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BIODIVERSITY RESEARCH

# Higher genetic diversity in introduced than in native populations of the mussel *Mytella charruana*: evidence of population admixture at introduction sites

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## ABSTRACT

**Aim** Levels of genetic diversity can be used to determine haplotype frequency, population size and patterns of invasive species distribution. In this study, we sought to investigate the genetic structure of the invasive marine mussel *Mytella charruana* and compare variation from invasive populations with variation found within three native populations.

**Location** Invaded areas in the USA (Florida, Georgia); native areas in Ecuador, Colombia and Brazil.

**Methods** We sequenced 722 bp of the mitochondrial COI gene from 83 *M. charruana* samples from four invasive populations (USA) and 71 samples from two natural populations (Ecuador, Columbia). In addition, we sequenced 31 individuals of a congeneric species, *Mytella guyanensis*, from Salvador, Brazil. We constructed the phylogenetic relationship among all haplotypes and compared diversity measures among all populations.

**Results** We found significantly higher levels of nucleotide diversity in invasive populations than in native populations, although the number of haplotypes was greater in the native populations. Moreover, mismatch distribution analyses resulted in a pattern indicative of population admixture for the invasive populations. Conversely, mismatch distributions of native populations resulted in a pattern indicative of populations in static equilibrium.

**Main conclusion** Our data present compelling evidence that the *M. charruana* invasion resulted from admixture of at least two populations, which combined to form higher levels of genetic diversity in invasive populations. Moreover, our data suggest that one of these populations originated from the Caribbean coast of South America. Overall, this study provides an analysis of genetic diversity within invasive populations and explores how that diversity may be influenced by the genetic structure of native populations and how mass dispersal may lead to invasion success.

**Keywords**
**Admixture, biological invasions, cytochrome-c-oxidase-subunit-I, invasive species, population genetics.**

## INTRODUCTION

Invasive species frequently cause ecological or economic harm (Pimentel *et al.*, 2000; Roman & Darling, 2007); however, they provide quintessential models for investigating interesting evolutionary questions and have been described as 'a grand experiment in evolution' (Ayala *et al.*, 1989; Sax *et al.*, 2007). To begin to understand the process of natural selection on introduced species, it is imperative that the evolutionary history of the species be known. To this end, the investigation


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of diversity patterns in neutral molecular markers is helpful when exploring various aspects of species invasions (e.g. Giraud *et al.*, 2002; Yonekura *et al.*, 2007). Patterns of genetic variation have proven indispensable for determining the likely source of a founding population (Saltonstall, 2002; Taylor & Keller, 2007; Le Roux & Wieczorek, 2009; but see Muirhead *et al.*, 2008). Identification of the source population can shed light on the range of natural environments in which invasive species are suitably adapted. Although identifying source populations is challenging, genetic characteristics of these populations can provide helpful insights into whether or not invasive populations representatively capture the diversity of native populations (Allendorf & Lundquist, 2003). In addition, understanding the ecological parameters of source propagules can shed light on the invasive organism's capacity for range expansion. In this study, we sought to investigate the genetic structure of an invasive marine mussel and compare variation from the invasive populations with variation found within natural populations.

In the past, the prevailing view was that invasive populations would display low genetic diversity because of founder effects and bottlenecks. Studies of invasive species possessing this pattern can be found throughout the literature; two aquatic examples are the spiny waterflea *Bythotrephes longimanus* (Colautti *et al.*, 2005) and the common guppy *Poecilia reticulata* (Lindholm *et al.*, 2005). Both studies found low levels of diversity and reduced heterozygosity in invasive populations, two major characteristics of population bottlenecks. More recently, however, researchers have documented successful invasions without a genetic bottleneck. This has led to the question of how introduced populations overcome the evolutionary problems associated with low genetic diversity in a foreign range yet still become established, referred to as a genetic paradox (Allendorf & Lundquist, 2003). Recent studies have reported equal or even increased levels of genetic diversity in invasive populations when compared with native populations (e.g. Kolbe *et al.*, 2004; Stepien *et al.*, 2005). These high levels of diversity are often attributed to multiple founding populations (e.g. Kolbe *et al.*, 2004; Stepien *et al.*, 2005; Taylor & Keller, 2007). Roman & Darling (2007) argue that invasions from multiple discrete source populations, or admixture, may be the standard rather than the exception with regard to species invasions. However, Zayed *et al.* (2007) report a study of the invasive bee *Lasioglossum leucozonium* in which the invasive population may have originated through the bottleneck of one singly-mated female and still prospered. Zayed *et al.* (2007) argue that the roll of chance cannot be forgotten when it comes to successful invasions. Indeed, recent studies have found that both bottleneck and admixture play a role in invasive species genetic variation (Taylor & Keller, 2007). Thus, there still remains a debate as to the role of admixture versus bottleneck in determining patterns of diversity among invasive species.

An ideal species in which to explore the genetic patterns of invasion is the tropical mussel *Mytella charruana*. *Mytella charruana* is native to the eastern Pacific Ocean from Guaymas, Sonora Mexico, south to Ecuador and west to the Galapagos Islands (Cardenas & Aranda, 2000). The charru mussel also occurs along the eastern coast of South America from Colombia to Argentina (Keen, 1971), and has been recently introduced to the south-east coast of North America (Carlton, 1992; Boudreaux & Walters, 2006). *Mytella charruana* was initially found in Jacksonville, Florida in 1986 covering a seawater intake pipe at the Northside Generator Power Plant (Lee, 1987). However, despite repeated searching, the species was not found the following year or any subsequent years until 2004, when *M. charruana* was found at New Smyrna Beach, Florida on an intertidal oyster reef in the Indian River Lagoon, *c.* 170 km south of Jacksonville (Boudreaux & Walters, 2006). Since then, close monitoring and detailed surveys have revealed a proliferation of this non-indigenous mussel along the Atlantic coast from central Florida up through South Carolina. This species has the potential to increase greatly in numbers, as native densities as high as 11,036 mussels $m^{-2}$ have been reported in native waters (Pereira *et al.*, 2003). The recent introduction, potential ecological threat and rapid spread make *M. charruana* a good model to study.

We examined the evolutionary history of the invasive mussel species *M. charruana* by comparing patterns of genetic diversity among native and invasive populations. We predicted that patterns of genetic diversity would indicate admixture of native populations within the invasive populations, causing equal levels of diversity in both invasive and native populations (equalizing the effects of both admixture and bottleneck). This *a priori* hypothesis arises from similar studies of other marine mussels (e.g. Marsden *et al.*, 1996; Holland, 2001; Astanei *et al.*, 2005; Stepien *et al.*, 2005; Therriault *et al.*, 2005; May *et al.*, 2006). In addition, we predicted that admixed populations would at least partially originate from the major South American port cities near Columbia and Venezuela where *M. charruana* could be transported via ballast water of oil tankers, and from Ecuador where *M. charruana* could be transported via ballast water of banana transport ships. This *a priori* hypothesis arises from the knowledge of primary shipping channels originating from the native habitat to the invasive region. Here, we attribute transport of the *M. charruana* invasion to ballast water dumping, as has been suggested by others for this species (Carlton, 1992; Boudreaux & Walters, 2006); however, other possible transport mechanisms such as hull fouling cannot be excluded.

Before addressing specific hypotheses regarding the invasion of *M. charruana*, we briefly and generally resolved the taxonomical classification of the species. Taxonomical identification is a critical step in predicting impacts and understanding bioinvasions (Lockwood, 1999; Holland, 2000; Le Roux & Wieczorek, 2009). *Mytella* is grouped within the subfamily Mytilinae with genera such as *Aulacomya*, *Brachidontes*, *Perna* and *Mytilus*, based on morphological characteristics. Although studies have explored the phylogenetic relationships of some of these species (Distel, 2000; Steiner & Hammer, 2000; Wood *et al.*, 2007), no molecular study has resolved the phylogentic

relationship of these genera with regard to *Mytella*. Furthermore, to add support to the morphologically described *Mytella* genus, we included a congeneric species, *Mytella guyanensis,* to determine if the genus *Mytella* is a monophyletic clade.

To investigate our hypotheses, we sequenced part of the mitochondrial DNA (mtDNA) cytochrome-c-oxidase-subunit-I (COI) gene from multiple individuals and sites in the invasive and native ranges of the species. We used these data to create a phylogeny of invasive and native *M. charruana* and native *M. guyanensis* and to infer a haplotype network, which allowed us to visualize within-species genetic diversity. Furthermore, we compared genetic variation within and between populations and tested for evidence of expansion in each of the populations. Finally, we discuss how our results fit with patterns of invasion from other species and determine what our results mean for the future of the *M. charruana* invasion in the southeast United States.

## METHODS

### Sample collection and DNA isolation

We collected 83 *M. charruana* samples from four invasive populations [New Smyrna Beach (NSB), FL ($n$ = 17); Jacksonville (JAX), FL ($n$ = 34); St. Marys, GA ($n$ = 16); and Sunbury, GA ($n$ = 16)], 41 samples from a natural population in Cartagena, Colombia and 30 samples from another natural population in Guayaquil, Ecuador (Fig. 1, Table 1). Moreover, we collected 31 individuals of a congeneric species, *M. guyanensis*, from Salvador, Brazil as a means to explore the population genetic patterns from another natural population (Table 1). Invasive samples were preserved in anhydrous calcium sulphate (Hammond Drierite Co., Xenia, OH, USA) and natural populations were preserved via air-drying. Desiccation of tissue samples enabled the extraction of high-quality DNA without the problems associated with carrying or shipping ethanol. DNA was extracted from the samples (adductor and mantle tissue) using Qiagen DNeasy Tissue Kit (Cat. No. 69504) and protocol (Qiagen, Hilden, Germany).

### Amplification and sequencing

For each sample, we amplified a 722 base pair (bp) fragment of mitochondrial COI gene using the polymerase chain reaction (PCR) method. Initially, we used the primers LCO1490, 5′-GGTCAACAAATCATAAAGATATTGG-3′ and HCO2198, 5′-TAAACTTCAGGGTGACCAAAAAATCA-3′ designed by Folmer *et al.* (1994), and obtained *c.* 740 bp of sequence. Using sequences obtained with these primers and sequences of *Perna perna* (GenBank, Accession no.: DQ351463.1; Zardi *et al.*, 2007), we designed our own novel primers: 5′-GTGTGGGGCTGGGTTAATAG-3′ and 5′-ATGATGGGCC-CACACTACAC-3′ to give us our final 722-bp fragment. PCR was performed in a MyCycler Thermal Cycler (Bio-Rad Laboratories, Inc., Hercules, CA, USA) using the following reaction components: 13.2 $\mu$L of de-ionized water, 1.6 $\mu$L of 25 mM $MgCl_2$, 0.4 $\mu$L of 10 mM dNTPs, 2 $\mu$L of 10x PCR buffer, 1 $\mu$L of 10 $\mu$M forward primer, 1 $\mu$L of 10 $\mu$M reverse primer, 0.2 $\mu$L of *Taq* DNA polymerase (5 units $\mu L^{-1}$) and 1 $\mu$L of DNA template (*c.* 100 ng $\mu L^{-1}$). PCRs were carried out under the following conditions: 95 °C for 4 min; followed by 35 cycles of 95 °C for 30 s, 45 °C for 30 s and 72 °C for 30 s; and finally an elongation period at 72 °C for 7 min. PCR product was cleaned using Exo-SAP-IT (USB Corp., Cleveland, OH, USA). Purified product was quantified using either a 2% agarose gel and ethidium bromide staining with a quantification size standard or a NanoDrop ND-1000 spectrophotometer (NanoDrop Technologies, Wilmington, DE, USA). Approxi-

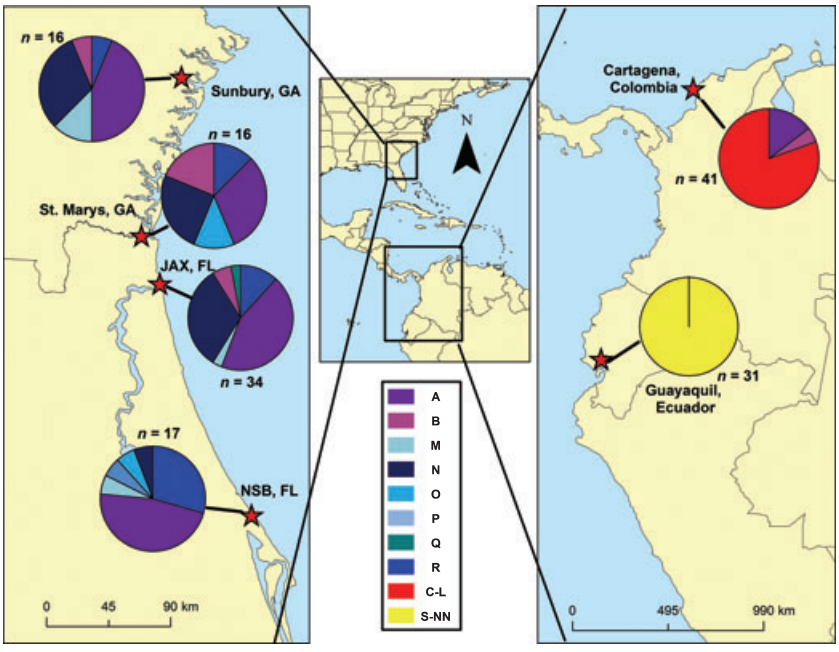


Figure 1 Distribution of *Mytella charruana* populations analysed in this study. Invasive populations of *M. charruana* have been found as far north as Sunbury, GA and as far south as New Smyrna Beach (NSB), FL. Different colours within pie graphs designate unique haplotypes and their frequencies within each population. Each colour represents the same haplotype among populations (see Fig. 3, for the genetic relationships among haplotypes).

Table 1 Estimates of *Mytella* spp. COI genetic diversity. Descriptive statistics comparing genetic variation among the seven populations (SD = standard deviation). United States sites represent invasive populations of *Mytella charruana*; Colombia and Ecuador represent natural *M. charruana* populations; and Brazil represents a natural *Mytella guyanensis* population. Sum of squared deviation and raggedness index indicate the goodness-of-fit of a model of sudden demographic expansion. Significant *P*-values indicate a poor fit to the sudden expansion model (see Fig. 4, for further explanation).

| | Populations | | | | | | |
|---|---|---|---|---|---|---|---|
| | NSB, FL | JAX, FL | St. Marys, GA | Sunbury, GA | Colombia | Ecuador | Brazil |
| Latitude/longitude | 28.9069° N 80.8206° W | 30.4195° N 81.4194° W | 30.7333° N 81.5386° W | 31.7699° N 81.2773° W | 10.407° N 75.734° W | 2.1964° S 80.0265° W | 12.968° S 38.509° W |
| No. samples | 17 | 34 | 16 | 16 | 41 | 30 | 31 |
| No. haplotypes | 6 | 6 | 5 | 5 | 12 | 22 | 18 |
| Nucleotide diversity, $\pi$ (SD) | 0.0090 (0.0009) | 0.0087 (0.0006) | 0.0090 (0.0010) | 0.0085 (0.0010) | 0.0044 (0.0005) | 0.0049 (0.0009) | 0.0024 (0.0005) |
| Gene diversity, $h$ (SD) | 0.721 (0.087) | 0.702 (0.052) | 0.825 (0.052) | 0.733 (0.079) | 0.911 (0.020) | 0.959 (0.026) | 0.804 (0.076) |
| Segregating sites | 17 | 18 | 16 | 17 | 18 | 27 | 23 |
| Mean pairwise diff. | 6.592 | 6.251 | 6.458 | 6.142 | 3.156 | 3.559 | 1.725 |
| Sum of squared dev. ($P$-value) | 0.132 (0.018) | 0.129 (0.016) | 0.070 (0.060) | 0.127 (0.010) | 0.007 (0.359) | 0.014 (0.069) | 0.002 (0.759) |
| Raggedness index ($P$-value) | 0.296 (0.006) | 0.235 (< 0.001) | 0.130 (0.085) | 0.272 (0.007) | 0.027 (0.552) | 0.066 (0.107) | 0.047 (0.649) |
| Tajima's $D$ ($P$-value) | 1.164 (> 0.10) | 1.412 (> 0.10) | 1.338 (> 0.10) | 0.788 (> 0.10) | −0.812 (> 0.10) | −1.784 (> 0.10) | −2.460 (< 0.01) |
| Fu and Li's $D^*$ ($P$-value) | 0.099 (> 0.10) | 1.256 (> 0.10) | 1.522 (< 0.02) | 0.130 (> 0.10) | 0.873 (> 0.10) | −1.100 (> 0.10) | −3.666 (< 0.02) |
| Fu and Li's $F^*$ ($P$-value) | 0.462 (> 0.10) | 1.539 (> 0.05) | 1.694 (< 0.05) | 0.362 (> 0.10) | 0.367 (> 0.10) | −1.556 (> 0.10) | −3.858 (< 0.02) |

mately 50 ng of the product was submitted for sequencing in both directions with the amplification primers. Dye terminator cycle sequencing reactions and capillary electrophoresis were performed on an ABI 3700 at the Nevada Genomics Center (University of Nevada, Reno).

### Exploring doubly uniparental inheritance

In most animals, mtDNA is solely maternally inherited; therefore, individuals are homoplasmic for mtDNA. However, a phenomenon known as doubly uniparental inheritance (DUI) has been observed in some bivalve species including freshwater and marine mussels (Skibinski *et al.*, 1994; Hoeh *et al.*, 1996; Theologidis *et al.*, 2008). These species possess distinct mtDNAs that are inherited either maternally or maternally and paternally. For example, in *Mytilus* spp., females inherit and pass on only the maternal mitotype ('F' type), while males are heteroplasmic and inherit both an F type and a paternal (M) type mtDNAs (e.g. Fisher & Skibinski, 1990; Zouros *et al.*, 1992). The M type is passed on through sperm, but is only maintained in male offspring. Presence of DUI can affect population genetic studies by producing falsely high levels of mtDNA diversity and divergence (Theologidis *et al.*, 2008).

Because of the large differences in the haplotypes recovered from the invasive populations (see Results), we explored the issue of DUI as the mechanism driving genetic diversity. To test this, we microscopically sexed and sequenced 19 female individuals from the JAX, FL population. By sequencing only females, we sought to determine whether we could uncover all of the most divergent haplotypes. We would expect to recover only F mitotypes from females, so if all the most divergent haplotypes were not recovered, we might have an indication of DUI as the source of diversity.

### Sequence alignment and Bayesian phylogenetic inference

Sequences were edited using SEQUENCHER 4.7 (Gene Codes Corp., Ann Arbor, MI, USA). Resulting sequences were aligned in CLUSTALW, available in MEGA 4 (Tamura *et al.*, 2007) using default parameters. Alignment was unambiguous and no internal stop codon was found; therefore, the verbatim CLUSTALW alignment was used for all analyses.

All distinct haplotypes were used for phylogenetic construction. A preliminary phylogeny was constructed using GenBank sequences of *Aulacomya atra*, *Mytilus* spp., *Perna* spp. and our *Mytella* spp. sequences. Of these Mytilinae genera, our results suggested that *Mytella* spp. was most closely related to *Perna* spp. (N.K. Gillis and E.A. Hoffman, unpublished data). Given these results, we used one *Perna perna* individual and one *Perna virdis* individual as outgroup species. Outgroup sequences were obtained from GenBank (Accession nos: DQ917588.1 and DQ91783.1; Wood *et al.*, 2007). The best-fit model of evolution was selected for COI using AIC (Akaike, 1973, 1974; Sakamoto *et al.*, 1986) implemented in MODEL-

TEST v2.2 (Nylander, 2004). This model utilized partitioning of data such that COI position 1 (COI1), COI position 2 (COI2), COI position 3 (COI3), and COI positions 1 and 2 (COI12) were partitioned separately.

Three combinations of partitioning schemes (1X: COI; 2X: COI12, COI3; 3X: COI1, COI2, COI3) were implemented and Bayesian metropolis-coupled Markov chain Monte Carlo estimations of phylogenies were performed for each using MRBAYES version 3.1.2 (Huelsenbeck & Ronquist, 2001). Each run comprised four chains (program default) that were sampled every 100 generations for 2,000,000 generations. For all analyses, the first 5000 samples were discarded as burnin. Theoretically, partitioning should improve the fit of the model to the data, thus resulting in a more accurate estimate of phylogeny (Ronquist & Huelsenbeck, 2003; Nylander *et al.*, 2004). However, partitioning also decreases the number of characters within each partition. This reduction of characters may result in random error in model parameters. The Bayes factors were analysed to select for the best-fit partitioning method. The harmonic mean estimated marginal likelihood of the stationary phase samples generated by the *sump* command in MRBAYES was used to calculate the 2 ln Bayes factors (Brandley *et al.*, 2005). A Bayes factor < 20 was taken to represent a strong partitioning scheme (Kass & Raftery, 1995).

### mtDNA variation and demographic analyses

Haplotype networks (*M. charruana* and *M. guyanensis*) were inferred using the 95% statistical parsimony algorithm implemented in TCS version 1.0 (Clement *et al.*, 2000) and described by Templeton *et al.* (1992). Within-population genetic diversity was compared by calculating haplotype diversity ($h$) and nucleotide diversity ($\pi$) using ARLEQUIN version 2.0 (Schneider *et al.*, 2000) and DNASP version 4.20.2 (Rozas & Rozas, 1999). Furthermore, mismatch distribution analysis (MDA; Rogers & Harpending, 1992) as implemented in ARLEQUIN, was conducted to explore the relatedness of the distribution of nucleotide site differences between the invasive and native populations and to test for evidence of range expansion. Differences between invasive and native populations for $h$ and $\pi$ were calculated using Welch's two-sample $t$-test in the statistical program R. Finally, we calculated Tajima's $D$ (Tajima, 1989), Fu & Li's (1993) $D^*$, and Fu & Li's (1993) $F^*$ using DNASP to test for evidence of demographic expansion.

## RESULTS

### Testing for the presence of DUI

Sequencing of 19 females from JAX, FL resulted in the recovery of five haplotypes. The three most common and divergent haplotypes of the invasive populations (A, N and R; Fig. 1) were all identified. Statistical analyses of the population of females produced the same trends as found in the other invasive populations (data not shown). Results suggest that the high levels of mtDNA diversity are not the result of DUI. All additional analyses were conducted without regard to the sex of the mussel, and these mussels were included with the remaining 15 unsexed JAX, FL samples.

### Selection and inference of Bayesian phylogeny

The best-fit model identified by AIC criteria for the 1X partitioning scheme was the General Time Reversible model with invariable sites and gamma distribution (GTR + I + Γ; Tavaré, 1986; 'COI'). The best-fit models identified for the 2X partitioning scheme were GTR + I for the combined COI position 1 and 2 dataset (COI12), and GTR + Γ for COI position 3 (COI3). The best-fit models chosen for the 3X partitioning scheme were GTR + Γ for COI position 1 (COI1) and position 3 (COI3), and F81 model (Felsenstein, 1981) for the second position dataset (COI2). Across all models, Bayes factors provided extremely strong support ($2\ \ln B_{10} > 210$) for the '2X' partitioned model as the best-fit to the combined dataset.

A total of 58 unique haplotypes (GenBank Accession nos: EU917142–EU917198, FJ940721) were identified and used for phylogenetic reconstruction (Fig. 2) from the three collective populations (native, invasive and native sister taxon). As shown in Fig. 2, Bayesian phylogenetic estimates under a 2X partitioning scheme recovered two strongly supported clades, with a posterior probability ($P$p) of 1.00 supporting the grouping of invasive *M. charruana* with Colombian and Ecuadorian samples and the separate grouping of all the Brazilian samples (*M. guyanensis*). The monophyletic clade of each species was well supported ($P$p = 0.98 and 1.00). There was no highly divergent structuring within the clades, and therefore, no well-resolved within-species phylogenetic patterns.

### mtDNA variation

Cytochrome-c-oxidase-subunit-I sequencing of *M. charruana* from four invasive populations yielded eight unique haplotypes ($n$ = 83; Table 1, Fig. 1). COI sequencing from the natural populations resulted in more unique haplotypes than the invasive populations (Colombia: 12, $n$ = 41; Ecuador: 22, $n$ = 30; Brazil: 18, $n$ = 31; Table 1, Fig. 1). Interestingly, two of the Colombian haplotypes were shared with the invasive populations (A and B; Figs 1 & 3). None of the Ecuadorian haplotypes were shared with the invasive populations. Average gene diversity of the invasive populations ($h = 0.7453 \pm 0.0547$) was significantly lower ($t$ = 5.2, $P$ = 0.01) than gene diversity of the two native *M. charruana* populations ($h = 0.9350 \pm 0.0339$); however, average nucleotide diversity of the invasive populations ($\pi = 0.0088 \pm 0.0002$) was significantly greater ($t$ = −14.6, $P$ = 0.01) and more than twice the average nucleotide diversity of the native populations ($\pi = 0.0047 \pm 0.0004$; Table 1).

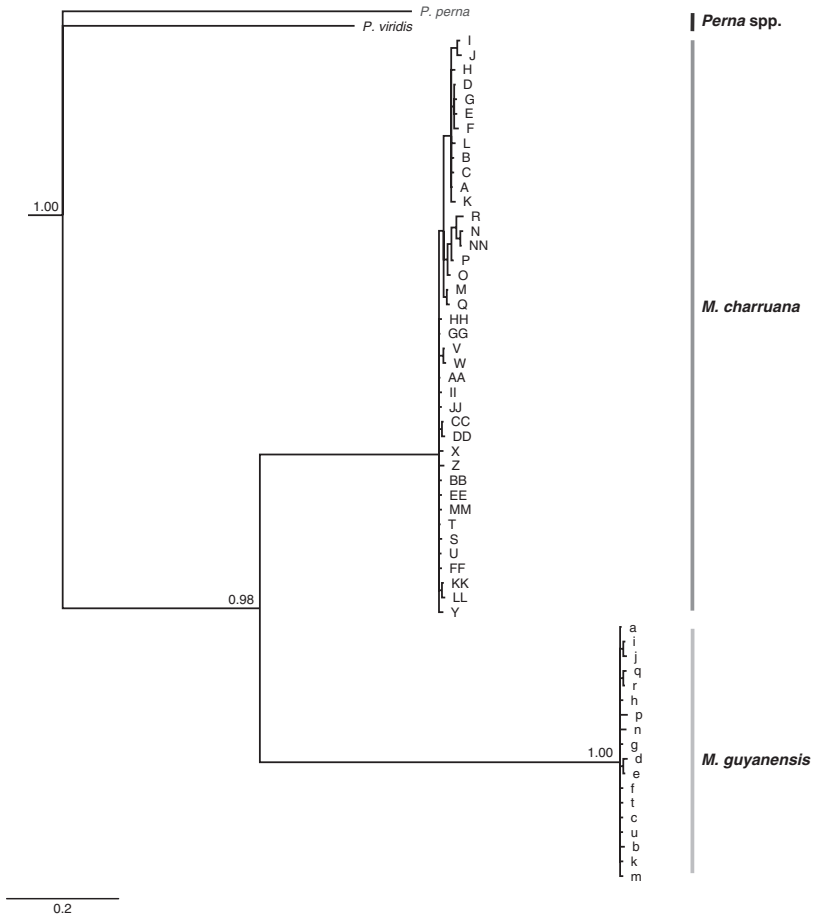


Figure 2 Phylogeny of sampled *Mytella* spp. Phylogeny was inferred using Bayesian metropolis-coupled Markov chain Monte Carlo estimation. Displayed is the 2X-partitioned model (1: COI positions 1 and 2; 2: COI position 3). Letters correspond to unique haplotypes found at various frequencies (see Fig. 3). Numbers represent posterior probabilities at major nodes.

All invasive populations shared three common and distinct haplotypes (A, $n = 35$; N, $n = 21$; R, $n = 12$) with uncorrected sequence divergences of 1.66% (A–N and A–R) and 0.83% (N–R; Fig. 3). The greatest uncorrected sequence divergences within the native Colombian and Ecuadorian populations were 1.11 and 2.22% respectively. However, the uncorrected sequence divergences between the most common haplotypes in these native populations were much lower than the divergence of common haplotypes in the invasive populations, e.g. in Colombia, A–G sequence divergence was 0.27%, and in Ecuador AA–GG sequence divergence was 0.42%. In summary, although the number of haplotypes was greater in the native populations, the mean nucleotide diversity was significantly lower than that of the invasive populations.

## Demographic analyses

The average number of segregating sites was lower (17) in the invasive populations than in the native populations (22.5) of *Mytella* spp. Conversely, the mean number of pairwise differences was much greater in the invasive populations individually (Table 1) and combined ($\theta_S = 6.227$) than in the native populations ($\theta_S = 3.156$; $\theta_S = 1.725$; and $\theta_S = 3.559$; Table 1). In addition, the raggedness indices of the invasive populations differed significantly from a smooth distribution (with the exception of St. Marys, $P = 0.085$); however, raggedness indices of the native populations were all non-significant, suggesting a smooth distribution. Moreover, the invasive populations revealed significant sum of squared deviation (SSD) values (with the exception of the St. Marys population, $P = 0.06$), thus rejecting a model of population expansion; however, the SSD values for all three native populations were not significantly different than the expected model of population expansion (Table 1). Overall, MDA revealed dichotomous patterns between native and invasive populations (Fig. 4).

Significance of neutrality tests varied depending on statistic and population. Fu and Li's $D^*$ and Fu and Li's $F^*$ showed no consistent pattern between the native and invasive populations (Table 1). Tajima's $D$ showed a nominal pattern of negative values for the native populations and positive values for the invasive populations. The negative values of Tajima's $D$ in the native populations suggest that demographic expansion has occurred in those populations. However, within the invasive populations, these neutrality tests are uninterpretable. This is because the admixed haplotype lineages do not have a history of coalescence in the populations where they are now co-occurring, so standard neutrality test interpretations are invalid.

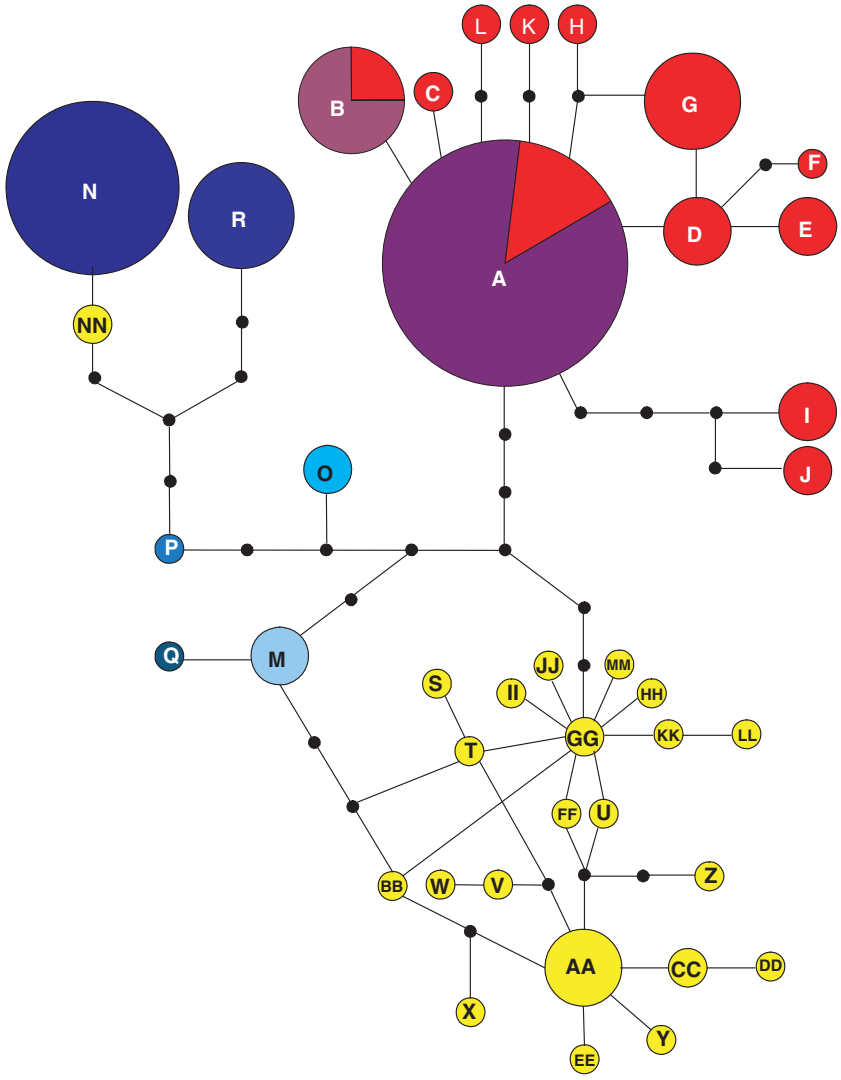


Figure 3 Relationships among haplotypes for native and invasive populations of *Mytella charruana*. Haplotype network was inferred by statistical parsimony. Individual labelled circles correspond to sampled haplotypes, while small black circles represent inferred unsampled haplotypes. The size of each circle corresponds to the frequency of that haplotype among all samples. Haplotype colours correspond to pie charts in Fig. 1; letters correspond to phylogeny in Fig. 2.

## DISCUSSION

In this study, we explored the genetics of a newly discovered relatively uncharacterized invasive mussel found along the south-eastern coast of the United States. Comparing mtDNA diversity among native mussel populations from Colombia, Ecuador and Brazil, as well as from the invaded range, we confirmed the monophyly of two different species within the *Mytella* genus, *M. charruana* and *M. guyanensis*. Moreover, we verified that the United States invasion is wholly comprised *M. charruana* and that these *Mytella* spp. are most closely related to mussels of the genus *Perna* (when compared with the Mytilinae sequences available on GenBank). Interestingly, *Perna viridis* has also invaded the southeastern United States within the last 10 years (Baker *et al.*, 2007). Our data also indicate that the two native *M. charruana* populations sampled in this study are entirely comprised non-overlapping haplotypes.

Theoretically, founding populations are expected to have lower genetic variation than native populations, characterized by reduced heterozygosity and a loss of rare alleles because of founder effects and bottlenecks (Holland, 2000; Allendorf & Lundquist, 2003; Colautti *et al.*, 2005). The success of invasive species despite bottlenecks has been referred to as a 'genetic paradox' (Allendorf & Lundquist, 2003). One proposed solution to this paradox is admixture of multiple source populations (Allendorf & Lundquist, 2003). Another proposed solution is that initial founder size, or high propagule pressure, causes equal levels of diversity between native and introduced populations (Allendorf & Lundquist, 2003). Indeed, a recent review of studies of invasive species indicated that founding is not necessarily linked to a paucity of genetic diversity (Roman & Darling, 2007). Studies investigating levels of genetic diversity among invasive bivalve species typically found that levels of genetic diversity did not decline with invasions [e.g. *Potamocorbula amurensis* (Duda, 1994), *Perna perna* (Holland, 2001), *Dreissena polymorpha* (Astanei *et al.*, 2005; May *et al.*, 2006), *Dreissena rostriformis bugensis* (Therriault *et al.*, 2005)]. Indeed, this was the basis of our hypothesis to test for equal levels of diversity between invasive and native populations. We

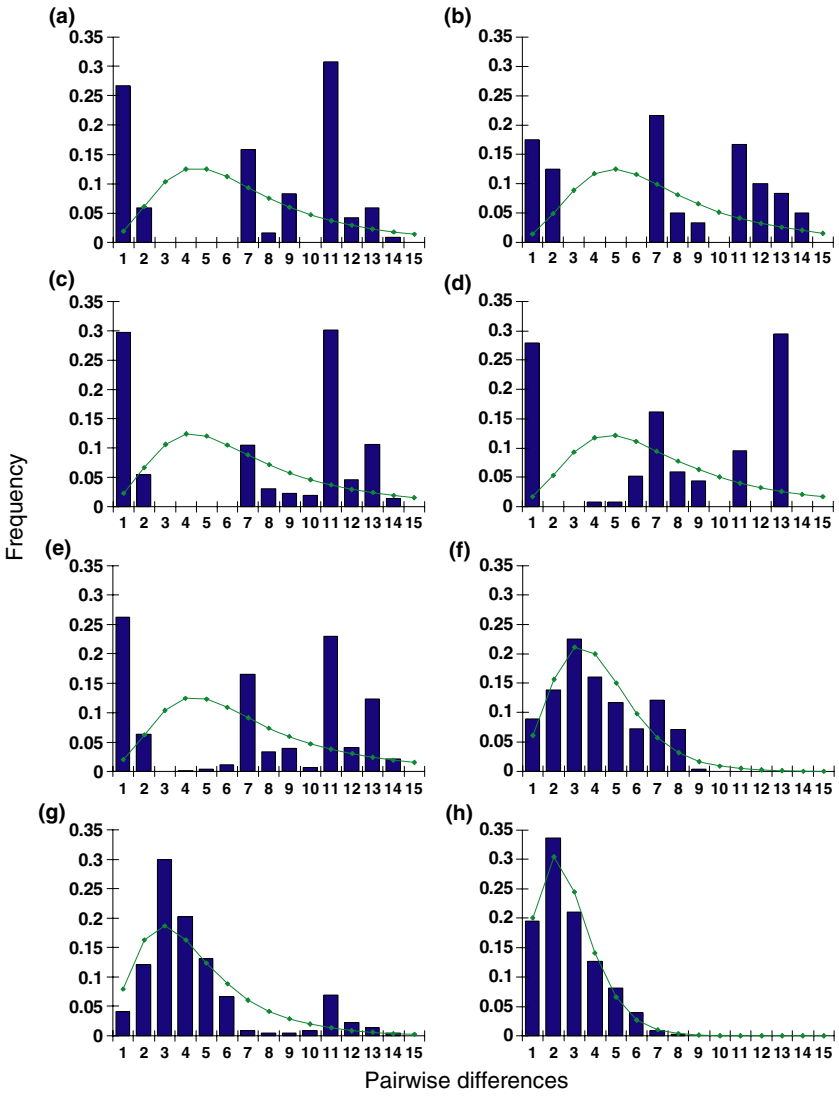


Figure 4 Mismatch distribution among COI haplotypes in invasive and native populations of *Mytella* spp. Bars are observed distributions and lines indicate the pattern expected under a model of sudden expansion. In all four invasive populations (a: Sunbury, GA; b: St. Marys, GA; c: JAX, FL; d: NSB, FL; e: pooled US populations), the observed and expected patterns are significantly different (Table 1). The multimodal distributions observed in the invasive populations are expected under a model of population admixture (Avise, 2000). The native Colombian (f), Ecuadorian (g) and Brazilian (h; *Mytella guyanensis*) populations exhibit a pattern more typical of a population in equilibrium (Avise, 2000). The native Ecuadorian population (g) shows evidence of population admixture, but is not significantly different ($P = 0.107$) than the expected pattern of a population at equilibrium.

found that gene diversity and number of haplotypes were greater in the native populations, a pattern indicative of bottlenecks in the invasive populations. Surprisingly, however, we found that invasive populations of *M. charruana* contain levels of nucleotide diversity even greater than those found in native populations, as evidenced by the occurrence of multiple common and highly differentiated mtDNA haplotypes occurring within invasive populations (Fig. 3). This combination of evidence for both bottleneck and admixture has not been documented in other bivalve invasions.

There are four possible explanations for the high levels of nucleotide diversity that we found: (1) DUI, (2) a highly diverse and large founding population, (3) multiple independent invasions, or (4) a combination of these explanations. Our data indicate that the most likely cause of the patterns of variation is multiple independent invasions. Why? First, during the course of this study, we ruled out DUI as the cause of the diversity. This is because all extreme haplotype variants (i.e. haplotypes A, N and R) occurred within a subset of only female mussels. Second, by comparing diversity within and between multiple native regions (Colombia and Ecuador), we characterized the diversity typical of some native populations. The divergence between common haplotypes in the invasive range is greater than that observed within either native population. Third, mismatch distribution data combined to form ragged, multimodal distributions for the invasive populations (Fig. 4), a pattern indicative of population admixture (Avise, 2000). Conversely, mismatch distribution data of native populations form smooth, unimodal distributions (Fig. 4), a pattern indicative of populations in static equilibrium (Avise, 2000). These data combine to form compelling evidence that the patterns of variation within the invasive populations of *M. charruana* in the southeast United States were caused by multiple independent invasions.

In addition to providing insights into how diversity is partitioned among populations, our data also provide insights into origins of the invasive populations. Here, we determined that native and invasive populations had strikingly different patterns of genetic variation. Native populations consisted of a relatively large number of closely related haplotypes. In

contrast, invasive populations had fewer, higher frequency haplotypes located in separate regions of the haplotype network (i.e. all invasive populations contained haplotypes A, N and R; Fig. 3). These patterns indicate that the *M. charruana* invasion resulted from bottlenecks of at least two distinct source populations (containing haplotypes A and N/R), and that the admixture of these distinct populations combined to form higher levels of nucleotide diversity in the invasive populations relative to the native populations studied here. Moreover, because our samples were collected within the first years of introduction, we characterized diversity of the actual founders. Thus, our data are not confounded by either post-colonization bottlenecking or post-expansion growth. Interestingly, one of the common invasive haplotypes (A) was also recovered in our native Colombian population. Moreover, there were no shared haplotypes between our invasive populations and our native Ecuadorian site as we expected. However, one of the Ecuadorian haplotypes (NN) was distinct from the remaining Ecuadorian haplotypes and similar to an invasive haplotype (N), indicating that the native haplotype N region may be invading the Ecuador site in addition to the southeast United States. Moreover, six haplotypes were only found in the invaded region indicating that our native sites undersampled the complete complement of *M. charruana* haplotypes. These results suggest that *M. charruana* was introduced to the United States from near the Colombian region as we predicted, but have yet to be introduced from the Ecuadorian region of South America.

Further information on ballast water transport and potential source populations can be gained by exploring vector strength data, which provide information concerning the number and location of ships that dump ballast water into ports of interest (Ruiz & Carlton, 2003). With regard to our study, we compiled data collected by the National Ballast Water Information Clearinghouse (http://invasions.si.edu/nbic/) and determined that ships containing source water from cities within the native range of *M. charruana* have dumped ballast water into the port of Jacksonville, Florida (21/6505 ballast tanks dumped in JAX originated from the native range of *M. charruana* from 2004–08). The low percentage (0.3%) included vessels originating from Trinidad, Venezuela, Mexico and Brazil, but none from Ecuador. Interestingly, the vector strength from San Juan, Puerto Rico is quite high (47% of all dumped water originated from San Juan), suggesting that San Juan may be a nexus for vector transport to Jacksonville.

What do our data mean for the future of the *M. charruana* invasion in the southeastern United States? The initial short-lived invasion discovered in Jacksonville, Florida (Lee, 1987) may have served as a sink population for several years. Empirical data indicate that many invasive populations exhibit a lag time during which they experience low survival as they adapt to overcome the genetic and ecological constraints typical of introduction (reviewed in Sakai *et al.*, 2001). Following this lag, invasive species often enter a growth phase in which they spread throughout their new habitat (Sakai *et al.*, 2001). Moreover, theoretical studies (e.g. Holt *et al.*, 2003) indicate that high migration into a suboptimal habitat may provide the genetic material necessary for local adaptation. Once adapted, the species can persist without further immigration, allowing further range expansion (Holt *et al.*, 2003). In our case, the invasive populations may currently be transitioning between lag and growth phases. Moreover, the constant influx of ships dumping ballast water from South America into North American ports in combination with the high diversity because of admixture and the broad salinity tolerances inherent in this species [they can survive in salinities ranging from 2–36 ppt (Pereira-Barros & Macedo, 1967; E.A. Hoffman and L.J. Walters, unpublished data)] provide ideal conditions for *M. charruana* to adapt and spread throughout their new environment.

In the future, our research will focus on estimating patterns of adaptive ecology within the invaded region. This will provide insight into how invasive species can rapidly evolve to survive in novel habitats. We are also interested in studying the shipping patterns between South America and the United States to determine the most likely route of the *M. charruana* introductions. Three means by which the invasions could have occurred include: (1) multiple ships from distinct areas having dumped ballast water into the United States ports, (2) a single ship may have stopped at multiple ports collecting ballast water at each stop before dumping the collective ballast water at a site, or (3) a single ship may have picked up ballast water from a port where population admixture had already occurred thus collecting all genetic variants at a single site and transporting them together. With our current data, we cannot predict which of these scenarios is most likely. Finally, we would like to identify and analyse more native populations to determine the unidentified source populations of *M. charruana* that founded the south-east United States populations. Studies linking propagule pressure and invasion pathways with invasion success indicate that recent human-mediated dispersal pathways often have increased levels of genetic diversity caused by mass dispersal (where dissemination of propagules occurs from many sources to many locations), which may assist invasion success (Wilson *et al.*, 2009). Overall, this information will help us to understand invasion patterns further, and thus, will aid in risk management of *M. charruana* and invasive species in general.

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