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Mitochondrial DNA and Color Pattern Variation in Three Western Atlantic *Halichoeres* (Labridae), with the Revalidation of Two Species

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Genetic surveys of widely distributed marine species often find previously undetected biodiversity. In the present study, populations of three species of *Halichoeres* were sampled across their entire geographical ranges: *Halichoeres cyanocephalus* and *Halichoeres maculipinna* were sampled on both sides of the Amazon freshwater outflow, the main biogeographic barrier in the tropical western Atlantic; and *Halichoeres garnoti* was sampled in the Caribbean and Bermuda. Genetic divergences between populations separated by the Amazon ranged from 2.3% in *H. cyanocephalus* to 6.5% in *H. maculipinna*. There is inconsistency between color differences and genetic partitions in the species surveyed. The color differences between populations of *H. cyanocephalus* and *H. maculipinna* correspond to deep genetic partitions at the cytochrome *b* locus. However, genetic similarity at this same locus was observed between populations of *H. garnoti* with striking color differences. Based on the combination of the observed genetic differences with diagnostic color differences, the Brazilian species *Halichoeres dimidiatus* (Agassiz) and *Halichoeres penrosei* Starks, 1913 are revalidated. In addition, a neotype is designated to *H. cyanocephalus* (Bloch, 1791), to clarify its taxonomic status and type locality. All species analyzed have a similar larval dispersal potential, but varying degrees of genetic divergences were observed, indicating that benthic stage ecology may also play a role in speciation in this group.

THE presence of potentially widely dispersing planktonic larva in most marine fishes has led to the assumption that such species are composed of genetically homogeneous populations (Warner, 1997). However, recent studies on larval behavior (Leis and McCormick, 2002) and oceanographic processes (Cowen, 2002) indicate that larvae often disperse far less than their potential. Moreover, habitat selection by larvae can also overcome the effects of long-distance dispersal and give rise to genetic differences in geographically close populations (Bierne et al., 2003). Consequently, genetic studies of widely distributed taxa often reveal the presence of previously overlooked species (Knowlton, 2000).

Early genetic surveys within the Caribbean region found no evidence of cryptic speciation in reef fishes (Shulman and Bermingham, 1995). However, when broader geographic areas were sampled, analyses of DNA variation demonstrated the presence of deeply divergent evolutionary partitions in several species, such as bonefishes (Colborn et al., 2001), blennies (Muss et al., 2001), surgeonfishes (Rocha et al., 2002), groupers (Carlin et al., 2003), and wrasses (Rocha, 2003a). Some of these genetic divergences are accompanied by slight color differences.

In reef fish taxonomy, color pattern is consistently used as a useful diagnostic character. When color differences are observed between populations that also show slight morphological differences, those populations are usually elevated to specific status (Randall, 1998). However, color alone is rarely used as a character to define species, especially in groups with highly variable intraspecific color patterns such as wrasses (Labridae). Despite the presence of bright color patterns and their wide variation observed in reef fishes, little is known about its evolutionary significance (McMillan et al., 1999). Sexual selection related to coloration appears to be present in damselfishes (Pomacentridae; Thresher and Moyer, 1983), but no correlation between mating success and color pattern was observed in the blue-head wrasse (Labridae; Warner and Schultz, 1992). In closely related species, coloration has been proposed to be useful in mate recognition. The sharpnose pufferfishes are an example of a group with little or no diagnostic external morphological characters, where color has been proposed as important in speciation and is routinely used as a species defining character (Allen and Randall, 1977; Moura and Castro, 2002).

Wrasses of the genus *Halichoeres* in the western Atlantic provide an excellent system to examine the relationship between genetic structure and color differentiation. The populations surveyed in this study are present on both sides of recognized biogeographic barriers, have a similar dispersal potential (pelagic larval phase

of 25–30 days; Sponaugle and Cowen, 1997; Wellington and Robertson, 2001) and exhibit slight color differences. Populations of *Halichoeres garnoti* in the Caribbean and Bermuda are separated by at least 1400 km of open-ocean (Smith-Vaniz et al., 1999), and populations of *Halichoeres cyanocephalus* and *Halichoeres maculipinna* in Brazil and the Caribbean are separated by the Amazon barrier (Joyeux et al., 2001; Rocha, 2003b). These populations have been considered to be the same species, but they show consistent color differences in three instances: (1) the juveniles of *H. cyanocephalus* in Brazil lack a blue spot on the midventral portion of the soft dorsal fin, which is present on juveniles of the Caribbean population; (2) terminal-phase males of *H. garnoti* at Bermuda have a red band on the upper posterior portion of the body, whereas males from the Caribbean have a black band at the same place; (3) the upper body of individuals from the Brazilian populations of *H. maculipinna* is usually brownish-pink, whereas individuals from the Caribbean have a yellowish-green upper body.

Samples of the above-mentioned species were collected across their entire geographical range in the tropical western Atlantic, and an analysis of mitochondrial DNA sequences was carried out aiming to assess the following questions: (1) Does genetic differentiation parallel the observed patterns of color differentiation in western Atlantic *Halichoeres*? (2) Do biogeographic barriers (the Amazon freshwater outflow and vast open-ocean distances) equally affect species with similar dispersal potential?

Materials and Methods

Specimens were collected with pole spears or hand nets while scuba diving or snorkeling in Bermuda, the Bahamas, Florida, Belize, St. Croix (U.S. Virgin Islands), Venezuela, Paraíba (northeastern Brazil), Espírito Santo (southeastern Brazil) and Trindade Island (Fig. 1) between 1997 and 2002. Tissue (muscle and/or gill) was stored in a saturated salt-DMSO buffer (Amos and Hoelzel, 1991).

Total genomic DNA was extracted using QIAGEN DNeasy extraction kits following the manufacturer's protocol. Extracted DNA was frozen in TE buffer (10 mM Tris-HCl, pH 7.5 and 1 mM EDTA, pH 8.0, diluted in water) and archived at −20 C. Primer names indicate the DNA strand (H = heavy and L = light strand) and the position of the 3′ end of the oligonucleotide primer relative to the human mitochondrial DNA sequence. A fragment of approximately 800 base pairs of the mtDNA cytochrome *b* gene was amplified with the primers L14725 (5′ GTG ACT TGA AAA ACC ACC GTT G 3′) and H15573 (5′ AAT AGG AAG TAT CAT TCG GGT TTG ATG 3′; Meyer, 1993). Based on initial sequences, the internal primers LI4768 (5′ ACC CAC CCA CTC CTT AAA ATC 3′), and H15496 (5′ TTG GAG ACC CAG ATA ATT TCA C 3′) were designed and worked consistently among *Halichoeres* species, yielding a product of 690–709 base pairs.

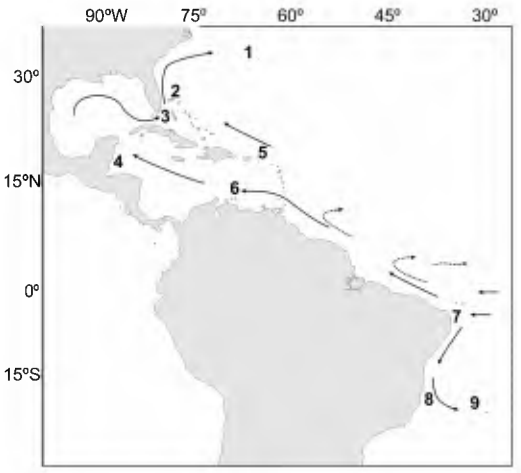

Fig. 1. Tropical western Atlantic. Numbers represent sampled locations: 1. Bermuda; 2. Bahamas; 3. Florida Keys; 4. Belize; 5. St. Croix; 6. Venezuela; 7. northeast Brazil; 8. southeast Brazil; 9. Trindade Island. Solid arrows indicate direction of mean surface oceanic currents; broken lines indicate subsurface currents.

Thermal cycling in polymerase chain reactions (PCR) consisted of an initial denaturing step at 94 C for I min 20 sec, then 35 cycles of amplification (40 sec of denaturation at 94 C, 30 sec of annealing at 52 C, and 55 sec of extension at 72 C), and a final extension of 2 min, 30 sec at 72 C. Excess primers were removed by incubation of PCR products with exonuclease I and shrimp alkaline phosphatase (USB Corp., Cleveland OH).

Sequencing reactions with fluorescently labeled dideoxy terminators (BigDye, Applied Biosystems, Inc., Foster City, CA) were performed according to manufacturer's recommendations and analyzed with an ABI 377 automated sequencer (Applied Biosystems, Inc., Foster City, CA). All samples were sequenced in the forward direction (with L14725 or LI4768 primers), and rare or questionable haplotypes were sequenced in both directions to ensure accuracy of nucleotide designations. Haplotypes of all species were deposited at GenBank (see Material Examined section).

Sequences were aligned and edited with Sequencher version 3.0 (Gene Codes Corp., Ann Arbor, MI). The computer program MODELTEST version 3.06 (Posada and Crandall, 1998) was used to determine the substitution model and the gamma distribution shape parameter that best fit the data through hierarchical likelihood ratio tests (hLRTs). The HKY (Hasegawa et al., 1985) substitution model was chosen for all datasets and the gamma distribution shape parameter varied from 0 in *H. cyanocephalus* to 0.1 in *H. maculipinna* to 0.4 in *H. garnoti*. The analyses were also run using the Tamura-Nei substitution model (Tamura and Nei, 1983) with no significant changes in tree topology. Relationships between unique haplotypes were reconstructed based on starting trees calculated using the neighbor-joining method (Nei, 1987) and further searched by 10^7 tree bisection and reconnection (TBR) iterations under the minimum evolution criterion with the software PAUP* version 4.0b10 (D. L. Swofford, Sinauer, Sunderland, MA, 2002, unpubl.). Additionally, maximum parsimony analyses were performed on datasets with deep phylogenetic breaks. Topological confidence was evaluated with 1000 bootstrap replicates (Felsenstein, 1985). Equal weighting of all three codon positions was used.

Population structure and gene flow were assessed with the program Arlequin v2.0 (S. Schneider, D. Roessli, and L. Excoffier, University of Geneva, Switzerland, 2000, unpubl.), which generated F_{ST}-values (a molecular analog of F_{ST} that takes into consideration sequence divergence among haplotypes). Genetic variation is described with nucleotide diversity (π, equation 10.19; Nei, 1987) and haplotype diversity (h, equation 8.5; Nei, 1987) within each location.

In addition to the genetic analysis, morphological comparisons were also carried out. Counts and measurements follow Randall and Lobel (2003). Measurements were taken with a digital caliper and recorded to the nearest tenth millimeter. Unless otherwise stated, institutional abbreviations follow Leviton et al. (1985). Specimens from the following museums were examined: MBML (Museu de Biologia Melo Leitão, Espírito Santo, Brazil), MCZ, MZUSP, SU, UF, UFES (Coleção Ictiológica, Universidade Federal do Espírito Santo, Brazil), UFPB (Coleção Ictiológica, Universidade Federal da Paraíba, Brazil), USNM and ZUEC (Museu de Historia Natural, Universidade Estadual de Campinas, Brazil).

Results

Halichoeres cyanocephalus.—A 701 bp fragment from the cytochrome *b* gene was analyzed from 17 individuals. A total of 26 polymorphic sites distributed among 10 haplotypes were identified. Mean nucleotide frequencies were A = 0.22, C = 0.31, G = 0.16, T = 0.31. The transition:transversion ratio was 5.5:1. The phylogenetic analysis (Fig. 2) assigned the haplotypes of *H. cyanocephalus* into two lineages (Brazil and Caribbean). Pairwise distances within lineages ranged from 1.17–2.83 nucleotide substitutions (d = 0.002–0.004). Although sample sizes are low, Brazilian and Caribbean populations are separated by at least 16 mutations (d = 0.023) and the F_{ST} between Brazil and Belize was 0.91 ($P < 0.01$). No morphological difference was detected between the lineages; however, a slight color difference is present (Fig. 5C–D).

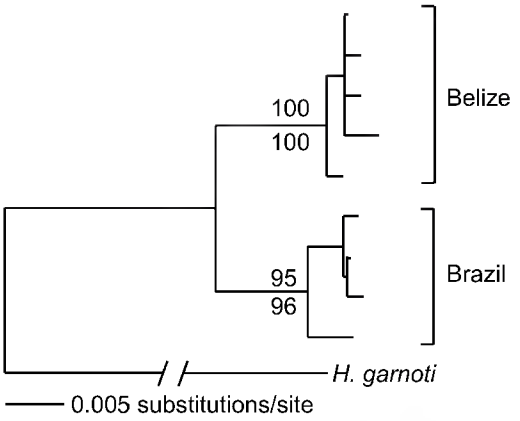

Fig. 2. Phylogenetic tree of *Halichoeres cyanocephalus* (Belize) and *Halichoeres dimidiatus* (Brazil) unique haplotypes. Neighbor-joining bootstrap support (> 50%) indicated on nodes, maximum-parsimony support below nodes. Branch lengths are according to indicated scale; the branch leading to the outgroup (*Halichoeres garnoti*) was reduced by 50%.

Halichoeres garnoti.—A 704 bp fragment from the cytochrome *b* gene was analyzed from 116 individuals. A total of 66 polymorphic sites were distributed among 55 haplotypes. Mean nucleotide frequencies were A = 0.22, C = 0.30, G = 0.16, T = 0.32. The transition:transversion ratio was 10.16:1. No geographic signal was detected in the phylogenetic analysis of *H. garnoti* (Fig. 3). Individuals with a red dorsum (indicated by arrows and the letter B in Fig. 3) were nested within the Caribbean lineage. Genetic distances among populations ranged from 0.06–2.45 nucleotide substitutions (d = 0.001–0.004). Haplotype diversity was relatively high and overall nucleotide diversity (π) was low in all populations (Table 1). No significant population structure was observed across the species' entire range from Bermuda to Venezuela (Table 2).

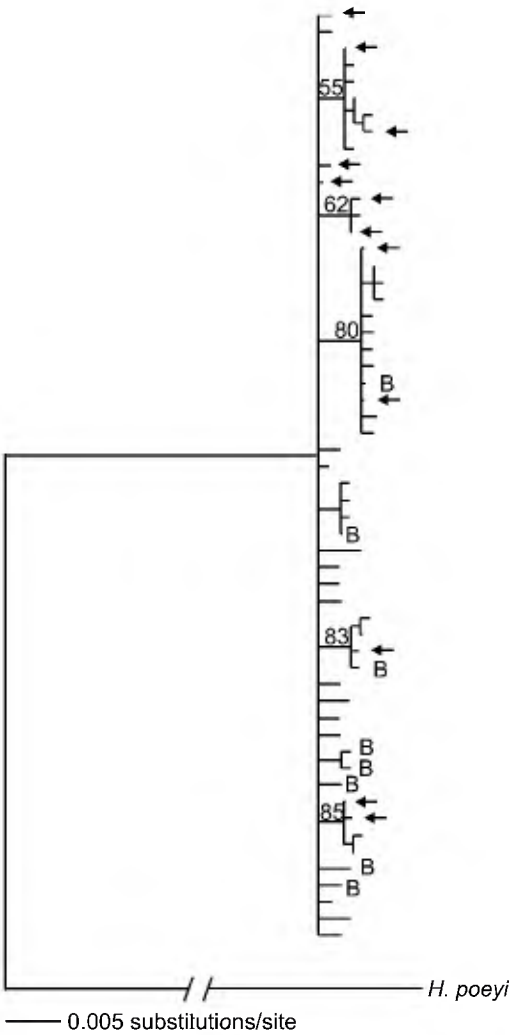

Fig. 3. Phylogenetic tree of all (55) *Halichoeres garnoti* unique haplotypes. No geographical signal was detected in the phylogeny. Arrows indicate haplotypes shared between Bermuda and one or more Caribbean location; haplotypes only found in Bermuda are marked with a B. Bootstrap support (> 50%) is indicated on nodes. Branch lengths are according to indicated scale; the branch leading to the outgroup (*Halichoeres poeyi*) was reduced by 50%.

Halichoeres maculipinna.—A 691 bp fragment from the cytochrome *b* gene was analyzed from 86 individuals. A total of 71 polymorphic sites distributed among 44 haplotypes were identified. Mean nucleotide frequencies were A = 0.22, C = 0.29, G = 0.17, T = 0.32. The transition:transversion ratio was 5.91:1. The phylogenetic analysis (Fig. 4) assigned the haplotypes of *Halichoeres maculipinna* into two lineages (Brazil and the North Atlantic) separated by 45.2–49.0 nucleotide substitutions (d = 0.065–0.071 sequence divergence). Genetic distances within lineages ranged from 0.01–2.05 nucleotide substitutions (d = 0.001–0.003). Haplotype diversity was relatively high and overall nucleotide diversity (π) was low in all populations of both lineages (Table 1).

The two lineages were allopatric, one northern (Florida, Bahamas, St. Croix, Belize, and Venezuela) and the other southern (coastal Brazil and Trindade Island), being separated by highly significant F_{ST}-values (F_{ST} = 0.958–0.984). No significant pairwise F_{ST}-values were observed within either lineage (Table 3). No morphological difference was detected between the lineages; however, a slight color difference is present (Fig. 5E–F).

Discussion

Halichoeres cyanocephalus.—The genetic divergence (d = 2.3%) between the Brazilian and Caribbean populations of *H. cyanocephalus* (Fig. 2) coincides with a subtle but consistent color difference: individuals in Belize, Florida, and the Virgin Islands have the color pattern shown in Figure 5D, whereas individuals in north and south Brazil have the pattern shown in Figure 5C. The Amazon is unlikely to be an effective barrier between Brazilian and Caribbean lineages, because the Brazilian lineage occurs over deep sponge bottoms off Brazil (Rocha et al., 2000) and French Guiana (Uyeno et al., 1983), the latter under the Amazon plume.

If not the Amazon barrier, what is causing genetic differentiation between these lineages? Rocha (2003b) suggests that it may be ecological speciation or speciation driven by "divergent selection on traits between populations or subpopulations in contrasting environments" (Schluter, 2001). Environmental differences between locations in the Caribbean Sea (characterized by clear waters all year round, relatively stable environmental conditions, and bottom sediments largely composed of calcium carbonate) and the Brazilian coast (a typical continental environment, with terrigenous substrates influenced by run off from rivers, and high turbidity caused by wind driven suspension of bottom sediments) may generate divergent selection pressures in the insular versus continental habitats, potentially promoting ecological speciation, even in the presence of small migration between populations.

Halichoeres garnoti.—As pointed out by Smith-Vaniz et al. (1999, pl. 12, figs. 78–80), there are noticeable color differences between Caribbean and Bermuda populations of this species (Fig.

TABLE 1. SAMPLE SIZE, NUMBER OF HAPLOTYPES, HAPLOTYPE DIVERSITY (h) AND NUCLEOTIDE DIVERSITY (π) OF THE POPULATIONS SURVEYED.

	N	Haplotypes	h	π
Halichoeres cyanocephalus				
Belize	12	6	0.68 ± 0.15	0.002 ± 0.001
NE Brazil	5	4	0.90 ± 0.16	0.004 ± 0.003
Total	17	10		
Halichoeres garnoti				
Bahamas	21	17	0.97 ± 0.03	0.008 ± 0.005
Belize	21	15	0.95 ± 0.03	0.009 ± 0.005
Bermuda	27	20	0.97 ± 0.02	0.008 ± 0.004
Florida Keys	3	1	0.00 ± 0.00	0.00 ± 0.00
St. Croix	23	18	0.98 ± 0.02	0.009 ± 0.005
Venezuela	22	16	0.97 ± 0.02	0.009 ± 0.005
Total	117	55		
Halichoeres maculipinna				
Bahamas	19	12	0.90 ± 0.06	0.003 ± 0.002
Belize	19	12	0.87 ± 0.07	0.002 ± 0.001
NE Brazil	5	4	0.90 ± 0.16	0.002 ± 0.001
Florida Keys	3	2	0.67 ± 0.31	0.001 ± 0.001
St. Croix	18	9	0.80 ± 0.09	0.002 ± 0.001
Trindade	5	3	0.70 ± 0.21	0.001 ± 0.001
Venezuela	17	11	0.88 ± 0.07	0.003 ± 0.002
Total	86	44		

5A–B). Unlike what occurs in other *Halichoeres* species (color differences are matched by fixed genetic differences in the pair *Halichoeres radiatus/brasiliensis* and the Brazilian and Caribbean lineages of *H. cyanocephalus* and *H. maculipinna*; Rocha and Rosa, 2001b; Rocha, 2003a) color differences are not accompanied by fixed genetic differences at the cytochrome *b* of *H. garnoti*. The population of *H. garnoti* in Bermuda shares 12 of its 20 haplotypes with one or more of the Caribbean locations (Fig. 3). Moreover, haplotypes endemic to Bermuda are randomly distributed across the tree on Figure 3.

Three hypothesis may explain the absence of genetic differentiation despite the presence of color differences: (1) the population at Bermuda is exposed to some environmental condition that causes their color to change phenotypically without an underlying genetic basis; (2) color differences are associated with differences at loci other than the cytochrome *b*; (3) the population at Bermuda is young, possibly postdating the last glacial maximum, such that color differences that appeared as a result of strong selection, drift or founder effect are not accompanied by fixed genetic differences at the cytochrome *b*.

The first two hypotheses remain untested; however, there is evidence favoring the third. During the last glacial maximum (25,000 to 15,000 yr B.P.), temperatures at Bermuda were 3–5 C lower than today (Sachs and Lehman, 1999), bringing mean annual temperatures below the 21 C (with much lower temperatures during the winter) threshold necessary for reef coral survival and leading to the likely exter-

TABLE 2. POPULATION PAIRWISE ΦST FOR *Halichoeres garnoti*. No comparisons are significant at $P = 0.05$.

Locations	1	2	3	4	5	6
1. Bermuda	0					
2. Florida	0.001	0				
3. Bahamas	−0.023	0.041	0			
4. Belize	0.011	−0.026	−0.004	0		
5. St. Croix	−0.006	0.141	−0.024	−0.005	0	
5. Venezuela	−0.013	−0.078	−0.016	0.018	−0.017	0

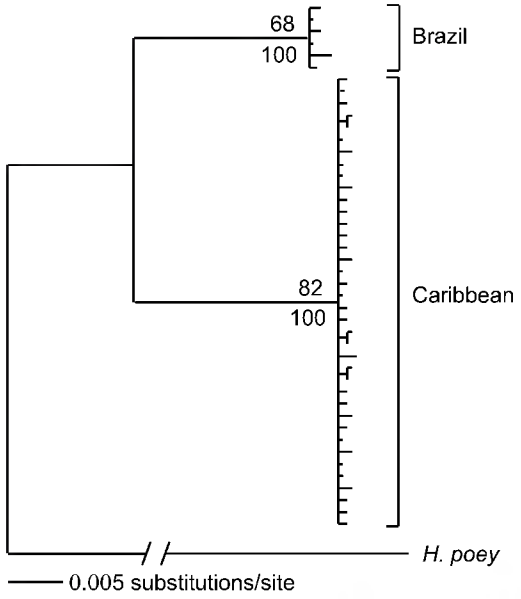

Fig. 4. Phylogenetic tree of *Halichoeres maculipinna* (Caribbean) and *Halichoeres penrosei* (Brazil) unique haplotypes. Neighbor-joining bootstrap support (> 50%) indicated on nodes, maximum-parsimony support below nodes. Branch lengths are according to indicated scale; the branch leading to the outgroup (*Halichoeres poeyi*) was reduced by 50%.

mination of most of the tropical reef fish fauna (Smith-Vaniz et al., 1999). Assuming that *H. garnoti* is a strictly tropical species (it does not occur in the northern Gulf of Mexico or in any coastal location north of Florida), it is probable that the Bermuda population is less than 15,000 years old. Thus, color differences may have a genetic basis but were not detected in neutral genes (such as the cytochrome *b*) because of insufficient time for lineage sorting (Avise, 2000).

Halichoeres maculipinna.—The pelagic larval duration (PLD) of *H. maculipinna* (30 days; Sponaugle and Cowen, 1997) is the longest among the Atlantic *Halichoeres*. However, this species showed the deepest genetic divergence observed in this study (d = 6.5%) between Brazilian and Caribbean populations, demonstrating the poor value of PLD as a predictor of genetic divergence. Despite the deep genetic split between Brazil and the Caribbean, careful examination of several individuals of *H. maculipinna* from locations at the two regions revealed no morphological differences. However, slight differences in coloration were detected (Fig. 5E–F).

The divergence observed between Caribbean and Brazilian lineages of *H. maculipinna* is the highest among all species examined. One possible explanation is that this high divergence is a result of strict habitat preferences. There are no field survey data that indicates that *H. maculipinna* crosses the Amazon barrier; it is a shallow water species (20–30 m maximum depth; Randall and Böhlke, 1965; Humann and Deloach, 2002) and was not collected under the Amazon plume (Collette and Rützler, 1977) or on deep sponge bottoms off northeastern Brazil (Rocha et al., 2000). Moreover, *H. maculipinna* has the weakest jaw musculature, the smallest mouth gap and the most specialized diet (fewer and smaller species of benthic invertebrates compared to congeners) among all Atlantic *Halichoeres* (Randall, 1967; Wainwright, 1988). As a specialized predator, *H. maculipinna* may be more sensitive to habitat differences than the other more generalist species surveyed here. In this case, stronger natural selection may lead to earlier and faster differentiation, possibly generating the higher observed divergences in this species.

Taxonomic implications.—The genetic divergence between the Caribbean and Brazilian populations observed in this study equals or exceeds divergences between recognized sister species pairs of other wrasses (Rocha, 2003a; Bernardi et al., 2004). In addition, there are diagnostic color characters that distinguish the Brazilian populations from their Caribbean counterparts

TABLE 3. POPULATION PAIRWISE ΦST FOR *Halichoeres maculipinna*. Asterisks indicate significance at P = 0.05.

Locations	1	2	3	4	5	6	7
1. Florida	0						
2. Bahamas	−0.101	0					
3. Belize	−0.075	0.006	0				
4. St. Croix	−0.105	0.010	−0.014	0			
5. Venezuela	−0.100	−0.002	0.013	0.009	0		
6. Coastal Brazil	0.973*	0.958*	0.969*	0.971*	0.959*	0	
7. Trindade	0.984*	0.961*	0.972*	0.974*	0.963*	0.001	0

Fig. 5. *Halichoeres garnoti* at the Bahamas (A) and Bermuda (B). *Halichoeres dimidiatus* at northeastern Brazil (C) and *Halichoeres cyanocephalus* at Belize (D) *Halichoeres penrosei* at northeastern Brazil (E) and *Halichoeres maculipinna* the Bahamas (F). All photos taken by Luiz Rocha, except 5C (by Gerald Allen) and 5D (by Jack Randall).

(Fig. 5). Randall (1998) suggested that the importance of diagnostic color characters in reef fish systematics is dramatically increased when those characters are observed in combination with (even minor) diagnostic counts or measurements. Here I propose that this principle should be extended to genetics: the coincidence of diagnostic genetic and color characters should warrant elevation of evolutionary lineages to specific status. Thus, the Brazilian populations of *H. cyanocephalus* and *H. maculipinna* should be considered valid species.

Bloch (1791) gave no type locality for *H. cyanocephalus*, and no types are known (Randall and Böhlke, 1965; Eschmeyer, 1998; Paepke, 1999; Parenti and Randall, 2000). The original description (Bloch, 1791:140) states [my translation]: "The residence of this fish is for me unknown. The original drawing is kept in the cabinet of Linkeschen (Heinrich Linck)." Moreover, the original description is inaccurate and incomplete because it is based on a single, apparently adult individual. Nonetheless, this name has been associated with the Caribbean species since at least the end of the 19th century (Jordan and Evermann, 1898).

To clarify the taxonomic status and the type locality of *H. cyanocephalus* (Bloch, 1791), and

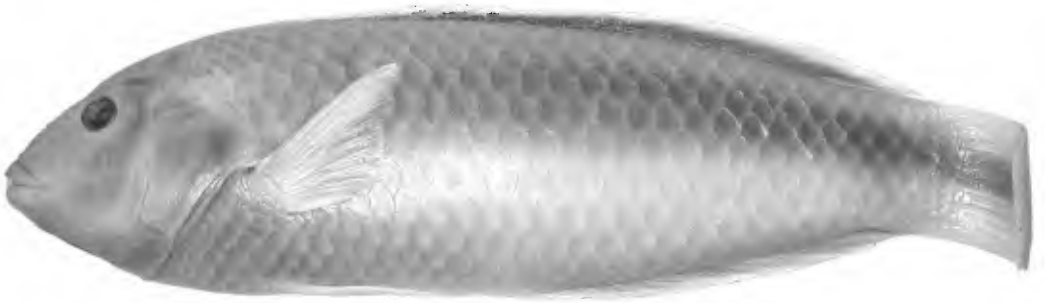

Fig. 6. Neotype of *Halichoeres cyanocephalus* (UF 224284). Photo by Luiz Rocha.

in accordance with article 75(3) of the International Code of Zoological Nomenclature (ICZN, 1999), I designate UF 224284 (202.2 mm SL) as a neotype. Detailed descriptions of *H. cyanocephalus* are available in the scientific literature (Randall and Böhlke, 1965) and in most Caribbean reef fish guides (Randall, 1996; Humann and Deloach, 2002).

Halichoeres cyanocephalus (Bloch, 1791)
Figures 5D, 6

Neotype.—UF 224284, adult from Haulover Cut, North Miami Beach, Florida, collected on 8 August 1967 by W. Zeiller, at a depth between 18 and 27 m. This specimen was chosen because it has the typical characters described by Randall and Böhlke (1965) and because it is the best preserved specimen of this taxon at the University of Florida.

Description of the neotype.—Dorsal IX, 12; anal III, 12; pectoral ii,11; total gill rakers on first arch 19 (seven on the upper limb); lateral-line scales 27 (20 + 2 + 5); anterior lateral-line scales with three pores; two pairs of enlarged canine teeth anteriorly in lower jaw, one pair on upper jaw. Standard length 202.2 mm; total length 227.5 mm; body depth 29.1% SL; caudal peduncle depth 14.6% SL; head length (HL) 28.3% SL; snout length 37.8% HL; upper jaw length 22% HL; eye diameter 14.1% HL; interorbital width 22.3% HL.

Color in alcohol.—Body light brown with a broad dark brown stripe on upper half, beginning at pectoral fin and extending into middle of caudal fin; a narrower stripe of lighter brown between the broad stripe and the dorsal fin. Faint indication of a dark bar on head, extending upward diagonally from upper posterior half of eye to upper portion of head. Dorsal fin dark brown on base, pale on margin; upper base of pectoral fin with a dark spot.

Distribution, habitat, and ecology.—Caribbean islands, North American coast from Florida to North Carolina and Central American coast from Colombia to Belize. Usually solitary, rare in shallow waters, but juveniles and small adults frequently observed between 5 and 15 m at Belize; mostly observed at depths over 30 m at the remaining locations.

The Brazilian species should now be considered valid as follows.

Halichoeres dimidiatus (Agassiz, in Spix and Agassiz, 1831)
Figures 5C, 7

This species was originally described as *Julis dimidiatus* by Agassiz, in Spix and Agassiz, 1831, and later placed in the synonymy of *Halichoeres cyanocephalus* (Bloch, 1791) by (Jordan and Evermann, 1898). Agassiz states that several specimens were preserved, but only one (MHNN 563, 153 mm SL) still exists (Kottelat, 1988). The type locality is given as Atlantic, off Brazil (Spix and Agassiz, 1831).

Diagnosis.—Dorsal IX, 12 (IX, 11 on remaining Atlantic *Halichoeres*, except *H. cyanocephalus*); anal III, 12; pectoral 13; total gill rakers 18 to 21; two pairs of enlarged canine teeth anteriorly in lower jaw. Juveniles and females blue with a bright yellow region dorsally from mouth to posterior base of dorsal fin; a single dark spot on caudal (a small ocellated dark spot at rear base of dorsal fin in addition to the caudal spot in *H. cyanocephalus*). Adults with a broad blue stripe on upper half of body ending at the beginning of the caudal fin (black and narrowing as it passes to end of caudal fin in *H. cyanocephalus*); lower half of body light blue; a diagonal dark band from eye to nape.

Distribution, habitat, and ecology.—From French Guyana (Uyeno et al., 1983) to the State of São

Fig. 7. Adult *Halichoeres dimidiatus*, approximately 150 mm SL, Guarapari, southeast Brazil. Photo by João Luiz Gasparini.

Paulo (24°S, 46°W), in southeastern Brazil (Menezes and Figueiredo, 1985). It is also present at Fernando de Noronha and Atol das Rocas (20°30′S, 29°20′W) but apparently absent from the remaining Brazilian oceanic islands of Trindade and St. Paul's Rocks (Gasparini and Floeter, 2001; Feitoza et al., 2003). Usually observed solitary; juveniles relatively common in shallow waters (3–20 m), adults in deeper waters (30–60 m).

Halichoeres penrosei Starks, 1913
Figures 5E, 8

The Brazilian form of *H. maculipinna* was described as *Halichoeres penrosei* by Starks (1913); the 55.7 mm SL holotype (SU 22211) is well preserved and was collected in tide pools at Natal, northeastern Brazil (5°S, 35°W), located at the middle of the range of the species. Digital photographs and a radiograph of the holotype are available for download at the California Academy of Sciences website (http://www.calacademy.org/research/ichthyology/Types/index.html).

Diagnosis.—Dorsal IX, 11; anal III, 11; pectoral 14; gill rakers 13–15; one pair of enlarged canine teeth anteriorly in lower jaw (two pair of canines on remaining Atlantic *Halichoeres* except *H. maculipinna*). Juveniles and females with wide black stripe through eye to base of tail, bordered above by prominent pinkish-brown line (bright yellow in *H. maculipinna*). White scales with orange spots on lower half of body (no spots on *H. maculipinna*) Adult males are primarily green with scales bordered by pink and a black spot on anterior portion of the dorsal fin. Narrow (< 0.5 mm width) orange stripes on lower half of head, from mouth to gill opening (stripes are > 1 mm wide in *H. maculipinna*).

Distribution, habitat, and ecology.—From Parcel Manuel Luiz (0°52′S, 44°15′W; Rocha and Rosa, 2001a) to the State of São Paulo (24°S, 46°W), in southeastern Brazil (Carvalho-Filho, 1999). It is present at Trindade Island (20°30′S, 29°20′W; Gasparini and Floeter, 2001), but apparently absent from the remaining Brazilian oceanic islands (Atol das Rocas, Fernando de Noronha,

Fig. 8. *Halichoeres penrosei*, approximately 50 mm SL, Guarapari, southeast Brazil. Photo by João Luiz Gasparini.

and St. Paul's Rocks; Feitoza et al., 2003). Common in coral and rocky reef tops to depths of 30 m and often observed solitary or in pairs.

Conclusions

Contrary to expectations of congruent population structure due to similar dispersal potential (25–30 days; Sponaugle and Cowen, 1997; Wellington and Robertson, 2001), the genetic effects of biogeographical barriers varied among species of Atlantic *Halichoeres*. Genetic divergences between Brazil and the North Atlantic ranged from 2.3% in *H. cyanocephalus/H. dimidiatus* to 6.5% in the pair *H. maculipinna/H. penrosei*. There is inconsistency between color differences and genetic partitions in the species surveyed. The color differences between the species pairs *H. cyanocephalus/H. dimidiatus* and *H. maculipinna/H. penrosei* correspond to deep genetic partitions at the cytochrome *b* gene. However, genetic similarity at this same gene was observed between populations of *H. garnoti* with striking color differences. Thus, caution against the use of color differences (when not supported by genetics and/or morphology) in the alpha systematics of fishes is herein suggested, a conclusion also reached by others (McMillan et al., 1999; Bernardi et al., 2004).

Genetic divergences ranged from 2.3–6.5% in lineages in Brazil and the Caribbean. A conventional molecular clock of 2% per million years for the cytochrome *b* gene (Avise, 2000) suggests that this divergence is between 0.6 and 1.65 millions of years (myr) old, much younger than the estimated age of the initial connection of the Amazon with the Atlantic, 10–12 myr old (Hoorn, 1994; Nittrouer et al., 1996; Costa et al., 2001). Thus, vicariance alone cannot explain the observed pattern, and dispersal from one area to the other after the formation of the Amazon is probably involved in speciation of western Atlantic *Halichoeres*.

The species pair with the highest divergence (6.5 %, *H. maculipinna/H. penrosei*) is also the more reef specialized and the one with the narrowest diet width. Although apparently being able to cross the Amazon barrier, a deep phylogenetic break (2.3 %) between Brazil and the Caribbean is present in the pair *H. cyanocephalus/H. dimidiatus*. These observations indicate that divergent environmental conditions (continental sediment-rich Brazilian waters versus insular, clear Caribbean waters) can be as important as vast distances of unsuitable habitat (either open ocean waters or the area influenced by the Amazon) in producing genetic partitions and that benthic-stage habitat preferences may be strongly influencing the biogeography of western Atlantic reef fishes.

Material Examined

Voucher catalog numbers followed by number of specimens and GenBank accession numbers in parentheses (when available).

Halichoeres cyanocephalus: Cuba: MCZ 14252 (3, syntypes of *Julis internasalis* Poey 1861); Florida: UF 65506 (1), UF 224284 (1, neotype of *H. cyanocephalus*); North Carolina: UF 88359 (2); Belize: UF 126324 (1, AY591376); St. John, U.S. Virgin Islands: UF 206250 (1); Jamaica: UF 229817 (1); Colombia: UF 231301 (1). Sequences without museum voucher: AY591377–AY591379.

Halichoeres dimidiatus: (all from Brazil); Parcel Manoel Luiz, Maranhão: MZUSP 53066 (2), UFPB 3890 (1), UFPB 3977 (1); Fernando de Noronha: MZUSP 14626 (1); Mamanguape, Paraíba: UFPB 4312 (1, AY591380); Cabedelo, Paraíba: UFPB 4325 (2); João Pessoa: UFPB 3790 (1), UFPB 4354 (2, AY591381, AY591382); Recife: MZUSP 47482 (1); Salvador, Bahia: USNM 357718 (1); Guarapari, Espírito Santo: MZUSP 51543 (1), ZUEC 3184 (1), ZUEC 3196 (1), MBML 354 (28); Angra dos Reis, Rio de Janeiro: MZUSP 66256 (2); Alcatrazes, São Paulo: MZUSP 47146 (1), USNM 357717 (1), ZUEC 2793 (1). Sequences without museum voucher: AY591383.

Halichoeres garnoti: Bermuda: UF 119713 (11, AY591372, AY591374), UF 119725 (1, AY591375), USNM 348309 (1); Bahamas: UF 13485 (5); Cayman Islands: UF 17620 (3); Colombia: UF 18830 (2); St. John U.S. Virgin Islands: UF 204900 (2); Belize: UF 209277 (4); Cuba: USNM 343625; Tobago: USNM 318896 (7). Sequences without museum voucher: AY591366–AY591371.

Halichoeres maculipinna: Bermuda: UF 119714 (2), UF 119731 (9, AY591359); St. John, U.S. Virgin Islands: UF 203752 (4); Bahamas: UF 206337 (5); Florida Keys: UF 219121 (12); Colombia: UF 223079 (12); Belize: USNM 329838 (1); Tobago: USNM 318864 (4). Sequences without museum voucher: AY591360–AY591365.

Halichoeres penrosei: (all from Brazil); Natal: SU 22211 (1, holotype); Espírito Santo: MZUSP 52303 (1), MBML 223 (2), 318(1), 395(1, AY591354), UFES 037 (1), 208(1); Ceará: MZUSP 65172 (1); Arembepe, Bahia: MZUSP 66259 (3); Salvador, Bahia: MZUSP 66268 (10 of 25). Sequences without museum voucher: AY591355–AY591358.

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