

INCREASED SAMPLING FOR INFERRING PHYLOGEOGRAPHIC PATTERNS IN *BOSTRYCHIA RADICANS*/*B. MORITZIANA* (RHODOMELACEAE, RHODOPHYTA) IN THE EASTERN USA¹

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Zuccarello and West (2003) reported on the phylogenetic diversity of algae identified as *Bostrychia radicans* (Montagne) Montagne and *B. moritziana* (Sonder ex Kützing) J. Agardh from around the world. They showed that the species complex consisted of seven distinct lineages, of which two lineages were common on the East Coast of the USA and eastern Gulf of Mexico. The distribution of haplotypes within these lineages on the East Coast of the USA showed a general north–south distribution. One haplotype of lineage 5 (B) was mostly collected in northern areas, while the other common haplotype (C) was more southerly in distribution. Samples in lineage 6 (haplotype D) were not found north of Sapelo Island, Georgia. Increased sampling from the eastern USA over 5 years later has revealed an altered pattern. Haplotype D is distributed in North Carolina and is common in some populations. Haplotype C is rare or absent in many sampled populations. Haplotype B is only observed in the northern sampled sites on both sides of the Florida peninsula. This disjunct distribution agrees with geological scenarios for a strait between the western Gulf of Mexico and southern Georgia in the Miocene/Pliocene, which closed in the late Pliocene. This paper highlights the importance of increased sampling to determine phylogeographic patterns and hypotheses of dispersal scenarios in algae.

Key index words: Atlantic USA; *Bostrychia moritziana*; *Bostrychia radicans*; genetic diversity; phylogeography; Rhodomelaceae; RUBISCO spacer

The understanding of species distribution at a global and local scale is important and the cataloging of species in particular locations can give us clues into the biodiversity of regions and indicate “biodiversity hot spots.” An appreciation of the distribution of genetic diversity can lead to insights into the history of

species and speciation processes (Avice 2000, Coyne and Orr 2004).

Research on the pattern of seaweed distribution around the world has been an ongoing enterprise since seaweed studies began (Lüning 1990). With the advent of molecular methods (e.g. DNA sequencing) and improved methodologies (e.g. cladistics), the distribution of genotypes (genetic variants) has led to the possibility of understanding historical patterns of seaweed movement on a species basis. More localized patterns of genetic diversity (population structure) have been studied in seaweeds using various types of markers. These studies are becoming more common and show that even for organisms (seaweeds) that release spores into the water column, populations can be very structured at small spatial scales (Faugeron et al. 2001, Zuccarello et al. 2001). This means that even in the “same” location (meters or tens of meters apart), samples can be genetically very different. Sampling, therefore, must be more intensive, even for studies that do not address local population structure but are interested in larger-scale patterns (e.g. phylogeography), as genotypes can be easily missed in such structured populations.

Zuccarello and West (2003) reported on the phylogenetic diversity of algae identified as *Bostrychia radicans* and *B. moritziana* from around the world. Using two organellar markers, the plastid-encoded RUBISCO spacer and the mitochondria-encoded *cox2-3* spacer, they showed that the species complex consisted of seven distinct lineages, not completely corresponding to morphological species groups. These two markers gave completely congruent phylogenetic patterns. Some lineages were collected in disparate locations around the world, and some were more localized (e.g. Atlantic Ocean for lineage 3). Zuccarello and West (2003) showed that two lineages were common on the East Coast of the USA (lineages 5 and 6). Plants from these two lineages were reproductively incompatible, suggesting cryptic species. Interestingly, even different haplotypes within the same lineages were reproductively incompatible, or partially incompatible. All these data suggest that diversity is seriously

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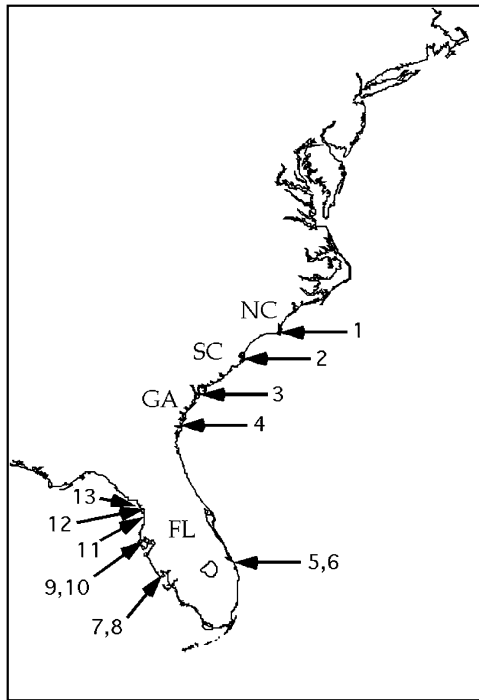


FIG. 1. Distribution of sampled locations along the southeastern USA. Numbers refer to populations in Table 1.

underrepresented within this species complex when based solely on morphological criteria.

The distribution of haplotypes also showed a north-south distribution. Samples of haplotype B within lineage 5 were from Connecticut to southern Georgia (Jekyll Island), plus a single sample from Texas. Samples of haplotype C within lineage 5 were very common in South Carolina and extended along the east coast of Florida as well as to Marco Island on the west coast. Samples of haplotype D within lineage 6 were only found in the southern distribution of this region (Sapelo Island, Georgia, to Clearwater, western Florida). Although the sampling in Zuccarello and West (2003) was widespread along the East Coast of the

USA, sample sizes from individual locations were small (one or two), and samples were missing from northwestern Florida.

Sampling was increased in June 2003, approximately 9 years after most of the samples from these areas were collected by Zuccarello and West (2003). Samples were collected haphazardly from locations (Table 1, Fig. 1), with at least a meter between each sample. At two sites, samples were collected in two different environments. At location 1 (Fort Fisher), samples were taken from the river's edge and also about 2–3 m back from the river's edge in saltgrass. In location 2 (Grice Marine Station), samples were collected on two different substrates (rock and *Spartina*) on either side of the point containing the marine station. RUBISCO spacer sequences were obtained from 131 silica-gel-dried individual plants (mean 10 samples per site; Zuccarello et al. 1999). Analysis methods followed Zuccarello and West (2003).

These new data (Table 1) show patterns that were not evident in the previous study (Zuccarello and West 2003). Two new haplotypes related to haplotype B were discovered (designated F and G, each one base pair different from haplotype B; Fig. 2). Haplotype C, which was collected at most sites in the southern half of the sampling range in Zuccarello and West (2003), was missing or rare in this new survey (e.g. haplotype C was one of two haplotypes collected at James Island in 1994, while in this study it was found once in 18 samples). Haplotype C was only common at Bonita Beach, Fort Myers, Florida (43%). Haplotype D was common all the way to the most northern site in this survey (22% of Fort Fisher, North Carolina, samples; 61% of James Island, South Carolina, samples); this is in contrast to it not being found north of southern Georgia before. Sampling from northwestern Florida, northeastern Gulf of Mexico, shows haplotype B to be common (28% of samples from locations 11–13), while previously it was collected only in Georgia and areas north, and a lone sample in the western Gulf of Mexico (Texas). Haplotype B was still not found in more southern areas of Florida, on either coast (locations 5–10).

The differences, changes in the distribution of haplotypes of *B. radicans*/*B. moritziana*, between this study and Zuccarello and West (2003) could be due to two, not mutually exclusive, possibilities. The difference is due to increased sampling intensity, or the range and frequency of these haplotypes has changed in the intervening 9 years between sampling effort.

It is clear that sampling effort must be intensified for any meaningful conclusions to be made about phylogeographic patterns. Although Zuccarello and West (2003) sampled more widely and possibly more intensely than other such studies, the inadequacy of their sampling effort to obtain accurate estimates of haplotype distribution is clear. The known patchiness of red algal genotypes has been documented (Zuccarello et al. 2001); this means that sampling one or few samples from a restricted area may give an inaccurate sample of the diversity in the area.

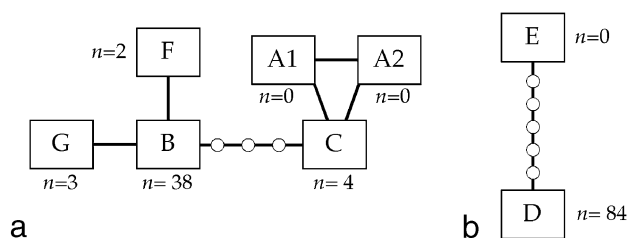


FIG. 2. Statistical parsimony network of RUBISCO spacer haplotypes. n = sample numbers. Lines indicate one base pair difference, circles represent missing haplotypes. (a) Lineage 5 samples (haplotypes A1 and A2 have only been found from Pacific Mexico to date—Zuccarello and West 2003), (b) lineage 6 haplotypes from the East Coast of the USA (haplotype E was not encountered in this study but has been found in southern Florida—Zuccarello and West 2003).

TABLE 1. Sample sites and RUBISCO spacer haplotype frequency along the East Coast of the USA and western Florida.

	Location, collection date	Latitude/longitude	N	Haplotype frequency	Additional information
1a	Fort Fisher, Cape Fear Estuary, NC, June 26, 2003	33.53N, 78.00W	9	B = 0.67 D = 0.33	Back from river's edge
1b			9	B = 0.89 D = 0.11	River's edge
2a	Grice Marine Station, James Island, SC, June 27, 2003	32.45.132N, 79.53.927W	11	B = 0.09 C = 0.09 D = 0.82	On rocks, south side
2b			7	B = 0.71 D = 0.29	On <i>Spartina</i> , northside
3	Dahwoo River, SC, June 27, 2003	32.38.125N, 80.20.451W	10	B = 0.6 D = 0.3 F = 0.1	On <i>Spartina</i>
4	Fort Pulaski, Tybee Island, GA, June 27, 2003	32.01.224N, 80.53.943W	8	B = 0.25 D = 0.63 F = 0.12	On <i>Spartina</i>
5	North Bridge, Fort Pierce, FL, June 28, 2003	27.28.705N, 80.18.780W	12	D = 1.00	On mangroves
6	Jensen Beach, FL, June 28, 2003	27.14.819N, 80.13.579W	9	D = 1.00	On <i>Rhizophora</i>
7	Bonita Beach, Fort Myers, FL, June 29, 2003	26.21.654N, 81.51.455W	7	C = 0.43 D = 0.57	On mangroves
8	Sanibel Island, Fort Myers, FL, June 29, 2003	26.28.092N, 82.03.464W	4	D = 1.00	On <i>Rhizophora</i> , exposed open water
9	Safety Harbor, Tampa Bay, FL, June 29, 2003	28.00.829N, 82.40.973W	8	D = 1.00	Small turf
10	Courtney Campbell causeway, Tampa Bay, June 29, 2003	27.58.301N, 82.36.741W	8	D = 1.00	Big plants
11	Bayport, FL, June 30, 2003	28.32.062N, 82.39.008W	5	B = 1.00	On rock and grass
12	Ozello, Homosassa Springs, FL, June 30, 2003	28.52.075N, 82.39.908W	12	B = 0.42 D = 0.33 G = 0.25	12 psu, on <i>Rhizophora</i>
13	Cedar Key, FL, June 30, 2003	29.08.461N, 83.02.251W	12	D = 1.00	On <i>Avicennia</i>
		Total	131		

Sampling location and date of collection. Latitude and longitude of site. Haplotype frequency (haplotypes B,C, F, G in lineage 5; haplotype D in lineage 6, see Zuccarello and West (2003) for more details). Environment of collection given as additional information, and other observations also listed.

If this sampling effort does reflect more accurately the distribution of genotypes in the eastern USA, two patterns emerge that are unusual. The first is the disjunct distribution of haplotype B. This haplotype is only found in the eastern USA from southern Georgia north (locations 1–4) and on the northwest coast of Florida (locations 11–12), while it is not observed in southern Florida. This, coupled with the previous data from Zuccarello and West (2003), where this haplotype was collected in the western Gulf of Mexico (one sample from Texas) and in all sites north of North Carolina, would suggest that this distribution is accurate. Data from this study and Zuccarello and West (2003) indicate that haplotype B is not collected in the lower Florida Peninsula. This unusual disjunct distribution has been reported for other marine organisms in this area (Bert 1986), and a geological explanation has been hypothesized. During the Miocene, a channel (variously called the Okefenokee Trough or Suwannee Seaway) separated partially submerged Florida from the rest of North America (Bandy and Lanier 1968, Olsen 1968). This strait slowly filled during the Miocene and early Pliocene (Brooks 1966), although it was believed to be reopened during the middle to late Pliocene (Winker and Howard 1977, Cronin et al. 1981). This passageway, during the late Pliocene or

possibly Miocene, would have facilitated dispersal along the north coast of the strait from the western Gulf of Mexico to the Georgia coast. The strait could have been a barrier to dispersal from mainland North America to the Florida Island and *vice versa*. Final closure of this Suwannee Seaway would have isolated western Gulf and eastern North American samples.

This scenario would also indicate that dispersal of haplotype B samples down into southern Florida has not occurred during the intervening millions of years. This could be possible if suitable habitats (mangroves) were already occupied by other *B. radicans*/*B. moritziana* lineages, or if the physiology of haplotype B samples is poorly adapted to more southerly habitats. Physiological differences are known to exist between different haplotypes of mangrove algae (Zuccarello et al. 2001) and have been shown in some of the *B. radicans*/*B. moritziana* samples collected from these locations (Karsten et al. 1994).

The second pattern that is different between this study and that of the Zuccarello and West (2003) is the increased distribution of haplotype D to more northern locations and the absence of haplotype C from many locations compared with the 2003 study. This difference again could be due to the low sampling effort in the 2003 study or a change in the abundance

and distribution of haplotypes in the intervening years. Haplotype D, which has close affinities to samples from South America (Zuccarello and West 2003), could have dispersed along the eastern USA possibly following the Atlantic Ocean Gulf Stream in the intervening years. More difficult to explain is the apparent absence, or low frequency, of haplotype C from areas where it was previously collected (e.g. location 2). This could be explained partly by the patchy nature of algal distribution and that we may have missed areas where haplotype C is present. It is also possible that climatic or even stochastic events have led to a reduction in the abundance of haplotype C in many locations.

Our new data set presents a different picture of the phylogeographic history of *B. radicans*/*B. moritziana* from the eastern USA from that of previous studies. Our data can be interpreted by evoking the paleogeographic history of Florida. This study highlights the importance of intensive sampling to properly address phylogeographic questions in algal biology.

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