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Diversity within red algal species: variation in world-wide samples of *Spyridia filamentosa* (Ceramiaceae) and *Murrayella periclados* (Rhodomelaceae) using DNA markers and breeding studies

GIUSEPPE C. ZUCCARELLO[1], BERNADETTE SANDERCOCK[2] AND JOHN A. WEST[2]

[1] *Marine Biological Association of the United Kingdom, The Laboratory, Citadel Hill, Plymouth PL1 2PB, UK*
[2] *School of Botany, The University of Melbourne, Victoria, 3010, Australia*

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Molecular and breeding studies on two pan-tropical marine red algae reveal vastly different levels of genetic variation and reproductive isolation. Sequenced DNA regions from the nuclear, mitochondrial and plastid genomes (partial LSU rRNA, *cox*2-3 spacer, RuBisCo spacer, respectively) revealed high genetic variation among individuals of *Spyridia filamentosa*. An *rbc*L analysis shows that *Spyridia* is a monophyletic genus distinct from other Ceramiaceae sampled, suggesting that the Ceramiaceae could be paraphyletic. There is complete congruence between all data sets of *S. filamentosa*, showing a well-supported phylogeographic pattern with samples from the Pacific distinct from Atlantic and Indian Ocean samples. One western Mediterranean sample is associated with Atlantic specimens, while an eastern Mediterranean sample has closer affinities to Pacific samples, possibly indicating a recent cryptic introduction into the eastern Mediterranean. Limited breeding studies imply that these samples are mostly reproductively isolated, whereas a successful cross demonstrated maternal inheritance of organellar DNA. These data indicate that *S. filamentosa* exists as several cryptic species. *Murrayella periclados* exhibits low levels of genetic variation and no phylogeographic structure, and almost complete reproductive compatibility between isolates. This suggests that all *M. periclados* samples share a recent common ancestor that may have dispersed relatively rapidly, or that rates of base pair substitution between these two species vary greatly. Rapid long-distance dispersal of *M. periclados* is not indicated by what is known of the biology of *M. periclados*, especially in comparison with *S. filamentosa*, which appears to be a much better candidate for long-distance dispersal. These data demonstrate that red algal morphospecies are not equivalent units of diversity, with implications for our view of red algal biodiversity and evolution.

Key words: *cox*2-3 spacer, cryptic species, large subunit rRNA, *Murrayella periclados*, *rbc*L, reproductive compatibility, RuBisCo spacer, *Spyridia filamentosa*, *Spyridia hypnoides*

Introduction

Molecular studies have greatly increased our knowledge of the patterns, history and genetic variability within species. Levels of genetic variation between presently recognized morphological species can differ greatly (Avise, 2000). This has led to the discovery of many 'cryptic species' and the conclusion that morphological lineages (morphospecies) are more inclusive than basal phylogenetic units (Hedin, 1997). This is especially true in organisms with simple morphological features and a limited number of variable and/or reliable characters, such as the algae. Genetic information, therefore, provides a superior tool for investigating the diversity and history of morphologically simple organisms (Koufopanou, 2001; Zuccarello & West, 2002).

Recently questions of intraspecific relationships between populations, or closely related species, have led to the relatively new discipline of 'phylogeography' (Bermingham & Moritz, 1998; and summarized in Avise, 2000). Phylogeography deals with the processes governing the geographic distribution and evolution of genealogical lineages within species. Phylogeographic studies have become quite common for assessing terrestrial and marine animal groups (Avise, 2000), but have been limited in marine red algae (van Oppen *et al.*, 1995; Lindstrom *et al.*, 1997; McIvor *et al.*, 2001).

Phylogeographic studies have revealed several intraspecific patterns with respect to genealogical

Correspondence to: G.C. Zuccarello. e-mail: giz@mba.ac.uk

Table 1. Isolates used in this study

Species	Collection details	#	COX	RS	LSU	rbcL	Genbank Accession £'s
Spyridia filamentosa	Cayo Enrique, Puerto Rico, 16 Mar 1989	PR2945	X	X	X		
S. filamentosa	Cayo Enrique, Puerto Rico, 16 Mar 1989	PR2946	X	X	X	X	AF458705-rbcL; AF458713-LSU; AF458719-COX; AF458738-RS
S. filamentosa	Media Luna Reef, Puerto Rico, 21 May 2000 (coll. D. Ballantine)	(PR.E315)	X	X	X		AF458720-COX
S. filamentosa	Siquijor, Philippines, 26 May 1988	PI2921	X	X	X		AF458726-COX
S. filamentosa	Calatagan, Batangas, Philippines, 11 May 1987	PI2846	X	X	X	X	AF458701-rbcL; AF458714-LSU; AF458725-COX; AF458735-RS
S. filamentosa	Mulege, Baja California Sur, Mexico, 8 Jan 1990	MX3022	X	X	X	X	AF458702-rbcL; AF458730-COX
S. filamentosa	Mulege, Baja California Sur, Mexico, 8 Jan 1990	MX3023	X	X	X	X	AF458704-rbcL; AF458711-LSU; AF458723-COX; AF458733-RS
S. filamentosa	Bahia Magdelena, BCS, Mexico, 7 Jan 1990	MX3028	X	X	X		
S. filamentosa	Pichilingue, La Paz, BCS Mexico, 6 Jan 1990	MX3031	X	X			
S. filamentosa	Bahia Balandra, BCS, Mexico, 6 Jan 1990	MX3032	X	X	X		AF458715-LSU; AF458729-COX; AF458736-RS
S. filamentosa	Bahia Balandra, BCS, Mexico, 6 Jan 1990	MX3036	X	X			
S. filamentosa	Mission Bay, San Diego, California, 17 Jul 2000	(CA.E332)	X	X	X		
S. filamentosa	Arno Bay, South Australia, 10 Feb 1999	SA3933	X	X	X		AF458724-COX
S. filamentosa	Blanche Harbour, South Australia, 10 Feb 1999	SA3936	X	X			
S. filamentosa	Botany Bay, New South Wales, Australia, 15 Sep 1998	NSW265	X	X	X		AF458727-COX; AF458734-RS
S. filamentosa	Pt. Peron, Western Australia (coll. J. Huisman)	(WA.E184)	X	X	X	X	AF458703-rbcL; AF458712-LSU; AF458722-COX; AF458737-RS
S. filamentosa	Pt. Peron, Western Australia, 17 Feb 2000 (coll. J. Huisman)	(WA.E232)	X	X			
S. filamentosa	Pt. Peron, Western Australia, 17 Feb 2000 (coll. J. Huisman)	(WA.E233)	X	X			
S. filamentosa	Ischia, Italy, 31 Aug 2001 (coll. W. Kooistra)	(IT.E696)	X		X		AF458716-LSU; AF458721-COX
S. filamentosa	Possidi, Greece, 22 Aug 2001	(GR.E697)	X	X	X		AF458728-COX
S. hypnoides	Media Luna Reef, Puerto Rico, 21 Jun 2000 (coll. D. Ballantine)	(PR.E310)	X	X	X	X	AF458707-rbcL; AF458717-LSU
S. hypnoides	Guajataca, Puerto Rico, 26 May 2000 (coll. D. Ballantine)	(PR.E313)	X	X	X	X	AF458706-rbcL; AF458731-COX; AF458739-RS
S. hypnoides	Media Luna Reef, Puerto Rico, 28 Dec 1999 (coll. D. Ballantine)	(PR.E214)	X	X	X		
S. hypnoides	Media Luna Reef, Puerto Rico, 1 Mar 2001 (coll. D. Ballantine)	(PR.E346)	X	X	X		
S. clavata	Guanica, Puerto Rico, 30 Apr 2001 (coll. D. Ballantine)	(PR.E668)	X	X	X		AF458718-LSU; AF458732-COX; AF458740-RS
Murrayella periclados	Cayo Caracoles, La Parguera, Puerto Rico, 20 Mar 1981	PR2513	X	X	X		AF458754-COX
M. periclados	La Parguera, Puerto Rico, 15 Feb 1998	PR3815	X				AF458753-COX
M. periclados	La Parguera, Puerto Rico, 15 Feb 1998	PR3816	X	X	X		AF458745-LSU
M. periclados	Misamis Oriental, Mindanao, Philippines, 20 May 1988	PI2887	X	X	X		AF458747-LSU
M. periclados	Olango Is., Cebu, Philippines, 15 Apr 1998	PI3828	X	X	X		AF458744-LSU; AF458755-COX
M. periclados	Olango Is., Cebu, Philippines, 15 Apr 1998	PI3829	X	X	X		AF458756-COX
M. periclados	Agana, Guam, 15 Aug 1989	GU2999	X	X	X		AF458757-COX
M. periclados	Haepiti, Moorea, French Polynesia, 8 Jul 1995	MO3506	X	X	X		
M. periclados	North Pala Lagoon, American Samoa, 29 Aug 1991	AS3180	X	X	X		AF458743-LSU
M. periclados	Yacura Is., Cuvu Bay, Viti Levu, Fiji, 6 Jun 1997	FJ3731	X	X	X		AF458763-RS
M. periclados	Lau Cala Bay, Suva, Viti Levu, Fiji, 3 Jun 1997	FJ3740	X	X	X		AF458741-LSU; AF458750-COX; AF458761-RS
M. periclados	Port Dickson, Selangor, West Malaysia, 14 May 98	MY3840	X		X		AF458758-COX; AF458764-RS
M. periclados	Pulai Bai, Sandakan, Sabah, Malaysia, 16 Aug 2000	MY4088	X		X		
M. periclados	Kranji, Singapore, 12 Jun 1989	SG2965	X	X	X		AF458748-LSU; AF458752-COX; AF458762-RS

Table 1 (*cont.*)

Species	Collection details	#	COX	RS	LSU	rbcL	Genbank Accession £'s
M. periclados	Bali Barat National Park, Indonesia, 8 May 1999	IN3959	X		X		
M. periclados	Maningrida, Arnhem Land, NT, Australia, 22 Aug 1999	NT3995	X	X	X		AF458746-LSU
M. periclados	Parra Açu, Maranhão, Brazil, 20 Nov 1996	BZ3650	X	X	X		AF458742-LSU; AF458751-COX
M. periclados	Ilha do Itaparica, Bahia, Brazil, 17 Nov 1996	BZ3656	X	X	X		
M. periclados	Fort Meyers Beach, Florida, USA, 19 Sep 2000	FL4118	X				AF458759-COX
Bostrychia moritziana	St. Lucia, Natal, South Africa, 22 Dec 1991	Bm.3275	X				AF458760-COX
B. moritziana	Beachwood, Natal, 4 Nov 1991	Bm.3204		X	X		AF458749-LSU; AF126695-RS
B. moritziana	Rio Guire, Edo Sucre, Venezuela, 11 Apr 1991	Bm.3149				X	AF458709-rbcL
B. tenuissima	Sydney, NSW, Australia, 4 Jan 1991	Bt.3111				X	AF458710-rbcL
Caloglossa leprieurii	James River, Cobhams Wharf, Virginia USA (coll. F. Ott)	Cl.3156				X	AF458708-rbcL

Collection site and date collected; #, JAW culture number (field samples in parentheses); X, DNA regions sequenced. GenBank Accession numbers in gene order, only variable sequences given. COX, *cox*2-3 spacer; RS, RuBisCo spacer; LSU, partial large subunit ribosomal RNA gene; *rbc*L, large subunit ribulose bisphosphate carboxylase.

groups that are morphologically indistinguishable (Avise, 2000). One pattern is that of large genetic gaps (major discontinuities) between allopatric entities, with the different lineages referred to as 'cryptic species' (e.g. Lee, 2000; Hoare *et al*., 2001). Cryptic species are especially evident among marine taxa (Knowlton, 1993) including red algae. The red algal species *Bostrychia radicans* (Montagne) Montagne and *B. moritziana* (Sonder *ex* Kützing) J. Agardh are separated by a single morphological character, monosiphonous branch tips, but belong to several separate well-defined lineages. These lineages are mainly allopatric and monosiphonous branch tips are distributed in several places within the phylogeny (Zuccarello *et al*., 1999*c*), suggesting that this character has arisen several times. This character is also variable in laboratory culture (Zuccarello & West, 1997). Isolates within separate lineages are unable to interbreed (Zuccarello *et al*., 1999*c*). In *Caloglossa leprieurii* (Montagne) G. Martens several lineages are also found that are reproductively isolated (Kamiya *et al*., 1998).

A second phylogeographic pattern is one in which genetic variation between phylogeographic groups is low, or non-existent. Such a phylogeographic pattern could be due to a recent rapid spread (high gene flow) of a species sharing a recent common ancestor, and has been seen in some marine organisms (Palumbi & Wilson, 1990).

Genetic data have also given us insights into recent introductions of species. Introductions of morphologically identical but genealogically distinct species (cryptic introductions) have been demonstrated in the red algae *Caulacanthus ustulatus* (Turner) Kützing (Rueness & Rueness, 2000; Zuccarello *et al*., 2002) and *Polysiphonia harveyi* Bailey (McIvor *et al*., 2001). In *C. ustulatus* a genetic variant has been found to have been introduced into France from the west Pacific although the alga (a different distinct genealogical lineage) already exists in Europe.

Reproductive isolation is the hallmark of the biological species concept (Mayr, 1942). Although its utility for the concept of red algal species has been questioned (Guiry, 1992), its importance in maintaining separate genealogical lineages in organisms is unquestioned, and it remains a central aspect of any integrative species concept (Harrison, 1998; Avise, 2000). Reproductive isolation has been shown to correlate with divergence in molecular markers (Kamiya *et al*., 1999; Zuccarello & West, 1997; Zuccarello *et al*., 1999*c*), with genetically similar samples able to hybridize. More divergent lineages are, in contrast, typically unable to hybridize.

Spyridia filamentosa (Wulfen) Harvey is found in many tropical and subtropical regions (Abbott & Hollenberg, 1976; Silva *et al*., 1987, 1996; Schneider & Searles, 1991; Adams, 1994; Stegenga *et al*., 1997; Womersley, 1998; Wynne, 1998; Yoshida, 1998), including mangrove habitats. Its reproductive biology in culture has been investigated (West & Calumpong, 1989). In certain tropical and subtropical localities it can be a dominant component of the macroalgal biomass (Cruz-Ayala *et al*., 1998).

Murrayella periclados (C. Agardh) Schmitz is also pan-tropical, mostly found in mangrove environments, but is less ubiquitous than *Spyridia filamentosa*. For example, *M. periclados* is not reported from Hawaii (Abbott, 1999), French Polynesia (Payri *et al*., 2000) or the eastern USA

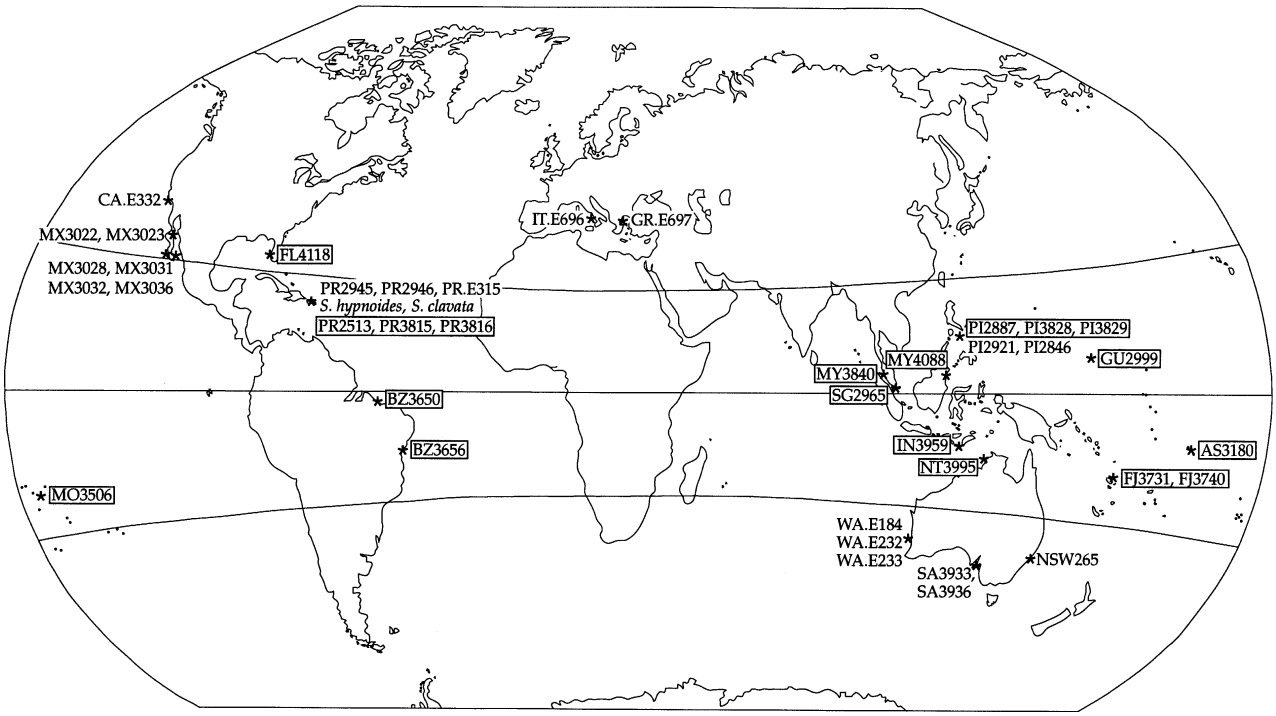

Fig. 1. World map showing collection locations of samples of *Spyridia filamentosa*, *S. hypnoides* and *S. clavata* and *Murrayella periclados* (in rectangles) used in this study. Sample abbreviations are in Table 1.

(Schneider & Searles, 1991; but see this study). Its *Polysiphonia*-type life cycle was investigated by Aponte & Ballantine (1987).

The present study was undertaken to: (1) compare the phylogeographic patterns, and elucidate the histories, of two pan-tropical red algal species (*Spyridia filamentosa*, *Murrayella periclados*) collected from around the world; (2) assess the levels of genetic variation within these two species using three molecular markers; and (3) determine levels of reproductive isolation between various isolates.

Materials and methods

Samples examined are listed in Table 1 and their geographic locations are represented in Fig. 1. Methods for collection, isolation and maintenance of cultures are presented in West & Zuccarello (1999). Field samples were dried in silica gel until DNA extraction. DNA extraction and amplification, single-stranded conformational polymorphism (SSCP) analysis and the sequencing of the plastid-encoded RuBisCo spacer were as described in Zuccarello *et al.* (1999*b*). Amplification and sequencing of the mitochondrial-encoded *cox*2-3 spacer followed Zuccarello *et al.* (1999*a*). Amplification of an approximately 950–1000 bp region of the nuclear large subunit of ribosomal RNA (LSU), corresponding to the middle third of the molecule (Y-fragment; Harper & Saunders, 2001) followed the procedure in Zuccarello & West (2002). Amplification and sequencing of the plastid-encoded large subunit of the ribulose bisphosphate carboxylase/oxygenase gene (*rbc*L) used amplification primers presented by Nam *et al.* (2000) and additional sequencing primers listed in Freshwater & Rueness (1994). The PCR procedure used for amplification of the *rbc*L was: an initial denaturation at 95 °C for 4 min, followed by 35 cycles of denaturing at 94 °C for 1 min, annealing at 45 °C for 1 min, and extension at 72 °C for 80 s. This was followed by a final 5 min extension at 72 °C. All PCR products were electrophoresed in 1–2% agarose to check product size and sequenced following procedures in Zuccarello *et al.* (1999*c*).

Sequences were assembled using the computer software supplied with the ABI sequencer and aligned with Clustal X (Thompson *et al.*, 1997); the alignment was refined by eye. Phylogenetic relationships were inferred with PAUP*4.0b (Swofford, 2001). Outgroups used were selected from available GenBank deposits or sequenced for this study. The *rbc*L sequences for the following species were downloaded from GenBank: *Pterocladiella capillacea* (Gmelin) Santelices *et* Hommersand (AB0176681); *Gracilariopsis tenuifrons* (Bird *et* Oliveira) Fredericq *et* Hommersand (AF212189); *Centroceras clavulatum* (C. Agardh) Montagne (AF259490); *Ceramium brevizonatum* H. Petersen (AF259491); *Ceramium diaphanum* (Lightfoot) Roth (U04020); *Antithamnion* sp. (X54532); *Aglaothamnion byssoides* (Arnott *ex* Harvey *in* W.J. Hooker) L'Hardy-Halos *et* Rueness (AF259489); *Callithamnion* sp. (U04019); *Polyneura latissima* (Harvey) Kylin (AF257438); *Phycodrys rubens* (Linnaeus) Batters (AF257428); *Spyridia hypnoides* (Bory) Papenfuss (U04025). For the LSU gene, sequences from GenBank were: *Polyneura latissima* (AF259475); *Phycodrys rubens* (AF259470); *Ceramium brevizonatum* (AF259415); *Centroceras clavulatum* (AF419113); *Pterothamnion villosum* (Kylin) Athanasiadis et Kraft (AF419114).

Maximum parsimony (MP) trees were constructed in PAUP*, using the heuristic search option, 500 random sequence additions, TBR branch swapping, unordered characters, gaps treated as missing data (except for

distance calculations of partial LSU; see below) and unweighted (except for successive weighting of *rbc*L data; see below). The program Modeltest version 3.06 (Posada & Crandall, 1998) was used to find the model of sequence evolution that best fits each data set by a hierarchical likelihood ratio test (hLRT) ($\alpha = 0{\cdot}05$) (Posada & Crandall, 2001). When the best sequence evolution model had been determined, maximum likelihood (ML) and distance searches were performed in PAUP* using the estimated parameters (substitution model, gamma distribution, proportion of invariable sites, transition–transversion ratio). Distance trees were constructed using neighbor-joining reconstruction (NJ). ML was also used to construct the most likely tree from the data set (2–5 random additions).

For the *rbc*L data a successive weighting strategy was employed (Farris, 1969). Based on an unweighted cladogram, each character (base position) is assigned a weight based on some measure of fit to this cladogram. We used successive weighting using the rescaled consistency index (Farris, 1989), as implemented in PAUP*. Trees were then generated using the reweighted data set (50 random sequence additions). This procedure was repeated until trees/weights converged and no change was observed between two successive rounds.

Distance matrices were calculated in PAUP* using the best sequence evolution model derived from Modeltest and uncorrected (p) distances.

Support for individual internal branches was determined by bootstrap analysis (Felsenstein, 1985), as implemented in PAUP*, and a decay index (Bremer, 1988). For bootstrap analysis, 1000 bootstrap data sets were generated from resampled data (5 random sequence additions), for both the MP and NJ analysis. Decay indices, on a strict consensus of the most parsimonious trees, were calculated with AutoDecay version 4.0.2 (Eriksson, 1998).

Haplotype networks (gene genealogies) were calculated using the networking algorithm developed by Templeton *et al*. (1992) (implemented in the computer program TCS 1.13; Clement *et al*., 2000) that produces an estimation of gene genealogies for DNA sequences. The relationship of haplotypes was estimated because in DNA sequences in which there are few substitutions, traditional phylogenetic methods perform poorly (Crandall & Templeton, 1993).

Data sets from different genomic regions were tested for incongruence using the incongruence length difference (ILD) test (Farris *et al*., 1994) as implemented in PAUP*. Combinations were made of the mitochondrial and plastid genome data (RuBisCo spacer and *cox*2-3 spacer) and of the data from all three genomes (RuBisCo spacer, *cox*2-3 spacer and partial LSU). The ILD test compares the incongruence length differences, i.e. the difference in the number of steps between separate and combined analyses of the original partitions (in our case, the different genomic regions), with a series of randomized partitions (1000) generated from the data (Farris *et al*., 1994). Incongruence between data sets (especially between organellar and nuclear genes) may be the result of: (1) differences in phylogenetic histories (Doyle, 1992); (2) differences in the power of phylogenetic resolution among the genes; or (3) differences in the rates or modes of evolution of the genetic regions (Bull *et al*., 1993). MP, NJ and ML reconstructions were performed on the combined data sets as described above.

Breeding studies were performed on unialgal cultures as outlined in Zuccarello *et al*. (1999*c*). Crosses were considered positive if carposporophytes released viable carpospores that germinated in a normal bipolar manner. Negative crosses were repeated three times. In all trials an unpaired female was used as a negative control.

Table 2. Identical DNA sequences for samples of *Spyridia* spp. and *Murrayella periclados*

Species	Partial LSU	*cox*2-3 spacer	RuBisCo spacer
S. filamentosa	MX3023 MX3028 CA.E332 NSW265 GR.E697 PI2921 SA3933	MX3023 MX3031 MX3028 MX3036 CA.E332	MX3023 MX3031 MX3028 MX3036 CA.E332 PI2921 GR.E697 SA3933 SA3936
	PR2946 PR2945 PR.E315	SA3933 SA36	WA.E184 WA.E232 WA.E233
	MX3022 MX3032	WA.E184 WA.E3232 WA.E233	PR2946 PR2945 PR.E315
		PR2946 PR2945	MX3032 MX3022
S. hypnoides	PR.E214 PR.E310 PR.E313 PR.E346	PR.E214 PR.E310 PR.E313 PR.E346	PR.E310 PR.E313 PR.E346
M. periclados	FJ3740 FJ3731 PI3829 MY3840	FJ3731 FJ3740 PI2887 AS3180 MO3506	FJ3750 NT3995 AS3180 MO3506 GU2999 PI2887 PI3828 PI3829 PR2513 PR3816 BZ3560
	BZ3650 BZ3656 MY4088	BZ3650 BZ3656 NT3995 IN3959	SG2965 BZ3656
	AS3180 GU2999	SG2965 MY4088	
	PI3828 MO3506	PR3815 PR3816	
	PR3816 PR2513		
	NT3995 IN3959		

Grouped by genic region and species. Isolate numbers (see Table 1) with identical sequences are in the same cell.

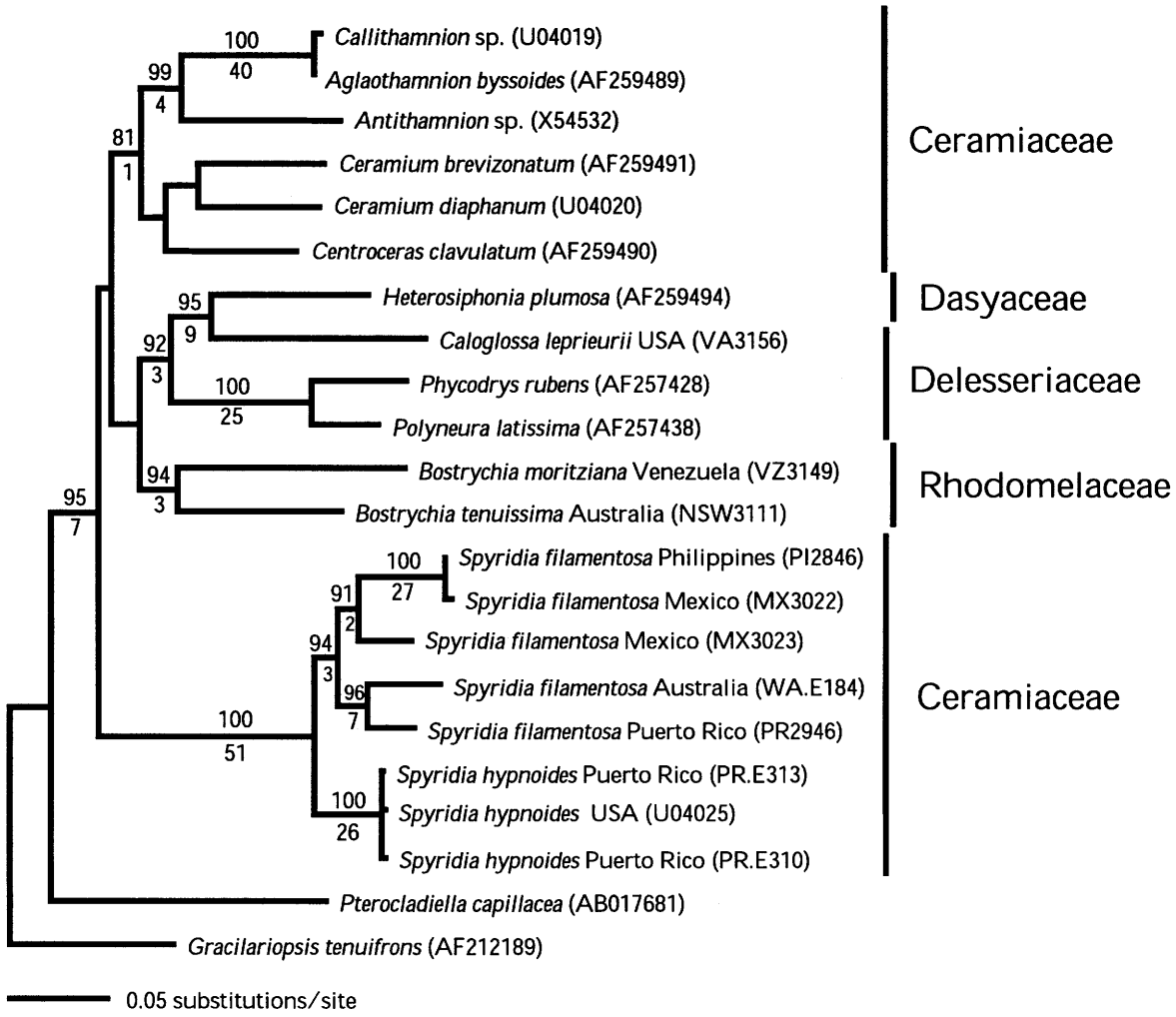

Fig. 2. Phylogeny of selected members of the Ceramiales, including *Spyridia* spp. samples, based on *rbc*L DNA sequences. Maximum-likelihood topology (hLRT-derived parameters, ML = 8932·60). Sample abbreviations are found in Table 1, GenBank samples are followed by the accession number. Values above branches are the percentage of bootstrap replicates (> 50%) derived from parsimony analysis. Values below branches are decay values. Outgroups are *Gracilariopsis tenuifrons* and *Pterocladiella capillacea*.

Results

Many of the sequences from different samples of *Spyridia* spp. and *Murrayella periclados* were identical, and these were not all used in the phylogenetic reconstructions and are presented in Table 2. All variable sequences were deposited in GenBank (Table 1).

Spyridia *spp.*

*rbc*L. The data set consisted of 1308 aligned characters, 411 of which were potentially informative. Unweighted MP produced six trees of 1645 steps (CI = 0·4517). Successive weighting produced 3 trees (score = 426·86). There were no substantial (or significant) topological differences among the trees inferred using unweighted and weighted parsimony methods. ML produced the topology shown in Fig. 2 (estimated evolution model: general-time-reversible (Lanave *et al.*, 1984), rate matrix: A → C = 2·898; A → G = 9·441; A → T = 8·670; C → G = 3·478; C → T = 27·057; G → T = 1·00; gamma distribution = 0·2354). The well-supported Ceramiales consists of a possibly paraphyletic Ceramiaceae (though this relationship has no (< 50%) bootstrap support), with *Spyridia* spp. forming a clade separate from other members of the Ceramiaceae. The other families, with less taxon sampling, show a monophyletic Rhodomelaceae and possibly paraphyletic Delesseriaceae. *Spyridia hypnoides* forms a strongly supported sister group to the *S. filamentosa* samples. Our *S. hypnoides* samples from Puerto Rico differ by 13 base pairs when compared with the eastern USA sample retrieved from GenBank (U04025). *Spyridia filamentosa* samples fall into two major groups: one containing samples from the Atlantic (PR2946) and the Indian Ocean (WA.E184), and the other containing Pacific Ocean samples (Mexico, Philippines). Within this Pacific lineage samples from Mexico (MX3022) and the Philippines (PI2846) are distinct from the other Mexican (MX3023) sample. MP and NJ topologies were essentially identical to the ML topology. Removal

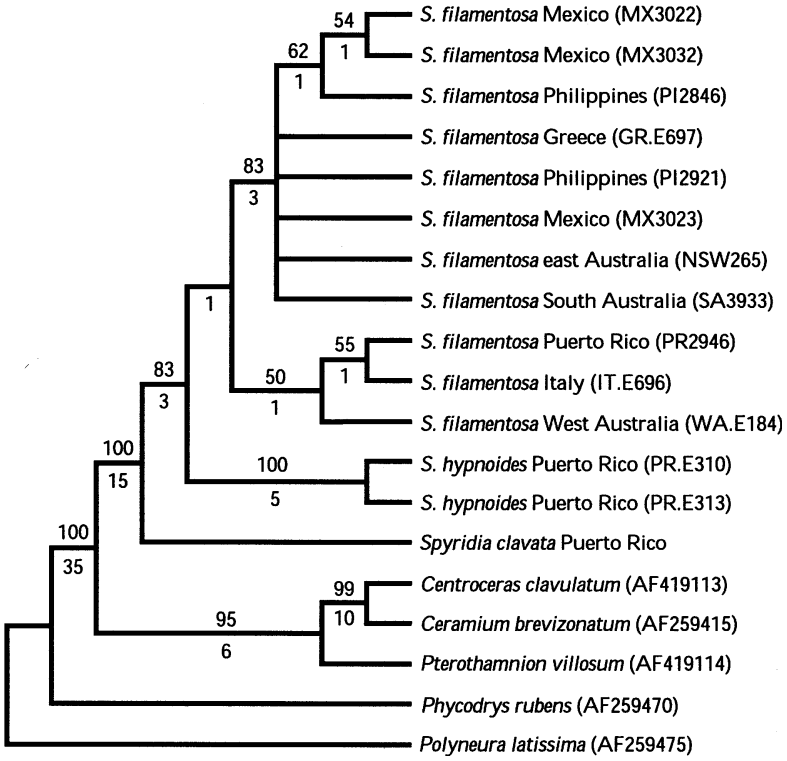

Fig. 3. Strict consensus topology of maximum parsimony trees based on partial LSU DNA sequence data (112 informative sites, 3 trees, 300 steps, CI = 0·8833). Values above branches are the percentage of bootstrap replicates (> 50%) derived from parsimony analysis. Values below branches are decay values. Outgroups are *Polyneura latissima* and *Phycodrys rubens*.

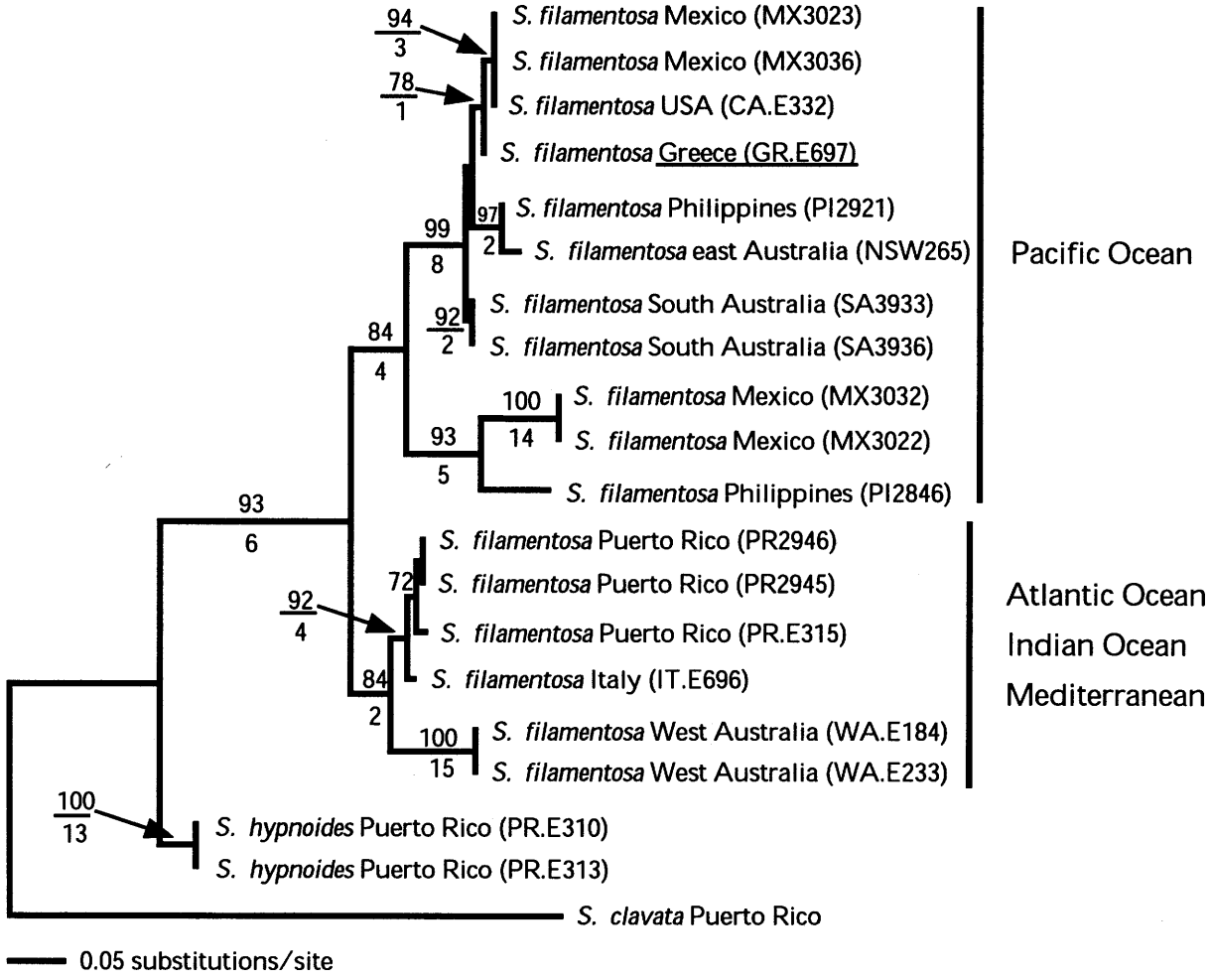

Fig. 4. Maximum likelihood reconstruction of *Spyridia* samples based on *cox*2-3 spacer DNA sequence (hLRT-derived parameters, ML = −1400·70). Values above branches are the percentage of bootstrap replicates (> 50%) derived from parsimony analysis. Values below branches are decay values. Samples with identical sequences were not all used (see Table 2). Outgroup was *Spyridia clavata*. The eastern Mediterranean sample is underlined.

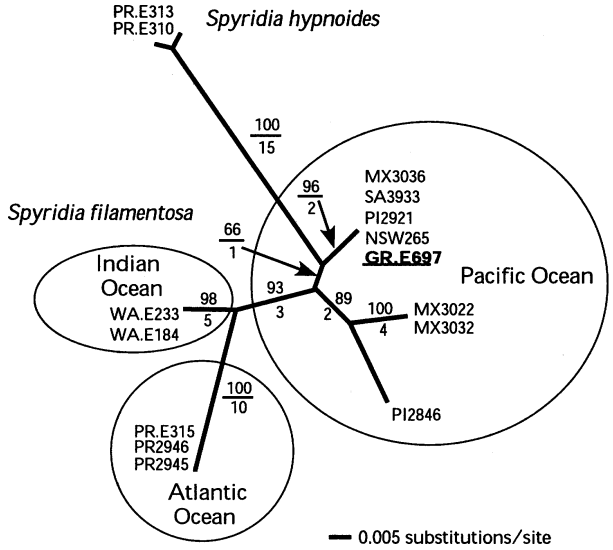

Fig. 5. Unrooted distance phylogram based on RuBisCo spacer DNA sequence (HKY85 distances, NJ reconstruction) of *Spyridia filamentosa* and *S. hypnoides* samples. Values above branches, or line, are the percentage of bootstrap replicates (> 50%) derived from parsimony analysis. Values below branches are decay values. The eastern Mediterranean sample is underlined.

of third codon positions did not significantly alter the topology of the MP tree and the relationships within *Spyridia* were unchanged.

Partial LSU. The data set consisted of 865 aligned characters, of which 112 characters were potentially informative. MP produced three trees of 300 steps; a strict consensus of these trees is shown in Fig. 3. Again, all *Spyridia* samples formed a strongly supported clade. *Spyridia clavata* Kützing formed a sister group to all other *Spyridia* samples. *Spyridia hypnoides* formed a well-supported clade (100%). Relationships within *S. filamentosa* samples were less resolved. A weakly supported group of samples (50%) from the Mediterranean Sea (Italy), Atlantic and Indian Ocean is seen, and forms a sister group to a moderately supported clade (83%) of samples from the Pacific Ocean. This clade, containing mostly Pacific samples, also includes a sample from the eastern Mediterranean (Greece). Some samples from Mexico (MX3022, MX3032) and the Philippines (PI2846) form a weakly supported clade (62%) distinct from the other Pacific samples. ML

Table 3. Distance matrix of DNA sequence data for *Spyridia* samples (*S. filamentosa*, *S. hypnoides*)

A.	1	2	3	4	5	6	7
1 MX3023	–	0·001	0·004	0·019	0·022	0·012	0·027
2 PI2846	0·001	–	0·003	0·019	0·014	0·012	0·025
3 MX3032	0·004	0·002	–	0·025	0·022	0·017	0·028
4 PR2946	0·012	0·012	0·015	–	0·006	0·007	0·032
5 IT.E696	0·014	0·010	0·014	0·005	–	0·009	0·036
6 WA.E184	0·009	0·009	0·011	0·006	0·007	–	0·030
7 S. hyp	0·016	0·015	0·016	0·018	0·020	0·017	–

B.	1	2	3	4	5	6	7	8	9	10	11	12	13
1 PR2946	–	0·016	0·023	0·139	0·188	0·174	0·291	0·209	0·279	0·197	0·294	0·274	0·294
2 PR.E315	0·014	–	0·022	0·139	0·216	0·213	0·331	0·209	0·279	0·225	0·336	0·310	0·313
3 IT.E696	0·020	0·020	–	0·124	0·196	0·193	0·303	0·203	0·254	0·205	0·352	0·323	0·343
4 WA.E184	0·074	0·074	0·069	–	0·291	0·379	0·340	0·345	0·327	0·349	0·563	0·502	0·383
5 MX3023	0·091	0·097	0·091	0·109	–	0·032	0·252	0·053	0·077	0·000	0·297	0·301	0·366
6 SA3933	0·089	0·098	0·092	0·121	0·026	–	0·234	0·040	0·059	0·020	0·294	0·261	0·363
7 PI2846	0·117	0·123	0·117	0·123	0·108	0·103	–	0·245	0·284	0·215	0·199	0·192	0·426
8 PI2921	0·097	0·097	0·094	0·118	0·040	0·031	0·107	–	0·019	0·040	0·325	0·310	0·400
9 NSW265	0·111	0·111	0·106	0·118	0·051	0·043	0·116	0·017	–	0·059	0·405	0·382	0·487
10 GR.E697	0·094	0·100	0·094	0·118	0·008	0·017	0·099	0·031	0·042	–	0·252	0·258	0·357
11 MX3032	0·117	0·123	0·123	0·144	0·114	0·112	0·088	0·120	0·131	0·105	–	0·006	0·474
12 MX3022	0·117	0·123	0·123	0·143	0·117	0·109	0·088	0·119	0·131	0·108	0·006	–	0·453
13 S. hyp	0·126	0·129	0·131	0·140	0·140	0·138	0·151	0·143	0·154	0·137	0·155	0·157	–

C.	1	2	3	4	5	6	7
1 MX3023	–	0·003	0·045	0·041	0·067	0·050	0·067
2 NSW265	0·003	–	0·048	0·044	0·071	0·054	0·070
3 PI2846	0·042	0·046	–	0·026	0·084	0·065	0·106
4 MX3032	0·039	0·042	0·025	–	0·078	0·060	0·085
5 PR2946	0·063	0·066	0·078	0·073	–	0·054	0·118
6 WA.E184	0·048	0·051	0·061	0·057	0·051	–	0·084
7 S. hyp	0·063	0·066	0·096	0·078	0·106	0·078	–

A, partial LSU; B, *cox*2-3 spacer; C, RuBisCo spacer. Only samples with variable sequences are compared (LSU = 820 bp compared; *cox*2-3 = 366 bp; RuBisCo spacer = 335 bp). Upper triangle represents HKY85 distances (for LSU data set: proportion of invariable sites = 0·9178, gamma distribution = 0·6655; for *cox*2-3 spacer data set: gamma distribution = 0·2585). Lower triangle represents uncorrected distances.

Table 4. Divergence levels within the species *Spyridia filamentosa* and *Murrayella periclados*, based on uncorrected distance values

Genetic region	Base pairs	% divergence
S. filamentosa		
Partial LSU	820	0·1–1·5
*cox*2-3 spacer	366	0·6–14·4
RuBisCo spacer	335	0·3–7·8
M. periclados		
Partial LSU	929[a]	0·1–1·0
*cox*2-3 spacer	336	0·3–1·8
RuBisCo spacer	370	0·3–1·0

Base pairs indicates the base pairs compared. % divergence indicates the range of divergence values.
[a]Includes gaps of equal length as independent characters.

Table 5. Crossing results between isolates of *Spyridia filamentosa*. Isolate descriptions are in Table 1

	PI2846♀	PR2946♀	MX3022♀	SA3933♀
PI2846♂	+[a]	−	−[a]	−
PR2946♂	−[a]	+[a]	−[a]	−[a]
MX3022♂	−[a]	−	+[a]	−
SA3933♂	−[a]		+[a]	+[a]

+, cystocarps formed and released carpospores; −, no functional cystocarps formed.
[a]pseudocystocarps formed.

and NJ trees did not differ significantly from the MP reconstruction.

*Cox*2-3 spacer. The data set consisted of 366 aligned characters, of which 96 characters were potentially informative. MP produced two trees of 209 steps (CI = 0·7751). ML (estimated evolution model: substitution model = HKY85 (Hasegawa *et al*., 1985); gamma distribution = 0·1953; Ti/Tv ratio = 3·7608) produced the topology depicted in Fig. 4. MP and NJ topologies were inconsequentially different. *Spyridia hypnoides* forms a sister group to a well-supported *S. filamentosa* clade. Increased sampling within *S. filamentosa* with this more variable genic region (Zuccarello & West, 2002) increases the support for relationships within this species. A Mediterranean/Atlantic/Indian Ocean and a Pacific Ocean clade are well supported (84 %). The Mediterranean/Atlantic/Indian Ocean clade is divided into two well-supported clades ($\geqslant$ 92 %): Indian Ocean samples (Western Australia) and the other samples, with the Mediterranean sample (Italy) distinct from the Puerto Rico samples. The Pacific Ocean clade again contains an eastern Mediterranean sample (Greece). Four well-supported groupings are found within this Pacific clade. One clade contains some samples from Pacific

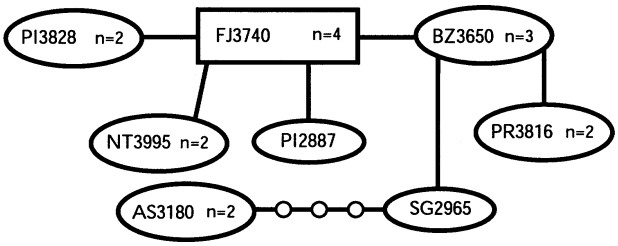

Fig. 6. Haplotype network for partial LSU haplotypes of *Murrayella periclados*. Line indicates one mutation step between haplotypes; open circles indicate missing intermediate; n, number of samples with identical haplotypes (see Table 2). The rectangle surrounds the dominant (most samples) haplotype.

Mexico (MX3022, MX3032) and the Philippines (PI2846), with the Philippine sample clearly distinct from the Mexican samples. The rest of the Pacific samples form a strongly supported clade (99 %) and within this clade three well-supported groups are found: (1) South Australian samples; (2) western Pacific samples (eastern Australia and Philippines (PI2921)) and (3) eastern Pacific samples (Mexico, California) and the eastern Mediterranean sample (Greece), which forms a sister group to the eastern Pacific samples.

RuBisCo spacer. This data set included 335 characters of which 46 were potentially informative. MP produced nine trees of 63 steps (CI = 0·9408). ML (estimated evolution model: substitution model = HKY85; Ti/Tv ratio = 3·4219) produced a topology identical to the MP tree. An unrooted NJ phylogram (HKY85 distances) is presented in Fig. 5; this phylogram has the same topology as the other tree reconstruction methodologies. Again the major groupings within *Spyridia filamentosa* are evident: (1) Atlantic samples, (2) Indian Ocean samples and (3) Pacific Ocean samples plus the eastern Mediterranean sample. Within this 'Pacific' group a separation between most Pacific samples (including MX3036 from Baja California) and two other Baja California samples (MX3022, MX3032) and a Philippines sample (PI2846) is again evident.

To assess the distribution and abundance of the two different Mexican lineages (MX3036 versus MX3022) multiple samples were collected from three separate locations in the Gulf of California (Bahia Balandra: 15 samples collected 19 March 2001; Santispac, Bahia Concepción: 19 samples collected 19 June 2001; Playa Armenta, Bahia Concepción: 18 samples collected 20 June 2001) and their RuBisCo spacer analysed using SSCP. All samples were identical to the sample of MX3036, part of the major east Pacific lineage.

The results from ILD tests indicated that the mitochondrial and plastid data sets are not significantly different from each other and could be

Table 6. Distance matrix of sequence data of *Murrayella periclados*

A.	1	2	3	4	5	6	7	8		
1 BZ3650	–	0·001	0·001	0·001	0·002	0·002	0·002	0·009		
2 PR3816	0·001	–	0·003	0·002	0·003	0·003	0·003	0·010		
3 SG2965	0·001	0·003	–	0·003	0·004	0·003	0·004	0·007		
4 FJ3740	0·001	0·002	0·003	–	0·001	0·001	0·001	0·008		
5 NT3995	0·002	0·003	0·004	0·001	–	0·002	0·002	0·009		
6 PI2887	0·002	0·003	0·003	0·001	0·002	–	0·002	0·009		
7 PI3828	0·002	0·003	0·004	0·001	0·002	0·002	–	0·009		
8 AS3180	0·009	0·010	0·006	0·008	0·009	0·009	0·009	–		
B.	1	2	3	4	5	6	7	8	9	10
1 PI3828	–	0·005	0·009	0·003	0·009	0·006	0·003	0·009	0·009	0·009
2 PI3829	0·005	–	0·014	0·009	0·018	0·014	0·009	0·018	0·018	0·009
3 SG2965	0·009	0·014	–	0·006	0·012	0·009	0·009	0·015	0·012	0·012
4 FJ3740	0·003	0·009	0·006	–	0·006	0·003	0·003	0·009	0·006	0·012
5 MY3840	0·009	0·018	0·012	0·006	–	0·003	0·009	0·009	0·012	0·009
6 BZ3650	0·006	0·014	0·009	0·003	0·003	–	0·006	0·006	0·009	0·009
7 FL4118	0·003	0·009	0·009	0·003	0·009	0·006	–	0·006	0·009	0·015
8 PR3815	0·009	0·018	0·015	0·009	0·009	0·006	0·006	–	0·015	0·015
9 PR2513	0·009	0·018	0·012	0·006	0·012	0·009	0·009	0·015	–	0·012
10 GU2999	0·009	0·009	0·012	0·006	0·012	0·009	0·009	0·015	0·012	–
C.	1	2	3	4						
1 3740.FJ	–	0·003	0·006	0·003						
2 3731.FJ	0·003	–	0·010	0·006						
3 3840.MY	0·006	0·010	–	0·006						
4 2965.SG	0·003	0·006	0·006	–						

A, partial LSU; B, *cox*2-3 spacer; C, RuBisCo spacer. Only samples with variable sequences are compared (LSU = 929 bp compared, including identical gaps considered as single independent characters; *cox*2-3 spacer = 336 bp; RuBisCo spacer = 370 bp). Upper triangle represents F81 (Felsenstein, 1981) distances (for LSU data set: Ti/Tv ratio = 0·8512). Lower triangle represents uncorrected distances.

combined in parsimony analysis ($p = 0{\cdot}7808$). This combined data set contained 704 characters, 143 of which were potentially informative, producing tree topologies similar to the individual data sets with increased bootstrap support at most nodes (data not presented).

A distance matrix was produced for the three individual data sets (nuclear partial LSU, mitochondrial *cox*2-3 spacer, plastid RuBisCo spacer), comparing *S. hypnoides* and *S. filamentosa* samples, using both distances derived from the estimated substitution model and uncorrected distances (Table 3). Maximum divergence between *S. hypnoides* and *S. filamentosa* was 2·0%, 15·7% and 10·6%, for uncorrected distances of the partial LSU, *cox*2-3 spacer and RuBisCo spacer, respectively. Maximum sequence divergence levels within *S. filamentosa* are (Table 4) 1·5%, 14·4% and 7·8% for the partial LSU, *cox*2-3 spacer and RuBisCo spacer data sets, respectively.

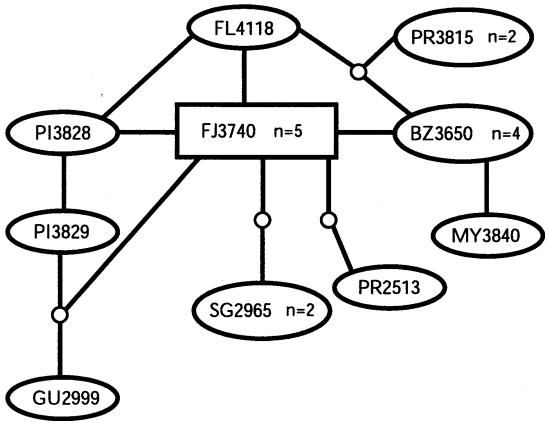

Fig. 7. Haplotype network for mitochondrial *cox*2-3 spacer haplotypes of *Murrayella periclados*. Line indicates one mutation step between haplotypes; open circles indicate missing intermediate; n, number of samples with identical haplotypes (see Table 2). Two lines leading to same haplotype indicate unresolved genealogies. The rectangle surrounds the dominant (most samples) haplotype.

Crossing experiments. Although growth was generally excellent in unialgal culture, crosses were difficult to perform with *S. filamentosa*. A few isolates continually reproduced sexually, but many were never sexually reproductive. Only four isolates could be used in crossing experiments (Table 5) and although isolates were all self-compatible (males and females from the same tetrasporophyte were sexually compatible) in all but one case crosses between isolates were unsuccessful despite spermatia being observed attached to trichogynes in all isolates. A successful cross was seen between a male South Australian sample (SA3933) and a

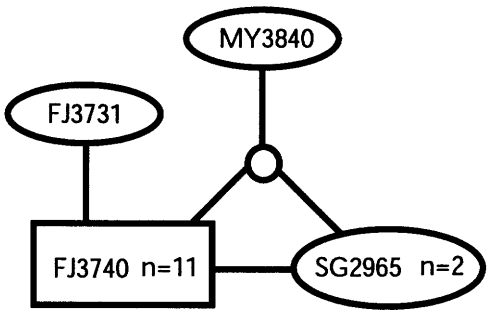

Fig. 8. Haplotype network of plastid RuBisCo spacer haplotypes of *Murrayella periclados*. Line indicates one mutation step between haplotypes; open circle indicates missing intermediate; n, number of samples with identical haplotypes (see Table 2). Two lines leading to same haplotype indicate unresolved genealogies. The rectangle surrounds the dominant (most samples) haplotype.

female Mexican sample (MX3022) (Table 5). Both these isolates are in the Pacific clade (see above) but are not phylogenetically the closest isolates tested (see for example Fig. 4). *Cox*2-3 spacer sequence divergence between the successfully crossed isolates is 10·9% (Table 3) while an unsuccessful cross between MX3022 and the Philippine sample (PI2846) has a divergence of 8·8%. SSCP analysis of the RuBisCo spacer of 9 tetrasporophyte germlings from the cross MX3022♀ and SA3933♂ and sequencing of the *cox*2-3 spacer of 2 sporelings (of the same 9) from this cross show that the organellar genomes were inherited maternally (identical SSCP mobility or DNA sequence to female organellar genome; data not shown). The reciprocal cross between MX3022♂ and SA3933♀ was unsuccessful, even after three repetitions. The development of the successful hybrids, after initial germination and growth, was not followed. In many successful and unsuccessful crosses pseudocystocarps were produced, i.e. with pericarps and presumed initial gonimoblast formation, but no carposporophyte development (Table 5). Pseudocystocarps were not observed in unpaired female isolates used as negative controls.

Murrayella periclados

Although samples of *M. periclados* were collected from world-wide localities, similar to *Spyridia filamentosa* (see Fig. 1), there was almost no sequence variation among samples and no phylogenetic structure (i.e. phylogenetic trees produced unresolved topologies with no or little bootstrap support (<50%) between the branches). Many samples, even from distant localities, had identical sequences (Table 2) for the DNA regions examined. A distance matrix shows that levels of divergence between variable isolates were low (Table 6). Divergence levels for *M. periclados* are summarized in Table 4. Maximum sequence divergence levels are 1·0%, 1·8% and 1·0% for the partial LSU, *cox*2-3 spacer and RuBisCo spacer data sets, respectively (compare this with the 1·5%, 14·4% and 7·8% divergence found within *S. filamentosa*). The number of variable characters and potentially parsimony-informative characters for phylogenetic analysis were: 13 variable characters, 10 of which were informative for the partial LSU (only when gaps of similar size and position were used in the data set); 11 variable and 5 informative for the *cox*2-3 spacer; 4 variable and 1 informative for the RuBisCo spacer.

Although phylogenetic trees were not informative with these minimal data, a haplotype matrix was produced to represent the genealogies for each genetic region analysed (partial LSU = Fig. 6; *cox*2-3 spacer = Fig. 7; RuBisCo spacer = Fig. 8). Ambiguities in the haplotype genealogies (i.e. two different branches leading to one haplotype; Figs 7 and 8) represent two equally likely evolutionary possibilities. Organellar genealogies (maternally inherited mitochondrial and plastid) are congruent as indicated by the ILD test ($p = 0{\cdot}965$) and by overlapping the two haplotype networks, except for isolates SG2965 (Singapore) and BZ3656 (Brazil). These two isolates share a unique plastid haplotype (Fig. 8, Table 2) though they differ in mitochondrial haplotypes by three steps; this is probably due to parallel mutations (homoplasy) in the plastid spacer. Character incongruence appears greater between the organellar haplotypes and the partial LSU (ILD test; $p = 0{\cdot}180$, and by comparing haplotype networks). For example, plastid haplo-

Table 7. Crossing results between isolates of *Murrayella periclados*

	PI2887♀	GU2999♀	FJ3731♀	PI3828♀	PI3829♀	IN3959♀
GU2999♂	+	+	+	+	+	+
MO3506♂	–[a]	–[a]	+	+	+	+
FJ3731♂	+	+	+	+	+	+
PI3828♂	+	+	+	+	+	+
PI3829♂	–[a]	–[a]	+	+	+	+
IN3959♂	–[a]	–[a]	+	+	+	+

Isolate descriptions are in Table 1. +, cystocarps formed and released carpospores that germinated.
[a]Cystocarps formed but carpospores failed to germinate. Pseudocystocarps were formed in all positive and negative crosses.

type SG2965 is designated as an intermediate nuclear haplotype between BZ3650 and AS3180 (Fig. 6), while AS3180 and BZ3650 share a more recent mitochondrial haplotype common ancestor than does BZ3650 with SG2965 (Fig. 7).

Crossing experiments. Cross results between six isolates of *M. periclados* are presented in Table 7. In most cases crosses were positive and in only a few cases did hybridization between isolates seem to be impaired. In these crosses designated as negative (Table 7), cystocarps appeared normal, i.e. they contained a fully developed carposporophyte and carpospores were released. These spores failed to germinate in three separate trials. In all crosses, including the successful crosses, pseudocystocarps were formed but were not observed in unpaired females used as controls.

Discussion

Our major conclusion is that there is a marked difference in the levels of genetic variation, and reproductive compatibility, between two pan-tropical red algal species collected from sites spanning a wide geographic area. These results clearly demonstrate that red algal species as currently recognized are not equivalent units of diversity.

Spyridia filamentosa consists of samples with high genetic variation and genealogical lineages that show strongly supported phylogeographic patterns. Samples from different ocean basins (Pacific, Atlantic, Indian) are for the most part clearly differentiated (see analysis of Mediterranean samples below) and, on the basis of limited tests, reproductively isolated (the only partially successful cross was between samples within the Pacific Ocean lineage). This would indicate that *S. filamentosa* consists of several cryptic species around the world. This pattern has also been found in other tropical red algae (Kamiya *et al*., 1998, 1999; Zuccarello *et al*., 1999*c*).

Our *S. filamentosa* data also reveal that all genomes are genealogically concordant (same partitions with all three genomes), which shows almost conclusively that the partitions accurately reflect phylogenetic divisions within the species and that these separations have existed for many generations. As the genomes are inherited in different manners (organellar being maternally inherited versus nuclear inherited bi-parentally), reciprocal monophyly (i.e. clearly distinct and concordant genealogies) takes more generations for nuclear genes, due to a larger effective population size of nuclear alleles (Palumbi *et al*., 2001).

Phylogeographic studies, mainly on animals, reveal several patterns of species distribution that can be correlated with hypothesized species histories. Deep genetic gaps producing well resolved phylogenies, and allopatric lineages, could indicate long-term barriers to gene exchange and dispersal (Avise, 2000). The *S. filamentosa* data, although admittedly based on a limited sample size, indicate that this species consists of two distinct lineages: an Atlantic/Indian Ocean lineage and a Pacific lineage. The Atlantic and Indian Ocean samples, though sharing a more recent common ancestor than they do with the Pacific samples, are also clearly distinct from each other. One sample from the western Mediterranean (Italy) is within the Atlantic clade, while another sample from the eastern Mediterranean (Greece) is within the Pacific clade. It is believed that many of the algae present in the Mediterranean were introduced or re-introduced into the Mediterranean from the Atlantic after the Messinian salinity crisis (approximately 5 mya), and the subsequent opening of the Strait of Gibraltar (Lüning, 1990). Few algae are believed to have survived this Messinian event. It is likely that the western Mediterranean sample is an Atlantic recolonizer.

Marine organisms associated exclusively or predominantly with the eastern Mediterranean are often thought to be recent Lessepsian migrants (through the Suez Canal) (Por, 1978) and some algae have been so classified (e.g. *Caulerpa racemosa* (Forsskal) J. Agardh (Piazzi *et al*., 1994; but see Famà *et al*., 2000)). The fact that our two *S. filamentosa* samples from the Mediterranean are in separate lineages may indicate that the eastern Mediterranean sample is a Lessepsian migrant, but its association with Pacific rather than Indian Ocean samples probably excludes this scenario (further sampling from the Red Sea and other Indian Ocean areas is necessary before firm conclusions can be reached). A likely explanation is that the eastern Mediterranean sample is another example of a cryptic anthropogenically introduced red algal species (McIvor *et al*., 2001). Increased sampling in the Mediterranean is required, possibly using SSCP of the RuBisCo spacer, to determine the distribution of these two *S. filamentosa* lineages. Ecophysiological studies could determine whether the introduced lineage might out-compete the endemic populations.

Within the Pacific lineage two major lineages are seen, each containing samples from Pacific Mexico and the Philippines. The fact that these two lineages contain samples found on both sides of the Pacific (Mexico and the Philippines) suggests that migration across the Pacific occurred at some time in the past. Samples from Mulege and Bahia Balandra (Gulf of California), Mexico, each contain individuals with divergent phylogenies. Increased sampling from Bahia Balandra, over 10 years later, did not reveal these two haplotypes, all samples

having a single haplotype (MX3036 group). Sampling from two areas near Mulege (Bahia Concepción) also revealed only one haplotype identical to MX3036. The inability to find the different plastid haplotypes when resampling years later could be due to the known patchiness, and subsequent sampling in the 'wrong' area, of plastid haplotypes in marine habitats (Zuccarello *et al*., 2001). Other possible explanations are an extreme rarity of these haplotypes, though they were found in a sample of two in the first collecting trip, or fluctuations in their abundance over intervening years. Further sampling in Pacific Mexico and the Philippines may reveal these two lineages again, and only increased sampling (numbers and locations) in these areas will be able to address these questions properly.

*rbc*L analysis of *Spyridia* samples along with other ceramialean samples suggests that the Ceramiaceae is paraphyletic. This apparent paraphyly has been noted before with *rbc*L sequence data (Freshwater *et al*., 1994; de Jong *et al*., 1998), and has been attributed to the ancestral nature of the Ceramiaceae (Hommersand, 1963) and/or poor taxon sampling. The consistent observation of a paraphyletic Ceramiaceae with increased taxon sampling suggests that this paraphyletic relationship may be valid. Our analysis of two other, morphologically distinct, *Spyridia* species (*S. hypnoides* and *S. clavata*) indicates that these both represent monophyletic groups distinct from *S. filamentosa*.

Murrayella periclados shows lower levels of genetic variation, compared with *S. filamentosa*, and no phylogeographic structure. Along with an overall greater ability for isolates to hybridize, this indicates that *M. periclados* is a conventional biological/evolutionary species. Phylogeographic patterns in which there is no or little genetic variation are indicative of species with a shallow genetic history (recent common ancestor) and high gene flow (frequent long-distance dispersal). In these species there is also a greater likelihood that homoplasy will falsely unite these closely related genotypes (Avise, 2000). Examples of such possible homoplasy are seen in comparisons of the plastid and mitochondrial haplotypes (see example in Results). A shallow genetic history and rapid dispersal, possibly anthropogenically, are possible reasons for the wide geographic distribution of many haplotypes of this species.

There is little in the biology of *Murrayella periclados* that would indicate that this plant is a good candidate for rapid dispersal, and this is especially true in comparison with *Spyridia filamentosa*, which shows strong phylogeographic structure. Populations of *M. periclados* are never as large as those noted for *S. filamentosa* (Cruz-Ayala *et al*., 1998), leading to a lower probability of transport in water ballast, for example. While *S. filamentosa* is common in bays and lagoons as a drifting alga, *M. periclado*s is attached and is confined to mangroves and hard substrata in quiet backwaters (Taylor, 1960). While *M. periclados* has no special means of vegetative propagation, *S. filamentosa* has special abscising brachyblasts that can rapidly produce many vegetative clones (West & Calumpong, 1989) that could be transported in water samples. This paradox between genetic variation and biology of *M. periclados* still needs to be explained.

Another explanation for the lowered level of genetic variation in *M. periclados* is a reduced mutation rate, up to 10 times lower than in *S. filamentosa* (Table 4). Although this cannot be ruled out it seems unlikely that such a large difference in mutation rate exists between algae within the same order.

Finally, our results for two pan-tropical morphospecies indicate very different evolutionary histories, dispersal scenarios and levels of variations. The several distinct lineages in *S. filamentosa* indicate that this alga consists of several cryptic species, while *M. periclados*, with little variation, conforms more closely to a biological/evolutionary species. Increased sampling of these species, continued breeding and ecophysiological culture studies and comparison with other species will further define the meaning of a red algal species.

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