

Contributions of molecular biology to understanding systematics and phylogeny in the order Bangiales

Wendy A. Nelson¹⁾ and Judy E.S. Broom²⁾

¹⁾Aquatic Biodiversity National Institute for Water and Atmospheric Research
Private Bag 14-901, Wellington, New Zealand
E-mail : w.nelson@niwa.co.nz

²⁾Department of Biochemistry, University of Otago
P.O. Box 56, Dunedin, New Zealand.

Abstract The data emerging from molecular sequencing studies are enabling new understandings of the order Bangiales through a re-examination of the systematics and phylogeny in this economically important group of red algae. Convergence in morphology and anatomy, not only within *Porphyra sens. lat.* but also spanning both the Bangiales and Erythropeltidales, has confounded both taxonomic distinctions and phylogenetic hypotheses based on these character sets. Examples are presented of new understandings about generic relationships within the order, and relationships between members of the orders Bangiales and Erythropeltidales based on the use of 18S rDNA sequence data. At a species level, taxonomic frameworks to distinguish *Porphyra* species are currently based around a range of characters including morphological, anatomical, biochemical, physiological and life history characteristics. Phenotypic plasticity in a number of species of *Porphyra* has further confused taxonomic treatments. The use of 18SrDNA sequence data has revealed unexpectedly high diversity in members of the Bangiales of the New Zealand region. Molecular sequence data have the potential to provide powerful tools for the circumscription and identification of species in the orders Bangiales and Erythropeltidales, enabling quicker and more direct identification than, for example, using characters from culture studies.

Key words: molecular biology, systematics, phylogeny, *Porphyra*, Bangiales, Erythropeltidales, 18SrDNA

Introduction

Until recently the red algal order Bangiales has been considered to contain 2 genera, *Bangia* and *Porphyra*, with about 8 species of *Bangia* and more than 130 species of *Porphyra* recognised worldwide (Guiry and Nic Dhonncha, 2002). The genus *Porphyra* is highly valued as a very nutritious food item in many countries in the world. Cultivation of *Porphyra* began in the 17th century and today in Japan the value of the nori industry is very significant (Oohusa, 1993). Identification of commercially important attributes and strain selection have contributed significantly to the quality of the product that is available, using conventional breeding and cultivation techniques as well as those based on cell culture techniques (Fujita and Rao, 1997). In New Zealand *Porphyra* has great cultural significance: *Porphyra* is known as karengo and is highly valued by Maori. In New Zealand there

are both traditional and commercial harvesting of wild stocks of karengo and a great deal of interest in the maintenance and sustainable use of the resource (Nelson and Conroy, 1989; Nelson *et al.*, 1990).

In addition to the cultural and commercial significance of *Porphyra*, the order Bangiales is scientifically important in a number of ways. The blades of *Porphyra* and the filaments of *Bangia* provide simple morphological systems for experimental research. The short generation time and small space required for culturing these algae as well as the small genome size, make them ideal tools for a variety of research programmes (Kitade *et al.*, 1998). Members of the Bangiales display an extraordinary complexity in the expression of life histories with two morphological phases and a diversity of sexual, asexual, and vegetative reproductive modes (Nelson and Knight, 1995; Notoya, 1997; Nelson *et al.*, 1999).

A significant feature of the Bangiales is the antiquity of this lineage. The oldest taxonomically resolved eukaryotic fossil (dated at 1.25 billion years) has a morphology that is virtually indistinguishable from modern members of *Bangia* (Butterfield *et al.*, 1990; Butterfield, 2000). It has been suggested that this fossil also provides the first evidence of eukaryotic sex. The antiquity of this lineage has the potential to provide insights into the evolution of red algae and into the phylogeography of the red algae.

Members of the Bangiales occur in both freshwater and marine environments and there are representatives present from the tropics to the poles. Marine populations of *Porphyra* and *Bangia* typically are found in the upper intertidal zone, capable of tolerating extreme conditions experienced in this rigorous environment - in temperature, desiccation and salinity variation. Given the persistence of this order over many millennia, these species provide useful experimental systems with which to examine the capacity of these organisms to survive significant variation in climate and environmental conditions.

Whilst *Porphyra* has received careful scientific study over many years in Japan, our work on *Porphyra* in New Zealand has been underway for a much shorter time. Field studies on the ecology and impact of harvesting practices (Nelson and Conroy, 1989; Nelson *et al.*, 1990) revealed the need for detailed systematic studies. Prior to 1990, a single species of *Porphyra* was reported to grow on rock and 2 species were known to be obligate epiphytes. Until recently few collections of *Bangia* had been made in the New Zealand region and a single species, *B. atropurpurea*, was recorded (Adams, 1994). Since that time our understanding has changed a great deal. Much of our progress has been an outcome of the insights provided by molecular sequencing data which have given us the capacity to recognise much greater diversity in members of the Bangiales than previously. It is possible to develop a workable taxonomic framework to distinguish species of *Porphyra* using a range of characters such as morphological, anatomical, biochemical, physiological, and life history characteristics. However, the taxonomic distinctions do not necessarily reflect phylogenetic relationships revealed by sequence analyses.

As our work has developed, it has become clear that fundamental questions about the order Bangiales need re-examining. There are unresolved issues at ordinal, generic and specific levels, and molecular research tools are essential components of research programmes addressing these problems. The phylogenetic analyses that can be undertaken using molecular data enable the development of a predictive framework that can assist research by directing the search for genes, biological products and potential mariculture species. Such analyses also provide a context for comparative studies and for the study of distributions and phylogeography. In this review examples are presented to illustrate the contribution of molecular sequencing data to research on the definition of the order Bangiales, the number of genera within the Bangiales, and the recognition of species.

Molecular methods employed in Bangiales research programme:

The majority of the molecular data used in the research discussed here is from the nuclear small subunit ribosomal RNA gene (nSSU, 18S-rDNA) which is a highly conserved region and has been shown to be phylogenetically informative for members of the Bangiales (Oliveira *et al.*, 1995; Müller *et al.*, 1998; Broom *et al.*, 1999; Kunimoto *et al.*, 1999). DNA extractions, amplification and sequencing were carried out as described by Broom *et al.* (1999), Broom *et al.* (2002), Nelson *et al.* (2003), Jones *et al.* (2004), using both thallus / filaments and conchocelis. Voucher specimens have been lodged in WELT, the Herbarium of the Museum of New Zealand Te Papa Tongarewa.

The variable V9 region of the nSSU locus has been found to be a useful indicator of overall sequence type as it reflects overall variation, is easy to amplify and can be sequenced in a single reaction (to 600bp). The region is used as an initial indicator of variability enabling a rapid check for whether a sample belongs to a previously recognised entity.

Sequence alignment and phylogenetic analyses were obtained following the methods given by Nelson *et al.* (2001), Broom *et al.* (2002), Jones *et al.* (2004). The parsimony ratchet search strategy (Nixon, 1999) has been employed in building maximum parsimony trees where large

datasets are being examined (>50 taxa). The datasets used in our research now include 34 unique sequence entities of *Porphyra* and 14 of *Bangia* from the New Zealand region. In addition the New Zealand sequences, we have also sequenced taxa from Australia (New South Wales, Victoria, Tasmania, Western Australia) (e.g. Farr *et al.*, 2003), the Falkland Islands and South Africa (Jones *et al.*, 2004). We compare the data we have obtained with sequences submitted to GenBank from other regions (Table 1). The outgroup chosen for rooted analyses consists of members of the Erythropeltidales and some Florideophyte taxa. However alignment with an outgroup necessitates removal of characters from variable regions which cannot be aligned with confidence between members of the ingroup and the outgroup. The use of ingroup only analyses and unrooted trees is preferable in some situations as it enables the inclusion of more informative characters (e.g. Broom *et al.*, 2002)

Definition of the Orders Bangiales and Erythropeltidales:

Prior to the application of molecular sequencing, three species endemic to the New Zealand region and obligately epiphytic on large brown algal hosts had been described as species of *Porphyra* - *P. subtumens*, epiphytic on *Durvillaea*, *P. cameronii* epiphytic on *Lessonia tholiformis* and *P. kaspar* epiphytic on *Sargassum johnsonii* (Nelson, 1993). The placement of these three species was based on their thallus morphology, holdfast system, form of growth, absence of monosporangia, presence of archeosporangia, and the formation of spermatia and sporangia in packets. Based on these criteria, ones used to characterise species of *Porphyra*, these three epiphytic species were placed in the order Bangiales. Molecular sequence data, however, revealed that these three species are closely related to one another and only distantly related to *Porphyra* (Fig. 1). Rather than falling within a clade of Bangiales molecular sequence data places these taxa within a clade of species belonging to the order Erythropeltidales (Nelson *et al.*, 2003). Two new genera, *Chlidophyllon* and *Pyrophyllon*, have been described to accommodate these three species from the New Zealand region that were previously placed in *Porphyra* (Nelson *et al.*, 2003). The molecular data are supported by

recent work on the polysaccharide chemistry of these taxa and *Porphyra* spp. (Hemmingson and Nelson, 2002).

These results raise questions about how the orders Bangiales and Erythropeltidales are defined, and the current reliance on morphological and anatomical characters of the gametophyte phase.

Generic boundaries:

Although molecular sequencing data have consistently supported the monophyletic origin of the Bangiales, a number of studies from the mid - 1990s onwards have revealed that *Porphyra* and *Bangia* are paraphyletic with respect to one another (Oliveira *et al.*, 1995; Müller *et al.*, 2000; Oliveira and Bhattacharya, 2000; Broom *et al.*, 2004). Species of "*Bangia*" and "*Porphyra*" are present on deeply divided clades on phylogenetic trees (Broom *et al.*, 1999, 2004; Müller *et al.*, 2000; Oliveira and Bhattacharya, 2000; Jones *et al.*, 2004). This situation raises the questions: (1) what constitutes "true" *Porphyra* and "true" *Bangia*, (2) how many other groupings of species warrant recognition as genera, and (3) how should genera within the Bangiales be defined?

In Fig. 2 a dataset of 80 *Porphyra* and 35 *Bangia* nSSU sequences is presented. *Porphyra purpurea* is the type species of the genus *Porphyra*, and at the moment there appears to be confusion surrounding the application of this name. In our phylogenetic analyses, sequences lodged in GenBank from specimens identified as *P. purpurea* come out at three different positions on phylogenetic trees and belong to quite separate clades (Fig. 2) Clarification of the concept / identity of the type species of *Porphyra* is a critical step that must be addressed prior to a reconsideration of generic boundaries within this order.

The type of the genus *Bangia* is *B. atropurpurea* (Roth) C. Agardh from a freshwater location in Bremen, Germany. The type is lost and an isoneotype has been selected by Brodie and Irvine (2003). The sequences of specimens of *Bangia* collected from freshwater localities from the northern hemisphere (both North America and Europe) group together in phylogenetic analyses (Broom *et al.*, 2004). The same, however, is not true for collections of *Bangia* from marine localities. The name *B. fus-*

Table 1. Entities, collection data and GenBank accession numbers for taxa included in sequence analyses (Figures 1 and 2). Taxa marked with an asterisk (*) were sequenced in our laboratory.

Entity	GenBank Accession No	Entity	GenBank Accession No
Figure 1 :			
<i>Mesostigma viride</i>	AJ250109	<i>Rhodella violacea</i>	AF168624
<i>Galdieria sulphuraria</i>	AF342747	<i>Boldia erythrosiphon</i>	AF168631
<i>Rhodosorus marinus</i>	AF342750	<i>Compsopogon coeruleus</i>	AF0087128
<i>Geminigera cryophila</i> nucleomorph	U53123	<i>Compsopogonopsis leptoclados</i>	AF087125
<i>Pyrenomonas salina</i> nucleomorph	X55032	<i>Flintiella sanguinaria</i>	AF342749
<i>Rhinomonas pauca</i> nucleomorph	U53131	<i>Erythrocladia</i> sp.	L26188
<i>Porphyra coleana</i> W.A. Nelson	AF136423*	<i>Erythrotrichia carnea</i>	L26189
<i>Bangia fuscopurpurea</i>	AF342745	Unidentified species MEK258	AY126433*
<i>Bangia atropurpurea</i> strain OM-1	D88387	<i>Smithora naiadum</i>	AF087126
<i>Porphyra yezoensis</i>	AB013178	Unidentified species LHF907	AY126432*
<i>Porphyra leucosticta</i>	L26199	Unidentified species CSW318	AY126430*
<i>Porphyra rakiura</i> W.A. Nelson	AF136425*	<i>Chlidophyllon kaspar</i> W.A. Nelson	AY126431*
<i>Porphyra cinnamomea</i> W.A. Nelson	AF136418*	<i>Pyrophyllon subtumens</i> W.A. Nelson	AY126434*
<i>Porphyra virididentata</i> W.A. Nelson	AF136421*	<i>Pyrophyllon cameronii</i> W.A. Nelson	AY126429*
Figure 2 :			
<i>Bangia</i> sp. BKE NZ	AY184343*	<i>P. umbilicalis</i> Hallig Grode	AF175549
<i>Bangia</i> sp. BTS NZ	AY184347*	<i>P. purpurea</i> Netherlands	AF175551
<i>Bangia</i> sp. BWP NZ	AY184348*	<i>Porphyra</i> sp. Wales	AF175554
<i>Bangia</i> sp. BMW NZ	AY184344*	<i>Porphyra</i> sp. ZDR980 SA	AY292629*
<i>Bangia</i> sp. BRM NZ	AY184346*	<i>Porphyra</i> sp. ZDR966 SA	AY292628*
<i>Bangia</i> sp. BNS Australia	AY184345*	<i>Porphyra</i> sp. ZGR SA	AY292631*
<i>Bangia</i> sp. MA USA	AF043362	<i>Porphyra</i> sp. ZCE SA	AY292627*
<i>Bangia fuscopurpurea</i> Mexico	AF169334	<i>Porphyra</i> sp. ZPP SA	AY292636*
<i>Bangia</i> sp. BFK NZ	AY184338*	<i>Porphyra</i> sp. ZBS SA	AY292626*
<i>Bangia fuscopurpurea</i> Greece	AF175533	<i>Porphyra</i> sp. ZIR901 SA	AY292632*
<i>Bangia</i> sp. NC USA	AF043363*	<i>Porphyra</i> sp. ZIR970 SA	AY292633*
<i>Bangia fuscopurpurea</i> NJ USA	AF169335	<i>P. purpurea</i> Netherlands	AF175550
<i>Bangia fuscopurpurea</i> France	AF175535	<i>P. umbilicalis</i> MA USA	AB013179
<i>Bangia</i> sp. BCH NZ	AY184335*	<i>P. cf leucosticta</i> NF Canada	AF175538
<i>Bangia</i> sp. BCP NZ	AY184336*	<i>Porphyra</i> sp. TAS Australia	AY909585*
<i>Porphyra</i> sp. OTA NZ	AY184351*	<i>Porphyra</i> sp. FIB Falkland I	AY909598*
<i>P. coleana</i> W.A. Nelson NZ	AF136423*	<i>Porphyra</i> sp. SBA NZ	AY909589*
<i>P. purpurea</i> NS Canada	L26201	<i>P. lucasii</i> Levring Australia	AY139685*
<i>Porphyra</i> sp. Brest, France	AF175548	<i>Porphyra</i> sp. GDM NZ	AY909597*

Table 1. Continued.

Entity	GenBank Accession No	Entity	GenBank Accession No
Figure 2 :			
<i>Porphyra</i> sp. OSK NZ	AY909593*	<i>P. lanceolata</i> Mexico	AY909594*
<i>Porphyra</i> sp. WLR NZ	AY292644/AY292645*	<i>P. pseudolanceolata</i>	AF175543
<i>Porphyra</i> sp. SIR NZ	AY909588*	<i>P. perforata</i> Mexico	AY909592*
<i>Porphyra</i> sp. LGW NZ	AY299973*	<i>P. kanakaensis</i>	AF175556
<i>Porphyra</i> sp. LGD30 NZ	AF136422*	<i>P. sp.</i> SY Japan	AB013182
<i>Porphyra</i> sp. GRB488 NZ	AY184350*	<i>P. pseudolinearis</i> Japan	AB013185
<i>Porphyra</i> sp. GRB178 NZ	AY909603*	<i>Porphyra</i> sp. DRB NZ	AY909599*
<i>Porphyra</i> sp. GRB145 NZ	AY184349*	<i>P. rakiura</i> NZ	AF136425*
<i>Porphyra</i> sp. GRB368 NZ	AY292639*	<i>P. abbottae</i>	AF175545
<i>Porphyra</i> sp. GRB580 NZ	AY909604*	<i>P. torta</i> Canada	AF175552
<i>Porphyra</i> sp. GRB108 NZ	AF136420*	<i>P. spiralis</i> Brazil	L26177
<i>Porphyra</i> sp. GRB287 NZ	AY909595*	<i>P. acanthophora</i> Brazil	L26197
<i>Bangia</i> sp. CA USA	AF043356	<i>Porphyra</i> sp. PTK NZ	AY292640/AY292641*
<i>Bangia</i> sp. BC Canada	AF043359	<i>Porphyra</i> sp. ZEK SA	AY292630*
<i>Bangia</i> sp. BGA NZ	AY184341*	<i>Porphyra</i> sp. SSR53 NZ	AF136427*
<i>Bangia fuscopurpurea</i> Haneda, Japan	AB053489	<i>Bangia</i> sp. Alaska/ Antarctica	AF043355
<i>Bangia fuscopurpurea</i> Enoshima, Japan	AB053488	<i>Bangia</i> sp. NH USA	AF043353
<i>Bangia</i> sp. Newfoundland, Canada	AF043357	<i>Bangia</i> sp. RI USA	AF043354
<i>B. fuscopurpurea</i> Ireland	AF175534	<i>Bangia</i> sp. BFM NZ	AY184339/AY184340*
<i>Bangia</i> sp. OR USA	AF043358	<i>Bangia</i> sp. BOH NZ	AY909600*
<i>Bangia</i> sp. Nth BC Canada	AF043360	<i>Bangia</i> sp. BHH NZ	AY184342*
<i>Bangia</i> sp. TX USA	AF043361	<i>Bangia</i> sp. BDS NZ	AY184337*
<i>B. fuscopurpurea</i> WA USA	AF169336	<i>Porphyra</i> sp. ZAE SA	AY292624/AY292625*
<i>P. nereocystis</i>	AF175542	<i>Porphyra</i> sp. FIA Falkland I	AY292637*
<i>P. miniata</i> NF Canada	AF175540	<i>Porphyra</i> sp. CHL Chile	AY913952*
<i>P. suborbiculata</i> Kjellman Japan, NZ, Australia	AF136424*	<i>Porphyra</i> sp. FIC Falkland I	AY292638*
<i>B. gloiopeltidicola</i> Japan	AB053490	<i>Porphyra</i> sp. TCH NZ	AY909583*
<i>P. yezoensis</i> Japan	AB013177	<i>Porphyra</i> sp. SSR91 NZ	AF136428*
<i>P. tenera</i> Japan	AB013175	<i>Porphyra</i> sp. WRO NZ	AY909586*
<i>P. dentata</i> Japan	AB013183	<i>Porphyra</i> sp. ROS125 NZ	AY184352/AY184353*
<i>P. haitanensis</i> Japan	AB013181	<i>P. virididentata</i> W.A. Nelson NZ	AF136421*
<i>P. katadae</i> Japan	AB013184	<i>Porphyra</i> sp. ROS54 NZ	AF136426*
<i>Porphyra</i> sp. GEP NZ	AY909596*	<i>Porphyra</i> sp. SMR NZ	AY909587*
<i>Porphyra</i> sp. STI NZ	AY909584*	<i>Porphyra</i> sp. RIB NZ	AY909590*
<i>Porphyra</i> sp. TNR NZ	AY909602*	<i>Porphyra</i> sp. AKL NZ	AY909601*
<i>P. amplissima</i> NS Canada	L36048	<i>P. cinnamomea</i> W.A. Nelson NZ	AH008010*
<i>Porphyra</i> sp. ZLI SA	AY292634/AY292635*	<i>Porphyra</i> sp. ROS204 NZ	AY292642/AY292643*
<i>Porphyra</i> sp. Marseille, France	AF175546	<i>Porphyra</i> sp. PTW NZ	AY909591

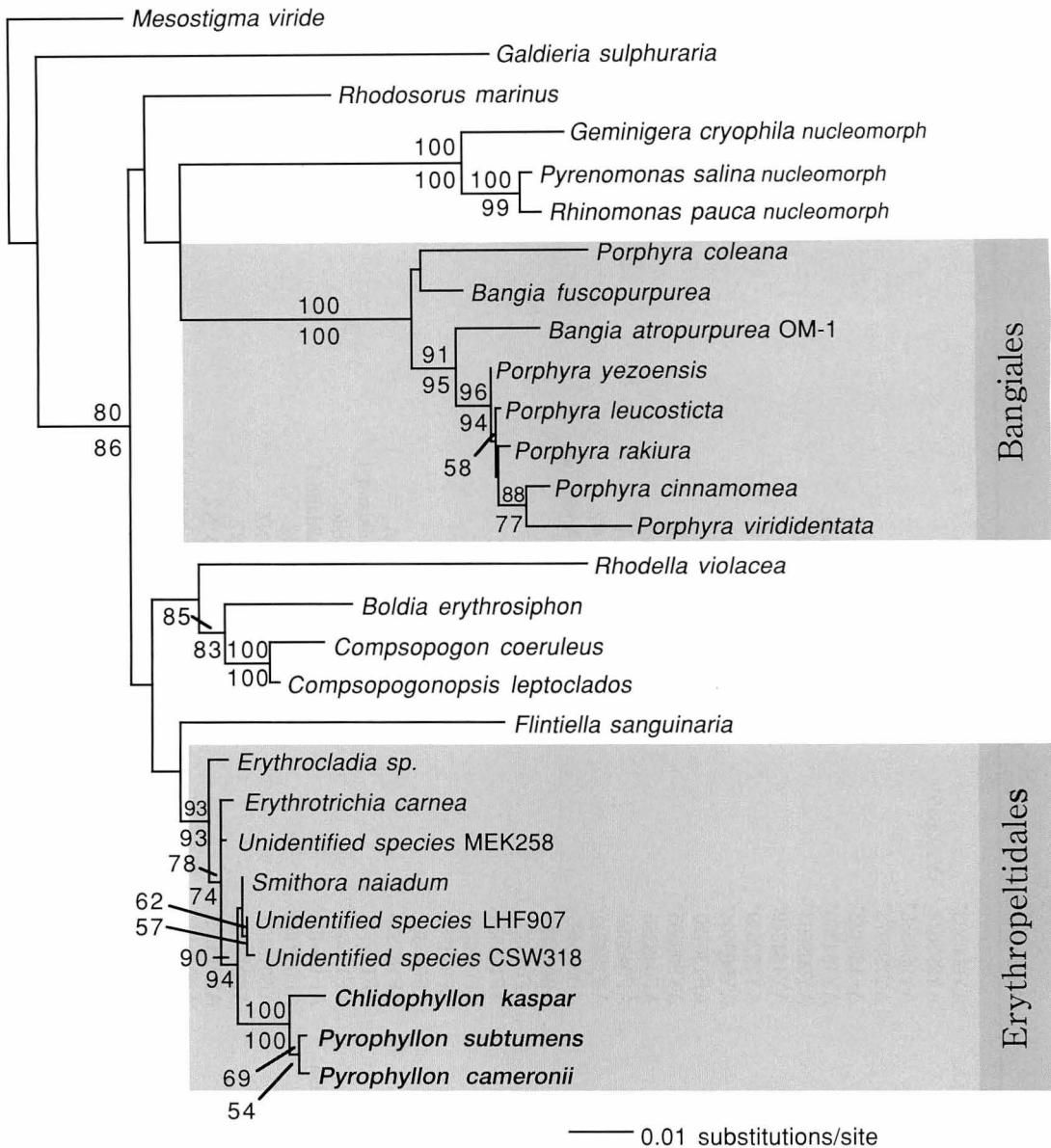


Figure. 1. Maximum likelihood phylogram of 27 red algal nSSU sequences outgroup rooted with the green algal *Mesostigma viride* estimated under the HKY-85 model of sequence evolution with rate heterogeneity using *Pyrophyllon cameranii*, *P. subtumens* and *Chlidophyllon kaspar* (shown in bold type) are resolved within the Erythropeltidales. Percentage bootstrap support values from 1000 replicates under maximum parsimony are shown above the branches, and from 1000 replicates under neighbour joining below. All analyses were completed using PAUP*4.0.

copurpurea has been widely applied to marine collections of *Bangia* although some authors have synonymised this species with *B. atropurpurea*. Sequences from GenBank from specimens identified as *B. fuscopurpurea* from the northern hemisphere, as well as marine *Bangia*

from a range of locations, appear in multiple positions on phylogenetic trees (Fig. 2 - e.g. clades B1, B2, B3). Thus the molecular data have revealed that freshwater *Bangia* are distinct phylogenetically from the very diverse marine filamentous Bangiales.

Resolution of the identity of type material and correspondence of type material with the sequences currently in GenBank is essential to the understanding of relationships within the order. In the application of molecular data to phylogenetic and systematic questions it is essential that voucher material is lodged in herbaria so that taxonomic concepts concerning entities and sequences are open to re-examination and further study in the light of new data.

The number of segregate genera that will be required within the Bangiales is not clear. However, in analyses that include taxa from the southern and northern hemispheres (New Zealand, South Africa, North Pacific, North Atlantic) there are clades emerging that are apparently monophyletic (Fig. 2). In addition to the three clades of marine "*Bangia*" (B1-B3), examples illustrated in Fig. 2 include a southern *Porphyra* clade containing entities from subantarctic and southern New Zealand, the Falkland Islands, southern Africa ("Southern Ocean"), a New Zealand clade of closely related *Porphyra* (Broom *et al.*, 2001) ("NZ"), and a clade of southern African *Porphyra* (Jones *et al.*, 2004) ("SA").

The evidence available to date suggests that biogeographic relationships vary between clades. Some clades contain taxa from widely separated geographic regions (e.g. *Bangia* clades highlighted in Fig. 2), while others appear to be much more restricted in distribution (e.g. *Porphyra* clades highlighted in Fig. 2). These differences may reflect hitherto unsuspected differences in dispersal potential of clade members as well as differences in age and geographic origins of the groups. Understanding phylogeographic relationships in the Bangiales is contingent on assembling and analysing data from all regions of the world.

Species concepts in the Bangiales:

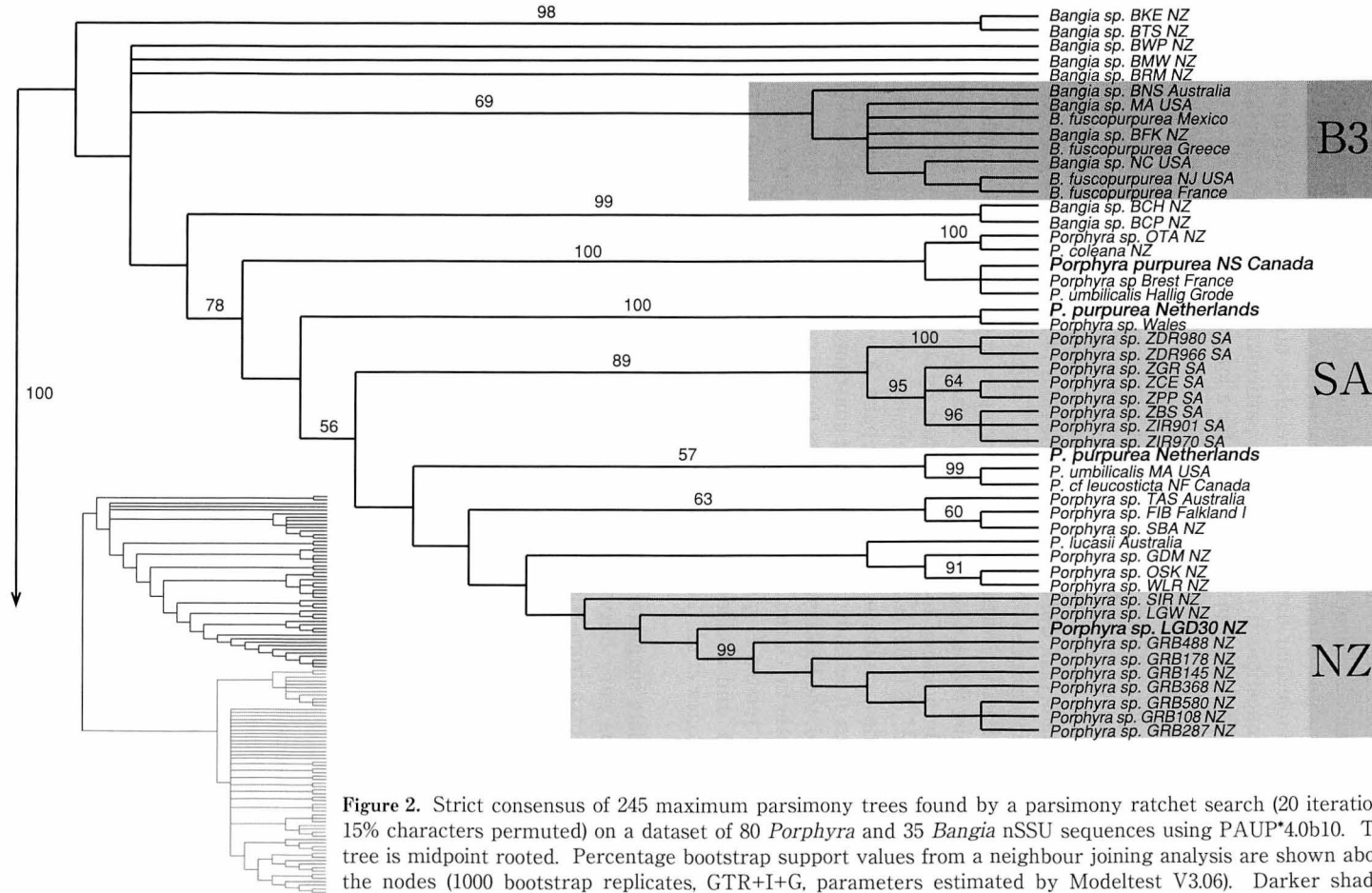
Species of *Porphyra* and *Bangia* are morphologically simple. Although morphology, life history characteristics, isozyme studies, polysaccharide chemistry, have provided very useful data, species identification within these genera has been hampered by the simple morphology they exhibit and the concomitant lack of distinguishing features (Lindstrom and Cole, 1993; Stiller and Waaland, 1993; Oliveira *et al.*, 1995; Broom *et al.*, 1999; Kunimoto *et al.*, 1999).

Taxonomic treatments are troubled by the problems of phenotypic plasticity exhibited by some taxa and convergent morphological characteristics shown by other taxa. The need for reliable, taxonomically informative characters, particularly for *Porphyra*, has been identified for a long time.

Sequence data are powerful tools to assist with defining species boundaries and relationships between species (Broom *et al.*, 1999, 2001, 2002; Nelson *et al.*, 2001). In the New Zealand region we have identified 35 *Porphyra* entities and 14 *Bangia* entities each with unique nSSU "signature" sequences, based on sequencing of more than 600 samples from the New Zealand region. These entities are distinguished by several to more than 160 base pair differences over a sequenced region of 1750 base pairs. A continuing challenge is identifying what constitutes the appropriate level of differences to warrant different taxonomic ranking (e.g. Broom *et al.*, 2001). Our current understanding suggests that there is no 'magic number', and that assessment of more informative genetic regions as well as traditional morphological markers is often required to resolve the status of entities defined by unique nSSU signatures.

Results of our studies in New Zealand demonstrate that morphological similarities and / or differences do not equate to membership of particular clades. For example, *Porphyra virididentata* W. A. Nelson (Fig. 4), a large broad ribbon growth form, found in the mid-lower intertidal zone and occurring strictly as a winter / spring annual, is most similar morphologically an undescribed species "LGD30" (Fig. 3), which shares the same growth form, seasonality and position on the shore, and overlaps with *P. virididentata* over part of its geographic range. However the nSSU sequences of these two species differ by 164 base pairs, and the two are resolved in different clades on the phylogenetic tree (Fig. 2). The species that is most closely related phylogenetically to *P. virididentata* is an undescribed species, "ROS54", that has a rosette growth form, and is found on upper intertidal shores year round.

Within other clades, however, there is a strong morphological similarity as seen for example in the members of the "New Zealand clade" highlighted in Fig. 2, entities which are



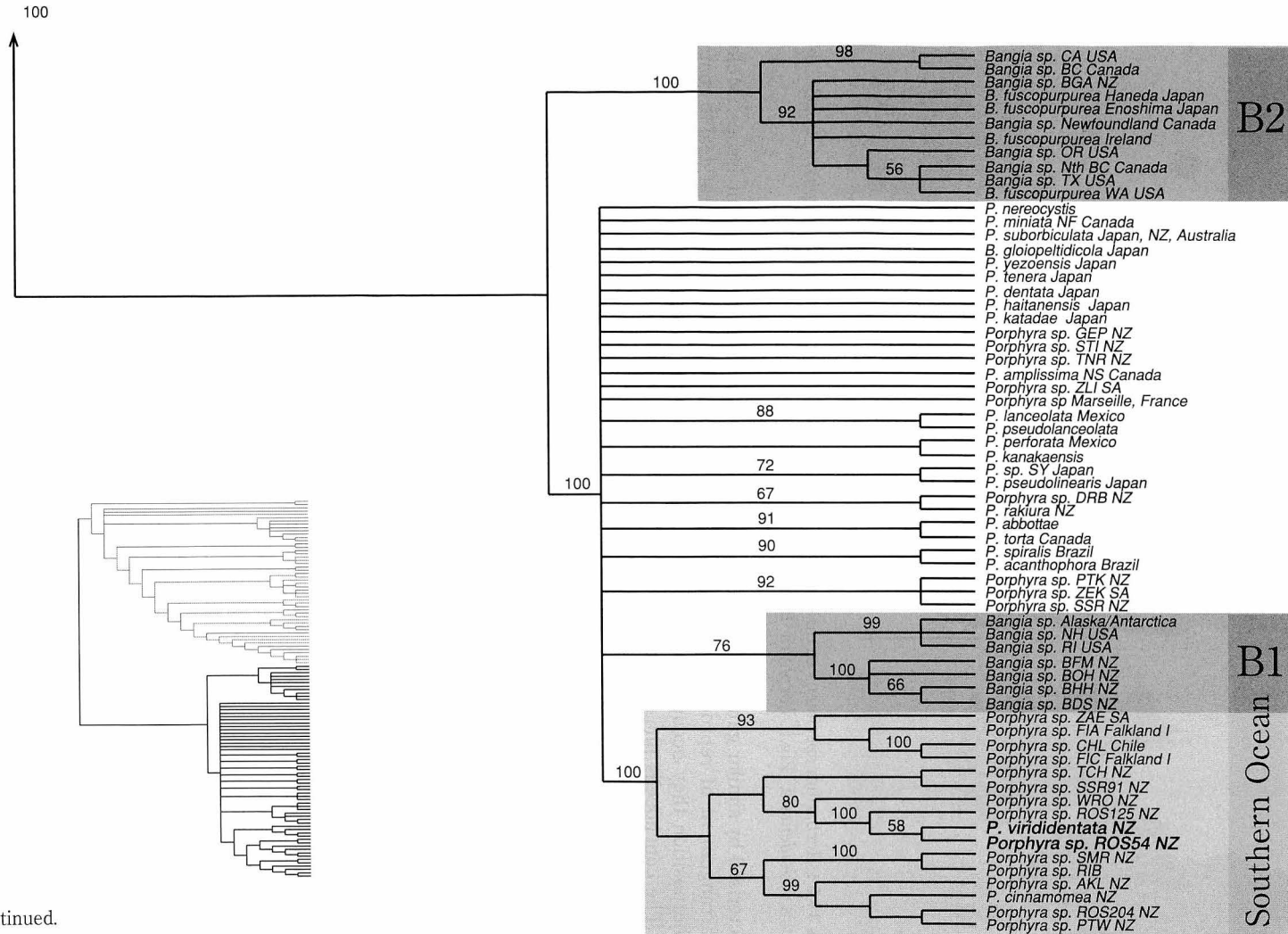
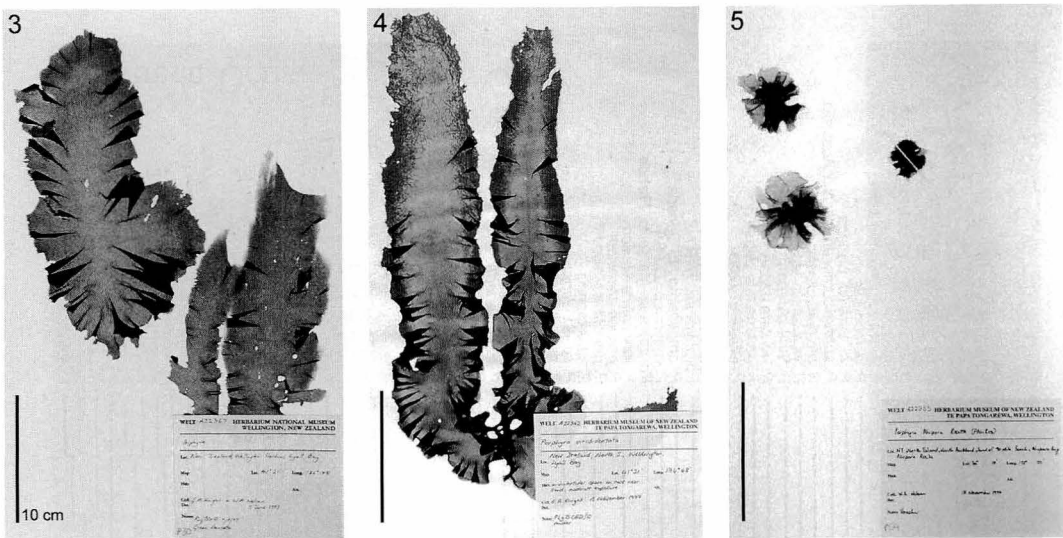


Fig. 2. Continued.



Figures 3 - 5 : Herbarium specimens of three species of New Zealand *Porphyra*. Fig. 3. *Porphyra* "LGD30", Fig. 4. *Porphyra virididentata*, Fig.5. *Porphyra* "ROS54".

characterised by similar gametophyte morphology, arrangement of reproductive structures, and the position they occupy in the intertidal zone. The conchocelis phase of these species, however, appears to provide some morphological characters by which entities can be distinguished (Knight and Nelson, 1999; Broom *et al.*, 2001).

Conclusions

Molecular sequence data have enabled new insights into the relationships and phylogeny of the Bangiophycidae and particularly the Erythropeltoidales and Bangiales. Generic concepts in the Bangiales need attention. It is clear that within the Bangiales additional genera must be circumscribed in order to reflect the genetic diversity and phylogenetic relationships within this group. This needs to be approached with caution and cannot be achieved with data from one geographic region of the world alone. It is essential that in the examination of generic boundaries as many sources of taxonomically informative data are examined as possible (morphology, anatomy, reproductive and life history characteristics, cell wall chemistry, as well as molecular data).

Research is currently occurring throughout the world on *Porphyra* spp. on the understanding of genes, biological products, strain selection and aquaculture improvement, stock con-

servation and management issues. Molecular sequencing data have a great deal to contribute to the study of the Bangiales: understanding the diversity of species in this group will lead the way for further development of nutritional attributes and the characteristics that contribute to breeding programmes and improved cultivars (e.g. Niwa and Aruga, 2003). In order for research on the applications of *Porphyra* and *Bangia* to be soundly based, it is essential that the work is being conducted on well defined species, and that, for example, breeding programmes use species that are phylogenetically closely related. Failure of specific research programmes may rest with the fact that the species being used are in fact only distantly related to each other. Although all species of bladed Bangiales are currently assumed to belong to a single genus, this taxonomic arrangement does not reflect phylogeny as taxa currently placed under "*Porphyra*" are widely divergent genetically.

Future directions for research on the Bangiales need to include the examination of the utility of other sequence data to provide insights into relationships within this group. Data from other regions of the world need to be gathered in order to get a more complete picture of the global situation and in order to develop sound generic and species concepts.

Acknowledgments

We would like to thank Dr M. Miyata for the opportunity for WN to attend and participate in the symposium, and for the hospitality of staff at the Chiba Natural History Museum. We acknowledge our colleagues Tracy Farr, Wyn Jones, Kate Neill and Christian Boedeker for their contributions to this research programme; the New Zealand Foundation for Research Science and Technology for funding research on the Bangiales (MNZX0202); and staff at the Herbarium of the Museum of New Zealand Te Papa Tongarewa.

References

- Adams, N.M. 1994. Seaweeds of New Zealand. Christchurch, Canterbury University Press. 360 p.
- Brodie, J.A., Irvine, L.M. 2003. Seaweeds of the British Isles. Volume 1. 3b. The Bangiophycidae. Intercept, Dorset.
- Broom, J.E.S., Farr, T.J., Nelson, W.A. 2004. Phylogeny of the *Bangia* flora of New Zealand suggests a southern origin for *Porphyra* and *Bangia* (Bangiales, Rhodophyta). *Molecular Phylogenetics and Evolution* 31: 1197-1207.
- Broom, J.E., Jones, W.A., Hill, D.F., Knight, G.A., Nelson, W.A. 1999. Species recognition in New Zealand *Porphyra* using 18S rDNA sequencing. *J. Appl. Phycol.* 11: 421-428.
- Broom, J.E., Nelson, W.A., Mutch, J., Farr, T.J., Jones, W.A. 2001. How much is enough? SSU variation as a signal of speciation in New Zealand *Porphyra*. *Phycologia* 40 (4 - Suppl.): 64.
- Broom J.E., Nelson, W.A., Yarish, C., Jones, W.A., Aguilar Rosas, R., Aguilar Rosas, L.E. 2002. A reassessment of the taxonomic status of *Porphyra suborbiculata* Kjellm., *Porphyra carolinensis* Coll et J.Cox and *Porphyra lilliputiana* W.A.Nelson, G.A. Knight et M.W.Hawkes (Bangiales, Rhodophyta) based on molecular and morphological data. *Eur. J. Phycol.* 37: 227-235.
- Butterfield, N.I., Knoll, A.H., Swett, K. 1990. A bangiophyte red alga from the Proterozoic of Arctic Canada. *Science* 250: 104-107.
- Butterfield, N.I. 2000. *Bangiomorpha pubescens* n.gen., n.sp.: implications for the evolution of sex, multicellularity, and the Mesoproterozoic / Neoproterozoic radiation of eukaryotes. *Paleobiology* 26: 386-404.
- Farr T.J., Nelson W.A., Broom J.E.S. 2003. A challenge to the taxonomy of *Porphyra* in Australia: the New Zealand red alga *Porphyra rakiura* (Bangiaceae, Rhodophyta) occurs in southern Australia, and is distinct from *P. lucasii*. *Australian Journal of Systematic Botany* 16: 569-575.
- Fujita, Y., Rao, U.S. 1997. Genetic improvement of *Porphyra* through cell culture techniques: present status and future prospects. *Nat. Hist. Res., Special Issue No. 3*: 71-81.
- Guiry, M.D., Nic Dhonncha, E. 2002. AlgaeBase. World-wide Web electronic publication. www.algae-base.org [22/08/2002]
- Hemmingson, J.A., Nelson, W.A. 2002. Cell wall polysaccharides are informative in *Porphyra* species taxonomy. *J. Appl. Phycol.* 14: 357-364.
- Jones, W.A., Griffin, N.J., Jones, D.T., Nelson, W.A., Farr, T.J., Broom, J.E. 2004. Phylogenetic diversity in South African *Porphyra* determined by Nuclear SSU sequence analysis. *Eur. J. Phycol.* 39: 197-211.
- Kitade, Y., Taguchi, G., Shin, J.-A., Saga, N. 1998. *Porphyra* monospore system (Bangiales, Rhodophyta): a model for the developmental biology of marine plants. *Phycol. Res.* 46: 17-20.
- Knight, G.A., Nelson, W.A. 1999. An evaluation of characters obtained from life history studies for distinguishing New Zealand *Porphyra* species. *Journal of Applied Phycology* 11: 411-419.
- Kunimoto M., Kito H., Yamamoto Y., Cheney D.P., Kaminishi Y., Mizukami Y. 1999. Discrimination of *Porphyra* species based on small subunit ribosomal RNA gene sequences. *J. Appl. Phycol.* 11: 203-209.
- Lindstrom S.C. & Cole K.M. 1993. The systematics of *Porphyra*: character evolution in closely related species. *Hydrobiologia* 260/261: 151-157.
- Müller, K.M., Sheath, R.G., Vis, M.L., Crease, T.J., Cole, K.M. 1998. Biogeography and systematics of *Bangia* (Bangiales, Rhodophyta) based on the Rubisco spacer, *rbcL* gene and 18S rRNA gene sequences and morphometric analyses. *Phycologia* 37: 195-207.
- Müller, K.M., Oliveira, M.C., Sheath, R.G., Bhat-tacharya, D. 2000. Ribosomal DNA phylogeny of the Bangiophycidae (Rhodophyta) and the origin of secondary plastids. *Am. J. Bot.* 88: 1390-1400.
- Nelson, W.A. 1993. Epiphytic species of *Porphyra* (Bangiales, Rhodophyta) from New Zealand. *Bot. Mar.* 36:525-534.
- Nelson, W.A., Brodie, J., Guiry, M.D. 1999. Terminology used to describe reproduction and life history stages in the genus *Porphyra* (Bangiales, Rhodophyta). *J. Appl. Phycol.* 11: 4407-410.
- Nelson, W.A., Broom, J.E., Farr, T.J. 2001. Four new species of *Porphyra* (Bangiales, Rhodophyta)

- from the New Zealand region. *Cryptogamie Algologie* 22: 263-284.
- Nelson, W.A., Broom, J.E., Farr T.J. 2003. *Pyrophyllon* and *Chlidophyllon* (Erythropeltidales, Rhodophyta), two new genera for obligate epiphytic species previously placed in *Porphyra*, and a discussion of the orders Erythropeltidales and Bangiales. *Phycologia* 42: 308-315.
- Nelson, W.A., Conroy, A.M. 1989. Effect of harvest method and timing on yield and regeneration of Karengo (*Porphyra* spp.) (Bangiales, Rhodophyta) in New Zealand. *J. Appl. Phycol.* 1: 277-283.
- Nelson, W.A., Knight, G.A. 1995. Endosporangia - a new form of reproduction in the genus *Porphyra* (Bangiales, Rhodophyta). *Botanica Marina* 38: 17-20.
- Nelson, W.A., O'Halloran, S.M.L., Conroy, A.M., Jorgenson, M.A. 1990. Phenology of the red seaweed *Porphyra* (karengo) at Kaikoura, New Zealand. *N.Z. Fish. Tech. Rep. No.* 20, 23 pp.
- Niwa, K., Aruga, Y. 2003. Rapid DNA extraction from conchocelis and ITS-1 rDNA sequences of seven strains of cultivated *Porphyra yezoensis* (Bangiales, Rhodophyta). *J. Appl. Phycol.* 15: 29-35.
- Nixon, K.C. 1999. The parsimony ratchet, a new method for rapid parsimony analysis. *Cladistics* 15: 407-414.
- Notoya, M. 1997. Diversity of life history in the genus *Porphyra*. *Nat. Hist. Res., Special Issue No.* 3: 47-56.
- Oohusa, T. 1993. Recent trends in nori products and markets in Asia. *J. Appl. Phycol.* 5: 155-159.
- Oliveira, M. C., Bhattacharya, D. 2000. Phylogeny of the Bangiophycidae (Rhodophyta) and the secondary endosymbiotic origin of algal plastids. *Am. J. Bot.* 87: 482-492.
- Oliveira, M. C., Kurniawan, J., Bird, C. J., Rice, E.L., Murphy, C.A., Singh, R. K., Gutell, R.R., Ragan, M. A. 1995. A preliminary investigation of the order Bangiales (Bangiophycidae, Rhodophyta) based on sequences of nuclear small-subunit ribosomal RNA genes. *Phycol. Res.* 43: 71-79.
- Stiller J.W., Waaland J.R. 1993. Molecular analysis reveals cryptic diversity in *Porphyra* (Rhodophyta). *J. Phycol.* 29: 506-517.
- (Manuscript received 10 January 2003 ; accepted 28 March 2003)

ウシケノリ目の系統と進化を理解するために分子生物学が果たす役割

ウエンディ・ネルソン¹⁾・シュディ・ブルーム²⁾

¹⁾ ニュージーランド国立水圏研究所 (NIWA)

水族生物多様性研究部門

Private Bag 14-901, ウェリントン,
ニュージーランド

E-mail: w.nelson@niwa.co.nz

²⁾ オタゴ大学生物化学研究部門

P.O. Box 56, デュネディン, ニュージーランド

分子生物学における塩基配列の遺伝子情報は、経済的に重要な紅藻のグループであるウシケノリ目の系統と進化の再検討において新しい解釈を可能にしている。これまで、広義な意味でのアマノリ属だけでなくウシケノリ目とエリスロペルテイス目に認める外部形態と内部構造の収斂は、分類学的な認識と系統発生学的な作業仮説に混乱を与えてきた。18SrDNAの塩基配列の情報をもとにしたウシケノリ目とエリスロペルテイス目に属する分類群間と、これら目内の属間の新しい系統関係の議論がその例である。アマノリ属の種の識別に用いる分類体系は、現在、形態学的、解剖学的、生化学的、生理学的及び生活史の特徴を羅列した一固まりの形質に基づいている。そして、アマノリ属の多数の種が示す表現型の環境への適応性は、形態形質に基づく分類学的な扱いを一層混乱させる要因となっている。18SrDNAの塩基配列にもとづく遺伝子情報は、ニュージーランド海域のウシケノリ目に予想以上の高い種の多様性があることを示した。すなわち、分子生物学的な塩基配列にもとづく遺伝子情報は、例えば培養実験などによって分かる種の特徴を用いるよりも、ウシケノリ目とエリスロペルテイス目に属する分類群に種の輪郭を与えて、より速く直接的に同定をおこなうための強力な手段となりうる。