Phylogeography of *Petrolisthes armatus*, an invasive species with low dispersal ability

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Theoretically, species with high population structure are likely to expand their range, because marginal populations are free to adapt to local conditions; however, meta-analyses have found a negative relation between structure and invasiveness. The crab *Petrolisthes armatus* has a wide native range, which has expanded in the last three decades. We sequenced 1718 bp of mitochondrial DNA from native and recently established populations to determine the population structure of the former and the origin of the latter. There was phylogenetic separation between Atlantic and eastern Pacific populations, and between east and west Atlantic ones. Haplotypes on the coast of Florida and newly established populations in Georgia and South Carolina belong to a different clade from those from Yucatán to Brazil, though a few haplotypes are shared. In the Pacific, populations from Colombia and Ecuador are highly divergent from those from Panamá and the Sea of Cortez. In general, populations were separated hundreds to million years ago with little subsequent gene flow. High genetic diversity in the newly established populations shows that they were founded by many individuals. Range expansion appears to have been limited by low dispersal rather than lack of ability of marginal populations to adapt to extreme conditions.

The population-genetic constitution of marine invasive species in their native range is increasingly being studied in efforts to determine the source of invasions into new areas (reviews in refs 1–5). Less attention has been focused on the insights that such studies can provide regarding the relationship between genetic structure within the species range and invasive properties. Both factors are related to dispersal ability, which, in turn, is dependent on the degree to which propagules can spread and to the capacity of a species to adapt to varying physical and biological environmental factors. A species may be unable to invade a certain area because it cannot reach it, or it may not do so because it cannot survive there. Its native populations will also be isolated from each other to the degree that propagules cannot overcome barriers within its native range. Such barriers can either hinder dispersal physically, or be environments that exceed the tolerance of propagules. Thus, native population structure and invasiveness should be related. Ultimately, the question is one regarding the factors that determine species ranges.

Mayr suggested that limits to range expansion are set by intraspecific gene flow. Populations at the range margin are unable to adapt to local conditions (and thus progressively invade new areas) because of the influx of genes from the species centre. Kirkpatrick and Barton constructed models that supported this proposal. Their reasoning would lead to the conclusion that species with high levels of genetic structure are more likely to expand their ranges. Existing data from invasive species, however, have indicated the opposite, i.e. that species with high rates of gene flow in their native range are more likely to expand to new areas. Böhning-Gaese, et al. found that in warblers of the genus *Sylvia* ability to disperse—as measured by capacity for long-distance flight—overwhelmingly explained the size of species ranges. In the marine realm, a meta-analysis by Gaither, et al. found that the best predictive variable of whether a species would become invasive is the inverse of genetic structure in its native range. Species with low pairwise (but not global) FST values between their native populations were more likely to be invasive. Thus, intraspecific phylogeography, of interest in any species, takes an additional dimension when the species is invasive, because it also addresses the basic biogeographical question of the factors that determine range expansion.

An invasive species with a wide native geographic range is the porcelain crab *Petrolisthes armatus* (Gibbes), a marine, crab-like anomuran decapod. This species has the largest geographical distribution of any neotropical porcelainid. In the Atlantic its range extends from central Florida and Bermuda to southern Brazil on the...
American coast, and from Senegal to Angola on the West African coast10–12. It is also present at Ascensión13,14. In the eastern Pacific it ranges from the northern part of the Gulf of California to southern Perú, but it seems to be absent from the eastern Pacific offshore islands15. Until the early 1990s, the range of this species in the western Atlantic did not extend any farther north than Cape Canaveral11,12. Rathbun15 listed a few specimens from Connecticut, but Chace13 expressed doubts regarding this report. Starting in the 1990s, *P. armatus* has expanded its populations to the shores of Georgia and South Carolina and has become abundant on rocky rubble, oyster reefs, and other intertidal habitats16, where it suppresses recruitment of other crabs and growth of small oysters17. Thus, the species is considered “invasive”18–20, although its recent spread is probably best classified as a range shift18, rather than an invasion.

The wide geographic distribution of *Petrolisthes armatus* is not due to an exceptionally long planktonic life. The planktonic phase, consisting of two Zoea stages, in both eastern Pacific and western Atlantic populations ends in a settling megalopa in 12–19 days in the laboratory21,22, a length of planktonic stage typical of other porcellanid species23–25. The wide distribution of the species and its potential to become invasive may be due to its wide habitat preference and physiological tolerance. The species occurs in the lower intertidal zone, under stones, in oyster and mussel beds, around mangrove roots, in corals, sponges and on dock pilings. It has also been dredged from rock, sand, and shell bottoms down to 18 m on the American coasts26 and down to 30 m in the eastern Atlantic24. *P. armatus* is by far the most abundant porcellanid in the rocky intertidal of the Panamanian Pacific coast, with densities up to 20 individuals per m². It tolerates salinities as low as 10‰ inside the Panamá Canal Locks, where it is common15. According to a 16S rDNA phylogeny by Hiller, *et al.*25, *P. armatus* from both American coasts forms a sister clade to one comprised of *P. robsouie* and *P. zacae*, the former adapted to withstand great changes in salinity25,30, and the later adapted to inhabit spaces formed by entangled roots of the mangrove *Rhizophora mangle*26.

*Petrolisthes armatus* reproduces throughout the year both in eastern Pacific27–29 and western Atlantic30,31. Females of Pacific *P. armatus* release larvae synchronously near the times of nocturnal high tides, probably as result of predation pressure by visual predators46. The larvae may use vertical migrations in order to be retained in estuaries37–39. Sexual maturity is reached in about a month after settlement.

Recent warming of coastal subtropical waters has facilitated a poleward range expansion of many organisms with tropical distribution18. It is not known whether the northward range shift of *P. armatus* along the coasts of Georgia and South Carolina was aided by warming waters, or whether larvae were not capable of spreading northward of Cape Canaveral until adults were transported by humans. Canning-Clode *et al.*20 studied the possible impact of cold water temperatures on the survivorship of *P. armatus*. In temperatures similar to the ones experienced by the southern and mid-Atlantic coast of the United States during abnormally cold-swells between January and March 2010, 39% of specimens survived.

Here we use DNA sequence data from the mitochondrial cytochrome oxidase I (COI) and 16S rDNA genes of *Petrolisthes armatus* collected from localities spanning a large part of its geographic range to address the following questions: (1) What does the intraspecific phylogeography of this species reveal about genetic connectivity of its populations? (2) Have there been barriers to gene flow within the species range? (3) How long ago were populations separated, and what has the rate of genetic exchange been after their separation? (4) Is the genetic structure of this invasive species consistent with the view that genetic structure is related to range expansions? (5) What is the origin of founders of newly established populations, and what have been the population genetic consequences of invasion of new areas?

**Results**

**Phylogenetic Analyses.** Although intraspecific genealogies are said to be better represented by networks than phylogenetic trees because ancestral haplotypes may still exist in the sample48, the split in *Petrolisthes armatus* from the eastern Pacific and the Atlantic cannot be more recent than the completion of the central American Isthmus, and thus their common ancestor could not be younger than 3 MY. Our purpose of reconstructing a phylogeny was to identify and date major clades within the species. For this reason, we employed a conservative approach of collapsing every node that did not receive adequate support in both our Maximum Likelihood and the two Bayesian analyses. This mostly meant that we preserved all nodes supported by >70% of the bootstrap iterations in RAxML, because this program produced much lower clade support than either MrBayes or BEAST. The mtDNA phylogeny of *P. armatus* from concatenated 16S and COI sequences (Figs 1 and 2) revealed that Atlantic haplotypes are monophyletic. All three approaches provided high support for one clade on the seaboard of the United States (which we will name the “NW Atlantic” clade) and for a second clade with all haplotypes from Bermuda, and from all southern locations (the “SW Atlantic” clade). All three approaches also provided high support for a separate eastern Atlantic clade. There was, however, one discrepancy between the topology produced by BEAST, on the one hand, and by RAxML and MrBayes on the other. Whereas RAxML and MrBayes showed the Atlantic clade as a tritomy, BEAST showed the eastern Atlantic clade to be reciprocally monophyletic sister of the SW Atlantic clade with a posterior probability of 95%. According to estimates from BEAST, these two clades were separated 3.1 million years ago (MYA) [95% HPDs (Highest Posterior Density) 1.7 to 4.8 MYA], whereas, if assumed to be part of the tritomy, the estimated age of their most recent common ancestor would be the same as the age of the Atlantic clade, 3.5 MYA (95% HPDs 2.0 to 5.4 MYA). Given the 95% HPDs, these differences are not significant. The geographic separation of the NW and SW Atlantic clades is not absolute. Two haplotypes at Colombia belong to the NW Atlantic clade, and four haplotypes at South Carolina belong to the SW Atlantic clade (Figs 1 and 3). These phylogeographically “oddball” haplotypes by and large are not related to potential recolonization after the 2010 cold spell that is thought to have decimated the populations of *P. armatus* in the NW Atlantic. Only six haplotypes at South Carolina (Fig. 1), were collected in November 2012, whereas the rest were captured long before this time.

Whereas the NW Atlantic clade shows little geographic partitioning, with haplotypes from the two sides of Florida and from South Carolina mixed, the SW Atlantic clade displays phylogeographic structure. Haplotypes
from Brazil are closely related to each other (Fig. 3), and appear to be ancestral to haplotypes from all other localities in the southern clade (Caribbean and Bermuda), except for three haplotypes in Bermuda, which form a monophyletic entity, nested among the Brazilian haplotypes (Fig. 1). The separation between Brazilian and Caribbean subclades is dated by BEAST as having occurred 2.5 MYA (95% HPDs 1.4 to 3.9 MYA). The Caribbean clade contains a subclade composed of the majority of haplotypes from Colombia, Panamá, Yucatán and Belize, with a few haplotypes from Venezuela, South Carolina, Trinidad and Bermuda. This clade was estimated to be 1.7 MY old (95% HPDs 0.9 to 2.7 MYA), and is nested within a polytomy that contains haplotypes from the Southeast Caribbean and from Bermuda. The small sample of African populations shows little phylogeographic structure (Figs 1 and 4). Haplotypes from three locations in the Gulf of Guinea are closely related to each other, but more distantly related to two haplotypes from Sierra Leone.

In the eastern Pacific (Fig. 2), haplotypes from Isla Puná and Salinas at the coast of Ecuador, and those from Colombia form a monophyletic unit with an estimated age of 1.8 MY (95% HPDs 0.7 to 3.5 MYA). However, the phylogeny of haplotypes from Panamá, Costa Rica and the Gulf of California is unresolved. Nevertheless the southern Pacific clade is separated from the rest of haplotypes by 33 mutations (Fig. 3). Given these phylogenetic
divisions, analyses of population genetics were conducted within each of the three regions, the western Atlantic, the eastern Atlantic and the eastern Pacific.

**Population genetics.** Hierarchical analysis of molecular variance (AMOVA) comparisons between clades (or geographically distant populations) illustrates the population genetic effect of population subdivision and few migrant haplotypes (Table 1). In the western Atlantic the gene genealogy suggested the existence of a barrier between Florida and Yucatán. Most of the variation (58%) was found on either side of the suspected barrier to genetic exchange, which is unsurprising given that these barriers were suggested by the phylogenetic analysis. Despite the existence of individuals of the northern clade in the South and vice-versa, and despite high $F_{ST}$ values between populations in the same region, $F_{CT}$ values between regions were high and significant. In the eastern Atlantic, the distance between Sierra Leone and the Gulf of Guinea may constitute a barrier to gene flow.
Accordingly, 65% of the variation was between the population in Sierra Leone, on the one hand, and the populations in Cameroon, Nigeria and São Tomé, on the other; the F_{CT} value was high, but, because of small sample size in Sierra Leone, not significant. In the eastern Pacific 69% of the variation was explained by the separation of populations South and North of Panamá, but, once again, the F_{CT} value was not significant.

Table 1. Analysis of Molecular Variance (AMOVA), comparing populations on either side of a barrier suspected on the basis of phylogeny (see text) in each of three regions where Petrolisthes armatus occurs. The comparison in the western Atlantic is between populations North and South of Florida (Florida populations were included in the North region), in the eastern Atlantic between Sierra Leone and three locations in the Gulf of Guinea, and in the eastern Pacific between North and South of Panamá (Panamá populations were included in the North region). Bold values indicate significant values on the basis of 10,000 reshufflings.
|                  | Northern clade | Western Atlantic | Southern clade |
|------------------|----------------|------------------|----------------|
| Bermuda          | —              | 2,105,620        | 2,174,240      |
| Georgia*         | NC             | 25,462           | 31,875         |
| S.Carolina       | —              | —                | 95,306         |
| E Florida        | 0.697***       | 0.092**          | 0.101***       |
| W Florida        | 0.673***       | 0.123***         | 0.085*         |
| Yucatán          | 0.529***       | 0.598***         | 0.739***       |
| Panamá           | 0.452***       | 0.506***         | 0.679***       |
| Colombia         | 0.311***       | 0.538***         | 0.624***       |
| Venezuela        | 0.111*         | 0.668***         | 0.606**        |
| Trinidad         | 0.214***       | 0.653***         | 0.630***       |
| Brazil           | 0.586***       | 0.762***         | 0.735***       |

Table 2. $F_{ST}$ values (below the diagonal) between populations in the same region, and times of separation of populations in years (above the diagonal) estimated from IMa2\(^1\), based on the concatenated fragment of 16S, COI-af, and COI-HL, except for comparisons involving Georgia (marked with an asterisk), which are based on COI-HL alone. ***$p < 0.001$, **$p < 0.01$, *$p < 0.05$, NS: not significant. NC: IMa2 failed to converge. Separation of clades is based on the phylogeny shown in Figs 1 and 2.

With the exception of immediately adjacent populations, pair-wise $F_{ST}$ values between populations in the western Atlantic were large and highly significant (Table 2), which, under Wright's\(^2\) island model would indicate a lack of gene flow over larger distances, assuming equilibrium between migration and genetic drift. Isolation by distance (IBD) analysis found a significant relationship between genetic dissimilarity and geographic distance, whether the two variables were measured in a linear ($r = 0.506$, $p < 0.0001$), or in a logarithmic ($r = 0.541$, $p < 0.0004$) scale. In contrast to the western Atlantic, genetic discontinuities between the populations in the eastern Pacific were mostly governed by the suggested barrier to gene flow between the coast of Colombia and the coast of Panamá. $F_{ST}$ values between populations within these two areas were large and significant, whereas within each area –despite long geographic distances between Panamá and the Sea of Cortez– they were indicative of gene flow between distant populations. Correlations between genetic and geographic distance in the eastern Pacific were not significant in either a linear ($r = 0.0496$, $p = 0.331$) or a logarithmic ($r = 0.0978$, $p = 0.263$) scale. Very small and non-significant $F_{ST}$ values between the Gulf of Guinea populations (Sierra Leone, with only two haplotypes was not included) indicated that *P. armatus* in this area is genetically uniform between Nigeria, Cameroon and São Tomé (Table 2). In the Isolation by Distance analysis the Mantel correlation coefficient was high ($r = 0.9849$), but because of the small number of populations and low sample size, not significant ($p = 0.1644$).

$F_{ST}$ statistics cannot distinguish between genetic similarity due to recent separation from similarity due to high gene flow. We employed a coalescent approach in IMa2 to date the time of the initial separation of populations and to estimate the magnitude and direction of subsequent gene flow. Based on the assumption that West Atlantic and eastern Pacific populations were separated for a minimum of 3 MY, and seeing that the average HKY\(^4\) distance (the model of DNA evolution used by IMa2) between their concatenated haplotypes is 4.04\%, we have calibrated estimated times of divergence and migration between populations on the assumption that the 1718 bp fragment experiences a substitution rate per lineage of 7.5–15 mutations per MY. In comparisons between the population at Georgia and the rest of the samples (for which only a 499 bp fragment of COI-HL was available), we used the divergence in this DNA fragment between West Atlantic and Pacific populations of 5.02\% to set the substitution rate prior for this fragment as 2–5 mutations per lineage per MY. Posterior probabilities of successful runs in IMa2 are supposed to rise to a peak at particular values of each parameter, then drop back down. In some pairwise comparisons, despite many chains, wide priors and long runs, posterior probabilities rose to a plateau and remained there, so that no unique value could be assigned to the relevant parameters. In the western Atlantic, times of initial separation estimated by IMa2 between populations (Table 2) ranged from tens of thousands to $>2$ million years. The most recent separations were between populations at South Carolina and Georgia, and between Georgia and East Florida, suggesting that the recently established northern populations may have been due to recent transfers. Obviously, given the recent introduction of *P. armatus* into Georgia and South Carolina, the estimates of separation of centuries to millions of years ago do not correspond to the actual time of transportation.
of individuals. These estimates of IMa2 are for the separation of the mixture of haplotypes transported into these localities relative to haplotypes in the rest of the species range. That populations at Georgia and South Carolina were the result of massive transfer, rather than having been started from a few individuals was also supported by their high genetic variability (Table 3). Both nucleotide and haplotype diversity in these populations were amongst the highest of all sampled populations with \( N > 10 \). Thus, the IMa2 results from the invaded localities reflect past history of the mixture of invaders. Estimates from the rest of the populations, however, are more likely to reflect population history.

According to IMa2 estimates, the population of *Petrolisthes armatus* in Bermuda, the geographically most isolated locality in the western Atlantic, has been separated from all other localities \( 1.6 \times 10^5 \) to \( 2.1 \times 10^6 \) years ago (Table 2) and has received little gene flow since then, except from Trinidad and the coast of Colombia (Table 4). The four haplotypes of the SW Atlantic clade found in South Carolina (Figs 1 and 3) were interpreted by coalescence as having arrived from Bermuda, as well as Venezuela and Yucatán (Table 4). To the extent that the analysis of the shorter COI-HL fragment can be trusted, there has also been some gene flow from the founder haplotypes of Georgia into Bermuda (Table 4). The separation of the population in eastern and western Florida from populations that belong to the southern Caribbean clade has also been \( > 10^5 \) years ago (Table 2). The split between populations on the two coasts of Florida was estimated as much more recent. Gene flow between the two Florida populations has been relatively high, whereas gene flow into southern populations was not significantly different from 0 (Table 3). However, there has been asymmetrical gene flow from the Caribbean coast of Colombia, as also seen in the phylogenetic analysis (Figs 1 and 3). All estimated times of separation of populations within the SW Atlantic clade were in the order of \( 10^5 \) years, except for those involving Brazil; the latter were more than a million years old with the exception of more recent isolation from Yucatán and Venezuela (Table 2). Most migration rates between Brazil and populations of the SW Atlantic clade were also not significantly different from 0 (Table 3). The contiguous Atlantic coasts of Panamá and Colombia registered both by \( F_{ST} \) values (Table 2) and by IMa2 estimates (Table 3) as containing a single, panmictic population. Populations at Yucatán and the Caribbean coast of Colombia appeared as exchanging a high number of propagules. Interestingly, the two NW Atlantic clade haplotypes found in Colombia (Figs 1 and 3) did not contribute to migration rates significantly different from 0 in IMa2.

In the eastern Pacific, estimated times of isolation between the population in the Sea of Cortez and any other population (including the one in Panamá) was \( > 1.5 \times 10^6 \) years (Table 2). Despite their geographic proximity, but in agreement with their distant phylogenetic affinity, populations on the coast of Panamá and of Colombia were also estimated to have been initially separated \( > 2 \times 10^5 \) years ago, longer than the isolation between populations at Panamá and the two localities at the coast of Ecuador (Salinas and Isla Puná). The most recent separation is

| Location       | N  | Nucleotide diversity | Haplotype diversity |
|----------------|----|----------------------|---------------------|
|                |    | Concatenated COI     | COI                 |
|                |    | Concatenated COI     | COI                 |
| West Atlantic  |    |                      |                     |
| Bermuda        | 14 | 0.010                | 0.019               |
| S.Carolina     | 22 | 0.009                | 0.007               |
| Georgia        | 139|                      | 0.009               |
| E Florida      | 26 | 0.008                | 0.011               |
| W Florida      | 24 | 0.009                | 0.013               |
| Yucatán        | 23 | 0.001                | 0.002               |
| Panamá         | 15 | 0.003                | 0.004               |
| Colombia       | 40 | 0.007                | 0.009               |
| Venezuela      | 9  | 0.010                | 0.018               |
| Trinidad       | 10 | 0.010                | 0.015               |
| Brazil         | 21 | 0.004                | 0.001               |
| East Atlantic  |    |                      |                     |
| Sea of Cortez  | 10 | 0.011                | 0.043               |
| Costa Rica     | 4  | 0.006                | 0.009               |
| Panamá         | 13 | 0.013                | 0.031               |
| Colombia       | 19 | 0.005                | 0.008               |
| Salinas        | 6  | 0.006                | 0.006               |
| Isla Puná      | 5  | 0.002                | 0.003               |
| East Atlantic  |    |                      |                     |
| Sierra Leone   | 2  | 0.002                | 0.002               |
| Cameroon       | 5  | 0.002                | 0.001               |
| Nigeria        | 4  | 0.002                | 0.004               |
| São Tomé       | 4  | 0.002                | 0.003               |

Table 3. Nucleotide and haplotype diversity\(^{107}\) based on Tamura and Nei\(^{109}\) distances in sampled populations of *Petrolisthes armatus*. Concatenated data are composed of a partial sequence of 16S and COI-af and COI-HL. COI data are limited to COI-HL distances.
between the Colombian and the Isla Puná populations. Rates of migration were generally not significantly different from 0, except for asymmetrical gene flow, from the population at Isla Puná to Salinas and from the coast of Colombia into Isla Puná (Table 3).

In contrast to the pattern seen in the western Atlantic, populations on the coasts of Nigeria, Cameroon and on the island of São Tomé contained haplotypes that mostly differ by only one mutation (Fig. 5), resulting in estimates of small FST values and recent separation (Table 2). Gene flow appeared to be along a North to South axis with a lower rate from São Tomé into Nigeria. Estimated migration in the opposite direction was not significantly different from 0.

### Discussion

Genetic structure of *Petrolisthes armatus* is characterized by deep phylogenetic divisions (i.e. clades) and large divergence between populations within the same clade. Clearly, this is a species with low ability to disperse, either within the western Atlantic or within the eastern Pacific, although our limited sample from the eastern Atlantic indicates higher genetic connectivity between the more closely spaced samples in this region. The degree of structuring within the West Atlantic and East Pacific is evident in the mtDNA phylogeny, in FST values and in the coalescent analysis. Yet a few errant haplotypes of one clade found in the region mainly inhabited by the other, along with the coalescence estimates of gene flow subsequent to initial separation, attest to infrenetrant long distance genetic exchange. What barriers and mechanisms of gene flow could account for the observed patterns?

Patterns of spread of genes between sedentary marine populations are obviously a function of the dispersal abilities of the species relative to potential barriers to planktonic transfer. The 12 to 19 day planktonic larva of *Petrolisthes armatus*\(^{41, 42}\) is conducive to high population structure, but it is not so limiting, as to be the entire explanation for the deep divisions seen in this species. Other tropical species in the same regions, which in the laboratory complete their planktonic cycle in approximately the same period of time, such as the sea urchin *Echinometra lucunter* with a larval life of about 10 days\(^{44}\), show much less genetic structure within the Caribbean. Only the sea anemone *Nematostella vectensis*\(^{47}\) show evidence of such high population differentiation between local populations. Shulman and Bermingham\(^{48}\) calculated that if larvae were transported as passive particles, it would take only 13 generations for genes of a species with a larva that stays in the plankton for two weeks to traverse through stepping stones the entire Caribbean following the main Caribbean Current, given average current vectors\(^{49-51}\). It is unlikely that *P. armatus* is lacking stepping stones, as, in addition to our

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Table 4. Rates of migration within each region estimated from the IMa2 algorithm\(^{111}\). Number of female propagules (2N\(m\)) received by the population listed in first column from population listed in first row (in the direction of the arrows). NC: IMa2 failed to converge. Values are based on the concatenated fragment of 16S, COI-af and COI-HL, except for comparisons between Georgia (marked with asterisk) and the rest of the populations, which are based on COI-HL alone. Migration rates that were not significantly different from 0 are shown as “0.000”. Bold values indicate significantly asymmetrical gene flow. Clades as defined in Fig. 2.

| Western Atlantic | Northern clade | Southern Clade |
|------------------|---------------|----------------|
| Bermuda          | ↓             | ↓              |
| Georgia*         | 1.133         | 0.000          |
| S.Carolina       | 0.000         | 0.000          |
| E Florida        | 0.000         | 0.000          |
| W Florida        | 0.249         | 0.000          |
| Yucatán          | 0.000         | 0.000          |
| Panamá           | 0.000         | 0.000          |
| Colombia         | 0.000         | 0.000          |
| Venezuela        | 0.000         | 0.000          |
| Trinidad         | 0.000         | 0.000          |
| Brazil           | 0.318         | 0.000          |

| Southern Clade | 2.989         | 0.000          |
|               | 0.000         | 0.000          |
|               | 0.000         | 0.000          |
|               | 1.249         | 0.000          |
|               | 0.000         | 0.000          |
|               | 0.000         | 0.000          |
|               | 0.000         | 0.000          |
|               | 0.000         | 0.000          |
|               | 0.000         | 0.000          |
|               | 0.000         | 0.000          |
|               | 0.000         | 0.000          |
|               | 0.000         | 0.000          |

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sampled localities, it is also found in the Greater and the Lesser Antilles\(^{10}\). The lack of long-distance dispersal indicated by the high population structure, therefore, is more likely due to local retention of larvae, which appears to be a characteristic of this species\(^{37,38}\). The tendency of larval retention, however, cannot be absolute in a species of such a wide range, so barriers to gene flow must also be present to account for the phylogeographic divisions.

An obvious such barrier is the Isthmus of Panamá, the timing of which we have used to date all other separations between populations. A multitude of lines of evidence has indicated that the final closure of the Isthmus, after a 12 MY process of narrowing water connections, occurred at approximately 3 MYA\(^{39}\). Some authors\(^{44,55}\) have agreed that there were water connections until this time, but they presented their conclusions so as to imply that there may have been previous interruptions of gene flow between the eastern Pacific and the western Atlantic\(^{56,57}\). Whether the water connections had narrowed at instances prior to 3 MYA bears little relevance to the phylogenetic reconstruction of *P. armatus*, because among five sister clades in the genus *Petrolisthes* with representatives on either side of the Isthmus of Panamá, eastern Pacific and Atlantic populations of *P. armatus* have diverged the least\(^{39,58,59}\), and are thus most likely to have been the most recently separated. This, however, does not necessarily mean that the trans-isthmian split was contemporaneous with the final isthmus closure, because it could have pre-dated it by any length of time. Our use of wide priors in the split between the Pacific and the Atlantic clades in the BEAST reconstruction has resulted in an estimated date of 3.5 MYA, which seems reasonable for a species with wide environmental tolerances, such as *P. armatus*. Based on this, we have estimated the split between western and eastern Atlantic clades at 3.1 MYA. The barrier that separated populations at these two regions was undoubtedly the long distance between American and African coasts. Scheltema\(^{60}\) estimated that, based on the speed of currents, larvae of marine organisms would require between 9 and 28 weeks to cross the contemporary tropical Atlantic. This distance barrier also accounts for distinct amphitropical clades in other porcellanids\(^{29}\), sea urchins\(^{61-63}\), and fishes\(^{64-66}\). Given the short length of larval life of *Petrolisthes*, the puzzle is not how the separation happened, but rather how the original colonization was effected. The only explanation for the expansion of *P. armatus* to the coast of Africa is that this species can occasionally raft, possibly on mangrove roots, on which adults and juveniles are frequently found. We can only speculate about ocean circulation in the Pliocene, but modern-day currents between the coast of Brazil and the coast of Africa\(^{67,68}\) are conducive to rafting transport; Ascension may have been a stepping stone.

The barrier that could account for the presence of two clades in the Caribbean, one predominantly on the coast of Florida, the other from Yucatán to south Brazil, is less obvious. The population at Yucatán shows the lowest nucleotide diversity among all sampled localities (Table 3), suggesting that effective population size may be smaller. Richards, *et al*\(^{69}\) found that a population of the brittle star *Ophiothrix suensoni* in the Gulf of Honduras was genetically isolated from the populations in the rest of the Caribbean, which they attributed to larval entrapment arising from the Mesoamerican gyre\(^{70,71}\). A similar explanation was offered by Jackson, *et al*\(^{72}\) for the isolation of populations of the Nassau Grouper, *Epinephelus striatus* in this area. However, our sample from Yucatán was from the northern side of the peninsula and, given that in *Petrolisthes armatus* there is significant isolation by distance in the western Atlantic, the geographic separation of the two clades may be due to distance alone. Although larval entrapment in the Yucatán area could be the reason haplotypes in this area are so similar to each other, the *F\(_ST\)* values between the Yucatán population and populations on the coast of Colombia and Panamá are significant but relatively small (Table 2), so this population is not completely closed. There is no need to postulate a barrier between Yucatán and Florida, because the large genetic distance between them could simply be a reflection of more gradual differentiation between stepping-stone populations off the Gulf of México shores, which were not sampled. What remains elusive is the original barrier that may have caused the differentiation of the NW and SW Atlantic clades. It is also difficult to explain why Bermuda was colonized only by the SW Atlantic clade. Presumably, the original colonization resulted from rafted individuals, carried by the Gulf Stream, which occasionally throws off cold rings that reach Bermuda\(^{69}\). The Gulf Stream, however, flows by the east coast of Florida, but we found no evidence of the existence of the NW Atlantic clade in Bermuda. Either the frequency of haplotypes of the northern clade in Bermuda is lower than ~0.07, so that it could not be detected with a sample of 14 individuals, or the colonization of Bermuda by southern haplotypes was a chance event that has not been repeated.

The two haplotypes of the NW Atlantic clade in Colombia and the four haplotypes of the southern clade in South Carolina were interpreted by coalescence as being the result of migration, not of incomplete lineage sorting. To the extent that this reconstruction is correct, their ancestors must have arrived through the Antilles, because no such odd haplotypes were detected in any other sampled locality. The average vector of currents on the average of 14 individuals, or the colonization of Bermuda by southern haplotypes was a chance event that has not been repeated.

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In the eastern Pacific, the monophyletic clade composed of haplotypes at the coasts of Ecuador and Colombia, highly differentiated from those at Panamá and the Sea of Cortez, is unusual. No other organism sampled genetically to date displays this genetic break between populations at Colombia and Panamá\(^{81}\). There is ample rocky habitat between Ecuador and Panamá, and there is little in patterns of ocean circulation to suggest that larvae...
could not spread through stepping stones\textsuperscript{82–84}. It will be interesting to find out whether other eastern Pacific species of \textit{Petrolisthes} show a similar genetic discontinuity.

The newly established populations of \textit{Petrolisthes armatus} in South Carolina and in Georgia are of particular interest, because they have earned the title of invasive species for this crustacean\textsuperscript{1,13–20}. Our data clearly show that these populations were initiated by the movement of individuals from Florida. Their haplotypic and molecular diversity is as high as that of native populations, a frequent characteristic of invasive populations when they are established by a large number of propagules\textsuperscript{1,85–87}. Transfer of large numbers of the oyster \textit{Crassostrea virginica} from the Gulf of México to the Chesapeake Bay during the early 1960s to replace oyster stocks infected with “MSX” disease has been associated with invasions by crustaceans inhabiting oyster beds from the Gulf to the Atlantic seaboard of the United States\textsuperscript{89}; the same may have been true for introductions into Georgia and South Carolina. A large number of recently introduced founders, however, confounds the detection of evolutionary processes within the populations with the mark left by the genetic constitution of haplotypes that founded them. This is particularly true when there are multiple invasions from different sources\textsuperscript{49,90}. At the time that our samples were taken at Georgia and South Carolina, a maximum of 22 years had elapsed since \textit{P. armatus} was noticed in these areas. Despite the obvious tendency of this species to form deep genetic divisions between its populations over time, ~250 generations have apparently not been enough for haplotypes to sort themselves to the degree that coalescence can correctly infer the history of isolation and migration. Comparisons of the Georgia population with those from the native range are further complicated by their being based on a short fragment of mtDNA, which is less likely to conform to the infinite sites mutation model assumed by the IMa2 algorithm. Population density of \textit{P. armatus} in Georgia and South Carolina fluctuates widely\textsuperscript{19}, as it also does in its native range\textsuperscript{82–84}, but the species appears well-established in areas occupied after its range shift\textsuperscript{91}. \textit{P. armatus} was present—though not as abundant—in South Carolina, even after the 2010 cold snap\textsuperscript{20}, which suggests that larvae as well as adults are able to tolerate temperate temperatures. It is apparently too early to tell whether local adaptation in this marginal environment will permit the species to expand its range farther north.

Conclusions

With sufficient variation, pronounced genetic structure is expected to allow local adaptation\textsuperscript{6,7}. Local adaptation is expected to promote range expansion\textsuperscript{94,95}. \textit{Petrolisthes armatus}, according to our mtDNA data, is a species characterized by high structure, although this structure may need thousands or millions of generations before it becomes established so that local adaptation can ensue. The signature of natural obstacles to migration remains in the genetics of both the newly established and the old populations. Two major barriers are identified by the phylogeny, the separation of Atlantic and Pacific populations by the completion of the Isthmus of Panamá, and the almost contemporaneous separation of two clades on the opposite shores of the tropical Atlantic. The subsequent separation of the North and South clades in the western Atlantic and a similar break in the eastern Pacific do not lend themselves to obvious explanations as to their causes, but further support the conclusion that dispersal in \textit{P. armatus} is limited. The recent range expansion from Florida to the northern Atlantic seaboard of the United States is most likely due to human transportation of oysters, but the ability of the species to survive in the new habitats shows that it possesses the necessary plasticity to tolerate new physical and biotic environments. Thus, the range limits of this species are more likely set by species characteristics related to low ability to disperse, rather than by narrow physiological tolerance. Given these characteristics, any subsequent spread of this invasive species is likely to be caused by human mediation.

Material and Methods

\textbf{Collections.} We collected, or obtained from museums, 276 individuals of \textit{Petrolisthes armatus} from both sides of the Atlantic and from the eastern Pacific (Fig. 6). All specimens from the Atlantic seaboard of the United States were collected before the extremely cold winter of 2010 (which is assumed to have caused high mortality of \textit{Petrolisthes armatus} in this area\textsuperscript{20}), except for 15 samples from Fort Pierce (collected in July 2010) and 11 samples from South Carolina (collected in November 2012). We additionally downloaded from GenBank 139 sequences of partial Cytochrome Oxidase I (COI) (Accession numbers FJ693377–693515) from Sapelo Island, Georgia, obtained by J.D. Robinson, E. Díaz-Ferguson, S. Pennings, T. Dale Bishop and J. Wares. These sequences were obtained from specimens collected in 2006–7 (J. Wares, per. com.).

\textbf{DNA extraction and sequencing.} DNA was extracted from chelipeds or walking legs using the DNeasy\textsuperscript{©} Blood & Tissue Kit (Qiagen), following the manufacturer's protocol for animal tissues. A 530 bp fragment of the ribosomal 16S rDNA was amplified using primers 16Sar-5’ (CGCTGTTTATCAAAAAACGAT) and 16Sbr-3’ (CCGGTGCTGAACCTACGATACG)\textsuperscript{96}; and was trimmed to 508 bp in the alignment. A 640 bp of Cytochrome Oxidase I (COI) was amplified using primers COIf-5’ (CCTGAGGAGGAGGAYCYCC) and COIa-3’ (AGTATAACGTCTGTTGTAGTGC)\textsuperscript{96}; and was trimmed to 532 bp in the alignment. A second 680 bp COI fragment was amplified using primers LCO1490 (GGTCAACAAATCATAAAGATATTG) and HCO2198 (TAAACTTACGGGTAGCAGCAAAAATCA)\textsuperscript{97}, and was trimmed to 678 bp in the alignment. We refer to the first COI fragment as COI-af and to the second as COI-HL. Double-stranded amplifications were performed in 25 ml volume reactions containing 5 µl of Taq buffer (5 ×), 2.5 µl of dNTP mix (8 mM), 1.2 µl of each primer (10 µM), 2.5 µl of MgCl\textsubscript{2} (25 mM), 0.2 µl of GoTaq\textsuperscript{®} Flexi DNA Taq Polymerase (Promega), 1 µl of DNA template, and 11.4 µl of ddH\textsubscript{2}O. Thermal cycling conditions consisted of an initial denaturation step at 96°C for 3 min, followed by 30 cycles of 95°C for 1 min, 50°C for 1 min, and 72°C for 1 min. An extension step at 72°C for 5 min followed the last cycle. Amplifications that resulted in unique PCR products were cleaned using the ExoSap-IT\textsuperscript{®} kit (USB Corporation) following the manufacturer's protocol. Samples that amplified multiple products were purified by cutting the band of the appropriate molecular weight out of a 2% low-melt agarose gel after electrophoresis in 1 × TAE buffer. PCR products were recovered by incubating the sample at 70°C for 10 min, and then at 45°C for 10 min.
60 min after adding 1 µl of GELase™ (Epicentre Biotechnologies). Clean PCR products were cycle-sequenced in both directions using the BigDye® Terminator v. 3.1 Cycle Sequencing Kit, and electrophoresed in an Applied Biosystems® 3130 Genetic Analyzer. DNA from one individual of Petrolisthes robsonae was also sequenced to be used as outgroup in the phylogenetic analyses. Sequences were aligned by eye using BioEdit98. Multiple attempts to identify an informative nuclear sequence failed.

Phylogenetic analyses. The Akaike Information Criterion [AIC, ref. 99], implemented in the program jModeltest2100, was used to select the model of nucleotide substitution that best fit each DNA region. These models were TIM3+I (I = 0.92) for 16S, GTR+I+G (I = 0.74, α = 1.29) for COI-af, and TIM+I+G (I = 0.18, α = 0.14) for COI-HL. The three fragments of each individual were concatenated and subjected to partitioned phylogenetic analysis after removal of redundant haplotypes, with the appropriate model applied to each partition.

Maximum Likelihood (ML) phylogenetic reconstruction was conducted in RAxML v. 8.2.6101, applying a GTR model with different values of α for each partition, as indicated by jModelTest2, and using the options for rapid bootstraps and automatic halting. Support values for the nodes were estimated from 456 bootstraps. Bayesian reconstruction was conducted in two Bayesian algorithms, MrBayes v.3.2.2102 and BEAST v. 1.8.2103, using as priors the models indicated by jModeltest2, but letting the programs estimate the parameters. Mr. Bayes was run in 4 chains and for 6×10⁷ steps, which allowed the average standard deviation of split frequencies to fall below 0.01, and the potential scale reduction factor to be equal to 1.00. Convergence was also determined in multiple runs, which produced the same topology. Node credibility values were determined by sampling every 600th tree after discarding 25,000 trees as a burnin. BEAST results came from eight separate runs of different numbers of steps, combined in Logcombiner v. 1.8.2 taking every 10th tree and a burnin of 10% per run, for a total of 26,085 trees. Tracer v. 1.6 verified that Effective Sample Size (ESS) of all estimates of the combined runs exceeded 200.

In addition to reconstructing the phylogeny, BEAST was used to estimate date of divergence between major clades. For this purpose, the separation between Atlantic and Pacific haplotypes was given an offset of 3 million years (MY) in a Lognormal Uncorrelated Relaxed clock. This is the generally accepted date of the completion of the Central American Isthmus53,59,104. However, as there are claims that only “narrow, shallow, and transient channels” connected the Caribbean with the Tropical Pacific as of 13 MYA (million years ago)54, the priors for this
calibration point were set as uniform, ranging from 0.2 to 10^{100} MY. RAxML and BEAST analyses were run on the CIPRES Science Gateway^{105}. A median-joining network^{106} of haplotypes in the same geographic region was constructed in PopArt v.1 (http://popart.otago.ac.nz) to visualize relationships between haplotypes and geography within subclades in which the ancestral sequences may still be present in the populations^{46}.

**Population genetic analyses.** For the purposes of population genetic analyses, 4 samples from the Pacific shore of Costa Rica were pooled with those from the adjacent coast of Panamá, and 3 samples from Belize were pooled with those from Yucatán. All analyses were based on the full concatenated sequences of 16S, COI-af and COI-HL except for those involving the invasive Georgia population, for which only sequences from COI-HL were available. Haplotype and molecular diversity^{107} and sequence-based pairwise F_{ST} (Φ_{ST}) values were calculated in Arlequin 3.5.1.2^{108} using Tamura and Nei^{109} distances. Analysis of Molecular Variance (AMOVA)^{110} was also carried out with this program, with significance determined in 10,000 reshufflings of haplotypes between populations. For a coalescent analysis, intended to determine times of separation of populations and subsequent direction of gene flow, we used IMA2 v. 2.0^{111}. In the MCMC mode of this program we used geometric heating with 60 chains and a burnin of 5 hours. The runs were continued until an ESS of >200 was obtained for each parameter. L mode, intended to determine whether gene flow was significantly different from 0 and significantly different in each direction, was run for 3 × 10^{7} steps. The uniform prior for mutation rate per lineage was estimated from assuming that divergence between Atlantic and Pacific populations of *P. armatus* reflects separation of 3 MY. The prior of the substitution rate was given a range equal to the mean and distributed symmetrically on either side of the mean. Comparisons between populations were made in pairwise fashion in separate runs. For isolation by distance analysis, Mantel^{112} correlations between Φ_{ST} and geographic distance by sea we employed BDWS^{113} with 10^{4} iterations.

**Data Availability.** Datasets generated during the current study are available in GenBank under accession numbers KY856997–857273 (for 16S rDNA) and KY857274–857550 (for COI) and in Dryad (https://doi:10.5061/dryad.pm1r2) (alignments).

**References**

1. Rius, M., Turon, X., Bernardi, G., Volckaert, F. A. M. & Viard, F. Marine invasion genetics: from spatio-temporal patterns to evolutionary outcomes. *Biological Invasions* **17**, 869–885, doi:10.1007/s10530-014-0792-0 (2015).
2. Geller, J. B., Darling, J. A. & Carlton, J. T. Genetic perspectives on marine biological invasions. *Annual Review of Marine Science* **2**, 367–393 (2010).
3. Holland, B. S. Genetics of marine bioinvasions. *Hydrobiologia* **420**, 63–71 (2000).
4. Dlugosch, K. M. & Parker, I. M. Founding events in species invasions: genetic variation, adaptive evolution, and the role of multiple introductions. *Molecular Ecology* **17**, 431–449, doi:10.1111/j.1365-294X.2007.03538.x (2008).
5. Lee, C. E. Evolutionary genetics of invasive species. *Trend. Ecol. Evol.* **17**, 386–391 (2002).
6. Mayr, E. *Animal Species and Evolution*. (Harvard University Press 1963).
7. Kirkpatrick, M. & Barton, N. H. Evolution of a species’ range. *American Naturalist* **150**, 1–23 (1997).
8. Bohning-Gaese, K., Caprano, T., Van Ewijk, K. & Veith, M. Range size: Disentangling current traits and phylogenetic and biogeographic factors. *American Naturalist* **167**, 555–567 (2006).
9. Gaither, M. R., Bowen, B. W. & Toonen, R. J. Population structure in the native range predicts the spread of introduced marine species. *Proceedings of the Royal Society B-Biological Sciences* **280**, doi:10.1098/rspb.2013.0409 (2013).
10. Werding, B., Hiller, A. & Lemaire, R. Geographic and depth distributional patterns of western Atlantic Porcellanidae (Crustacea: Decapoda) (eds Daniel, Simberloff & Marcel, Rejmánek) 285–390 (Univ of California Press 2000).
27. Gore, R. H. The larval development of the commensal crab Polygonyx gibbesi Haig, 1956 (Crustacea: Decapoda). The Biological Bulletin 135, 111–129 (1968).
28. Hernández, G. et al. Larval development of Clastotoecus nodosus (Street, 1872)(Crustacea: Decapoda: Porcellanidae), under laboratory conditions. Scientia Marina 67, 419–428 (2003).
29. Hiller, A., Kraus, H., Almon, M. & Werding, B. The Petrolisthes galathinus complex: Species boundaries based on color pattern, morphology and molecules, and evolutionary interrelationships between this complex and other Porcellanidae (Crustacea: Decapoda: Anomura). Mol. Phylogenet. Evol. 40, 547–569 (2006).
30. Haig, J. Eastern Pacific expeditions of the New York Zoological Society. Porcellanidae crabs (Crustacea: Anomura) from the west coast of tropical America. Zoologica, New York Zoological Society 53, 57–8 (1968).
31. Werding, B. & Hiller, A. Description of a new species of Petrolithes from the western Pacific (Decapoda, Anomura, Porcellanidae). Crustaceana 77, 257–264 (2004).
32. Diaz-Ferguson, E. & Vargas-Zamora, J. A. Abundance of Petrolisthes armatus (Crustacea: Porcellanidae) on a tropical estuarine intertidal rocky beach, Gulf of Nicoya estuary, Costa Rica. Rev. Biol. Trop. 49, 97–101 (2001).
33. Oliveira, E. & Masunari, S. Estrutura populacional de Petrolisthes armatus (Gibbes) (Decapoda, Anomura, Porcellanidae) da Ilha do Farol, Mattinhos, Paraná, Brasil. Revista Brasileira de Zoologia 12, 355–371 (1995).
34. Brito de Oliveira, D., Costa Silva, D. & Martinelli-Lemos, J. M. Larval and adult density of the porcellanid crab Petrolisthes armatus (Anomura: Petrolisthes) in an Amazon estuary, northern Brazil. Zoologica (Curitiba) 30, 592–600 (2013).
35. Christy, J. Timing of larval release by intertidal crabs on an exposed shore. Bull. Mar. Sci. 39, 176–191 (1986).
36. Morgan, S. G. & Christy, J. H. Adaptive significance of the timing of larval release by crabs. American Naturalist 114, 457–479 (1979).
37. Tilburg, C. F., Seay, J. E., Bishop, T. D., Miller, H. L. & Mele, C. Distribution and retention of Petrolisthes armatus in a coastal plain estuary: the role of horizontal and vertical movement in larval transport. Estuaries, Coastal and Shelf Science 88, 260–266 (2010).
38. de Mello Junior, M., Schwamborn, M. R., Neumann-Leitão, S. & Paranaguà, M. N. Abundance and instantaneous transport of Petrolisthes armatus (Gibbes, 1850) planktonic larvae in the Catatumbo inlet, northeast Brazil. Anais da Academia Brasileira de Ciência 84, 95–102 (2012).
39. de Mello Junior, M., Melo, M. P., Paranagua, M. N., Neumann-Leitão, S. & Schwamborn, R. Composition of decapod larvae in a northeastern Brazilian estuarine inlet over a full tidal cycle. Latin American Journal of Aquatic Research 44, 401–410, doi:10.1590/s0716-99502016000500021 (2016).
40. Posada, D. & Crandall, K. A. Intraspecific gene genealogies: trees grafting into networks. Trends. Ecol. Evol. 16, 37–45 (2001).
41. Wright, S. Evolution in mendelian populations. Genetics 16, 97–159 (1931).
42. Haegewa, M., Kishimoto, H. & Yano, T. Dating of the human-ape splitting by a molecular clock of mitochondrial DNA. J. Mol. Evol. 22, 160–174 (1985).
43. McCartney, M. A., Keller, G. & Lessios, H. A. Dispersal barriers in tropical oceans and speciation of Atlantic and eastern Pacific Echinometra sea urchins. Molecular Ecology 9, 1391–1400 (2000).
44. Chaves-Fonnegra, A., Feldheim, K. A., Secord, J. & Lopez, J. V. Population structure and dispersal of the coral-excavating sponge Cliona delitrix. Molecular Ecology 24, 1447–1466, doi:10.1111/mec.13134 (2015).
45. Reitzel, A. M., Darlington, J. A., Sullivan, J. R. Global population genetic structure of the starlet anemone Nematostella vectensis: multiple introductions and implications for conservation policy. Biological Invasions 10, 1197–1213, doi:10.1007/s10530-007-9196-8 (2008).
46. Andras, J. P., Rypien, K. L. & Harvell, C. D. Range-wide population genetic structure of the Caribbean sea fan coral. Gorgonia ventilata. Molecular Ecology 22, 56–73, doi:10.1111/mec.12104 (2013).
47. Zardus, J. D. & Hadfield, M. G. Multiple origins and incursions of the Atlantic barnacle Chthamalus proteus in the Pacific. Molecular Ecology 14, 3719–3733, doi:10.1111/j.1365-294X.2005.02701.x (2005).
48. Shulman, M. J. & Bermingham, E. Early life history, ocean currents, and the population genetics of Caribbean reef fishes. Evolution 49, 897–910 (1995).
49. Lessios, H. A., Robertson, D. R. & Cubit, J. D. Spread of Polyonyx gibbesi Haig, 1956 (Crustacea: Decapoda) from the western Pacific (Decapoda, Anomura, Porcellanidae). Crustaceana 77, 257–264 (2004).
50. Richardson, P. L. Caribbean Current and eddies as observed by surface drifters. Deep-Sea Research Part II-Topical Studies in Oceanography 52, 429–463, doi:10.1016/j.dsr2.2004.11.001 (2005).
51. Roberts, C. M. Connectivity and management of Caribbean coral reefs. Science 278, 1454–1457 (1997).
52. Wüst, G. Stratification and circulation in the Antillean-Caribbean basins. Vol. 1 (Columbia University Press 1964).
53. O’Dea, A. et al. Formation of the Isthmus of Panama. Science Advances 2, ea150883 (2016).
54. Montes, C. et al. Middle Miocene closure of the Central American Seaway. Science 348, 226–229, doi:10.1126/science.aaa2815 (2015).
55. Bacon, C. D. et al. Biological evidence supports an early and complex emergence of the Isthmus of Panama. Proceedings of the National Academy of Sciences of the United States of America 112, 610–615 (2015).
56. Lessios, H. A. & Marko, E. Early and progressive migration across the Isthmus of Panama is robust to missing data and biases. Proceedings of the National Academy of Sciences 112, E5767–E5768 (2015).
57. Lessios, H. A. Appearance of an early closure of the Isthmus of Panama is the product of biased inclusion of data in the metaanalysis. Proceedings of the National Academy of Sciences 201514719 (2015).
58. Stillman, J. H. & Reeb, C. A. Molecular phylogeny of eastern Pacific porcelain crabs, genera Petrolithes and Pachycheles, based on the mtDNA 16S rDNA sequence: Phylogeographic and systematic implications. Mol. Phylogenet. Evol. 19, 236–245 (2001).
59. Lessios, H. A. The Great American Schism: Divergence of marine organisms after the rise of the Central American Isthmus. Annual Review of Ecology Evolution and Systematics 39, 63–91 (2008).
60. Scheltema, R. S. On dispersal and planktonic larval of benthic invertebrates: an eclectic overview and summary of problems. Bull. Mar. Sci. 39, 290–322 (1986).
61. Lessios, H. A. et al. Phylogeography and benthic evolution in Arbacia, a sea urchin genus with an unusual distribution. Molecular Ecology 21, 130–144, doi:10.1111/j.1365-294X.2011.05303.x (2012).
62. Lessios, H. A., Kessing, B. D. & Pearse, J. S. Population structure and speciation in tropical seas: global phylogeography of the sea urchin Diadema. Evolution 55, 955–975 (2001).
63. Bowen, B. W., Bass, A. L., Muss, A., Carlin, J. & Robertson, D. R. Phylogeography of two Atlantic squirrelfishes (Family Holocentridae): exploring links between pelagic larval duration and population connectivity. Marine Biology 149, 899–913 (2006).
64. Schultz, J. K. et al. Global phylogeography and seasecape genetics of the lemon sharks (genus Negaprion). Molecular Ecology 17, 5336–5348 (2008).
65. Rocha, L. A. et al. Recent invasion of the tropical Atlantic by an Indo-Pacific coral reef fish. Molecular Ecology 14, 3921–3928 (2005).
66. Muss, A., Robertson, D. R., Stepień, C. A., Wietz, P. & Bowen, B. W. Phylogeography of Ophioleucus: the role of ocean currents and geography in reef fish evolution. Evolution 55, 561–572 (2001).
67. Molinari, R. L. Observations of eastward currents in the tropical South Atlantic Ocean: 1978–1980. Journal of Geophysical Research: Oceans 87, 9707–9714 (1982).
68. Lumpkin, R. & Garzoli, S. L. Near-surface circulation in the tropical Atlantic Ocean. Deep-Sea Research Part I-Oceanographic Research Papers 52, 495–518, doi:10.1016/j.dsr.2004.09.001 (2005).

69. Richards, V. P., DeBiasse, M. B. & Shivji, M. S. Genetic evidence supports larval retention in the Western Caribbean for an invertebrate with high dispersal capability (Ophiothrix suensonii: Echinodermata, Ophiuroidea). Coral Reefs 34, 313–325, doi:10.1007/s00338-014-1257-z (2015).

70. Paris, C. B., Cherubin, I. M. & Cowen, R. K. Surfing, spinning, or diving from reef to reef: effects on population connectivity. Marine Ecology Progress Series 347, 285–300, doi:10.3354/meps089685 (2007).

71. Sheng, J. Y. & Tang, L. Q. A numerical study of circulation in the western Caribbean Sea. Journal of Physical Oceanography 33, 2049–2069, doi:10.1175/1520-0485(2003)033<...1049:asoci:2.0.co;2 (2003).

72. Jackson, A. M. et al. Population structure and phylogeography in Nassau Grouper (Epinephelus striatus), a mass-aggregating marine fish. Plos One 9, doi:10.1371/journal.pone.0097508 (2014).

73. Avise, J. C. Molecular population structure and the biogeographic history of a regional fauna: a case history with lessons for conservation biology. Oikos 63, 62–76 (1992).

74. Collin, R. The effects of mode of development on phylogeography and population structure of North Atlantic Crepidula (Gastropoda: Calyptraeidae). Molecular Ecology 10, 2249–2262 (2001).

75. Lee, T. & O'Foighil, D. Placing the Floridian marine genetic disjunction into a regional evolutionary context using the scorched mussel, Brachidontes exactus, species complex. Evolution 59, 2139–2158 (2005).

76. Gold, I. R. & Richardson, L. R. Population structure in greater amberjack, Seriola dumerili, from the Gulf of Mexico and the western Atlantic ocean. Fish. Bull. 96, 767–778 (1998).

77. Pelc, R. A., Warner, R. R. & Gaines, S. D. Geographical patterns of genetic structure in marine species with contrasting life histories. J. Biogeogr 36, 1881–1890 (2009).

78. Lessios, H. A., Kane, J. & Robertson, D. R. Phylogeography of the pantropical sea urchin Tripneustes: contrasting patterns of population structure between oceans. Evolution 57, 2026–2036 (2003).

79. Trovant, B. et al. Scorched mussels (Brachidontes spp., Bivalvia: Mytilidae) from the tropical and warm-temperate southwestern Atlantic: the role of the Amazon River in their speciation. Ecology and Evolution 6, 1778–1798, doi:10.1002/ece3.2016 (2016).

80. Rocha, L. A., Bass, A. L., Robertson, D. R. & Bowen, B. W. Adult habitat preferences, larval dispersal, and the comparative phylogeography of three Atlantic surgeonfishes (Teleostei: Acanthuridae). Molecular Ecology 11, 243–252 (2002).

81. Lessios, H. A. & Baums, I. B. In Coral Reefs of the Eastern Pacific (eds Glynn, P. W., Enos, I. C. & Manzello, D.) Ch. 16, (Springer-Verlag 2016).

82. Devis-Morales, A., Schneider, W., Montoya-Sánchez, R. A. & Rodríguez-Rubio, E. Monsoon-like winds reverse oceanic circulation in the Panama Bight. Geophysical Research Letters 35 (2008).

83. Rodriguez-Rubio, E., Schneider, W. & del Rio, R. A. On the seasonal circulation within the Panama Bight derived from satellite observations of wind, altimetry and sea surface temperature. Geophysical Research Letters 30 (2003).

84. Forshbergh, E. D. On the climatology, oceanography and fisheries of the Panama Bight. Inter-American Tropical Tuna Commission Bulletin 14, 46–385 (1969).

85. Roman, J. & Darling, J. A. Paradox lost: genetic diversity and the success of aquatic invasions. Trends in Ecology & Evolution 22, 454–464, doi:10.1016/j.tree.2007.07.002 (2007).

86. Lockwood, J. L., Cassey, P. & Blackburn, T. The role of propagule pressure in explaining species invasions. Trend. Ecol. Evol. 20, 223–228 (2005).

87. Kolbe, J. J. et al. Genetic variation increases during biological invasion by a Cuban lizard. Nature 431, 177–181, doi:10.1038/nature02807 (2004).

88. Carlton, J. T., Newman, W. A. & Bettini-Pitombo, F. B. In the Wrong Place-Alien Marine Crustaceans: Distribution, Biology and Impacts Vol. 6 Invading Nature, Springer Series in Invasion Ecology (eds Galil, B. S., Clark, P. F. & Carlton, J. T.) 159–213 (Springer 2011).

89. Roman, J. Diluting the founder effect: cryptic invasions expand a marine invader's range. Proceedings of the Royal Society B-Biological Sciences 273, 2453–2459 (2006).

90. Rock, D. G., Zhan, A., Lejeune, C., MacIsaac, H. J. & Cristescu, M. E. Looking at both sides of the invasion: patterns of colonization in the violet tunicate Botryllus violaceus. Molecular Ecology 20, 503–516, doi:10.1111/j.1365-294X.2010.04971.x (2011).

91. Hellebone, A. L. & Hay, M. E. Propagule pressure of an invasive crab overwhelm native biotic resistance. Marine Ecology Progress Series 342, 191–196, doi:10.3354/meps342191 (2007).

92. Boudreaux, M. L., Stiner, J. L. & Walters, L. J. Biodiversity of sessile and motile macrofauna on intertidal oyster reefs in Mosquito Lagoon, Florida. Journal of Shellfish Research 25, 1079–1089 (2006).

93. Glancy, T. P., Frazer, T. K., Cichra, C. E. & Lindberg, W. J. Comparative patterns of occupancy by decapod crustaceans in seagrass, oyster, and marsh-edge habitats in a Northeast Gulf of Mexico estuary. Estuaries 26, 1291–1301, doi:10.1002/eat2.02836 (2003).

94. Lambrinos, J. G. How interactions between ecology and evolution influence contemporary invasion dynamics. Ecology 85, 2061–2070, doi:10.1890/03-0313 (2004).

95. Bridle, J. R. & Vines, T. H. Limits to evolution at range margins: when and why does adaptation fail? Trends in Ecology & Evolution 22, 140–147 (2007).

96. Palumbi, S. In Molecular Systematics (eds Hillis, D. M., Moritz, C. & Mable, B. K.) 205–247 (Sinauer Associates 1996).

97. Folmer, O., Black, M., Hoeh, W., Lutz, R. & Vrijenhoek, R. DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. Molecular Marine Biology and Biotechnology 3, 294 (1994).

98. Hall, T. A. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucl. Acids. Symp. Ser. 41, 95–98 (1999).

99. Akahe, H. A new look at the statistical model identification. IEEE Trans. Autom. Contr. 19, 716–723 (1974).

100. Darriba, D., Taboada, G. L., Doallo, R. & Posada, D. MrBayes 3.2: efficient Bayesian phylogenetic inference and model choice across a large model space. Systematic Biology 61, 539–542 (2012).

101. Drummond, A. J., Suchard, M. A., Xie, D. & Rambaut, A. Bayesian Phylogenetics with BEAUti and the BEAST 1.7. Molecular Biology and Evolution 29, 1969–1973 (2012).

102. Ronquist, F. MrBayes 3.2: Efficient Bayesian phylogenetic inference and model choice across a large model space. Systematic Biology 61, 539–542 (2012).

103. Bandelt, H. J., Forster, P. & Rohrl, A. Median-joining networks for inferring intraspecific phylogenies. Molecular Biology and Evolution 16, 37–48 (1999).

104. Nei, M. Molecular evolutionary genetics. (Columbia University Press 1987).

105. Excoffier, L. & Lischer, H. E. L. Arlequin suite ver 3.5: A new series of programs to perform population genetics analyses under Linux and Windows. Molecular Ecology Resources 10, 564–567 (2010).
109. Tamura, K. & Nei, M. Estimation of the number of nucleotide substitutions in the control region of mitochondrial DNA in humans and chimpanzees. *Molecular Biology and Evolution* **10**, 512–526 (1993).

110. Excoffier, L., Smouse, P. E. & Quattro, J. M. Analysis of molecular variance inferred from metric distances among DNA haplotypes: Application to human mitochondrial DNA restriction data. *Genetics* **131**, 479–491 (1992).

111. Hey, J. & Nielsen, R. Multilocus methods for estimating population sizes, migration rates and divergence time, with applications to the divergence of *Drosophila pseudoobscura* and *D. persimilis*. *Genetics* **167**, 747–760 (2004).

112. Mantel, N. The detection of disease clustering and the generalized regression approach. *Cancer Res.* **27**, 209–220 (1967).

113. Jensen, J. L., Bohonak, A. J. & Kelley, S. T. Isolation by distance, web service. *BMC Genetics* **6**: 13. v.3.15 [http://ibdws.sdsu.edu](http://ibdws.sdsu.edu) (2005).

**Acknowledgements**

We are indebted to B. Werding (Justus-Liebig Universität Giessen), G. Paulay, A. Bemis and J. Slapcinsky (Florida Museum of Natural History), N. Voss (Rosenstiel School of Marine and Atmospheric Science), R. Lemaitre, K. Reed and G. Keel (National Museum of Natural History), F. Sanford (Coe College), D. Knott (College of Charleston), W. Sterrer (Bermuda Natural History Museum), M. Tavares (Museu de Zoologia da Universidade de São Paulo), I. Miranda (Universität Regensburg) and P. Wirtz for providing specimens, to L. Geyer, L. Rivera and A. Calderón (STRI) for their support in the lab, and to J. Wares (University of Georgia) for providing information on the individuals sampled at Georgia. B. Werding critically read the manuscript.

**Author Contributions**

A.H. and H.A.L. designed this study, collected and analysed data, and wrote the manuscript.

**Additional Information**

**Competing Interests:** The authors declare that they have no competing interests.

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