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PLACING THE FLORIDIAN MARINE GENETIC DISJUNCTION INTO A REGIONAL EVOLUTIONARY CONTEXT USING THE SCORCHED MUSSEL, *BRACHIDONTES EXUSTUS*, SPECIES COMPLEX

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Abstract.—The well-documented Floridian Gulf/Atlantic marine genetic disjunction provides an influential example of presumed vicariant cladogenesis along a continental coastline for major elements of a diverse nearshore fauna. However, it is unclear if this disjunction represents a local anomaly for regionally distributed morphospecies, or if it is merely one of many such cryptic phylogenetic splits that underlay their assumed genetic cohesiveness. We aimed to place the previously characterized scorched mussel Gulf/Atlantic genetic disjunction into a regional phylogenetic perspective by incorporating genotypes of nominal conspecifics sampled throughout the Caribbean Basin as well as those of eastern Pacific potential geminate species. Our results show it to be one of multiple latent regional genetic disjunctions, involving five cryptic Caribbean species, that appear to be the product of a long history of regional cladogenesis. Disjunctions involving three stem lineages clearly predate formation of the Isthmus of Panama and of the Caribbean Sea, although four of the five cryptic species have within-basin sister relationships. Surprisingly, the Atlantic clade was also found to be widespread in the southern Caribbean, and ancestral demography calculations through time for Atlantic coast-specific genotypes are consistent with a northward range extension after the last glacial maximum. Our new data seriously undermine the hypothesis of a Floridian vicariant genesis and imply that the scorched mussel Gulf/Atlantic disjunction represents a case of geographic and temporal pseudocongruence. All five Caribbean Basin cryptic species exhibited an intriguing pattern of predominantly allopatric distribution characterized by distinct geographic areas of ecological dominance, often adjoining those of sister taxa. This pattern of distribution is consistent with allopatric speciation origins, coupled with restricted postspeciation range extensions. Several lines of indirect evidence favor the hypothesis that the predominantly allopatric distributions are maintained over evolutionary time scales, primarily by postrecruitment ecological filters rather than by oceanographic barriers to larval-mediated gene flow.

Key words.—Bivalvia, Caribbean, cryptic species, genetic disjunction, phylogeny, pseudocongruence.

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Comparative molecular studies can yield important and novel insights into marine biodiversification processes by placing the genetic structuring of taxa into an inferred historical context (Grosberg and Cunningham 2001). Of particular interest are studies in which previously unsuspected genetic discontinuities, common to diverse faunal elements, have been uncovered (Lavery et al. 1996; Borsa et al. 1997; Chase et al. 1998; Gopurenko et al. 1999), and the most prominent such case concerns the marine fauna of peninsular Florida (reviewed by Avise 1992, 2000; Cunningham and Collins 1994, 1998; Palumbi 1994).

From a marine biogeographic perspective, peninsular Florida provides one of the most intriguing and best-studied nearshore evolutionary landscapes. The southern section of the Floridian landmass projects into tropical marine waters, thereby isolating disjunct warm temperate water bodies on its northeastern (Atlantic) and northwestern (Gulf of Mexico) flanks. Briggs (1970, 1974), on the basis of faunistic similarity, grouped both Atlantic and Gulf warm temperate faunas into the Carolinian zoogeographic province. Many, but by no means all, Carolinian morphospecies presently have a distinctly allopatric distribution along the Floridian coastline (Avise 1992, 2000; Cunningham and Collins 1994, 1998). Their last direct contact may have occurred during Pleistocene glacial maxima (Cronin 1988; Pielou 1991; Wares 2002) or earlier via the Miocene Suwannee Seaway, a former marine connection across the northern flank of the peninsula (Bert 1986; Bert and Harrison 1988; Webb 1990; Cunningham et al. 1991; Felder and Staton 1994; Randazzo and Jones 1997).

Replicate genetic characterizations of nominal species (including continuously distributed taxa) found on either flank of the Floridian peninsula have revealed cryptic phylogenetic disjunctions among diverse Gulf/Atlantic Carolinian marine faunal elements (Avise 2000). Although some of the studied taxa showed no obvious genetic structuring along the peninsular coastline (Gold and Richardson 1998; Avise 2000; Kirkendale et al. 2004), many yielded a Gulf/Atlantic genetic disjunction in which genetic divergence levels among the two disjunct populations far surpassed that observed within either population (Avise 2000). Such disjunct patterns have been detected in a wide variety of nearshore species (Bert 1986; Saunders et al. 1986; Avise et al. 1987; Bert and Harrison 1988; Cunningham et al. 1991; Sarver et al. 1992; Felder and Staton 1994; Bert and Arnold 1995; Duggins et al. 1995; Ó Foighil et al. 1996; Schizas et al. 1999; Avise 2000; Collin 2001, 2002), with by far the most intensively studied exemplar being the continuously distributed American oyster, *Crassostrea virginica* (Reeb and Avise 1990; Karl and Avise 1992; Hare and Avise 1996, 1998; Hare et al. 1996; McDonald et al. 1996).

The well-documented Gulf/Atlantic genetic disjunction is important because it has become the most influential example of how vicariance events may profoundly mold the genetic structuring of a diverse nearshore marine fauna in an osten-

sibly continuous coastal environment (Avise 2000). However, the general applicability of the Gulf/Atlantic study system is limited because its findings have not been placed within a regional geographic and phylogenetic perspective. Does it represent a local anomaly that has no equivalent in and little phylogenetic relevance to other parts of the geographic range of regionally distributed morphospecies? Or might it represent but one of a nested series of cryptic phylogenetic splits that underlay their assumed genetic cohesiveness?

These are pertinent questions because a large fraction of the Carolinian fauna is not restricted to this particular zoogeographic province. In many cases, such as the scorched mussel, *Brachidontes exustus*, the nominal range of constituent morphospecies extends from the Atlantic coast of the southeastern United States, throughout the Gulf of Mexico and the Caribbean, and also includes Bermuda (Abbott 1974; Sterrer 1986). This expansive regional range encompasses a much more complex evolutionary landscape in which the Suwannee Seaway closure represents but one local element of a dynamic geological history that has profoundly reconfigured continental, oceanic, and archipelagean interfaces (Droxler et al. 1998; Iturralde-Vinent and MacPhee 1999).

Until recently, the prevailing paradigm for Caribbean marine taxa was that they typically exhibited extensive within-Basin distributions in association with little apparent genetic structuring (Mitton et al. 1989; Lacson 1992; Hateley and Sleeter 1993; Shulman and Bermingham 1995; Lessios et al. 2001, 2003; Rocha et al. 2002; Williams and Reid 2004). However, more recent reef fish studies have begun to challenge this consensus by uncovering latent genetic structuring, despite extended pelagic larval development, that either has a clear allopatric signature, as in the cleaner goby, *Elacatinus* (Taylor and Hellberg 2003a), or else is consistent with parapatric ecological speciation, as in *Halichoeres* wrasses (Rocha et al. 2005). The *Elacatinus* data has been the subject of contrasting genes versus oceanography interpretations, with the former proposing that the geographic partitioning of discrete lineages is maintained by genetically mediated aspects of this species' larval or postlarval ecology (Taylor and Hellberg 2003b; Warner and Palumbi 2003), and the latter emphasizing the role of oceanographic features in promoting passive local larval retention (Colin 2003).

Brachidontes mussels date at least from the Jurassic (Coan et al. 2000) and are common constituents of regional intertidal faunas, typically attaching to hard substrates using byssal threads (Abbott 1974; Sterrer 1986). They have a planktotrophic larval mode of development, which may persist for up to 40 days in laboratory conditions (Campos and Ramorino 1980; Fields and Moore 1983). We have recently characterized the genetic structure of Floridian *B. exustus* populations using mitochondrial and nuclear markers (Lee and Ó Foighil 2004). Both sets of markers recovered the expected disjunction involving sister clades distributed on alternate flanks of peninsular Florida and lineage-specific mitochondrial molecular clocks placed its origin in the Pliocene. Our primary novel result, however, was the discovery that the Gulf/Atlantic disjunction represents but one of three cryptic, nested genetic discontinuities represented in Floridian scorched mussel populations. The most pronounced phylogenetic split distinguished the Gulf and Atlantic sister clades from two additional cryptic sister clades present in samples taken from the southern Florida tropical marine zone. Floridian populations of *B. exustus* were composed of four cryptic taxa, a result consistent with the hypothesis that the Gulf/Atlantic disjunction in this morphospecies is but one of multiple latent regional genetic breakpoints.

The goal of this present study was to place the Gulf/Atlantic scorched mussel genetic disjunction into a regional phylogenetic perspective by incorporating genotypes of nominal conspecifics sampled throughout the Caribbean Basin as well as those of eastern Pacific potential geminate species. Our results indicated that this regional morphospecies is composed of three stem lineages that predate closure of the Isthmus of Panama and incorporates five cryptic Caribbean species that display largely allopatric within-basin distributions. The Atlantic clade was found to be widespread in the southern Caribbean, and the Atlantic coast population appears to have resulted from a northward range extension after the last glacial maximum. The scorched mussel Gulf/Atlantic disjunction appears to represent a case of geographic and temporal pseudocongruence (Cunningham and Collins 1994) and may not be a product of Floridian vicariance.

Materials and Methods

Samples

Nominal specimens of *B. exustus* were sampled from 22 localities throughout the Caribbean Basin (Table 1). It is now clear that *B. exustus* is a cryptic species complex (Lee and Ó Foighil 2004) and, whenever possible, at least 15 individuals per locality were genotyped to ensure that the primary local genotypes were sampled. Additional *Brachidontes* species were collected, including *B. modiolus*, a morphologically distinct Caribbean congener; two Panamian eastern Pacific species, *B. adamsianus* and *B. semilaevis*; and a variety of other global congeners. We used regional *Geukensia* and *Ischadium* taxa as outgroups in addition to representatives of four other mytilid genera (*Lithophaga*, *Modiolus*, *Mytilus*, and *Septifer*). A summary of the sampling locations and of voucher specimen information is outlined in Table 1. Detailed sampling records have been deposited together with the voucher specimens in the Mollusk Collection of the University of Michigan's Museum of Zoology. Specimens were preserved in 95% ethanol prior to molecular characterization.

Molecular Data

Total genomic DNA was isolated from the posterior adductor muscle to avoid male gonadal tissue enriched with paternally transmitted ''male'' mitochondrial genomes prevalent in Mytilidae (Rawson and Hilbish 1995; Quesada et al. 1996; Lee and Ó Foighil 2004). We are confident that this measure was successful due to the lack of heteroplasmy observed in our direct sequences and to the topological congruence among our mitochondrial and nuclear markers (Lee and Ó Foighil 2004). The extraction was done using a DNeasy Tissue Kit (Qiagen, Valencia, CA) according to the manufacturer's instructions. Three target fragments were amplified and directly sequenced: mitochondrial cytochrome *c* oxidase subunit I (COI), nuclear ribosomal large subunit (28S), and

TABLE 1. Samples collected for this study with voucher specimen information (University of Michigan Museum of Zoology catalog number).

Taxa	Locality	Code	N^1	N^2	N^3	Catalog no.
Brachidontes exustus	Panacea, FL	PA	15	1	1	300111
	Cedar Key, FL	CK	16	1	1	300112
	Bradenton, FL	BR	3	1	1	300113
	Marco, FL	MA	15	1	1	300114
	Boca Chica Key, FL	BC	17	5	6	300118, 300120
	Horseshoe, FL	HS	15	1	3	300115, 300121
	Key Biscayne, FL	KB	8	2	3	300122-3
	Sebastian Inlet, FL	SI	15	1	1	300116
	Wassaw Island, GA	WI	15	2	2	300117
	New Providence, Bahamas	BA	15	2	3	300119
	Bermuda Island, U.K.	BE	15	1	2	300151
	Habana, Cuba	CU	16	1	2	300154
	St. Ann's Bay, Jamaica	JA	15	1	1	300155
	Fajardo, Puerto Rico	PR	16	1	1	300156
	Barbados	BB	16	1	2	300158-9
	Chaguaramas, Trinidad	CT	10	2	2	300167
	Maracas Bay, Trinidad	MT	9	1	3	300160
	Isla de Margarita, Venezuela	VE	15	1	3	300161-5
	Isla Blanquilla, Venezuela	VE	1	1	1	300152
	Querepare, Venezuela	VE	2			300166
	Bocas del Tora, Panama	BT	27	4	4	300168-70
	Veracruz, Mexico	VC	17	1	1	300171
B. adamsianus	Cuastecomate, Mexico	CM	3			300174
	Puerto Vallarta, Mexico	PV	15	1	1	300175
	Bique Beach, Panama	BI	7	1	1	300176
	Naos, Panama	NA	9	1	2	300177
	Isla Jicarón, Panama				1	300178
	Isla Secas, Panama				2	300179
B. puniceus	Cape Verde	CV	5	2	2	300153, 300157
B. semilaevis	Chumical, Panama	CH	15	2	2	300180
B. modiolus	Key Biscayne, FL		1	1	1	300124
	Long Key, FL		2	1	2	300125
	Barbados		3			300173
	Isla Cubagua, Venezuela		3			300172
B. mutabilis	Okinawa, Japan				AB103124*	
B. rodriguezi	Mar del Plata, Argentina				2	300181
B. semistriatus	Kwazulu-Natal, South Africa				2	300186
B. variabilis	Hong Kong				1	300185
B. sp. 1	Darwin Harbor, Australia				1	300182
B. sp. 2	Darwin Harbor, Australia				1	300183
	Hong Kong				1	300184
Geukensia demissa	Wassaw Island, GA			1	1	300131
	Atlantic Coast, USA		U56844*			
	North Falmouth, MA				AY145405*	
G. granosissima	Bradenton, FL		4	1	1	300129
	Marco, FL		2	1	1	300130
Ischadium recurvum	Panacea, FL		6	1	1	300126
	Cedar Key, FL		3	1	1	300127
	Everglades City, FL		1	1	1	300128
Lithophaga curta	Ooiso, Japan				AB103123*	
Modiolus nipponicus	Manazuru, Japan				AB103125*	
Mytilus edulis					Z29550*	
Septifer virgatus	Nagasakibana, Japan				1	300187
Total			373	47	78	

* Sequence obtained from GenBank.
[1] *N*: number of individuals typed for COI.
[2] *N*: number of individuals typed for ITS1.
[3] *N*: number of individuals typed for 28S.

nuclear ribosomal first internal spacer (ITS1). Polymerase chain reactions (PCRs) were performed to amplify a 660 nucleotide (nt) portion of COI, 771 nt (aligned length) of 28S, and 637 nt (aligned length) of ITS1 using primer pair LCO1490/HCO2198 (Folmer et al. 1994), D23F/D6R (Park and Ó Foighil 2000), and primers annealing to flanking regions of the 18S and 5.8S genes (White et al. 1996), respectively. The target fragments were amplified with GoTaq DNA Polymerase (Promega, Madison, WI) and a negative control (no template) was included in each amplification run. For all reactions, a touchdown protocol (Palumbi 1996) was utilized. After 2 min denatuation at 95°C, an initial annealing temperature of 65°C was decreased by 2°C per cycle (30 sec denaturing at 95°C, 40 sec annealing, and 1 min extension

at 72°C) until the final gene-specific annealing temperature (45°C for COI and 52°C for 28S and ITS1) was reached and subsequently maintained for an additional 30 cycles. Double-stranded products were isolated on 1% agarose gels, excised over UV light, and extracted using a QIAquick gel extraction kit (Qiagen). Both strands of the amplified fragments were directly cycle-sequenced, using the PCR primers, by the University of Michigan's Sequencing Core Facility. All novel DNA sequences have been deposited in GenBank (COI: AY825105–AY825222, 28S: AY825079–AY825104, ITS1: AY825223–AY625245). Previously generated sequences (AY621835–AY622009, Lee and Ó Foighil 2004) representing Floridian genetic variation were also included and a few sequences, mainly for outgoup taxa, were retrieved from the GenBank database (see Table 1).

Phylogenetic Analyses

Sequence chromatograms were edited by comparing both strands for all taxa using Sequence Navigator 1.0.1 (Applied Biosystems, Foster City, CA). COI sequences were aligned easily due to an absence of indels. The nuclear ribosomal DNA fragments were aligned with Clustal X (Thompson et al. 1997) using default parameters and then adjusted manually where necessary. A partition-homogeneity test (Farris et al. 1995) was performed (100 random replications) using PAUP*4.0b10 (Swofford 2003) to evaluate character congruence among 28S and ITS1 datasets.

Phylogenetic relationships among Caribbean and putative geminate *Brachidontes* taxa were analyzed with COI and combined nuclear rDNA (28S + ITS1) datasets using Floridian *Geukensia* and *Ischadium* species as outgroups (Distel 2000). In addition, a global *Brachidontes* phylogeny was constructed from 28S dataset using four mytilid genera (*Mytilus*, *Modiolus*, *Lithophaga*, and *Septifer*) as outgroups. Maximum parsimony (MP) analyses were conducted with PAUP* using heuristic search option (100 random stepwise additions and tree bisection-reconnection branch-swapping). Nodal support was estimated through bootstrap analysis (Felsenstein 1985) using 100 replications with 10 random additions per each bootstrap replicate. Because of the extensive computational time, the maximum number of trees to be saved was limited to 5000 and bootstrap values were accessed with the fast stepwise-addition option for heuristic searches (10,000 replicates) when the COI dataset was analyzed.

Bayesian analysis was also performed on each dataset using MrBayes 3.0b4 (Ronquist and Huelsenbeck 2003) under the best-fit substitution model (GTR + I + Γ for COI and 28S datasets and GTR + I for 28S and ITS1 combined dataset) determined by hierarchical likelihood ratio tests (hLRTs) as implemented in Modeltest 3.06 (Posada and Crandall 1998). Model parameters were treated as unknown and were estimated for each analysis. The COI third-codon position was set up to have different gamma-distributed rate variation (Γ) and proportion of invariant sites (I) than the first and second position in the analysis of COI dataset. Random starting trees were used and analyses were run for 1 million generations, sampling every 100 generations for each dataset. Posterior probability values were estimated by generating a 50% majority rule consensus tree after the burn-in period of 2000 using PAUP*.

Within-Clade Genetic Structure and Migration Rates

Genetic structures of major nominal *B. exustus* COI clades recovered were characterized using Arlequin 2.001 (Schneider et al. 2000). Genetic variation within each clade was estimated using haplotype diversity (*H*; Nei 1987) and nucleotide diversity (π, the mean of pairwise sequence differences; Tajima 1983). Tajima's (1989) *D*-statistic (10,000 permutations) was computed to test for selective neutrality of mitochondrial COI sequences. The fraction of the total genetic variation distributed among populations was estimated with the analysis of molecular variation (AMOVA; Excoffier et al. 1992) based on simple pairwise distance. Populations represented by a single mussel, in addition to the Cape Verde population (which is geographically isolated and phylogenetically divergent from its sister populations), were removed from the analyses to avoid biased estimates. A parsimony network was constructed for each clade using the statistical parsimony (Templeton et al. 1992) method in TCS 1.13 (Clement et al. 2000).

We estimated patterns of gene flow among populations within each of the major nominal *B. exustus* COI clades using Migrate 1.7.6 (Beerli and Felsenstein 2001), which estimates directional and asymmetric gene flow using a Markov chain Monte Carlo maximum-likelihood procedure. Populations with small sample sizes ($\leq$5) were not included in this analysis. A maximum-likelihood (ML) corrected transition to transversion ratio of 6.732, calculated from the Bayesian tree using PAUP*, and empirical base frequencies were used. Each search run involved 10 short chains with 100,000 sampled genealogies and three long chains with 1 million sampled genealogies. For both the short and long chains, 10,000 genealogies were discarded as initial burn-in. A static heating scheme, four chains with temperatures 1.0, 1.2, 1.5, and 3.0, was used. Three initial searches were conducted for each clade with different random seeds and F_{ST}-based estimates of Θ ($=2N\mu$ for the mtDNA genome, where N is the effective population size and μ is the mutation rate) and migration rates ($M = 2mN$, where m is migration rate per generation in a population) to check consistency of the results. Three additional runs were performed with parameter estimates from the previous run as starting values.

Estimation of Sequence Divergence Rates and Divergence Times

We estimated *Brachidontes* specific mitochondrial COI mutation rates by calibrating rates of third-codon position sequence divergence among two putative transisthmian geminates identified by phylogenetic analyses of mitochondrial COI and combined nuclear rDNA datasets. Prior to rate calculation, evidence of substitution saturation was investigated by plotting the pairwise third-codon position differences (uncorrected) against GTR + I + Γ-corrected pairwise distances for all codon positions (Fig. 1). Third positions saturated rapidly, suggesting that saturation started to occur slightly prior to the divergence of geminate 2 and that by the time when geminate 1 diverged third positions appeared to be

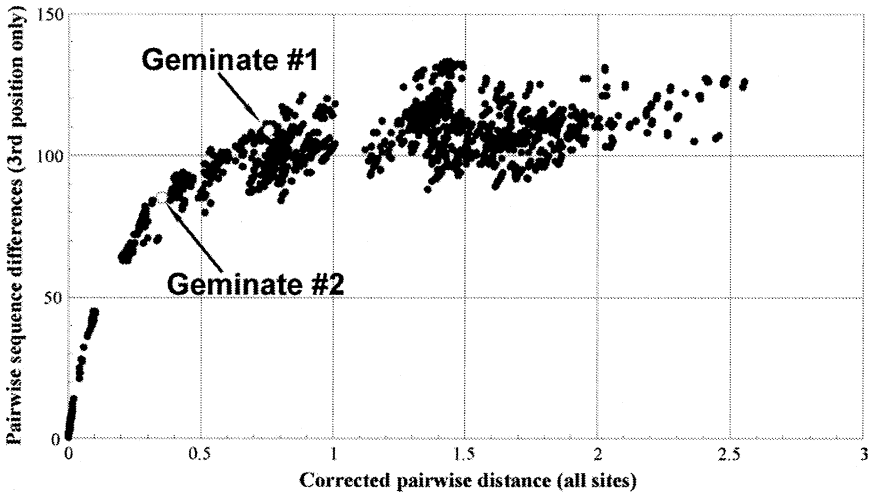

FIG. 1. Plots of third-codon position substitution saturation for the mitochondrial COI dataset, including outgroup taxa. The uncorrected pairwise sequence differences for third codon positions (y-axis) are plotted against GTR + I + Γ-corrected pairwise distances for all codon positions (x-axis). Plots for two putative geminate pairs (unfilled circles) are based on average pairwise distances.

completely saturated. To test the rate consistency of a Poisson-distributed molecular clock, likelihood ratio tests (Felsenstein 1981) were conducted using the HKY substitution model, which was chosen by hLRTs using Modeltest, and a molecular clock could not be rejected for COI third-codon positions ($\delta = 58.035$, df = 56, $P > 0.10$). In these analyses, the COI data matrix was pruned to 58 haplotypes representing all of the clades recovered in the analysis of the total COI dataset, including outgroups, to minimize computational time requirements.

In a calculation of third-position divergence rates, net nucleotide divergences (*d*, Nei and Li 1979) among two geminate pairs were calculated based on HKY corrected pairwise sequence divergences using all COI haplotypes to account for ancestral within-lineage variation (Edward and Beerli 2000). These estimates were then calibrated at two dates: 3.6 million years (Coates et al. 1992) and at 2.7 million years (Marshall 1988). These calibration dates are likely to span the actual cessation of gene flow for intertidal *Brachidontes* because the former is based on the differentiation of nearshore molluscan paleofaunas and the latter on the intercontinental exchange of land mammals. COI third positions diverged at rates of 51.93–69.24% and 36.58–48.77% per million years based on geminate 1 and 2 calibrations, respectively, yielding estimates of 25.96–34.62% and 18.29–24.38% per million years per lineage. The mutation rates calibrated by geminate 2 (18.29–24.38% per million years per lineage) were further used to estimate divergence times among *B. exustus* clades because the best estimate is likely from the least divergent geminate clade (Knowlton and Weight 1998; Lessios et al. 2001) and it exhibits less saturation (Fig. 1). Divergence times were determined by calculating net third-position divergences among *B. exustus* major COI clades.

Estimation of Ancestral Population Parameters

COI haplotypes of the *B. exustus* Atlantic clade were divided into two datasets, one with all haplotypes and the other with unambiguous northern haplotypes only (see Results). Ancestral population parameters, Θ ($=2N\mu$ for the mtDNA genome, where N is the effective population size and μ is the mutation rate) and g (the exponential growth rate of the population) were jointly calculated for the each dataset using COI third-codon positions only with Fluctuate 1.4 (Kuhner et al. 1998). Analyses were repeated 10 times for each dataset using 10 short chains of 5000 steps each and five long chains of 25,000, with sampling increments of 20, and the mean values were calculated. Each analysis used a ML-corrected transition-to-transversion ratio, estimated using PAUP* under the HKY substitution model. COI third-position divergence rates obtained above were converted into mutation rates per generation, assuming a *Brachidontes* generation time of 3 years (Morton 1988). These estimates were used to generate the relative effective population size at any time t with the equation $N_t = \Theta^{e-(g\mu)t}$ (Kuhner et al. 1998).

RESULTS

Phylogenetic Analyses

A *Brachidontes* phylogeny constructed from the nuclear 28S dataset is shown in Figure 2. It incorporates 78 genotypes generated from nine nominal and two unidentified *Brachidontes* species, in addition to outgroups (Table 1). Both MP and Bayesian analyses recovered a well-supported *Brachidontes* clade sister to *Geukensia*/*Ischadium* taxa. Nominal *B. exustus* genotypes were not monophyletic, being present in three distinct tip clades, each of which also contained a non-Caribbean congener: eastern Pacific *B. semilaevis*, one of two polyphyletic eastern Pacific *B. adamsianus* lineages, and eastern Atlantic *B. puniceus*. The Caribbean congener, *B. modiolus*, which shares much of its distribution with nominal *B. exustus*, was not included in this species complex. Instead, *B. modiolus* grouped with diverse congeners from the eastern Pacific (*B. adamsianus* second polyphyletic lineage), western South Atlantic (*B. rodriguezi*) and a variety of western Pacific lineages (*B. mutabilis*, *B.* sp. 1, and *B.* sp. 2) in the Bayesian

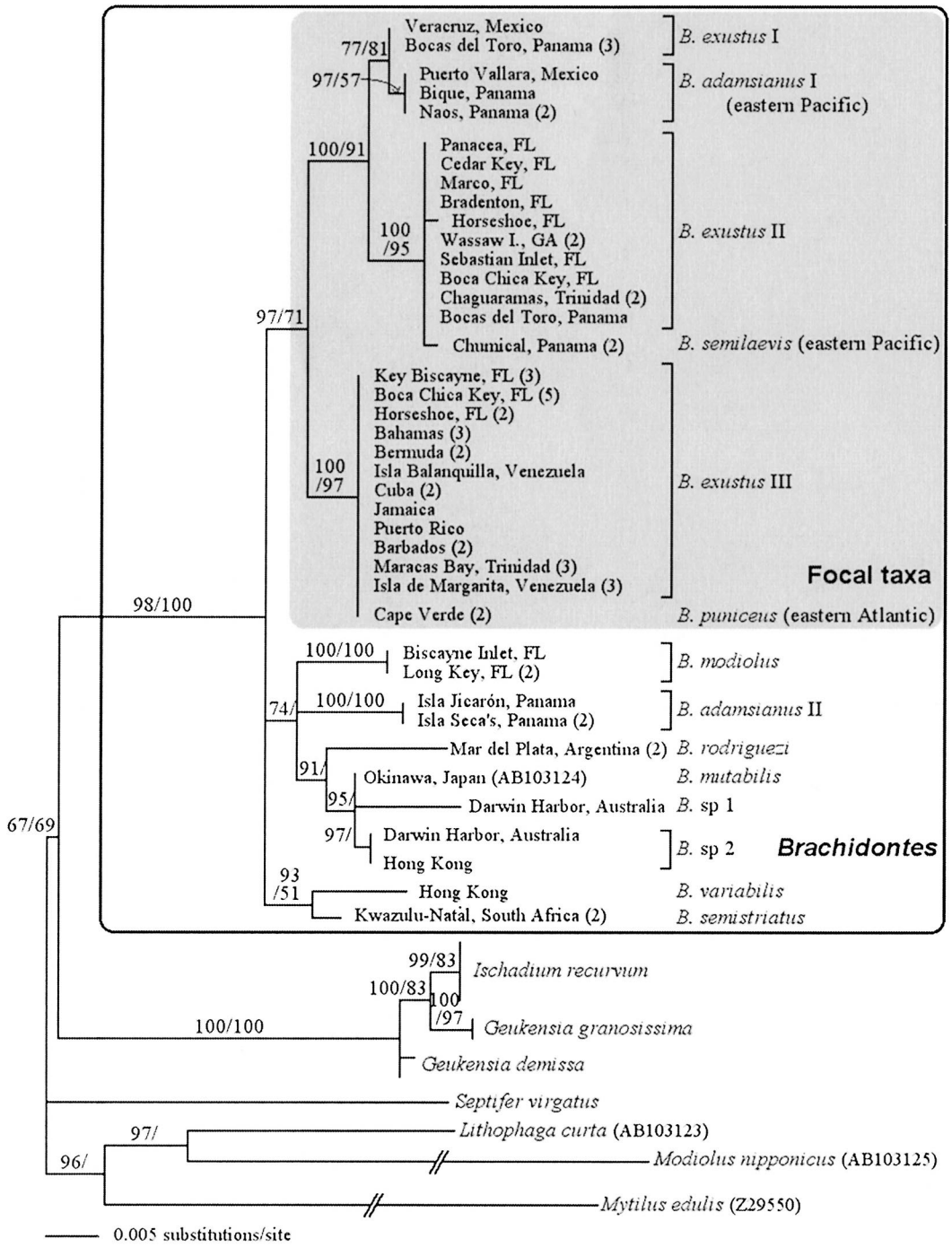

FIG. 2. Bayesian tree for globally collected *Brachidontes* taxa based on nuclear 28S sequence data using representatives of four mytilid genera (*Lithophaga*, *Modiolus*, *Mytilus*, and *Septifer*) as outgroups. Nodal support values (Bayesian posterior probability/parsimony bootstrap) are indicated above the branches. Numbers in parentheses following sampling locations indicate multiple individuals sharing the same genotype.

analysis. The polyphyletic status of *B. exustus* has already been established (Lee and Ó Foighil 2004), and Figure 2 demonstrates that cryptic species complexes are also prevalent in other global populations of *Brachidontes*, including those in the eastern Pacific (Panama), Hong Kong, and Northern Australia (Darwin). The taxonomic details of these complexes remain to be determined, however, the global gene tree (Fig. 2) shows that the *B. exustus* cryptic species complex is part of a phylogenetically distinctive and, according to available data, almost exclusively Neotropical lineage.

An extensive mitochondrial COI database was constructed for the focal taxa (Fig. 2) and cross-referenced using nuclear ribosomal markers. The mitochondrial database consisted of 150 nominal *B. exustus* COI haplotypes (representing 293

individuals, Table 2) obtained by combining a previous Florida/Bahamas dataset (Lee and Ó Foighil 2004) with 69 new genotypes sampled from 12 Caribbean/Bermudan localities (Table 1). Multiple individuals of the three non-Caribbean sister species recovered in 28S gene tree (*B. adamsianus* [the sister polyphyletic lineage only], *B. semilaevis*, and *B. puniceus*), and of the coexisting congener *B. modiolus*, were also characterized for COI. A subsample of 32 nominal *B. exustus* individuals representing the primary COI tree topological features and the main sampling locations were further genotyped for two nuclear ribosomal fragments (28S and ITS1; Table 1). The two nuclear datasets were not significantly incongruent ($P = 0.58$ in the partition-homogeneity test) and were analyzed as a combined dataset.

Bayesian and MP analyses were conducted on both combined nuclear and mitochondrial datasets using *Geukensia* and *Ischadium* species as outgroups (Fig. 3). These analyses recovered the same three stem 28S lineages (Fig. 2), albeit with much more tip resolution (especially so for the mitochondrial topologies), to reveal seven well-supported nuclear/mitochondrial terminal clades encompassing five nominal *B. exustus* lineages and three non-Caribbean nominal congeners. A novel western Caribbean lineage formed a robust clade with the eastern Pacific *B. adamsianus* (cryptic species I; Fig. 2) in both mitochondrial and nuclear analyses and these taxa putatively represent a transisthmian geminate species pair (Fig. 3, geminate 1). The other four nominal *B. exustus* clades were previously encountered in Floridian *Brachidontes* samples (Lee and Ó Foighil 2004) and clustered in two discrete tip clades (Fig. 3). As in the earlier study, the Gulf and Atlantic lineages were robustly sister to each other; however, *B. semilaevis* could now be identified as their putative transisthmian geminate (Fig. 3, geminate 2). The remaining Bahamas and Antilles (i.e., Key Biscayne clade of Lee and Ó Foighil, 2004) nominal *B. exustus* sister clades lacked putative geminates but the former surprisingly incorporated Cape Verde *B. puniceus* nominal congeners, most clearly for the nuclear rDNA markers (Fig. 3, indicated by asterisks).

Not all features of the nuclear and mitochondrial gene tree topologies (Fig. 3) were congruent. The primary discrepancy concerned the placement of *B. modiolus*. While it was robustly sister to the focal *Brachidontes* taxa in nuclear trees (Figs. 2, 3), *B. modiolus* formed weakly supported derived mitochondrial COI clades together with *B. exustus* Bahamas and Antilles clades: (*B. modiolus* (Bahamas, Antilles)) in the Bayesian tree (Fig. 3) and (Antilles (Bahamas, *B. modiolus*) in the MP strict consensus (not shown). Support values for the nominal *B. exustus* Bahamas/Antilles mitochondrial COI clade were much higher (Bayesian posterior probability of 100 and MP bootstrap of 80), when COI haplotypes of *B. modiolus* were excluded from the analyses.

TABLE 2. Number of individuals sequenced (number of haplotypes) by nominal *Brachidontes exustus* COI clades and collection localities. See Table 1 for the locality code. *N*, total number of individuals sequenced; *Nh*, number of unique haplotypes.

mt *B. exustus* clades	Number of individuals sequenced (number of haplotypes) by localities																								
	PA	CK	BR	MA	BC	HS	KB	SI	WI	BA	BE	CU	JA	PR	BB	CT	MT	VE	BT	VC	*N*	*Nh*
W. Caribbean																			17(12)	17(3)	34	15
Bahamas																						
Bahamas A					11(9)	4(4)	5(5)			14(10)	14(9)							1(1)			49	34
Bahamas B					5(4)	1(1)				1(1)	1(1)										8	7
Antilles																						
Antilles A							3(2)					16(6)	15(6)	16(8)	16(9)		6(6)	11(6)			83	32
Antilles B																	3(3)	6(6)			9	8
Gulf	15(11)	16(12)	3(3)	15(14)		10(6)										10(5)					59	39
Atlantic					1(1)			15(8)	15(3)										10(3)		51	15
Total	15(11)	16(12)	3(3)	15(14)	17(14)	15(11)	8(7)	15(8)	15(3)	15(11)	15(10)	16(6)	15(6)	16(8)	16(9)	10(5)	9(9)	18(13)	27(15)	17(3)	293	150

Caribbean Basin Phylogeography of Nominal Brachidontes exustus Clades

Western Caribbean clade

As the informal name implies, this discrete lineage was restricted to western Caribbean continental samples, being encountered in large numbers at one site each in Mexico and

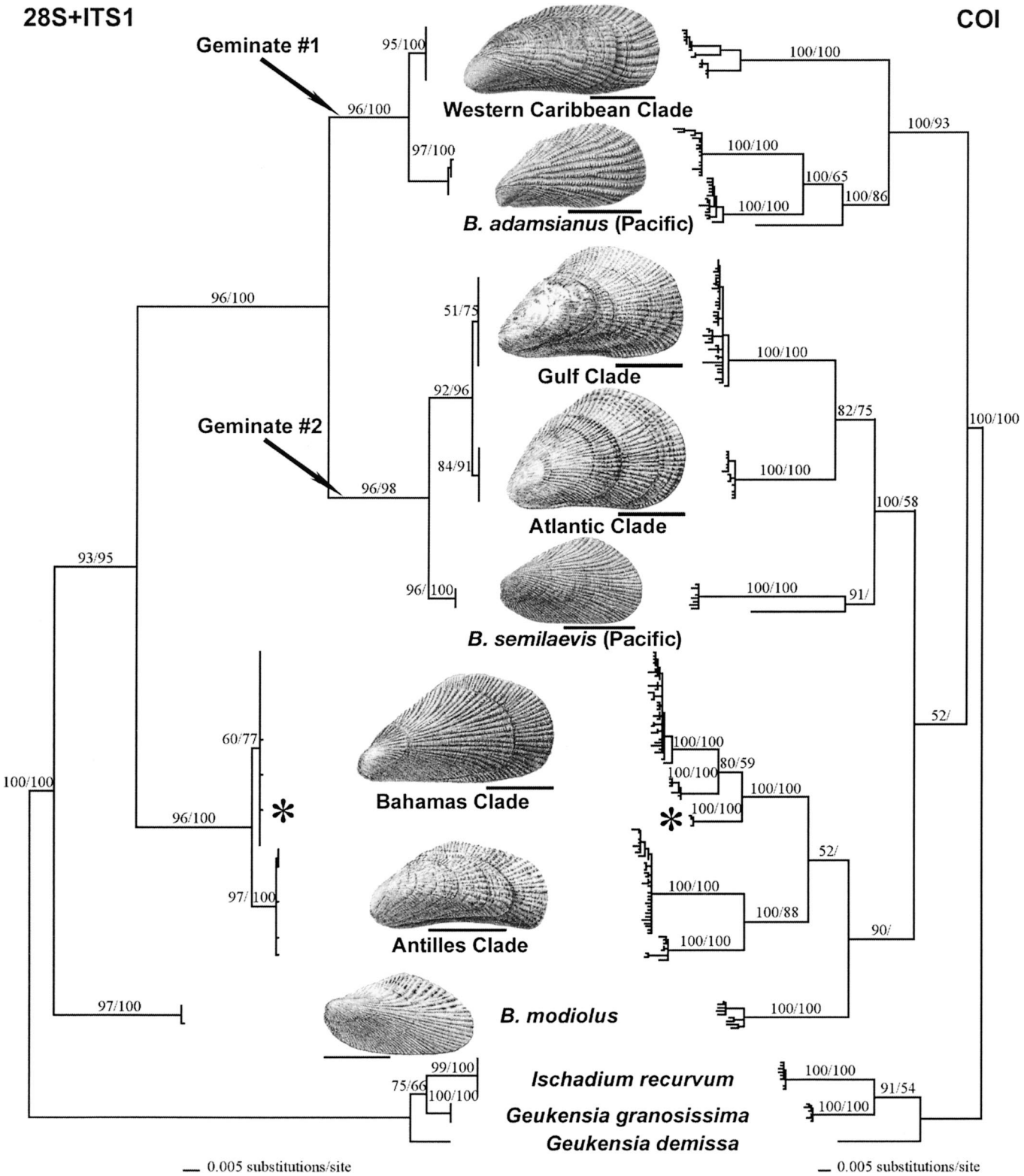

FIG. 3. Bayesian trees for focal *Brachidontes* taxa (see Fig. 2; the Caribbean *B. modiolus*, which shares much of its distribution with nominal *B. exustus* was also included) based on combined (28S and ITS1) nuclear ribosomal (left) and mitochondrial COI (right) datasets using *Ischadium* and *Geukensia* spp. as outgroup taxa. Numbers above the branches represent the Bayesian posterior probabilities/parsimony bootstrap values (>50 only) for the supported nodes. Exemplar *Brachidontes* shell phenotypes are inserted, and scale bars represent 5 mm. Genotypes for *B. puniceus* from Cape Verde are marked by asterisks, and inferred transisthmian geminate pairs are indicated by arrows.

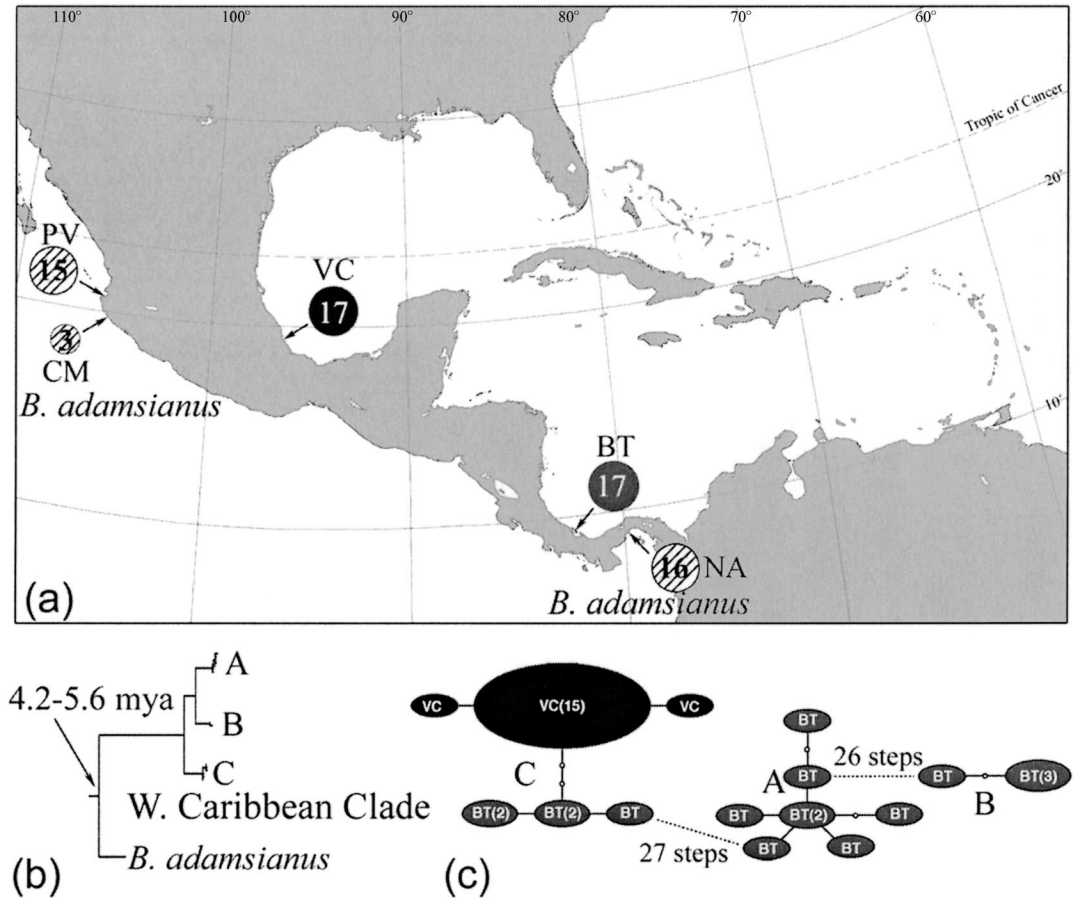

FIG. 4. *Brachidontes exustus* western Caribbean clade. (a) Map showing sampling locations (see Table 1 for locality code); (b) portion of COI Bayesian tree showing sister group relationships and inferred transisthmian divergence times; and (c) statistical parsimony network of western Caribbean COI genotypes. Three western Caribbean COI tip clades are labeled in capital letters in the Bayesian tree (b) and in the parsimony network (c). In the parsimony network, the observed COI haplotypes are represented by ovals sized according to their relative abundance (numbers >1 given in parentheses) and labeled and color-coded to reflect sampling location. Smaller unlabeled circles represent inferred missing haplotypes. Branch lengths connecting tip clades are from a minimum spanning tree computed using Arlequin 2.001 (Schneider et al. 2000).

Panama (Fig. 4a). Its sister taxon, the putatively geminate *B. adamsianus*, occurred in the eastern Pacific and contained three well-defined mitochondrial tip clades (Fig. 3). Our mitochondrial COI calibrations dated this transisthmian split at 4.2–5.6 million years ago (Fig. 4b), and this represents a minimal estimation calculated using the basal *B. adamsianus* tip clade (Fig. 3) that showed the smallest net divergence from the western Caribbean sister taxon. Although the western Caribbean clade did not exhibit nuclear marker polymorphism (Fig. 3), its COI genotypes formed three distinct subclades, each of which was composed of a few haplotypes (Figs. 4b, c). This profile was reflected in low haplotype diversity but relatively high nucleotide diversity (Table 3). Northern and southern samples lacked common haplotypes (Fig. 4c), exhibited significant among-population genetic subdivision (Table 4), and showed no evidence of significant interpopulational gene flow (Table 5). The much lower levels of mitochondrial diversity in the northern sample (three haplotypes [one predominant] in the subclade C), relative to that of the southern sample (12 haplotypes [none predominant] in all three subclades), appeared to be consistent with founder effect expectations.

TABLE 3. Haplotype (H) and nucleotide diversity (π) with sampling variance and estimates of Tajima's D-statistic with associated level of significance ($^*P < 0.05$) calculated for the major mitochondrial COI nominal *Brachidontes exustus* clades recovered.

COI *B. exustus* clades	Haplotype diversity (H)	Mean number of pairwise differences (π)	Tajima's D
Western Caribbean clade	0.802 ± 0.069	16.529 ± 7.546	1.1888
Bahamas clade	0.987 ± 0.006	20.380 ± 9.126	−0.395
Antilles clade	0.890 ± 0.025	15.623 ± 7.037	−0.805
Gulf clade	0.963 ± 0.015	5.425 ± 2.651	−1.809*
Atlantic clade	0.761 ± 0.047	2.954 ± 1.572	−0.679

Bahamas and Antilles clades

These two sister clades (Figs. 2, 3) were previously encountered in the earlier Floridian study, although at very different frequencies (Lee and Ó Foighil 2004). The expanded

TABLE 4. Analysis of molecular variation results for major COI nominal *Brachidontes exustus* clades recovered.

COI *B. exustus* clades	Source of variation	df	Percentage of variation	Fixation indices
Western Caribbean clade	among populations	1	53.57	
	within populations	32	46.43	$F_{ST} = 0.5357$*
Bahamas clade[1]	among populations	4	6.88	
	within populations	51	93.12	$F_{ST} = 0.0688$
Bahamas clade (partitioned)[1]	Bermuda vs. non-Bermuda	1	0.01	$F_{CT} = 0.0001$
	among populations within groups	3	6.88	$F_{SC} = 0.0688$
	within populations	51	93.11	$F_{ST} = 0.0689$
Antilles clade	among populations	6	23.89	
	within populations	85	76.11	$F_{ST} = 0.2389$*
Antilles clade A only	among populations	6	26.26	
	within populations	76	73.74	$F_{ST} = 0.2626$*
Antilles clade B only	among populations	1	−17.63	
	within populations	7	117.63	$F_{ST} = -0.1763$
Gulf clade	among populations	4	14.78	
	within populations	54	85.22	$F_{ST} = 0.1478$*
Atlantic clade[2]	among populations	3	70.85	
	within populations	46	29.15	$F_{ST} = 0.7085$*
Atlantic clade (partitioned)[2]	northern vs. southern groups	1	76.96	$F_{CT} = 0.7696$
	among populations within groups	2	1.77	$F_{SC} = 0.0770$
	within populations	46	21.27	$F_{ST} = 0.7873$*

* $P < 0.05$.
[1] A single mussel from Venezuela and five haplotypes from Cape Verde were removed from the analyses.
[2] A single individual sample from Boca Chica Key, FL, was removed from the analyses.

sampling of Caribbean populations revealed a striking pattern of predominantly allopatric north/south within-basin distributions and our mitochondrial COI calibrations dated this sister taxon split at 3.4–4.6 million years ago (Figs. 5a,b).

The Bahamas clade predominated in the Florida Keys, Bahamas, and Bermuda sampling sites, being exclusively present in the latter two samples. Surprisingly, this clade also had an amphi-Atlantic distribution and incorporated the nominal Cape Verdean congener *B. puniceus*, unambiguously so for the nuclear markers (Figs. 2, 3) and as a well-supported tip sister clade for the mitochondrial marker (Figs. 3, 5c). It is also noteworthy that a single Bahamas clade specimen (confirmed with both mitochondrial and nuclear markers) was recovered in the southern Caribbean on the offshore Venezuelan Isla Blanquilla, more than 1900 km from its nearest sampled clade member in the Bahamas (Fig. 5a, Table 2).

Pronounced levels of Bahamas clade mitochondrial genetic variation (Table 3) resolved into three well-defined tip clades (Fig. 5b). One tip clade had a distinct geographic signature, being exclusive to the geographically isolated Cape Verde population (Figs. 5a–c). The other two tip clades coexisted throughout the Florida Keys, Bahamas, and Bermuda populations (Figs. 5a–c, Table 2). Unlike the case for other Caribbean nominal *B. exustus* taxa, these latter populations showed no significant genetic subdivision, remarkably so in the case of the Bermudan population, where only 0.01 % of variation could be attributed to its isolation by more than 1500 km of open ocean (Table 4). In concordance with the AMOVA results, relatively high levels of gene flow were detected by ML estimation among the Florida Keys, Bahamas, and Bermuda populations (Table 5).

One of the more enigmatic results of the earlier study (Lee and Ó Foighil 2004) concerned a rare nominal *B. exustus* Key Biscayne clade, sister to the Bahamas clade, represented by three subadult specimens sampled in a marginal *Brachidontes* habitat in Key Biscayne, southern Florida. Our expanded regional sampling (Fig. 5a) showed that this clade was widespread in the Antilles, being exclusively present in samples from Cuba, Jamaica, Puerto Rico, Barbados, and one location in Trinidad. Therefore, we now refer to it as the ''Antilles clade.'' The majority of samples collected from Venezuela also belonged to this clade (Table 2).

The Antilles clade contained a well-defined phylogenetic mitochondrial dichotomy, dated by the COI calibrations at 2.1–2.9 million years ago (Fig. 5b), but not reciprocated when cross-referenced with the nuclear markers (Fig. 3). The two mitochondrial tip clades were unequally represented, both numerically and spatially, across the Antilles clade's range. One (A) was predominant in all sampling sites and a much rarer tip clade (B) was present only in the two southernmost sites, Trinidad and Venezuela (Table 2). Although tip clade A contained a few common widely distributed haplotypes (Fig. 5d), its constituent populations were significantly subdivided (Table 4). Interestingly, haplotype diversity was higher in southern populations (Barbados, Trinidad, Venezuela) than in the Greater Antilles populations, and southern haplotypes were often several mutational steps away from the centrally positioned haplotypes in the network, whereas most of the Greater Antilles haplotypes were just one step away (Fig. 5d). A bidirectional but asymmetric gene flow pattern among most populations was indicated by ML estimation, and levels were especially high among the Greater Antilles locations (Table 5).

Gulf and Atlantic clades

The previous Floridian study (Lee and Ó Foighil 2004) recovered a Gulf/Atlantic genetic disjunction in which reciprocally monophyletic sister groups, the Gulf and Atlantic clades, were found on the respective flanks of the Floridian

TABLE 5. Maximum likelihood estimates of gene flow among populations of major *Brachidontes exustus* COI clades. The analysis was carried out using an unrestricted migration matrix model with variable subpopulations size. The maximum likelihood estimates (95% profile confidence intervals) are shown for population sizes ($\Theta = 2\mu N_f$) and number of immigrant females per generation ($2mN_f$), where N_f is the female effective population size, μ is the mutation rate per generation per site, and m is the migration rate per generation in a population. See Table 1 for the population codes.

			Migration rate ($2mN_f$)					
Clade	Population	$\Theta = 2\mu N_f$	BT	VC				
Western Caribbean clade	BT	0.0265 (0.0169–0.0445)		8.68×10^{-8} (6.51×10^{-8}–2.17×10^{-4})				
	VC	0.0003 (0.0002–0.0005)	0.08 (4.58×10^{-3}–0.37)					
			BC	BA	BE			
Bahamas clade A[1]	BC	0.0093 (0.0059–0.0216)		1.49×10^{-10} (1.12×10^{-10}–0.49)	2.47×10^{-15} (1.88×10^{-15}–0.49)			
	BA	0.0138 (0.0059–34.5283)	5.91 (1.36–9.30×10^{8})		2.53×10^{-9} (1.90×10^{-9}–1.51)			
	BE	0.0081 (0.0031–0.0197)	4.31 (1.37–10.62)	1.73×10^{-15} (1.31×10^{-15}–1.24)				
			CU	JA	PR	BB	MT	VE
Antilles clade A[2]	CU	0.0003 (0.0002–0.0005)		0.14 (0.01–0.62)	1.69 (0.90–2.83)	0.14 (0.01–0.62)	2.11 (1.21–3.37)	2.90×10^{-17} (2.25×10^{-17}–0.27)
	JA	0.0002 (0.0001–0.0004)	1.36 (0.49–2.92)		0.82 (0.20–2.11)	0.27 (0.02–1.20)	0.54 (0.09–1.68)	2.17 (0.99–4.05)
	PR	0.0017 (0.0008–0.0042)	7.07 (3.40–12.74)	13.35 (7.97–20.74)		1.58×10^{-16} (1.23×10^{-16}–1.51)	1.66×10^{-16} (1.28×10^{-16}–1.51)	1.58×10^{-16} (1.23×10^{-16}–1.51)
	BB	0.0136 (0.0067–0.0341)	15.59 (8.35–26.15)	8.39×10^{-12} (6.29×10^{-12}–2.50)	1.30 (0.07–5.72)		1.27×10^{-15} (9.86×10^{-16}–2.50)	1.28×10^{-15} (9.91×10^{-16}–2.50)
	MT	0.0023 (0.0016–0.0036)	0.16 (0.01–0.71)	2.07×10^{-15} (1.56×10^{-15}–0.31)	2.18×10^{-16} (1.69×10^{-16}–0.31)	2.18×10^{-16} (1.69×10^{-16}–0.31)		7.11×10^{-13} (5.34×10^{-13}–0.31)
	VE	0.0044 (0.0028–0.0074)	4.40×10^{-16} (3.41×10^{-16}–0.52)	2.13×10^{-12} (1.60×10^{-12}–0.52)	1.46×10^{-11} (1.10×10^{-11}–0.52)	0.27 (0.20–1.18)	0.27 (0.02–1.18)	
			PA	CK	MA	HS		
Gulf clade[3]	PA	0.0014 (0.0005–0.0056)		3.47×10^{-11} (2.60×10^{-11}–1.38)	30.30 (22.18–36.83)	5.00 (2.07–9.72)		
	CK	0.0045 (0.0025–0.0090)	0.41 (0.02–1.82)		6.63 (4.20–10.36)	8.63 (5.42–12.85)		
	MA	0.0605 (0.0283–0.1679)	69.97 (46.01–95.97)	62.48 (43.12–86.94)		5.93×10^{-15} (4.60×10^{-15}–3.75)		
	HS	0.0092 (0.0067–0.0143)	8.83×10^{-16} (6.85×10^{-16}–0.36)	4.32×10^{-13} (3.24×10^{-13}–0.36)	1.04×10^{-15} (8.06×10^{-16}–0.36)			
			SI	WI	BT	CT		
Atlantic clade[4]	SI	0.0967 (0.0279–19.4814)		23.16 (9.19–399.31)	1.26×10^{-14} (9.70×10^{-15}–7.40)	88.53 (56.02–129.63)		
	WI	0.0031 (0.0007–7.8131)	26.15 (15.72–40.13)		3.94×10^{-16} (3.04×10^{-16}–2.78)	2.12×10^{-9} (1.59×10^{-9}–2.78)		
	BT	0.0002 (0.0001–0.0003)	4.25×10^{-13} (3.19×10^{-13}–0.24)	2.85×10^{-17} (2.18×10^{-17}–0.07)		4.98×10^{-17} (3.79×10^{-17}–0.07)		
	CT	0.0010 (0.0004–0.0027)	5.43×10^{-16} (4.10×10^{-16}–0.71)	1.14×10^{-16} (8.77×10^{-17}–0.71)	10.26 (6.99–40.46)			

[1] Five haplotypes from Horseshoe Key, four from Key Biscayne, and one from Venezuela were not included in the analyses.
[2] Three haplotypes from Key Biscayne were not included in the analyses.
[3] Three haplotypes from Bradenton were not included in the analyses.
[4] One haplotype from Boca Chica Key was not included in the analyses.

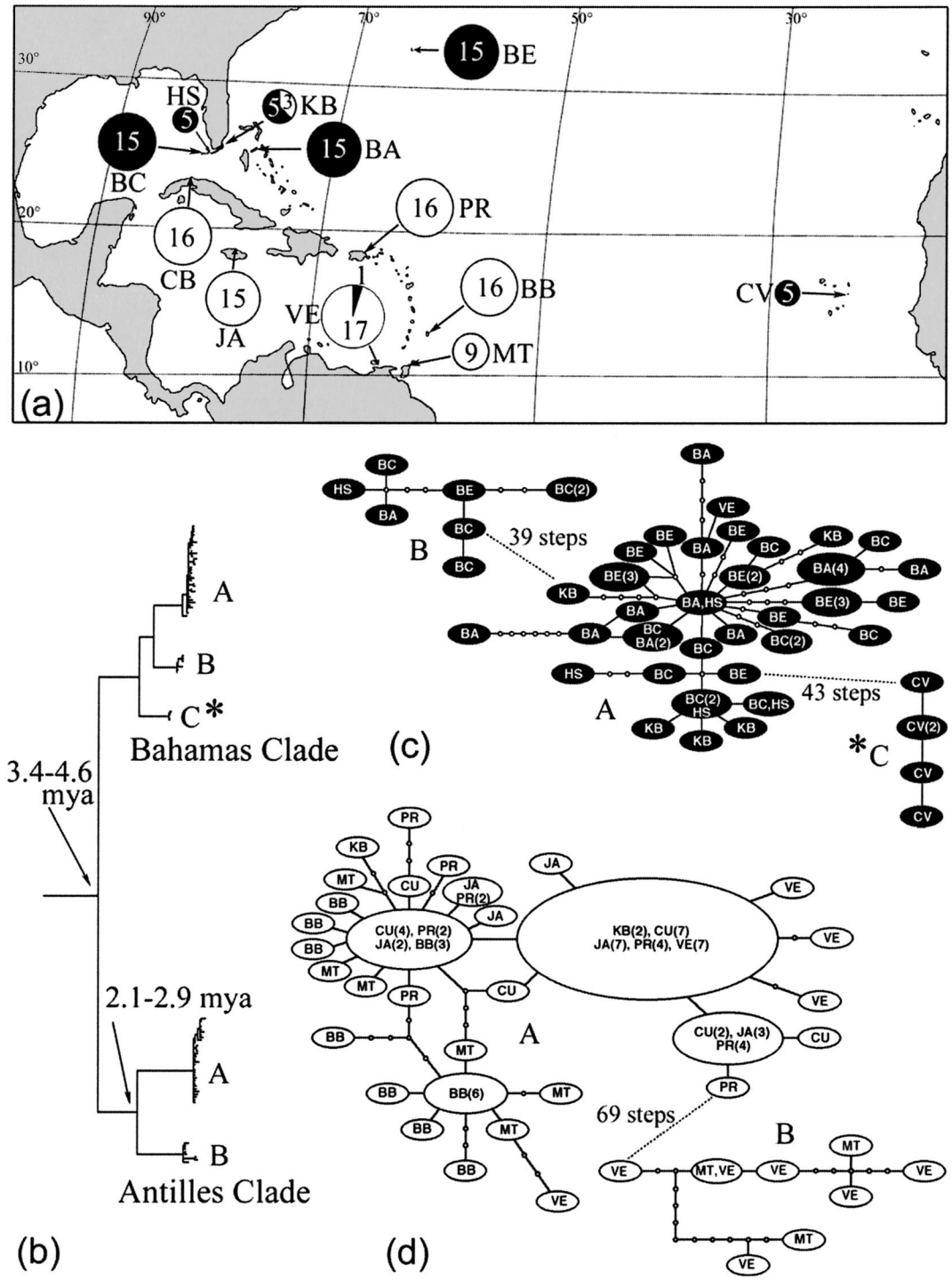

FIG. 5. *Brachidontes exustus* Bahamas and Antilles sister clades. (a) Map showing sampling locations (black circles, Bahamas clade; white, Antilles clade; see Table 1 for locality code); (b) portion of COI Bayesian tree showing sister group relationships and their inferred divergence times; and (c) statistical parsimony networks of the Bahamas clade and (d) of the Antilles clade. COI tip clades are labeled in capital letters in the Bayesian tree (b) and in the parsimony networks (c, d). In the parsimony network, the observed COI haplotypes are represented by ovals sized according to their relative abundance (numbers >1 given in parentheses) and labeled and color-coded to reflect sampling location. Smaller unlabeled circles represent inferred missing haplotypes and genotypes for *B. puniceus* from Cape Verde are marked by asterisks. Branch lengths connecting tip clades are from a minimum spanning tree computed using Arlequin 2.001 (Schneider et al. 2000).

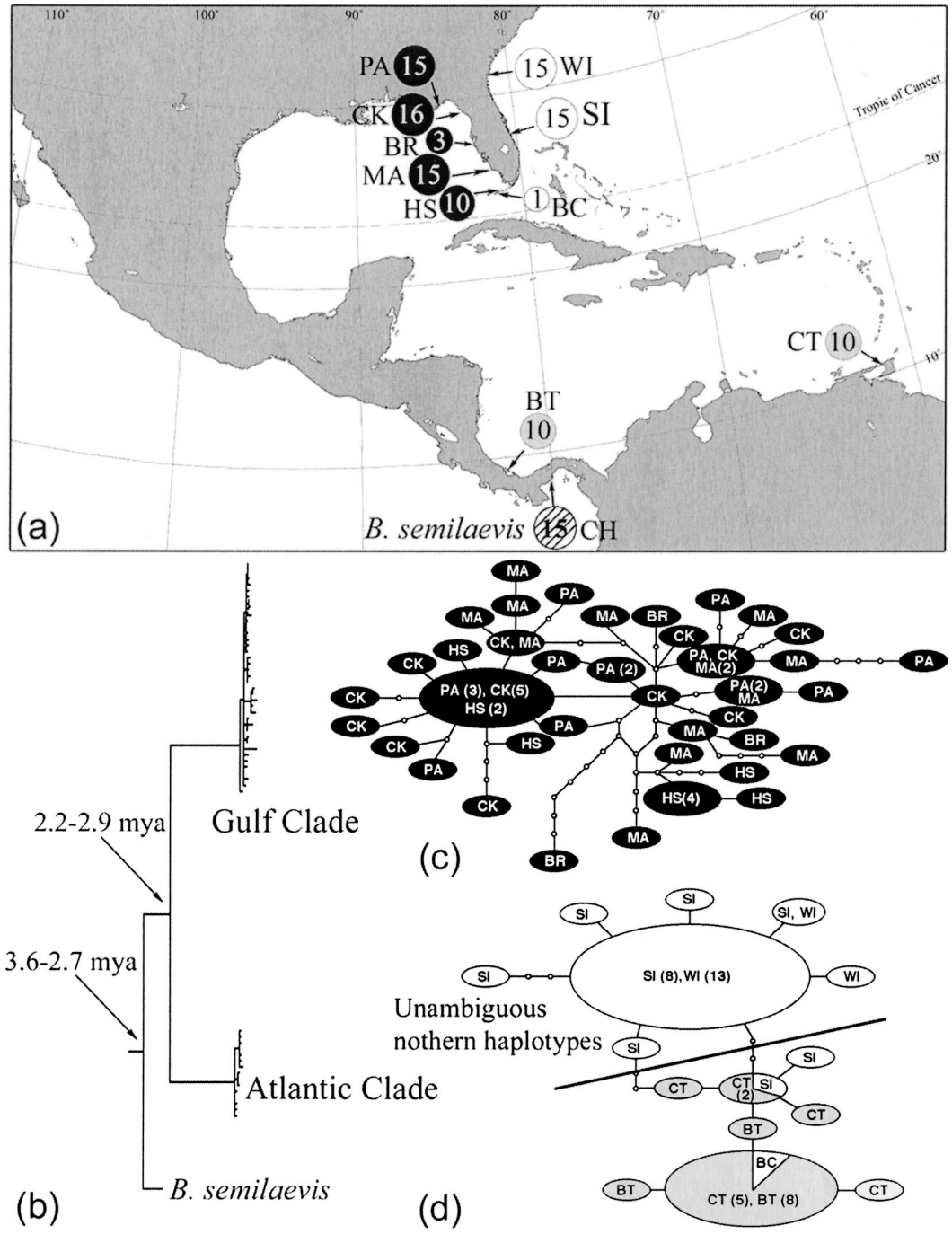

FIG. 6. *Brachidontes exustus* Gulf and Atlantic sister clades. (a) Map showing sampling locations (black circles, Gulf clade; white, Atlantic clade northern samples; gray, Atlantic southern samples; see Table 1 for locality code); (b) portion of COI Bayesian tree showing sister group relationships and their inferred divergence times; and (c) statistical parsimony networks of the Gulf clade and (d) of the Atlantic clade (white indicates individuals collected from the northern sampling sites and gray for those from the southern sites). In the parsimony network, the observed COI haplotypes are represented by ovals sized according to their relative abundance (numbers >1 given in parentheses) and labeled and color-coded to reflect sampling location. Smaller unlabeled circles represent inferred missing haplotypes. Branch lengths connecting tip clades are from minimum spanning tree computed using Arlequin 2.001 (Schneider et al. 2000).

peninsula. A lineage specific mitochondrial COI calibration, based on the split of these sister lineages with their putative transisthmian geminate species *B. semilaevis* (Fig. 3), dated the Gulf/Atlantic disjunction to 2.2–2.9 million years ago (Fig. 6b).

Extending the sampling effort throughout the Caribbean Basin did not reveal any additional members of the Gulf clade. However, Atlantic clade populations (confirmed for both mitochondrial and nuclear markers) were recovered from two sites in the southern Caribbean: Bocas del Toro,

TABLE 6. Estimated ancestral population parameters, Θ ($=2N\mu$ for the mtDNA genome, where N is the effective population size and μ is the mutation rate per generation) and g (the exponential growth rate of the population), for the Atlantic clade (all haplotypes) and for the unambiguous northern haplotypes (±SD). Estimates are based on 10 replicate analyses of COI third-codon positions. The times at which the effective population size was 1% of its current size were calculated based on g and μ (mutation rates per generation calibrated for a cession of transisthmian gene flow at 3.6 million years [Coates et al. 1992] and at 2.7 million years [Marshall 1988]). The 95% confidence intervals are presented after each estimate.

Atlantic clade	Θ	g	Time to 1% relative N
All haplotypes	0.064 ± 0.055	639.8 ± 471.4	88,554 (60,793–162,981) 118,073 (81,057–217,307)
Unambiguous northern haplotypes only	1.564 ± 2.287	6483.6 ± 1613.7	8738 (7571–10,332) 11,651 (10,094–13,776)

Panama (where they coexisted with western Caribbean clade members on mangrove roots), and Chaguaramas, Trinidad (Fig. 6a, Table 2). Both of these new locations are more than 1600 km south of the previous southernmost sampling record, a solitary individual present in an otherwise exclusively Bahamas clade Florida Keys sample (Boca Chica Key; Fig. 6a).

Figure 6d shows an unrooted gene network incorporating all Atlantic clade mitochondrial genotypes. Its relatively truncated topology reflected the fact that the Atlantic clade had the lowest nucleotide (H) and haplotype (π) diversities of all the five nominal *B. exustus* taxa (Table 3). Regional subdivisions were clearly evident among northern (white) and southern (gray) populations: the network had two distinct topological domains, separated by three inferred mutational steps, and each domain was dominated numerically by mussels sampled in one regional population. However, although AMOVA analyses indicated that 76.96% of the Atlantic clade variation was due to the north-south subdivision, this value was not statistically significant (Table 4), and a surprisingly high rate of Trinidad-to-Florida gene flow was obtained by ML estimation (Table 5). These indicators of north-south genetic connectivity stem from the placement of three northern individuals within the predominantly southern topological domain, two of which (including the sole Florida Keys, BC, specimen) shared the same COI genotypes with southern mussels (Fig. 6d).

In the earlier study of Floridian *B. exustus* diversity, relative estimates of N through time for the Atlantic clade were consistent with its local persistence through the last ice age maximum (Lee and Ó Foighil 2004). Incorporation of the new southern genotypes allows us to recalculate ancestral demographic structure for both the entire clade and for a parsed Floridian dataset containing only those haplotypes forming an unambiguously northern genealogy (Fig. 6d). Table 6 shows the parameters Θ and g, calculated for the total Atlantic clade (all haplotypes) and for the unambiguously northern clade, using Fluctuate (Kuhner et al. 1998), and the time to 1% N (relative to present-day estimates), for the two clock calibrations. Plots of the relative estimates of N through time are presented in Figure 7. When haplotypes from southern sampling sites and the three northern haplotypes nested in the southern group were excluded from the dataset, the estimated growth rate (g) for the truncated northern dataset was an order of magnitude larger than the rate calculated for all Atlantic clade haplotypes (Table 6), suggesting accelerated population growth in the former. The range of estimated

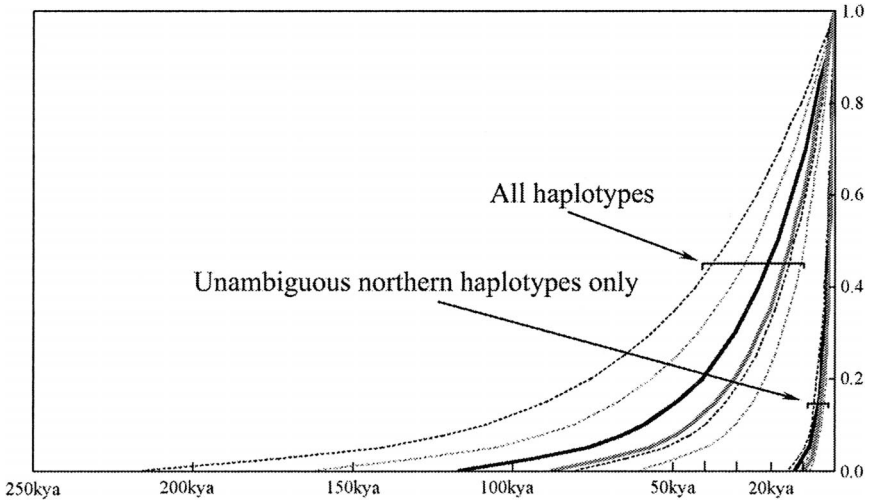

FIG. 7. Patterns of growth for the *Brachidontes exustus* Atlantic clade, calculated separately for all COI haplotypes recovered and also for a parsed subset of haplotypes comprising an unambiguously northern genealogy (Fig. 6d), based on estimates of the parameter g (exponential growth rate of the population) generated jointly with Θ ($= 2N\mu$ for the mtDNA genome, where N is the effective population size and μ is the mutation rate per generation) using Fluctuate (Kuhner et al. 1998). The x-axis represents time (thousands of years) and the y-axis represents the ancestral effective population sizes relative to current size. Two growth trajectories (with 95% confidence intervals: dashed lines) are based on *Brachidontes*-specific COI third-codon mutation rates per generation, calibrated for a cessation of gene flow at 3.6 million years ago (Coates et al. 1992; black lines) and at 2.7 million years ago (Marshall 1988; gray lines).

ages (including 95% confidence intervals) by which *N* drops to 1% of its present-day size was from 60,000 to 217,000 years ago for the entire Atlantic clade and from 7000 to 14,000 years ago for the unambiguously northern group. This latter estimate postdates the end of the last glacial maximum (Williams et al. 1998) and is considerably more recent than the earlier figure of 52,000 to 120,000 years ago based on all northern haplotypes (Lee and Ó Foighil 2004).

Discussion

We were interested in establishing how the Gulf-Atlantic genetic disjunction for the Caribbean *B. exustus* species complex scaled relative to the phylogenetic structuring experienced over the rest of its collective geographic range. Our results show it to be merely one of multiple latent regional genetic disjunctions involving five sibling species that appear to be the product of a long history of regional cladogenesis. We were unable to reliably distinguish these five cryptic taxa on morphological grounds only and relied on a conservative phylogenetic diagnosis, using the criterion of reciprocal monophyly for both nuclear and mitochondrial markers. Gene tree topologies and lineage-specific molecular clock calibrations reveal that the generative cladogenetic events were temporally and spatially heterogeneous. Disjunctions involving the three stem lineages clearly predate formation of the Isthmus of Panama and the Caribbean Sea (Fig. 3). However, of the five Caribbean cryptic species, only the western Caribbean clade has a transisthmian sister taxon and may therefore be putatively a direct vicariant product of American Seaway closure. The remaining four Caribbean taxa have within-basin sister relationships although molecular clock estimations place the Bahamas/Antilles disjunction at/before closure of the Isthmus (3.4–4.6 million years ago) and the Gulf/Atlantic split postclosure (2.2–2.9 million years ago).

New Insights into the Scorched Mussel Gulf/Atlantic Disjunction

Our earlier study (Lee and Ó Foighil 2004), based on Floridian and Bahamian scorched mussel samples, concluded that the Gulf/Atlantic genetic disjunction for this morphospecies represented a case of temporal pseudocongruence (Cunningham and Collins 1994). In other words, although it also appeared to be a product of Floridian vicariance, the Pliocene cladogenic event underlying the scorched mussel disjunction was considerably older than the Pleistocene genetic disjunctions of many coexisting taxa (Avise 2000). Our new data allow placement of the scorched mussel Gulf/Atlantic disjunction into a comprehensive regional phylogenetic context and seriously undermine the hypothesis of a Floridian vicariant genesis, implying that the pseudocongruence may be both biogeographic and temporal in nature.

The unexpected discovery of Atlantic clade populations in the southern Caribbean opens up the possibility that the Gulf/Atlantic split may not have originated within the temperate Carolinian zoogeographic zone. The unrooted mitochondrial COI network (Fig. 6d) indicates that a minority of northern haplotypes was newly introduced from southern source populations. Northward Atlantic clade trans-basin migration may be an ongoing process because a high rate of directional gene flow was estimated from Trinidad to Florida (Table 5). However, the large bulk of the northern haplotypes form an exclusively northern subclade and the network topology (Fig. 6d) is equivocal concerning its potential source/founder evolutionary relationship with its predominantly southern sister subclade. Nevertheless, several lines of indirect evidence indicate that the northern subclade may represent a founder population in a relatively marginal environment. Mussels from southern sites grow much larger than northern samples—most Panamian specimens collected in this study were more than twice the shell length of the largest specimen recovered from northern populations (25 mm). The latter represent the highest-latitude populations (Florida to North Carolina) found in this morphospecies (Abbott 1974), and this detail is pertinent because there was a significant southward displacement of nearshore North American Atlantic coast faunas during the last ice age maximum (Cronin 1988; Pielou 1991; Wares 2002). It is therefore interesting to note that the range of estimated ages in which *N* for the exclusively northern subclade drops to 1% of its present-day size was from 7000 to 14,000 years ago (including 95% confidence intervals; Fig. 7, Table 4). This time frame is well short of the end of the last glacial maximum 20,000 years ago (Williams et al. 1998), indicating that the *B. exustus* Atlantic clade may have established its current Florida–North Carolina distribution much later than its divergence from the sister Gulf clade (2.18–2.91 million years ago).

As with any molecular clock–based estimation, our calculations of ancestral population size through time for the Atlantic northern subclade contain a number of sources of potential error; for example, biogeographic-based mutation rate calibrations tend to give inflated estimates relative to fossil record calibrations (T. M. Collins 1996; Hillis et al. 1996; Marko 2002). If the onset of mitochondrial COI genetic divergence among ancestral *B. semilaevis* and Gulf/Atlantic clade geminate lineages occurred significantly before closure of the Isthmus of Panama, our lineage-specific mutation rate calibrations would be overestimates (Knowlton and Weigt 1998; Marko 2002) and the ancestral population size values underestimates. Such a scenario is entirely possible, given that the fossil record indicates a Middle Miocene onset of uplift-associated marine faunal change (L. S. Collins 1996; Vermeij 2001) and that our lineage-specific rates are unusually high (Lee and Ó Foighil 2004). It should be kept in mind, however, that mytilid mitochondrial genomes exhibit high rates of mutation relative to other marine invertebrates (Wares and Cunningham 2001). Fortunately, the inference of a recent rapid decline in ancestral effective population size in the Atlantic northern subclade is robust to a wide range of molecular clock calibrations. For instance, a recent study of reef-dwelling arcid bivalve transisthmian geminate species pairs, which used fossil-calibrated mitochondrial COI divergences, found that one of the most divergent pairs may be more than 30 million years old (Marko 2002). We consider it unlikely that transisthmian divergences for *Brachidontes* geminates are in this age bracket: Knowlton and Weigt (1998) found that the best (most recently diverged) transisthmian taxa for such estimates are mangrove associates and our putative *Brachidontes* geminates are common in this habitat. Nevertheless, even if a calibration of 30 million years is

applied, slowing the inferred mutation rate by an order of magnitude, the estimated Atlantic northern subclade effective population size still plunges to about 35% of its current size by 20,000 years, a value consistent with a hypothesis of local extirpation and subsequent postglacial recolonization (Marko 2004).

It is also possible that our ancestral demography calculations through time for the Atlantic northern subclade are overestimates due to an undersampling of southern Caribbean populations. Our two southern sampling sites, in Panama and Trinidad, where Atlantic clade individuals were recovered are more than 2200 km apart and we managed to obtain and genotype a mere 20 Atlantic clade individuals over this extensive geographic range. It would not be surprising if some of the exclusively northern haplotypes (Fig. 6d) were also eventually found to be present in southern populations, a result that would act to erode the network of exclusively northern genotypes and accelerate the inferred rate of northern population decrease through time.

Based on the available mitochondrial COI data, we consider it likely that the Atlantic clade northern populations stem from multiple, temporally heterogeneous, post–ice age maximum colonization events by southern tropical Caribbean source populations. A minority of these colonizations appear to have been recent; however most northern COI genotypes stem from an inferred earlier wave of colonization that we date to 7000–14,000 years ago (Fig. 7, Table 6). The generality of this surprising pattern of trans-Caribbean scorched mussel Gulf/Atlantic Floridian pseudocongruence is not clear at present, but it may well be a recurring biogeographic theme among some members of the large fraction of Carolinian morphospecies that share the scorched mussel's nominal distribution range from North Carolina to the southern Caribbean (Abbott 1974).

Allopatric Speciation Drives Scorched Mussel Cladogenesis

Our results increase the number of Caribbean *Brachidontes* species from two to six and are consistent with numerous molecular studies of invertebrate species complexes that have prompted significantly amplified estimates of marine biodiversity (Knowlton 2000; Thorpe et al. 2000). Viewed regionally, only *B. modiolus* approximated the common Caribbean condition of extensive within-basin distribution associated with little apparent genetic structuring (Mitton et al. 1989; Lacson 1992; Hateley and Sleeter 1993; Shulman and Bermingham 1995; Lessios et al. 2001, 2003; Rocha et al. 2002; Williams and Reid 2004). In contrast, the five cryptic *B. exustus* species had an intriguing pattern of within-basin distribution characterized by distinct geographic areas of ecological dominance, often adjoining those of sister taxa, and, in general, our individual sampling locations were dominated by a particular species. Only two of 21 sampling sites contained large numbers of more than one coexisting cryptic species and in both cases the coexisting taxa were not sister species: western Caribbean clade + Atlantic clade in Bocas del Toro, Panama, mangrove habitat and Gulf clade + Bahamas clade in Horseshoe site, Florida Keys (Table 2). This pattern of distribution is consistent with allopatric speciation origins for all five cryptic taxa, coupled with restricted post-speciation range extensions (Meyer 2003; Williams and Reid 2004). Allopatric speciation is a common mode of marine diversification, even in species with extended pelagic larval development (Colborn et al. 2001; Lessios et al. 2001; Meyer 2003; Williams and Reid 2004) and may result from either vicariant division or from founder dispersal (Paulay and Meyer 2002). With the exception of the western Caribbean clade, which appears to be the product of transisthmian vicariance (Figs. 3, 4), and the incipient founder differentiation of the Cape Verdean, Bahamas, clade population (Fig. 5), it is not possible to distinguish among these two allopatric mechanisms for the remainder of the study taxa.

Maintenance of Allopatry

One of the most salient questions in the ecology and evolution of nearshore tropical marine faunas concerns the degree of connectivity among geographically discrete populations (Warner and Palumbi 2003). Do they primarily represent closed systems with very little population exchange, even on relatively modest geographic scales (Cowen et al. 2000)? Or might they be best described as open systems (Mora and Sale 2002)? Evidence can be found for a spectrum of connectivity, from extensive (in the absence of biogeographic barriers) for species with broad larval dispersal potential (Lessios et al. 2001; Rocha et al. 2002) to almost nonexistent in many nominal taxa with reduced pelagic larval development (Kirkendale and Meyer 2004; Meyer et al. 2005). Of particular interest are taxa, such as the scorched mussel species complex, in which pronounced latent genetic structuring persists despite extended pelagic larval development (Barber et al. 2002; Taylor and Hellberg 2003a; Williams and Reid 2004). With the exception of the cleaner goby, *Elacatinus* (Taylor and Hellberg 2003a), this pattern has not previously been documented in the Caribbean fauna, in the absence of distinct habitat partitioning (Rocha et al. 2005). The sampling focus of the *Elacatinus* study loosely approximated that of the Bahamas and Antilles mussel sister clades (Fig. 5). The primary genetic disjunctions recovered in comparable samples of both studies were congruent in that they distinguished northern Bahamian from Antillean lineages, although mitochondrial genetic divergence levels were much less in the goby than in the mussels (Taylor and Hellberg 2003a).

Although the most prominent instances of scorched mussel genetic structuring involved that of the cryptic species themselves (Figs. 4–6), four of the five cryptic species also showed significant genetic differentiation of their constituent populations (Table 4), implying that local recruitment dynamics predominate. The exception is the Bahamas clade in that it lacked significant genetic differentiation among its western Atlantic/Caribbean populations, despite their incorporation of two divergent mitochondrial clades and an isolated oceanic island population on Bermuda (Fig. 5, Table 4). The Bermudan shallow water marine fauna represents a moderately impoverished oceanic extension of the Caribbean fauna (Sterrer 1986, 1998) and the nearest source populations are approximately 1500 km to the southwest in southern Florida and the Bahamas. Based on net transport of drift bottles, this distance represents a minimum passage of 21–30 days for

passive pelagic transport of larvae (Jackson 1986) and our results indicate that an oceanic dispersal filter of this geographic/temporal scale is well within the larval dispersal capabilities of scorched mussels. Nevertheless, the lack of Bermudan genetic distinctiveness is surprising, given that populations of the sister Antillean clade exhibit significant genetic substructure (Table 4), despite being separated by a series of much smaller individual dispersal filters, and that Pleistocene fossil *Brachidontes* have been found on Bermuda (Richards et al. 1969). There is some evidence for a partial turnover of the Bermudan marine malacofauna on ecological time scales (Abbott and Jensen 1967; Sterrer 1986). We hypothesize that the Bermudan scorched mussel population may have a history of intermittent persistence and that the present-day population may be much younger than the fossil record would indicate.

The *Elacatinus* Caribbean data has been the subject of contrasting genes versus oceanography interpretations (Colin 2003; Taylor and Hellberg 2003b; Warner and Palumbi 2003). We cannot distinguish among these possibilities directly for scorched mussel lineages or rule out a role for undocumented human-mediated introductions. However, several lines of indirect evidence favor the hypothesis that the predominantly allopatric distributions are maintained over evolutionary time scales, primarily by postrecruitment ecological factors rather than by oceanographic barriers to larval-mediated gene flow. For instance, data supporting evolutionary recent trans-basin gene flow among the geographically disjunct Atlantic clade northern and southern populations (Fig. 6d, Tables 5) undermines the case that oceanographic barriers to larval dispersal explains the apparent absence of this clade in central Caribbean insular habitats (Fig. 6a). In addition, the geographic scale of the Bahamas/Antilles sister taxon disjunction (145 km between Cuba and the Florida Keys), which may have persisted for the past 3.4–4.6 million years, was dwarfed by within-clade scales of more evolutionarily recent realized gene flow: the Bahamas clade range expansion across the Atlantic (Figs. 5a–c) and the basin-spanning distribution of the most common Antilles clade haplotype (Fig. 5d). Perhaps the most compelling evidence is that cryptic scorched mussel species were not restricted to their core distributional areas and were encountered (coexisting in low frequency with the local dominant) in other parts of the Basin, for example, three subadult Antilles clade individuals in Key Biscayne (Fig. 5, Table 2), one Bahamas clade individual in Venezuela (Fig. 5, Table 2), and one Atlantic clade southern genotype in the Boca Chica Florida Key (Fig. 6, Table 2). These individuals were typed for both nuclear and mitochondrial markers and appear to represent genuine allorecruits rather than mere introgressed mitochondrial genomes. The Key Biscayne Antillean clade subadults are particularly interesting because, in our earlier study of Floridian/Bahamian scorched mussel populations (Lee and Ó Foighil 2004), we failed to recover any Antilles clade adults—a result consistent with allorecruitment of these three individuals from a geographically distinct source population, such as Cuba (Fig. 5). Subadult specimens were genotyped in the Key Biscayne sample simply due to the very low numbers of scorched mussels ($N = 8$; Table 2) encountered in this apparently marginal location (Lee and Ó Foighil 2004) and the resulting need to process every individual retrieved. Given these observations, a genes not oceanography (Taylor and Hellberg 2003b; Warner and Palumbi 2003) hypothesis for the maintenance of scorched mussel allopatry predicts that careful genotyping of subadult recruits in southern Florida would reveal that Antilles clade member have a widespread, low-frequency presence in southern Floridian juvenile scorched mussel cohorts. In summary, although local autorecruitment dynamics appear to routinely predominate for most of the five cryptic taxa (Table 4), our data collectively imply that postrecruitment environmental exclusion processes have played, and are playing, a key role in the long-term maintenance of within-basin scorched mussel allopatry.

At present, we can only speculate as to the nature of the environmental exclusion mechanism(s), although there seems to be a good fit with continental and oceanic nearshore tropical distribution pattern expectations (Abbott 1960; Vermeij 1987; Williams and Reid 2004; Meyer et al. 2005). The western Caribbean (Fig. 4a) and Gulf and Atlantic clades (Fig. 6a) were almost exclusively found in relatively high-nutrient, lower-salinity continental habitats, whereas the Bahamas and Antilles sister taxa appeared to be restricted to more oceanic, higher-salinity, and lower-productivity conditions, typically on offshore islands (Fig. 5a). This putative environmental effect can be observed over small geographic distances in the Florida Keys, where there are marked habitat and faunal distinctions among the more continental inner (bayside) and the more oceanic outer (oceanside) habitats (Mikkelsen and Bieler 2000; Bieler and Mikkelsen 2004). We sampled two adjacent sites in the lower Keys, a Bahamas clade-dominated oceanside location (Boca Chica Key) that lacked Gulf clade specimens, and a bayside location (Horseshoe, West Summerland Key) in which the majority of individuals typed belonged to the Gulf clade and Bahamas clade specimens were in the minority (Table 2).

The Bahamas and Antilles sister clades inhabit ostensibly similar and adjacent island habitats in their respective northern and central/southern Caribbean core ranges (Fig. 5a). Paradoxically, there are no obvious environmental factors that could plausibly operate as differential postrecruitment exclusion mechanisms and these two intertidal suspension-feeding cryptic mussel species are unlikely to differ significantly in trophic modes. Our data are consistent with the hypothesis that this narrow geographic disjunction (145 km from Cuba to the Florida Keys) may have persisted since their presumed allopatric speciation 3.4–4.6 million years ago. One speculative possibility is that latent coevolved biotic interactions, which also developed in allopatry, may operate to critically reduce the survivorship/reproduction of allorecruits. The possible nature of such putative coevolved biotic interactions remains obscure, but this hypothesis may be testable using caged and uncaged reciprocal transplant experiments.

Clade-specific environmental exclusion mechanisms also likely played a decisive role in determining which of the five regional cryptic scorched mussel species colonized the newly available Atlantic coast (Florida to North Carolina) habitat following the northward expansion of nearshore temperate faunas at the end of the last ice age (Cronin 1988; Pielou 1991; Wares 2002). Although our results show that the At-

lantic clade northern populations stem from multiple, temporally heterogeneous colonization events, starting at 7000–14,000 years ago by a southern Caribbean clade (Figs. 6, 7), they do not indicate why only this clade was successful in becoming established. A simple, pelagic larval-mediated dispersal model would predict that the actual source lineage in the southern Caribbean represented the least likely colonizer among the five candidate cryptic species, especially so relative to the Gulf, Bahamas, and Antilles clades. This is because its relatively remote southern Caribbean location (i.e., the newly available habitat) would inevitably lead to disproportionately severe dilution/mortality for its larvae as they dispersed across the Caribbean Basin, yielding much lower representation in Atlantic coast scorched mussel colonizing larval cohorts (Cowen et al. 2000) compared to the more geographically proximate Gulf, Bahamas, and Antilles clades. As discussed above, continental environmental conditions may have prevented Atlantic coast establishment of the Bahamas/Antilles sister clades. However, the apparent failure of the geographically proximate continental Gulf clade to colonize the Atlantic flank of Florida, thereby leaving it open for long-distance colonization by a sister tropical southern Caribbean species, is quite enigmatic, but hints at a narrower window of ecological specialization in the former. It would seem that not all scorched mussels are created equally.

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