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

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Abstract

Diversity and phylogenetic relationships of New Zealand representatives of the red algal order Gelidiales have been examined using *rbc*L 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, Pterocladiella, 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 caulacantheum* 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 *Pterocladiella* 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 *rbc*L 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.
Capreolia implexa Guiry et Womersley	AY648012	Hooper Pt, Spirits, Bay, North I.	15 Nov 1996	Nelson	A26812
Capreolia implexa	AY648009	Castlepoint, Wairarapa, North I.	31 Mar 1998	Nelson	A22316*
Capreolia implexa	AY648010	Evans, Bay, Wellington, North I.	13 Nov 1997	Nelson & Knight	A26811
Capreolia implexa	AY648008	Cable Bay, Nelson, South I.	8 May 1997	Nelson	A26821
Capreolia implexa	AY648011	Gentle Annie Pt, Westland, South I.	9 Mar 2000	Nelson & Russell	A26818
Capreolia implexa	AY648013	Ringaringa, Stewart I.	9 Oct 1998	Nelson & Broom	A26817
Gelidium caulacantheum J. Agardh	AY648017	Puheke, Northland, North I.	8 May 2001	Nelson & Farr	A25776
Gelidium caulacantheum	AY648015	Matauri Bay, Northland, North I.	28 Oct 2003	Nelson	A26819
Gelidium caulacantheum	AY648016	Piha Beach, Auckland, North I.	5 Apr 2000	Nelson & Farr	A26816
Gelidium caulacantheum	AY648020	Tauranga Harbour, North I.	31 July 2003	Nelson	A26824
Gelidium caulacantheum	AY648014	Castlepoint, Wairarapa, North I.	31 Mar 1998	Nelson	A22317*
Gelidium caulacantheum	AY648018	Puponga, South I.	28 Sep 1996	Nelson	A26823
Gelidium caulacantheum	AY648019	Queen Charlotte Sound, South I.	30 Dec 2001	Broom	A26822
Gelidium sp. “Northland”	AY648024	Te Ngaire, Northland, North I.	9 May 2001	Nelson & Farr	A25789
Gelidium sp. “Fiordland”	AY648023	Fiordland, South I.	13 Oct 2000	Wing & Goebel	A25798
Gelidium microphyllum (Crosby Smith) Kylin	AY648022	Ringaringa, Stewart I.	9 Oct 1998	Nelson	A26813
Gelidium longipes J. Agardh	AY648021	Northland, North I.	7 May 2001	Nelson	A25764
Pterocladia lucida (Turner) J. Agardh	AY648025	Maketu, North I	31 July 2003	Nelson	A26820

of Hillis et al. (1996). PCR products spanning the *rbc*L-*rbc*S region were amplified using primers from Freshwater and Rueness (1994); for sequencing of *Gelidium longipes* samples, primer F-492 (cgt atg gat aaa ttt ggt cg) was replaced by primer F-492ga (cgt atg gat aa**g** ttt gg**a** c). This primer matches the *G. longipes rbc*L sequence, and was much more effective for sequencing PCR products derived from this taxon. It differs from F-492 by two nucleotide substitutions (indicated in bold) and in the removal of the terminal guanine residue. In some instances a nested amplification strategy was used to amplify the target. Amplification using primers F-57 and R-*rbc*S-start was followed by amplification using F-57 with R-752, and F-492 R-*rbc*S-start, diluting the original amplification product 1:100. This yielded two overlapping products spanning the *rbc*L gene and associated spacer. Amplifications were performed in a Stratagene Robocyler (Stratagene Corporation, La Jolla, Ca) with parameters as follows: an initial denaturation of 30 s at 94 °C was followed by 35 cycles of 25 s at 94 °C, 45 s at 45 °C, 1 min 30 s at 72 °C and a final extension for 10 min at 72 °C. Amplification products were purified by PEG precipitation and sequenced on an ABI 377 automated sequencer according to standard methods. Sequences were aligned with 36 Gelidiales *rbc*L sequences downloaded from GenBank (Table 2) using Se-Al v2.0a11 (Rambaut, 1996). Where *rbc*L/*rbc*S spacer sequences were available, these were also included in the alignment. Three taxa, from the Bonnemaisoniales (*Delisea pulchra* (Grev.) Mont.) and Ceramiales (*Laingia hookeri* (Lyall ex Harv.) Kylin and *Hypoglossum hypoglossoides* (Stackh.) Collins & Herv.), were chosen as outgroup taxa.

Phylogenetic analysis

Maximum parsimony (MP) and neighbour joining (NJ) phylogenetic trees were constructed using PAUP*4.0b10 (Swofford, 2002). MP trees were obtained by a heuristic search strategy (100 replicates of random-order stepwise sequence addition followed by tree-bisection-reconnection (TBR) branch swapping) with gaps treated as unknown bases. A less computer intensive search strategy of 3 replicates of random-order sequence addition followed by subtree-pruning-regrafting (SPR) branch swapping returned an identical consensus tree, and this search strategy was used to find MP trees for 100 bootstrap replicates. Modeltest 3.06 (Posada & Crandall, 1998) was used to identify an appropriate model of sequence evolution for the

Table 2. Published samples used in this study. Taxa are in the order that they appear in Figure 1

Sample	Origin	GenBank accession no.
Delisea pulchra		U26812
Hypoglossum hypoglossoides		AF257368
Laingia hookeri		AF257371
Pterocladia lucida	Owhiro Bay NZ	U01048
Pterocladia lucida	SA Australia	AF305808
Pterocladia lucida	Lord Howe Is	AY352423
Pterocladiella melanoidea	Spain	U01046
Pterocladiella bartlettii	Costa Rica	AF305806
Pterocladiella bartlettii	TX USA	AF305807
Pterocladiella caloglossoides	Australia	AY352422
Pterocladiella beachiae	Costa Rica	AF305811
Pterocladiella caerulescens	Hawaii	AF305805
Pterocladiella capillacea	Japan	AB023849
Pterocladiella capillacea	Owhiro Bay NZ	U24156
Pterocladiella capillacea	Venezuela	U01893
Pterocladiella capillacea	CA USA	U01896
Pterocladiella capillacea	Ireland	U01891
Pterocladiella capillacea	SA Australia	U01898
Gelidiella acerosa	Hawaii	L22457
Gelidiella ligulata		AB017678
Ptilophora pectinata	NSW Aus	AY344043
Ptilophora subcostata	Japan	U16835
Ptilophora scalarimosa	Philippines	AF305804
Ptilophora hildebrandtii	South Africa	AF522359
Ptilophora mediterranea	Greece	AF522360
Ptilophora pinnatifida	South Africa	AF522361
Ptilophora leliaertii	South Africa	U16834
Ptilophora helenae	South Africa	AY344045
Ptilophora rhodoptera	South Africa	AF522365
Ptilophora coppejansii	South Africa	AF522366
Ptilophora diversifolia	South Africa	AF305803
Gelidium divaricatum	Japan	U16828
Gelidium caulacantheum	Porirua NZ	U00103
Capreolia implexa	Australia	L22456
Gelidium caulacantheum	Piha NZ	U01043
Gelidium sp.	NSW Australia 1	AY352420
Gelidium sp.	NSW Australia 2	AY352418
Gelidium asperum	Vic Australia	AY350782
Gelidium australe	SA Australia	AY350783
Gelidium micropterum	SA	U00446
Onikusa pristoides	South Africa	U01044
Gelidium microdonticum	Costa Rica	AF305799
Gelidium isabelae	South Africa	AY350778
Gelidium sesquipedale	Spain	L22071
Gelidium sp.	South Africa	AY350775

(*Continued on next page*)

Table 2. (*Continued*)

Sample	Origin	GenBank accession no.
Gelidium pulchellum	Ireland	U01969
Gelidium latifolium	France	U01818
Gelidium latifolium	Ireland	U01821
Gelidium floridanum	FL USA	U00107
Gelidium allanii	Doubtless Bay NZ	L22458
Gelidium sp.	Lord Howe Is	AY350777
Gelidium tenuifolium	Japan	AB030628
Gelidium elegans	Japan	AB030623
Gelidium pacificum	Japan	AB030627
Gelidium omanense	Oman 1	AY346460
Gelidium pluma	Hawaii USA	AF522367
Gelidium omanense	Oman 3	AY346462
Acanthopeltis japonica	Japan	AB017673
Gelidium vagum	Japan	AB017680
Gelidium rex	Chile	AF305801
Onikusa japonicum	Japan	AB017676
Gelidium chilense	Chile	AF305800
Gelidium capense	South Africa	L22461
Gelidium pusillum	CA USA	U00984
Gelidium coulteri	CA USA	U00105
Gelidium pusillum	Japan	AB017679
Gelidium crinale	Spain	AF308792
Gelidium pusillum	NC USA	U00981
Gelidium pusillum	Puerto Rico	U00983
Gelidium crinale	Canary Is	AF308793
Gelidium crinale	WA Australia	AY350780
Gelidium crinale	NSW Australia	AY350781

dataset. The model selected was the General Time Reversible model allowing for invariant sites and with rate heterogeneity modeled by four gamma-distributed rate classes ($GTR + I + \Gamma$). Parameters estimated by Modeltest were used to calculate genetic distances and build a neighbour joining (NJ) tree; support for clades under NJ was estimated by 1000 bootstrap replicates.

Bayesian trees were constructed using MrBayes v3.01 (Ronquist & Huelsenbeck, 2003) to run four Metropolis-coupled MCMC chains (one cold and three incrementally heated, temperature parameter = 0.2). Three independent MrBayes analyses were run under the $GTR + I + \Gamma$ model of sequence evolution, each for 1,000,000 generations. Model parameters were treated as unknown and were estimated in each analysis. Random starting trees were used and the trees were sampled every 100 generations. Appropriate burn-in values were determined by inspection of plots of log-likelihood against generation time for each run, and trees obtained before this value were discarded. The remaining trees were used to calculate 50% majority rule consensus trees in PAUP*4.0b10, in which each clade posterior probability value is represented by the proportion of trees containing that clade.

The maximum likelihood tree was estimated under an heuristic search strategy starting from one of the trees found in the third Bayesian analysis after burn-in, and proceeding via TBR branch swapping for 70000 iterations, using the model of sequence evolution identified by Modeltest.

Results

Sequences generated in the course of this study were deposited in GenBank under Accession nos AY648008-25 (Table 1). The phylogenetic matrix consisted of 1491 characters, of which 514 were parsimony informative, 852 constant and 125 parsimony-uninformative. Relatively few *rbc*L/*rbc*S spacer sequences were available, and since these contributed few parsimony-informative characters (3 out of 514) their inclusion had little effect on the analysis. Maximum parsimony analysis identified 84 trees of length 2838. Trees obtained from the three Bayesian analyses converged to similar -ln likelihoods and consensus trees of the three independent analyses had an identical topology. For simplicity only the results of the third run are shown here. All four methods of analysis produced trees with very similar structures. Figure 1 shows the maximum likelihood phylogram with support values from the other analyses overlaid.

The analyses presented here provide strong support for clades that equate to the genera currently recognised in this order, and New Zealand taxa are resolved in clades that correspond to the genera *Pterocladia, Pterocladiella, Capreolia* and *Gelidium.*

Pterocladia: The four *Pterocladia* samples shown on the tree form a well supported clade within which the two New Zealand samples group together as do the two Australian samples.

Pterocladia lucida (Turner) J. Agardh 1852: 483
NZ distribution: Three Kings, North, northern South, and Chatham Is.
Basionym and type locality: *Fucus lucidus* Turner 1819: 98, pl.238; BM; southern Australia.
Synonyms: *Pterocladiastrum robustum* Akatsuka 1986: 57, *Ptilophora pectinata* (A. & E.S.Gepp) R.E. Norris sensu Adams 1994: 154.

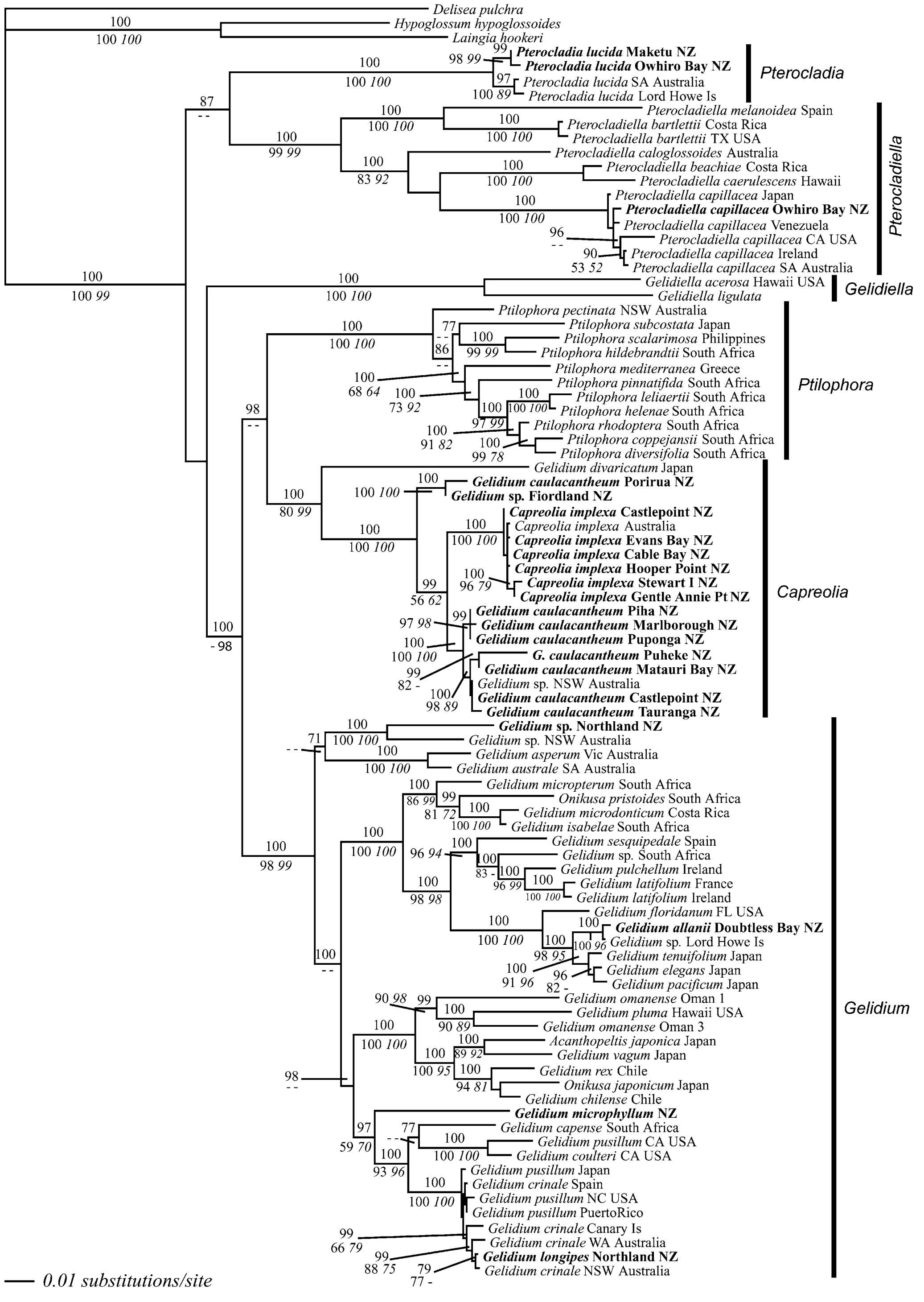

Figure 1. Maximum likelihood phylogram (–ln likelihood 16022.19) found by PAUP*4.0b10. Support values are shown on each node: Bayesian posterior probability values above the line, and MP and NJ bootstrap values below, NJ in italics. Support values are shown only on those nodes supported by >70% on at least one analysis. New Zealand taxa are in bold type.

Remarks: In New Zealand *P. lucida* is morphologically very variable: Moore (1945) discussed five growth forms although she considered all belonged to a single variable species. V.W. Lindauer in unpublished manuscripts had referred previously to one of the growth forms as *P. lucida* var. *sublittoralis*. Adams (1994) transferred the specimens with this distinctive growth form, all collected from subtidal locations, to *Ptilophora pectinata,* noting that only tetrasporangial material had been seen in the New Zealand collections. The basionym of *Ptilophora pectinata* is *Pterocladia lucida* f. *pectinata* A. & E.S. Gepp (1906), based on material from New South Wales, and this form was raised to species status as *Pterocladia pectinata* by Lucas (1931). Subtidal material consistent with the morphological concept employed by Adams, and with the illustration in Norris (1987: 247, Figure 8), and initially identified as *Ptilophora pectinata,* was included in our sequencing study (Figure 1: *P. lucida* Maketu, Table 1: voucher = WELT A26820). The sequence data, however, placed this material corresponding to "*sublittoralis*" of Lindauer and *P. pectinata* of Adams, with *Pterocladia lucida* from both New Zealand and Australia, and not with the *Ptilophora* clade.

Akatsuka (1986) established a new genus and species, *Pterocladiastrum robustum*, for the growth forms of *Pterocladia lucida* named by Moore (1945) as "Robust" and "Poor Knights". The new taxon was established on the basis of the "pattern of surface cells coupled with distinctive tetrasporangial pinnules", characters that have not been widely accepted as reliable at a generic level. Adams (1994) recorded this monotypic genus although expressed reservations about its distinctiveness, and Womersley & Guiry (1994) in a taxonomic treatment of *Pterocladia lucida* in southern Australia rejected *P. robustum*. In AlgaeBase (Guiry & Nic Dhonncha, 2004) *Pterocladiastrum* is listed as a synonym of *Ptilophora pectinata*, which we show above to be a synonym of *Pterocladia lucida* for New Zealand specimens.

Pterocladiella: This genus has a worldwide distribution, with a single New Zealand representative. The sequence data place the New Zealand sample of *P. capillacea* within a clade of closely related specimens sourced from throughout the world (Figure 1), a result that is in agreement with the findings of Freshwater et al. (1995) and Shimada et al. (2000).

Pterocladiella capillacea (Gmelin) Santelices et Hommersand 1997: 117–118.
NZ distribution: Kermadec, Three Kings, North, northern South, and Chatham Is.
Basionym and type locality: *Fucus capillaceus* Gmelin 1768: 146, pl. 15, Figure 1; Lectotype (original illustration); Mediterranean.
Synonyms: *Pterocladia lindaueri* Fan 1961: 335.

Remarks: Fan (1961) distinguished *Pterocladia lindaueri,* described from a collection from the northern North I., on the basis of unequal locules in the cystocarps. Cystocarps of unequal size also occur in *Pterocladiella capillacea,* and material from the type locality of *P. lindaueri* is indistinguishable from *P. capillacea*. Santelices and Hommersand (1997) concluded that material described by Fan was likely to have been material of *P. capillacea*. These species are considered here to be synonymous.

Capreolia: A clade containing *Capreolia implexa* is clearly differentiated in the phylogenetic analyses. Samples of taxa in this clade included in this study were sourced from a wide geographic spread (northern North Island to Stewart Island) in order to assess diversity within New Zealand. The results suggest that there are at least three species of *Capreolia* in New Zealand, including two or three taxa currently grouped under *G. caulacantheum*.

Gelidium caulacantheum J. Agardh 1876: 548.
NZ distribution: North, South, and Chatham Is.
Type and type locality: LD; Berggren collections from both the Bay of Islands and Tauranga, North I.

Remarks: Within New Zealand, a number of names have been applied to small, intertidal species commonly found in turfs (e.g. *Gelidium subulifolium* (Harv.) V.J.Chapm., *G. subuliferum* Laing), as well as two forms (f. *laxiforme*, f. *fasciculatum*) and one variety (var. *pygmaeum*) of *G. caulacantheum* established by Chapman (1969). At present these are all grouped as *G. caulacantheum* sensu Adams (1994).

Capreolia implexa Guiry et Womersley 1993: 267.
NZ distribution: Three Kings, North, South, Stewart, and Chatham Is.
Type and type locality: MEL; Sandringham, Port Phillip, Victoria, Australia.

Remarks: Sequence data place New Zealand and Australian material of *C. implexa* within the same clade. The name *G. pusillum* (Stackhouse) LeJolis was previously applied to *C. implexa* in New Zealand (Adams, 1994).

Gelidium: Four samples sequenced here are grouped in the *Gelidium* clade.

"*Gelidium* Northland" is known from a single, sterile collection and is thus unable to be characterised and described at present. The sequence data indicate that this species is clearly distinct from all other New Zealand taxa.

Gelidium allanii V.J. Chapm. 1969: 98.
NZ distribution: northern North I.
Type and type locality: AKU in AK; Waitata Rocks, Bay of Islands.

Remarks: Molecular sequence analyses place this species within the *Gelidium* clade, well separated from other New Zealand Gelidiales. Previous research on *Gelidium allanii* (Nelson et al., 1994) revealed that this species, which is found on both the east and west coasts of the northern North I., has a restricted distribution and possesses an unusual pyruvulated agar.

Gelidium microphyllum (Crosby Smith) Kylin 1934: 56.
NZ distribution: southern North, South, Stewart, Chatham, Snares, Antipodes, Auckland, and Campbell Is.
Basionym, type and type locality: *Nitophyllum microphyllum* Crosby Smith in Laing 1902: 344; CHR; Green Island Beach, Dunedin.

Remarks: The placement of *G. microphyllum* within the *Gelidium* clade is well supported. This species is restricted to cold waters around the New Zealand mainland and subantarctic islands. Although specimens similar to *G. microphyllum* have been found at the Kermadec Islands, they are unlikely to be conspecific as the Kermadecs (29–32°S) are tropical to subtropical.

Gelidium longipes J. Agardh 1876: 547.
NZ distribution: northern North I.
Type and type locality: LD; Berggren, Bay of Islands.

Remarks: Material from the type locality of *G. longipes* grouped in a clade containing specimens identified as both *G. pusillum* and as *G. crinale*, both European species reported to have cosmopolitan distributions but for which species concepts remain poorly understood (Freshwater & Rueness, 1994). Although this endemic taxon has a very restricted distribution in New Zealand (Nelson & Farr, 2003), the relationship with material from other parts of the world is clearly seen by the results presented here. Clarification of the species concepts of European *Gelidium* and a further examination of species–level diversity in this group are required before nomenclatural change is undertaken.

Excluded taxon: Levring (1949) described *Gelidium ceramoides* from material collected from Kaka Point, Otago, and subsequently it has been found infrequently from only Otago and Stewart I. (Nelson & Phillips, 2001). Preliminary microscopic examination and culture studies clearly establish that this species is not a member of the Gelidiales: material collected from the type locality was sequenced and found to be unrelated to members of the Gelidiales, and more closely related to Gigartinales sensu lato. This species is the subject of a further study.

Discussion

Molecular sequence data have enabled an examination of the phylogenetic relationships of New Zealand species of Gelidiales. New Zealand taxa are found across the order, belonging to well resolved clades corresponding to four genera. Interpretation of morphological variability in *Pterocladia lucida* in New Zealand has been problematic: this study has clarified the identity of material recently transferred to *Ptilophora pectinata* as more correctly placed in *Pterocladia lucida*. The genus *Capreolia* is clearly differentiated in the phylogenetic analyses, and the results of this study indicate that there are likely to be at least three species of *Capreolia* in New Zealand. The relationship between *Gelidium caulacantheum* and *Capreolia implexa* has been recognised previously (Bailey & Freshwater, 1997; Freshwater et al., 1995, Freshwater & Bailey, 1998). *Capreolia* was established by Guiry and Womersley (1993) on the basis of its biphasic life history in which fertilised carpogonia develop directly into tetrasporophytes. Further research is required on this group to clarify species boundaries and to reconsider the criteria used to distinguish the genus, particularly life history characters. Fertile material of *G. caulacantheum* indicates that at least some of the material resolved in this clade does not have the same life history as *C. implexa*. The sequence of the Japanese *G. divaricatum* is resolved as a sister taxon to the *Capreolia* clade. The generic placement of this species warrants attention.

Within the *Gelidium* clade in our analyses there are four New Zealand species that are distinguished by *rbc*L sequence data: *G. allanii, G. longipes, G. microphyllum* and *G.* "Northland". From examination of herbarium collections, it is clear that there are additional undescribed taxa in the New Zealand region, currently represented by fragmentary and incomplete specimens. In particular it is likely that there will be greater diversity in this order when fuller collections, including samples purpose-collected for molecular sequencing, can be made from the northern islands of the New Zealand archipelago (Kermadec Islands) and from the subantarctic islands (Antipodes, Bounty, Auckland and Campbell Is).

In the data presented here *Pterocladia* is an Australasian genus, and the *Capreolia* clade reveals an Australasian radiation. The clade equating to the genus *Gelidium* contains four phylogenetically distinct New Zealand taxa. Increased taxon sampling from throughout the southern hemisphere may illuminate further relationships within this clade.

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