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RESEARCH ARTICLE

Evidence for panmixia despite barriers to gene flow in the hooked mussel, *Ischadium recurvum* (Mytilidae; Brachidontinae) along the North American coastline

Frank M. Fontanella, Yvette Garner, Jasmine Starnes and Megan Whitaker

Department of Biology, University of West Georgia, Carrollton, GA, USA

ABSTRACT

The discovery of phylogeographic patterns within broadly distributed marine species can be particularly challenging because absolute physical barriers to dispersal can be inconspicuous. Genetic boundaries often lie where ocean currents meet, forming sharp physical and ecological gradients, which may act as barriers to successful migrants. In eastern North America, coastal species often show phylogeographic differentiation associated with two recognized genetic barriers: the Gulf/Atlantic and the Virginia/Carolina discontinuities. We examined 185 specimens of the intertidal hooked mussel *Ischadium recurvum* collected from 15 locations along the eastern coastline of North America to examine phylogeographic, migration and historical demographic patterns associated climate change associated with Pleistocene glacial patterns. Hypothesis testing using Bayes factors in Migrate-n rejected the presence of phylogeographic breaks consistent with either maritime discontinuity and favoured a panmictic population model. The migration rate from the Gulf to the Atlantic was approximately three times higher than the migration from the Atlantic to the Gulf whereas the Carolina–Virginia migration rates were nearly equal. The summary statistics (Tajima's *D*, Fu's *Fs*) were significant and the demographic analyses (mismatch distributions, Bayesian skyline plot) were consistent with patterns of population expansion following glacial retreat during the Pleistocene epoch.

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Mitochondrial DNA; panmixia; gene flow; *Ischadium recurvum*

Introduction

Traditionally, levels of biological diversity have been considered and measured in numbers of species. Applying phylogeographic methods to studies of natural populations make clear that high levels of geographically structured genetic diversity exist within species due to the effects of environmental changes. The observed depth of genetic divergence has led to a reevaluation of the level at which conservation effort should be directed to maintain independently evolving lineages, with an emphasis on the preservation of divergent evolutionary lineages or evolutionarily significant units.

Phylogeographic patterns within broadly distributed species often occur at biogeographical province boundaries (Briggs and Bowen 2012). The discovery of these discontinuities is particularly challenging in marine environments because absolute physical barriers to dispersal are usually absent. Genetic boundaries often lie where water masses carried by ocean currents meet, forming sharp physical (often thermal) and ecological gradients, which may act as barriers to successful migrants. Examples come from coastlines around the world, including North America (Bowen and Avise 1990; Hare and Avise 1998; Kelly et al. 2006), South America (Sanchez et al. 2011; Brante et al. 2012) and South Africa (Teske et al. 2009, 2011).

In eastern North America, coastal species often show phylogeographic differentiation associated with two recognized genetic barriers: the Gulf/Atlantic and the Virginia/Carolina discontinuities (Figure 1). The Gulf/Atlantic discontinuity has been explained by the fact that the Florida peninsula emerged in the Miocene Epoch and has been interpreted as forming an efficient barrier to gene flow due to its extension from temperate to subtropical waters (Avise 1992). Geological events in the Pliocene through Pleistocene Epochs alternately submerged and exposed the Florida peninsula, possibly reducing gene flow between Gulf and Atlantic coast populations (Avise 1992). Typically, Atlantic populations show an asymmetric distribution extending further north along the Atlantic coast caused by larvae being dispersed by the Gulf Stream Current. As the Gulf Stream reaches Cape Hatteras, North Carolina, the cold Labrador Current that flows from the north directs the Gulf Stream away from the coast and out into the Atlantic Ocean. This region marks one of the sharpest thermal boundaries in the world's coastal oceans where strong currents meet and interact with oceanic climates and physiographic factors (Gray and Cerame-Vivas 1963) to yield one of the steepest ecological gradients along the western Atlantic coast. Unsurprisingly, this area marks the southern range boundary for scores of taxa within the Virginian Province (Cape Cod, MA, to Cape Hatteras, NC) and the northern boundary for taxa in the Carolinian Province

CONTACT Frank M. Fontanella ffontane@uwg.edu Department of Biology, University of West Georgia, 1601 Maple Street, Carrollton, GA 30118, USA

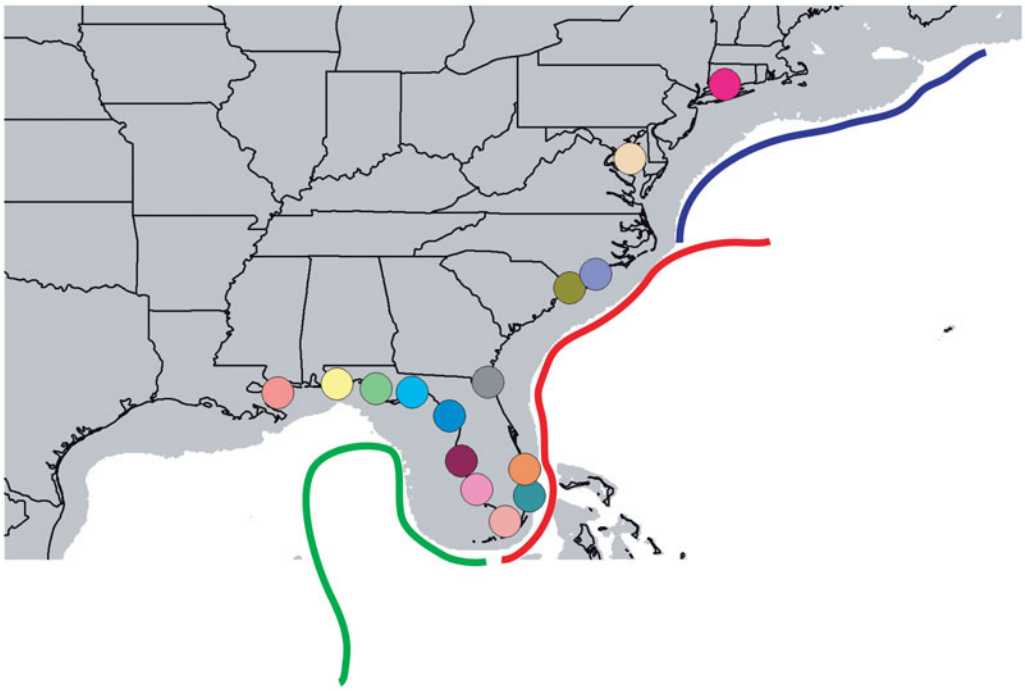

Figure 1. Map of the eastern United States showing location of samples used in this study. Lines represent approximate locations of the three major oceanic currents; Loop current in the Gulf of Mexico, Gulf Stream current along the southern Atlantic and the Labrador current along the northern Atlantic coastlines. Light grey area represents the coastline during the Last Glacial Maximum derived from CCSM models.

(Cape Hatteras to Cape Canaveral or West Palm Beach, FL; Briggs 1974; Briggs and Bowen 2012). Subsequently, additional genetic breaks associated with these diverging currents have been discovered for coastal species that span the eastern coastline of North America (Young et al. 2002; McCartney et al. 2013; Boehm et al. 2015).

The intertidal hooked mussel *Ischadium recurvum*, extends along the Atlantic coast of North America, ranging from Cape Cod, Massachusetts, into the Gulf of Mexico (GOM) and south to the West Indies (Abbott and Morris 1995). This epibenthic species is found in moderate to low wave energy environments attached to rocks, oyster reefs, and boat docks. Spawning occurs from late May to early November resulting in development of planktotrophic larvae. While information on their larval development is lacking, the larvae of the closely related genus *Brachidontes* can live up to 40 days under laboratory conditions (Campos and Romorino 1980; Fields and Moore 1983).

Here, we investigate the phylogeographic patterns of the hooked mussel *I. recurvum* throughout their native ranges in Florida and along the Atlantic coast. We aim to determine whether this species displays the phylogeographic patterns observed in other coastal and marine taxa that span multiple phylogeographic barriers.

Materials and methods

One hundred and eighty five specimens of *I. recurvum* were collected from 15 locations along the Florida and Atlantic coastlines (Figure 1). Tissues from all specimens were preserved in 95% ethanol and stored at 4 °C until DNA was extracted. Total genomic DNA was extracted from adductor muscle using QIAGEN DNeasy Blood and Tissue Kit (Hilden, Germany) extraction kits following the manufacturer's protocol. We amplified a 693 base pair (bp) fragment of the mitochondrial cytochrome c oxidase subunit I (COI) gene using polymerase chain reaction. PCR reactions were carried out in 25 µl reactions using 0.75 µM of each primer, 1 U Taq polymerase (Qiagen, Hilden, Germany), 10 µl master Amp^{TM} 2× PCR premix (Epicenter Biotechnologies, Madison, WI), approximately 100 ng of DNA template and ddH_2O to the final volume. The thermal regime was 5 min at 95 °C, followed by 35 cycles of 60 s at 94 °C for denaturation, 30 s at 47 °C for annealing and 60 seconds at 68 °C for extension, followed by a 5 min final extension at 68 °C. PCR products were purified with EXOSAPiT (USB Corporation, Cleveland, OH) and sequenced in both directions with the original primers in 5 µl sequencing reactions with BigDye® Terminator v3.1 (Applied Biosystems, Foster City, CA) following the manufacturer's recommendations. Reactions were purified with the CleanSeq Dye-terminator removal kit (Agencourt, Brea, CA) and analysed on an ABI Prism 3730 sequencer (Applied Biosystems, Perkin-Elmer, San Diego, CA). Sequences were assembled, edited, and aligned using SEQUENCHER 4.7 (Gene Codes, Corp., Ann Arbor, MI) and an open reading frame was verified for each gene. The alignments were unambiguous and we did not detect any gaps present for the specimens sequenced in this study.

Haplotype diversity (Hd) and nucleotide diversity (π) were calculated to measure DNA polymorphisms using DNAsp v 6.0 (Rozas et al. 2017). These measures are appropriate for this type of study because they do not depend on sequence length or sample size. We used Migrate-n (Beerli and Felsenstein 1999; Beerli 2009) to investigate ancient gene flow across the Gulf-Atlantic and Cape Hatteras discontinuities. Migrate-n incorporates a coalescent approach to

estimate migrate rates between two populations and performs best when two populations are used. Migrate-n assumes that the data are in equilibrium, that population sizes and migration rates are constant through time, and that populations are randomly sampled. Often, one or more of these assumptions is violated, however, the software has been shown to be robust to violations in simulated studies (Beerli 2009; Beerli and Palczewski 2010). We used the Bayesian inference model to estimate θ, M and the number of migrants per generation (Nm) using the formula $Nm=\theta \times M$ for Gulf Atlantic and Carolina Virginia Province migration patterns. In addition, we tested the hypothesis of unidirectional gene flow for each region by constraining $M=0$, and a panmictic model which assumes that all individuals belong to a single population. For each hypothesis, we estimated starting values of θ and M with an F_{ST} calculation and used uniform priors (for θ, minimum = 0.0, maximum = 0.3, delta = 0.01; for M, minimum = 0.0, maximum = 1000.0, and delta = 100.0). Parameter space was searched using four parallel chains with static heating (temperatures: 1.0, 1.5, 3.0, 100,000.0). We ran each chain for 20 million generations and sampled every 100 generations. For each chain, we used a burn-in of 20,000. We inspected histograms of estimated θ and M posterior values (bin number = 1500) to assess convergence. In general, Nm values >1 indicate that the effect of migration is greater than the effect of genetic drift. The marginal likelihoods of the models were evaluated using Bayes factors (Beerli and Palczewski 2010). The lack of a phylogeographic break would be indicated if the panmictic model was favoured over the models assuming the existence of phylogenetic breaks.

For phylogenetic analyses, the best-fitting model of nucleotide substitution was selected using the Akaike information criterion (AIC) following the procedure outlined by and implemented in jModelTest v 2.1.4 (Darriba et al. 2012). These results give a best fit for the Hasegawa, Kishino and Yano (HKY) nucleotide substitution model with gamma-distributed rate heterogeneity among sites (gamma shape = 0.024).

Bayesian phylogenetic analysis was carried out with MrBayes v. 3.2.6 (Ronquist and Heulsenbeck 2003) with 3×10^8 steps, sampling every 3000th tree from two runs. Convergence was assessed as having been reached when the average standard deviation of split frequencies became <0.01, and the potential scale reduction factor (Gelman and Rubin 1992) 1.00 for all parameters. The runs were also visually checked for lack of trends in Tracer v1.6 (Rambaut et al. 2014). The first 25% of trees were discarded from each run as burn-in, and a 50% majority rule tree was constructed. Trees were visualized using the FigTree v1.1.2 program, available at http://tree.bio.ed.ac.uk/software/figtree/. Maximum likelihood (ML) analysis was conducted in RAxML v.8.2 (Stamatakis 2014) using the HKY + G model and rapid bootstrapping for 10,000 iterations. We chose *Geukensia demissa* and *G. granossisima* as outgroup taxa based on Lee and Foighil (2004). Support values for clades with less than 90% Bayesian support and ML support are not shown.

Nucleotide diversity can be calculated using the average number of pairwise nucleotide differences (π) or by calculating the number of segregating sites (S). Under the null hypothesis of population stability, the difference between these two values, Tajima's D (Tajima 1989) and Fu's F_s (Fu 1997), can be used to infer demographic history of a population. If populations have been stable over time, both statistics are expected to be close to zero (Tajima 1989; Fu 1997). Significant deviation from zero (positive or negative) permits for the rejection of the null hypothesis of population stability. Under the assumption of neutrality, negative values are expected in populations that have undergone recent increases because rare alleles are more numerous than expected. Positive values occur if rare alleles are eliminated from populations following genetic bottlenecks (Tajima 1989). Mismatch distributions were conducted to further investigate the possibility of demographic change. The validity of the expansion models was calculated by constructing 10,000 coalescent simulations in DnaSP.

Although Tajima's D and mismatch distributions are able to provide insights into whether or not population growth has been expansive, they are not able to provide information about the shape of population growth over time. Therefore, to estimate the shape of population growth through time we constructed Bayesian skyline plots (BSPs) implemented in BEAST v1.8.4 (Drummond et al. 2012) under the appropriate model. Genealogies and model parameters for each lineage were sampled every 1000th iteration for 1×10^7 generations under a relaxed lognormal molecular clock with uniformly distributed priors and a pre-burn-in of 100. Demographic plots were visualized using Tracer v 1.6 (Rambaut et al. 2014). The rate of divergence for *I. recurvum* was calibrated using the value estimated by Luttikhuizen et al. (2003) from the fossil record for Mytilidae which translates to a mutation rate of 0.0026 per nucleotide site per million years (Schenekar and Weiss 2011). We used a generation time of 3 years derived from the closely related genus *Brachidontes* (Morton 1988).

Results

A total of 23 haplotypes were found in the 185 *I. recurvum* individuals examined. The unique sequences (haplotypes) were submitted to GenBank (accession nos. MH041466–MH041483). The translated amino acid sequences did not contain stop codons or indels. The presence of an open reading frame lack of intern stop codons suggests that the amplicon was a fragment from the functional mitochondrial COI gene region and not a nuclear pseudogene or DNA contaminant (Buhay 2009; Siddall et al. 2009).

Haplotype diversity ranged from 0.43 to 1.0 with a slightly higher diversity found in the GOM populations, while nucleotide diversity (π) was equal between the two coasts (Table 1). The Migrate-n analyses inferred the number of migrants moving towards the Atlantic coast (23.465) was approximately three times the number of individuals moving towards the Gulf Coast (Nm = 7.923), while the Carolina–Virginia Province showed nearly equal amounts of migrants (Nm = 4.02 and 3.86, respectively) (Figure 2). These results suggest the effects of migration (regardless of direction) are more powerful than the effects of drift. We rejected the presence of a phylogeographic break at either location and the possibility of unidirectional gene flow based on the Bayes factors comparisons in

Table 1. Sample size (*N*), number of haplotypes (HN), haplotype diversity (Hd), nucleotide diversity (π), average number of pairwise differences (*k*) and results of Tajima's *D* and Fu's F_s statistic for each sampling location of *I. recurvum*.

	N	HN	Hd	π	*K*	Taj. *D*	Fu's *Fs*
Gulf of Mexico							
Bradenton, FL	43	5	0.59	0.0017	0.952	−0.44 CI (−1.44, 1.74)	−0.39 CI (−3.10, 4.40)
New Orleans, LA	6	2	0.60	0.0008	0.600	1.44 CI (−1.2, 1.64)	0.79 CI (−1.35, 2.50)
Fort Myers, FL	9	3	0.75	0.0014	1.0	1.23 CI (−1.60, 1.75)	0.35 CI (−1.99, 3.28)
Pensacola, FL	25	8	0.80	0.0025	1.527	−0.88 CI (−1.73, 1.94)	−2.64 CI (−3.36, 3.96)*
Cedar Key, FL	13	4	0.72	0.0020	1.615	0.86 CI (−1.76, 1.86)	0.55 CI (−2.42, 4.21)
St. Andrews, FL	18	5	0.76	0.0016	1.039	0.53, CI (−1.71, 1.85)	−01.15 CI (−2.19, 4.36)
Panacea, FL	12	10	0.97	0.0054	3.485	−1.28 CI (−1.71, 2.35)	−5.14 CI (−3.34, 4.68)*
Atlantic							
Stuart, FL	13	5	0.692	0.0022	1.436	−0.38 CI (−1.68, 1.87)	−0.86 CI (−2.33, 3.80)
Jacksonville, FL	12	10	0.970	0.0056	3.458	−0.19 CI (−1.18, 1.68)	−0.45 CI (−2.11, 3.70)
Hampstead, NC	7	6	0.952	0.0033	2.190	−0.53 CI (−1.52, 1.75)	−3.02 CI (−2.47, 3.49)*
New Haven, CT	8	2	0.429	0.0012	0.857	0.41 CI (−1.53, 1.60)	1.65 CI (−1.74, 2.47)
Myrtle Beach, SC	6	6	1.0	0.0040	2.467	−0.35 CI (−1.33, 1.44)	−3.77 CI (−1.81, 2.99)*
Anne Arundel, MD	6	2	0.533	0.0013	1.067	1.03 CI (−1.33, 1.75)	1.72 CI (−1.45, 3.00)
Pompano Beach, FL	8	2	0.429	0.0006	0.429	0.33 CI (−1.44, 1.60)	0.54 CI (−1.09, 2.21)
All samples		23	0.793	0.0027	1.528	−1.85 CI (−1.55, 1.93)*	−15.11 CI (−6.3 6.44)*

*Significantly different at a $p = .05$.

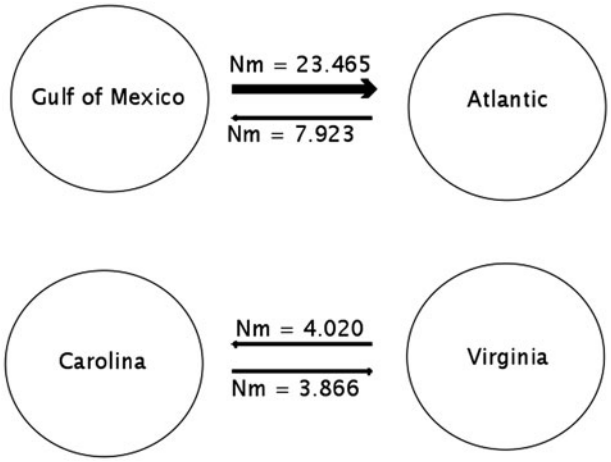

Figure 2. Number of migrants (Nm) calculated from θ and *M* estimates from Migrate-n. Arrow width is proportional to the approximate level of gene flow.

Table 2. Phylogeographic models compared using Bayes factors.

Model	Bezier Lnl	LBF	Bayes factor model rank	Model probability
Gulf/Atlantic Full	−514.0	−14.31	3	0
Gulf→Atlantic	−502.6	0	2	0
Atlantic→Gulf	−689.5	−38.56	6	0
Virginia/Carolina Full	−603.1	−26.48	4	0
Virginia→Carolina	−700.2	−4358	7	0
Carolina→Virginia	−655.4	−30.64	5	0
Panmictic→model	−211.32	86.05	1	1.000

The Bezier approximated marginal likelihood, natural log Bayes factors, model rank, and model probability are reported for each model.

Migrate-n. The panmictic model was selected as the best model for each species, indicating it is more likely that the sampled locations represent one panmictic population than multiple separate populations (Table 2).

The Bayesian analysis produced a 50% majority-rule consensus tree with a harmonic mean of 1652.97 following a burn-in of 20,000 generations. As both the ML and BI analyses produced highly congruent estimates of the phylogenetic patterns, only the Bayesian consensus phylogram is presented with the posterior probabilities and non-parametric bootstrap values for the shared branches (Figure 3).

Tajima's *D* was not significant for any of the individual populations while Fu's *Fs* was significant for the Pensacola, FL and Panacea, FL populations from the Gulf, and the Hampstead, NC and Myrtle Beach, SC populations from the Atlantic (Table 1). Ramirez-Soriano et al. (2008) showed that Fu's *Fs* is one of the more powerful tests for detecting expansion on nonrecombining genomic regions. However, given the small sample sizes we recommend the results for these individual sampling localities be interpreted cautiously. Tajima's *D* and Fu's *Fs* were significant for all samples (−1.85, −15.11, respectively) and the mismatch distribution analysis (Figure 4) recovered a unimodal pattern ($\tau = 1.52$, $R^2 = 0.03$, $p < .05$). The effective sample size (ESS) for the BSP analysis was greater than 200, suggesting that the 10 million generations were sufficient to determine the demographic history. Unimodal distributions with low raggedness values, significantly negative *D* and *Fs* values and the BSP are indicative of population expansion occurring within the last 25,000 years (Figure 4).

Discussion

Based on the results of the phylogenetic analyses and our hypothesis testing using Bayes factors in Migrate-n, our data reject the presence of phylogeographic breaks consistent with either maritime discontinuity in *I. recurvum*. The Bayesian analyses inferred a single well-supported monophyletic ingroup that lacked geographic structure associated with the sample sites or either genetic discontinuity (Figure 3). Although the migration analyses depicted bi-directional migration patterns for each hypothesis, the migration rate from the Gulf to the Atlantic was approximately three times higher than the migration from the Atlantic to the Gulf whereas the Carolina–Virginia migration rates were nearly equal (Figure 2). Finally, the hypothesis testing using Bayes factors rejected the models assuming phylogeographic breaks and favoured a panmictic population model (Table 2).

The lack of genetic population structure, therefore, invites the question as to what factors may be responsible for panmixia in this species. While there is little information available as to the migration rate and dispersal distances of

Figure 3. Bayesian 50% majority-rule consensus tree for the 185 *I. recurvum* samples and outgroup taxa. Support values (posterior probabilities above branches, nonparametric bootstrap proportions below) are shown for nodes with a posterior probability >95%.

I. recurvum, they produce planktotrophic larvae that likely spend several days to weeks in the water column (Campos and Romorino 1980; Fields and Moore 1983). Larval exchange between the GOM and the Atlantic may be promoted by the Loop Current moving larvae down the western coast of Florida and into the Atlantic. Avise (2000) suggested that the Gulf Stream may promote "leakage" of Gulf haplotypes into the Atlantic Coast of Florida, a hypothesis consistent with the results of our Gulf-Atlantic migration analyses. Subsequently, not all marine species exhibiting an Atlantic-Gulf Coast distribution display genetic differentiation between the two regions. Panmictic populations across this region have been recovered for the Spanish mackerel (*Scomberomorous maculatus*), the scamp (*Mycteroperca phenax*) (Zatcoff et al. 2004), the little brown seastar (*Echinaster spinulosus*) (Fontanella 2016), and black mangroves (*Avicennia germinans*) (Hodel et al. 2016).

Similarly, we did not recover evidence of a genetic break or migration barrier associated with the Virginian–Carolinian zoogeographic regions. Haplotypes were shared between populations spanning the region and the migration analyses inferred similar number of migrants between regions. These findings are consistent with other species with trans-Cape Hatteras distributions including: the summer flounder, *Paralichthys dentatus* (Jones and Quattro 1999); the horseshoe crab, *Limulus polyphemus*; the American oyster, *Crassostrea virginica*; and the black sea bass, *Centropristis striata* (Avise 1992).

The demographic analyses showed signatures of rapid population expansion consistent with a post-Pleistocene expansion model. The mismatch distribution was unimodal, Tajima's *D* and Fu's *Fs* test were significant, all of which are indicative of recent population expansion (Figure 4 and Table 1, respectively). The BSP inferred a strong expansion curve, with the population expansion occurring after the Last Glacial Maximum (LGM) (Figure 4). The lack of population structure coupled with the recent population expansion has been found for several other marine organisms (Benzie et al. 2002; McMille-Jackson and Bert 2004; Chatti et al. 2012) and suggests that the marine environment is highly dynamic through time, affecting the distribution and population expansion of some species. The most likely explanation for the historical increase in population size and range expansion is due to an increase in available niche space as the Florida Shelf became submerged following the retreat of the Laurentide ice sheet at the end of the last glacial cycle (~21,000 years ago) (Anderson et al. 1988). The leading edge model of population expansion predicts that populations would have undergone rapid expansion as previously unsuitable habitat became colonized. Such rapid or step-wise colonizations would be characterized by low levels of genetic diversity as each new founding population represented only a fraction of the ancestral population's genetic diversity (Nichols and Hewitt 1994; Hewitt 2000). Given that sea level has increased by more than 150 m since the LGM (Anderson et al. 1988), a small population restricted to a refuge of suitable habitat in deeper waters may have expanded into the increasingly available niche space. Thus, the leading-edge effect (Hewitt 1996) resulting in recent range expansion combined with some level of contemporary larval mixing seems to be the main source of panmixia in this species.

Mitochondrial DNA (mtDNA) has been the marker of choice for inter and intraspecific studies, largely because these sequences typically show enough resolution to reveal

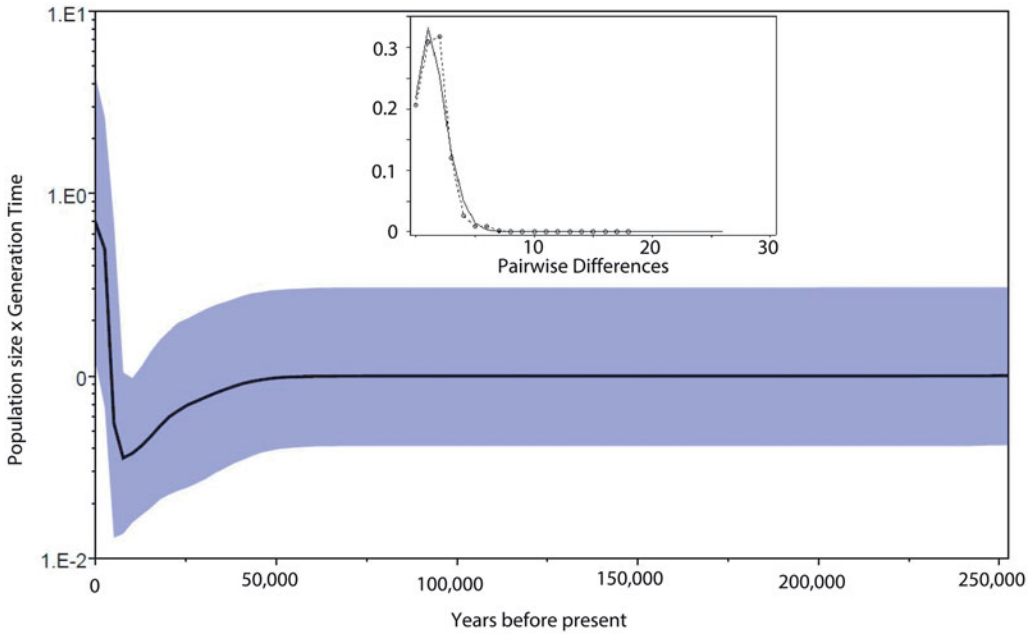

Figure 4. Mismatch distributions (above) and Bayesian skyline plot (below) depicting the demographic history for all *I. recurvum* samples in this study. For the mismatch distributions, open circles represent the observed distribution of pairwise differences and the solid line represents the expected distribution assuming population expansion. For the skyline plots, the solid line represents the median value for the log of the population size (log N_e) and the dashed lines represent the upper and lower 95% credible intervals. The *x*-axis represents time in thousands of years.

genetic variation among recently diverged lineages and the identification of barriers to gene flow. The gene region coding for the COI has become one of the most frequently used markers due to its high degree of variability (Avise 2000; Bucklin et al. 2011). Our study shows that along the North American coastline, *I. recurvum* lacks the mitochondrial diversity typically associated with broad ranging marine species despite the presence of well-defined phylogeographic barriers. The limited population structure, high level of migration across potential barriers and evidence of recent population expansion suggests that *I. recurvum* represents a single, well-established population throughout this region. Given that our study is restricted to a portion of the range, further study incorporating populations from the West Indies would be required before a full evaluation of the conservational status of *I. recurvum* can be evaluated.

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Disclosure statement

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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