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MULTIPLE CRYPTIC SPECIES: MOLECULAR DIVERSITY AND REPRODUCTIVE ISOLATION IN THE *BOSTRYCHIA RADICANS/ B. MORITZIANA* COMPLEX (RHODOMELACEAE, RHODOPHYTA) WITH FOCUS ON NORTH AMERICAN ISOLATES[1]

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Red algae of the *Bostrychia radicans/B. moritziana* complex are common in warm temperate areas of North America. Phylogenetic analysis of both plastid and mitochondrial DNA sequence data revealed seven distinct evolutionary lineages among worldwide samples. Although only two haplotypes (plastid and mitochondrial) were found in Pacific Mexico, four plastid and 11 mitochondrial haplotypes were found in a similar latitudinal spread along the Atlantic coast of the United States. On the U.S. Atlantic coast only one plastid haplotype was found in northern samples (Connecticut to North Carolina), whereas further south several plastid haplotypes were found. Phylogenetic analyses suggested that this single plastid haplotype found among northern samples could be the result of a northward range expansion possibly since the last glacial maximum. Crossing data of samples within the same evolutionary lineage showed that samples with the same plastid haplotypes were generally sexually compatible; samples with different plastid haplotypes were reproductively isolated. Samples from Pacific Mexico were partially reproductively compatible with some samples from the Atlantic USA (plastid haplotype C) and were more closely related to these samples than these U.S. samples were to other U.S. Atlantic samples. Compatible solute types mirrored the plastid haplotype, with plastid haplotype B having only sorbitol, whereas all other haplotypes also contained dulcitol. Samples from Atlantic USA, with different plastid haplotypes (e.g. B vs. C), but within the same evolutionary lineage, were reproductively isolated from each other. Data indicate that reproductive isolation occurs between and within supported evolutionary lineages and that the number of cryptic species is high.

***Key index words:* *Bostrychia moritziana*, *Bostrychia radicans*, *cox*2-3 spacer, hybridization, phylogeography, reproductive isolation, Rhodophyta, RUBISCO spacer, SSCP**

***Abbreviations:* *cox*2-3 spacer, cytochrome oxidase 2-cytochrome oxidase 3 intergenic spacer; RUBISCO spacer, RUBISCO large and small subunit intergenic spacer; SSCP, single-stranded conformational polymorphism**

Cryptic diversity in marine organisms is increasingly being revealed as molecular studies have expanded (Knowlton 1993, Palumbi 1994; in seaweeds: McIvor et al. 2001, Gabrielson et al. 2003, van der Strate et al. 2002a, Zuccarello et al. 2002). Combined with these studies is a greater awareness of the geographic distribution of these genetic variants and an improved understanding of the speciation processes in marine organisms (Avise 2000).

Phylogeographic studies of marine and freshwater animals have been especially well investigated along the east and south coasts of the United States (see summaries in Avise 2000). These studies have shown, with exceptions, a strong biogeographic discontinuity (phylogenetic disjunction) among marine animals from either side of the Florida peninsula, with a transitional boundary in the east central coastline of Florida (Reeb and Avise 1990, Sarver et al. 1992). Molecular phylogeographic studies of marine algal species, at the intraspecific level, along these coasts and around a Florida transition zone are very preliminary (Zuccarello and West 1997).

The genus *Bostrychia* (Rhodomelaceae, Ceramiales) is widespread in tropical and warm temperate environments around the world. It is often found in estuarine systems associated with mangrove or salt marsh vegetation. The genus has been studied extensively in terms of its physiology, especially tolerance to salinity variation and compatible solute content (Karsten and Kirst 1989, Karsten et al. 1992, 1993, 1994, 1995) and biogeography (Zuccarello and West 1995, 1997, 2002, West and Zuccarello 1999, Zuccarello et al. 1999d).

Bostrychia radicans (Montagne) Montagne is one of the most widespread species within the genus (King and Puttock 1989). The taxonomy of these specimens is complex due to morphological plasticity and a lack of reliable defining characters. The presence or ab-

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sence of monosiphonous lateral branch tips (King and Puttock 1989), a phenotypically plastic character (Zuccarello and West 1995), has been used to differentiate between *B. radicans* and *B. moritziana* (Sonder ex Kützing) J. Agardh. However, these characters do not correlate with molecular monophyletic units (Zuccarello and West 1997). Therefore it was suggested that compoundly branched monosiphonous laterals may be a better character to distinguish monophyletic species. This idea was later challenged (Zuccarello et al. 1999d), because organisms with monosiphonous compoundly branched apices are found in several distinct evolutionary lineages, although the data were not able to resolve the relationships between these supported lineages.

Although morphological diagnostic characters are missing to separate lineages, molecular data do show that distinct molecular lineages do exist and the molecular data do not group these samples by area of origin. Previous limited molecular phylogenetic data on *B. radicans*, mostly from the Americas, placed three Pacific Mexico isolates, two Venezuelan isolates, and one Atlantic U.S. isolate (NJ2880, NJ, USA) within one phylogenetic lineage, based on the plastid-encoded RUBISCO spacer DNA sequence data, whereas another U.S. isolate (FL2928, Gulf of Mexico), along with Peruvian and some Brazilian isolates, was placed in a separate and distinct lineage (Zuccarello and West 1997). Crossing data in that study showed that isolates from the two separate lineages were reproductively isolated, though isolates within a lineage were generally reproductively compatible, including the Pacific Mexico isolates with certain isolates from Venezuela.

A later study, focusing on *B. moritziana* morphotypes from the southern hemisphere, with increased sampling compared with the previous studies (Zuccarello and West 1995, 1997), both within and between different geographic regions distinguished five major lineages of the *B. moritziana/B. radicans* complex (Zuccarello et al. 1999d). This study also found that isolates collected in close proximity may be morphologically identical but evolutionarily very divergent.

Phylogeographic studies of marine organisms have been aided by methods to rapidly score genotypes, especially of non-recombining "haploid" genomes such as mitochondria or plastids. Although these methods (e.g. single-stranded intergenic spacer [SSCP], microsatellites) are well established (Lessa and Applebaum 1993) and have been applied to a variety of organisms, their use in seaweeds is just commencing (Coyer et al. 2002, van der Strate et al. 2002b). The ability of the SSCP method to distinguish between "mutant" DNA fragments relies on the principles that the molecular conformation of single-stranded DNA is nucleotide-sequence specific; that this conformation is altered by point substitutions, insertions, and deletions; and that the altered conformation affects the mobility of the molecule to migrate through a polyacrylamide gel (Sunnucks et al. 2000). In red seaweeds this method has been used to distinguish between plastid-encoded RUBISCO spacer haplotypes within and between populations of *Caloglossa leprieurii* (Montagne) G. Martens (Zuccarello et al. 2000, 2001), to distinguish between isolates of *B. tenuissima* King et Puttock with different polyol contents (Zuccarello et al. 1999c), and to screen isolates in a biogeographic study of *B. moritziana* (Zuccarello et al. 1999d). Recently, a mitochondrial marker has been developed for intraspecific studies in red algae, the cytochrome oxidase 2-cytochrome oxidase 3 intergenic spacer (*cox*2-3 spacer) (Zuccarello et al. 1999a), that show higher levels of mutation than the plastid-encoded RUBISCO spacer (Zuccarello and West 2002).

The discovery of distinct molecular/evolutionary lineages within morphospecies and their relationship to our definition of species ("species concepts") in red algae is still being debated. A commonly debated species concept is the biological species concept (Mayr 1942); this concept assumes that biological species exhibit some form of reproductive isolation (pre- or postzygotic). Reproductive isolation certainly contributes to maintaining separate genealogical lineages in organisms and is still a key aspect of many integrative species concepts (Harrison 1998, Avise 2000). Isolates of *B. radicans* from Atlantic and Pacific North America have been studied with respect to their hybridization patterns. Isolates from North America were freely sexually compatible along large areas of the Pacific coast (Zuccarello and West 1995). In contrast, samples from Atlantic North America (eastern United States) exhibited greater reproductive isolation (Zuccarello and West 1995), although phylogenetic relationships of the samples at the time were not known. Later evidence showed a strong correlation between reproductive isolation and genetic distance (Zuccarello and West 1997, Zuccarello et al. 1999d).

Species of the genus *Bostrychia* have a unique set of compatible solutes that are found in no other Ceramiales (Karsten et al. 1992). These sugar alcohols (sorbitol and dulcitol) are important in osmoregulation (Karsten and Kirst 1989). Isolates from North America showed variation in the types of compatible solutes produced and other physiological parameters (Karsten et al. 1993), with some isolates producing sorbitol and dulcitol, whereas others produced only sorbitol.

This study was initiated to increase sampling of *B. radicans/B. moritziana* from the Pacific and Atlantic North American coasts to 1) assess phylogenetic relationships of these samples plus other samples from around the world to determine worldwide levels of plastid and mitochondrial haplotype diversity within this species complex, 2) compare reproductive isolation with levels of genetic diversity to understand the history of reproductive isolation within these marine algae, and 3) correlate molecular relationships with compatible solute production.

MATERIALS AND METHODS

Samples. *Bostrychia radicans* and *B. moritziana* samples used in this study are presented in Table 1. Conditions for collection,

TABLE 1. *Bostrychia radicans/B. moritziana* isolates used.

Collection location and date	Latitude/ Longitude	Culture code	Polyol type	RUBISCO Haplo.	RUBISCO Seq.	*cox*2-3 Haplo.	Accession Number	
							RUBISCO	*cox*2-3
Mulege, Baja California Sur, Mexico. 8 Jan 1991	26° 53′ N	MX3019	SD	A1	X			
	112° 01′ W	MX3020	SD[a]	A1				
		MX3021	SD	A1				
Estero Coyote, BCS, Mexico. 26 Mar 1991	26° 42′ N	MX3124	SD	A1	X	Z1	AY260866	AY257648
	113° 35′ W							
Bahia Magdelena, BCS, Mexico. 7 Jan 1991	24° 48′ N	MX3026	SD[a]	A1				
	112° 19′ W	MX3027	SD	A2	X	Z1	AY260867	
		MX3029	SD[a]					
		MX3037	SD[a]	A1			Z1	
Bahia Balandra, BCS, Mexico. 6 Jan 1990	24° 10′ N	MX3030	SD	A1	X			
	110° 18′ W	MX3034	SD[a]	A1				
		MX3035	SD	A1	X			
		MX3038	SD[a]	A1				
Teacapan, Sinaloa, Mexico. 22 Mar 1992	22° 21′ N	MX3253	SD	A1	X			
	105° 39′ W							
San Blas, Nayarit, Mexico. 22 Mar 1992	21° 13′ N	MX3249	SD	A1	X			
	105° 16′ W							
Barra de Navidad, Jalisco, Mexico. 19 Mar 1992	19° 12′ N	MX3258	SD[b]	A1		Z1		
	104° 41′ W							
Rio Pijijiapan, Chiapas, Mexico. 24 Mar 1993	15° 32′ N	MX3380	SD[c]	A1	X	Z1		
	93° 14′ W							
R. San Juan, Chiapas, Mexico. 25 Mar 1993	15° 10′ N	MX3381	SD	A1		Z2		AY257649
	92° 47′ W							
Buena Vista, Guatemala. 22 Mar 1993	13° 53′ N	GU3367	SD[c]	A1	X	Z1		
	90° 45′ W							
Pueblo Viejo, Guatemala. 22 Mar 1993	13° 53′ N	GU3371	SD[c]	A1	X	Z1		
	90° 42′ W							
Stamford, CT, USA. 13 Aug 1991	41° 03′ N	CT3175	S	B	X	Y		AY257650
	73° 32′ W	CT.E208	?	?		Y		
Long Island, NY, USA. 22 Oct 1995	40° 53′ N	NY3449	S	B	X			
	72° 22′ W	NY3450	S	B		Y		
Mullica R., NJ, USA. 17 Jul. 1973	39° 32′ N	NJ2881	S	B	X	Y		
	74° 24′ W							
Cape Charles, VA, USA. (Göttingen 100-70)	37° 19′ N	VA3148	S	B	X	X		
	76° 13′ W							
York R., Croaker, VA, USA. 6 Nov 1991	37° 16′ N	VA3228	S	B	X	X		AY25765
	76° 42′ W							
Cape Fear Estuary, NC, USA. 27 Jan 1991	33° 53′ N	NC3115	S	B	X	V		
	78° 00′ W	NC3116	S	B	X	V	AF126713	AY257652
Belle Baruch Marine Lab, SC, USA. 15 Oct 1995	33° 24′ N	SC3536	SD	C	X	S		
	79° 16′ W							
Isle of Palms, SC, USA. 21 Jul 1991	32° 47′ N	SC3171	SD	C	X	S	AF126714	
	79° 47′ W	SC3172	SD[d]	C		S		
Charleston, SC, USA. 9 Oct 1991	32° 44′ N	SC3195	SD	C	X	S		
	79° 57′W							
James Island, SC, USA. 17 Jun 1994	32° 43′ N	SC3406	SD	C		S		AY257653
	80° 00′ W	SC3409	S	B		Y		
Dawhoo R. Landing, SC, USA. 17 Jun 1994	32° 32′ N	SC3410	S	B	X	Y		
	80° 18′ W							
Tybee Is., GA, USA. 18 Jun 1994	32° 05′ N	GA3411	S	B	X	Y		
	80° 52′ W							
Sapelo Is., GA, USA. 15 Dec 1990	31° 26′ N	GA3094	SD	D	X	N	AY260868	AY257654
	81° 15′ W							
Jekyll Is., GA, USA. 18 Jun 1994	31° 4′ N	GA3418	SD	B	X	W		AY257655
	81° 22′ W							
Cedar Key, FL, USA. 19 Sep 2000	29° 08′ N	FL4124	?	D	X	N		
	83° 03′ W							
Ft. Pierce, FL, USA. 19 Jun 1994	27° 29′ N	FL3419	SD	D	X	N		
	80° 19′ W	FL3420	SD	C	X	R		AY257656
Tampa, FL, USA. 16 Mar 1988	27° 56′ N	FL2928	SD	D	X	L		AY257657
	82° 57′ W							
Clearwater, FL, USA. 19 Sep 2000	27° 58′ N	FL4125	?	D	X	N		
	82° 49′ W							
Marco Island, FL, USA. 19 Sep 2000	25° 59′ N	FL4112	?	C	X	Q		
	81° 43′ W							
Miami R., Miami, FL, USA. 14 Jun 1994	25° 47′ N	FL3416	SD	E	X	M	AY260869	AY257658
	80° 11′ W							
Plantation, Florida Keys, FL, USA. 15 Jun 1994	25° 00′ N	FL3427	SD	C	X	Q		AY257659
	80° 22′ W							

(*continued*)

TABLE 1. Continued.

Collection location and date	Latitude/ Longitude	Culture code	Polyol type	RUBISCO Haplo.	RUBISCO Seq.	*cox*2-3 Haplo.	Accession Number RUBISCO	Accession Number *cox*2-3
Port Aransas, TX, USA. Sep 1974	27° 51′ N 97° 04′ W	TX3492	S	B	X	T		AY257660
Soro, Edo Sucre, Venezuela. 11 Apr 1991	10° 34′ N 62° 27′ W	VZ3136	SD	VZ1	X	P	AY260870	AY257661
Punta Piedra, Edo Sucre, Venezuela. 9 Apr 1991	10° 22′ N 64° 20′ W	VZ3142	SD	VZ2	X	O	AY260871	AY257662
Ilha do Cardoso, São Paulo, Brazil. 4 Apr 1990	26° 52′ N 47° 57′ W	BZ3051	?	BZ5	X	U	AY260872	AT257663
Ilha do Cardoso, São Paulo, Brazil. 24 Nov 1989	26° 52′ N 47° 57′ W	BZ3017	SD	BZ	X	K	AF126708	AY257664
Puerto Pizarro, Tumbes, Peru. 10 Feb 1990	3° 30′ S 80° 01′ W	PE3043	SD	PE	X		AF126710	
Morib, Selangor, Malaysia. 12 May 1998	2° 16′ N 101° 27′ E	MY3845	SD	n.a.	X	n.a.	AY260873	AY257665
Port Alma, Queensland, Australia, 24 Jul 1998	23° 36′ S 150° 51′ E	AU3881	?	n.a.	X		AF126715	
Fort Meyers Beach, FL, USA. 19 Sep 2000	26° 21′ N 81° 54′ W	M4119.FL	?	n.a.	X		AY260874	
Buenaventura, Colombia. 10 Sep 1991	3° 54′ N 77° 06′ W	M3189.CO	?	CO[f]	X	n.a.	AF126705	AY257666
Palmerston, Northern Territory, Australia. 21 Jun 1997	2° 32′ S	M3751.NT	?	PAL[f]			AF126699	
West Sawang, Sulawesi, Indonesia. 16 Nov 1994		M3453.IN	?	IN[f]	X		AF126700	
Rio Cariaco, Edo Sucre, Venezuela. 10 Apr 1991	10° 20′ N 63° 58′ W	M3150.VZ	SD[c]	n.a.	X	n.a.	AF126703	AY257667
Ilha do Cardoso, São Paulo, Brazil. Feb 1998	26° 52′ N 47° 57′ W	BZ3826	?	n.a.	X		AF126704	
Trinidade, Rio de Janeiro State, Brazil. 4 Mar 2001	23° 21′ S 44° 43′ W	M4146.BZ	?	n.a.	X		AY260875	
Plage de Foué, New Caledonia. 2 Feb 1998	21° 06′ S 165° 22′ E	M3818.NC	SD	NC2[f]	X	n.a.	AF126697	AY257668
Plage de Foué, New Caledonia. 2 Feb 1998	21° 06′ S 165° 22′ E	M3819.NC	?	AU[f]	X	n.a.	AF126698	AY257669
Beachwood, Natal, South Africa. 4 Nov 1991	28° S	M3204.SA	?	SA2[f]	X		AF126695	
		M3264.SA	?	SA1[f]	X	n.a.		AY257670
Tooradin, Western Port Bay, VIC, Australia. 25 Oct 1986	38° 13′ S 145° 22′ E	M2749AU	SD[a]	AU[f]	X	n.a.	AF126691	AY257671
Farasan Island, Red Sea, Saudi Arabia. 8 Jul 2000	16° 56′ N 42° 02′ E	M4069.S.Ar	?	n.a.	X	n.a.	AY260876	AY257672
Kota Kinabalu, Sabah, Malaysia. 17 Aug 2000	05° 52′ S 116° 01′ E	M4109MY	?	n.a.	X	n.a.	AY260877	AY257673
Sikuati Beach, Sabah, Malaysia. 13 Aug 2000	06° 53′ S 116°42′ E	M4074.MY	?			n.a.	AY260878	AY257674

Collection location, date of collection, and latitude and longitude given. Culture number = location two-letter abbreviation followed by four-digit J. A. West culture designation; M prefix, followed by culture number, indicates *Bostrychia moritziana* morphotype. Polyol-type (data from this study [Table 6] unless noted): SD, D-sorbitol and D-dulcitol; S, D-sorbitol only; RUBISCO Haplo., RUBISCO haplotype designations; RUBISCO Seq., RUBISCO spacer sequenced; *cox*2-3 Haplo., *cox*2-3 haplotype designations; GenBank Accession number for variable RUBISCO spacer and *cox*2-3 spacer haplotypes. n.a., not applicable.

[a]Karsten et al. (1992).
[b]West et al. (1993).
[c]Karsten et al. (1993).
[d]Karsten et al. (1994).
[e]Pedroche et al. (1995).
[f]Previously published *B. moritziana* haplotype designations, Zuccarello et al. (1999d).

isolation, and culture have been reported elsewhere (West and Calumpong 1988, West and Zuccarello 1999).

Phylogenetic analysis. Methodologies for DNA extraction, PCR amplification and haplotype scoring, via SSCP, and direct sequencing of the RUBISCO spacer are presented in Zuccarello et al. (1999b). Amplification and sequencing of *cox*2-3 spacer followed Zuccarello et al. (1999a). All PCR products were electrophoresed in 2% agarose to check product size and yield.

Sequences were assembled using the computer software supplied with the ABI 377 sequencer (Applied Biosystems, Foster City, CA, USA), aligned with Clustal X (Thompson et al. 1997) and refined by eye. Phylogenetic relationships were inferred with PAUP*4.0b10 (Swofford 2002). In all phylogenetic analyses gaps were considered as missing data. In the minimum spanning network, gaps of common size and position were considered as single mutational events. Outgroups were difficult to align to these samples. The closest sister group to *B. radicans* and *B. moritziana* is *Stictisiphonia kelanensis* (Grunow ex Post) King et Puttock (unpublished data). Placing this sample in the phylogenetic reconstruction and realigning the data accordingly did not alter any relationships that are presented here. All

phylogenetic reconstructions presented are based on mid-point rooting, without an outgroup selected.

Maximum parsimony trees were constructed in PAUP* using the heuristic search option, 500 random sequence additions, unweighted and unordered characters, and treé bisection reconnection (TBR) branch swapping. Distance trees were constructed using the Kimura-2 parameter estimate of distance and neighbor-joining reconstruction. Maximum likelihood (ML) was also used to construct the most-likely tree from the data set (two random sequence additions). ML parameters were estimated using the hierarchical likelihood ratio test. The program Modeltest version 3.06 (Posada and Crandall 1998) was used to find the model of sequence evolution that best fit each data set ($\alpha = 0.05$). When the best sequence evolution model was determined, ML tree searches were performed in PAUP* using the estimated parameters (gamma distribution (G), proportion of invariable sites (I), and transition/transversion ratio (Ti/Tv).

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 (five random sequence additions), for both the parsimony and distance analysis. Decay indices, on a strict consensus of the most parsimonious trees, were calculated with AutoDecay, version 4.0.2 (Eriksson 1998). Tree topologies based on ML distance data are presented, although all reconstructions were essentially identical. All unique sequences are deposited at GenBank.

Minimum spanning networks were constructed by hand comparing both the most parsimonious trees, generated in PAUP*, for the respective data sets and the sequence variation itself. Branch points in the network were inferred when two equally parsimonious solutions were possible.

Hybridization. Crosses between select isolates were performed as indicated in Zuccarello and West (1995). Data from Zuccarello and West (1995, Fig. 3) are redrawn here (Table 2), because these data are referred to often. Crosses between male and female gametophytes (haploid) were only scored when the control cross (male and females from same diploid), performed at the same time as outcrosses, was positive. In certain cases this was not possible, because only one sex was available; in these case at least one positive outcross had to occur. Crosses were considered positive if carpospores (diploid zygote products) were released from two or more carposporophytes and the carpospores subsequently germinated in a normal bipolar manner. Certain F_1 progeny were followed, and whether the free-living diploid (tetrasporophyte) became reproductive and released tetraspores (meiotic spores) was scored. In certain crosses pseudocystocarps (partially formed pericarps, but little or no carposporopyte development; Zuccarello and West 1995) were produced and noted.

Polyol analysis. Polyol analysis followed methodologies outlined previously (Karsten et al. 1991, 1994).

RESULTS

Phylogeny and haplotype distribution. SSCP analysis identified six RUBISCO spacer haplotypes from North America. Two haplotypes were scored for the Pacific North American samples (Mexico and Guatemala, Fig. 1, haplotypes A1 and A2), whereas four haplotypes were scored for the Atlantic North American samples (USA, Fig. 2, haplotypes B, C, D, E). A large subset of haplotypes with similar mobility in the SSCP gels were sequenced (Table 1), and no sequence variation was found that was not reflected in a band mobility shift in the SSCP gel. Phylogenetic analysis of these plastid haplotypes, plus other samples of *B. radicans* and *B. moritziana* from around the world, were incorporated into an aligned data set of 338 characters (available upon request). Maximum parsimony analysis produced 42 most-parsimonious trees of 202 steps (CI = 0.7255; RI = 0.8593) (not shown); this topology was consistent with the neighbor-joining topology, as was the ML topology (Fig. 3) (estimated evolution model: substitution model = HKY85 [Hasegawa et al. 1985], Ti/Tv ratio = 2.1439, I = 0.5695, G = 65.776; –log-likelihood = 1418.5136). Bootstrap resampling and phylogenetic reconstruction revealed seven major lineages with good bootstrap support. Although support for the lineages is generally high, relationships between lineages remained unresolved. The first five of these lineages were reported previously (Zuccarello et al. 1999d).

Sequencing of the *cox*2-3 spacer was also performed, mostly concentrating on North American samples. Thirteen *cox*2-3 spacer haplotypes were found in North America. Two haplotypes were found in Pacific North American samples (haplotype Z1 and Z2), and 11 haplotypes were found in Atlantic North America (L, M, N, Q, R, S, T, V, W, X, Y). Phylogenetic analysis of these mitochondrial haplotypes, plus other samples of *B. radicans*/*B. moritziana* from around the world, were incorporated into an aligned data set

TABLE 2. Cross results between isolates of *Bostrychia radicans* (redrawn from Zuccarello and West 1995).

RUBISCO			B	B	B	B	B	C	C	C	D	D
	*cox*2-3		Y	Y	X	V	V	S	S	S	N	L
		Isolate	CT3175	NJ2880	VA3228	NC3115	NC3116	SC3171	SC3172	SC3195	GA3094	FL2928
B	Y	CT3175	+							−		
B	Y	NJ2880		+				−	−	−	−	−
B	X	VA3228		−	+	−		−[p]	−	−	−	−
B	V	NC3115		−[p]	−	+		−	−	−	−	−
B	V	NC3116			−	−	+	−	−	−	−	−
C	S	SC3171	−		−[p]	−	−[p]	+	+	+	−	−
C	S	SC3195	−		−[p]	−	−[p]	+	+	+	−	−
A1	Z1	MX3030		−		−[p]			+[a]		−	−
A2	Z1	MX3027		−		−			+[a]		−	−
A1	Z1	MX3020		−		−			+[a]			−

Isolate designations in Table 1. RUBISCO spacer and *cox*2-3 spacer haplotypes (see Table 1) shown in first and second row/column, respectively. Females, horizontal rows; males, vertical columns; +, cystocarps produced, carpospores released and germinated; −, cystocarps produced; [p], pseudocystocarps produced.

[a]Tetrasporophytes produced but tetraspores abortive.

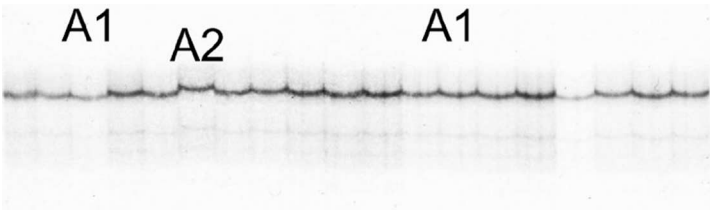

FIG. 1. Sample SSCP gel of amplified RUBISCO spacer of specimens from Pacific North America. Specimens are aligned north to south (left to right). Haplotypes A1 and A2 are marked.

of 449 characters (available upon request). Maximum parsimony analysis produced 18 most-parsimonious trees of 550 steps (CI = 0.6873; RI = 0.8576) (not shown); this topology was consistent with the neighbor-joining topology, as was the ML topology (estimated evolution model: substitution model = K81 [Kimura 1981], I = 0.2856, G = 1.828, –log-likelihood = 2867.97107) (Fig. 4). The phylogeny obtained revealed six distinct lineages, corresponding to lineages 1 and 3–7 of the RUBISCO spacer tree. Lineage 2 samples, of the RUBISCO spacer, were not analyzed because no amplification product was produced with the *cox*2-3 spacer primer pairs on these samples, possibly due to primer mispairing. Relationships within lineage 5 are better resolved with the *cox*2-3 spacer data set than the RUBISCO spacer data set (Fig. 4).

Lineage 1 contained samples from the Indo-Pacific, identified as *B. moritziana*. Over 100 samples were analyzed from a wide area, and all share very similar RUBISCO spacer sequences (Zuccarello et al. 1999d). Lineage 2 contained samples of *B. moritziana* also from the western Pacific, morphologically identical to samples from lineage 1, sympatrically distributed but reproductively isolated from lineage 1 samples (Zuccarello et al. 1999d). Lineage 3, although not strongly supported in the RUBISCO analysis, contained samples from South America that were mostly found in freshwater (M3150.VZ, M4146.BZ) with a *B. moritziana* morphology, plus an estuarine sample from southern Brazil. Lineage 4 contained one sample of *B. moritziana* from Pacific Colombia. The last lineage also reported previously was lineage 5. This lineage contained samples from the Pacific and Atlantic Americas, mostly from North America. These samples were identified as *B. radicans* in previous studies, although some isolates have monosiphonous branches that are characteristic of *B. moritziana* (Zuccarello and West 1995, 1997). Lineage 6 contained samples identified as *B. radicans* from diverse locations around the world (Australia, Malaysia), including North America. Lineage 7 contained samples, fitting the *B. moritziana* morphology, from several widespread locations (west Florida, Malaysia, Saudi Arabia).

Because this article focuses on the relationships between phylogenetic structure and reproductive compatibility of North American samples found in lineages 5 and 6, a minimum spanning network was produced from these isolates, plus isolates from South

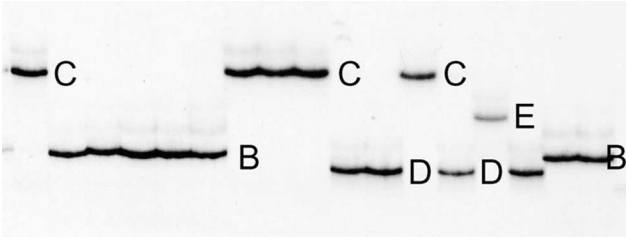

FIG. 2. Sample SSCP gel of amplified RUBISCO spacer of specimens from Atlantic North America. Haplotypes B, C, D, and E are marked.

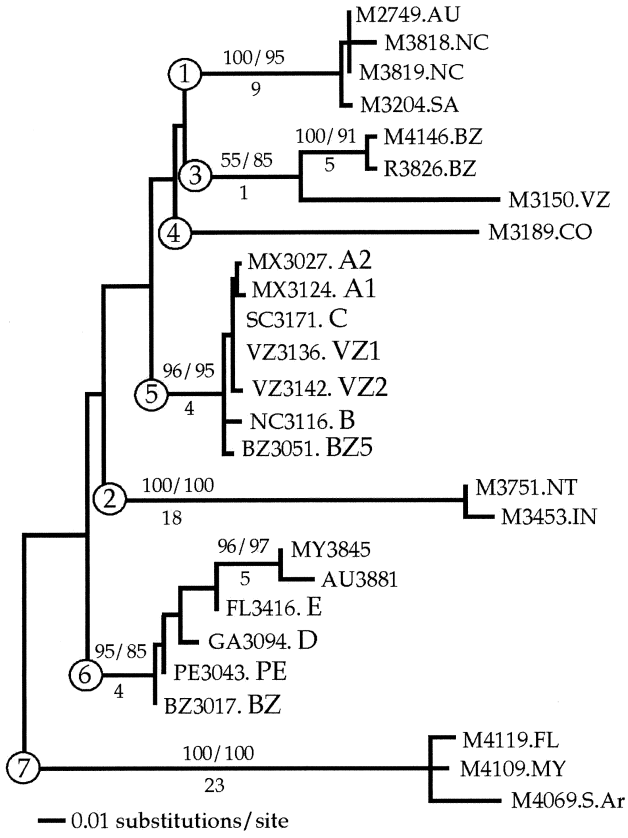

FIG. 3. Topology of ML derived tree from RUBISCO spacer data set of specimens of the *Bostrychia radicans/B. moritziana* complex. Mid-point rooting. Parsimony-derived bootstrap values (1000 bootstrap replicates, 5 random sequence additions) above branches, left of diagonal. ML derived bootstrap values (100 replicates) above branches, right of diagonal. Decay values below branches. Lineages 1–7 indicated. For information on sample culture codes (including four numerals) or haplotype abbreviations, see Table 1.

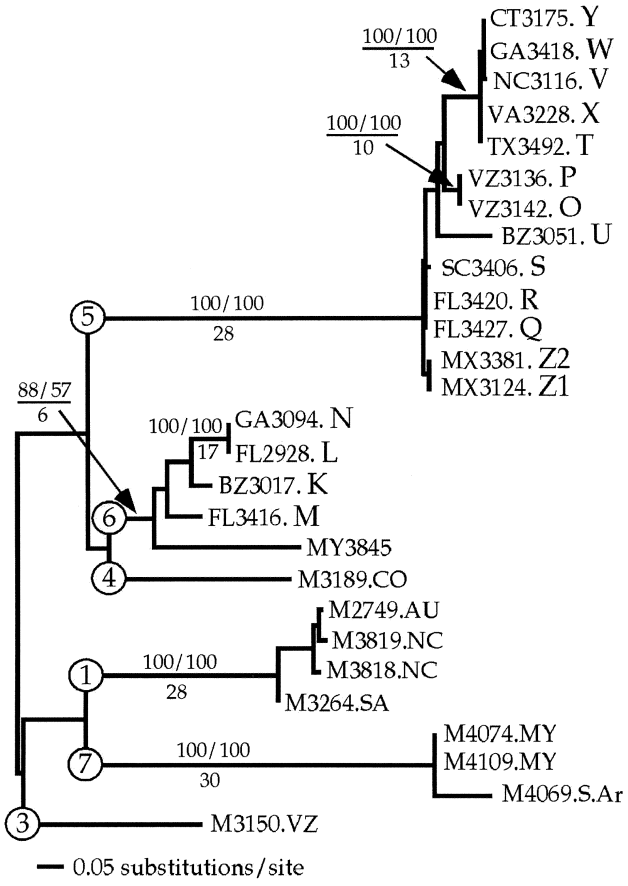

FIG. 4. Topology of ML derived tree from *cox*2-3 spacer data set of specimens of the *Bostrychia radicans/B. moritziana* complex. Mid-point rooting. Parsimony-derived bootstrap values (1000 bootstrap replicates, 5 random sequence additions) above branches, left of diagonal. ML derived bootstrap values (100 replicates) above branches, right of diagonal. Decay values below branches. Lineages 1–7 (lineage 2 missing, see text) indicated. Position of haplotypes indicated. For information on abbreviations of samples, see Table 1.

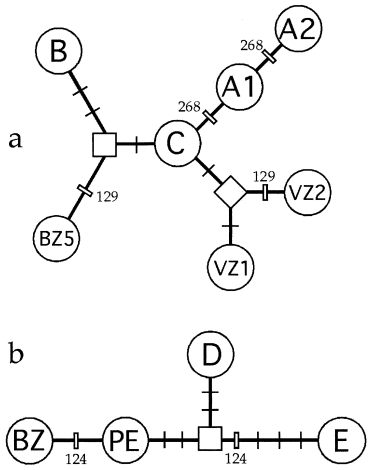

FIG. 5. Parsimony network of plastid RUBISCO spacer haplotypes referred to in text and Table 1. (a) Haplotypes from lineage 5; (b) haplotypes from lineage 6. Circles indicate haplotypes, and squares indicate hypothetical haplotype branch points. Cross line indicates position of single base pair substitutions. Homoplasious substitutions indicated by open rectangle and base pair position.

America within these lineages. Plastid haplotype variation is shown in Fig. 5. Within lineage 5, haplotypes A1 and A2 (from the Pacific) differ from haplotype C at only 1 bp, and haplotype A1 was far more common in Pacific Mexico (Table 1) and was the inferred ancestral haplotype for haplotype A2. Haplotype C differed from the Venezuelan haplotypes (VZ1, VZ2) by 2 bp. Mutational steps between haplotype C and the BZ5 (from Brazil) and B haplotypes was of 2 and 3 bp, respectively. Haplotype B is found sympatric with haplotype C (at James Island, SC, USA, Table 1). Haplotypes D and E from the Atlantic USA were found in lineage 6 as were samples from South America. Haplotype BZ (Brazil), collected from the same island as haplotype BZ5 in lineage 5, differs from PE (Peru) by 1 bp. The two U.S. haplotypes differ from each other by 6 bp, whereas haplotype D had more sequence similarity to the South American samples than to haplotype E (Fig. 5).

A minimum spanning network of the *cox*2-3 spacer haplotypes within lineages 5 and 6 is presented in Figure 6 and clearly shows the relationships of, and mutational steps between, the haplotypes. Haplotypes Z1 and Z2, from the Pacific, were between two and seven mutational steps from haplotypes S, R, and Q from the southeast United States. This group of haplotypes was 23 bp different from the Venezuelan haplotypes P and O. Mitochondrial haplotypes T, V, W, X, and Y (corresponding to samples with plastid haplotype B) are quite divergent from the other U.S. haplotypes (S, R, Q). Haplotypes N and L, within lineage 6, differed by 1 bp, whereas the other haplotypes within this lineage were quite divergent from each other (Fig. 6). For all instances in which data are available for both plastid and mitochondrial markers, plastid haplotypes perfectly overlapped the mitochondrial haplotypes (Fig. 6). The haplotype localities along the U.S. Atlantic and Gulf of Mexico coasts are shown in Figure 7.

Hybridization data. Crossing results are presented in Tables 2, 3, 4, and 5. Results from Zuccarello and West (1995) are redrawn in Table 2. Plastid and mitochondrial designations have been added to the isolate identification.

Table 2 shows that crosses between different isolates within plastid haplotype B were unsuccessful. Most of these crosses are between isolates with mitochondrial haplotype X or Y and haplotype V, the most divergent mitochondrial haplotype (Fig. 6). A cross between isolates with mitochondrial haplotype V both collected from the Cape Fear Estuary (NC3115 vs. NC3116) also proved unsuccessful, although these isolates were self-compatible. A cross between isolates with haplotype X and Y was also unsuccessful. All crosses between isolates with plastid haplotype C and

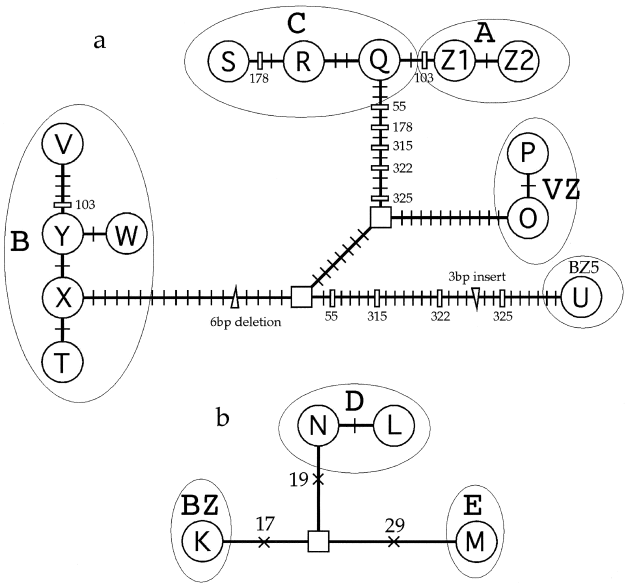

FIG. 6. Parsimony network of mitochondrial *cox*2-3 spacer haplotypes referred to in text and Table 1. (a) Haplotypes from lineage 5; (b) haplotypes from lineage 6. Circles indicate haplotypes, and squares indicate hypothetical haplotype branch points. Cross line indicate single base pair substitutions. Triangle indicates a common indel. Homoplasious substitutions indicated by open rectangle and base pair position. X on line gives number of mutations including common indels. Oval over mitochondrial haplotypes indicates plastid haplotype overlap.

mitochondrial haplotype S were successful, as were crosses between samples with plastid haplotype D (mitochondrial haplotype L and N). All crosses between isolates with different plastid haplotypes were negative, except for crosses between haplotype C (Atlantic USA) and haplotypes A1 and A2 (Pacific Mexico). Although these crosses were successful the tetrasporophytes derived from these carposporophytes (carpospores) produced only abortive tetrasporangia.

To further investigate the inability of some isolates within plastid haplotype B to cross, a second set of crosses were performed concentrating on isolates with this haplotype (Table 3). Most crosses within haplotype B were successful; however, unsuccessful crosses worked in some reciprocal cross combination. The one isolate with relative low crossing success (VA3148) has been maintained in culture for over 25 years.

Table 4 shows that crosses between isolates with plastid haplotype B were successful. Again crosses between plastid haplotype C isolates and Pacific Mexico isolates were successful, though this time tetrasporophytes were not followed, except for this case crosses between different plastid haplotypes were not successful.

Table 5 shows that crosses between isolates with the same plastid haplotype were successful, whereas crosses between isolates with different plastid haplotypes were unsuccessful, except for two cases. One is the previously mentioned crosses between haplotype C (Atlantic USA) and A1 (Pacific Mexico) and the reciprocal cross. Interestingly, this set of crosses shows the only case in which a C haplotype and an A1 haplotype were incompatible. The other successful interplastid haplotype cross was between an isolate with plastid haplotype C and B (SC3409 female and FL3420 male), though the reciprocal cross did not work. The diploid hybrid was not followed.

Even though this was a small cross data set from all potential isolates, due to logistic constraints, the results show that crosses within plastid haplotypes are generally successful. The only exception in crossing failure within plastid haplotypes is within plastid haplotype B in which crossing success is reduced but not completely inhibited (Tables 2 and 3).

Polyol analysis. Polyols were scored for 35 new samples used in this study (Table 6). Results are summarized in Table 1 along with published data (Karsten et al. 1992, 1993, 1994). The only haplotype to lack dulcitol was haplotype B, except for one isolate (GA3418) in which the dulcitol level was extremely low.

DISCUSSION

SSCP analysis is able to score accurately all plastid haplotypes (RUBISCO spacer) as shown in other studies (Zuccarello et al. 1999b,c,d). Phylogenetic reconstruction using plastid and mitochondrial data sets gives congruent relationships with seven well-supported lineages (although we lack mitochondrial data on samples from plastid lineage 2, we consider failure

TABLE 3. Cross results between isolated of *Bostrychia radicans* from Atlantic USA.

RUBISCO			B	B	B	B	B	C
	*cox*2-3		Y	Y	X	V	W	S
		Isolate	NY3449	NJ2881	VA3148	NC3116	GA3418	SC3406
B	Y	NY3449	+	+	−P	+	−P	−
B	Y	NJ2881	+	+	+	−P	+	−
B	Y	SC3410	+	+	−P	+	+	−
B	X	VA3228	+	−P	+	+	+	
B	V	NC3116	+	+	−P	+	+	
B	W	GA3418	+	+	+	+	+	−
C	S	SC3406	−P	−P	−P	−P	−P	+

Isolate designations in Table 1. RUBISCO spacer *cox*2-3 spacer haplotypes (see Table 1) shown in first and second row/column, respectively. Females, horizontal rows; males, vertical columns; +, cystocarps produced, carpospores released and germinated; −, cystocarps produced; P, pseudocystocarps produced.

TABLE 4. Cross results between *Bostrychia radicans*.

RUBISCO			A1	C	B	B	B
	*cox*2-3		?	R	Y	Y	W
		Isolate	MX3253	FL3420	SC3411	NY3450	GA3418
A1	?	MX3253	+	+	−	−	−
C	R	FL3420	+	+	−	−	−
B	Y	SC3411	−	−	+	+	+
B	Y	NY3450	−	−	+	+	+
B	W	GA3418	−	−	+	+	+

Isolate designations in Table 1. RUBISCO spacer and *cox*2-3 spacer haplotypes (see Table 1) shown in first and second row/column, respectively. Females, horizontal rows; males, vertical columns; +, cystocarps produced, carpospores released and germinated; −, cystocarps produced.

of *cox*2-3 spacer amplification of these specimens to be a synapomorphy for this lineage). Differences in phylogenetic reconstruction are all in poorly supported relationships (i.e. relationships between lineages). The first five lineages are the same as those proposed in a previous study that concentrated on Indo-Pacific samples (Zuccarello et al. 1999d). Previous studies have shown that crosses between these lineages were unsuccessful (Zuccarello et al. 1999d).

These morphologically undifferentiated but very diverse evolutionary lineages raises the issue of species definitions in red algae. Species have been defined mostly on morphological criteria, though it has been shown that morphological species concepts do not reflect reproductively compatible groups nor do they reflect monophyletic lineages (Guiry 1992, Zuccarello and West 1995, Zuccarello et al. 1999d). The large degree of sequence variation, congruence between genetic markers, robust bootstrap support for the lineages, and lack of reproductive compatibility between many of the above lineages would indicate that these seven lineages share many of the criteria used to define species, barring morphologically definable characters. This suggests that the *B. radicans/B. moritziana* complex consists of seven cryptic species. Cryptic species in other marine algae have been identified based on similar considerations (Zuccarello et al. 2002). Similarly cryptic species were inferred in the green alga *Cladophoropsis membranacea* (Hoffman Bang ex C. Agardh) Børgesen based on monophyletic groups and loss of amplifiable microsatellite loci (van der Strate et al. 2002a).

But what is the evolutionary status of samples within these lineages? Sequence similarity in neutral markers, even relatively quickly evolving markers such as the RUBISCO spacer, does not necessarily indicate

TABLE 6. Polyol content of *Bostrychia radicans* and *B. moritziana* isolates from Pacific and Atlantic North America.

Isolate	Plastid	D-Dulcitol	D-Sorbitol
MX3027	A2	166.4 ± 7.3	164.6 ± 14.8
MX3019	A1	225.6 ± 17.6	244.2 ± 20.7
MX3030	A1	253.9 ± 12.6	230.1 ± 10.8
MX3035	A1	182.4 ± 9.3	179.8 ± 4.8
MX3124	A1	301.6 ± 14.1	279.8 ± 10.9
MX3249	A1	247.8 ± 9.9	233.1 ± 12.0
MX3253	A1	299.5 ± 23.9	248.4 ± 12.0
MX3381	A1	269.7 ± 77.4	195.5 ± 43.9
SC3171	C	118.3 ± 10.5	272.4 ± 17.1
SC3195	C	157.5 ± 6.6	283.0 ± 15.3
SC3406	C	175.2 ± 20.3	241.8 ± 72.1
FL3420	C	225.4 ± 14.1	167.6 ± 9.0
FL3427	C	266.8 ± 7.5	207.9 ± 6.7
SC3536	C	107.1 ± 7.7	329.5 ± 16.3
CT3175	B	n.t.	626.3 ± 13.5
NJ2881	B	n.t.	522.9 ± 10.9
NY3449	B	n.t.	555.1 ± 14.5
NY3450	B	n.t.	431.3 ± 7.8
VA3148	B	n.t.	472.3 ± 18.1
VA3228	B	n.t.	632.7 ± 33.6
NC3115	B	n.t.	510.4 ± 9.8
NC3116	B	n.t.	471.5 ± 19.5
SC3409	B	n.t.	461.7 ± 83.5
SC3410	B	n.t.	459.0 ± 20.5
SC3411	B	n.t.	411.4 ± 16.5
GA3418	B	30.6 ± 3.3	350.1 ± 17.0
TX3492	B	n.t.	599.2 ± 64.6
GA3094	D	156.5 ± 20.0	193.9 ± 5.3
FL3419	D	394.2 ± 37.3	284.5 ± 17.0
FL2928	D	354.9 ± 7.5	278.7 ± 4.9
FL3416	E	324.9 ± 12.5	265.1 ± 11.4
VZ3136	VZ1	368.1 ± 13.8	277.1 ± 13.7
VZ3142	VZ2	344.4 ± 17.8	258.2 ± 6.8
PE3043	PE	299.6 ± 1.5	275.8 ± 3.2
BZ3017	BZ	255.9 ± 22.2	225.4 ± 19.3
MY3845	n.a.	204.9 ± 30.8	150.3 ± 34.2
M3818.NC	n.a.	176.4 ± 21.1	116.4 ± 26.5

For further descriptions of isolates, see Table 1. Polyol concentration (μmol·g dry weight^{-1}) ± SD. n.t., no trace.

TABLE 5. Cross results between isolates of *Bostrychia radicans*.

RUBISCO			B	B	C	C	D	D	A1
	*cox*2-3		Y	T	S	R	N	L	Z1
		Isolate	SC3409	TX3492	SC3406	FL3420	FL3419	FL2928	MX3124
B	Y	SC3409	+	+P	−P	−	−P	−P	−P
B	T	TX3492	+P	+	−P	−	−P	−	−P
C	S	SC3406	−P	−P	+P	+P	−	−	−P
C	R	FL3420	+P	−	+P	+	−	−	+P
D	L	FL2928	−P	−	−	−	+P	+	−
A1	Z1	MX3124	−P	−P	−P	+	−	−	+P

Isolate designations in Table 1. RUBISCO spacer and *cox*2-3 spacer haplotypes (see Table 1) shown in first and second row/column, respectively. Females, horizontal rows; males, vertical columns; +, cystocarps produced, carpospores released and germinated; −, no cystocarps produced; P, pseudocystocarps produced.

reproductively compatible populations or morphological homogeneity. Morphologically distinguishable *Porphyra* species have been shown to differ by only a single nucleotide in the RUBISCO spacer (Brodie et al. 1996). Although *B. radicans* samples are morphologically indistinguishable, we find that samples that differ by as little as 1 bp in the RUBISCO spacer can be partially reproductively incompatible. The only successful cross (syngamy and carposporophyte development) between plastid haplotypes in lineage 5 is between haplotypes A (Pacific) and C (Atlantic), and even in this cross tetrasporophytes were reproductively sterile (aborted tetrasporangia). So plastid haplotypes seem to correlate well with reproductive isolation. This was also seen in the red algae *Spyridia filamentosa* (Wulfen) Harvey in which the only compatible isolates were ones with similar plastid haplotypes (Zuccarello et al. 2002). A possible reason for this link between a neutral plastid marker and reproductive compatibility is that evolutionary changes (mutation rate) in this marker have a similar rate to changes in mating compatibility loci (which are completely unknown in red algae).

Reproductive isolation has been used as one of the characters in defining species, and certainly reproductively isolated populations will follow distinct evolutionary trajectories (i.e. respond independently to selective forces). So does the list of possible cryptic species increase, with samples within lineages (e.g. haplotypes B and C) being also considered cryptic species? Certainly these "evolutionary units" must be appreciated in questions of biodiversity and in ecological studies, because it has been shown that these molecularly distinguishable groups (cryptic species) can be physiologically different (Zuccarello et al. 2001).

It could be assumed that two north–south coastlines of similar latitudinal spread (Pacific and Atlantic North America) containing the same species would have similar levels of biodiversity. Two *B. radicans* plastid and mitochondrial haplotypes (plastid haplotypes A1 and A2, mitochondrial haplotypes Z1 and Z2) were found along the entire coast of Pacific Mexico to central Guatemala. Four plastid haplotypes and 11 mitochondrial haplotypes were found on the Atlantic coast of the United States. These Atlantic haplotypes were found in two separate phylogenetic lineages. This genetic uniformity along Pacific Mexico is also reflected in crossability of isolates in which all isolates from Pacific Mexico hybridize, but this is not the case from the Atlantic USA (Zuccarello and West 1995; this study). These data indicate differing underlying levels of diversity in these two areas. This could be due to either 1) a recent origin of this species along the North American Pacific coast, probably from a small founder population from the Atlantic, and a rapid range expansion, at least from Guatemala to northern Mexico (approximately 3000 km), probably following climatic changes after the last glacial maximum (Pleistocene); or 2) spread of a selectively favored (selective sweep, Maynard-Smith and Haigh 1974) mitochondrial and plastid haplotype, and any other maternally inherited-hitchhiking elements, possibly adapted to this more northerly colder environment. The genetic fingerprint of a rapid expansion of a small founder population, under neutrality, and a selective sweep to fixation in a constant-size population can be similar (Avise 2000). We presently favor the former explanation as 1) northward range expansions, because the Pleistocene have been reported in other marine organisms from the eastern Pacific (Hellberg et al. 2001), or 2) the complete sexual compatibility of all isolates tested from this area indicates a close nuclear (assuming a multilocus nuclear control of reproductive compatibility) homogeneity of isolates. Further study of various nuclear loci (e.g. microsatellites) may be able to unravel the levels of genetic variation found in these North American Pacific populations. Although it is known that samples from Pacific South America (Peru, PE3043) belong to a different genetic lineage, further sampling between this locality and the present samples will give insight into whether this low genetic variation is found along the entire Pacific central American coast and possibly the origin of the northern samples.

The phylogeographic pattern of genetic variants along the Atlantic USA coast is also interesting (Fig. 7). North of North Carolina only one plastid haplotype is found (B). It is possible that range expansion also occurred along this coast as waters warmed after the last glacial maximum. Haplotype B is very different from the other haplotypes in lineage 5 based on *cox*2-3 spacer sequence. A long genetic separation between the two groups is likely, with the range expansion following climatic changes. Haplotype B may be more tolerant of cold temperatures, although this needs to be investigated.

Lineage 6 samples from North America are distantly related to lineage 5 samples, though occasionally they are found sympatrically (Fort Pierce, Florida). The origin of these North American samples is as yet unresolved, although it appears that samples from South America are closely related. Further sampling with the Gulf of Mexico, including the Caribbean, is needed.

Within each plastid haplotype group that contained more than one sample, there are at least two mitochondrial haplotypes indicating that mutations in the *cox*2-3 spacer are more frequent than in the plastid spacer, as has been shown previously (Zuccarello et al. 1999a, Zuccarello and West 2002). The *cox*2-3 spacer, therefore, reveals a more detailed phylogenetic history than can be gathered from the plastid spacer. This has bearing on studies of cryptic variation and population studies in marine algae, because different markers will give different insights into levels of variation within and between geographic regions and populations. Although the plastid haplotypes often correlate with reproductively isolated samples, the mitochondrial haplotypes do not. For the most part samples with different mitochondrial haplotypes, within a plastid haplotype,

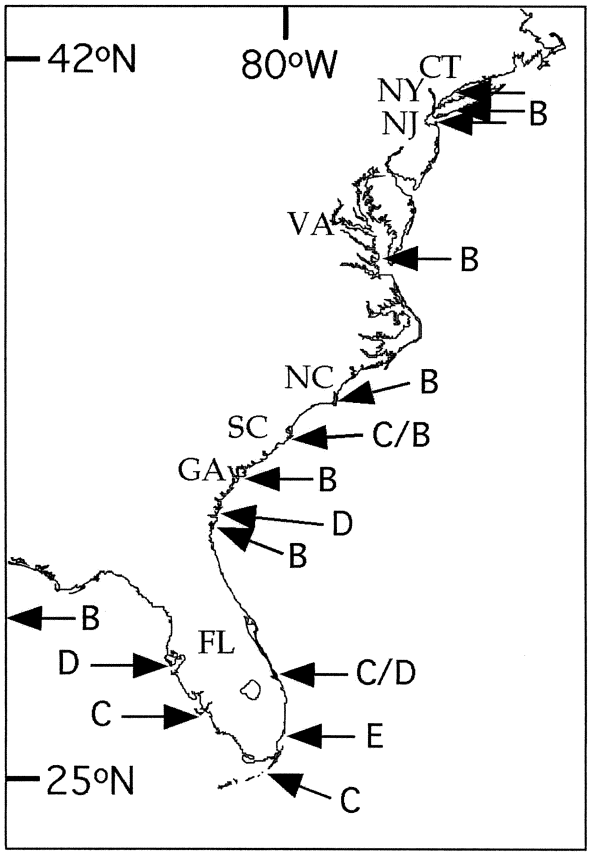

FIG. 7. Map of the eastern United States showing location and position of plastid haplotypes.

hybridize. This seems to be less the case with samples within plastid haplotype B, which contains the most mitochondrial diversity, as often these samples do not hybridize. Crossability experiments must be taken with caution as results can differ, over trials and over the years, though in this case general trends are relatively clear cut. To understand population genetic structure of this species in Atlantic North America the *cox*2-3 spacer would be more fruitful that the RUBISCO spacer (Zuccarello et al. 2001), and such a study is underway.

The plastid haplotype groups are also correlated with the compatible solute (polyol) data. Most samples produce both sorbitol and dulcitol, common in *B. radicans* and *B. moritziana* from around the world (Karsten et al. 1992). Only plastid haplotype B samples have only sorbitol. Though compatible solute production has been used as a chemotaxonomic marker (Karsten et al. 1999), many cases of different compatible solutes in the same species have been found (Karsten et al. 1995, 1999), and in a few rare cases isolates in culture have been known to "switch" from producing only one polyol to producing both (Karsten et al. 1994). Differences in compatible solute production have also been shown to correlate with plastid haplotypes of *Bostrychia tenuissima* from eastern Australia (Zuccarello et al. 1999c). In that study all samples of the same haplotype, which only produced sorbitol, were found mainly in southern populations (colder habitats). A similar situation is seen in this study with sorbitol-producing samples (plastid haplotype B) found in the more northerly regions, indicating that the dulcitol enzymatic pathway may be more sensitive to colder environmental conditions or that dulcitol is less effective, as a compatible solute, at colder temperatures (Karsten et al. 1994, 1995). Interestingly, the most southerly sample with haplotype B (GA3418, from Jekyll Island, GA, USA) produces both compatible solutes, again suggesting a genetic–environment interaction in polyol production. Factors such as the physiological effect of compatible solutes on growth and reproduction in different environments, sensitivity of enzymatic pathways to environmental changes must be further investigated where different haplotypes, with different polyols, are found in the same locality (e.g. James Island, SC, USA).

In conclusion, our data show that specimens with a *B. radicans/B. moritziana* morphology are found in seven distinct lineages, showing high levels of genetic variation but morphological stasis, and support the contention that this complex consists of at least seven cryptic species. Samples from North America are mainly found in two lineages, with samples reproductively isolated between lineages. The levels of genetic (organellar) diversity of the species are not comparable between the Atlantic coast of North America and Pacific North America, with higher levels in the Atlantic, indicating a markedly different evolutionary/biogeographic history of the *B. radicans/B. moritziana* floras. Samples within lineage 5, from North America, with different plastid haplotypes are also reproductively isolated showing that, in this case, there is a good correlation between plastid haplotypes and reproductive isolation. These data would indicate that these reproductively isolated samples could also be considered biological species and increases the number of cryptic species within this well-studied red algal species complex.

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Avise, J. C. 2000. *Phylogeography: the History and Formation of Species.* Harvard University Press, Cambridge, MA, 447 pp.

Bremer, K. 1988. The limits of amino acid sequence data in angiosperm phylogenetic reconstruction. *Evolution* 42:795–803.

Brodie, J., Hayes, P. K., Barker, G. L. & Irvine, L. M. 1996. Molecular and morphological characters distinguishing two *Porphyra* species (Rhodophyta, Bangiophycidae). *Eur. J. Phycol.* 31:303–8.

Coyer, J. A., Peters, A. F., Hoarau, G., Stam, W. T. & Olsen, J. L. 2002. Inheritance patterns of ITS1, chloroplasts and mitochon-

dria in artificial hybrids of the seaweeds *Fucus serratus* and *F. evanescens* (Phaeophyceae). *Eur. J. Phycol.* 37:173–8.
Eriksson, T. 1998. AutoDecay, version 4.0 (program distributed by the author). Bergius Foundation, Royal Swedish Academy of Sciences, Stockholm.
Felsenstein, J. 1985. Confidence intervals on phylogenies: an approach using the bootstrap. *Evolution* 39:783–91.
Gabrielson, T. M., Brochmann, C. & Rueness, J. 2003. Phylogeny and interfertility of North Atlantic populations of "*Ceramium strictum*" (Ceramiales, Rhodophyta): how many species? *Eur. J. Phycol.* 38:1–13.
Guiry, M. D. 1992. Species concepts in marine red algae. *In* Round F. E. & Chapman D. J. [Eds.] *Progress in Phycological Research.* Biopress Ltd., Bristol, pp. 251–78.
Harrison, R. G. 1998. Linking evolutionary pattern and process. *In* Howard D. J. & Berlocher S. H. [Eds.] *Endless Forms: Species and Speciation.* Oxford University Press, Oxford, pp. 19–31.
Hasegawa, M., Kishino, K. & Yanno, T. 1985. Dating the human-ape splitting by a molecular clock of mitochondrial DNA. *J. Mol. Evol.* 22:160–74.
Hellberg, M. E., Balch, D. P. & Roy, K. 2001. Climate-driven range expansion and morphological evolution in a marine gastropod. *Science* 292:1707–10.
Karsten, U., Bock, C. & West, J. A. 1995. Low molecular weight carbohydrate patterns in geographically different isolates of the eulittoral red alga *Bostrychia tenuissima* from Australia. *Bot. Acta* 108:321–6.
Karsten, U. & Kirst, G. 1989. The role of inorganic ions and D-sorbitol in the osmotic adjustment of the estuarine red alga *Bostrychia radicans* Mont. (Ceramiales, Rhodomelaceae). *Crypto. Bot.* 1:226–9.
Karsten, U., Thomas, D. N., Weykam, G., Daniel, C. & Kirst, G. O. 1991. A simple and rapid method for extraction and separation of low molecular weight carbohydrates from macroalgae using high-performance liquid chromatography. *Plant Physiol. Biochem.* 29:373–8.
Karsten, U., West, J. A. & Ganesan, E. K. 1993. Comparative physiological ecology of *Bostrychia moritziana* (Ceramiales, Rhodophyta) from freshwater and marine habitats. *Phycologia* 32:401–9.
Karsten, U., West, J. A. & Zuccarello, G. C. 1992. Polyol content of *Bostrychia* and *Stictosiphonia* (Rhodomelaceae, Rhodophyta) from field and culture. *Bot. Mar.* 35:11–9.
Karsten, U., West, J. A., Zuccarello, G. C. & Kirst, G. O. 1994. Physiological ecotypes in the marine alga *Bostrychia radicans* (Ceramiales, Rhodophyta) from the east coast of the USA. *J. Phycol.* 30: 174–82.
Karsten, U., West, J. A., Zuccarello, G. C., Nixdorf, O., Barrow, K. D. & King, R. J. 1999. Low molecular weight carbohydrate patterns in the Bangiophyceae (Rhodophyta). *J. Phycol.* 35:967–76.
Kimura, M. 1981. Estimation of evolutionary distance between homologous nucleotide sequences. *Proc. Natl. Acad. Sci. USA* 78:454–8.
King, R. J. & Puttock, C. 1989. Morphology and taxonomy of *Bostrychia* and *Stictosiphonia* (Rhodomelaceae/Rhodophyta). *Aust. Syst. Bot.* 2:1–73.
Knowlton, N. 1993. Sibling species in the sea. *Annu. Rev. Ecol. Syst.* 24:189–216.
Lessa, A. P. & Applebaum, G. 1993. Screening techniques for detecting allelic variation in DNA sequences. *Mol. Ecol.* 2:119–29.
Maynard-Smith, J. & Haigh, J. 1974. The hitch-hiking effect of a favourable gene. *Gen. Res.* 23:23–35.
Mayr, E. 1942. *Systematics and the Origin of Species from the Viewpoint of a Zoologist.* Columbia University Press, New York, 334 pp.
McIvor, L., Maggs, C. A., Provan, J. & Stanhope, M. J. 2001. *rbcL* sequences reveal multiple cryptic introductions of the Japanese red alga *Polysiphonia harveyi. Mol. Ecol.* 10:911–9.
Palumbi, S. R. 1994. Genetic divergence, reproductive isolation, and marine speciation. *Annu. Rev. Ecol. Syst.* 25:547–72.
Pedroche, F. F., West, J. A., Zuccarello, G. C., Sentíes, G. & Karsten, U. 1995. Marine red algae of the mangroves in South Pacific México and Pacific Guatemala. *Bot. Mar.* 38:111–9.
Posada, D. & Crandall, K. A. 1998. Modeltest: testing the model of DNA substitution. *Bioinformatics* 14:817–8.
Reeb, C. A. & Avise, J. C. 1990. A genetic discontinuity in a continuously distributed species: mitochondrial DNA in the American oyster, *Crassostera virginica. Genetics* 124:397–406.
Sarver, S. K., Landrum, M. C. & Foltz, D. W. 1992. Genetics and taxonomy of ribbed mussels (*Geukensia* spp.). *Mar. Biol.* 113: 385–90.
Sunnucks, P., Wilson, A. C. C., Beheregaray, L. B., Zenger, K., French, J. & Taylor, A. C. 2000. SSCP is not so difficult: the application and utility of single-stranded conformation polymorphism in evolutionary biology and molecular ecology. *Mol. Ecol.* 9:1699–710.
Swofford, D. L. 2002. *PAUP*. Phylogenetic Analysis Using Parsimony (*and Other Methods).* Version 4. Sinauer Associates, Sunderland, MA.
Thompson, J. D., Gibson, T. J., Plewniak, F., Jeanmougin, F. & Higgins, D. G. 1997. The Clustal X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acids Res.* 24:4876–82.
van der Strate, H. J., Boele-Bos, S. A., Olsen, J. L., van de Zande, L. & Stam, W. T. 2002a. Phylogeographic studies in the tropical seaweed *Cladophoropsis membranacea* (Chlorophyta, Ulvophyceae) reveal a cryptic species complex. *J. Phycol.* 38:572–82.
van der Strate, H. J., van de Zande, L., Stam, W. T. & Olsen, J. L. 2002b. The contribution of haploids, diploids and clones to fine scale population structure in the seaweed *Cladophoropsis membranacea* (Chlorophyta). *Mol. Ecol.* 11:329–45.
West, J. A. & Calumpong, H. P. 1988. Mixed-phase reproduction of *Bostrychia* (Ceramiales, Rhodophyta) in culture. I. *B. tenella* (Lamouroux) J. Agardh. *Jpn. J. Phycol.* 36:292–310.
West, J. A., Zuccarello, G. C., Pedroche, F. F. & Karsten, U. 1993. Marine red algae of the mangroves in Pacific Mexico and their polyol content. *Bot. Mar.* 35:567–72.
West, J. A. & Zuccarello, G. C. 1999. Biogeography of sexual and asexual populations in *Bostrychia moritziana* (Rhodomelaceae, Rhodophyta). *Phycol. Res.* 47:115–23.
Zuccarello, G. C., Bartlett, J. & Yeates, P. H. 2000. Differentiation of *Caloglossa leprieurii* (Rhodophyta) populations in northern and eastern Australia using plastid haplotypes. *Eur. J. Phycol.* 35: 357–63.
Zuccarello, G. C., Burger, G., West, J. A. & King, R. J. 1999a. A mitochondrial marker for red algal intraspecific relationships. *Mol. Ecol.* 8:1443–7.
Zuccarello, G. C., Sandercock, B. & West, J. A. 2002. Diversity in red algal species: variation in world-wide samples of *Spyridia filamentosa* (Ceramiaceae) and *Murrayella periclados* (Rhodomelaceae) using DNA markers and breeding studies. *Eur. J. Phycol.* 37:403–17.
Zuccarello, G. C. & West, J. A. 1995. Hybridization studies in *Bostrychia.* 1. *B. radicans* (Rhodomelaceae, Rhodophyta) from Pacific and Atlantic North America. *Phycol. Res.* 43:233–40.
Zuccarello, G. C. & West, J. A. 1997. Hybridization studies in *Bostrychia.* 2. Correlation of crossing data and plastid DNA sequence data within *B. radicans* and *B. moritziana* (Ceramiales, Rhodophyta). *Phycologia* 36:293–304.
Zuccarello, G. C. & West, J. A. 2002. Phylogeography of the *Bostrychia calliptera/B. pinnata* complex (Rhodomelaceae, Rhodophyta) and divergence rates based on nuclear, mitochondrial and plastid DNA markers. *Phycologia* 41:49–60.
Zuccarello, G. C., West, J. A., Kamiya, M. & King, R. J. 1999b. A rapid method to score plastid haplotypes in red seaweeds and its use in determining parental inheritance of plastids in the red alga *Bostrychia* (Ceramiales). *Hydrobiologia* 401:207–14.
Zuccarello, G. C., West, J. A., Karsten, U. & King, R. J. 1999c. Molecular relationships within *Bostrychia tenuissima* (Rhodomelaceae, Rhodophyta). *Phycol. Res.* 47:81–5.
Zuccarello, G. C., West, J. A. & King, R. J. 1999d. Evolutionary divergence in the *Bostrychia moritziana/B. radicans* complex (Rhodomelaceae, Rhodophyta): molecular and hybridization data. *Phycologia* 38:234–44.
Zuccarello, G. C., Yeates, P. H., Wright, J. T. & Bartlett, J. 2001. Population structure and physiological differentiation of haplotypes of *Caloglossa leprieurii* (Rhodophyta) in a mangrove intertidal zone. *J. Phycol.* 37:235–44.