

A mitochondrial marker for red algal intraspecific relationships

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Abstract

Intraspecific studies of red algae have relied on nuclear or plastid markers rather than mitochondrial data to address questions of systematics, biogeography or population genetics. In this study, primers were developed that spanned the noncoding intergenic region between the mitochondrial cytochrome oxidase subunit 2 and cytochrome oxidase subunit 3 genes. These primers were demonstrated to be successful on a variety of red algae in different orders: Gracilariales, Bonnemaisoniales and Ceramiales (families: Delesseriaceae, Ceramiaceae and Rhodomelaceae). Amplification products were between 450 and 320 bp in length, with variation in length shown among geographically distant isolates within a species. The region was variable within a single species, as shown for *Bostrychia moritziana* and *B. radicans*, and within populations of *Caloglossa leprieurii*. In the latter species, four mitochondrial haplotypes were observed in isolates from a single locality in Woollooware Bay, New South Wales, Australia. Analysis of hybrids between different mitochondrial haplotypes of *B. moritziana* revealed that the mitochondria are maternally inherited in this species. This is the first report of a mitochondrial marker that is variable within red algal populations and may lead to a better understanding of the population ecology of these important marine organisms.

Keywords: *Bostrychia*, *Caloglossa*, maternal inheritance, mitochondria, mitochondrial marker, Rhodophyta

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Introduction

Nuclear and plastid DNA markers have been used in a variety of studies on the evolution, biogeography and systematics of red algae. Molecular studies in red algae have helped resolve questions at various ranks, ranging from interordinal to intraspecific levels of investigation (Maggs *et al.* 1992; Freshwater & Rueness 1994; Freshwater *et al.* 1994; van Oppen *et al.* 1995a; Pakker *et al.* 1996; Lindstrom *et al.* 1997; Saunders & Kraft 1997; Zuccarello & West 1997; Woolcott & King 1998). During the last few years, studies have focused on within-population differentiation using allozymes (Pearson & Murray 1997; Sosa & Garcia-Riena 1993; Sosa *et al.* 1996), randomly amplified polymorphic DNA (van Oppen *et al.* 1995b) and microsatellites (Wattier

et al. 1997). More recently, techniques for studying within-population variation using plastid markers have been available (Zuccarello *et al.* 1999). Nonetheless, there is still a need for single-locus DNA markers, which require minimal optimization, are of useful size and high variability, and are applicable at the population level in a variety of algal taxa.

Although mitochondrial markers have proven extremely useful in population genetic and phylogeographical studies in animals (see Avise 1994) their use in plants and algae has been hampered by a lack of highly variable markers. Plant mitochondrial DNA (mtDNA) evolves slowly in nucleotide sequence, although rapidly with respect to gene order (Palmer & Herbon 1988). This slow nucleotide sequence variation has led to its low utility in intraspecific studies. The rate of sequence variation in red algal mtDNA, and hence its utility at the intraspecific level, is unknown. The use of noncoding regions of

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mtDNA in red algae should yield highly variable single-locus markers, which will aid in population studies within this protistan lineage.

Through the work of the Organelle Genome Megasequencing Program (OGMP, [HTTP://megasun.bch.umontreal.c/ogmproj.html](http://megasun.bch.umontreal.c/ogmproj.html)) and other independent laboratories, complete or partial mitochondrial genomic DNA data are available for many protistan organisms. For the Rhodophyta, two mitochondrial genomes have been completely sequenced: those for *Porphyra purpurea* (Wahlenberg) C. Agardh (Burger *et al.* 1999) and *Chondrus crispus* Stackhouse (LeBlanc *et al.* 1995a). Partial mitochondrial data are also available for a third species, *Cyanidium caldarium* (Tilden) Geitler (Viehmann 1995). Most research, building on such studies, has been concerned with genome organization, and operon and gene evolution (LeBlanc *et al.* 1995b, 1997; Burger *et al.* 1996; Lang *et al.* 1996; Viehmann *et al.* 1996; Gray *et al.* 1998). This research has allowed conserved gene orders to be analysed and for markers, anchored in conserved genes, to be designed that span intergenic spacers. One conserved gene pair is the cytochrome oxidase subunit 2 (*cox2*) and 3 (*cox3*) genes. These genes are separated by an intergenic spacer varying in length from ≈ 50 bp in *Cyanidium* to ≈ 190 bp in *Porphyra*.

We developed primers to amplify and sequence the mitochondrial *cox2-cox3* intergenic spacer and tested these primers on a number of distantly related red algal species. Sequence variability within the *cox2-cox3* spacer was examined and used to assess the potential use of this noncoding region in intraspecific and population-level studies in red algae. Also, crossing experiments were conducted and, using the *cox2-cox3* spacer as a marker, we investigated mitochondrial inheritance in the red alga *Bostrychia*.

Materials and methods

Algal samples were collected, identified and placed in unialgal culture following techniques outlined in West & Calumpong (1988). Algae from three different red algal Orders were tested: Gracilariales (*Gracilaria bangmeiana* Zhang & Abbott); Bonnemaisoniales (*Asparagopsis armata* Harvey); and Ceramiales, with three families represented: Delesseriaceae [*Caloglossa leprieurii* (Montagne) Martens]; Ceramiaceae (*Griffithsia monilis* Harvey); and Rhodomelaceae [*Symphyclocladia marchantioides* (Harvey) Falkenberg; *Bostrychia moritziana* (Sonders ex Kuetzing) J. Agardh; and *B. radicans* (Montagne) Montagne].

An aligned sequence matrix of the *cox2* and *cox3* genes and the intervening spacer were produced from published sequences of three rhodophycean genera: *Porphyra purpurea* (GenBank accession no. AF114794); *Chondrus crispus* (GenBank accession no. Z47547) and *Cyanidium caldarium* (GenBank accession no. Z48930). Conserved

regions at the 3' end of the *cox2* and the 5' end of the *cox3* genes were used to design two degenerate primers: (i) a forward primer (*cox2-for*) 5'-GTACCWCTTTDRG-RRKDAAATGTGATGC-3'; and (ii) a reverse primer (*cox3-rev*) 5'-GGATCTACWAGATGRAAWGGATGC-3'. DNA extraction of algal material was carried out as described by Goff & Moon (1993). Amplification reactions were performed in 50- μ L volumes using the following final concentrations: 1 \times polymerase chain reaction (PCR) buffer (PromegaTM), 100 μ M of each dNTP, 2.5 mM of MgCl₂, 3 pmol of each primer, 0.1% bovine serum albumin (BSA; Sigma), 1 U of *Taq* polymerase (PromegaTM) and 1 μ L of template DNA. Optimal amplification conditions were: initial denaturation at 94 °C for 4 min, followed by 5 cycles of 93 °C/45 °C/72 °C for 1 min each, 30 cycles of 93 °C/55 °C/72 °C for 30 s each and a final cycle at 72 °C for 5 min. Amplified products were electrophoresed in 2% agarose. Automated sequencing was performed on an ABI Prism 377 DNA SequencerTM (Perkin-Elmer) after cycle sequencing of the purified PCR product with dye-labelled dideoxynucleotides. Hybridizations between isolates of *B. moritziana* were performed as described in Zuccarello & West (1995) using isolates 2749 (J. A. West culture collection designation) from Tooradin (Western Port Bay, Victoria, Australia), 3738 (Montfort Boys Town, Viti Levu, Fiji), 3264 (Beachwood, Natal, South Africa) and 3275 (St. Lucia, Natal, South Africa).

Results

The *cox2-cox3* spacer region from three different red algal orders was successfully amplified (Fig. 1). Sequences lodged in GenBank include: *Gracilaria bangmeiana* (accession no. AF109786); *Asparagopsis armata* (accession no. AF109788) and *Symphyclocladia marchantioides* (accession no. AF109787). These sequences showed alignable regions within the 3' end of the *cox2* gene but were not

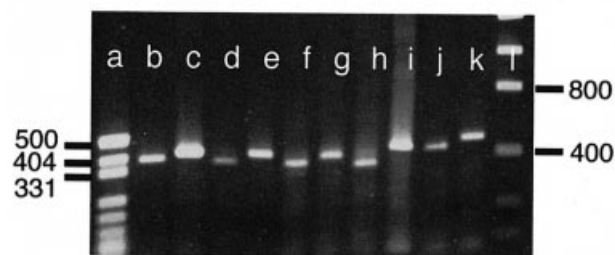


Fig. 1 Amplified *cox2-cox3* spacer region separated by agarose gel electrophoresis (2%) and visualized following ethidium bromide staining. Lanes a and l, molecular-weight ladders; lane b, *Gracilaria bangmeiana*; lane c, *Symphyclocladia marchantioides*; lane d, *Griffithsia monilis*; lane e, *Asparagopsis armata*; lane f, *Caloglossa leprieurii* (W5); lane g, *Bostrychia moritziana* (isolate BP27); lane h, *B. moritziana* (3204); lane i, *B. moritziana* (3416); lane j, *B. radicans* (3017); and lane k, *B. radicans* (3136).

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C.1.W.2 TATACCTGGCGATTAAATCAAACATCTCTATTTTTTAAACGCGAAGGGTTGTATTACGG
C.1.W5 .....T.....T.....T.....T.....A.....A.....T.....
C.1.W19 .....T.....G.....T.....T.....T.....T.....A.....A.....T.....
C.1.W18 .....T.....G.....T.....T.....T.....T.....A.....A.....T.....

C.1.W.2 TCAATGTAGTAAAATATGTGGTATAAAATCATGGATTTATGCCAATAGTTATAGAAGCGGT
C.1.W5 .....T.....T.....T.....T.....T.....T.....T.....T.....T.....
C.1.W19 .....T.....T.....T.....T.....T.....T.....T.....T.....T.....
C.1.W18 .....T.....T.....T.....T.....T.....T.....T.....T.....T.....

C.1.W.2 TAAATTACCTGATTATATTTATTGAATATCTAACAAAATTAATGAGGACTAATTATGAAA
C.1.W5 .....T.....T.....T.....T.....T.....T.....T.....T.....T.....
C.1.W19 .....T.....T.....T.....T.....T.....T.....T.....T.....T.....
C.1.W18 .....T.....T.....T.....T.....T.....T.....T.....T.....T.....

C.1.W.2 TTATCATTTTTAAATTAGTGTCTCATTTTTATTATTATTTTTGTTTTTATTATTAGAC
C.1.W5 .....A.....A.....A.....A.....A.....A.....A.....A.....A.....
C.1.W19 .....A.....A.....A.....A.....A.....A.....A.....A.....A.....
C.1.W18 .....A.....A.....A.....A.....A.....A.....A.....A.....A.....

C.1.W.2 TTTAAAAATAAAAAACGACAAAAAATACCTTAAACATTTTTTTGAAAAAAAAGTTAG
C.1.W5 .....T.....T.....T.....T.....T.....T.....T.....T.....T.....
C.1.W19 .....T.....T.....T.....T.....T.....T.....T.....T.....T.....
C.1.W18 .....T.....T.....T.....T.....T.....T.....T.....T.....T.....

C.1.W.2 TATGTTGCTACCAAAAATTTACAAA
C.1.W5 .....I+.....
C.1.W19 .....I+.....
C.1.W18 .....A.....A.....
    
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Fig. 2 DNA sequence of the *cox2-cox3* spacer region in isolates of *Caloglossa lepreurii* collected from Woolooware Bay, New South Wales. Dots denote that the nucleotide is identical to that shown in the top sequence. Arbitrary haplotype designations: W.2; W5; W19; and W18.

alignable between each other in the spacer region. Other sequences are presented in Figs 2 and 3, and will be published together with population studies to follow. Amplified products varied in size between ≈ 450 and 320 bp in length, with size variation seen within species (*Bostrychia moritziana* and *B. radicans*) (Fig. 1). All sequencing revealed an easily alignable 3' end of the *cox2* gene in the amplification product. The 5' end of the *cox3* was harder to recognize, possibly owing to the fact that the primer was designed close to the start of this gene. However, an alignable sequence was found, corresponding to the first few nucleotides of the *cox3* gene. Sequencing revealed an AT-rich region (56–80%), with several long tracts of A or T residues within the intergenic region (*cox2-cox3* spacer).

Isolates of *Caloglossa lepreurii* from one population at Woolooware Bay, New South Wales, were amplified and sequenced. The amplified product was 326 bp in length and revealed four distinct mitochondrial haplotypes (Fig. 2), differing by 2 bp in one case (haplotype W.2 vs. W5) to greater than 20 bp (haplotype W.2 vs. W18).

Successful crosses were carried out between four different isolates of *B. moritziana* and, in two cases, reciprocal crosses were also obtained. Sequences for the *cox2-cox3* spacer region of these isolates is shown (Fig. 3). All four isolates differed in their *cox2-cox3* spacer sequence. In all cases the sequence of the female was transmitted to the diploid offspring.

Discussion

A primer combination has been designed that has been used to amplify a noncoding mitochondrial region in diverse red algal orders. This mitochondrial marker has

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2749 AGTACCTGGTCGTTAAAACCAAACTCTCTTTTTTACAAAAGAGAAGGACTTTACTACGG
2749X3738 .....T.....T.....T.....T.....T.....T.....T.....T.....T.....
3738 .....T.....T.....T.....T.....T.....T.....T.....T.....T.....
3738X2749 .....T.....T.....T.....T.....T.....T.....T.....T.....T.....
3738X3264 .....T.....T.....T.....T.....T.....T.....T.....T.....T.....
3264 .....T.....C.....T.....T.....T.....T.....T.....T.....T.....
3264X3738 .....T.....C.....T.....T.....T.....T.....T.....T.....T.....
3738X3275 .....T.....C.....T.....T.....T.....T.....T.....T.....T.....
3275 .....T.....T.....T.....T.....T.....T.....T.....T.....T.....

2749 ACAATGCAGCGAAATATGCGGAGTTAATCATGGTTTTATGCCAATGTTGTGAAGCCGT
2749X3738 .....T.....T.....T.....T.....T.....T.....T.....T.....T.....
3738 .....T.....T.....T.....T.....T.....T.....T.....T.....T.....
3738X2749 .....T.....T.....T.....T.....T.....T.....T.....T.....T.....
3738X3264 .....T.....T.....T.....T.....T.....T.....T.....T.....T.....
3264 .....G.....T.....C.....T.....T.....T.....T.....T.....T.....
3264X3738 .....G.....T.....C.....T.....T.....T.....T.....T.....T.....
3738X3275 .....G.....T.....C.....T.....T.....T.....T.....T.....T.....
3275 .....G.....T.....C.....T.....T.....T.....T.....T.....T.....

2749 ACGTTTGCCAAATATGTTGCATGGTTATCAAGCAAATTTGATTCAACTAATGAGAAATA
2749X3738 .....A.....A.....A.....A.....A.....A.....A.....A.....A.....
3738 .....A.....A.....A.....A.....A.....A.....A.....A.....A.....
3738X2749 .....A.....A.....A.....A.....A.....A.....A.....A.....A.....
3738X3264 .....A.....A.....A.....A.....A.....A.....A.....A.....A.....
3264 .....A.....A.....A.....A.....A.....A.....A.....A.....A.....
3264X3738 .....A.....A.....A.....A.....A.....A.....A.....A.....A.....
3738X3275 .....A.....A.....A.....A.....A.....A.....A.....A.....A.....
3275 .....A.....A.....A.....A.....A.....A.....A.....A.....A.....

2749 TCATTAGTAAAATAATAATTTAATTTTACTACTTATCTTTGTTTTCAATAAACAAA
2749X3738 .....G.....G.....G.....G.....G.....G.....G.....G.....G.....
3738 .....G.....G.....G.....G.....G.....G.....G.....G.....G.....
3738X2749 .....G.....G.....G.....G.....G.....G.....G.....G.....G.....
3738X3264 .....G.....G.....G.....G.....G.....G.....G.....G.....G.....
3264 .....G.....GT.....G.....G.....G.....G.....G.....G.....G.....
3264X3738 .....G.....GT.....G.....G.....G.....G.....G.....G.....G.....
3738X3275 .....G.....G.....G.....G.....G.....G.....G.....G.....G.....
3275 .....G.....GT.....G.....G.....G.....G.....G.....G.....G.....

2749 AGAATAAGTAGTAAAAAACTAAAAAAAATCTTAAAATTTTTACAAAAAATGATTCAAA
2749X3738 .....T.....C.....G.....T.....G.....T.....G.....T.....G.....T.....
3738 .....T.....C.....G.....T.....G.....T.....G.....T.....G.....T.....
3738X2749 .....T.....C.....G.....T.....G.....T.....G.....T.....G.....T.....
3738X3264 .....T.....C.....G.....T.....G.....T.....G.....T.....G.....T.....
3264 .....T.....C.....GG.....G.....T.....G.....T.....G.....T.....
3264X3738 .....T.....C.....GG.....G.....T.....G.....T.....G.....T.....
3738X3275 .....T.....C.....G.....T.....G.....T.....G.....T.....G.....T.....
3275 .....T.....C.....G.....T.....G.....T.....G.....T.....G.....T.....

2749 AATTAACTAATTTATTTTATGAATAATTCATCTTATTTCTAGAAAATATACAAA
2749X3738 .....T.....C.....T.....C.....T.....C.....T.....C.....T.....
3738 .....T.....T.....T.....T.....T.....T.....T.....T.....T.....
3738X2749 .....T.....T.....T.....T.....T.....T.....T.....T.....T.....
3738X3264 .....T.....T.....T.....T.....T.....T.....T.....T.....T.....
3264 .....T.....T.....C.....T.....A.....T.....T.....T.....T.....
3264X3738 .....T.....T.....C.....T.....A.....T.....T.....T.....T.....
3738X3275 .....T.....T.....C.....T.....A.....T.....T.....T.....T.....
3275 .....T.....T.....C.....T.....A.....T.....T.....T.....T.....
    
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Fig. 3 DNA sequence of the *cox2-cox3* spacer region in isolates and hybrids of *Bostrychia moritziana*. For full details of the isolate numbers, see the Materials and methods. Hybrids: 2749 × 3738, diploid hybrid between female 2749 and male 3738; 3738 × 2749, diploid hybrid between female 3738 and male 2749; 3738 × 3264, diploid hybrid between female 3738 and male 3264; 3264 × 3738, diploid hybrid between female 3264 and male 3738; and 3738 × 3275, diploid hybrid between female 3738 and male 3275.

also been shown to be variable within species and even within populations.

Mitochondrial markers have proven useful in phylogenetic and population studies within animals owing to their haploid nature, uniparental inheritance and lack of recombination. The mitochondrial control region has been used frequently and very successfully in systematic, phylogeographic and population studies because of its high mutation rate (Avisé 1994). No region in the mtDNA of higher plants or algae has been found with a level of variation similar to the animal mitochondrial control region.

During the last few years, the mitochondrial genomes of two red algae [*Porphyra purpurea* (Bangiales) and *Chondrus crispus* (Gigartinales)] have been completely sequenced and, additionally, a 10-kb contiguous mitochondrial

region of the red alga (*Cyanidium caldarium*, Viehmann 1995). These sequences will enable the detection of conserved gene orders in red algae and amplification of intergenic regions, which presumably would yield unconstrained, highly variable DNA sequence data. The gene order of cytochrome oxidase subunit 2 (*cox2*) followed by cytochrome oxidase subunit 3 genes (*cox3*), is one that is conserved within the red algal mitochondrial genome. Based on amplification primers homologous to conserved regions in the *cox2* and *cox3* genes, the results here show that this gene position is also conserved in the red algal orders Gracilariales, Bonnemaisoniales and Ceramiales.

The *cox2-cox3* spacer is variable within a population of the mangrove red alga *Caloglossa leprieurii*, with four mitochondrial haplotypes found to date (Fig. 2). These algal samples were collected within a 10-m radius at a site in Woolooware Bay (part of Botany Bay, New South Wales). The spatial variability of mitochondrial haplotypes within this population are under investigation. The size of the spacer region appears to lend itself to a rapid scoring of mitochondrial haplotypes using single-stranded conformational polymorphism, as has been successfully used in plastid haplotype scoring (Zuccarello *et al.* 1999), or using other mutation detection methods such as denaturing gradient gel electrophoresis (DGGE) and temperature gradient gel electrophoresis (TGGE). These methods are currently being optimized for this mitochondrial region.

The *cox2-cox3* spacer region has the potential to be useful in population and demographic studies of red algae. Although the size of this region is not as large as the animal mitochondrial control region (≈ 350 bp vs. 1 kb) it appears to show sufficient DNA sequence variability for population genetic and, possibly, phylogeographical studies. Future study of the value of this region in demographic studies of red algae is warranted and will reveal the full utility of this region.

Hybridization data between gametophytes of *Bostrychia moritziana* with different mitochondrial haplotypes revealed that mtDNA is transmitted maternally (Fig. 3). The possibility of paternal inheritance of red algal mitochondria exists because ultrastructural studies of red algal spermatia have shown mitochondria within mature or maturing spermatia in species within the Ceramiales (Kugrens & West 1972; Broadwater & Scott 1983; Broadwater *et al.* 1991). It is not known in *Bostrychia* whether maternal inheritance is a result of swamping (many-fold excess of maternal vs. paternal mitochondria), degradation of male mtDNA, or selective destruction or inactivation of male mitochondria. Hoekstra (1990) and Hurst (1994) discuss theories proposed to account for the evolution and maintenance of uniparental inheritance of organellar DNA.

Uniparental inheritance, and maternal inheritance of organelle DNA, is the dominant form of extranuclear

DNA inheritance in plants; however, there are exceptions (Reboud & Zeyl 1994) and 'leakage', the alternative form of cytoplasmic inheritance, can occur in rare cases. The present data set is too small to exclude alternative forms of inheritance. Previous analysis of plastid inheritance in *B. moritziana*, and the closely related species *B. radicans*, revealed that plastids are also inherited maternally (Zuccarello *et al.* 1999). Further study will determine the generality of maternal inheritance of organellar DNA in red algae.

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References

- Awise JC (1994) *Molecular markers, Natural History and Evolution*. Chapman & Hall, New York.
- Broadwater S, Scott J (1983) Fibrous vacuole associated organelles (FVAOs) in the Florideophyceae: a new interpretation of the 'appareil cinétique'. *Phycologia*, **22**, 225–233.
- Broadwater S, Scott JL, West JA (1991) Spermatangial appendages of *Spyridia filamentosa* (Ceramiales, Rhodophyta). *Phycologia*, **30**, 189–195.
- Burger G, Lang BF, Reith M, Gray MW (1996) Genes encoding the same three subunits of respiratory complex II are present in the mitochondrial DNA of two phylogenetically distant eukaryotes. *Proceedings of the National Academy of Sciences of the USA*, **93**, 2328–2332.
- Burger G, Saint-Louis D, Gray MW, Lang BF (1999) Complete sequence of the mitochondrial DNA of the red alga *Porphyra purpurea*: Cyanobacterial introns, and shared ancestry of red and green algae. *The Plant Cell*, in press.
- Freshwater DW, Rueness J (1994) Phylogenetic relationships of some European *Gelidium* (Gelidiales, Rhodophyta) species, based on *rbcl* nucleotide sequence analysis. *Phycologia*, **33**, 187–194.
- Freshwater DW, Fredericq S, Butler BS, Hommersand MH, Chase MW (1994) A gene phylogeny of the red algae (Rhodophyta) based on plastid *rbcl*. *Proceedings of the National Academy of Sciences of the USA*, **91**, 7281–7285.
- Goff LJ, Moon DA (1993) PCR amplification of nuclear and plastid genes from algal herbarium specimens and algal spores. *Journal of Phycology*, **29**, 381–384.
- Gray MW, Lang BF, Cedergren R *et al.* (1998) Genome structure and gene content in protist mitochondrial DNAs. *Nucleic Acids Research*, **26**, 865–878.
- Hoekstra RF (1990) Evolution of uniparental inheritance of cytoplasmic DNA. In: *Organizational Constraints on the Dynamics of Evolution* (eds Smith JM, Vida G), pp. 269–278. Manchester University Press, Manchester.
- Hurst LD (1994) Cytoplasmic genetics under inbreeding and outbreeding. *Proceedings of the Royal Society of London Series B Biological Sciences*, **258**, 287–298.
- Kugrens P, West JA (1972) Ultrastructure of spermatial development

- in the parasitic red algae *Levringiella gardneri* and *Erythrocyctis saccata*. *Journal of Phycology*, **8**, 331–343.
- Lang BF, Goff LJ, Gray MW (1996) A 5S rRNA gene is present in the mitochondrial genome of the protist *Reclinomonas americana* but is absent from red algal mitochondrial DNA. *Journal of Molecular Biology*, **261**, 607–613.
- LeBlanc C, Boyen C, Richard O, Bonnard G, Grienenberger J-M, Kloareg B (1995a) Complete sequence of the mitochondrial DNA of the rhodophyte *Chondrus crispus* (Gigartinales). Gene content and genome organization. *Journal of Molecular Biology*, **250**, 484–495.
- LeBlanc C, Kloareg B, Goer SL-D, Boyen C (1995b) DNA sequence, structure, and phylogenetic relationship of the mitochondrial small-subunit rRNA from the red alga *Chondrus crispus* (Gigartinales, Rhodophytes). *Journal of Molecular Evolution*, **41**, 196–202.
- LeBlanc C, Richard O, Kloareg B, Viehmann S, Zetsche K, Boyen C (1997) Origin and evolution of mitochondria: what have we learnt from red algae? *Current Genetics*, **31**, 193–207.
- Lindstrom SC, Olsen JL, Stam WT (1997) Postglacial recolonization and the biogeography of *Palmaria mollis* (Rhodophyta) along the Northeast Pacific coast. *Canadian Journal of Botany*, **75**, 1887–1896.
- Maggs CA, Douglas SE, Fenety J, Bird CJ (1992) A molecular and morphological analysis of the *Gymnogongrus devoniensis* (Rhodophyta) complex in the north Atlantic. *Journal of Phycology*, **28**, 214–232.
- van Oppen MJH, Draisma SGA, Olsen JL, Stam WT (1995a) Multiple trans-Arctic passages in the red alga *Phycodrys rubens*: evidence from nuclear rDNA ITS sequences. *Marine Biology (Berlin)*, **123**, 179–183.
- van Oppen MJH, Olsen JL, Stam WT (1995b) Genetic variation within and among North Atlantic and Baltic populations of the benthic alga *Phycodrys rubens* (Rhodophyta). *European Journal of Phycology*, **30**, 251–260.
- Pakker H, Klerk H, van Campen J-H, Olsen JL, Breeman AM (1996) Evolutionary and ecological differentiation in the pantropical to warm-temperate seaweed *Digenea simplex* (Rhodophyta). *Journal of Phycology*, **32**, 250–257.
- Palmer JD, Herbon LA (1988) Plant mitochondrial DNA evolves rapidly in structure, but slowly in sequence. *Journal of Molecular Evolution*, **28**, 87–97.
- Pearson EA, Murray SN (1997) Patterns of reproduction, genetic diversity, and genetic differentiation in California populations of the geniculate coralline alga *Lithothrix aspergillum* (Rhodophyta). *Journal of Phycology*, **33**, 753–763.
- Reboud X, Zeyl C (1994) Organelle inheritance in plants. *Heredity*, **72**, 132–140.
- Saunders GW, Kraft GT (1997) A molecular perspective on red algal evolution: focus on the Florideophycidae. *Plant Systematics and Evolution (Suppl.)*, **11**, 115–138.
- Sosa PA, Garcia-Riena G (1993) Genetic variability of *Gelidium canariensis* (Rhodophyta) determined by isozyme electrophoresis. *Journal of Phycology*, **29**, 118–124.
- Sosa PA, Cabrera-Perez MA, Garciareina G (1996) Genetic variation of *Gracilaria cervicornis* (Rhodophyta) gametophytes from the Canary Islands. *European Journal of Phycology*, **31**, 143–147.
- Viehmann S (1995) *Die analysis des mitochondrialen Genoms der rotalge Cyanidium caldarium*. *Neue Aspekte Zur Evolution der Mitochondrien*. PhD Thesis. Institut für Pflanzenphysiologie, Justus Liebig Universität, Giessen, Germany.
- Viehmann S, Richard O, Boyen C, Zetsche K (1996) Genes for two subunits of succinate dehydrogenase form a cluster on the mitochondrial genome of Rhodophyta. *Current Genetics*, **29**, 199–201.
- Wattier R, Dallas JF, Destombe C, Saumitou-Laprade P, Valero M (1997) Single locus microsatellites in Gracilariales (Rhodophyta): high level of genetic variability within *Gracilaria gracilis* and conservation in related species. *Journal of Phycology*, **33**, 868–880.
- West JA, Calumpong HP (1988). Mixed-phase reproduction of *Bostrychia* (Ceramiales, Rhodophyta) in culture. I. *B. tenella* (Lamouroux) J. Agardh. *Japanese Journal of Phycology*, **36**, 292–310.
- Woolcott GW, King RJ (1998) *Porphyra* and *Bangia* (Bangiaceae, Rhodophyta) in warm temperate waters of eastern Australia: morphological and molecular analyses. *Phycological Research*, **46**, 111–123.
- Zuccarello GC, West JA (1995) Hybridization studies in *Bostrychia*. 1: *B. radicans* (Rhodomelaceae, Rhodophyta) from Pacific and Atlantic North America. *Phycological Research*, **43**, 233–240.
- Zuccarello GC, West JA (1997) Hybridization studies in *Bostrychia*. 2: Correlation of crossing data and plastid DNA sequence data within *B. radicans* and *B. moritziana* (Rhodophyta, Ceramiales). *Phycologia*, **36**, 293–304.
- Zuccarello GC, West JA, Kamiya M, King RJ (1999) A rapid method to score plastid haplotypes in red seaweeds and its use in determining parental inheritance of plastids in the mangrove red alga *Bostrychia* (Ceramiales). *Hydrobiologia*, in press.

The work described in this paper is part of ongoing research into the evolutionary biology of mangrove-associated red algae conducted by Joe Zuccarello, John West and Robert King. Gertraud Burger is a senior researcher with the Organelle Genome Megasequencing Program and is interested in the evolution of mitochondria in diverse protists.
