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Characterisation of the enigmatic, endemic red alga *Gelidium allanii* (Gelidiales) from northern New Zealand – morphology, distribution, agar chemistry

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Abstract

Gelidium allanii Chapman is endemic to northern New Zealand with a highly localised distribution. This species has remained little known, having been collected from only one locality for more than 40 years. We compare this species with other New Zealand members of the Gelidiaceae, in particular *Gelidium caulacantheum* and *Pterocladia capillacea*, presenting new data on morphology, distribution and agar chemistry to more fully characterise *G. allanii*. *G. allanii* possesses a very distinctive, highly pyruvated agar. New Zealand records of two Australian species *G. australe* and *G. asperum* are examined and it is concluded that there is no evidence for their occurrence in New Zealand.

Introduction

Gelidium has considerable commercial significance as a source of the phycocolloid agar, with substantial harvests of wild stocks occurring in Spain, Morocco, Portugal, Korea, Japan and Mexico (McHugh, 1991). Although there are about 100 species in this geographically widespread genus, many have been described without reference to morphological variation, based on only a few herbarium specimens (Santelices, 1988). This has resulted in confused specific limits both within *Gelidium* and with respect to the closely related genus *Pterocladia* (e.g. Hommersand & Fredericq, 1988; Norris, 1992; Santelices, 1988, 1990, 1991; Santelices & Stewart, 1985; Stewart, 1992). As Santelices (1988) noted, the practice of some authors to describe and name morphological varieties of *Gelidium* and *Pterocladia* has increased the taxonomic confusion already existing in these two genera.

Agar is a term used to describe gelling polysaccharides that occur naturally in certain red seaweeds. The purest form of agar is agarose, a linear polymer consisting of alternating 3-linked β-D-galactopyranosyl and 4-linked 3,6-anhydro-α-L-galactopyranosyl residues, a disaccharide repeating unit that is known as agarobiose. The polymer may also be partially methylated (Fig. 1A), and can also have varying levels of pyruvate ketal (Fig. 1B), 3,6-anhydrogalactosyl residues, sulphate esters, and branching sugar residues as naturally occurring substituents.

The structures of agars produced by certain members of the Gelidiaceae have been the focus of several previous studies. In 1966, Araki found 6-*O*-methylgalactose in agars from all four members of the Gelidiaceae he studied, and pyruvate ketals in two of them. Izumi (1971) quoted a pyruvate content of 2.28% for *Gelidium amansii* agar. In a survey of eight agarophytes Young *et al.*. (1971) found a range of agar pyruvate contents from 0.04% for *Gelidium sesquipedale* to 2.92% for *Gracilaria compressa*. Agar extracted from *Pterocladia capillacea* (as *P. pinnata*) from Barbados had a pyruvate content of 0.65%. Miller and Furneaux (1982) studied four New Zealand species of Gelidiaceae: *Pterocladia lucida*, *P. capillacea*, *Gelidium*

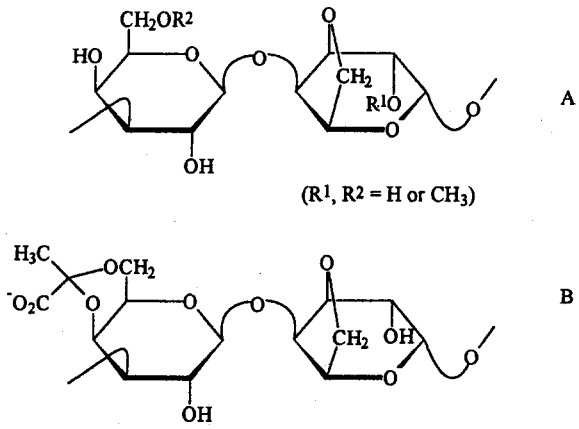

Fig. 1. Structures of: partially methylated agarose (A); pyruvated agarose (B).

caulacantheum and *G. pusillum*. The agars were analysed qualitatively by ^{13}C NMR spectroscopy, hydrolysis and paper chromatography. Pyruvic acid but no methylated sugars were detected. Recent advances in the chemical methods used for the analysis of algal polysaccharides (Stevenson & Furneaux, 1991) have enabled low levels of methylation to be readily detected in small amounts of agar. In addition, the acid-labile 3,6-anhydrogalactosyl units of agar can now be detected and the presence of an *O*-methyl group on them determined. Numerous samples may now be rapidly analysed in detail and this may provide useful taxonomic information.

We initiated a study of some New Zealand representatives of *Gelidium* to examine their agar chemistry and the distribution of wild stocks. A species with a high pyruvate content was revealed in preliminary analyses of collections made in northern New Zealand, including a sample (Hokianga Harbour, 1989, coll. B. Terzaghi) analysed by K. Guiseley. As a highly pyruvated agar may be of use commercially, for example as the gel matrix in high electroendosmosis (EEO) electrophoresis, these results led to a larger survey of Gelidiaceae agar chemistry. However, the need to identify plants being used for analyses, and to determine their wider distribution, revealed confusion in the systematics of New Zealand species of *Gelidium*, as well as very little information about their distribution and ecology. Chapman (1969) recorded 15 taxa in the genus *Gelidium* in New Zealand, placed in 9 species with 3 varieties, 3 forms and 1 ecad. The northern species which we considered in this study were *G. allanii*, *G. caulacantheum*, *G. australe* sensu Chapman (1969) and *Pterocladia capillacea*, using both characteristics of agar chemistry and morphology to compare these species. We also examined the use of the names *G. asperum* (C. Agardh) Greville and *G. australe* J. Agardh in New Zealand.

The identity of *Gelidium allanii* has remained unclear from the time of its description. For more than forty years it was known only from the type locality in the Bay of Islands, and collections were made infrequently, with virtually all of these lodged in the Lindauer herbarium housed at AKU. Although specimens were distributed in 1942 by V. W. Lindauer as No. 137 of the *Algae Nova-Zelandicae Exsiccatae*, initially labelled as *G. setchellii* sp.nov. with a type written description and later as *G. allanii* sp. ined., it was not until Chapman (1969) that this species was formally described.

Gelidium asperum was recorded for New Zealand by Agardh (1851:475), later cited by Harvey (1855:244) and by Laing (1939:140), who considered 'No doubt most of the forms from New Zealand placed under *G. corneum* by earlier collectors belong either to this species or to *Pterocladia capillacea*', citing a Lindauer record from the Bay of Islands. Laing (1939) also noted in reference to *Gelidium* 'The whole genus requires re-investigation for New Zealand. Other undetermined species exist here, one very close to the Australian *G. australe*.' The name *G. asperum* has not been used subsequently in published accounts of New Zealand algae, although Chapman (1969) gave it as a synonym of *G. australe*.

The first published record of *Gelidium australe* J. Agardh for the New Zealand flora was in Chapman (1969), although in a letter distributed in 1947 with the XI and XII fascicles of his Exsiccatae, Lindauer requested that the identification of specimen No. 137, distributed as *G. setchellii* sp.nov. and then as *G. allanii* sp.ined., be amended to *G. australe* J. Agardh. Chapman (1969) gave the New Zealand distribution of *G. australe* as Piha (west coast Auckland), and commented that 'It is not unlikely that this species is more widely distributed on the west coast of New Zealand'.

Materials and methods

Herbarium collections

Herbarium collections housed at WELT, AKU, AK, CHR and NSW of *G. allanii*, *G. asperum*, *G. australe*, *G. caulacantheum*, and *Pterocladia capillacea*

were examined. (Herbarium abbreviations given in Holmgren *et al.*, 1990.) Specimens from the Agardh herbarium (LD) of *G. australe* (including the lectotype), and *G. asperum*, as well as material from the Harvey herbarium (TCD) originally identified as *Gelidium corneum*, collected from New Zealand, were examined and compared to type material of *G. allanii* (AKU000047).

Field collections

Thirty sites (Fig. 2) in the northern North Island, New Zealand were sampled on four field trips (November 1991, August 1992, November 1992, February 1993) and collections were made for anatomical/morphological study and polysaccharide analyses of *Gelidium allanii*, *G. caulacantheum*, *G. 'australe'* sensu Chapman (Piha), and *Pterocladia capillacea* as well as observations of distribution and habitats.

Chemistry

Twenty-three agar samples were prepared from *Pterocladia* and *Gelidium* seaweeds collected at various sites and analysed for constituent sugars and pyruvic acid content. Agar was extracted from seaweed samples with aqueous sodium dihydrogen phosphate according to the method of Miller and Cape (1979) then purified by dialysis and freeze-thawing. Any portion of seaweed taken for analysis was only extracted once.

Pyruvic acid ketals were determined by hydrolysing agar (7 mg) in oxalic acid (0.02 M, 2.1 cm^3) for 2 h at 120 °C in screw-capped test tubes. The released pyruvic acid was quantified as its 2,4-dinitrophenylhydrazone derivative by the method of Sloneker and Orentas (1962).

The constituent sugar composition of native agar samples was determined by capillary GLC analysis (Falshaw & Furneaux, 1994) of alditol acetate derivatives prepared by the reductive hydrolysis procedure of Stevenson and Furneaux (1991) that employs 4-methylmorpholine borane as an *in situ* reductant during hydrolysis with aqueous trifluoroacetic acid. Substitution/linkage analysis was performed as for constituent sugar analysis on agar samples permethylated by treatment with potassium dimsyl- dimethylsuphoxide-methyl iodide (Stevenson & Furneaux, 1991). ^{13}C NMR spectroscopy was performed using a Bruker AC 300 spectrometer (75 MHz, 0.885 s acquisition time, 0.5 s delay time and 90 ° pulse width). The spectrum was recorded on a 3% w/v solution in D_2O-H_2O (50:50) at 90 °C. Chemical shifts are quoted relative to internal DMSO as internal standard at 39.4 ppm.

^1H NMR spectrometry was performed on the above machine (300 MHz, 2.7 s acquisition time, 1 s delay time and 30 ° pulse width). The spectrum was recorded on a 2% w/v solution in D_2O at 90 °C. Chemical shifts are quoted relative to DMSO as internal standard at 2.7 ppm.

Results

Herbarium collections

Specimens of *G. allanii* examined from AK, AKU, CHR and WELT were all collected from either the type locality in the Bay of Islands, or from Cable Bay in Doubtless Bay. A voucher of material collected from the Hokianga Harbour, sent to Dr K. Guiseley, had been lodged at WELT and this was identified as *G. allanii*. Specimens of *G. allanii* were discovered amongst collections of *Pterocladia capillacea* from the Poor Knights Islands (WELT A9918 now as WELT A20822 – collected January 1978). In the Lindauer Herbarium (in AKU), there are only four separate collections of *G. allanii* made between 1937 and 1948. No collections have been located of this species from the Bay of Islands between 1950 (WELT A2139, coll. U. V. Dellow) and 1982 (WELT A13469, coll. W. A. Nelson). The first collection of *G. allanii* outside the Bay of Islands was made in 1986 (WELT A17073 - Cable Bay, Doubtless Bay, W. A. Nelson). Thus, herbarium collections of *G. allanii* are limited in number and were found to be from only four locations.

One specimen has been located (AKU 101830 – Piha) that is consistent with Chapman's (1969:100–101, pl. 34) concept of *Gelidium australe* in New Zealand and on which there is a determination in his handwriting. This specimen clearly differs from both *G. australe* and *G. asperum* based on comparisons with herbarium specimens (including the lectotype of *G. australe* – LD 33188) and the detailed account provided by Womersley (1994) who expressed doubt about the validity of the New Zealand record of *G. australe*. Specimen AKU 101830 is a rather large and sparsely branched specimen, falling into the current understanding of the endemic *Gelidium caulacantheum*, a species described by Agardh as being terete and filiform. This species is considered to be phenotypically variable (Dromgoole & Booth, 1985), par-

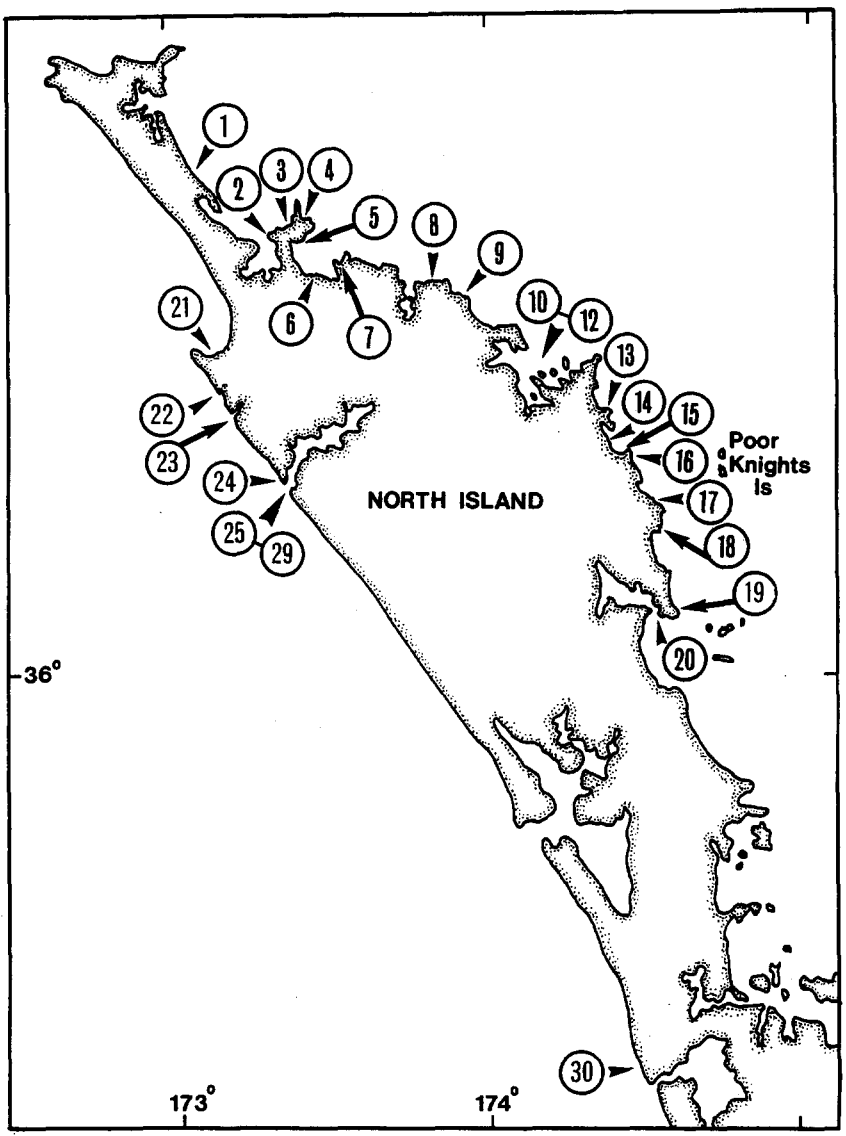

Fig. 2. Northern North Island showing collection sites; 1. Henderson Point, Great Exhibition Bay; 2.* Rangiputa, Rangaunu Harbour; 3. Puheke, Rangaunu Bay; 4. Matai Bay, Doubtless Bay; 5.* Whatuwhiwhi, Doubtless Bay; 6.* Cable Bay, Doubtless Bay; 7. Hihi-Waitetoki, Doubtless Bay; 8. Tauranga Bay; 9. Matauri Bay; 10. Opito Bay-Tikorangi Island, Bay of Islands; 11.* Oneroa Bay/Waitata Rocks/Flat Rocks, Bay of Islands; 12. Rawhiti Point, Bay of Islands; 13. Whangaruru North, Bland Bay; 14. Oakura; 15. Helena Bay; 16. Mimiwhangata; 17. Sandy Bay; 18. Matapouri; 19. Ocean Beach, Bream Head; 20. Urquhart's Bay, Whangarei Harbour; 21. Reef Point, Ninety Mile Beach; 22. Herekino (south shore); 23. Whangape (north shore); 24. Hokianga, North Head; 25. Hokianga Harbour, South Head; 26. Omapere; 27. Opononi; 28.* Pakanae; 29.* Koutu Point; 30. Piha. Also, Poor Knights Islands, Aorangi I.*. * = sites where *G. allanii* has been located.

ticularly with respect to interbranch length as well as the degrees of branching present. Specimens collected from Tauranga and Ahipara, housed in the Lindauer Herbarium and labelled as *G. australe*, are also specimens of *G. caulacantheum*. In WELT and AK plants identified as *G. australe* by Lindauer, collected from the Waitata Rocks, and Flat Rocks, Bay of Islands, are all specimens of *G. allanii*. Thus, specimens in New Zealand herbaria (AK, AKU, WELT) previously identified as *G. australe* have been found to be either specimens of *G. allanii*, or large sparsely branched specimens of *G. caulacantheum*.

Distinguishing characters

Diagnostic characters that enable the separation of the taxa discussed in this paper are summarised in Table 1, and *G. allanii*, *P. capillacea* and *G. caulacantheum* are illustrated in Fig. 3. According to Chapman (1969), *Gelidium allanii* could be confused easily with *P. capillacea* when sterile. However, these species can be distinguished by both the shape of apices and by their apical cells. The branches of *G. allanii* are tapered and the apical cell is very prominent. This apical cell arrangement enables collections of *G. allanii* and *Pterocladia capillacea* to be readily distinguished with a dissecting microscope, in both fresh and herbarium specimens. The tips of *P. capillacea* have a rounded, often blunt appearance and the apical cells lie close to the apical margin as illustrated by Stewart (1992). Although there are similarities in the habit of these species, plants of *Gelidium allanii* have a distinctive, silky texture enabling them to be distinguished by touch from *P. capillacea* growing adjacently in tidal pools. In fertile female material *P. capillacea* exhibits unilocular cystocarps whereas *G. allanii* possesses bilocular cystocarps, diagnostic of the genus. *G. caulacantheum* can be readily distinguished from both *P. capillacea* and *G. allanii* by its stature, colour and terete axes.

G. asperum and *G. australe* occur in subtidal habitats in Australia, a feature which distinguishes them from all New Zealand species of *Gelidium* which occupy solely intertidal habitats. Key characters used to distinguish *G. australe* and *G. asperum* (Womersley, 1994) are summarised in Table 1. Both of these Australian species are morphologically distinct from the northern New Zealand Gelidiaceae.

Distribution

Localities – Prior to this study the only confirmed populations of *G. allanii* were at the type locality and Cable Bay, Doubtless Bay (Figs 2–6). *G. allanii* was located at only six of the thirty sites visited during this work - the type locality (Fig. 2–11), two sites in the Hokianga Harbour (Fig. 2–28,29; at Koutu Point only drift plants were found), two sites in Doubtless Bay (Fig. 2–5,6) and one in Rangaunu Harbour (Fig. 2–2). At all sites the plants were strictly localised and the populations varied in density from sparse with only scattered individuals, to locally abundant patches.

Habitat – At three of the sites, *G. allanii* plants occurred submerged, strongly attached, around the fringes of large mid-intertidal pools, either growing on rock faces or in association with *Corallina* and near to clumps of *P. capillacea*. At one site *G. allanii* was found growing on calcareous worm tubes, and at another on a limestone reef. Individuals were also observed on decorator crabs. Proximity to calcium carbonate substrates was the feature common to all sites.

Chemistry

Portions (1–10 g) of each collection of air-dried seaweed were removed for analysis. Each portion was extracted once and the resulting polysaccharide analysed. To determine sample variation, more than one portion of some collections were analysed. Constituent sugar analysis involves the hydrolysis of polysaccharide with *in situ* reduction to produce alditols. These are then acetylated to produce volatile alditol acetates for analysis by GC-MS. For example, 4-linked, 2-*O*-methyl-, 3,6-anhydrogalactopyranosyl units are analysed as 1,4,5-tri-*O*-acetyl-, 3,6-anhydro-2-*O*-methyl-galactitol, known as 2-MeAnGal. All the extracts analysed revealed components consistent with agar, *i.e.* total 3,6-anhydrogalactosyl units (represented as 2-MeAnGal + AnGal) and total galactosyl units (*i.e.* Gal + 6-MeGal + 2-MeGal) were present in a ratio close to 1:1 (Table 2). Low levels of 2-*O*-methylation on 3,6-anhydrogalactosyl units and 6-*O*-methylation on galactosyl units were observed for all samples. Extracts from collections labelled *G. 'australe'* from Piha were indistinguishable from those of *G. allanii* from three sites and *G. caulacantheum* from Ngakuta Bay (Miller & Furneaux, 1982), Waitata Rocks, Long Beach and Opito Bay. Agar from *G. caulacantheum* collected at Piha and a cultured sample of *G. caulacantheum* had higher methylation levels. The extract from a collection at Henderson Point had slightly lower levels. Although agars from separate portions of certain collections were obtained in variable amounts, the analyses were very similar. Thus the differences in structure observed between collections from different sites must have some other explanation such as seasonal, environmental or biological factors. Agar from *Pterocladia capillacea* had a lower 6-*O*-methyl-galactose content than the other species.

The pyruvic acid content of all the *P. capillacea*, *G. caulacantheum* and *G. 'australe'* samples was around 1% (Table 2). All the *G. allanii* samples had pyruvic acid contents of around 5.5%. This corresponds to approximately one pyruvic acid ketal per six agarobiose units. These levels are much higher than

Table 1. Distinguishing characters of taxa

Characters/Taxa	*Gelidium allanii*	*Pterocladia capillacea*	*Gelidium caulacantheum*	*Gelidium australe**	*Gelidium asperum**
Terete/compressed	Compressed	Compressed	Terete	Terete to compressed	Terete
Apex/branch tips	Acute to acuminate	Rounded to obtuse	Acute	Acute	Acute
Apical cell	Very prominent	Flush with apex margin	Visible, not protruding		
Position of tetrasporangia	Sori form elongate patches on both surfaces of pinnule with sterile margins	Sori ovate to elongate on compressed pinnules: sterile margins	Sori surrounding pinnule, slightly swollen	Stichidia clavate to tapering	Stichidia clavate
Cystocarps	Bilocular	Unilocular	Bilocular	Bilocular	Bilocular
Ostioles per locule	(1)2–5	1(−3)	1	1	1
Distribution	Intertidal; local, restricted Northland	Intertidal; widespread North I. South I. to Kaikoura and Jackson Bay, Chatham Is	Intertidal; widespread North I. South I. Chatham Is	Subtidal Western Australia to Victoria, Tasmania	Subtidal South Australia to Victoria, Tasmania
Colour	Red to dark crimson	Dark red-purple	Dark red brown to dull purple	Medium to dark red brown	Dark red brown
Branching	Bi-tripinnate; many axes	Regularly pinnate; pyramidal outline	Pinnate; bushy	Regularly pinnate; densely branched, several - numerous axes.	Irregularly branched; in several axes.

* Data from Womersley (1994)

Table 2. Constituent sugar and pyruvic acid analyses of agars (first extract) from various *Gelidium* and *Pterocladia* collections.

Sample		WELT	Site	Date	Sugar[a] (nommalised mol. %) 2-MeAnGal	AnGal	6-MeGal	Xyl	2-MeGal	Gal	% Yield	% Pyruvic Acid
Pterocladia capillacea		A20800	6	XI. 91	2	47	1	1	0	49	15.0	1.1
		A20802	26	XI. 91	2	46	1	1	0	50	15.0	0.8
		A20801	24	X. 91	1	45	1	1	0	52	17.8	1.0
Gelidium caulacantheum			b	IX. 80	2	44	4	1	0	49	30.0	1.2
		A20824	11	VIII.92	1	48	6	1	0	44	7.1	1.4
					1	47	5	1	0	46	12.0	1.2
		A20803	11	XI. 92	1	46	4	1	0	48	12.6	1.2
					2	48	3	0	0	47	18.0	1.2
		A20804	c	I.93	7	43	7	0	1	42	20.4	1.4
					5	45	6	0	Tr	44	33.0	1.3
					6	41	7	0	1	45	18.3	1.3
		A20815	30	VII. 92	4	43	9	1	0	43	12.7	1.6
					4	45	8	0	0	43	12.9	1.4
		A20805	1	II. 93	1	47	2	1	0	49	27.0	1.1
		A20806	10	II. 93	1	45	4	1	0	49	19.9	1.0
Gelidium 'australe'		A20807	30	XI. 92	2	45	4	1	0	48	8.0	1.2
		A20814	30	VIII. 92	2	48	4	1	0	45	13.1	1.1
Gelidium allanii		A20813	6	XI. 91	2	48	5	0	0	45	15.5	5.0
		A20811	6	VIII. 92	2	46	4	1	0	47	13.0	5.5
	T	A20812	6	XI. 92	2	47	5	1	0	45	19.2	5.7
	F	A20823	6	XI. 92	1	47	4	1	0	47	18.1	5.4
		A20808	28	II. 93	2	47	4	1	0	46	27.9	5.7
		A20810	2	II. 93	1	48	4	1	0	46	22.7	5.2

Tr = Trace, T = Tetrasporic, F = Female

[a] AnGal determined as 1, 2, 4, 5-tetra-*O*-acetyl-3,6-anhydrogalactitol, Gal as galactitol hexa-acetate etc.

[b] Sample of Miller & Furneaux (1982),

[c] Cultured sample ex. Porirua Harbour

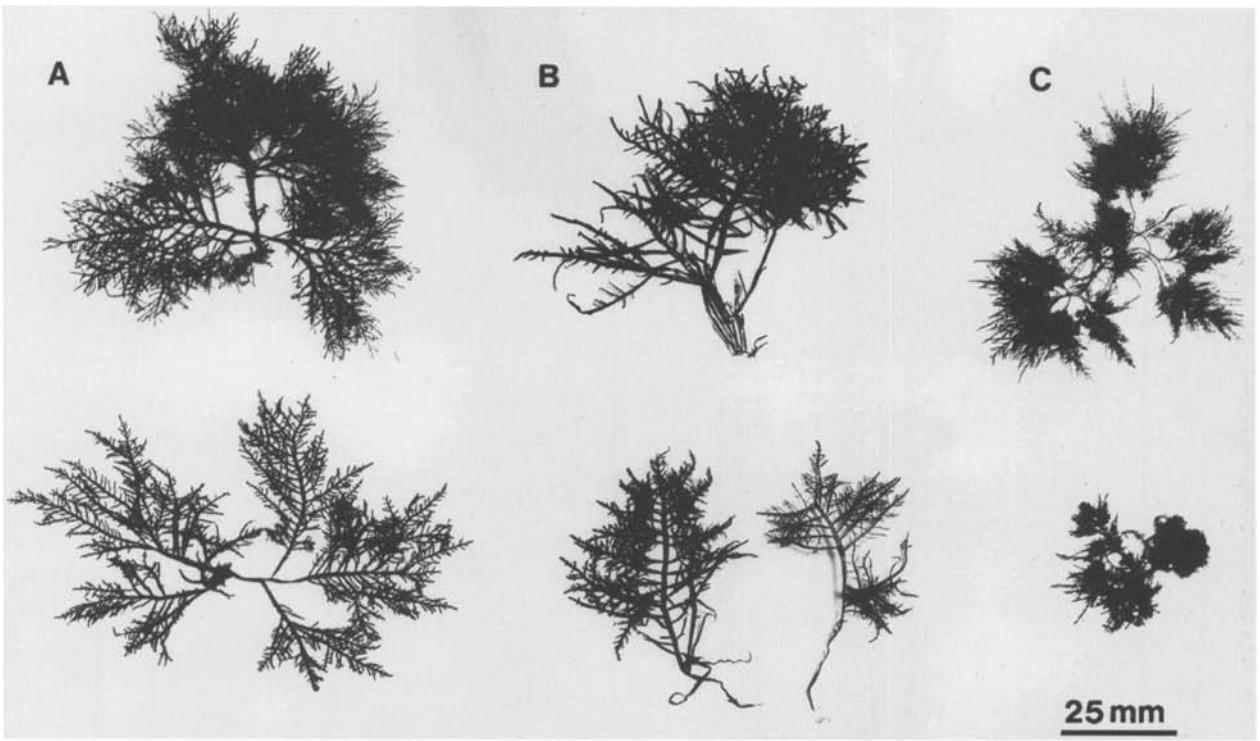

Fig. 3. Three species of Gelidiaceae from northern New Zealand – *G. allanii* (A); *P. capillacea* (B); *G. caulacantheum* (C) (left to right).

Table 3. Substitution/linkage analysis of agar from tetrasporophytic *Gelidium allanii* (Cable Bay XI. 92)

Constituent sugar and deduced substitution[a]	Normalised mol %
3-Gal	38
3,4,6-Gal	8
3,6-Gal	2
4-AnGal	47
4-Gal	1
3,4-Gal	3
T-Xyl	1

[a] 3, 6-Gal means a 3, 6-disubstituted and/or linked galactopyranosyl residue, analysed as 1,3,5,6-tetra-*O*-acetyl-2,4-di-*O*-methyl-galactitol; T-Xyl means a terminal sugar unit, analysed as 1,5-di-*O*-acetyl-2,3,4-tri-*O*-methyl-xylitol, etc. See text for further explanation.

those reported previously for other agars. Pyruvic acid is, therefore, a useful taxonomic marker for identifying samples of *G. allanii*. There was no difference in the analysis of female and tetrasporic samples of *G. allanii* (Table 2).

The unusual agar from *G. allanii* was analysed in more detail. The ^{13}C NMR spectrum of this agar (Fig. 4) shows twelve strong signals corresponding to those expected for the agarobiose repeating unit shown in Fig. 1A ($R^1 = R^2 = H$). In addition, smaller signals are visible which correspond to C1, C3, C4, C5 and C6 of a β-D-galactopyranosyl unit substituted with a pyruvate ketal at the 4- and 6- positions (Lahaye & Yaphe, 1989) as in Fig. 1B. The presence of pyruvate is confirmed by a signal at 25.4 ppm corresponding to the methyl group of the pyruvate ketal. Further weak signals are observed for C5 and C6 of a 6-*O*-methyl-β-D-galactosyl unit (Fig. 1A: $R^2 = CH_3$). The *O*-CH_3 signal is at 59.0 ppm. The level of 2-*O*-methylation (Fig. 1A: $R^1 = CH_3$) is too low to be detected by this method.

The ^1H NMR spectrum shows a strong signal at 1.45 ppm corresponding to the methyl protons of the pyruvate ketal and a signal at 5.22 ppm corresponding to the anomeric proton of the 3,6-anhydrogalactosyl residue adjacent to a pyruvate substituted D-galactosyl residue (Fig. 5). The spectrum is similar to that

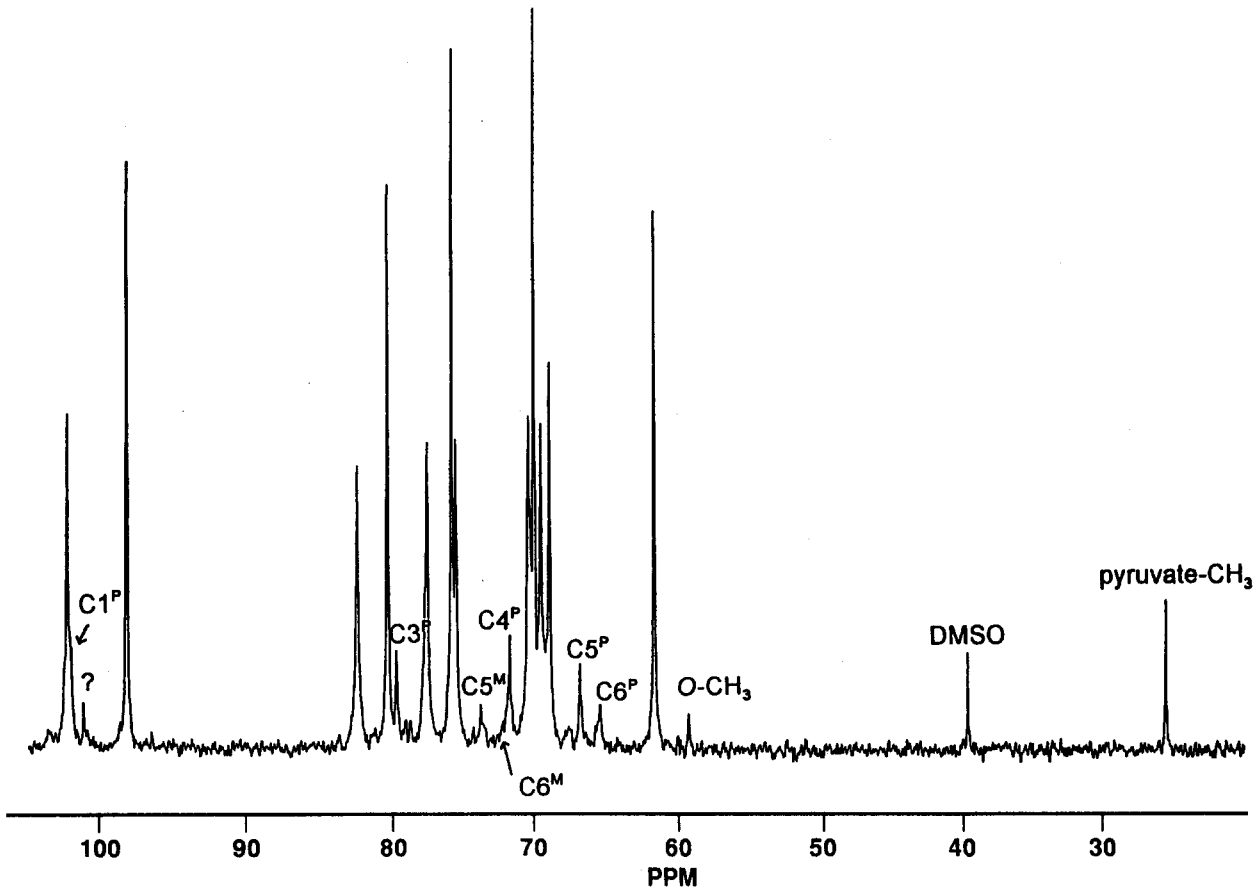

Fig. 4. ^{13}C NMR spectrum of *G. allanii* agar. Resonances due to galactosyl residues bearing a 4,6-*O*-pyruvate ketal or 6-*O*-methyl ether substituent are marked as P or M, respectively.

obtained by Murano *et al.* (1992) for *Gracilaria dura* except that the above signals are more intense for *Gelidium allanii* agar. Signals at 3.42 and 3.51 ppm corresponding to the methyl protons in 6-*O*-methylgalactose and 2-*O*-methylgalactose, respectively, are also visible (Fig. 5).

Substitution/linkage analysis involves methylation of an agar sample prior to hydrolysis/reduction (Stevenson & Furneaux, 1991). Subsequent acetylation occurs on sites in the sugar ring previously linked or substituted with sulphate ester, pyruvate ketal or branching sugar units. GC-MS analysis of the derived partially methylated alditol acetates allows structural information to be obtained. Thus, the major components for *G. allanii* agar were 3-Gal (*i.e.* 1,3,5-tri-*O*-acetyl-2,4,6-tri-*O*-methylgala ctitol) corresponding to 3-linked galactopyranosyl residues and 4-AnGal (*i.e.* 1,4,5-tri-*O*-acetyl-3,6-anhydro-2-*O*- methylgalactitol) corresponding to 4-linked 3,6-anhydrogalactopyranosyl residues (Table 3) as expected for agar (Fig. 1A). In addition, 3,4,6-Gal (corresponding to 3-linked galactopyranosyl residues substituted at the 4-, and 6-, positions with a pyruvate ketal) was observed at a level corresponding to one pyruvated galactosyl unit per 6 agarobiose units (Fig. 1B). This is consistent with the results obtained from pyruvic acid analysis. A small amount of 3,6-Gal was also observed. This is likely to be derived from 3-linked galactosyl units substituted at the 6- position with single xylose branches (detected as T-Xyl) or a sulphate ester group. A small amount of 3,4-Gal also detected may be 3-linked and 4-substituted, or 4-linked and 3-substituted. Since the substitution/linkage analysis involves permethylation of the sample any native *O*-methyl groups present are masked.

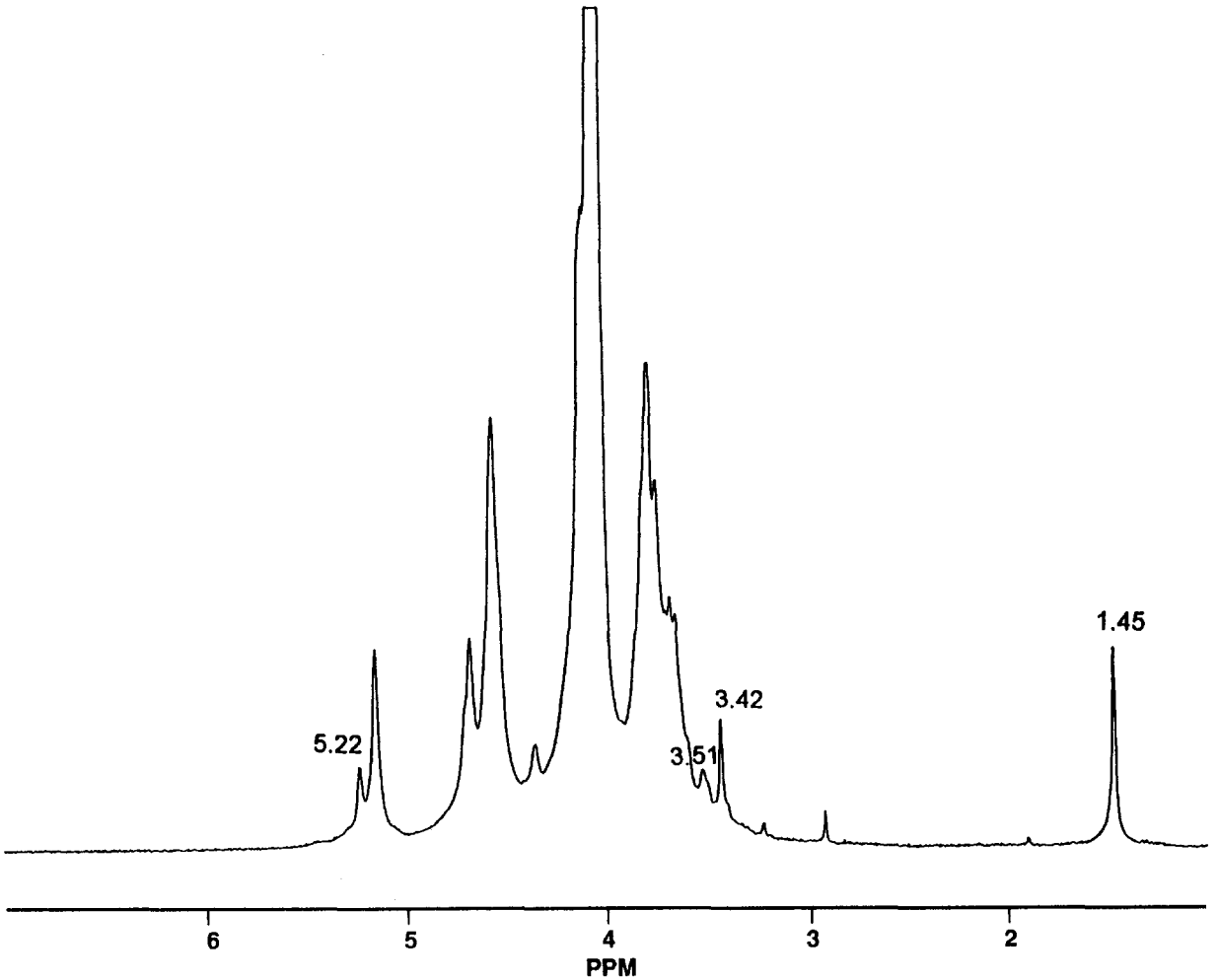

Fig. 5. ^{1}H NMR spectrum of *G. allanii* agar. (See text for explanation.)

Discussion

The identity of *G. allanii* has remained unclear since its first discovery in the 1930s. When this study began we developed several hypotheses about the restricted distribution of this species:

1. that it is not distinct from either *Pterocladia capillacea* or some other *Gelidium* species already recorded for the New Zealand flora;
2. that it is an adventive species, having arrived through shipping contacts (the Bay of Islands has been a centre of much shipping activity from the earliest period of European contact: Nelson & Adams, 1987);
3. that it is an endemic alga with a very restricted distribution.

An examination of both herbarium and new collections of *G. allanii* clearly established that this taxon is distinct from all other Gelidiaceae in northern New Zealand. The new sites where this species was located argue against an adventive origin. Although the Hokianga Harbour has also been the site of early shipping contacts, and Cable Bay of modern shipping contact (the site of submarine telecommunication cable), the highly local distribution of this species and its presence in remote stretches of coastline give support to our understanding that this is an endemic species with a very restricted distribution.

The use of the names *G. asperum* and *G. australe* for New Zealand plants has complicated the under-

standing of the systematics of northern New Zealand *Gelidium* species in the past. Our understanding from this study is that there is no evidence for their presence in northern New Zealand and that previous records have been based on misidentified material. As currently understood the species *Gelidium caulacantheum* is very variable: the chemical evidence from our work suggests that this species complex may require further taxonomic attention. The results of sugar analyses indicate significant variation in methylation between samples from different sites (Table 2). This is in line with preliminary results on this group based on rbcL nucleotide sequence analysis (Freshwater & Rueness, 1994) in which *Gelidium* sp. (Piha) (= *G. 'australe'* sensu Chapman) was found to be related to but distinct from *G. caulacantheum* collected from Porirua Harbour.

G. allanii is the largest species of *Gelidium* occurring in New Zealand and contains a highly pyruvated, potentially useful agar. However, the restricted distribution and the small size of populations of this species preclude any harvesting of this species from wild stocks. If commercial use is to develop, it would be necessary to develop an economic culturing technique appropriate to this species. Melo *et al.* (1991) have reviewed the farming techniques that have been developed for other species of *Gelidium*. A major issue facing the expansion of *Gelidium* culture to fullscale farming rests on whether growth rates obtainable are commercially viable. With such small wild populations it would be necessary for cultivation of this species to develop a method of propagation requiring as little field-derived material as possible. Techniques which enable the amplification of plant material – cell propagation and callus culture – would be appropriate options to explore in order to protect the resource present in the wild. However, the application of such techniques to a commercial operation would require a commercial product of sufficient value to warrant the substantial costs involved. If this species is to be investigated commercially, it will be necessary to know more about its ecology, demography and reproductive biology.

The observation that *G. allanii* grows only in areas with calcium carbonate substrata is interesting. Santelices (1988) noted that although the preference of certain species of both *Gelidium* and *Pterocladia* for coralline algae substrata has been reported on a number of occasions, no studies have been conducted on this aspect of the ecology of the Gelidiales and the nature of the association remains unknown. Salinas (1991) refers to the ability of *G. sesquipedale* to reattach to calcareous polychaete worm tubes, and notes 'the observed links between the existence of growth of *Gelidium* and calcareous substrata may account for population structures. It may also clarify the difficulties which this species has in recolonising habitats in which calcareous organic substrata (*Lithophyllum* and *Mesophyllum*) have been destroyed'.

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References

Agardh JG (1851) Species, Genera et Ordines Algarum. Volume 2, part 1, 351 pp. Lund.

Araki C (1966) Some recent studies on the polysaccharides of agarophytes. Proc. int. Seaweed Symp. 5: 3–17.

Chapman VJ (1969) Marine Algae of New Zealand. Part III: Rhodophyceae 1: Bangiophycidae and Florideophycidae (Nemalionales, Bonnemaisoniales, Gelidiales). Cramer, Lehre, 113 pp, 38 pls.

Dromgoole FI, Booth WE (1985) The structure and development of hairs on the thallus of *Gelidium caulacantheum* J. Ag. N.Z.J. mar. Freshwat. Res. 19: 43–48.

Falshaw R, Furneaux RH (1994) Carrageenan from the tetrasporic stage of *Gigartina decipiens* (Gigartinaceae, Rhodophyta). Carbohyd. Res. 252:171–182.

Freshwater DW, Rueness J (1994) Phylogenetic relationships of some European *Gelidium* (Gelidiales, Rhodophyta) species, based on *rbc*L nucleotide sequence analysis. Phycologia 33: 187–194.

Harvey WH (1855) Algae. In Hooker JD (ed.), The Botany of the Antarctic Voyage of H. M. Discovery ships Erebus and Terror in the years 1839–1843. II. Flora Novae-Zelandiae. Part II. London, Reeve: 211–266, pls 107–121.

Holmgren PK, Holmgren NH, Barnett LC (eds) (1990) Index Herbariorum Part 1: The Herbaria of the World. Regnum Vegetabile 120: 1–693.

Hommersand MH, Fredericq S (1988) An investigation of cystocarp development in *Gelidium pteridifolium* with a revised description of the Gelidiales (Rhodophyta). Phycologia 27: 254–272.

Izumi K (1971) Chemical heterogeneity of the agar from *Gelidium amansii*. Carbohydr. Res. 17: 227–230.

Lahaye M, Yaphe W (1989) The chemical structure of agar from *Gracilaria compressa* (C. Agardh) Greville, *G. cervicornis* (Turner) J. Agardh, *G. damaecornis* J. Agardh and *G. domingensis* Sonder *ex* Kuetzing (Gigartinales, Rhodophyta). Bot. mar. 32: 369–377.

Laing RM (1939) New Zealand seaweeds. Reference List No. II. The Rhodophyceae - Part A (Bangiales, Nemalionales, Cryptonemiales, and Gigartinales). Trans. Proc. r. Soc. N.Z. 69: 134–164.

McHugh DJ (1991) Worldwide distribution of commercial resources of seaweeds including *Gelidium*. Hydrobiologia 221: 19–29.

Melo RA, Harger BWW, Neushul M (1991) *Gelidium* cultivation in the sea. Hydrobiologia 221: 91–106.

Miller IJ, Cape CF (1979) Extraction of agar from *Pterocladia lucida*. N.Z.J. Sci. 22: 253–255.

Miller IJ, Furneaux RH (1982) Agars from New Zealand red algae in the family Gelidiaceae: a structural study. N.Z.J. Sci. 25: 15–18.

Murano E, Toffanin R, Zanetti F, Knutsen SH, Paoletti S, Rizzo R (1992) Chemical and macromolecular characterisation of agar polymers from *Gracilaria dura* (C. Agardh) J. Agardh (Gracilariaceae, Rhodophyta). Carbohydr. Polym. 18: 171–178.

Nelson WA, Adams NM (1987) Marine algae of the Bay of Islands area – A list of species. National Museum of N.Z. Misc. Series 16: 47 pp.

Norris RE (1992) A proposed phylogenetic scheme for the Gelidiales. In Abbott IA (ed.), Taxonomy of Economic Seaweeds III. California Sea Grant College Program, University of California, La Jolla, California: 151–171.

Salinas JM (1991) Spray system for re-attachment of *Gelidium sesquipedale* (Clem.) Born. et Thur. (Gelidiales: Rhodophyta). Hydrobiologia 221: 107–117.

Santelices B (1988) Synopsis of biological data on the seaweed genera *Gelidium* and *Pterocladia* (Rhodophyta). FAO Fisheries Synopsis 145: 55 pp.

Santelices B (1990) New and old problems in the taxonomy of the Gelidiales (Rhodophyta). Hydrobiologia 204/205: 125–135.

Santelices B (1991) Intrageneric differences in cystocarp structure in *Gelidium* and *Pterocladia*. Hydrobiologia 221: 1–17.

Santelices B, Stewart JG (1985) Pacific species of *Gelidium* and other Gelidiales (Rhodophyta) with keys and descriptions to the common or economically important species. In Abbott IA, Norris JN (eds), Taxonomy of Economic Seaweeds I. California Sea Grant College Program, University of California, La Jolla, California: 17–31.

Sloneker JH, Orentas DG (1962) Pyruvic acid, a unique component of an exocellular bacterial polysaccharide. Nature 194: 478–479.

Stevenson TT, Furneaux RH (1991) Chemical methods for the analysis of sulphated galactans from red algae. Carbohydr. Res. 210: 277–298.

Stewart JG (1992) Separation of California species of *Gelidium* and *Pterocladia*: an evaluation of vegetative characters. In Abbott IA (ed.), Taxonomy of Economic Seaweeds III. California Sea Grant Program, University of California, La Jolla, California: 183–191.

Womersley HBS (1994) The Marine Benthic Flora of Southern Australia – Part IIIA. ABRS, Canberra, 508 pp.

Young K, Duckworth M, Yaphe W (1971) The structure of agar Part III. Pyruvic acid, a common feature of agars from different agarophytes. Carbohydr. Res. 16: 446–448.