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Chemotaxonomy of New Zealand red algae in the family Gigartinaceae (Rhodophyta) based on galactan structures from the tetrasporophyte life-stage

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ABSTRACT

The identification of the polysaccharides from tetrasporophytic plants of nine endemic New Zealand species belonging to the Gigartinaceae, *'Gigartina' ancistroclada, 'G.' grandifida, Gigartina dilatata, G. divaricata, G. macrocarpa, G. marginifera, G. pachymenioides, G. sp. 'Lindauer 164'* and *Sarcothalia livida* using infra-red spectroscopy in conjunction with constituent sugar and glycosyl linkage/substitution analysis is reported. All nine species contain galactans with structures consistent with λ-type carrageenans. Differences in the structures of the galactans in these and a further six previously studied species indicate chemotaxonomically distinct groupings that correspond to *Sarcothalia*, *'Sarcothalia'* and *Gigartina* genera plus some outliers. These distinct, chemotaxonomic groupings are aligned to those determined by *rbc*L sequence analysis reported in the literature.

1. Introduction

Correct identification of individual species of seaweed is important not only for understanding biodiversity but also for commercial uses, where one species may contain a desired compound while a similar, but unrelated, species may not. The taxonomy of the genus *Gigartina* based on morphology has 'always been recognised as difficult' both in New Zealand and globally.[1] A number of attempts have been made to understand morphological relationships within the New Zealand members of the family Gigartinaceae with differing results.[1–5] The most recent and comprehensive morphological examination of the genus *Gigartina* in New Zealand was by Adams in 1994, who listed 20 separate species.[5] Adams proposed three groups of species based on morphological differences defined as

- Group A—branched species with tetrasporangial sori discrete and dotted all over the fronds.
- Group B—branched species with tetrasporangial sori in patches expanded over the upper fronds and
- Group C—species with large, leafy blades.[5]

The New Zealand *Gigartina* species corresponding to each of these three groups are listed in Table 1.

The number of possible groupings based on various morphological characters proposed by the different authors above makes it difficult to produce definitive relationships between species or to determine the existence of separate genera. However, seaweed taxonomy has been revolutionised in recent years by the use of molecular biology through *rbc*L sequence analysis. A number of New Zealand members of the family Gigartinaceae have been analysed and categorised using *rbc*L sequence analysis,[6–10] and a summary of the results is shown in Table 1. As a consequence of these *rbc*L sequence studies, some New Zealand members of the family Gigartinaceae have been transferred to the genera *Sarcothalia* or *Chondracanthus* and, in addition, other taxa have been assigned to as yet unnamed genera, *'Gigartina'* or *'Sarcothalia'*. While there is incomplete overlap in the placement of species based on morphology and *rbc*L sequence analysis, there are good correlations between the species in branched group A and the genus *Sarcothalia*, and the species in branched group B and the genus *Gigartina*, but not for bladed group C (Table 1).

Polysaccharide structure can be a useful taxonomic tool for red algae. Most species of red algae contain galactans with 4-linked galactosyl units, the configurations of which have taxonomic significance. Some genera (e.g., *Gelidium* and *Gracilaria*) produce agars which contain 4-linked L-galactosyl units, while others, such as the genus *Gigartina*, produce carrageenans which contain 4-linked D-galactosyl units. Other aspects of polysaccharide structure may also have taxonomic significance. For example, differences in agar structure have been used successfully to identify new species of red seaweed, for example, *Curdiea balthazar*[11,12]

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Table 1
Grouping of New Zealand members of the family Gigartinaceae based on various taxonomic characters

Author(s)	Adams[5]	Hommersand et al.[6-9] Nelson & Broom[10]	Falshaw & Furneaux (this work) Falshaw & Furneaux[18-20] Falshaw et al. [21]
Taxonomic Character	**Morphology**	***rbc*L Sequence Analysis**	**Lambda-Carrageenan Structure**
Group 1	**A – Branched**	***'Sarcothalia'***	**Some G2S,6S; High D2S,6S**
		'G'. ancistroclada	*'G.' ancistroclada*
		'S.' decipiens	*'S.' decipiens*
	G. ancistroclada		
	G. decipiens	***Sarcothalia***	**High G2S; High D2S,6S**
	G. tuberculosa	n.d.	n.d.
	G. dilatata	n.d.	*G. dilatata*
	G. marginifera	n.d.	*G. marginifera*
	G. livida	*S. livida*	*S. livida*
		S. lanceata	*S. lanceata*
		S. circumcincta	n.d.
			G. pachymenioides
Group 2	**C – Blades**		**VERY Complex (includes DA)**
	G. lanceata		
	G. circumcincta		
	G. pachymenioides	n.d.	
	G. sp. "subantarctic"	n.d.	n.d.
	G. atropurpurea	*'G.' atropurpurea*	*'G.' atropurpurea*
Group 3	**B – Branched**	***Gigartina***	**High G2S,6S; High D2S**
	G. langii	n.d.	n.d.
	G. sp. "Bounty Is."	n.d.	n.d.
	G. clavifera	*G. clavifera*	*G. clavifera*
	G. divaricata	*G. divaricata*	*G. divaricata*
	G. macrocarpa	*G. macrocarpa*	*G. macrocarpa*
	G. sp. "Lindauer 164"	*G. sp. "Lindauer 164"*[a]	*G. sp. "Lindauer 164"*
	G. sp. "Three Kings Is."	*G. sp. "Three Kings Is."*	n.d.
	G. alveata		
	G. chapmanii	***'Gigartina'***	**Low D**
		'G.' grandifida	*'G.' grandifida*
		Outlier	
		'G.' alveata	
Group 4		***Chondracanthus***	**High G2S; High D2S**
			'G.' alveata
		C. chapmanii	*C. chapmanii*

[a] named as *G. tuberculosa*[9]
n.d – not determined

and to differentiate between morphologically similar species of red seaweed in the genera *Gelidium*[13] and *Gracilaria.*[14] Carrageenans have a linear structure of alternating 3-linked β-D-galactopyranosyl (G) and 4-linked α-D-galactopyranosyl (D) units. Various hydroxyls may be sulfated (S), pyruvated (P) or occasionally methylated (M). An additional, common feature is the existence of the 4-linked units in the form of the 3,6-anhydride (A). Variations in carrageenan structure occur between different species. While minor differences in polysaccharide structure may occur due to seasonal and environmental conditions, the overall structure of the polysaccharide is generally quite stable. This consistency is the basis of the commercial carrageenan industry. For example, various species in the Gigartinaceae contain different ratios of kappa- and iota-carrageenans.[15] In members of the Gigartinaceae, carrageenan structure also depends on the life-stage of the species, that is, gametophytes contain κ-type carrageenans, while tetrasporophytes contain λ-type carrageenans.[16,17] Results of previous studies on the structure of the carrageenans from the tetrasporophyte phase of six endemic New Zealand *Gigartina* species indicate significant structural differences that may have taxonomic significance.[18–21] A detailed study of the carrageenans from *Gigartina atropurpurea* has shown almost no changes in carrageenan structure between seasons (unpublished data). This finding adds weight to the use of carrageenan structure as a chemotaxonomic tool.

The idealised structure of λ-carrageenan consists of 3-linked β-D-galactopyranosyl 2-sulfate units alternating with 4-linked α-D-galactopyranosyl 2,6-disulfate units [(G2S-D2S,6S)$_n$], Figure 1A. In ξ-carrageenan, the 4-linked unit lacks the 6-sulfate ester, and it is thus [(G2S-D2S)$_n$] (Fig. 1B). π-Carrageenan has the same sulfation pattern as ξ-carrageenan, but the 3-linked units have pyruvate acetal substituents on the 4- and 6-positions, that is, [(GP,2S-D2S)$_n$] (Fig. 1C). However, unusual units and substitution patterns have also been found in the λ-type carrageenans from

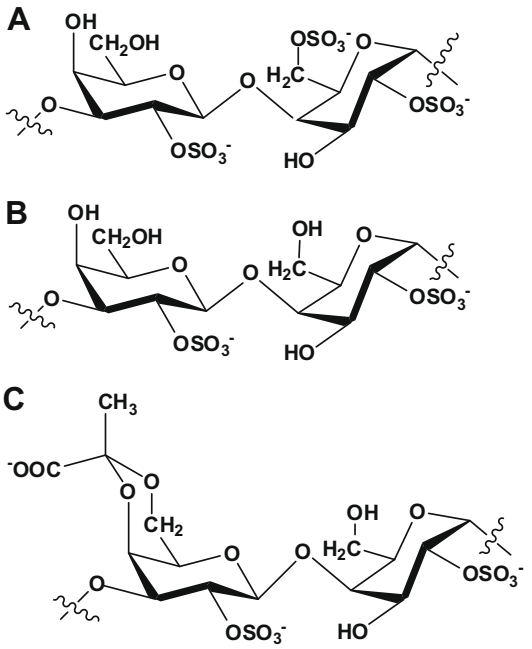

Figure 1. Idealised disaccharide repeat units for λ -type carrageenans. A = λ; B = ξ; C = π.

the tetrasporophyte phase of New Zealand members of the Gigartinaceae, see below.

Of the six endemic New Zealand members of the Gigartinaceae previously subjected to chemical analysis, the polysaccharides from the tetrasporophyte phase of *Sarcothalia lanceata* and *'S.' decipiens* were both very close to the ideal structure of λ-carrageenan. However, in the *'S.' decipiens* polysaccharide, approximately 15% of the 3-linked β-D-galactopyranosyl units contained an additional 6-sulfate that is, (G2S,6S) units.[18,20] The tetrasporophyte phases of *Chondracanthus chapmanii*, *Gigartina clavifera* and *'Gigartina' alveata* also contained λ-type carrageenans, but each polysaccharide contained significant amounts of D2S units as in ξ-carrageenan.[19,20] *G. clavifera* also contained a high proportion (25%) of 3-linked units with an additional 6-sulfate, that is (G2S,6S) units.[19] In contrast, the polysaccharide from the tetrasporophyte phase of *'G.' atropurpurea* was very complex and contained a substantial amount of unsulfated 4-linked 3,6-anhydro-α-D-galactopyranosyl units not previously found in lambda-type carrageenans.[21] Now, the same analytical techniques have been used to characterise the polysaccharides from the tetrasporophyte phase of nine further endemic New Zealand Gigartinaceae: *'G.' ancistroclada, G. dilatata, G. divaricata, 'G.' grandifida, G. macrocarpa, G. marginifera, G. pachymenioides, G.* sp. *'Lindauer 164'* and *Sarcothalia livida.* These new results and their taxonomic implications will be discussed here in relation to the species analysed previously.

2. Experimental

2.1. Materials

Specimens of the material studied have been deposited in the Herbarium of the Museum of New Zealand, Te Papa Tongarewa. Tetrasporophytic specimens of the following species were collected from various sites around New Zealand as follows: *Gigartina macrocarpa*—Rangiputa, Great Exhibition Bay, Rangaunu Harbour on 7 October 1995 (WELT A21523); *G. marginifera*—Ocean Beach, Bream Head on 6 October 1995 (WELT A21178); *'G.' ancistroclada*—Cathedral Cove, SE Otago on 19 February 1996 (WELT A21964); *G. dilatata* (WELT A21963) and *G.* sp. 'Lindauer 164' (WELT A21867)—Ringaringa, Stewart Island on 20 February 1996; *S. livida*—Papatowai, SE Otago on 18 February 1996 (WELT A21866); *G. pachymenioides*—Deadman's Beach, Stewart Island on 21 February 1996 (WELT A21865); *G. divaricata*—Auckland Islands on 11 December 1996 (WELT A21968); *'G.' grandifida*—Wharekauri, Chatham Island on 16 May 1999 (WELT A22885). Samples were air-dried and stored in the dark at room temperature before analysis.

2.2. Isolation of polysaccharides

Samples were prepared and analysed according to the methods described in Falshaw and Furneaux.[18] Briefly, the polysaccharides were extracted using 0.05 M $NaHCO_3$ (60 mL/g weed) at 90 °C for 4 h. The cooled extract, in each case, was treated with amyloglucosidase to digest any floridean starch present, reheated, filtered then dialysed and lyophilised. The yield from the first extraction of *G. divaricata* was lower than that expected for a *Gigartina* species, so a second extraction was undertaken on the residue from the first extraction.

2.3. Analysis of polysaccharides

2.3.1. Infrared spectroscopy

Infrared spectroscopy was performed using a Perkin–Elmer 1605 FTIR spectrophotometer. Samples were analysed as films, prepared by drying 0.4% solutions on silanised glass dishes.

2.3.2. Methylation

A portion of each polysaccharide was converted to the triethylammonium salt form by dialysis against triethylammonium hydrochloride (0.1 M, pH 7, 2×), then distilled water and was lyophilised. A portion of each of these samples (approx. 1 mg) was then methylated using potassium methylsulfinylmethanide in DMSO and methyl iodide. The methylated samples were purified by dialysis [$H_2O \times 1$; Et_3NHCl (0.1 M, pH 7) × 1; $H_2O \times 2$] and recovered by lyophilisation according to the method of Stevenson and Furneaux.[22]

2.3.3. Constituent sugar and glycosyl linkage/substitution analyses

Constituent sugar analyses of the polysaccharides and glycosyl linkage/substitution analyses of the methylated polysaccharides prepared in Section 2.3.2 were performed, using the reductive hydrolysis method described in Stevenson and Furneaux.[22] Combined reduction and acid hydrolysis were achieved using *N*-methylmorpholine borane in aqueous trifluoroacetic acid followed by acetylation with a 1:1 mixture of acetic anhydride and trifluoroacetic acid to prepare (partially methylated) alditol acetate derivatives. The derivatives were analysed by GLC and GLC–EIMS under the conditions described in Stevenson and Furneaux,[22] and Falshaw and Furneaux.[18] C-1-Deuterated alditol acetate derivatives were prepared and analysed by GLC–EIMS according to the method of Falshaw and Furneaux.[18]

2.3.4. Pyruvate acid analysis

Pyruvate acetal substitution was detected as the 2,4-dinitrophenylhydrazone derivative of pyruvic acid following the procedure of Nelson et al.[13]

3. Results

The amyloglucosidase-treated, lyophilised extracts from the tetrasporophyte phase of nine endemic New Zealand Gigartinaceae

Table 2
Yield, neutral constituent sugar and pyruvic acid analyses of the polysaccharides from tetrasporophytes of nine members of the family Gigartinaceae in New Zealand

	'G'. grandifida[b]	*G. divaricata/2*	*G. divaricata/1*	*G.* sp. *'Lindauer 164'*	*G. macrocarpa*	*'G'. ancistroclada*	*G. pachymenioides*	*G. dilatata*	*S. livida*	*G. marginifera*
Yield (%)	66	26	32	52	48	58	60	60	57	59
Pyruvic acid content (%)	1.8	3.1	1.7	2.3	2.3	n.d.[c]	Tr	Tr	Tr	Tr
Constituent sugar (normalised mole%)[a]										
AnGal	1	0	0	Tr	0	0	1	0	0	0
Gal	93	100	100	98	99	100	98	97	97	99
Xyl	1	0	0	1	0	0	1	2	1	1
Glc	5	Tr	Tr	1	1	0	Tr	1	2	Tr

Tr—trace.
[a] AnGal determined as 1,2,4,5-tetra-*O*-acetyl-3,6-anhydrogalactitol, Gal as galactitol hexaacetate, etc.
[b] Extract not treated with amyloglucosidase.
[c] n.d.—not determined due to insufficient sample.

were fluffy white solids. Yields from air-dried seaweed are shown in Table 2. The combined yield from the two extractions of *G. divaricata* was similar to the yield of single extracts from the other eight species.

Infrared spectra of all the samples tested showed an intense band at 1250 cm^{-1}, characteristic of sulfate esters in general.[23] The spectra from *G. dilatata, G. marginifera*, *G. pachymenioides* and *S. livida* extracts also showed a broad band centred at 830 cm^{-1} with a shoulder at 820 cm^{-1}. The corresponding band in the FTIR spectra of extracts from tetrasporic *G. divaricata, G. macrocarpa, 'G.' grandifida* and *G.* sp. *'Lindauer 164'* was much narrower with one discernible maximum at 833 cm^{-1}.

Constituent sugar analyses shown in Table 2 revealed that the extracts from tetrasporophytes of all the nine species of Gigartinaceae examined consisted almost exclusively of galactose.

The results of the glycosyl linkage/substitution analyses are shown in Table 3. The main species obtained for the *'G.' ancistroclada*, *G. dilatata, G. marginifera*, *G. pachymenioides* and *S. livida* extracts were 2,3-Gal (i.e., 1,2,3,5-tetra-*O*-acetyl-4,6-di-*O*-methyl-galactitol) and 2,4,6-Gal (i.e., 1,2,4,5,6-penta-*O*-acetyl-3-*O*-methyl-galactitol). However, the *'G.' ancistroclada* extract also contained 12% 2,3,6-Gal (i.e., 1,2,3,5,6-penta-*O*-acetyl-4-*O*-methyl-galactitol). Some 2,6-Gal (i.e., 1,2,5,6-tetra-*O*-acetyl-3,4-di-*O*-methyl-galactitol) was observed in the analysis of the *G. pachymenioides* extract.

Glycosyl linkage/substitution analysis of extracts from tetrasporophyte *G. divaricata, G. macrocarpa* and *G.* sp. *'Lindauer 164'* revealed six predominant units (>5 mol %). These were 2,3-Gal, 2,4,6-Gal and 2,3,6-Gal as observed for the other species described above but also 2,3,4,6-Gal (i.e., 1,2,3,4,5,6-hexa-*O*-acetyl-galacti-

Table 3
Glycosyl-linkage/substitution analysis of the polysaccharides from tetrasporophytes of nine members of the family Gigartinaceae in New Zealand (*normalised mole%*)

Constituent sugar and deduced substitution[a]	Possible unit type	*'G'. grandifida*	*G. divaricata/2*	*G. divaricata/1*	*G.* sp. *'Lindauer 164'*	*G. macrocarpa*	*'G'. ancistroclada*	*G. pachymenioides*	*G. dilatata*	*S. livida*	*G. marginifera*
3-Linked units											
3-Gal	G	1	1	1	2	0	2	2	1	1	Tr
2,3-Gal	G2S	13	14	22	22	24	42	38	44	46	48
2,3,6-Gal[b]	G2S,6S	22	15	14	16	14	6	2	0	2	2
3,6-Gal	G6S	1	0	0	0	0	1	0	1	0	0
3,4,6-Gal	GP	0	0	0	0	1	0	0	0	0	0
2,3,4,6-Gal[d]	GP,2S	11[c]	13[c]	8[c]	8[c]	7[c]	0	0	0	0	0
Total 3-linked		*48*	*43*	*45*	*48*	*46*	*51*	*42*	*46*	*49*	*50*
4-Linked units											
2,4-AnGal	DA2S	2	1	1	0	0	2	2	3	1	2
4-Gal	D	4	11	7	10	7	2	0	1	0	Tr
2,4-Gal	D2S	26	21	24	21	26	3	5	1	1	0
4,6-Gal		1	1	0	0	1	0	0	0	0	0
2,4,6-Gal[b]	D2S,6S	16	16	17	17	16	39	36	43	43	43
Total 4-linked		*49*	*50*	*49*	*48*	*50*	*46*	*43*	*48*	*45*	*45*
Terminal/ambiguous units											
2,3,4-Gal		3	6	4	1	1	1	3	2	1	4
3,4-Gal		0	0	0	0	0	0	0	1	0	1
2,6-Gal		0	1	2	2	2	1	12	1	5	0
2-Gal		0	0	0	1	1	0	0	0	0	0
2,3,4,6-Gal[d]		—	—	—	—	—	1	0	2	0	0
Total Terminal/ambiguous units		*3*	*7*	*6*	*4*	*4*	*3*	*15*	*6*	*6*	*5*

[a] 2,4-Gal means a 2,4-disubstituted and/or linked galactopyranosyl unit, analysed as 1,2,4,5-tetra-*O*-acetyl-3,6-di-*O*-methyl-galactitol etc.
[b] Enantiomeric partially methylated alditol acetates (2,3,6-Gal and 2,4,6-Gal) differentiated by deuterium labelling and determined by GC–MS analysis.[18]
[c] Assumed to be from 2-sulfated 4-linked-galactopyranosyl units with a pyruvate acetal substituent on the 4- and 6-positions due to the pyruvic acid content (Table 2).
[d] 2,3,4,6-Gal could result from either a 3-linked 4′,6′-pyruvylated β-D-galactopyranosyl 2-sulfate (GP2S) unit or some other type of galactopyranosyl unit that is incompletely methylated. 2,3,4,6-Gal is assigned to GP2S, where there is additional evidence of pyruvate ketal substitution from colorimetric assay, otherwise it is assigned as an ambiguous unit.

tol), 4-Gal (i.e., 1,4,5-tri-*O*-acetyl-2,3,6-tri-*O*-methyl-galactitol) and 2,4-Gal (i.e., 1,2,4,5-tetra-*O*-acetyl-3,6-di-*O*-methyl-galactitol), Table 3. The second extract from *G. divaricata* contained more 2,3,4,6-Gal, and correspondingly less 2,3-Gal, than the first extract.

Glycosyl linkage/substitution analysis of the extract from tetrasporophyte *'G.' grandifida* showed that it contained five predominant units (>5 mol %) that is, 2,3-Gal, 2,3,6-Gal, 2,3,4,6-Gal, 2,4-Gal and 2,4,6-Gal, Table 3.

Extracts from tetrasporophyte *G. divaricata, G. macrocarpa, 'G.' grandifida* and *G.* sp. *'Lindauer 164'* each contained a notable amount of pyruvate acetal groups, while the extracts from tetrasporic *G. dilatata, G. marginifera, G. pachymenioides* and *S. livida* each contained only a trace amount, based on colorimetric pyruvic acid analysis, Table 2. There was insufficient *'G.' ancistroclada* extract to undertake this analysis.

4. Discussion

Constituent sugar analysis of all the polysaccharide extracts (Table 2) showed that they contained predominantly galactose. However, the species could be divided into three groups based upon glycosyl linkage/substitution analysis, pyruvic acid content and IR spectral data.

The first group contained *G. dilatata, G. marginifera, G. pachymenioides* and *S. livida*. Glycosyl linkage/substitution analysis of all these extracts yielded two major partially methylated alditol acetate derivatives that were consistent with 2-sulfated 3-linked-galactopyranosyl units and 2,6-disulfated 4-linked-galactopyranosyl units (Table 2). It is noteworthy that *S. livida* and *G. pachymenioides* extracts contain 5 and 12 mol % of 2,6-Gal, respectively. The origin of 2,6-Gal had been discussed previously and has been tentatively assigned to non-reducing terminal 2,6-disulfated units generated by chain cleavage of the polysaccharide during the methylation procedure.[20] This explanation seems unlikely to account for the particularly high molar proportion of 2,6-Gal in the latter extract, so further work will be required to identify its origin definitively.

The IR spectrum for each species in this group contained a broad band centred at 830 cm^{-1} with a shoulder at 820 cm^{-1}, characteristic of equatorial 2- and 6-sulfate ester groups, respectively.[23] Species in this group also contained only trace amounts of pyruvate acetal groups.

The second group contained *G. divaricata, G. macrocarpa, G.* sp. *'Lindauer 164'* and *'G.' grandifida*. The peak centred around 833 cm^{-1} in the IR spectrum for extracts from each member of this group was much narrower than that for the first group, indicating the presence of equatorial 2-sulfate but less 6-sulfate than for the first group. All these extracts contained a number of predominant major partially methylated alditol acetate derivatives (Table 2). Most of these derivatives are consistent with 2-sulfated species, while the presence of less 2,4,6-Gal than for the first group is consistent with fewer 6-sulfated 4-linked units although there were possibly more 6-sulfated 3-linked units (2,3,6-Gal). Very similar results had also been found previously for *G. clavifera*.[19] Species in this group also contained a notable amount of pyruvate acetal groups. Interestingly, the second extract from *G. divaricata* contained almost twice the amount of pyruvate acetal groups (as determined by both colorimetric and glycosyl linkage/substitution analysis) than the first extract.

Glycosyl linkage/substitution analysis of *'G.' ancistroclada* closely matched with the first group with the exception that it contained 12% of 2,6-disulfated 3-linked-galactopyranosyl units. It was alone in having a significant proportion of this residue, so it was considered as a separate group. An IR spectrum was not recorded due to lack of sample.

The glycosyl linkage/substitution composition of the three groups of polysaccharides from the tetrasporic life-stage of the nine endemic New Zealand species of Gigartinaceae analysed herein is shown graphically in Figure 2 along with the glycosyl linkage/substitution results from previous studies on the carrageenans from tetrasporophytes of six other endemic New Zealand Gigartinaceae.[18–21] The taxonomic relationships between these species are explored below.

Two endemic New Zealand species, originally known as species of *Gigartina*, *G. lanceata* and *G. livida*, were re-classified by Hommersand et al. and placed in the genus, *Sarcothalia*,[4] a re-classification later supported by *rbc*L sequence evidence.[8] The polysaccharide extract from tetrasporic plants of *S. lanceata* had previously been shown to have a structure close to that for idealised λ-carrageenan (i.e., alternating G2S-D2S,6S).[20] The present

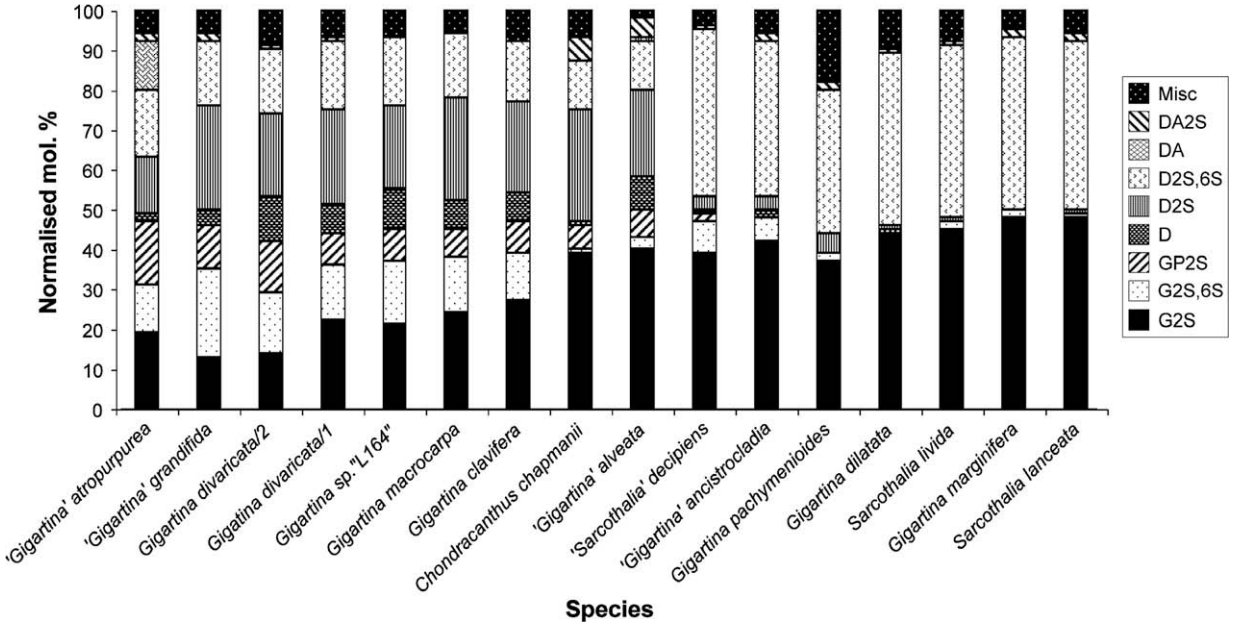

Figure 2. Relative proportions of sugar units in polysaccharide extracts from tetrasporophytes of 15 endemic New Zealand Gigartinaceae.

results show that the polysaccharide extract from tetrasporic plants of *S. livida* also has a similar structure, so this may be a chemotaxonomic indicator for the genus, *Sarcothalia*. In addition, the present results show that the same derivatives predominated in the extracts from tetrasporic *G. dilatata, G. marginifera* and *G. pachymenioides*. Sequence data have not yet been published for *G. dilatata, G. marginifera* and *G. pachymenioides*. However, we predict that *G. dilatata, G. marginifera* and *G. pachymenioides* belong to the *Sarcothalia* genus based on the structures of their galactans. Sequence analyses of the *rbc*L gene would also allow inter-specific differences to be determined, which is not possible chemotaxonomically, as there are insufficient differences between these galactan structures to be useful in differentiating them at species level.

Another similarity between *S. livida*, *G. dilatata* and *G. marginifera* is that they are all branched species with discrete tetrasporangial sori dotted all over the fronds and so were also grouped together by Adams.[5] Adams also included two other endemic New Zealand species, *'G.' ancistroclada* and *'S.' decipiens*, in the same group (see Table 1) but Hommersand et al. considered these two species to be slightly different from either *Gigartina* or *Sarcothalia* species and they used these generic names in inverted commas to indicate this relationship.[4,6]

The structure of the polysaccharide extract from tetrasporophytes of *'S.' decipiens* was previously found to be similar to those reported now for *S. livida, G. dilatata, G. marginifera* and *G. pachymenioides* extracts, but with one key difference; 15% of its 3-linked galactopyranosyl units contained an additional 6-sulfate.[18] The structure of the polysaccharide extract from tetrasporic plants of *'G.' ancistroclada* is very similar to that from *'S.' decipiens* in that 12% of its 3-linked galactopyranosyl units contain an additional 6-sulfate. Thus, the carrageenans from both tetrasporic *'G.' ancistroclada* and *'S.' decipiens* are similar, but not identical, to those of *Sarcothalia* species. Again, a galactan structure with a significant level of additional 6-sulfate on the 3-linked galactopyranosyl units may be a useful chemotaxonomic indicator for '*Sarcothalia*' species.

Glycosyl linkage/substitution analysis of polysaccharide extracts from tetrasporic plants of three other endemic New Zealand *Gigartina* species (*G. divaricata, G. macrocarpa* and *G.* sp. *'Lindauer 164'*) all yielded similar proportions of three pairs of partially methylated alditol acetate derivatives: 2,3-Gal and 2,4-Gal; 2,3,6-Gal and 2,4,6-Gal; and 2,3,4,6-Gal and 4-Gal (Table 3) that are consistent with G2S and D2S; G2S,6S and D2S,6S; and GP2S and D units, respectively. Hommersand et al. placed *G. divaricata, G. macrocarpa* and *G.* sp. *'Lindauer 164'* in the genus *Gigartina*[9] so the presence of these units in similar proportions maybe a chemotaxonomic indicator for the genus *Gigartina*. Further evidence to support this hypothesis comes from the structure of the polysaccharide extract from tetrasporic plants of another endemic New Zealand *Gigartina* species, *G. clavifera*, reported previously.[19] Tetrasporic *G. clavifera* also contains a carrageenan structurally similar to those from *G. divaricata, G. macrocarpa* and *G.* sp. *'Lindauer 164'*, and was also placed in the genus, *Gigartina*, by Hommersand et al.[9] Another similarity is that the four species *G. divaricata, G. macrocarpa, G.* sp. *'Lindauer 164'* and *G. clavifera* were also grouped together by Adams as they are all branched species with tetrasporangial sori in patches expanded over the upper fronds.

Glycosyl linkage/substitution analysis of the extract from tetrasporic *'G.' grandifida* showed that it was similar to *G. divaricata, G. macrocarpa, G.* sp. *'Lindauer 164'* and *G. clavifera* in that it contained five of the same predominant units, but *'G.' grandifida* contained less of the derivative 4-Gal. Also, the proportions of the predominant units differed. For *'G.' grandifida*, there were similar amounts of derivatives defined as G2S and D2S6S, and also G2S,6S and D2S (Table 3). While there are similarities between the galactan from tetrasporic *'G.' grandifida* and those of the true *Gigartina* species, there are also differences. This suggests that *'G.' grandifida* is not a true *Gigartina* species but may belong to a closely related genus. Recent *rbc*L sequence evidence supports this view.[10]

The polysaccharide extracts from tetrasporic plants of two other endemic New Zealand species of Gigartinaceae (*'G.' alveata* and *Chondacanthus chapmanii*) were also found previously to contain λ-type carrageenans, but in each of these species, the polysaccharides contained significant amounts of D2S units as in ξ-carrageenan.[19,20] The biggest difference in structure between the two species was the level of unsulfated 4-linked galactopyranosyl (D) units (8% for *'G.' alveata* but 1% for *C. chapmanii*). Interestingly, *rbc*L sequence analysis of these two species indicates that they are not closely related; Hommersand et al. transferred *G. chapmanii* to *Chondracanthus* and concluded that *'G.' alveata* is genetically unrelated and belongs to an undescribed monotypic genus.[8]

The polysaccharide from the tetrasporophyte of the endemic New Zealand species *'G.' atropurpurea* had also been reported previously.[21] It has a very complex structure with some similarities to those polysaccharides from the *Gigartina* species, but it also contains a substantial amount (11%) of unsulfated 4-linked 3,6-anhydro-galactopyranosyl units not previously found in lambda-type carrageenans.[21] This suggests that *'G.' atropurpurea* is chemotaxonomically distinct from the *Sarcothalia*, '*Sarcothalia*' and *Gigartina* genera. This view is supported by *rbc*L sequence evidence as Hommersand et al. considered that *'G'. atropurpurea* was not a true *Gigartina* but was in a separate, but as yet unnamed, genus.[9]

5. Conclusions

The use of modern analytical techniques has facilitated the identification of the polysaccharides from tetrasporophytic plants of nine endemic New Zealand Gigartinaceae. All nine species contain galactans, and their structures have been compared with chemical data obtained previously for six other endemic New Zealand species in the Gigartinaceae.[18–21] Differences in the structures of these 15 galactans indicate three major groupings with chemotaxonomic significance, plus some other outliers. The three major groupings correspond to the genera *Sarcothalia*, '*Sarcothalia*' and *Gigartina* proposed by Hommersand et al. as a result of *rbc*L sequence analysis.[4]

Chemotaxonomic characters of galactans in the following genera are

- *Sarcothalia*—close to idealised λ-carrageenan (i.e., alternating G2S and D2S,6S units);
- *'Sarcothalia'*—deviates from idealised λ-carrageenan by having approximately 15% G2S,6S units and
- *Gigartina*—similar amounts of G2S and D2S; G2S,6S and D2S,6S; and GP2S and D units.

Thus, the chemical structure of these galactans is a useful taxonomic marker and consequently, we predict that *rbc*L sequence analysis of *G. dilatata, G. marginifera* and *G. pachymenioides* will confirm that they are members of the genus *Sarcothalia*.

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