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Molecular divergence and morphological diversity among four cryptic species of **Plocamium** (Plocamiales, Florideophyceae) in northern Europe

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### Molecular divergence and morphological diversity among four cryptic species of *Plocamium* (Plocamiales, Florideophyceae) in northern Europe

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(Received 7 December 2004; accepted 20 May 2005)

The Plocamiaceae currently includes two genera: the free-living *Plocamium* and the adelphoparasite, *Plocamiocolax*. *Plocamium* includes ca. 40 species that are widely distributed throughout the world's oceans. Most species are clearly defined anatomically and biogeographically; however, the type species *Plocamium cartilagineum* (type locality: considered northern Europe) is an exception. Although reportedly cosmopolitan in distribution, our investigations unequivocally indicate that there is substantial genetic diversity among isolates from different geographic locations and that there are at least eight divergent cryptic species currently included in *P. cartilagineum*. In this investigation we focused on populations of *P. cartilagineum* from northern Europe. We used a combination of molecular and morphological characters in order to establish boundaries between four cryptic species in this geographical area. Our molecular results not only resolve these four entities as distinct, but also indicate that they are among the most divergent of all *Plocamium maggsiae* sp. nov. in northern Europe. A preliminary assessment of '*P. cartilagineum*' in other geographical areas is provided.

Key words: cryptic species, Florideophyceae, large-subunit rDNA, molecular systematics, phylogeny, Plocamiaceae, *Plocamium*, Rhodophyta, small-subunit rDNA

### Introduction

Originally described by Kützing (1843, p. 442, as Plocamieae), the family Plocamiaceae is characterized by a unique combination of characters variously reminiscent of other red algal orders. The Plocamiaceae share the following characters with some members of the Ceramiales: ramisympodial growth (Norris et al., 1984), producing a predictable axial pattern of thallus development; uniaxial construction; four periaxial cells, the firstformed lateral and the subsequent pair transverse; direct fusion of the fertilized carpogonium with the supporting cell (Gordon-Mills & Kraft, 1981); a single outwardly-directed gonimoblast initial on which a number of gonimolobes arise to form clusters in which most cells develop into carposporangia; and tetrasporangia developed in highly differentiated stichidia. The Plocamiaceae and some members of the Gigartinales share: the disctype pattern of spore germination (cf. Fritsch,

1945); three-celled carpogonial branches; a pseudoparenchymatous pericarp formed by cortical cell divisions; and zonate, rather than cruciate or tetrahedral, cleavage pattern of tetrasporangia. Recently, SSU (small-subunit ribosomal DNA) data used in combination with anatomical and morphological observations have supported the elevation of the Plocamiaceae to ordinal status, the Plocamiales, comprising two genera (Saunders & Kraft, 1994). Species of the first genus, Plocamiocolax Setchell, are parasitic on various species of the second genus, Plocamium Lamouroux.

*Plocamium* is characterized by erect, bilaterally compressed thalli composed of repeatedly pinnate branches bearing alternating groups of two to more than ten branchlets (ramuli). There is a distinct apical cell and thalli are pseudoparenchymatous with the cortex composed of small, isodiametric cells surrounding a medulla of larger cells. Cystocarps are either scattered along frond margins or borne in special short fertile branches, and lack a specialized pore for carpospore release. Tetrasporangia are borne in specialized stichidia

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of various morphologies (Dixon & Irvine, 1977; Womersley, 1994).

*Plocamium* includes ca. 40 species (in a recent review of the genus Wynne (2002) recognized 36), which are widely distributed throughout the world's oceans (South & Adams, 1979; Ricker, 1987; Gabrielson & Scagel, 1989; Adams, 1994; Womersley, 1994; Abbott, 1999). Most species are clearly defined anatomically (based on the number of ramuli in the alternating series, and features of the tetrasporangial stichidia and cystocarps) and biogeographically. In this respect the type species, Plocamium cartilagineum (L.) Dixon (type locality: considered to be northern Europe) is an exception. Plocamium cartilagineum is reportedly cosmopolitan in distribution (Dixon, 1967) and is characterized by having ramuli alternating in series of 2-6. Tetrasporangial stichidia are simple or terminally branched, replacing ramuli, and cystocarps are sessile. This concept of P. cartilagineum sensu lato encompasses plants of considerable morphological variability. Preliminary investigations have uncovered substantial morphological and genetic diversity among isolates from different geographic locations indicating that cryptic species may currently be included within P. cartilagineum (Saunders & Kraft, 1994; Fredericq et al., 1996; Goff et al., 1996). We define cryptic species as genetically cohesive biological units that have not been recognized as distinct species owing to a perceived (i.e. they differ morphologically, but not in features interpretable and/or recognized by taxonomic experts as species level attributes) or real absence of diagnostic morphological variation.

In northern Europe, P. cartilagineum is the only species currently recognized (Dixon & Irvine, 1977). There is, however, a substantial range in plant size and habit, from small compact morphs to large loosely constructed individuals. This morphological variability raises the distinct possibility that cryptic species are included within the morphological concept of P. cartilagineum within the type region. To resolve the evolutionary affinities and geographic limits of the species we sequenced the partial large subunit (LSU) ribosomal RNA gene for multiple collections of P. cartilagineum from around the world, as well as for many other *Plocamium* spp. We uncovered eight cryptic species within P. cartilagineum sensu *lato*, four of these from northern Europe. We then characterized the European species based on biogeography/ecology, morphology, and anatomy to provide a suite of characters for identification of the taxa in the field, as well as to establish a link between the genetic groups and the type material for species names previously applied to Plocamium in Europe.

#### Materials and methods

Samples were collected as indicated in Table 1 and DNA extracted according to Saunders (1993). The LSU was polymerase chain reaction (PCR) amplified as three overlapping fragments with previously published primer combinations (Harper & Saunders, 2001) for one representative of each 'species' uncovered within the Plocamiaceae, whereas only the Y fragment of Harper & Saunders (ca. 950 bp) was amplified for replicate isolates within each 'species'. The Wizard<sup>TM</sup> PCR preps DNA purification System (Promega, Madison, WI) was used to clean amplified products for subsequent DNA sequencing. Applied Biosystems' (ABI; Foster City, CA) dRhodamine Terminator Cycle Sequencing Ready Reaction Kit was used to sequence the PCR products, and reactions were electrophoresed in an ABI Prism 310 Genetic Analyzer.

The LSU sequences determined here were aligned by eye with the assistance of the computer package SeqPup (Gilbert, 1995). The alignment included sequences from Sarcodia ciliata Zanardini (Withall & Saunders, unpubl.), Sarcodia marginata J. Agardh (Withall & Saunders, unpubl.), Sarcodia sp. (AF419127), and Trematocarpus fragilis (C. Agardh) De Toni (Withall & Saunders, unpubl.) as outgroup taxa (Saunders & Kraft, 2002), a previously published sequence for Plocamium cartilagineum from Ireland (AF419141), and 19 newly determined LSU sequences representing a variety of species/isolates of Plocamium, as well as Plocamiocolax pulvinata Setchell (Table 1). The sequence alignment contained 24 taxa and 2851 sites of which 122 were removed as ambiguously aligned or because they were complementary to the external PCR primer sequence. An appropriate model for this alignment was estimated with the computer program Modeltest (v 3.06; Posada & Crandall, 1998). A general timereversible model with a gamma distribution and invariant sites resulted. Distance analyses were completed in PAUP (v. 4.0b10; Swofford, 2001) using the Modeltest parameters under minimum evolution (random additions = 100, TBR branch swapping). MR. BAYES (v. 2.01; Huelsenbeck & Ronquist, 2001) was used to complete Bayesian inference under the previously determined model. Four Markov chains were used with the temperature set to 0.2, and 10<sup>6</sup> generations were completed with sampling each 100 generations. Log-likelihood values for the sampled trees stabilized around 30000 generations and the final 6000 trees (4000 burn-in) were used to calculate the posterior probabilities. For unweighted parsimony a heuristic approach was used in PAUP with 100 random sequence additions, TBR branch swapping, and all characters treated as unordered (gaps equal missing data). For distance and parsimony analyses bootstrap replicates (2000 with 20 random additions) were assessed as an indicator of robustness (Felsenstein, 1985).

Herbarium-mounted specimens were scanned using an Epson Expression 636 scanner, except for type collections, which were photographed under interior lighting with a Nikon Coolpix 995 digital camera. Vegetative structures were excised, rehydrated, and mounted in 60% Karo in 5% formalin and seawater.

Location Voucher <sup>a</sup>		) Sep	Current name Habitat & Habit <sup>b</sup>		Collector <sup>c</sup>	Date	Group <sup>d</sup>	GenBank <sup>e</sup>	
Denmark		2 26							
Faeroe I.	FI1	1:0	P. cartilagineum (L.) Dixon	Drift	R. Nielson	6 Nov 01	EUR1	T33	
"	FI2	0	P. cartilagineum	Drift; cystocarpic	R. Nielson	6 Nov 01	EUR1	T33	
"	FI3	IA	P. cartilagineum	Drift	R. Nielson	6 Nov 01	EUR1	T33	
"	FI4	na	P. cartilagineum	Drift	R. Nielson	6 Nov 01	EUR1	T33	
"	FI5	on	P. cartilagineum	Drift: tetrasporangial	R. Nielson	6 Nov 01	EUR1	T33	
1	FI6	pint	P. cartilagineum	Drift; tetrasporangial	R. Nielson	6 Nov 01	EUR1	T33	
France		ildi	C						
Cap Blanc Nez	GWS1187	B	P. cartilagineum	Drift	GWS	14 Aug 01	EUR1	T33	
ı <sup>*</sup>	GWS1188	na:	P. cartilagineum	Drift	GWS	14 Aug 01	EUR1	T33	
1	GWS1189	ter	P. cartilagineum	Drift	GWS	14 Aug 01	EUR1	T33	
1	GWS1191	Sis	P. cartilagineum	Drift	GWS	14 Aug 01	EUR1	T33	
1	GWS1192a		P. cartilagineum	Drift	GWS	14 Aug 01	EUR1	T33	
1	GWS1192b	Ξ.	P. cartilagineum	Drift	GWS	14 Aug 01	EUR1	T33	
'	GWS1227	dec	P. cartilagineum	Drift	GWS	14 Aug 01	EUR1	T33	
Cap Griz Nez	GWS 254	oai	P. cartilagineum	Low intertidal epiphytic; cystocarpic	GWS	21 Aug 97	EUR1	AY881708	
Pointe du Grouin	GWS1196	h	P. cartilagineum	Drift: cystocarpic	GWS	15 Aug 01	EUR1	T33	
	GWS1199	õ	P cartilagineum	Drift	GWS	15 Aug 01	EUR1	T33	
1	GWS1200		P cartilagineum	Drift cystocarpic fruticose	GWS	15 Aug 01	EUR1	T33	
	GWS1200		P cartilagineum	Drift epiphytic: tetrasporangial bryozoans	GWS	15 Aug 01	EUR1	T33	
	GWS1201 GWS1202		P cartilagineum	Drift: fruticose portions	GWS	15 Aug 01	EUR1	T33	
,	GWS1202		P cartilagineum	Drift, eniphytic: cystocarnic, fruticose	GWS	16 Aug 01	FUR1	T33	
,	GWS1210		P cartilagineum	Drift, epiphytic; tetrasporangial fruticose	GWS	16 Aug 01	FUR1	T33	
1	GWS1214		P cartilagineum	Drift, epiphytic; tetrasporangial, fruticose	GWS	16 Aug 01	EUR1	T33	
Can Griz Nez	GWS 271		P cartilagingum	Low intertidal rock: evetocarpic	GWS	21 Aug 07	EUR2	T33	
	GWS 272		P cartilagingum	Low intertidal rock: tetrasporangial fruticose portions bryozoans	GWS	21 Aug 97	EUR2	AV881700	
Pointo du Grouin	GWS1102		D cantilagineum	Drift: tatrasporangial	GWS	21 Aug 97	EUR2	T22	
	GWS1193		P cantilagineum	Drift: tetrasporangial frutigosa	GWS	15 Aug 01	EUR2	T 3 3	
1	GWS1194		P. cantilagineum	Drift, tetrasporangial, fruticose	CWS	15 Aug 01	EUR2	T 3 3	
1	GWS1193		P. cartilagineum	Drift, tetrasporangial, fruticose portions	GWS	15 Aug 01	EUR2	135 T22	
1	CWS1197		P. cantilagineum	Drift, aninhyticu tetrasporangial frutioasa partiana hrvazaana	CWS	15 Aug 01	EUR2	T 3 3	
1	GWS1196		P. carillagineum	Drift, epipinyuc, tetrasporangiai, fruncose portions, oryozoans	GWS	15 Aug 01	EUR2	133	
1	GWS1203		P. cartilagineum	Drift, cystocarpic	GWS	15 Aug 01	EUR2	133	
1	GWS1209		P. carillagineum	Drift, epipinyuc; cystocarpic, iruticose portions, bryozoans	GWS	16 Aug 01	EUR2	133	
1	GWS1211		P. cartilagineum	Drift; tetrasporangiai, iruticose portions	GW2	16 Aug 01	EUK2	133	
	GWS1212		P. cartilagineum	Drift; tetrasporangiai, iruticose portions	GW2	16 Aug Ul	EUK2	133	
	GWS1213		P. cartilagineum	Drift; tetrasporangial, fruticose	GWS	16 Aug 01	EUR2	133	
ap Griz Nez	GWS 255		P. cartilagineum	Low intertidal rock	GWS	21 Aug 97	EUR3	AY881710	
	GWS 270		P. cartilagineum	Low intertidal rock	GWS	21 Aug 97	EUR3	133	
ointe du Grouin	GWS1204		P. cartilagineum	Low intertidal rock	GWS	16 Aug 01	EUR3	133	
	GWS1205		P. cartilagineum	Low intertidal rock; slightly fruticose in portions	GWS	16 Aug 01	EUR3	T33	
	GWS1206		P. cartilagineum	Low intertidal rock; slightly fruticose in portions	GWS	16 Aug 01	EUR3	T33	
	GWS1207		P. cartilagineum	Low intertidal rock	GWS	16 Aug 01	EUR3	T33	
	GWS1208		P. cartilagineum	Low intertidal rock	GWS	16 Aug 01	EUR3	T33	

# Table 1. Collection information for formation for the study.

(continued)

	2007							
Table 1. Continued.	mber 2							
Location	voucher	Current name	irrent name Habitat & Habit <sup>b</sup> Collector <sup>c</sup>		Date	Group <sup>d</sup>	GenBank <sup>e</sup>	
Ireland & Northern Ireland	2 26							
Ballangar	GWS1839	P cartilagineum	Low intertidal rock: tetrasporangial	GWS	30 Jul 03	EUR1	Т33	
"	GWS1840	P. cartilagineum	Low intertidal, epiphytic: tetrasporangial, fruticose	GWS	30 Jul 03	EUR1	T33	
Donegal	GWS123	P. cartilagineum	Drift, epiphytic: tetrasporangial	C. Maggs	2 Sept 01	EUR1	T33	
"	GWS123	P. cartilagineum	Drift: tetrasporangial. fruticose portions	C. Maggs	2 Sept 01	EUR1	T33	
Coral Strand	GWS1836	P. cartilagineum	Subtidal, epiphytic: tetrasporangial, fruticose portions	GWS	29 Jul 03	EUR1	T33	
"	GWS183	P. cartilagineum	Subtidal, epiphytic; fruticose	GWS	29 Jul 03	EUR1	T33	
"	GWS183	P. cartilagineum	Drift: tetrasporangial, fruticose	GWS	29 Jul 03	EUR1	T33	
Mullaghmore Head	GWS181	P. cartilagineum	Low intertidal rock: tetrasporangial, fruticose portions	GWS	28 Jul 03	EUR1	T33	
"	GWS182	P cartilagineum	Drift epiphytic cystocarpic thick	GWS	28 Jul 03	EUR1	T33	
Portaferry	GWS178	P. cartilagineum	Subtidal, epiphytic: tetrasporangial	GWS	24 Jul 03	EUR1	T33	
"	GWS180	P cartilagineum	Subtidal rock: tetrasporangial bryozoans	GWS	24 Jul 03	EUR1	T33	
"	GWS1803:	P cartilagineum	Subtidal rock tetrasporangial	GWS	24 Jul 03	EUR1	T33	
Spiddal	GWS182	P cartilagineum	Drift epiphytic tetrasporangial & cystocarpic thick bryozoans	GWS	29 Jul 03	EUR1	T33	
"	GWS182	P. cartilagineum	Drift, epiphytic; tetrasporangial, bryozoans	GWS	29 Jul 03	EUR1	T33	
"	GWS1836	P. cartilagineum	Drift: cvstocarpic	GWS	29 Jul 03	EUR1	T33	
Donegal	GWS123	P cartilagineum	Drift: cystocarpic bryozoans	C Maggs	2 Sept 01	EUR2	T33	
Mullaghmore Head	GWS1816	P. cartilagineum	Drift, epiphytic: tetrasporangial, bryozoans	GWS	28 Jul 03	EUR2	T33	
Spiddal	GWS1826	P cartilagineum	Drift epiphytic : cystocarpic bryozoans	GWS	29 Jul 03	EUR2	T33	
"	GWS1827	P. cartilagineum	Drift, epiphytic: cystocarpic, fruticose, bryozoans	GWS	29 Jul 03	EUR2	T33	
"	GWS1828	P cartilagineum	Drift epiphytic: cystocarpic, fruticose portions, bryozoans	GWS	29 Jul 03	EUR2	T33	
Doaghbeg Co Donegal	GWS1754	P cartilagineum	Intertidal rock	C Maggs	9 Mar 03	EUR4	T33	
St. John's Pt. Co. Down	GO167	P cartilagineum	Intertidal rock	C. Maggs	14 Nov 93	EUR4	AF419141	
"	MAGGS1	P cartilagineum	Intertidal rock	C. Maggs	24 Mar 02	EUR4	T33	
Scotland (Oban)	SCT1	P cartilagineum	Subtidal	T. Chopin	12 Oct 01	EUR1	T33	
"	SCT2	P cartilagineum	Subtidal	T. Chopin	12 Oct 01	EUR1	T33	
"	SCT3	P cartilagineum	Subtidal	T. Chopin	12 Oct 01	EUR1	T33	
"	SCT4	P cartilagineum	Subtidal	T. Chopin	12 Oct 01	EUR1	T33	
Norway	GWS1234	P cartilagineum	Cystocarpic	I Rueness	31 Aug 01	EUR1	T33	
Australia	0.00120.		cystocal pro		or ring or	2010	100	
Queenscliff	GWS1564	P angustum (I Agardh)	Drift	GWS	7 Dec 02		AY881711	
2	0.00000	J.D. Hooker <i>et</i> Harvey		0.15	, 200 02			
"	GWS926	P cartilagineum	Drift	GWS	4 Dec 00	AUS1	AY881712	
"	GWS1583	P cartilagineum	Drift	GWS	8 Dec 02	AUSI	T33	
Point Lonsdale Reef	GWS955	P cartilagineum	Subtidal	GWS	10 Dec 00	AUS2	AY881713	
Bicheno	GWS1471	<i>P</i> cirrhosum (Turner)	Subtidal on rock	GWS	26 Nov 02	110.02	AY881714	
bieneno	0.0011/1	M.J. Wynne	Sublidui on rock	6115	201107-02		111001/11	
Lord Howe I.	GWS1025	P. hamatum J. Agardh	Subtidal on coral	GWS	11 Mar 01		AY881715	
Port Elliot	GWS446	P. leptophyllum Kützing	Subtidal	GWS & G.T. Kraft	29 Oct 98		AY881716	
Queenscliff	GWS928	P. mertensii (Greville) Harvey	Drift	GWS	4 Dec 00		AY881717	
Warrnambool	GWS938	P. patagiatum J. Agardh	Drift	GWS	6 Dec 00		AY881718	
"	GWS937	P. preissianum Sonder	Drift	GWS	6 Dec 00		AY881719	
Lord Howe I.	GWS1038	Plocamium. sp.	Subtidal on cobble	GWS	12 Mar 01		AY881720	

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	2007							
BC Canada	ber							
Wizard Chan	KVL 72 B	Plocamiocolax	Dredged 20 m	KVL	9 May 01		AY881721	Ĵ
Wizara Chan.	br 1	nulvinata Setchell	Diedged 20 m	IT V L	<i>y</i> 1010y 01		111001/21	Ψ.
Bradys Beach	KVLIIK	P cartilagineum	Dredged	KVL	23 May 01	EPAC	AY881722	oti
"	KVL 112	P. cartilagineum	Dredged	KVL	23 May 01	EPAC	T33	<i>c</i> ]
Cape Beale	KVL 120	P cartilagineum	Exposed rock	GWS	6 June 01	EPAC	T33	20
"	KVL 12P	P. cartilagineum	Exposed rock	GWS	6 June 01	EPAC	T33	š
"	KVL 12	P. cartilagineum	Exposed rock	GWS	6 June 01	EPAC	T33	m
"	KVL 123	P. cartilagineum	Exposed rock	GWS	6 June 01	EPAC	T33	ЦЦ
Wizard Chan.	KVL 65	P. cartilagineum	Dredged 20 m	KVL	9 May 01	EPAC	T33	m
"	KVL 67 🖳	P. cartilagineum	Dredged 20 m	KVL	9 May 01	EPAC	T33	IS
"	KVL 68 🔒	P. cartilagineum	Dredged 20 m	KVL	9 May 01	EPAC	T33	dc
"	KVL 69 🛱	P. cartilagineum	Dredged 20 m	KVL	9 May 01	EPAC	T33	·
"	KVL 70 🖉	P. cartilagineum	Dredged 20 m	KVL	9 May 01	EPAC	T33	
"	KVL 71 b	P. cartilagineum	Dredged 20 m	KVL	9 May 01	EPAC	T33	
Dixon I.	KVL 50 5	P. violacium Farlow	Exposed rock	KVL	8 May 01		T33	
"	KVL 51 5	P. violacium	Exposed rock	KVL	8 May 01		T33	
"	KVL 52 🙆	P. violacium	Exposed rock	KVL	8 May 01		T33	
"	KVL 53	P. violacium	Exposed rock	KVL	8 May 01		T33	
"	KVL 54 🖁	P. violacium	Exposed rock	KVL	8 May 01		T33	
"	KVL 55 🛓	P. violacium	Exposed rock	KVL	8 May 01		T33	
"	KVL 56 6	P. violacium	Exposed rock	KVL	8 May 01		T33	
"	KVL 57	P. violacium	Exposed rock	KVL	8 May 01		T33	
"	KVL 58	P. violacium	Exposed rock	KVL	8 May 01		T33	
Seppings I.	KVL 113	P. violaceum	Exposed rock	KVL	25 May 01		T33	
"	KVL 114	P. violaceum	Exposed rock	KVL	25 May 01		T33	
"	KVL 115	P. violaceum	Exposed rock	KVL	25 May 01		T33	
"	KVL 116	P. violaceum	Exposed rock	KVL	25 May 01		AY881723	
"	KVL 117	P. violaceum	Exposed rock	KVL	25 May 01		T33	
"	KVL 118	P. violaceum	Exposed rock	KVL	25 May 01		T33	
"	KVL 119	P. violaceum	Exposed rock	KVL	25 May 01		T33	
Oregon, USA								
Indian Beach	KVL 103	P. cartilagineum	Exposed rock	GWS	14 May 01	EPAC	T33	
	KVL 100	P. oregonum Doty	Exposed rock	GWS	14 May 01		T33	
"	KVL 101	P. oregonum	Exposed rock	GWS	14 May 01		AY881724	
	KVL 105	P. oregonum	Exposed rock	GWS	14 May 01		T33	
	KVL 106	P. oregonum	Exposed rock	GWS	14 May 01		T33	
	KVL 107	P. oregonum	Exposed rock	GWS	14 May 01		T33	
"	KVL102	P. violaceum	Exposed rock	KVL	14 May 01		T33	
Cheju I., Korea	011	D 16 1 1	COUDA 10		10 7			
Chagwido,	CH172	P. telfairiae	SCUBA 10 m	E.S. Bae, Y.S. Oh & H-G. Choi	18 Jan 01	WPAC	AY881725	
		(W. Hooker <i>et</i> Harvey)						
N 1	01177	Harvey in Kützing	CCLIDA 5		10 1 01		<b>T</b> 22	
Marado "	CH175	P. telfairiae	SCUBA, 5m	E.S. Bae, Y.S. Oh & H-G. Choi	19 Jan 01		133	
Ionon (Ilinochime)	CH1/0	P. tetjatriae	SUUBA, 3 m	E.S. Bae, Y.S. Un & H-G. Choi	19 Jan Ul	WDAC	AY881/26	
Japan (Hirosnima)	UK119	r. cartuagineum	Intertidal	J.п. Оак & Y.S. Keum	so june 00	WPAC	133	

<sup>*a*</sup>Vouchers are in the phycological collections of Saunders at UNB. <sup>*b*</sup>Habit data provided for European species only. <sup>*c*</sup>GWS = G.W. Saunders, KVL = K.V. Lehmkuhl. <sup>*d*</sup>LSU defined 'species'. <sup>*e*</sup>GenBank number indicates that the LSU was completed for that collection (LSU for G0167 was obtained from GenBank), T33 indicates that only the hypervariable region was sequenced.

Where necessary to reveal anatomical detail, material was stained with 1% aniline blue (6.3% acetic acid) prior to mounting. Photomicrographs were taken using a Leica DFC480 digital camera mounted on a Leica DM5000B light microscope and edited with Photoshop<sup>TM</sup> on a Macintosh computer.

### **Results & discussion**

#### Molecular investigations

A hypervariable region of the LSU (primer T33, Y fragment; Harper & Saunders, 2001) was sequenced for: 86 isolates of P. cartilagineum (69 European, 3 Australian, 1 northwest Pacific, and 13 northeast Pacific plants); isolates of the following additional Plocamium spp.: P. angustum (J. Agardh) Hooker & Harvey (n = 1), P. cirrhosum (Turner) M.J. Wynne (n=1), P. hamatum J. Agardh (n=1), P. leptophyllum Kützing (n=1), *P. mertensii* (Greville) Harvey (n = 1), *P. oregonum* Doty (n=5), *P. patagiatum* J. Agardh (n=1), *P. preissianum* Sonder (n = 1), *Plocamium* sp. (n = 1); Lord Howe Island species of Saunders & Kraft, 1994), P. telfairiae (W. Hooker & Harvey) Harvey (n=3), and *P. violaceum* Farlow (n=17); and Plocamiocolax pulvinata. If the partial sequence was identical to an LSU sequence already in our database the individual was assigned to that 'species'. Each time a novel sequence for the primer T33 was acquired, the remaining LSU region was determined for that individual and an additional 'species' was available for comparison with subsequent partial sequences. Using this approach 20 'species' were uncovered, eight of which were initially assigned to *P. cartilagineum*. The European isolates of *P. cartilagineum* fell into four groups, and we have tentatively designated these P. cartilagineum EUR1 (n = 42), P. cartilagineum EUR2 (n=17), P. cartilagineum EUR3 (n=7), and P. cartilagineum EUR4 (n=3). Three Australian isolates of P. cartilagineum (P. cartilagineum AUS1 (n=2), P. cartilagineum AUS2) form the fifth and sixth groups, and thirteen northeast Pacific isolates (P. cartilagineum EPAC) formed a seventh group. The northwest Pacific isolate of P. cartilagineum was not associated with any other groups of P. cartilagineum and was identical in LSU sequence to one isolate of P. telfairiae (we designate this group P. cartilagineum WPAC). Two other isolates of *P. telfairiae* have an LSU sequence distinct from P. cartilagineum WPAC. The remaining eleven groups were equivalent to P. pulvinata and ten additional species of *Plocamium*, which are listed above.

Using PAUP, pair-wise nucleotide differences were calculated for the 20 groups in the multiple alignment considering all substitutions, deletions, and insertions. Interspecific differences varied from 10 nucleotide changes (P. patagiatum and P. mertensii) to 89 nucleotide changes (P. preissianum and P. violaceum). Surprisingly, the European groups of *P. cartilagineum* were among the most divergent (26-45 nucleotide differences), values greater than those observed between some genera in other florideophyte families (e.g. Harper & Saunders, 2002). The two Australian isolates of P. cartilagineum were more divergent in their LSU than two endemic species, P. patagiatum and P. mertensii (30 versus 10 nucleotide differences, respectively). The northeast Pacific isolates of *P. cartilagineum* are generally more similar in LSU sequence to other northeast Pacific species (P. violaceum, P. oregonum, and P. pulvinata) than to geographically remote isolates of P. cartilagineum.

One representative from each of the 20 groups was included in an LSU alignment for minimum evolution distance, parsimony, and Bayesian inference analyses. Fig. 1 presents the minimum evolution distance tree with superimposed distance (upper) and parsimony (mid) bootstrap values (2000 replicates with 20 random additions), and Bayesian inference posterior probability values (lower). Unweighted parsimony resulted in a single most parsimonious tree with a length of 568 (consistency index = 0.695, retention index = 0.781) with a similar topology to distance and Bayesian analyses. Plocamium cartilagineum EUR1 and EUR4, although relatively divergent, formed a lineage distinct from the other isolates, whereas EUR2 and EUR3 formed a grade to the four species of the Plocamiaceae in the northeast Pacific (Fig. 1), which included P. cartilagineum (EPAC) from this area. *Plocamium cartilagineum* (WPAC) joined P. hamatum and P. preissianum with moderate support, while AUS1 and AUS2 associated with P. angustum and P. cirrhosum isolates, also from Australia.

### Plocamium in northern Europe

The 69 northern European isolates of *Plocamium* resolved as four groups, each represented by a distinct LSU sequence, despite the fact that *P. cartilagineum* is the only species currently recognized in the flora. These data were confirmed by relatively high levels of divergence in the plastid *rbc*L gene as well (data not shown). There have been indications that *P. cartilagineum* is not cosmopolitan based on sequences obtained by a number of groups investigating other questions (Saunders & Kraft, 1994; Fredericq *et al.*, 1996; Goff *et al.*, 1996). Our data support this notion and provide the first published evidence for multiple species in northern Europe. It would be clearly useful to have a series of characters that

Cryptic Plocamium spp.



0.005 substitutions/site

Fig. 1. Minimum evolution distance tree with superimposed distance (upper) and parsimony (mid) bootstrap values (2000 replicates with 20 random additions), and Bayesian inference probability values (lower).

unambiguously distinguish between these four entities. Diagnostic characters are also needed to assist in matching the genetic groups with previously described taxa, so that appropriate specific epithets can be assigned and synonyms clarified. In the light of our molecular data, we can gain a retrospective understanding of each genetic group. Characters such as biogeography, ecology and habitat, as well as morphological features are particularly attractive because they do not require detailed microscopic analyses to make identifications. We also completed anatomical investigations to determine whether there are differences among the groups that may have signaled the diversity that has become evident through molecular analyses, and to provide additional defining characters. Through this exercise we have successfully identified a series of morphological and anatomical features to distinguish the four species, although non-tetrasporangial isolates of EUR1 and EUR2 remain difficult to distinguish.

### EUR1 = *Plocamium cartilagineum* (L.) Dixon *sensu stricto*

BASIONYM: *Fucus cartilagineus* Linnaeus 1753: 151. LECTOTYPE: Leiden (Herb. Lugdb. Bat. 910.184.14).

TYPE LOCALITY: "in Oceano australiore" (more likely northern Europe *fide* Dixon, 1967).

### Synonyms:

Fucus plocamium Gmelin 1768: 153 nom. illegit.

Fucus coccineus Hudson 1778: 586 nom. illegit.

Plocamium vulgare Lamouroux 1813: 50 nom. illegit.

Plocamium coccineum Lyngbye 1819: 39 nom. illegit.

*Plocamium coccineum* var. *subtile* Lyngbye 1819: 39.

Delesseria plocamium C. Agardh 1822: 181 nom. illegit.

Plocamium lyngbyanum Kützing 1843: 450.

Plocamium binderianum Kützing1843: 450.

DISTRIBUTION: Throughout northern Europe.

REPRESENTATIVE SPECIMENS: Listed in Table 1 (EUR1).

**OBSERVATIONS:** *Plocamium cartilagineum* EUR1 (Figs 2-14) is the most widely distributed species and was collected throughout northern Europe, generally subtidal and epiphytic on other algae (Table 1). A few exceptions were collected in the lowest intertidal on algae and rock, but in the latter cases the possibility of an algal turf being the actual substratum cannot be excluded. Thalli are loosely but richly branched and usually red to pink in colour. It is the largest species of *Plocamium* in northern Europe, with plants reaching 10-12 cm in height and some main axes 1-2 mm in width (Fig. 2), but with considerable variability in size. The ramuli alternate in series of (2)3-4(5) and can be strongly incurved near the growing tips, although the lowermost ramulus in each series (quiescent indeterminate axes) can ultimately reflex outwards acquiring a slight sigmoidal shape. As is common in *Plocamium*, there is a tendency for the terminal ramulus in a series to develop into an indeterminate branch, and for some of the subordinate ramuli to undergo lesser degrees of development. However, EUR1 has a common tendency for some of the subordinate ramuli (not necessarily in serial order) to undergo development equivalent to, or greater than, the terminal ramulus, and at times the apparent basal ramulus of a series (supposed quiescent leading axis from the subtending series) can also develop an indeterminate branch. This is an artifact that derives from the terminal ramulus in a series becoming dominant and displacing the leading indeterminate axis to the side, which, when combined with erosion of the unbranched basal ramulus, gives the appearance that the basal lateral in the series is highly developed (Figs 3-4). The previous developmental attributes are present throughout basal and distal thallus regions in EUR1 and impart fuller branching to specimens of P. cartilagineum than observed in the other European species. Apical cells are generally rounded and cut off subtending cells that can initiate cortication in the first cell file (Fig. 5). In the leading indeterminate axis, the transverse periaxial cells initiate divisions in the third to fourth cell file, but retain an orthostichous appearance until the 8-12<sup>th</sup> cell file (Fig. 5). The cell files initially display a chevron appearance, which is evident from the second to eighth (or slightly more) cell file, the cortication masking the central axial filament throughout the axes (Fig. 5). In surface view (mid-thallus at the base of the first series of ramuli) the main axis has incomplete cortication with a mix of larger inner and smaller outer cortical cells evident (Fig. 6). In cystocarpic specimens, developing procarps are widely distributed along the abaxial and adaxial margins and the resulting cystocarps are sessile. Tetrasporangial stichidia are occasionally simple but more typically compound, and replace ramuli in the series (Fig. 7), as well as forming adventitiously in axils on some plants. In some thalli the majority of ramuli, as well as their growing tips, will convert to stichidial development resulting in truncated branches and yielding fruticose plants (Fig. 8). The extent of this development varies widely within and between tetrasporangial plants and can provide morphologies that are rather atypical of this species (Fig. 8). In some plants truncated or underdeveloped ramuli appear to form in the absence of stichidial development, with the tips appearing dark and assuming a gall-like appearance (Fig. 9). It is possible that this is in response to a pathogen, and where this occurs in combination with tetrasporangial development highly fruticose plants result (Fig. 8), which can produce unusually complex stichidia in lieu of typical branches (Fig. 10). Thus the fruticose morphology associated with *Plocamium* in Europe seems to be the result of tetrasporangial and/or pathogen-affected development of ramuli and stichidia in EUR1. Further, GWS001202 and GWS001837 (overall fruticose morphology, no obvious reproduction), and GWS001200 and GWS001210 (fruticose with cystocarps) all exhibit greyish tips with gall-like growths indicating that this phenomenon is not confined to tetrasporangial development, supporting pathogen involvement. Tetrasporangia are 37-60 µm in length when viewed in section (Fig. 11), relatively small compared to EUR2.

COMMENTS: Linnaeus (1753) described *Fucus cartilagineus* in his *Species Plantarum* and apparently intended the name to refer to an assemblage with a cosmopolitan distribution because he includes a citation of Guettard (1747) that reported a European (English Channel and Atlantic coasts) distribution for the taxon, but lists *Oceano australiore* or southern seas as the distribution. Our investigations certainly dispute the cosmopolitan distribution for *P. cartilagineum sensu lato*,

Cryptic Plocamium spp.



**Figs 2–7.** Morphology and anatomy of *Plocamium cartilagineum* EUR1 (GWS000254 unless noted). Fig. 2. Gross morphology of a cystocarpic specimen. Centimetre rule. Figs 3, 4. In normal development (Fig. 3) the leading axis of a series (*a*) becomes quiescent and is overtopped by the uppermost ramulus, which continues as the indeterminate axis (*b*). The terminal ramulus in the subtending series (*c*) generally undergoes considerable development with subtending ramuli (e.g., *d*) undergoing progressively less development. This pattern occurs in EUR1, but commonly subordinate ramuli can develop equal to or greater than the terminal ramulus. More interestingly, in EUR1 (Fig. 4) there is a tendency for the terminal ramuli of a series (*c*) to dominate development over the indeterminate axis (*b*), displacing the latter to the side, which, with the erosion of '*a*', gives the appearance of a highly developed basal ramulus in a series. What was a subordinate ramulus (*d*) continues to develop and appears terminal in its series. The previous processes combine to produce a more richly branched thallus for EUR1. Scale = 3 mm. Fig. 5. Morphology of the growing tip of a leading axis. Scale = 12.5 µm. Fig. 6. Surface view of cortex mid-axis below the first series of alternating ramuli at a growing tip. Scale = 20 µm. Fig. 7. Compound stichidia replacing ramuli in a tetrasporangial isolate (GWS001235). Scale = 80 µm.



Figs 8-14. Morphology and anatomy of Plocamium cartilagineum EUR1 (GWS001838 unless noted). Fig. 8. Fruticose morphology associated with, or in this case dominating the appearance of, tetrasporangial isolates of EUR1. Centimetre rule. Fig. 9. Truncated ramuli that had a greyish hue and terminated in atypical rounded or gall-like tips (arrows). Scale = 160 µm. Fig. 10. A complex stichidium with the greyish hue and blunt apices discussed previously, atypical for EUR1 overall, but common in the fruticose morphology. Scale = 320 µm. Fig. 11. Tetrasporangial stichidium in section with mature zonate tetrasporangia. Scale = 40 µm. Fig. 12. Digital photograph of lectotype collection from Leiden. Centimetre rule. Fig. 13. Rehydrated tip from lectotype with the EUR1 chevron pattern and an orthostichous pattern to the transverse cortical derivatives. Scale = 12.5 µm. Fig. 14. Rehydrated lectotype in surface view, mid-axis, below the first series of alternating ramuli. Scale =  $20 \,\mu m$ .

IC1 CM

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ilo

.H.

Cacini

pinnatis, laciniis linearibus coloratis: Roy Theoides rubens varie diffection . Ray syn. 3. 37.1!

and it would thus appear that Linnaeus' concept must be confined to a species in either the Northern or Southern Hemisphere. Linnaeus' original treatment is based on the phrase-name of Van Royen (1740), referable to a single collection 1967; designated lectotype) in (Dixon, the Rijksherbarium, Leiden (Herb. Lugdb. Bat. 910.184.14), which unfortunately lacks geographical details. The complication here is that Van Royen's flora included both Dutch and exotic material (cf. Dixon, 1967). Despite the uncertainty as to the origin of Van Royen's collection, Dixon (1967) argued that Linnaeus confused the type locality for Fucus cartilagineus and concluded that the lectotype is from northern Europe, not the southern seas. Dixon's conclusion stems largely from Linnaeus' citation of the works of Ray (1724) and Guettard (1747) in his description of Fucus cartilagineus, which indicated a distribution along the English Channel and Atlantic coasts of France, respectively. We have viewed the lectotype sheet. The thalli are similar to EUR1 and EUR2 in gross morphology, more akin to the former in being branched extensively along the entire thallus (Fig. 12), rather than sparsely branched in the lower portions as in EUR2. They were host to bryozoans and a crustose coralline alga, which we observed commonly on EUR1 and EUR2, consistent with Dixon's conclusion on their geographical origin. The plants appear to show fruticose morphology in parts, although this is more the result of poor pressing than any of the phenomena discussed here, but a few axes were truncated with slightly rounded (gall-like) tips, as noted for EUR1 (Fig. 9). In rehydrated material, a chevron pattern was evident, there was a strong orthostichous pattern of the transverse periaxial derivatives (Fig. 13), and the cortication was loose and incomplete on the main axes near the base of the first series of ramuli (Fig. 14), observations again matching EUR1. Interestingly, Kützing (1866) clearly illustrated compound stichidia for P. cartilagineum (as P. coccineum) and differentiated this from his P. subtile (considered equivalent to EUR2, discussed below), which has simple stichidia in his drawings (cf. Kützing 1866; pl. 41, 42). He also documented (Kützing 1866; pl. 41) the gall-like tips for P. cartilagineum under P. coccineum var. tenue.

It is generally accepted that S. G. Gmelin's herbarium no longer exists (Dixon, 1960; Dixon & Irvine, 1970; cf. Wynne, 2002). We were therefore unable to access the material upon which our first synonym (*F. plocamium*) was based. Careful inspection of the drawing of *Fucus plocamium* in Gmelin (1768) suggests that perhaps he is depicting our EUR3, although his plants are reportedly too

large and more referable to EUR1 in this regard. Regardless, Gmelin included Linnaeus' epithet and description in his account rendering F. plocamium a superfluous and illegitimate epithet. As a consequence, all taxa that have been subsequently based on Fucus plocamium are also illegitimate. This is the case with *Plocamium vulgare* Lamouroux, a change in specific epithet introduced to avoid a tautology when Lamouroux erected Plocamium on the basis of F. plocamium, and Delesseria plocamium C. Agardh. The situation surrounding Hudson's various applications of *Fucus cartilagi*neus and Fucus coccineus is complex (cf. Dixon, 1967). Most significantly, Hudson (1778) included a reference to Gmelin's F. plocamium in his description rendering F. coccineus a superfluous and illegitimate name. This was earlier placed in synonymy with P. cartilagineum (cf. Dixon, 1967). *Plocamium coccineum* Lyngbye is based on F. *coccineus* and is, therefore, also an illegitimate synonym of P. cartilagineum.

We also recognize P. binderianum and P. lyngbyanum as synonyms based on Kützing's (1843) descriptions and our observations of the respective type material (cf. Wynne, 2002). For *P. binderianum*, the type locality (Helgoland) is in agreement with our distribution range for P. cartilagineum. Although the tetrasporangial stichidia have the tendency to be recurved in the type material (Kützing, 1843), they are usually complex and replace ramuli or develop adventitiously in axils. In addition the colour, relatively flattened main axes, presence of truncated branches with gall-like tips, and the lack of recurved vegetative branches are reasons to include this taxon as a synonym of P. cartilagineum. In P. lyngbyanum, the thallus size (up to 19 cm), collection locality (Faeroe Islands), broad flattened axes, extensive development of subordinate laterals and displacement of leading axes in lower thallus regions, and lack of distinctly aculeate ramuli (characteristic of *P. uncinatum* (see EUR2 below)) justify synonymy of this taxon with *P. cartilagineum*. We have not observed the type material of P. coccineum var. subtile Lyngbye, but considering the type location (Faeroe Islands) this variety is best considered a synonym of P. cartilagineum. Wynne (2002) listed additional synonyms of Southern Hemisphere distribution including Р. brachiocarpum Kützing locality (type New Zealand), P. confervaceum Bory de Saint-Vincent (type locality Chile), P. cruciferum Harvey in Hooker f. (type locality Antarctica), and pusillum Sonder (type locality Australia). Р. Based on our results we consider that these taxa are probably not synonyms of the Northern Hemisphere P. cartilagineum and are available, where applicable, for cryptic species in the

Southern Hemisphere following further investigation (see below).

EUR2 = *Plocamium subtile* Kützing

BASIONYM: *Plocamium subtile* Kützing 1866: 15, pl. 42.

TYPE LOCALITY: "In mari atlantico." Probably northwest coast of France; type indicates St. Malo. Synonyms:

Delesseria plocamium var. uncinatum C. Agardh 1822: 181.

*Plocamium coccineum* var. *subtile* Kützing 1843: 449. *nom. illegit.* (non *Plocamium coccineum* var. *subtile* Lyngbye)

Plocamium coccineum var. uncinatum (C. Agardh) Kützing 1849: 884. nom. illegit.

*Plocamium uncinatum* (C. Agardh) Kützing 1866: 16.

*Plocamium cartilagineum* (Linnaeus) P. Dixon var. *uncinatum* (C. Agardh) Guiry ex Benhissoune, Boudouresque, Perret-Boudouresque et Verlaque 2002: 398,

*Plocamium cartilagineum* (Linnaeus) P. Dixon var. *uncinatum* (C. Agardh) M.J. Wynne 2002: 350. *nom. illegit.* 

DISTRIBUTION: Thus far known with certainty only from the northern coast of France and Ireland; possibly Helgoland, Germany.

REPRESENTATIVE SPECIMENS: Listed in Table 1 (EUR2).

**OBSERVATIONS:** Plocamium subtile EUR2 (Figs 15-27) was collected predominantly from the north coast of France, but also from various locations around Ireland. Because these are the only two areas for which we have reasonable numbers of samples, it is possible that EUR2 is more widely distributed on European coasts. It was most commonly collected in the drift without any attachment to an algal substratum, and as attached plants on rocks in the low intertidal (Table 1) distinguishing it weakly from EUR1. However, at least a few individuals from the drift were unequivocally epiphytic, particularly the Irish plants. There was a noteworthy trend in the presence/absence of bryozoans growing on the thalli assigned to the various groups with bryozoans obvious on eight of 17 isolates for EUR2, but only on 4 of 42 collections of P. cartilagineum EUR1. Whether the infestation of bryozoans was due to the habitat in which the various isolates were living, or whether there is a specific affinity by the animals for a particular species of *Plocamium* requires further investigation.

*Plocamium subtile* (Fig. 15) is generally darker red in colour with a tendency for pressed plants to retain a more terete aspect to their main axes, which are dark red to black. The thalli are generally smaller than observed for *P. cartilagineum*, ranging from 6-10 cm when reproductive, but a few of our collections have axes up to 12 cm in length. Ramuli alternate in series from (1)2-5 with some plants having a particularly sparse appearance owing to a predominance of series at the lower end of this range (Fig. 16). In P. subtile, the terminal ramuli commonly (more so toward the distal portions of a plant) formed indeterminate axes, while the proximal ramuli in a series were less likely to develop and were typically subordinate to the former if they did. Furthermore, the tendency for the terminal or subordinate ramuli in a series to displace and overtake the leading indeterminate axis, as documented in P. cartilagineum (Figs 3-4), also occurred rarely in *P. subtile*. The resultant thalli were generally more sparsely branched, having a more obvious distichous habit of pectinately arranged ramuli and more distinct percurrent axes (Fig. 15). Ramuli are moderately to strongly incurved near the growing tips, the lowermost in each series tending to straighten with further development, but generally failing to reflex as in P. cartilagineum, and thus having a less sigmoidal aspect. However, the basal ramulus (as well as others) in a series can become highly reflexed and acquire a hooked shape (Fig. 17) contributing to a fruticose morphology in some thalli.

Apical cells were generally rounded, occasionally acute, and cut off subtending cells that initiate cortication in the first cell file (Fig. 18). Development of the transverse periaxial cells was observed in the third to fourth cell file, but these derivatives were soon masked in the overall cortication of the thallus surface generally failing to produce an obvious orthostichous pattern (Fig. 18). Additionally, the cell files were closely compacted and, in combination with the greater cortication, resulted in the chevron pattern observed in P. cartilagineum being less apparent in this species (Fig. 18). The surface cortication at the base of the first series of ramuli was more developed in *P. subtile* with the surface displaying a more uniform appearance (Fig. 19). A feature of considerable irregularity, both within and among isolates of *P. subtile*, was the occurrence of sporadic to regularly spaced serrations on the abaxial (rarely adaxial) margins of the basal (occasionally other lower) ramuli (Fig. 20). This feature was not confidently observed in any of the P. cartilagineum isolates. As noted for P. cartilagineum, many isolates of P. subtile displayed the fruticose habit with thicket-like branches (Table 1) in distal thallus regions, or, in the case of this species, substantially across the thallus (Fig. 21). The fruticose morphology was not as tightly correlated with tetrasporangial isolates in this species (Table 1), and also differed ontogenetically.

TYPE: Leiden.

Cryptic Plocamium spp.

Figs 15-20. Morphology and anatomy of Plocamium subtile EUR2 (GWS001198 unless noted). Fig. 15. An example of P. subtile displaying the relatively sparse branching in lower thallus regions, more regular distichous habit and pectinate branching, and percurrent nature of the major axes. Centimetre rule. Fig. 16. An atypical representative (GWS001203) of this species with sparse branching and spinose appearance owing to a predominance of series alternating with one or two ramuli. Centimetre rule. Fig. 17. Strongly curved and elongated lower ramulus characteristic of thalli (GWS001195) in this species with a fruticose or thicket-like habit. Scale =  $320 \,\mu m$ . Fig. 18. Growing tip displaying the compact cell files, and absence of a distinct chevron pattern and orthostichous persistence of the transverse cortical derivatives. Scale = 20 µm. Fig. 19. Cortical surface at base of first series of ramuli. Cells are highly compact and relatively uniform in size. Scale =  $20 \,\mu m$ . Fig. 20. Serrations along the abaxial margin in an isolate (GWS000271) of EUR2. Scale =  $80 \,\mu m$ .





**Figs 21–27.** Morphology and anatomy of *Plocamium subtile* EUR2. Fig. 21. An example of *P. subtile* (GWS001827) displaying the fruticose habit. Centimetre rule. Fig. 22. In some fruticose isolates of EUR2 (e.g., GWS001827) adventitious development of marginal serrations (and other adventitious growth as well, see Fig. 17) contributed greatly to the thallus habit. Scale =  $160 \mu m$ . Fig. 23. Typical tetrasporangial stichidia in an isolate of *P. subtile* (GWS001211) derived by conversion of ramuli directly into simple structures. Scale =  $160 \mu m$ . Fig. 24. 'Complex' stichidia were usually obvious variations on the typical simple development characteristic of this species (GWS001212). Scale =  $160 \mu m$ . Fig. 25. Section through a stichidium with mature zonate tetrasporangia (GWS001212). Scale =  $40 \mu m$ . Fig. 26. Digital photograph of the type for *P. subtile* from Kützing's collections in Leiden. As noted on the sheet, this was the collection used in the diagrams included by Kützing (1866, pl. 42) in describing this new species and the collection location is indicated as St. Malo, consistent with the distribution for EUR2. Centimetre rule. Fig. 27. A close-up of the simple tetrasporangial stichidia replacing ramuli that characterize this species (type). Scale = 2 mm.

In some isolates this was simply a matter of distal thallus regions displaying increased development in subordinate ramuli of the series and these at times overtopping leading indeterminate axes, as is common across entire thalli in P. cartilagineum. However, this developmental aspect was coupled with a second phenomenon not observed in isolates of *P. cartilagineum*, which resulted in highly fruticose thalli (Fig. 21). In these plants the basal ramuli (as well as other laterals) of a series elongate and develop an aculeate morphology and/or their serrations would undergo adventitious development (coupled with other adventitious growth) to produce convoluted branches and spines (Fig. 22). A pathogenic component was not apparent in this development for our collections but cannot be excluded.

Cystocarps were sessile and distributed along the abaxial and adaxial thallus margins as in *P. cartilagineum*. The tetrasporangial stichidia were derived directly from converted ramuli and their growing tips (adventitious stichidia were not noted) and were almost exclusively simple (Fig. 23). Only in cases where the uppermost lateral and the growing tip underwent almost synchronous conversion was the appearance of compound stichidia manifest, but the derivation of this as an aggregate of simple stichidia was almost always apparent (Fig. 24) in P. subtile. Juvenile compound stichidia, as commonly observed for P. cartilagineum, were not observed. Lastly, in section the in situ tetrasporangia ranged from 60 to 90 µm in length (Fig. 25), substantially larger than those observed in the previous species.

COMMENTS: At the species level P. subtile and *P. uncinatum* have equal priority. The latter is based on Delesseria plocamium var. uncinatum C. Agardh. The type collection (Lund; Typus 27915) has a vegetative habit consistent with small fruticose samples of EUR2, the ramuli branch from the main axis at markedly wide angles and are distinctly recurved and tapered, and comes from the northern coast of France. Unfortunately, the vegetative attributes are variable within and between collections, and species, in northern Europe. *Plocamium subtile*, also described by Kützing in 1866, agrees well in vegetative detail (Fig. 26) with EUR2 and is from the northern coast of France, but additionally has clearly visible reproductive features (Fig. 27). The most useful attribute for distinguishing P. cartilagineum from *P. subtile* is the predominantly compound rather than simple stichidia, respectively (Fig. 27), in tetrasporangial isolates. These differences are clearly shown in Kützing (1866; cf. pl. 41, 42) and associated with the respective species in that work. For these reasons P. subtile is selected over *P. uncinatum.* It is important to establish that Kützing (1866) did not consider *P. subtile* as an elevation of his earlier illegitimate *Plocamium coccineum* var. *subtile* Kützing (1843) (non *Plocamium coccineum* var. *subtile* Lyngbye), for which he listed the type locality as Helgoland, Germany. Because we have not viewed the type collection for this entity, we can only tentatively include it as a synonym of this species. If it is a synonym, it would represent a range extension of this species to Helgoland.

Because there are no available names for EUR3 and EUR4, we describe two new species of European *Plocamium*.

EUR3 = *Plocamium nanum* G.W. Saunders et Lehmkuhl, sp. nov.

Thallus maturus typice 25–50 mm in altitudine, ramuli 3–6 ramosi in seriebus alternantibus; axes majores teretes, similes in diametro in partibus distalibus et basalibus thalli; epilithicus, littoralis, generatum inter fucos; reproductio ignota.

Mature thallus typically 25–50 mm in height; 3–6 branched ramuli in alternating series. Main axes subterete, similar in diameter in distal and basal portions of the thallus. Epilithic, intertidal, generally among fucoids. Reproduction unknown.

ETYMOLOGY: Named for its small size relative to other northern European species.

HOLOTYPE: G.W. Saunders, 21.xiii.1997 (Herb. UNB – GWS000255, sterile); Fig. 28.

TYPE LOCALITY: Cap Griz Nez, France.

DISTRIBUTION: Thus far collected only from the northern coast of France.

REPRESENTATIVE SPECIMENS: GWS000270, GWS001204, GWS001205, GWS001206, GWS001207, GWS001208.

**OBSERVATIONS:** *Plocamium nanum* (Figs 28–31) was collected only from the north coast of France, exclusively from intertidal rocks and generally under a fucoid canopy (Table 1). We acknowledge that we have sampled relatively few European locations, and the distribution may be wider than reported here. Thalli of this species (Fig. 28) were smaller than the previous two (25–50 mm in height) and are reminiscent of a small P. subtile but more wiry in overall appearance. Another key difference is the number of laterals in the alternating series greater (3)4-5(6),which is generally in P. nanum. The major axes are largely subterete throughout the thallus and change only minimally in diameter from lower to upper portions of the plant (300-450 µm). Some samples of this species (Table 1) can have slightly fruticose or thicket-like regions, which are caused by extensive gall-like growths and the resultant truncated branches (Fig. 29), as described for *P. cartilagineum* above. Growing tips (Fig. 30) have a more ordered appearance to their apical cell files than was



**Figs 28–34.** Morphology and anatomy of *Plocamium nanum* (EUR3; GWS000255 unless noted) and *P. maggsiae* (EUR4; Maggs1). Fig. 28. Holotype of *P. nanum*. Centimetre rule. Fig. 29. An isolate of *P. nanum* (GWS001205) with slightly fruticose portions, which were caused by gall-like proliferations and the resultant truncated branches. Scale =  $80 \mu m$ . Fig. 30. Growing tip displaying a slight chevron pattern and orthostichous file of the transverse cortical derivatives. Scale =  $20 \mu m$ . Fig. 31. Cortex surface mid-thallus at the base of the first series of ramuli. Cells are moderately compact and relatively uniform in size, slightly smaller than in the previous two species. Scale =  $20 \mu m$ . Fig. 32. Holotype of *P. maggsiae*. Centimetre rule. Fig. 33. Ramuli commonly had a scalloped aspect to their abaxial margin, and sporadic serrations on both margins, which often produced short spinose branches. Scale =  $80 \mu m$ . Fig. 34. Growing tip displaying an elongate aspect, and an absence of a chevron pattern and orthostichous file of the transverse, as is characteristic for this species. Scale =  $20 \mu m$ .

observed for P. subtile (Fig. 18), but did not attain pattern the distinct chevron noted for Р. cartilagineum (Fig. 5). An orthostichous trace of the transverse cortical derivatives was visible well past the recognizable cell files, as in P. cartilagineum. As with the previous vegetative features, P. nanum was also intermediate in the degree of compactness of the outer cortex at the base of the terminal series of ramuli (Fig. 31), although the cortical cells were generally smaller (compare to Figs 6 and 19). No reproductive structures were observed for this species.

EUR4 = *Plocamium maggsiae* G.W. Saunders et Lehmkuhl, sp. nov.

Thallus maturus typice inter 35–45 mm in altitudine, ramuli 5–10 ramosi in seriebus alternantibus; epilithicus in zona infima littorali, tegetes in areis rimae abstagnis superis formantes; reproductio ignota.

Mature thallus typically between 35–45 mm in height, 5–10 branched ramuli in the alternating series. Epilithic in lower intertidal, forming turfs in areas of seepage from upper pools. Reproduction unknown.

ETYMOLOGY: Named for Dr. Christine Maggs in recognition of her many contributions to our understanding of the European algal flora and her mentorship of the senior author during his formative years.

HOLOTYPE: C. Maggs, 24.iii.2002 (Herb. UNB – Maggs1, sterile); Fig. 32.

TYPE LOCALITY: St. John's Point, County Down, Northern Ireland.

DISTRIBUTION: Collected from type locality and Doaghbeg (Fanad), County Donegal, Ireland.

Representative specimens: G0167, GWS001754. **OBSERVATIONS:** Plocamium maggsiae (Figs 32-34) is the most distinctive European species in both its habitat and habit. It was collected in the lower intertidal (but not in pools) as turf-like mats in seepage areas from higher intertidal pools at moderately exposed sites (C. Maggs, pers. comm.). *Plocamium maggsiae*, with a thallus size of 35-45 mm in height, is smaller than P. cartilagineum and P. subtile but best distinguished from the other three species by ramuli alternating in series of (3)5-8(10) (Fig. 32). The basal and other lower ramuli in each series are variously, strongly incurved, outcurved or straight, and have irregularly scalloped margins (Fig. 33) with sporadic serrations, which often developed into short projections, all of which combined to give the thalli a spinose habit when viewed microscopically. The growing tips are generally more elongate than in the other three species (Fig. 34), lack a distinct chevron pattern, and are orthostichous for the transverse cortical derivatives, to only five or six cell rows. No reproductive structures were observed for this species.

The following key is provided to facilitate separation of the northern European species.

- Plants predominantly subtidal, epiphytic or less commonly epilithic, >6 cm in height, ramuli alternating in series of one to five, reproductive thalli common... 2
- 1b. Plants intertidal, epilithic, <6 cm in height, ramuli generally alternating in series of three to ten, reproduction unknown... 3</li>
- 2a. Plants richly branched throughout; growing tips with chevron pattern to cell files, transverse periaxial derivatives obviously orthostichous, surface cortication incomplete; tetrasporangial stichidia usually compound, tetrasporangia 37–60 µm in length...

P. cartilagineum

2b. Plants increasingly branched distally; growing tips lacking distinct chevron, transverse periaxial derivatives failing to form orthostichous rows, surface cortication complete; ramuli sometimes aculeate and/or developing serrations; tetrasporangial stichidia usually simple, tetrasporangia 60–90 μm in length...

P. subtile

- 3a. Plants forming tufts among fucoids; ramuli alternating in series of 3–6, transverse periaxial derivatives forming an obvious orthostichous row...
   *P. nanum*
- 3b. Plants forming turfs in tidepool seepage; ramuli alternating in series of 3–10, irregular in morphology, sometimes with scalloped margins and sporadic serrations; growing tips elongate compared to other species...

P. maggsiae

### Other epithets associated with Plocamium cartilagineum in Europe

Throughout our investigation we have encountered specific epithets that have been associated with P. cartilagineum but do not fit into the groups designated here. These include: P. brachiocarpum Kützing (1849: 885); P. coccineum var. fenestratum Kützing (1843: 450); P. fenestratum (Kützing) Kützing (1849: 301); P. irregulare Meneghini (1844: 300); and P. mediterraneum Meneghini (1844: 300). For the sake of completeness and for future reference we briefly outline our reasoning for excluding them from our groups. De Toni (1900) placed P. brachiocarpum (Type locality: New Zealand) in synonymy with P. cartilagineum. Given our molecular data, it is very unlikely that P. cartilagineum exists in the southern oceans and, therefore, this synonymy is probably incorrect. We examined the type of P. brachiocarpum (from Leiden) and the ramuli in each series have a

distinctive acropetal pattern. The basal ramuli are usually simple, the next in the series has minor serrations on the adaxial margin, the following is poorly developed vegetatively, and so on until the terminal ramulus, which is generally fully developed as a lateral branch. In the same work, De Toni (1900) concluded that P. mediterraneum, P. fenestratum (based on Fucus coccineus var. fenestratum), and P. irregulare should be considered synonyms of Delesseria plocamium var. uncinatum (our EUR2 = Plocamium subtile). These taxa must all be considered because they are from southern Europe and, based on our collection locations, we cannot determine the southern limit of the distribution for any of our groups. We obtained a number of representative collections for P. fenestratum from Leiden (including type material from Triëst; W. Prud'homme van Reine, pers. comm.), and confirmed that these isolates are morphologically different from members of any of the northern species. The thalli of P. fenestratum are more delicate in construction, and the ramuli alternate in a series of seven to ten. In addition, entire axes tend to be highly recurved lending an overall wavy aspect to the thalli; a character that we did not see in our northern collections.

Plocamium irregulare and P. mediterraneum also have southern European distributions (Adriatic and Mediterranean, respectively). The first is characterized by ramuli alternating in series of one to many in an irregular pattern along the main axis, and by elongate, pedicellate cystocarps (Meneghini, 1844). We have not seen this combination of characters in any of our isolates, and it is likely that this represents a separate species endemic to southern Europe. Plocamium mediter*raneum* is characterized by ramuli that are rarely reflexed and that alternate in series of 2-4 (Meneghini, 1844). According to this description, this species most resembles P. nanum, although it differs in the number of ramuli alternating in series. A full molecular characterization of isolates of these morphologies is warranted to determine how they relate to the northern species.

### Northeast Pacific

The fourteen northeast Pacific '*Plocamium cartilagineum*' isolates sequenced for the LSU included individuals of variable size and morphology (thallus size ranging from 35 to 110 mm, ramuli alternating in series of 3–5), from various habitats (mainly subtidal with three isolates collected from very exposed, intertidal rock) and from various northeast Pacific localities (Catalina Island, CA, to Bamfield, BC). All are identical in their LSU and grouped more closely with other northeast Pacific species (P. violaceum, P. oregonum, and Plocamiocolax pulvinata) than to geographically remote isolates of P. cartilagineum. Our results are consistent with earlier phylogenies based on the internal transcribed spacer (ITS) of the ribosomal cistron (Goff et al., 1996). Northeast Pacific isolates have historically been considered a separate species, P. pacificum Kylin (1925). Californian plants were reportedly more robust, more cartilaginous, and less compressed than plants elsewhere (Harvey, 1853). Not all authors agree, and Gabrielson & Scagel (1989) did not support the recognition of a separate northeast Pacific species. Based on our molecular data and morphological observations, we support the resurrection of P. pacificum as a distinct species of Plocamium, endemic to the northeast Pacific. Interestingly, Farlow (1881, p. 151) attributed this species to Kützing, as P. californicum, without listing a reference. Unfortunately, we have found no further reference to this epithet, which clearly predates Kylin's (1925) P. pacificum.

Plocamiocolax pulvinata is an adelphoparatsite on northeast Pacific isolates of Plocamium pacificum. Our LSU data agree with published ITS molecular data (Goff *et al.*, 1996), suggesting that the parasite is more closely related to the non-host *P. violaceum* than to its host *P. pacificum*. Further, recognition of *Plocamiocolax* as a distinct genus renders *Plocamium* paraphyletic based on our molecular results. It is, however, premature to sink the former until generic concepts within the Plocamiaceae are fully evaluated – a task outside the scope of the current study.

Two morphs of the northeast Pacific species P. violaceum exist, the smaller having been treated as a discrete species, P. tenue Kylin (Kylin, 1925). Plocamium violaceum was described by Farlow (1877, p. 240) based on two Californian specimens now housed at the Farlow Herbarium (FH) (collectors: Dr C.L. Anderson and Mr D. Cleveland), and is characterized by a markedly large, incurved lower ramulus. Kylin (1925) erected P. tenue based on two collections from the San Juan Islands, Washington (Brown Island and south of False Bay) and distinguished it from P. violaceum by its smaller, less conspicuous, simple branches (<4 mm in length) and the smaller diameter of the main axis (rarely >0.5 mm). Plocamium tenue is also said to grow in dense stands, whereas P. violaceum generally forms isolated tufts (Doty, 1947, p. 178).

We sequenced partial ITS for isolates fitting *P. violaceum* (n=3) and *P. tenue* (n=4) morphologically; all isolates have identical sequences (data not shown). We therefore do not support the discrimination of *P. tenue* from *P. violaceum*,

at least in the Bamfield region where we acquired our isolates. Collections of the former from the type locality must be studied to resolve this issue.

### Northwest Pacific

Plocamium cartilagineum is reported from the northwest Pacific (Yano et al., 2004). However, based on the LSU sequence of our isolate, it differs from representatives of this species in other parts of the world. It is most closely associated with one of our isolates of P. telfairiae. Plocamium diversity warrants further investigation in this region as two additional isolates of *P. telfairiae* differed in sequence from the previous group, indicating that cryptic species may be included within *P. telfairiae*, one of these encompassing 'P. cartilagineum' from the northwest Pacific. Our results are consistent with those of Yano et al. (2004) in indicating that the genus *Plocamium* in the northwest Pacific is in need of a detailed taxonomic investigation. Yano et al. (2004) also identified two groups containing 'P. cartilagineum' and/or P. telfairiae isolates (as noted here), additionally including P. recurvatum Okamura within these complexes.

### Australia

In Australia, preliminary molecular data have indicated that some isolates of 'P. cartilagineum' are more closely related to other sympatric species of *Plocamium* than to geographically remote isolates (Saunders & Kraft, 1994). Our LSU data show similar patterns in that two Australian isolates of 'P. cartilagineum' are more divergent in their LSU than two endemic species, P. patagiatum and P. mertensii. Our limited treatment of isolates from this region does not allow us to comment on the extent of the diversity hidden within 'P. cartilagineum' in Australia. We can only note that there are at least two cryptic entities, neither associated with P. cartilagineum sensu stricto, suggesting that a similar situation with respect to cryptic species in 'P. cartilagineum' exists in Australia as in Europe and further investigation is necessary.

### Conclusion

To date, species of *Plocamium* have been defined largely in morphological terms. While this approach has been successful in the majority of cases, *P. cartilagineum sensu lato* presents a challenge as it encompasses plants of considerable and apparently continuous morphological variability. In order to deal with this problem, we have broadened the traditional morphological approaches to taxonomy by first designating taxa using molecular fingerprints, and then by morphological/anatomical features. We have sequenced the large subunit (LSU) ribosomal DNA from multiple collections of *P. cartilagineum* to resolve the evolutionary affinities of the cryptic entities and present unequivocal evidence that *P. cartilagineum* is not a cosmopolitan species. This study sets a foundation for future studies directed at resolving further this species complex.

During presentation and review of these results two philosophical questions were raised: do morphologically cryptic species warrant recognition; and if two species cannot be distinguished morphologically, is any purpose served by recognizing them as distinct? In our opinion, the answer to both these questions is "yes". Species do not evolve specifically to render their identification easier for scientists. It is fortunate that in most (known at least) cases selective pressures do manifest obvious visual differences between species, but this is an indirect fortuitous benefit and not a direct selective 'decision' on the part of diverging species. Cryptic species may be morphologically similar, but they can be, as is the case here, molecularly (and thus physiologically and biochemically) very divergent and fully deserving of specific status. Failure to recognize unique species as distinct, regardless of their morphological attributes, reduces the accuracy of species inventories for regions, decreases our ability to recognize species at risk and invasive species, and limits our capacity to understand the contributions of these species to ecosystems and their potential benefits (e.g. as food or pharmaceuticals) to humanity.

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