

Phylogenetic diversity of New Zealand Gelidiales as revealed by *rbcL* sequence data

W.A. Nelson^{1,*}, T.J. Farr¹ & J.E.S. Broom²

¹National Institute of Water and Atmospheric Research Ltd (NIWA), Private Bag 14901, Wellington, New Zealand; ²Department of Biochemistry, University of Otago, PO Box 56, Dunedin, New Zealand

*Author for correspondence: e-mail: w.nelson@niwa.co.nz

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Abstract

Diversity and phylogenetic relationships of New Zealand representatives of the red algal order Gelidiales have been examined using *rbcL* sequence data. Extensive field collections have been made from throughout the New Zealand region. Six genera have been reported previously from New Zealand (*Capreolia*, *Gelidium*, *Pterocladia*, *Pterocladiaella*, *Pterocladiastrum*, *Ptilophora*). This research has revealed species with very restricted local distributions, as well as the discovery of several undescribed, cryptic taxa. The common and widespread *Gelidium caulacanthum* is confirmed to be more closely related to *Capreolia* than to other species of *Gelidium*. The generic concept of *Capreolia*, based on life history characters, will need to be modified to accommodate additional species possessing “*Gelidium*” life histories. A species endemic to New Zealand, *Gelidium ceramoides*, has been found to differ significantly from all other members of the Gelidiales and requires reclassification in another genus and order. Examination of field collections and herbarium specimens in addition to molecular sequence data have led us to conclude that specimens previously placed in the genera *Ptilophora* and *Pterocladiastrum* belong within *Pterocladia lucida*.

Introduction

The red algal order Gelidiales contains a number of commercially valuable agarophyte genera and is thus economically important. In New Zealand there has been an agar industry for ca. 60 years primarily based on the use of *Pterocladia lucida* (Turner) J. Agardh (Schiel & Nelson, 1990). Interest in potential new sources of valuable polysaccharides has stimulated research on the Gelidiales in New Zealand.

Definition of genera and species in the Gelidiales has been considered problematic for a very long time, in part because of the morphological variability that appears to be a feature of many of the taxa. Although there has been considerable effort expended in the search for reliable taxonomic characters in *Gelidium* (e.g. Stewart, 1976; Rodriguez & Santelices, 1987; Santelices, 1990) to date there has been little success. Over the past 15 years research on the Gelidiales has

seen a focus on regional taxonomic studies employing traditional morphological and anatomical techniques (e.g. Santelices, 1994; Lee & Kim, 1995). Molecular sequence data are providing fresh insights into the phylogenetic relationships within the Gelidiales as well as an improved understanding of generic boundaries (Freshwater et al., 1995; Bailey & Freshwater, 1997; Freshwater & Bailey, 1998; Patwary et al., 1998; Shimada et al., 1999; Tronchin et al., 2002, 2003).

Chapman (1969) recorded two genera of Gelidiales from the New Zealand region, recognising 15 taxa within the genus *Gelidium* (including nine species with three varieties, three forms and one ecad) and three species of *Pterocladia*. Chapman also listed three species as ‘species excludendae’. Adams (1994) recorded four genera, six species of *Gelidium*, three species of *Pterocladia*, and one species each of *Ptilophora* and *Pterocladiastrum*, although she expressed doubt about the recognition

of two of these species (*Pterocladia lindaueri* Fan and *Pterocladiastrum robustum* Akatsuka). Guiry and Womersley (1993) erected the genus *Capreolia* for a mat-forming species found in the mid-intertidal zone on both exposed and sheltered coasts from south-eastern Australia and New Zealand, placing material that had been previously identified as *Gelidium pusillum* (Stackhouse) Le Jolis into the monotypic *C. implexa* Guiry and Womersley. Santelices and Hommersand (1997) established the genus *Pterocladia* for species previously assigned to *Pterocladia* that possessed cystocarps with unequally developed locules. Nelson et al. (1994) and Nelson and Farr (2003) examined the endemic species *Gelidium allanii* and *G. longipes* respectively.

Over a period of 6 years, collections of Gelidiales have been made throughout the New Zealand region from the Three Kings Islands (34°S 172°E) to Stewart Island (47°S 168°E) and including the Chatham Islands (44°S 167°W). In this paper we report on the results of molecular sequencing techniques applied to a selection of these collections. The value of *rbcL* sequence data for developing hypotheses about phylogenetic relationships of genera within this order has been established

(Freshwater et al., 1995; Shimada et al., 1999; Tronchin et al., 2002, 2003) although a more variable marker may be required to elucidate relationships at the species level. Our objective in this study was to survey diversity and examine current understandings of New Zealand representatives of the order.

Materials and methods

Field material was sorted and treated in three ways. Samples were preserved in 3–5% formalin/seawater for anatomical and morphological examination, material for extraction of molecular sequence data was dried in silica gel, and fresh material was pressed as herbarium sheets to serve as voucher specimens (Table 1) which are lodged in the herbarium of the Museum of New Zealand Te Papa Tongarewa (WELT, Holmgren et al., 1990).

Molecular biology methods: DNA extraction and PCR amplification

DNA was extracted using the Chelex method of Goff and Moon (1993) or the CTAB extraction method

Table 1. GenBank accession numbers, collection information and voucher numbers for samples from the New Zealand region sequenced in the course of this study (Vouchers marked * differ in the collection date from the sample sequenced)

Sample	GenBank accession no	Location/Strain	Date	Collectors	WELT no.
<i>Capreolia implexa</i> Guiry et Womersley	AY648012	Hooper Pt, Spirits, Bay, North I.	15 Nov 1996	Nelson	A26812
<i>Capreolia implexa</i>	AY648009	Castlepoint, Wairarapa, North I.	31 Mar 1998	Nelson	A22316*
<i>Capreolia implexa</i>	AY648010	Evans, Bay, Wellington, North I.	13 Nov 1997	Nelson & Knight	A26811
<i>Capreolia implexa</i>	AY648008	Cable Bay, Nelson, South I.	8 May 1997	Nelson	A26821
<i>Capreolia implexa</i>	AY648011	Gentle Annie Pt, Westland, South I.	9 Mar 2000	Nelson & Russell	A26818
<i>Capreolia implexa</i>	AY648013	Ringaringa, Stewart I.	9 Oct 1998	Nelson & Broom	A26817
<i>Gelidium caulacanthum</i> J. Agardh	AY648017	Puheke, Northland, North I.	8 May 2001	Nelson & Farr	A25776
<i>Gelidium caulacanthum</i>	AY648015	Matauri Bay, Northland, North I.	28 Oct 2003	Nelson	A26819
<i>Gelidium caulacanthum</i>	AY648016	Piha Beach, Auckland, North I.	5 Apr 2000	Nelson & Farr	A26816
<i>Gelidium caulacanthum</i>	AY648020	Tauranga Harbour, North I.	31 July 2003	Nelson	A26824
<i>Gelidium caulacanthum</i>	AY648014	Castlepoint, Wairarapa, North I.	31 Mar 1998	Nelson	A22317*
<i>Gelidium caulacanthum</i>	AY648018	Puponga, South I.	28 Sep 1996	Nelson	A26823
<i>Gelidium caulacanthum</i>	AY648019	Queen Charlotte Sound, South I.	30 Dec 2001	Broom	A26822
<i>Gelidium</i> sp. "Northland"	AY648024	Te Ngairi, Northland, North I.	9 May 2001	Nelson & Farr	A25789
<i>Gelidium</i> sp. "Fiordland"	AY648023	Fiordland, South I.	13 Oct 2000	Wing & Goebel	A25798
<i>Gelidium microphyllum</i> (Crosby Smith) Kylin	AY648022	Ringaringa, Stewart I.	9 Oct 1998	Nelson	A26813
<i>Gelidium longipes</i> J. Agardh	AY648021	Northland, North I.	7 May 2001	Nelson	A25764
<i>Pterocladia lucida</i> (Turner) J. Agardh	AY648025	Maketu, North I	31 July 2003	Nelson	A26820