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Population structure of carite (*Scomberomorus brasiliensis*) in waters offshore of Trinidad and northern Venezuela

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ABSTRACT

Population structure of carite (Serra Spanish mackerel, *Scomberomorus brasiliensis*) in waters offshore of Trinidad and northern Venezuela was assessed by analysis of allele and genotype distributions at 16 nuclear-encoded microsatellites and mitochondrial (mt)DNA haplotype distribution among individuals from five localities in the southern Caribbean Sea. Tests of homogeneity (microsatellites) revealed a genetically discrete subpopulation (stock) of carite in the Cariaco Basin along the northern coast of Venezuelan mainland. This subpopulation contained a small, highly divergent clade of four mtDNA haplotypes that possibly represent recent admixture of formerly isolated subpopulations. Spatial autocorrelation analysis of the microsatellite data was consistent with the notion that carite from Isla de Margarita (Venezuela) and Trinidad may warrant management as distinct stocks, while spatial autocorrelation analysis and tests of selective neutrality of mtDNA variation suggested that carite off the south coast of Trinidad differ from carite along the west and north coasts. The implication of these findings is that there may be a number of spawning stocks of carite in the southern Caribbean Sea.

1. Introduction

Serra Spanish mackerel (*Scomberomorus brasiliensis*), referred to as 'carite' in Trinidad, is a relatively large, reef-associated, coastal pelagic species distributed in the western Atlantic Ocean and the Caribbean Sea from the Yucatán Peninsula and Belize to as far south as Lagoa Tramandai, Rio Grande de Sul, Brazil (Collette et al., 1978). The species comprises one of the most important commercial fisheries in Trinidad. From 1976 to 1989, carite constituted 25% of fish earnings in Trinidad, with an annual average value of TT$6 million (Hodgkinson-Clarke, 1990); while for the years 1987 to 1991 landings of carite were valued at TT$15 to TT$25 million (Henry and Martin, 1992). Between 1996 and 2006, carite comprised 19% of the average annual landings by the Trinidadian artisanal fleet and 25% of the landed fish value; during this period, annual landings and ex-vessel value averaged over 1800 metric tons and TT$25.9 m, respectively (unpublished data from the Fisheries Division, Ministry of Agriculture, Land and Marine Resources, Trinidad). Stock assessments in 1991 categorized carite in waters off Trinidad as fully exploited (Henry and Martin, 1992). More recent assessments (Martin and Nowlis, 2004), using length frequency data from 1995 to 2002 and total catch weight and effort data from artisanal gillnets, indicated that carite biomass was below maximum-sustainable-yield (MSY) biomass and that the carite stock appeared to be experiencing fishing mortality rates above MSY fishing-mortality-rate levels.

In this study, population structure of carite in waters offshore of Trinidad and from two localities in Venezuela was assessed via analysis of genetic variation at 16 nuclear-encoded microsatellites and in a 590 base-pair (bp) fragment of a mitochondrial protein-coding gene. The study was in response to needs by the Fisheries Division, Ministry of Agriculture, Land and Marine Resources, Trinidad, to assess whether carite in waters offshore of Trinidad are composed of a single or multiple stocks. Knowledge of stock structure is important, particularly in an overexploited species, to ensure that sub-regional exploitation does not unknowingly extirpate unique genetic resources inherent in sub-regional populations (Begg et al., 1999; Hilborn et al., 2003). There also are possible jurisdictional issues given that ocean fishing is a vital industry and conflicts between or among countries over availability of fishing rights in the Caribbean Sea already have occurred (Blake and Campbell, 2007). Of potential concern relative to carite are the waters to the south and west of Trinidad where carite are taken by fishers from both Trinidad and Venezuela. Currently, the two countries have an agreement (http://diplowizard.diplomacy.edu/tara/GetXdoc.asp?IDconv=2172) that allows fishers to engage freely in fishing activities without the need

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for a fishing license from either country to within two nautical miles from the coast of either country.

2. Materials and methods

A total of 526 carite were sampled between January 2007 and April 2008 from commercial catches taken at seven localities (Fig. 1). Sampling dockside was carried out by personnel from the Fisheries Division, Ministry of Agriculture, Land, and Marine Resources, Trinidad. Carite fishing in all these areas is largely artisanal in small fiberglass vessels propelled by outboard engines. Fishers use two types of nets, monofilament or demersal and multifilament or drifting, and fish at distances 2–5 km from the coastline at depths of 10–15 m; most generally fish within a 5 km radius from their home port. Five of the localities were in Trinidad: Las Cuevas on the North Coast (158 individuals); Orange Valley, Claxon Bay, and San Fernando on the West Coast (100 individuals); and Erin on the South Coast (130 individuals). Two of the localities were in Venezuela: off North Point on the Isla de Margarita (109 individuals) and in the Cariaco Basin between Cumana and the Gulf of Santa Fe on the north coast of the Venezuelan mainland (29 individuals). Carite taken at North Point were sampled in Porlamar on the east coast of Isla de Margarita; carite taken along the north Venezuelan coast were sampled in Cumana (Fig. 1). Sampling areas for carite in Orange Valley, Claxon Bay, and San Fernando on the west coast of Trinidad tended to overlap; consequently, fish from these three localities were pooled into a single sample (West Coast). Duplicate caudal fin clips (5 mm^3) were taken from each fish, fixed twice in 95% ethanol, and sent to College Station, Texas. Cardiac muscle tissue was sampled from a few fish and fixed in the same way.

Whole genomic DNA was extracted from each fish, with the phenol chloroform extraction procedure described in Sambrook et al. (1989). All 526 carite were assayed for allelic variation at 16 nuclear-encoded microsatellites. Polymerase-chain-reaction (PCR) primers, repeat motifs, and specific annealing temperatures for each microsatellite may be found in Renshaw et al. (2009). Microsatellite amplification products were electrophoresed with an ABI 377 automated sequencer (Applied Biosystems Inc., Foster City, CA), following manufacturer instructions. Resulting chromatograms were analyzed in GENESCAN (v. 3.1.2, Applied Biosystems); alleles were scored with GENOTYPER (v. 2.5, Applied Biosystems).

A 590 bp fragment of the mitochondrially encoded NADH-dehydrogenase subunit 4 (ND-4) gene was PCR amplified and sequenced from two carite, with PCR primers NAP-2 (Arevalo et al., 1994) and ND4LB (Bielawski and Gold, 2002) used for both amplification and sequencing. PCR amplifications and sequencing were carried out with protocols outlined in Pruett et al. (2005). Internal primers, *Sbr*ND4F (5′-CCACACTTATGCTCGTCC-3′) and *Sbr*ND4R (5′-GCTTTGGGAAGTCATAGGT-3′) were then designed and used to amplify and sequence a slightly smaller (581 bp) fragment of the ND-4 gene from 130 carite. PCR amplifications with the redesigned primers were carried out in 25 μl reaction volumes containing ~100 ng of DNA, 1× reaction buffer (50 mM KCl, 10 mM Tris–HCl at pH = 9.0, 0.1% Triton-X 100), 2 mM $MgCl_2$, 0.3 μM of each primer, 250 μM of each dNTP, and 0.25U *Taq* DNA polymerase (Invitrogen). The PCR protocol consisted of an initial denaturation at 95 °C for 5 min, followed by 45 cycles of denaturation at 95 °C for 30 s, annealing at 50 °C for 45 s, extension at 72 °C for 2 min, and a final extension at 72 °C for 20 min. Amplified products were sequenced with the BigDye Terminator Kit® ver 1.1 (Applied Biosystems); sequenced products were separated and visualized on an ABI 3100 capillary sequencer (Applied Biosystems). Sequences were aligned and edited with SEQUENCHER 3.0 (Gene Codes Corporation). Specimens with sequences containing unique mutations were re-sequenced for confirmation.

Summary statistics for microsatellite data, including number of alleles, allelic richness, expected heterozygosity (unbiased gene diversity), and the inbreeding coefficient F_{IS}, measured as Weir and Cockerham's (1984) *f*, were obtained for each sample locality, with F-STAT (Goudet, 1995; version 2.9.3.2, http://www2.unil.ch/popgen/softwares/fstat.htm). Homogeneity among samples in allelic richness and unbiased gene diversity was tested with Friedman rank tests as implemented in procedure FREQ of SAS software (SAS Institute, Cary, NC). Tests of conformance of genotypes at each microsatellite to Hardy–Weinberg (HW) equilibrium expectations and tests of genotypic equilibrium between pairs of microsatellites were carried out for each sample locality, with an exact probability test as implemented in GENEPOP (Raymond and Rousset, 1995; Rousset, 2008, v. 4.0, http://kimura.univ-montp2.fr/~rousset/Genepop.htm). The exact probability in each test was estimated with a Markov chain approach (Guo and Thompson, 1992) that employed 5000 dememorizations, 500 batches and 5000 iterations per batch. Sequential Bonferroni correction (Rice, 1989) was applied for all multiple tests performed simultaneously. Occurrences of null alleles, large allele dropout, or stuttering were evaluated for each microsatellite in each sample, with the software MICROCHECKER (van Oosterhout et al., 2004; http://www.microchecker.hull.ac.uk/).

Summary statistics for mtDNA, including number of haplotypes, haplotype (nucleon) diversity, and nucleotide diversity, were obtained for each sample, with ARLEQUIN (Schneider et al., 2000; version 3.11, http://cmpg.unibe.ch/software/arlequin3/). Haplotype richness was estimated according to El Mousadik and Petit (1996) and with the software RAREFAC available at http://www.pierroton.inra.fr/genetics/labo/Software/Rarefac/. Homogeneity between pairs of samples in number of mtDNA haplotypes and in haplotype diversity was tested with a bootstrap resampling approach (after Dowling et al., 1996) in which the probability that the number of different haplotypes or haplotype diversity observed in one locality would be observed in a random sample of the same size in another locality was estimated. POP TOOLS (a free-ad in software for Excel, available at http://www.cse.csiro.au/poptools/index.htm) was used to randomly sample the number of fish sampled in one locality from another locality. Random sampling was performed 1000 times and the average number of observed haplotypes and their upper (0.95) and lower (0.05) percentiles recorded. Selective neutrality of variation in mtDNA in each of the five samples was tested with Fu's (1997) F_S statistic and Fu and Li's (1993) D^* and F^* statistics, as implemented in the DNASP package (Rozas et al., 2003; v. 5.00.07, http://www.ub.edu/dnasp/). Significance of F_S, D^*, and F^* was assessed from 10,000 coalescent simulations (after Rozas et al., 2003), based on the observed number of segregating sites in each sample. A statistical parsimony network (Templeton et al., 1992) of known and inferred (extinct and/or non-sampled) mtDNA haplotypes was generated with TCS v. 1.21 (Clement et al., 2000).

Homogeneity of allele (genic) and genotype distributions (microsatellites) and mtDNA haplotype distribution across sample localities was tested with exact tests, as implemented in GENEPOP, and analysis of molecular variance (AMOVA), as implemented in ARLEQUIN. Exact probabilities were estimated with a Markov chain method and employing the same parameters as used in tests of HW and genotypic equilibrium (see above). Significance of Φ_{ST} among localities from AMOVA was assessed by permutation (1,023 replicates for both microsatellites and mtDNA). Results of exact tests and AMOVA indicated significant heterogeneity among the five sample localities in both allele and genotype distributions and in mtDNA haplotype distribution. Exact tests were then used to test homogeneity of genotype (microsatellites) and haplotype (mtDNA) distributions between pairs of localities. Exact probabilities were estimated with a Markov chain method and employing the same

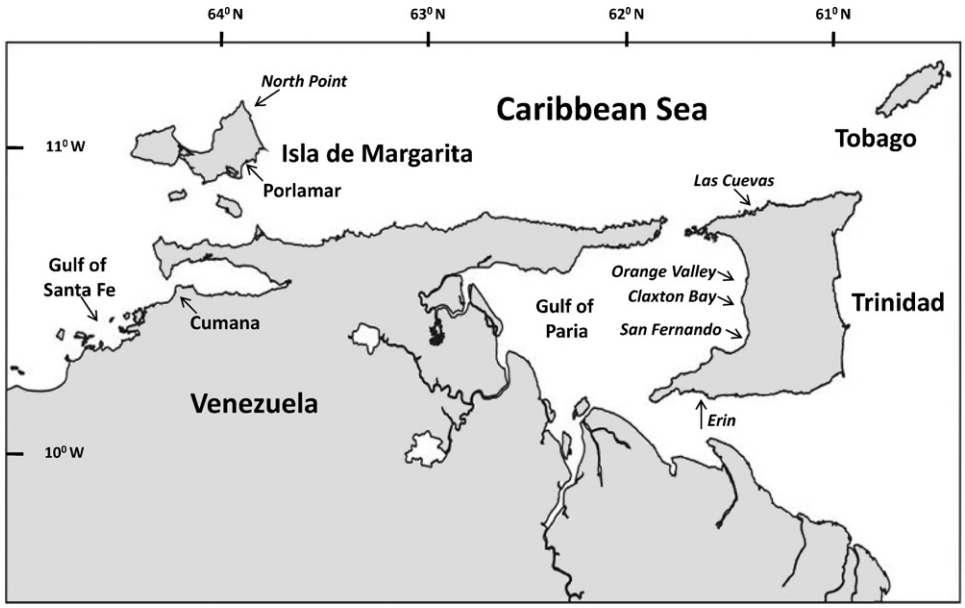

Fig. 1. Approximate sample localities for carite (*Scomberomorus brasiliensis*) in the southern Caribbean Sea.

parameters as used in tests of HW and genotypic equilibrium (see above).

Both microsatellite and mtDNA data were used to examine whether there was a relationship between genetic divergence and geographic distance, i.e., an isolation-by-distance effect, with multilocus spatial autocorrelation analysis (Smouse and Peakall, 1999; Peakall et al., 2003), as implemented in GenAlEx 6.0 (Peakall and Smouse, 2006). The spatial autocorrelation coefficient (r) was computed with linear, one-dimensional geographic distances between pairs of sample localities and the multilocus genetic distance outlined in Smouse and Peakall (1999). With isolation by distance, the estimated value of r among proximal localities (small distance classes) will differ significantly from zero and decrease with increasing distance between localities (larger distance classes). The distance class at which r no longer differs significantly from zero provides an approximation of the distance at which genetic divergence (population structure) can be detected (Peakall et al., 2003). Because estimation of spatial autocorrelation is influenced by the size of distance classes, r was computed based on a series of increasing, shore-line distances between pairs of localities (Peakall et al., 2003), with the first distance class reflecting the distance over which fish were obtained within each sample locality. Because the distribution of pairwise geographic distances between pairs of localities studied was uneven (Table 2), distance classes were selected in order to maximize pairs of localities within different distance classes. Significance of r was determined with random permutations of genotypes among localities. The distribution of r values under the null hypothesis of random spatial distribution of genotypes was used to determine the probability of significance of observed values of r according to a one-tailed test (i.e., r is significant if it lies beyond the upper 95% bound of the distribution). Significance of r also was tested by generating bootstrap 95% confidence intervals for r. Bootstrap values were obtained by sampling, with replacement, pairwise comparisons within a given distance class. Bootstrap resampling was performed 1000 times and significance of r inferred when the 95% CI did not overlap zero.

3. Results

Summary statistics, including number of alleles, allelic richness, and unbiased gene diversity for each of the 16 microsatellites, by locality, are given in Appendix A. The average number of alleles per microsatellite among localities ranged between 3.6 and 28.6 and averaged 13.4. Allelic richness and unbiased gene diversity among localities averaged 10.28 (range = 3.13–21.30) and 0.672 (range = 0.199–0.948), respectively. Neither allelic richness ($X^2 = 2.75$, $P = 0.600$) nor unbiased gene diversity ($X^2 = 3.43$, $P = 0.488$) differed significantly among the five localities. Significant departures from Hardy–Weinberg (HW) equilibrium expectations before, but not after, Bonferroni correction were found at seven microsatellites; in all but one case (*Sbr*28–two samples), departures from HW equilibrium occurred in only one of the five localities. Significant departure from Hardy–Weinberg equilibrium expectations both before and after Bonferroni correction was found for microsatellite *Sbr*14 in fish from the West Coast of Trinidad ($F_{IS} = 0.127$, $P = 0.000$) and for microsatellite *Sbr*18 in fish from Cumana ($F_{IS} = 0.160$, $P = 0.000$). Analysis with Microchecker indicated occurrence of null alleles at *Sbr*14 in fish from Cumana and the West Coast of Trinidad and at *Sbr*18 in fish from Cumana and the North Coast of Trinidad. As no other instances of possible null alleles at these two microsatellites in other areas was indicated by Microchecker, both *Sbr*14 and *Sbr*18 were used in all subsequent analyses. A total of 61 of 600 pairwise tests of genotypic disequilibrium were significant before Bonferroni correction; twelve of these (2%) remained significant after Bonferroni correction. Eleven of the 'significant' occurrences of genotypic disequilibrium involved different pairs of microsatellites at a single locality; one pair (*Sbr*14 and *Sbr*18) displayed 'significant' genotypic disequilibrium in two localities (Cumana and Isla de Margarita). Considering the absence of consistent, significant genotypic disequilibrium involving the same pair of microsatellites, all 16 microsatellites were treated as genetically independent.

Exact tests of homogeneity of allele and genotype distributions among localities were significant ($P = 0.000$ for both distributions) over all microsatellites (Table 1). Results from molecular analysis of variance (Amova) of microsatellite genotypes also indicated significant genetic heterogeneity as the genetic variance component (F_{ST}) attributable to variation among localities was 0.002 and differed significantly ($P = 0.000$) from zero. Exact tests of pairwise comparisons (Table 2) indicated that only comparisons between fish from Cumana and fish from the other four localities differed significantly ($P = 0.000$) following Bonferroni correction. Probability

Table 1
Probability of genic and genotypic homogeneity at 16 microsatellites among carite (*Scomberomorus brasiliensis*) from five localities in the southern Caribbean Sea. Probability values are based on exact tests; significance was assessed via a Markov-chain method.

Microsatellite	Genic homogeneity	Genotypic homogeneity
*Sni*13	0.272	0.377
*Sni*26	0.888	0.092
*Sni*29	0.448	0.444
*Sca*49	0.069	0.107
*Sca*61	0.042	0.087
*Sbr*6	0.158	0.216
*Sbr*9	0.284	0.286
*Sbr*14	0.006	0.038
*Sbr*16	0.085	0.130
*Sbr*18	0.046	0.097
*Sbr*19	0.886	0.894
*Sbr*24	0.000	0.000
*Sbr*26	0.074	0.095
*Sbr*28	0.754	0.773
*Sbr*35	0.010	0.014
*Sbr*36	0.172	0.233
Overall	0.000	0.000

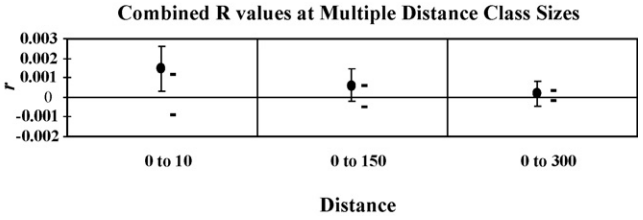

Fig. 2. Correlogram showing spatial autocorrelation (*r*) among carite (*Scomberomorus brasiliensis*) sampled from five localities in the southern Caribbean Sea. Estimates of *r* (ovals) are computed for increasing distance class sizes; 95% confidence error bars for *r* (plain error bars) as determined by bootstrapping over population pairs; upper and lower bounds (dashed error bars) of a 95% CI for *r* generated under the null hypothesis of a random distribution of carite among localities.

values for pairwise comparisons between fish from the south coast of Trinidad and fish from the west and north coasts of Trinidad were less than 0.05 (0.010 and 0.035, respectively) but were not significant following Bonferroni correction. Finally, spatial autocorrelation analysis, with microsatellite data from all five areas (Fig. 2), revealed that *r* values in the first distance class differed significantly from zero ($0.002 \geq r \geq 0.001$), becoming non-significant at distances between areas greater than 150 km. GenAlEx runs with the fish from Cumana excluded gave essentially the same result (data not shown). The distance (150 km) at which spatial autocorrelation is no longer significant provides an approximation of the distance at which genetic divergence (population structure) can be detected (Peakall et al., 2003). Based on the matrix of estimated geographic distances between individual areas (Table 3), results of the spatial autocorrelation analysis suggest that carite off the north and south coasts of Trinidad may have less gene exchange with one another than either does with carite off the west coast on Trinidad.

Summary statistics for mtDNA sequences, including the number of haplotypes and haplotype (nucleon) and nucleotide diversity, are given in Appendix A. The geographic distribution of the 41 different mtDNA haplotypes (GenBank Accession Numbers FJ415772–FJ415812) among the 130 individuals assayed is given in Appendix B. Haplotype #4 was the most common, occurring in 36–52% of the individuals in each locality. The number of haplotypes per locality ranged from 9 to 15, with haplotype richness ranging from 9.0 to 14.0; haplotype diversity ranged from 0.731 to 0.870. The 95% bootstrap confidence intervals for the number of mtDNA haplotypes and for haplotype diversity for each of the five localities overlapped, indicating homogeneity in the number of haplotypes and haplotype diversity among localities. Nucleotide diversity ranged from 0.0049 to 0.0121, with nucleotide diversity in fish from Cumana being nearly twice that of fish from the other four localities. As nucleotide diversity represents the average number of nucleotide differences per site between any two DNA sequences chosen randomly from a sampled population (Nei and Li, 1979), this finding indicates that fish from Cumana contain more divergent mtDNA haplotypes than fish from the other four localities. Results of tests of selective neutrality of mtDNA within each locality are shown in Table 4. Fu's (1997) F_S statistic was negative in each locality except Cumana and differed significantly from zero in fish from the west coast of Trinidad ($P = 0.043$) and the south coast of Trinidad ($P = 0.002$). Fu and Li's (1993) D^* and F^* statistics also were negative except for D^* in fish from Cumana; none of the D^* and F^* statistics differed significantly from zero.

An exact test of homogeneity in mtDNA haplotype distribution among localities was significant ($P = 0.028$). Results from Amova also indicated significant genetic heterogeneity among the five localities; the genetic variance component (F_{ST}) attributable to variation among localities was 0.042 and differed significantly ($P = 0.002$) from zero. Exact tests of pairwise comparisons of mtDNA haplotype distributions are given in Table 5. Pairwise comparisons of mtDNA haplotype distributions between fish from Cumana and fish from the north and south coasts of Trinidad differed significantly before, but not after, sequential Bonferroni correction; all remaining pairwise comparisons were non-significant. Estimates of the spatial autocorrelation coefficient (*r*), based on mtDNA data, did not differ significantly from zero in any distance class.

Table 2
Probability values from pairwise exact tests of homogeneity in microsatellite genotype distributions among carite (*Scomberomorus brasiliensis*) from five localities in the southern Caribbean Sea. Asterisks indicate significance before, but not after Bonferroni, correction; boldface indicates significance following sequential Bonferroni correction.

	North Coast	West Coast	South Coast	Isla de Magarita	Cumana
North Coast	–	0.297	0.035*	0.781	**0.000**
West Coast		–	0.010*	0.613	**0.000**
South Coast			–	0.086	**0.000**
Isla de Magarita				–	**0.000**
Cumana					–

Table 3
Approximate pairwise distances (in km) between sample localities of carite, *Scomberomorus brasiliensis*.

	North Coast	West Coast	South Coast	Isla de Magarita	Cumana
North Coast	–	105	190	290	360
West Coast		–	85	305	375
South Coast			–	395	460
Isla de Magarita				–	125
Cumana					–

Table 4
Fu's (1997) F_S and Fu and Li's (1993) D^* and F^* measures of selective neutrality, based on variation in a 581 base-pair fragment of the mitochondrially encoded ND-4 gene, for carite (*Scomberomorus brasiliensis*) from five localities in the southern Caribbean Sea. Probabilities of significance were estimated from coalescent simulations (Rozas et al., 2003).

	F_S	P	D^*	P	F^*	P
North Coast	−1.315	0.271	−0.172	>0.100	−0.352	>0.100
West Coast	−3.759	0.043	−0.686	>0.100	−1.042	>0.100
South Coast	−6.514	0.002	−1.468	>0.100	−1.777	>0.100
Isla de Margarita	−1.567	0.249	−0.369	>0.100	−0.784	>0.100
Cumana	0.563	0.623	0.037	>0.100	−0.149	>0.100

Table 5
Probability values from pairwise exact tests of homogeneity in mtDNA haplotype distributions among carite (*Scomberomorus brasiliensis*) from five localities in the southern Caribbean Sea. Boldface indicates significance before but not after sequential Bonferroni correction.

	North Coast	West Coast	South Coast	Isla de Magarita	Cumana
North Coast	–	0.436	0.422	0.385	**0.028**
West Coast		–	0.403	0.189	0.089
South Coast			–	0.193	**0.028**
Isla de Magarita				–	0.087
Cumana					–

The parsimony network of the mtDNA haplotypes (Fig. 3) revealed that 37 of the mtDNA haplotypes occurred in clades of varying size. Four of the mtDNA haplotypes (#s 6, 8, 10, and 11), all found in fish from Cumana, however, were well displaced from the remainder and differed, on average, from all other carite mtDNA haplotypes by approximately 2.9%. A total of 12 or more base-pair substitutions separated this small clade of four haplotypes (containing six individuals) from the nearest (inferred) neighboring clade, suggesting that the six fish in this clade could be representatives of a different taxon. To examine this possibility, both global and sample-pairwise exact tests and AMOVA were rerun, but with the six individuals from the divergent mtDNA clade removed from the dataset. For microsatellites, probability values for both global and pairwise exact tests on the revised dataset were virtually identical to those obtained with the original dataset, i.e., there was significant heterogeneity overall, with carite from Cumana differing significantly from carite at other four localities and with carite from the south coast of Trinidad differing marginally (i.e., before, but not after, Bonferroni correction) from those sampled from the west ($P = 0.010$) and north ($P = 0.036$) coasts of Trinidad. Results from AMOVA ($F_{ST} = 0.002$, $P = 0.002$) also were virtually the same. For mtDNA, removal of the six individuals from the mtDNA data set resulted in a non-significant F_{ST} of −0.005 ($P = 0.597$) in AMOVA; all pairwise exact tests also were non-significant. Multidimensional scaling of the microsatellite data, as implemented in procedure MDS of SAS software (SAS Institute, Cary, NC), was carried out to assess whether the six individuals in the divergent mtDNA clade were outliers relative to their microsatellite genotypes. All six individuals fell within the central 'cloud' of composite microsatellite genotypes for carite from all five localities (data not shown), indicating that these six individuals are not separated in their nuclear-microsatellite genotypes from the other carite studied.

4. Discussion

Homogeneity testing (exact tests and analysis of molecular variance) revealed significant differences among carite from the five localities in both microsatellite allele and genotype distributions. Pairwise exact tests (microsatellites) revealed that carite from Cumana differed significantly ($F_{ST} > 0$) from those in the remaining four localities both before and after Bonferroni correction for multiple tests; F_{ST} values in pairwise comparisons between carite from the south coast of Trinidad and those from the north and west coasts differed significantly from zero before, but not after, Bonferroni correction. Estimates of microsatellite (genetic) variation, measured as allelic richness and gene diversity, did not differ among carite from the five localities.

Initial analyses of mtDNA variation gave nearly identical results; an exact test and an analysis of molecular variance indicated significant heterogeneity in mtDNA haplotype distribution among the five localities, while pairwise exact tests indicated significant heterogeneity (before, but not after, Bonferroni correction) between fish from Cumana and fish from both the North and South coasts of Trinidad. Estimates of mtDNA variation, measured as haplotype richness and haplotype diversity, did not differ among carite from the five localities. Nucleotide diversity, however, among carite from Cumana was nearly twice that of carite from the other four localities, indicating occurrence of very divergent haplotypes within the sample of carite from Cumana. A parsimony network of mtDNA haplotypes revealed the presence of a small clade of four haplotypes (six fish) from Cumana that differed by 12 or more base-pair substitutions from the nearest (inferred) neighboring clade. When fish with these four haplotypes were removed from the dataset, tests of homogeneity for microsatellite gene and genotype distributions remained unchanged (i.e., carite from Cumana still differed significantly from the rest), whereas mtDNA haplotypes distributions among localities became homogeneous.

The average degree of sequence difference between the clade comprised of the four divergent mtDNA haplotypes (found at the same locality) and all remaining carite mtDNA haplotypes was approximately 2.9% as compared to an average sequence difference of 0.6% among the latter. Composite microsatellite genotypes of the six fish that possessed the divergent mtDNA haplotypes, however, did not differ from composite microsatellite genotypes of all other carite sampled, indicating that the six fish likely do not represent a genetically isolated taxon. The pattern where discontinuous mtDNA haplotypes co-occur at a given geographic site (Category II of Avise et al., 1987) is not common but has been observed in populations of other vertebrates, including fishes (Avise et al., 1984; Taberlet et al., 1992; Świslocka et al., 2008; Tominaga et al., 2009) and is generally hypothesized to stem either from recent admixture of formerly isolated populations or from hybridization and introgression of mtDNA from one species into another (Avise et al., 1987; Taberlet et al., 1992). Of the two possibilities, the former would seem the more likely in our study, as the same mtDNA fragment of the closest relative in the region, the king mackerel *Scomberomorus cavalla*, differs from that of carite by roughly 17.6% (M.A. Renshaw, unpublished data). Identification of the population or stock from which the divergent mtDNA lineage arose awaits further sampling; based on geologic and hydrographic considerations (see below), the cryptic population, if it still exists, would most likely be to the west,

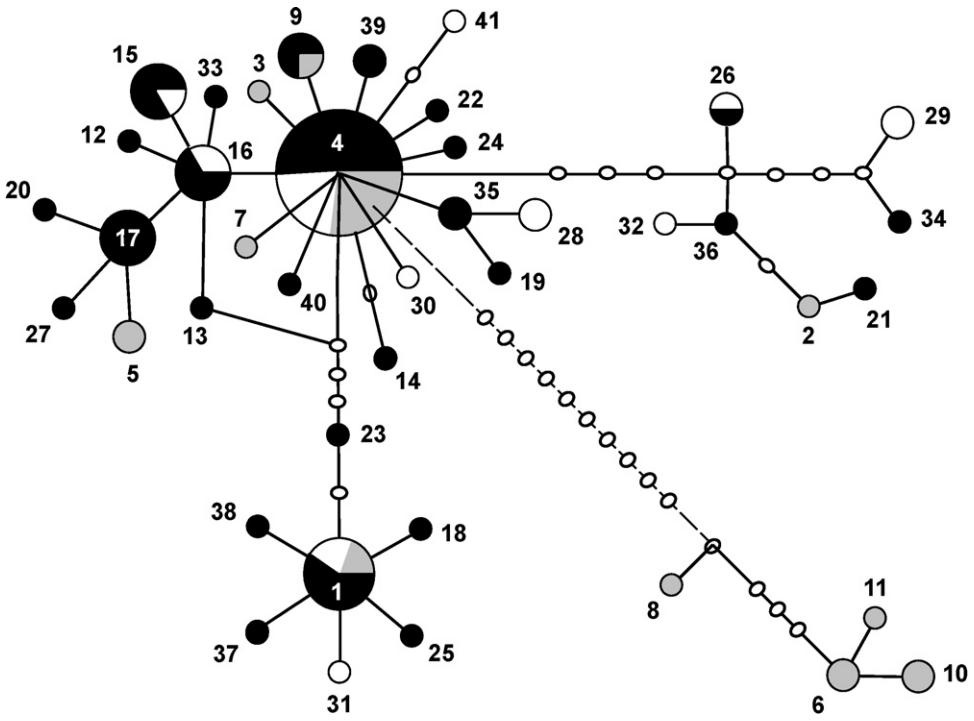

Fig. 3. Parsimony network of ND-4 mtDNA haplotypes in carite (*Scomberomorus brasiliensis*) from the southern Caribbean Sea. Black represents mtDNA haplotypes, by proportion within circles, found in fish sampled from waters off Trinidad; white represents mtDNA haplotypes, by proportion in circles, found in fish sampled from waters off Isla de Margarita; and gray represents mtDNA haplotypes, by proportion within circles, found in fish sampled from waters off the mainland of Venezuela (Cumana). Sizes of circles are scaled to reflect their relative frequencies. Small, unnumbered circles represent undetected mtDNA haplotypes. Lines between haplotypes represent one base-pair substitution regardless of length.

particularly as the other carite examined in this study were from the east.

The divergent mtDNA lineage notwithstanding, results of homogeneity testing of the microsatellite data indicate that carite sample from Cumana represents a distinct population or stock relative to carite from the other four localities. Occurrence of a population or stock of carite in this area, distinct from carite to the east, is not altogether surprising given the geology and hydrology of the region. Briefly, the area between Cumana and the Gulf of Santa Fe forms the southern portion of the Cariaco Basin, an east-west, pull-apart basin (Schubert, 1982) located on the continental shelf off the north coast of Venezuela. The basin is semi-isolated from the rest of the Caribbean Sea by a 150 m deep sill located between the Isla de Margarita to the east and Cabo Codera to the west (Smoak et al., 2004). Connections between the Cariaco Basin and the open Caribbean Sea are only through two shallow channels, one to the north and one to the west, both of which are reduced in times of lower sea level (Lane-Serff and Pearce, 2009). In addition, water circulation inside the basin is restricted and displacement from the basin is controlled largely by prevailing winds from the east-northeast (Okuda, 1982), which effectively would 'push' fish at any life-history stage to the west, not east towards Isla de Margarita and Trinidad.

Results of spatial autocorrelation analysis of the microsatellite data indicated an isolation-by-distance effect, with or without the sample from Cumana, with the autocorrelation becoming non-significant at approximately 150 km. The average distances between the three localities in Trinidad and the two localities in Venezuela are ~330 km (Isla de Margarita) and ~400 km (Cumana), consistent with the hypothesis that the carite sampled in waters off Trinidad are partially isolated genetically from the carite sampled in waters off Venezuela. In addition, the distance between the localities off the south and north coasts of Trinidad is ~190 km, beyond the distance at which spatial autocorrelation became zero. This was consistent with pairwise exact tests (microsatellites) where carite from the South Coast differed from carite from the west and north coasts before (but not after) Bonferroni correction. Carite from the south coast of Trinidad also appear to have undergone a different demographic event than carite sampled elsewhere as tests of selective neutrality of mtDNA variation were non-significant for each locality sampled except for the south coast (Fu's $F_S = -6.514$, $P = 0.002$). The F_S approach detects population growth and/or genetic hitch-hiking. As mtDNA, in the genetic sense, is a single locus, the significant, negative F_S value observed in carite from the south coast indicates there has been either a 'selective sweep' or a recent reduction in female effective size (female because mtDNA is inherited through the maternal parent) in the area, resulting in an excess of rare mtDNA variants over those expected under a neutral model (Kaplan et al., 1989). Collectively, the analyses of microsatellite data and the neutrality tests of mtDNA suggest that carite off the south coast of Trinidad may be partially isolated genetically from carite along the west and north coasts and may require management as a distinct stock. However, as fish from different areas were neither aged as to cohort nor sampled in the same year, we cannot exclude the possibility that temporal variation in allele and genotype (microsatellite) and haplotype (mtDNA) distributions could impact the results and confound patterns of spatial divergence. Future assessment of the temporal reproducibility of the spatial genetic patterns observed would certainly be warranted.

Relatively little has been reported about carite spawning and migration in the southern Caribbean Sea. The species is not thought to migrate extensively (Collette and Nauen, 1983), with one report (Batista and Fabrè, 2001) suggesting that carite migration in Trinidadian waters covers about 70 nautical miles (~130 km). Sturm (1974) observed peak spawning between October and April in low salinity waters in the Gulf of Paria off the southwest coast of Trinidad and hypothesized occurrence of a post-spawning, 'clockwise' feeding migration northward to higher salinity waters off the north coast. This hypothesized migration pattern could lead to an

isolation-by-distance effect from south to north if the number of successful migrants decreased along a northward gradient and/or if there were some degree of philopatry to the spawning grounds off the southwestern coast. Results of homogeneity testing and spatial autocorrelation analysis of the microsatellite data are consistent with the above and indicate the possibility that there may be a number of carite spawning locations within the region.

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Appendix A.

Summary statistics for 16 nuclear-encoded microsatellites and a 581 base-pair sequence of the mitochondrially encoded ND-4 gene for carite (*Scomberomorus brasilienses*) from five localities in the southern Caribbean Sea.

Microsatellite	South Coast Trinidad	West Coast Trinidad	North Coast Trinidad	Isla de Margarita	Cumana
*Sni*13					
n	130	100	158	109	29
#*A*	5	6	5	6	3
A_R	3.95	3.55	4.11	3.80	3.00
H_E	0.245	0.160	0.281	0.247	0.101
P_{HW}	0.120	1.000	0.079	0.692	1.000
F_{IS}	0.058	−0.061	0.055	−0.003	−0.024
*Sni*26					
n	130	100	158	109	29
#*A*	15	16	18	18	13
A_R	11.73	12.67	13.41	13.75	13.00
H_E	0.851	0.864	0.846	0.854	0.742
P_{HW}	0.857	0.335	0.385	0.056	0.824
F_{IS}	0.005	0.004	−0.025	0.033	−0.069
*Sni*29					
n	130	100	158	109	29
#*A*	4	4	4	3	3
A_R	3.19	3.29	3.17	3.00	3.00
H_E	0.237	0.349	0.275	0.357	0.251
P_{HW}	1.000	0.718	0.516	0.821	1.000
F_{IS}	−0.071	−0.087	0.035	−0.055	−0.098
*Sca*49					
n	130	100	158	109	29
#*A*	6	5	6	7	3
A_R	3.84	3.79	3.52	4.62	3.00
H_E	0.440	0.448	0.386	0.447	0.581
P_{HW}	0.765	0.333	0.828	0.103	0.099
F_{IS}	−0.031	0.041	0.001	0.076	0.051
*Sca*61					
n	130	100	158	109	29
#*A*	5	8	6	8	4
A_R	3.52	5.20	4.44	4.55	4.00
H_E	0.193	0.207	0.201	0.198	0.194
P_{HW}	0.250	0.040	0.096	0.164	1.000
F_{IS}	0.005	0.178	0.089	0.072	−0.067
*Sbr*6					
n	130	100	158	109	29
#*A*	20	19	20	21	12
A_R	15.79	15.97	15.43	16.18	12.00
H_E	0.926	0.927	0.925	0.924	0.900
P_{HW}	0.568	0.033	0.937	0.815	0.317
F_{IS}	0.036	0.083	0.001	0.017	0.042
*Sbr*9					
n	130	99	158	109	29
#*A*	11	12	13	11	12
A_R	9.59	9.65	10.36	9.34	12.00
H_E	0.860	0.853	0.863	0.852	0.862
P_{HW}	0.349	0.326	0.323	0.283	0.194
F_{IS}	−0.038	0.006	0.018	0.085	−0.040
*Sbr*14					
n	130	100	158	109	29
#*A*	30	28	32	34	19
A_R	22.12	21.28	21.84	22.27	19.00
H_E	0.952	0.950	0.950	0.951	0.938
P_{HW}	0.434	0.000	0.156	0.105	0.115
F_{IS}	0.039	0.127	0.001	0.064	0.045

Appendix A (*Continued*)

Microsatellite	South Coast Trinidad	West Coast Trinidad	North Coast Trinidad	Isla de Margarita	Cumana
*Sbr*16					
n	130	100	158	109	29
#*A*	16	15	21	15	12
A_R	11.02	11.71	12.23	10.68	12.00
H_E	0.869	0.880	0.867	0.868	0.839
P_{HW}	0.086	0.331	0.008	0.545	0.077
F_{IS}	0.097	−0.011	0.080	0.060	−0.069
*Sbr*18					
n	130	96	158	109	29
#*A*	26	26	27	29	18
A_R	19.54	19.13	19.78	20.22	18.00
H_E	0.942	0.942	0.943	0.946	0.944
P_{HW}	0.874	0.413	0.065	0.575	0.000
F_{IS}	0.020	0.038	0.074	−0.009	0.160
*Sbr*19					
n	130	100	158	109	29
#*A*	23	19	25	20	16
A_R	14.69	14.95	15.80	15.08	16.00
H_E	0.879	0.887	0.886	0.867	0.865
P_{HW}	0.201	0.644	0.090	0.004	0.137
F_{IS}	−0.006	−0.071	0.007	0.111	0.163
*Sbr*24					
n	130	100	158	109	29
#*A*	9	9	11	10	5
A_R	6.57	6.23	6.73	7.09	5.00
H_E	0.405	0.491	0.509	0.461	0.475
P_{HW}	0.433	0.841	0.513	0.852	0.162
F_{IS}	0.013	−0.100	0.005	−0.014	−0.016
*Sbr*26					
n	130	100	158	109	29
#*A*	18	21	18	15	11
A_R	13.02	12.94	12.45	12.17	11.00
H_E	0.872	0.847	0.868	0.854	0.877
P_{HW}	0.992	0.126	0.079	0.213	0.739
F_{IS}	−0.006	0.044	0.045	0.012	0.018
*Sbr*28					
n	130	99	158	109	29
#*A*	9	8	10	10	7
A_R	6.01	6.22	5.84	6.44	7.00
H_E	0.421	0.414	0.412	0.455	0.528
P_{HW}	0.005	0.001	0.661	0.321	0.638
F_{IS}	0.160	0.195	0.064	−0.049	−0.111
*Sbr*35					
n	130	100	158	109	29
#*A*	19	15	19	18	14
A_R	13.75	12.45	13.07	13.90	14.00
H_E	0.892	0.867	0.889	0.901	0.813
P_{HW}	0.082	0.060	0.025	0.099	0.988
F_{IS}	0.052	−0.026	0.096	−0.028	−0.145
*Sbr*36					
n	130	100	158	109	29
#*A*	7	7	7	8	6
A_R	5.27	5.89	5.24	5.40	6.00
H_E	0.711	0.733	0.694	0.700	0.700
P_{HW}	0.376	0.002	0.178	0.717	0.526
F_{IS}	−0.092	0.072	0.060	−0.114	−0.084
MtDNA					
n	25	25	25	26	29
#*H*	15	13	9	10	11
H_R	14.0	12.0	8.0	9.0	9.1
H_D	0.870	0.867	0.813	0.767	0.731
π_D	0.0064	0.0067	0.0049	0.0069	0.0121

For microsatellites (MSAT): *n* is sample size, #*A* is number of alleles, A_R is allelic richness, H_E is gene diversity (expected heterozygosity), P_{HW} is probability of conforming to expected Hardy–Weinberg genotypic proportions, and F_{IS} is an inbreeding coefficient measured as Weir and Cockerham's (1984) *f*.
For MTDNA: *n* is sample size, #*H* is number of haplotypes (alleles), H_R is haplotype richness, H_D is haplotype (nucleon) diversity, and π_D is nucleotide diversity.

Appendix B.

Spatial distribution of mtDNA haplotypes among carite (*Scomberomorus brasiliensis*) from five localities in the southern Caribbean Sea.

Haplotype #	North Coast	West Coast	South Coast	Isla de Margarita	Cumana
#1	4	2	0	2	2
#2	0	0	0	0	1
#3	0	0	0	0	1
#4	10	9	9	12	15
#5	0	0	0	0	2
#6	0	0	0	0	2
#7	0	0	0	0	1
#8	0	0	0	0	1
#9	1	1	1	0	1
#10	0	0	0	0	2
#11	0	0	0	0	1
#12	1	0	0	0	0
#13	1	0	0	0	0
#14	1	0	0	0	0
#15	2	2	1	1	0
#16	3	0	1	2	0
#17	2	1	3	0	0
#18	0	0	1	0	0
#19	0	0	1	0	0
#20	0	0	1	0	0
#21	0	0	1	0	0
#22	0	0	1	0	0
#23	0	0	1	0	0
#24	0	0	1	0	0
#25	0	0	1	0	0
#26	0	0	1	1	0
#27	0	0	1	0	0
#28	0	0	0	2	0
#29	0	0	0	2	0
#30	0	0	0	1	0
#31	0	0	0	1	0
#32	0	0	0	1	0
#33	0	1	0	0	0
#34	0	1	0	0	0
#35	0	2	0	0	0
#36	0	1	0	0	0
#37	0	1	0	0	0
#38	0	1	0	0	0
#39	0	2	0	0	0
#40	0	1	0	0	0
#41	0	0	0	1	0

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