Phylogeography of the shrimp *Palaemon floridanus* (Crustacea: Caridea: Palaemonidae): a partial test of meta-population genetic structure in the wider Caribbean

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**Abstract**

Marine organisms with a pelagic stage are often assumed to display minor population structure given their extended larval development and subsequent high long-distance dispersal ability. Nonetheless, considerable population structure might still occur in species with high dispersal ability due to current oceanographic and/or historical processes. Specifically, for the wider Caribbean and Gulf of Mexico, theoretical and empirical considerations suggest that populations inhabiting each of the following areas should be genetically distinct: Panama, Belize, Southwest Florida (Tampa), and Southeast Florida (Fort Pierce). This study tests the hypothesis of significant genetic differentiation in *Palaemon floridanus* populations across the wider Caribbean and Gulf of Mexico. Population level comparisons were conducted using sequences of the mtDNA COI. In agreement with predictions, AMOVA and pairwise $F_{ST}$ values demonstrated population differentiation among most pairs of the studied populations. Only Panama and East Florida populations were genetically similar. An isolation-with-migration population divergence model (implemented in IMA2) indicated that population divergence with incomplete lineage sorting can be invoked as the single mechanism explaining genetic dissimilarity between populations from the east and west coast of Florida. Historical demographic analyses indicated demographic expansion of *P. floridanus* in some localities [recent in Panama and ancient in East Florida and the wider Caribbean (entire dataset)] but constant population in other localities (in Belize and West Florida). This study rejects the idea of panmixia in marine species with high long-distance dispersal ability. Contemporary and historical processes might interact in a complex manner to determine current phylogeographic patterns.

**Introduction**

Exploring genetic structure is important for understanding the processes governing species distribution (Sotka et al. 2004), for guiding the establishment of sound conservation strategies (Goerlitz et al. 2003; Palumbi 2004; Baums 2008; Carpenter et al. 2011), for the efficient management of exploited populations (Palumbi 2004; de Oliveira-Neto et al. 2008; Marko et al. 2011), for revealing the spread of invasive species (Geller et al. 2008; Freshwater et al. 2009) and, ultimately, for shedding light into the evolutionary process (Knowlton et al. 1993; Palumbi 1994; Knowlton & Weight 1998; Avise 2000). In contrast to terrestrial species, marine organisms were originally assumed to display high population connectivity and subsequent high gene flow (Avise 2000; Cowen et al. 2000). This traditional view was based on the observation that many marine organisms have wide
geographical ranges and extended larval periods (compared with terrestrial species), and thus marine species were expected to have considerable long-distance dispersal ability. Furthermore, geographical barriers are less obvious in marine than in terrestrial environments (Cowen et al. 2000). Oceanic currents were expected to favour rather than constrain population inter-connectivity and thus promote genetic homogenization over relatively large geographical scales.

The long-standing view of high levels of contemporary gene flow and little genetic differentiation among populations of marine organisms has been examined for more than 30 years now (Avise 2000; Cowen et al. 2000, 2006; Patarnello et al. 2007; Hellberg 2009) but the accumulated body of literature provides limited support for it. Little or no population differentiation has been reported for various species of marine vertebrates and invertebrates with large geographic ranges and extended larval periods (fishes: Zardoya et al. 2004; Costagliola et al. 2004; invertebrates: Silberman et al. 1994; McMillen-Jackson & Bert 2004; Naro-Maciel et al. 2011). On the contrary, a considerable number of other studies have demonstrated remarkable levels of genetic differentiation among populations, even in species with considerable dispersal ability (fish: Taylor & Hellberg 2003; invertebrates: Hohenlohe 2004; Baums et al. 2005; Magoulas et al. 2006; Arnaud-Haond et al. 2008; Reuschel et al. 2010). Thus, the paradigm of marine populations as open panmictic systems has been seen as a faulty generality for at least 10 years and an increasing number of studies during the last decades have revealed conditions that might be relevant in driving genetic structure in marine species (Avise 2000; Marko & Hart 2011).

Important factors driving connectivity and population structure in the marine environment include biological traits such as type of development (direct versus planktonic), duration of the pelagic larval period (Teske et al. 2007), larval behaviours (Cowen et al. 2000, 2006), isolation by distance (Planes & Fauvelot 2002), generation times (Rolán-Alvarez et al. 1995), local adaptation (Rigino & Nachman 2001), and oceanographic processes reducing (or favouring) gene flow among localities (Cowen et al. 2000, 2006; White et al. 2010). Nonetheless, the relative importance of these factors in shaping marine phylogeographic patterns remains unresolved. Furthermore, it is not yet possible to make strong generalizations across high taxonomic levels with respect to the effect of particular factors on phylogeographic patterns. For instance, even congeneric or confamiliar species with comparable potential for dispersal have been shown to display remarkable differences in the extent of genetic structure over the same geographic range (Bird et al. 2007). Clearly, additional studies on the phylogeography of marine organisms are needed to improve our understanding of historical and contemporary processes driving genetic structure in the marine environment.

Of particular interest to this study is the exploration of population differentiation in species with long-distance dispersion ability. The tropical/subtropical shrimp Palaemon floridanus represents an example for the exploration of the role of oceanographic conditions and distance in driving population structure in the marine environment. The native distribution range of this shrimp is considerable and includes the Gulf of Mexico, the Caribbean basin, and the Northwestern Atlantic Ocean (herein considered part of the wider Caribbean) (Chace 1942; Coen et al. 1981; Streth & Chace 1995; J. Antonio Baeza, personal observations). Palaemon floridanus dwells in a wide variety of intertidal and shallow subtidal habitats throughout its range of distribution, including mangrove roots, among rocks in rocky intertidal pools, in crevices in intertidal fossilized coral terraces, and among shallow water red algae, and it is also present in man-made structures such as rock jetties and around wharf pilings (Chace 1942; Coen et al. 1981; Streth & Chace 1995; J. Antonio Baeza, personal observations). The long-distance dispersal ability of this species most probably is high given its ‘extended’ larval development, which includes eight zoal stages and between eight and 16 larval instars (Knowlton & Vargo 2004). In the laboratory, larval development lasts 18–23 days (Knowlton & Vargo 2004). Considering the above, high gene flow might be expected in P. floridanus. Nonetheless, historical and oceanographic processes reducing gene flow among populations are expected to produce genetic differentiation in this shrimp, at least among distantly located populations (e.g. North versus South Caribbean) (Avise 2000; Cowen et al. 2000, 2006).

The wider Caribbean represents a basin with complex geographical history and highly diverse flow regimes, with areas that might either constrain or favour genetic admixture, as suggested by high-resolution biophysical models (Cowen et al. 2000, 2006) and empirical studies (Avise 2000; Wise et al. 2004; Duran & Rützler 2006; Solis et al. 2006; Taylor & Hellberg 2006; Eytan & Hellberg 2010). Biophysical models predicting connectivity in the wider Caribbean, including South Florida, take into account the interaction between advection and diffusion properties of water circulation in the region and larval traits (i.e. survival and behaviour) (Cowen et al. 2000, 2006). For species with a larval period similar to that of P. floridanus, biophysical models predict genetic dissimilarity between northern (e.g. West and East Florida, Belize) and Southern (e.g. Panama) Caribbean populations, given the existence of the semi-permanent Panama-Colombia Gyre off the coast of Costa Rica, Panama, and Colombia which produces larval retention in the Southwestern Caribbean.
and thus prevents larval export from Southern to Northern Caribbean populations (Cowen et al. 2000, 2006). Also, populations from Belize are predicted to be isolated, and thus genetically different, from other populations in the wider Caribbean, including those from Florida and Panama (Cowen et al. 2000, 2006). In agreement with the prediction above, genetic dissimilarity between Belizean and other populations from other Caribbean localities (including the Northwestern Atlantic) and Gulf of Mexico have been reported before in several species of vertebrates and invertebrates (Duran & Rützler 2006; Taylor & Hellberg 2006; Eytan & Hellberg 2010). Lastly, the same models predict genetic homogeneity for conspecific populations from the east and west coast of Florida (Cowen et al. 2000, 2006). Nonetheless, several empirical studies have not supported this last prediction and have demonstrated distinct Atlantic and Gulf coast lineages in at least 10 species of invertebrates and vertebrates with larval periods similar to or longer than that of P. floridanus. Genetic breaks occur at various points along the Southern Florida peninsula in the studied species (see Avise 2000; Wise et al. 2004; Solits et al. 2006 and references therein). Such breaks appear to be driven by the geographic history of the region, which includes multiple glacial periods, rather than by current oceanographic conditions (Avise 2000). In general, taking into account theoretical predictions and empirical studies, genetic differentiation is expected between the southernmost and northernmost populations of P. floridanus as well as between the east and west coast of Southern Florida.

The aim of this study is to test for significant genetic differentiation in P. floridanus across the wider Caribbean and Gulf of Mexico. Specifically, populations from Panama, Belize, Southwestern Florida, and Southeastern Florida should be genetically dissimilar. For this purpose, population-level comparisons were conducted using sequences of COI mitochondrial DNA. The COI gene is well suited for population genetic studies (see Hellberg 2009 for a discussion of suitable genetic markers) (Fig. 1). Little is known about the demographic history of the Caribbean fauna. Thus, population expansion in P. floridanus was explored to increase knowledge of historical processes in the region. We also explored the role of historical and contemporary processes in explaining putative genetic heterogeneity between populations from the east and west coast of Florida.

Material and Methods

Shrimp collections and sampling rationale

A total of 78 shrimps Palaemon floridanus were collected from four different localities in the wider Caribbean and Gulf of Mexico; Panama (South Caribbean), Belize (Central Caribbean), West Florida (Eastern Gulf of Mexico) and East Florida (Northwestern Atlantic) during 2008 (Fig. 1). The rationale for choosing and sampling the four localities above was to have a simple setup, taking into account logistic, monetary, and time limitations, with which to test for population dissimilarity among locations within the wider Caribbean and Gulf of Mexico, as predicted by biophysical models and empirical studies (Cowen et al. 2006; Solits et al. 2006). In Panama and Belize, shrimps (n = 20 and 18 per locality, respectively) were collected from among roots of intertidal mangroves at the Bocas del Toro Research Station, Bocas del Toro Island (9°21′3″ N, 82°15′27″ W), and at Twin Cays (16°49′45″ N, 88°06′15″ W), located near Carrie Bow Caye, respectively. On the east coast of Florida, shrimps (n = 20) were collected from among intertidal rocks fouled with algae and sponges located at the west border of the Indian River Lagoon (27°26′47″ N, 80°19′21″ W), near the Smithsonian Marine Station at Fort Pierce, Fort Pierce. On the west coast of Florida, shrimps (n = 20) were collected from among rocks in a man-made rocky jetty in Tampa Bay near the University of Tampa (27°56′51″ N, 82°27′59″ W). Shrimps were either preserved in the field in 95% alcohol immediately after collection or occasionally from abdominal muscle tissue using the Qiagen® DNeasy® Blood and Tissue Kit following the manufacturer’s protocol. The polymerase chain reaction
(PCR) was used to amplify a 658-base pair (bp) region (excluding primers) of the COI mtDNA with a modified version of the Folmer’s primers HCO2198 (5’- TAAAC TTCAAGGTGACCAAAAAAAYCA -3’) and LCO1490 (5’-G GTCACAATCATAAAAAGAYTYGG-3’) (Folmer et al. 1994; modified by Chris Meyer at the Laboratory of Analytical Biology, National Museum of Natural History, Smithsonian Institution). Standard PCR 25-µl reactions (2.5 µl of 10 × Taq buffer, 2 µl of 50 mm MgCl₂, 2.5 µl of 10 mm dNTPs, 2.5 µl each of the two primers (10 mm), 0.625 U Taq, 1.25 µl of 20 mm BSI and 8.625 µl double distilled water) were performed on a Per- tier Thermal Cycler (DYAD®) under the following conditions: initial denaturation at 96 °C for 4 min followed by 40 cycles of 94 °C for 45 s, 52 °C for 1 min, and 72 °C for 1 min, followed by chain extension at 72 °C for 10 min. PCR products were purified with ExoSapIT (a mixture of exonuclease and shrimp alkaliphosphatase, Amersham Pharmacia, Cleveland, Ohio) and then sent for sequencing with the ABI Big Dye Terminator Mix (Applied Biosystems, Carlsbad, California) to the Laboratory of Analytical Biology of the National Museum of Natural History, Smithsonian Institution. The final set of consensus sequences were confirmed by sequencing both strands and a consensus sequence for the two strands was obtained using the software SEQUENCHER 4.5 (Gene Codes Corp, Ann Arbor, Michigan). The final set of consensus sequences was aligned with SEQUENCHER 4.5. All haplotypes obtained during this study were deposited in GenBank (accession numbers: KC019174-KC019188).

Population genetic analyses

The software ARLEQUIN version 3.5.1.3 (Excoffier et al. 2005) was used to assess diversity at each sampling locale. The standard diversity indices herein calculated for each locality were number of haplotypes, haplotypic diversity (Nei 1987), nucleotide diversity (Tajima 1983; Nei 1987), and average number of pairwise differences between haplotypes (Tajima 1983, 1993). To test for genetic variance within and among populations, an analysis of molecular variance (AMOVA; Excoffier et al. 1992) was conducted in the same software using uncorrected haplotype pairwise differences as a measure of divergence. To evaluate differentiation between locations (Slatkin 1995), population pairwise FST values were calculated using the observed number of unweighted haplotype pairwise differences and the number of haplotypes. Significance of the different FST values was determined through 10,000 permutations. We predicted significantly different FST values among all of the different studied populations.

To explore the relationship between genetic and geographical distance in P. floridanus, a Mantel test was conducted in the software IBD (Bohonak 2002). The significance of the Mantel test was determined with 10,000 permutations. Prior to the analysis, average pairwise differences between populations were calculated with ARLEQUIN version 3.5.1.3. Measurements of distances between pairs of localities in the Caribbean and Gulf of Mexico were conducted using the ‘path ruler’ tool in Google Earth (http://earth.google.com/). Calculations of the shortest distance between two localities were conducted avoiding islands and land masses.

Divergence of and migration between Florida populations

Populations from the two coasts of Florida shared haplotypes but were genetically dissimilar, as suggested by FST values (see Results). Three scenarios might explain the above results: (i) contemporary gene flow solely, (ii) population divergence (with incomplete lineage sorting) from an ancestral polymorphic population solely or (iii) a combination of gene flow and divergence with incomplete lineage sorting. In an attempt to distinguish between the different scenarios above, we further explored the relationship between populations from the two coasts of Florida using the software IMA2 (Hey & Nielsen 2007).

IMA2 is a coalescent-based method (Kingman 1982) that uses one or more loci from two or more populations and Markov chain Monte Carlo (MCMC) simulations of gene genealogies to estimate the posterior density of various parameters that are part of an ‘isolation with migration’ (IM) model (Hey & Nielsen 2007; Hey 2010). The parameters estimated by IMA2 are the time of divergence (t = mutation scaled time since divergence), effective population sizes, and migration rates (m₁ and m₂ = mutation scaled migration rate) between the studied populations. By contrast to other classical methods (e.g. AMOVA, FST, see above), the IM model in IMA2 does not assume that mutation, drift, and migration are in an evolutionary equilibrium (Hey & Nielsen 2007; Hey 2010). Thus, this analysis is appropriate for estimating parameters for recently separated populations such as West and East Florida that may share haplotypes due to gene flow solely, ancestral polymorphism solely or a combination of the above. The IM model implemented in IMA2 assumes that each population is panmictic and that the genealogical relationship between two populations is not affected by migration from other populations (Hey & Nielsen 2007; Hey 2010). Most probably, the two assumptions above are broken in natural situations. However, IMA2 is still capable of distinguishing between complete isolation and...
divergence with gene flow (Hey & Nielsen 2007; Hey 2010).

For the analysis, we used the following strategy. First, we conducted a succession of relatively short preliminary runs employing the 'MCMC mode'. These short runs were used to determine priors for subsequent longer runs. In each succeeding run, we reduced the upper bounds on parameter priors to sample the posterior more intensively. The final runs included previous priors and were run twice (with different starting points) to guarantee that the independent runs converged. These final runs consisted of a burn-in period of 10,000 generations and a post-burn-in period of 10,000,000 generations. Each run included 40 chains. In all runs, we used the HKY model of nucleotide substitution. Also, we used exponential priors for the migration rate parameters with a mean value of 0.5. Exponential priors (instead of uniform priors) were used because we expected low gene flow between the two studied populations (see Results). Exponential distributions proceed from zero to positive infinity and have their highest density at zero (Hey 2010). These exponential priors were chosen in an attempt to represent more realistically the different scenarios discussed above (i, ii, and iii).

In the final runs (codes are available upon request from the author), we recorded the posterior probability distribution, the maximum-likelihood estimate, and the credibility interval [i.e. 95% highest posterior density interval (HPD)] for each parameter. We used the 95% HPD interval to assess whether divergence had occurred between the two studied populations and whether migration had taken place between the two populations after initial divergence. Also, we used the likelihood ratio tests provided by IMA2 to evaluate whether migration rates between populations were significantly different from zero (Hey 2010). If the two studied populations shared haplotypes only because of contemporary migration, then the m parameters should be significantly different from zero and the t parameter should not differ significantly from zero. In turn, if the populations shared haplotypes not because of contemporary migration but due to population divergence with incomplete lineage sorting, then the m parameters should not be significantly different from zero and the t parameter should differ significantly from zero.

Parameter estimates were similar between the first and second half of each final run and visual inspection of the plots between likelihood and time did not show any long-term trend, indicating that the likelihoods had stabilized prior to the 10,000th generation. Furthermore, the two final runs gave very similar results, indicating that likelihood convergence has been achieved prior to the 10,000th generation.

### Historical demography

To examine historical demography in *Palaemon floridanus*, Tajima’s D statistic was calculated (in ARLEQUIN) and mismatch distribution and Bayesian skyline analyses were performed for each studied locality separately (Tajima’s D statistics and mismatch distribution analyses) and/or for the entire dataset (mismatch distribution and Bayesian skyline analyses). First, evidence of recent population expansion was tested using Tajima’s D statistic (Tajima 1989). A negative value of D indicates the presence of an excess of low frequency haplotypes, as would be expected under a sudden expansion scenario (Ariscou & Excoffier 1996). Significance of the different D values was determined using 10,000 permutations.

Mismatch distributions (i.e. the distribution of nucleotide site differences between pairs of individuals; Rogers & Harpending 1992; Harpending 1994; Schneider & Excoffier 1999) were calculated for the four studied populations and for the entire dataset in ARLEQUIN version 3.5.1.3. A stable population is expected to exhibit a multimodal mismatch distribution. In turn, an expanding population after an episode of low effective population size should display a unimodal mismatch distribution. Thus, it is possible to test the goodness of fit of the observed distribution against that of a hypothetical expanding population. Each observed mismatch distribution was compared with that expected under the model of sudden demographic expansion using a least squares approach with 10,000 bootstrap replicates. The raggedness index statistic (RHI, Harpending et al. 1993) was calculated for each population and for the entire dataset. The magnitude of this later statistic increases the longer a population has been stable. Lastly, the parameter τ (a unit of mutational time) obtained during the mismatch distribution analysis in ARLEQUIN version 3.5.1.3 was used to estimate time since population expansion using the formula $T = \tau/2\mu$ (Harpending 1994), implemented in the website http://www.uni-graz.at/zoolww/mismatchcalc/ (Schenekar & Weiss 2011). In the formula, T is time in years since expansion and μ is the cumulative substitution rate per generation across the DNA fragment under study (Schenekar & Weiss 2011). A divergence rate (per My) estimate of 1.4% (see Knowlton & Weight 1998) and a generation time of 1.5 years were used for the calculations. The generation time we used is based on preliminary studies on the life history of *P. floridanus* and is similar to that reported for other Palaemonidae shrimps (Carini & Hughes 2004; Chaves-Campos et al. 2010).

Lastly, a skyline plot characterizing the historical demography of *P. floridanus* in the four studied populations and in the wider Caribbean (entire dataset) was generated in the software BEAST v.1.4 (Drummond &
For the skyline plot analysis, the HKY evolutionary model was employed for consistency because it is the one implemented in IMa2 (see below). All tip dates were set to zero, a strict molecular clock was used (substitution rate = 1.4; Knowlton & Weight 1998), and starting trees were randomly generated. The tree prior was set to assume the coalescent Bayesian skyline demographic process with uniform smoothing. This model tuning was preferred over others given its good performance in numerous scenarios without strong priors (Ho & Shapiro 2011). For the analysis, independent MCMC chains were run for 20,000,000 generations each, convergence was checked with TRACER v.1.5 (Rambaut & Drummond 2007), and the estimates were based on the last 8000 sampled trees (burn-in was set at 2,000,000 steps and parameters were sampled every 2000 steps). The same analysis was run twice using different random seeds to confirm convergence and the results of these two runs were combined with LOGCOMBINER v1.5.3. Finally, the software TRACER was used to plot the skyline (Rambaut & Drummond 2007; Ho & Shapiro 2011).

Results
In 658 aligned sites, a total of 15 different haplotypes were found in the 78 individuals. The number of haplotypes and polymorphic sites was similar among East Florida, Belize, and Panama (Fig. 2). West Florida presented approximately half of the number of haplotypes observed at the remaining of the studied localities. The number of polymorphic sites was also comparatively low at this locality. Haplotype diversity was lower at West Florida than at East Florida, Belize, and Panama. No evident differences in haplotype diversity occurred among East Florida, Belize, and Panama (Fig. 2). Lastly, no differences in nucleotide diversity and the mean number of pairwise differences were observed among the different localities (Fig. 2).

Population genetic structure
The AMOVA used to test for hierarchical population structure revealed a mean value of overall FST of 0.18 or 0.24 depending on the use of pairwise differences or number of haplotypes for the calculations, respectively (Table 1). Molecular variation was greater within than among populations (82% and 18%, respectively). However, this comparatively small variability among populations was significant and denoted significant genetic structure (P < 0.05, Table 1). Pairwise FST values were significant for all comparisons of population pairs but one. The only non-significant FST value was that obtained for the comparison between East Florida and Panama (Table 2).

The Mantel test revealed no isolation by distance in Palaemon floridanus; the slope of the relationship between geographical distance and genetic distance did not differ significantly from a slope of zero (P = 0.2992).
Divergence of and migration between Florida populations

Posterior probabilities of $t$ (time to divergence) between *Palaemon floridanus* populations from West and East Florida peaked at 2.103 and the 95% HPD credibility values did not include zero probabilities (Fig. 3). Thus the West and East populations from Florida diverged in the past. In turn, the $m$ parameters in both directions had posterior probabilities that peaked at zero (migration from East Florida to West Florida) or were very close to zero (from West Florida to East Florida) (Fig. 3). The 95% HPD values did include zero and the LLR test indicated that gene flow between East Florida and West Florida was not significantly different from zero ($P > 0.05$). Thus, the COI gene fragment revealed no gene flow (no secondary contact) since initial divergence between the two coasts of Florida. Overall, these results suggest that the genetic pattern (shared haplotypes but significant $F_{ST}$ value) observed between the two coasts of Florida is due to splitting of an ancestral population and incomplete lineage sorting, and not to contemporary gene flow between populations.

Demographic history of *Palaemon floridanus*

None of the Tajima’s D results was significant ($P > 0.05$). Thus, the D values do not support a recent population expansion scenario for *P. floridanus* in the wider Caribbean. On the contrary, the mismatch distributions calculated for the different localities and for the wider Caribbean (entire dataset) were unimodal and did not differ statistically from those expected for populations experiencing a demographic expansion ($P > 0.05$ in all cases) and from those expected for populations experiencing a spatial expansion ($P > 0.05$ in all cases). The different HRI statistics calculated for all the different localities and for the entire dataset were also not significant (Fig. 4), again denoting sudden population expansion in *P. floridanus*. The value of $\tau$ for the entire dataset was 2.162, corresponding to an initiation of demographic expansion of 234,694 years before present for *P. floridanus* in the wider Caribbean.

The Bayesian skyline plot calculated separately for the different localities indicated dissimilar demographic histories within the Caribbean. No expansion was observed for populations from West Florida and Belize. In contrast, demographic expansion of *P. floridanus* in East Florida and Panama occurred, respectively, within the last 25,000 and 320,000 years before present (Fig. 5). Lastly, the Bayesian skyline plot calculated for the entire dataset indicated a demographic expansion of *P. floridanus* in the wider Caribbean occurring within the last 320,000 years before present (Fig. 6). Also, a slight but perceivable inflection point on the relationship between time and effective population size occurred 25,000–35,000 years before present (Fig. 5).

**Discussion**

This study demonstrates moderate genetic differentiation in a marine invertebrate with high long-distance dispersal that inhabits the wider Caribbean and Gulf of Mexico. The observed population structure in *Palaemon floridanus* is intermediate between two extremes previously reported for the region. These extremes range from species organized into a single and large, highly connected, panmictic

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Table 1. Pairwise Slatkin linearized $F_{ST}$ values among locales.

<table>
<thead>
<tr>
<th></th>
<th>Belize</th>
<th>East FL</th>
<th>Panama</th>
<th>West FL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Belize</td>
<td>0</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>East FL</td>
<td>0.1615</td>
<td>0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Panama</td>
<td>0.1679</td>
<td>0.0146</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>West FL</td>
<td>0.1114</td>
<td>0.3085</td>
<td>0.3355</td>
<td>0</td>
</tr>
</tbody>
</table>

Significance levels established with 10,000 permutations. Significance at the $P < 0.05$ level is indicated in bold.

Table 2. AMOVA results using pairwise differences as distance method and from haplotype frequencies.

<table>
<thead>
<tr>
<th>source of variation</th>
<th>df</th>
<th>sum of squares</th>
<th>variance components</th>
<th>percentage of variation</th>
<th>fixation index</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>based on pairwise differences</td>
<td>3</td>
<td>10.717</td>
<td>0.1486 Va</td>
<td>18.00</td>
<td>$F_{ST}$: 0.18,004</td>
</tr>
<tr>
<td>among populations</td>
<td>74</td>
<td>50.078</td>
<td>0.6767 Vb</td>
<td>82.00</td>
<td></td>
</tr>
<tr>
<td>within populations</td>
<td>77</td>
<td>60.795</td>
<td>0.8253</td>
<td></td>
<td></td>
</tr>
<tr>
<td>total</td>
<td></td>
<td>60.795</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>based on haplotype frequencies</td>
<td>3</td>
<td>6.594</td>
<td>0.0977 Va</td>
<td>24.92</td>
<td>$F_{ST}$: 0.24,922</td>
</tr>
<tr>
<td>among populations</td>
<td>74</td>
<td>21.778</td>
<td>0.2943 Vb</td>
<td>75.08</td>
<td></td>
</tr>
<tr>
<td>within populations</td>
<td>77</td>
<td>28.372</td>
<td>0.3919</td>
<td></td>
<td></td>
</tr>
<tr>
<td>total</td>
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<td>28.372</td>
<td></td>
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<td></td>
</tr>
</tbody>
</table>

Significance levels established with 10,000 permutations. Each location was considered as a separate group and significance at the $P < 0.05$ level is indicated in bold.
population (e.g. the spiny lobster *Panulirus argus*; Naromaciel et al. 2011) to others with strong genetic differentiation, even among populations separated by small geographic distances (e.g. the coral *Flavia fragum*; Goodbody-Gringley et al. 2010). This study also represents a partial test of meta-population structure in *P. floridanus*. Populations from Panama (South Caribbean), Belize (North Caribbean), Tampa (West Florida), and Fort Pierce (East Florida) were predicted to be genetically different each one from another. In agreement with expectations, $F_{ST}$ values indicated population differentiation among most of the studied populations. Thus, the partial agreement between our results and connectivity predictions from biophysical oceanographic models suggests that contemporary meso-scale oceanographic processes (e.g. semi-permanent Panama Colombia Gyre) can be invoked to explain phylogeographic patterns in the wider Caribbean (e.g. they might drive genetic dissimilarity between South and North Caribbean populations in *P. floridanus*). On the other hand, no evidence of isolation-by-distance was detected among the studied populations. A correlation between genetic dissimilarity and distance would have further supported the notion that contemporary oceanographic processes are mostly responsible for the observed population structure. Also, the Panama population of *P. floridanus* did not differ genetically from that in East Florida. The absence of isolation-by-distance in our data and the existence of genetically similar populations that are separated by 1000s of kilometers indicates that factors other than oceanographic conditions (e.g. historical processes, migration via shipping vectors or via rafting using floating objects like algal clumps) also play a role in determining population structure in *P. floridanus*. In the following, we discuss three outstanding yet not completely understood phylogeographic issues highlighted by

![Fig. 3. IMA2 analysis of Palaemon floridanus populations from the West and East coast of Florida using the COI gene fragment.](image)

![Fig. 4. Mismatch distributions for Palaemon floridanus COI sequences.](image)
the results from this study: (i) genetic dissimilarity between West and East Florida populations, (ii) unexpected genetic similarity between geographically distant population in the wider Caribbean (e.g. Panama and East Florida in P. floridanus), and (iii) the historical demography of marine invertebrates, including P. floridanus, in the wider Caribbean and Gulf of Mexico.

Genetic dissimilarity between West and East Florida

In Palaemon floridanus, populations from the west and east coasts of Southern Florida were genetically dissimilar, in disagreement with predictions from biophysical models (Cowen et al. 2006) but in general agreement with that reported for other organisms inhabiting the two coasts of Florida (Avise 2000; Soltis et al. 2006). Distinct Atlantic and Gulf coast lineages have been demonstrated before in at least 10 species of invertebrates and vertebrates with larval periods similar to or longer than that of P. floridanus (see Avise 2000; Wise et al. 2004; Soltis et al. 2006; and references therein). This Atlantic/Gulf coast genetic discontinuity in the species studied above appear to be explained by historical (i.e. isolation and differentiation either during Pleistocene or Pliocene glaciations; Soltis et al. 2006) rather than contemporary processes (e.g. ocean currents; Cowen et al. 2000, 2006). Importantly, the details of the historical processes explaining such genetic discontinuities remain obscure (Avise 2000; Soltis et al. 2006).

In P. floridanus, three scenarios might explain the observed genetic pattern (i.e. shared haplotypes but genetic dissimilarity as indicated by AMOVA): contemporary gene flow solely (explaining shared haplotypes between the two populations), population divergence from an ancestral polymorphic population but with incomplete lineage sorting or a combination of gene flow and ancestral polymorphism. In an attempt to distinguish between the three scenarios above, we further explored the relationship between these two populations using model-based population genetic methods that do not assume that mutation, drift, and migration are in an evolutionary equilibrium (Hey & Nielsen 2007; Hey 2010). Our results using the software IMA2 indicated that the two studied populations have indeed diverged in the distant past and that contemporary migration (e.g. after initial divergence) was not significantly different from zero. Thus, population divergence with incomplete lineage sorting can be invoked as the most relevant mechanism explaining our empirical results. Additional sampling of populations extending throughout the two coasts of Florida and other localities within the Gulf of Mexico might help resolve the geographic position of a (putative) genetic break in the studied shrimp (e.g. Cape Canaveral, see Avise 2000) and might also enable one to explore whether the Mississippi River represents another historical geographical barrier for this species, as shown before for other crustaceans inhabiting the Gulf of Mexico (e.g. Callichirus islagrande; Bilodeau et al. 2005). Overall, our results highlight the value of using model-based population genetic methods that do not assume that mutation, drift, and migration are in an equilibrium when attempting to resolve complex demographic histories (Hey & Nielsen 2007; Hey 2010; Marko & Hart 2011).

Genetic similarity between Panama and East Florida

In Palaemon floridanus, the Panama population did not differ genetically from that in East Florida, as shown by
Historical demography of *Palaemon floridanus* in the wider Caribbean

Different conclusions about the demographic history of *P. floridanus* have been reached depending upon methodologies used for detecting population expansion. On the one hand, the non-significant values of the Tajima’s D statistics indicate that the population of *P. floridanus* throughout the wider Caribbean has been stable during recent times. On the other hand, the mismatch distributions were unimodal for each population separately and for the complete dataset, and the HRI statistics were low and non-significant, indicating past population expansion in *P. floridanus*. The observed differences between the statistical methodologies might be due to their differing power to detect past population growth. A study that compared the ability of several tests to detect population expansion demonstrated that the Tajima’s D test is more powerful than the HRI test when expansion has occurred relatively recently, but that the HRI test is more powerful than the Tajima’s D test when population expansion has occurred far in the past (Ramos-Onsins & Rozas 2002). Given that population expansion in *P. floridanus* from the wider Caribbean occurred ~320,000 years before present, as indicated both by the τ statistic and the skyline plot analysis (see below), more weight is given to the results of the mismatch distribution and the skyline plot analyses hereafter.

The population expansion of *P. floridanus* in the wider Caribbean might be the consequence of increasing tropical and subtropical habitat availability resulting from global climate change and sea level rise after the Last Glacial Maximum (LGM). Population expansion starting near the time of the LGM has been detected in other Caribbean crustaceans (e.g. in two distinct lineages of the spiny lobster *Panulirus argus*; Naro-Maciel et al. 2011) with high long-distance dispersal ability and has been reported for marine invertebrates from other geographical regions as well (e.g. temperate regions: *Emerita analoga*; Dawson et al. 2011). Nonetheless, the estimation of the time at which population expansion of *P. floridanus* began in the region based on the τ value of the mismatch distribution analysis and the skyline plot was 235,000 years before present, a rather long period of time that encompasses both glacial and interglacial climates. Phylogeographic studies in other species from other localities have also indicated population expansion that predates the LGM within the Caribbean (the coral reef bennies *Acanthemblemaria aspera* and *A. spinosagobie*; Eytan & Hellberg 2010). The conditions explaining such steady increase in effective population size in *P. floridanus* from the wider Caribbean remain to be addressed.

Importantly, when the different populations of *P. floridanus* are analyzed separately, skyline plot analyses indicate that different localities in the wider Caribbean and Gulf of Mexico exhibit different demographic histories. Populations from Belize and West Florida have remained stable for long periods of time, whereas Panama has experienced a recent population expansion and East Florida an older expansion in *P. floridanus*. The above again indicates dissimilar timings and magnitudes of demographic changes in the studied species in the wider Caribbean and Gulf of Mexico. However, we interpret these results with caution because our estimates are based in a single locus (see Eytan & Hellberg 2010) and given that the population size at the tip of the plot did overlap with the upper 95% confidence interval at the root of the plot. Future studies including both mitochondrial and nuclear markers would allow us to provide a more complete...
picture of the historical demography of *P. floridanus* in the wider Caribbean.

**Outlook**

Overall, the interaction between contemporary (*e.g.* ocean currents, recent dispersal via shipping vectors) and historical processes (*e.g.* vicariance) may have determined the phylogeographic pattern herein reported for *P. floridanus*. We argue in favour of new studies using additional molecular markers, more intensive geographic sampling, and non-equilibrium population genetic approaches (Hey 2010; Marko & Hart 2011) to keep improving our understanding of the putatively complex demographic history of this shrimp and most probably other species in the same geographical region.

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