	28.v.1997	K. Gunnarsson	D	*P. "miniata"*
Bangia atropurpurea	129	Lœsø, Denmark	25.v.1994	R. Nielsen	C	*B. atropurpurea*
	JB72	Rhosneigr, Anglesey	14.iv.1995	E. Jones & J. Brodie	Sg	*B. atropurpurea*
P. drachii	Pdr	The Smalls, Pembrokeshire, Wales	30.viii.1994	F. Bunker (subtidal, on kelp frond)	H	*P. drachii*
P. leucosticta	Pleuc	Flamborough Head, Yorkshire, England	23.viii.1994	J. Brodie	D	*P. leucosticta*
	P. gu	Guernsey, Channel Islands	6.vi.1995	G. Barker	Sg	*P. leucosticta*
	P. sp.	Sherkin Island, Co. Cork, Ireland	?.v.1995	H. Fazakerley	D	*P. leucosticta*
	JB87	Martin's Haven, Pembrokeshire, Wales	16.vi.1996	J. Brodie	Sg	*P. leucosticta*
	JB95	Lleyn Peninsula, Wales	15.viii.1996	E. Murray (subtidal, in kelp forest)	Sg	*P. leucosticta*
P. "insolita"	059	Helgoland, Germany	No date	I. Bartsch	Cc	*P. umbilicalis*
	061	Helgoland, Germany	26.ii.1991	I. Bartsch	Cc	*P. "insolita"*
P. sp.	Pmu	Muckinish, Co. Clare, Ireland	25.xi.1996	H. Fazakerley	D	*P. "insolita"*
P. *"yezoensis"*	055	Helgoland, Germany	26.ii.1991	P. Kornmann (as *P. yezoensis*)	Cb	*P. "insolita"*
"P. miniata var. *abyssicola"*	Pab	Ore Bay, Orkney, Scotland	8.ix.1926	L. Lyle	H	*P. leucosticta*
P. "ochotensis"	IB1, IB2 990	Helgoland, Germany	10.ix.1991	I. Bartsch (3 individuals)	D	*P. dioica*
P. thulaea	Pth	Godthaab, Greenland	28.viii.1958	T. Christensen 8464 (Flora of Greenland, BM isotype)	H	*P. amplissima*
Pacific						
P. "yezoensis"	Pye	Oshoro Bay, Japan	17.v.1996	M. Masuda	H	*P. "yezoensis"*
P. "tenera"	Pte	Fukuyama, Japan	1968	T. Fujiyama	D	*P. "yezoensis"*
P. "pseudolinearis"	P2	Hokkaido, Japan	?.i.1995	K. Kogame (Bartsch culture no. 074)	Cc	*P. "pseudolinearis"*

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                  1                                                                                                  99
P. purpurea       CCAACAGCCAACGTCTAGTTCAATGACTACTTACAACTGC-TTAAACTAGCAAAGTATAAGTAGAATTAACTTATAAAAATAAGGAGCATAGAATAGTG
P. purpurea H     ........T..............................-.....................................................................
P. dioica         ..................T...................-.....................................................................
P. amplissima     ........A..........T...................-.....................................................C.....
P. linearis       ........A..........T........T...T......-.........G.....................................................
P. umbilicalis    ........A........A..T............T......-.........G.....................................................
B. atr D          ........A..........T............A......-......................A.....................C.A.....
B. atr W          ........A..........T............AC.....-......................A.....................C.A.....
P. 'miniata'      ........A..........T............C.....-.....................................C..........C.....
P. 'pseudolinearis' ........A...A.......T...........G.T.A...-.....TC......T.G.....................C.....
P. drachii        ........A...A.......T...........G.T.A...-G...TT.......T.G.....................C.....
P. leucosticta    ........A...A.......T............T.A.A.T.....-...TC..TCG.....G..............C.....
P. 'yezoensis'    ........A...A.......T............TGA.A.T.....-...TC..T.G.....G..............C.....
P. 'insolita'     ........A...A.......T............T.A.A.T.....-G..TC..T.G.....G..............C.....
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FIG. 1. Sequence alignment of *Porphyra* and *Bangia* species *rbc*L–*rbc*S regions. Underlined: stop codon *rbc*L and start codon *rbc*S; . = same base; - = space; H = Hayling Island, D = Denmark, W = Wales.

1). In the absence of any discernible morphological differences between *P. purpurea* from Hayling Island and other *P. purpurea* isolates, we feel that the single transition in the *rbc*L region does not, in this case, warrant separation of *P. purpurea* into two species.

Specimens provisionally identified as *Porphyra "miniata"* are clearly distinguishable morphologically (Brodie and Irvine, pers. observ.) and molecularly from the other species of *Porphyra* in this region, but type material has not been available for comparison. The records of *P. miniata* in Great Britain appear to be based on misidentifications of *P. amplissima.*

Porphyra drachii was first described by Feldmann (1979) on the basis of specimens collected from the fronds of *Laminaria hyperborea* off the coast of Brittany by Pierre Drach in 1948. The first published record of this species in Great Britain was in 1997 (Table 1). Searches so far failed to locate the type material, but a comparison of our specimens with plants collected by J. Cabioch from Santec, Brittany, France, and considered by her to be genuine *P. drachii* (proposed Neotype; Cabioch, pers. comm.) appear to confirm that they are conspecific.

Porphyra amethystea remains a mystery, as we have not been able to obtain a sequence from type material. *Porphyra abyssicola* and *P. helenaea* also require further study.

The one base-pair difference in the *rbc*L–*rbc*S spacer between *B. atropurpurea* from Denmark and Wales suggests that there might be more than one species in the northern Atlantic. Nevertheless, our data confirm that *B. atropurpurea s. l.* (Danish and Welsh) and *Porphyra* spp. are congeneric, as suggested by Oliveira et al. (1995), whose ssu-rDNA data showed that it nested stably within the *Porphyra* group. The type species of the genus *Bangia* Lyngbye (1819:82) is *B. fuscopurpurea,* based on marine material collected from limestone rocks at Dunraven Castle near Bridgend, south Wales, by W.W. Young and described by Dillwyn (1807: table 92) as *Conferva fuscopurpurea.* The type has not been located, and visits to the type locality over the past two years have failed to find any *Bangia* at the site. Before making a formal transfer, we need to obtain morphological and sequence data from material that can be designated as a neotype of *B. fuscopurpurea,* on which a nomenclatural change could be based. We have made no observations on freshwater material and so cannot speculate on the affinity of *B. atropurpurea* (Roth) C. Agardh (1824:76), of which *B. fuscopurpurea* is usually regarded as a synonym.

The data have also helped to resolve the debate about the taxonomic significance of the number of cell layers that make up the *Porphyra* blade (Brodie et al. 1997). Species have sometimes been distinguished on the basis of whether the blades are mono- or distromatic. Isolates of *P. amplissima* used in this study consisted of either monostromatic (Pstr, Table 1) or distromatic (JB89, JB91, Pmi, Table 2) blades. Sometimes both one and two cell layers can be observed within a single, vegetative thallus (Brodie, pers. observ.). This is also in accord with the observations of Stiller and Waaland (1993) and Oliveira et al. (1995), who found no correlation between phylogenetic position and the number of cell layers.

The distance matrix (Table 3) shows that the species fall into two distinct groupings: an Atlantic group (mean distance = 0.04644, SD 0.01937, n = 36) containing *P. purpurea, P. dioica, P. amplissima, P. linearis, P. umbilicalis, P. "miniata",* and *B. atropurpurea* (Denmark and Wales) (Table 2), and a Pacific group (mean distance = 0.05720, SD 0.03011, n = 10), which includes *P. "insolita" from Helgoland, P. leucosticta* and *P. drachii* from Great Britain, and *P. "pseudolinearis"* and *P. "yezoensis"* from Japan. The mean distance between the two groups is 0.14089, SD 0.01855, n = 45.

Porphyra leucosticta is widespread in Great Britain and Ireland and is also reported from other northern European and Mediterranean countries, but its alignment with the Pacific group raises the question of its origin. One possibility is that it is an alien species in the North Atlantic. If this is the case, its presence around much of the coast suggests that it is well established; it must have arrived at least 135 years ago, as there are herbarium records dating back to 1863. It was first described by Thuret in Le Jolis in 1863, and we have found no British specimens collected earlier than this subsequently identified as *P. leucosticta.* It was reported from Denmark (Rosenvinge 1909), Helgoland (Herbarium Kuck-

TABLE 3. *Distance matrix of* Porphyra *and* Bangia *species based on* rbc*L*–rbc*S sequence data. The italic area indicates the* Atlantic group*; the bold area indicates the* Pacific group. *The specific name is represented by the first three letters; H = Hayling Island, D = Denmark, and W = Wales.*

	P. pur	*P. purH*	*P. dio*	*P. amp*	*P. lin*	*P. umb*	*P. "min"*	*B. atrD*	*B. atrW*	*P. "pse"*	*P. dra*	*P. leu*	*P. yez*	*P. "ins"*
P. pur	*0.0000*	*0.0103*	*0.0103*	*0.0313*	*0.0528*	*0.0528*	*0.0528*	*0.0639*	*0.0751*	0.1216	0.1337	0.1476	0.1476	0.1476
P. pur H		*0.0000*	*0.0207*	*0.0313*	*0.0528*	*0.0528*	*0.0528*	*0.0639*	*0.0751*	0.1216	0.1337	0.1476	0.1476	0.1476
P. dio			*0.0000*	*0.0207*	*0.0420*	*0.0420*	*0.0420*	*0.0528*	*0.0639*	0.1097	0.1216	0.1352	0.1352	0.1352
P. amp				*0.0000*	*0.0420*	*0.0420*	*0.0207*	*0.0313*	*0.0420*	0.1097	0.1216	0.1352	0.1352	0.1352
P. lin					*0.0000*	*0.0207*	*0.0639*	*0.0639*	*0.0751*	0.1337	0.1460	0.1476	0.1476	0.1476
P. umb						*0.0000*	*0.0639*	*0.0639*	*0.0751*	0.1337	0.1460	0.1476	0.1476	0.1476
P. "min"							*0.0000*	*0.0528*	*0.0103*	0.0980	0.1097	0.1230	0.1230	0.1230
B. atr D								*0.0000*	*0.0420*	0.1460	0.1585	0.1732	0.1732	0.1732
B. atr W									*0.0000*	0.1460	0.1585	0.1732	0.1230	0.1732
P. "pse"										**0.0000**	**0.0313**	**0.0759**	**0.0759**	**0.0646**
P. dra											**0.0000**	**0.0874**	**0.0874**	**0.0874**
P. leu												**0.0000**	**0.0207**	**0.0207**
P. yez													**0.0000**	**0.0207**
P. "ins"														**0.0000**

uck 1897—at BAH Helgoland), Cherbourg, northern France (Thuret in Le Jolis 1863), and Sweden (Kylin 1907). Furthermore, published (but unconfirmed) records suggest that it is widespread on both sides of the Atlantic, in the east from Iceland and Norway to Spain and the Azores, the Mediterranean, and the Black Sea (Athanasiadis 1987), and in the west from Canada (Labrador) to the United States (New Hampshire) (South and Tittley 1986). Whether all reports of *P. leucosticta* belong to a single species remains to be resolved. Kornmann (1961) speculated that it might be the same species as *P. tenera* from the north Pacific, as there are great similarities in morphology and life history of both species. Another possibility is that an ancestor originating in the Pacific crossed the Bering Straits and Canadian Arctic archipelago to the Atlantic Ocean during a period of raised palaeoclimate temperatures (Van den Hoek 1982). Further examination of specimens from the Arctic and Mediterranean would provide valuable data here. The alignment of *P. drachii* with the Pacific group suggests that it also could be an alien.

The concept of the occurrence of alien species among the east Atlantic representatives of the genus is not new. Kornmann (1986) reported the presence of a species in Helgoland that he identified as *P. yezoensis* Ueda, following Kurogi (1959, 1961, 1972), and concluded that it was an immigrant from the Pacific (East Asia). Similarly, Kornmann and Sahling (1991) provisionally identified a species first observed in 1959 on Helgoland as another East Asian species, *P. "ochotensis",* because of similarities in carpospore arrangement (Kurogi 1972). Specimens identified as *P. "ochotensis"* from Helgoland (Table 2) have the same RUBISCO spacer sequence as *P. dioica,* but whether the Helgoland and the Pacific plants are conspecific is not yet clear. The discovery in 1988 of a third unidentified species of *Porphyra* on Helgoland initially led Kornmann and Sahling (1991) to speculate that this was another Pacific (East Asian) immigrant, but their failure to identify it with any species from that region caused them to describe it as a new species, *P. insolita,* which they suggested had hitherto gone unrecognized on other European coasts. Material in culture identified by Kornmann as *P. "yezoensis"* from Helgoland does not have the same RUBISCO spacer sequence as our sample of *P. "yezoensis"* from Japan (although closely related) but has the same sequence as *P. "insolita"* 061 from Helgoland (leg. Bartsch) (Table 2). Kornmann and Sahling's initial speculation that *P. "insolita"* is an introduction from the Pacific would appear to be reasonable, although its identity remains uncertain. The type of *P. insolita* cannot be found.

Other evidence of a link between Pacific and Atlantic species of *Porphyra* has been proposed by Lindstrom and Cole (1992b, 1993), who found a number of closely related "species pairs" in these two geographical regions. Stiller and Waaland (1996), using RFLPs, found that *P. rediviva* Stiller and Waaland was most closely related to *P. purpurea,* although the two species differed in chromosome number, season of first appearance, and habitat. Stiller and Waaland (1996) argued that their data suggested prolonged genetic separation between these species. Their material of *P. purpurea* included samples supplied by C. Bird (one of which was European material from Hayling Island, United Kingdom); she had previously established, by molecular analysis, that her east Canadian specimens were conspecific with British Isles *P. purpurea* (Bird, unpubl.). Unfortunately, we do not always know whether specimens passing under the same name as those we have examined are in fact conspecific.

Of the other species in the Pacific group in Table 2, the material identified as *P. "tenera"* has the same sequence as *P. "yezoensis"* and possibly is a misidentification; sequence data from an authentic sample of *P. tenera* would confirm this. The sample of *"P. tenera"* came from a sheet of compressed nori (Hoshi-nori) dated 1968; these sheets are now usually prepared from dried *P. yezoensis* (Miura 1975). In 1955, cultivators began transplanting *P. yezoensis* from its natural habitats, and it has replaced *P. tenera* in many areas of Japan.

The relationship between the northeast and northwest Atlantic species requires resolution. *Porphyra amplissima, B. atropurpurea, P. leucosticta, P. linearis, P. miniata, P. purpurea* and *P. umbilicalis* are listed for the northwest Atlantic (South and Tittley 1986, Bird and McLachlan 1992), but conspecificity with their northeast Atlantic counterparts is for the most part not yet determined.

Whether the RUBISCO spacer is a useful tool with which to assess species-level taxonomy probably depends on the genus involved, the time scale of separation of species, and the level of resolution required. The antiquity of *Porphyra* and *Bangia* (Xiao et al. 1998) and the data presented above indicate that analysis of the RUBISCO spacer region is the method of choice for the preliminary screening of these genera, and their spacer has proved a valuable indicator for discriminating between species of *Porphyra* which have been notoriously difficult to identify. As the differences between sequences are relatively few, greater resolution within the Bangiophycidae might be obtained using other regions of the genome.

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