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Molecular evidence for sequential colonization and taxon cycling in freshwater decapod shrimps on a Caribbean island

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Abstract

Taxon cycling, i.e. sequential phases of expansions and contractions in species' distributions associated with ecological or morphological shifts, are postulated to characterize dynamic biogeographic histories in various island faunas. The Caribbean freshwater shrimp assemblage is mostly widespread and sympatric throughout the region, although one species (Atyidae: *Atya lanipes*) is geographically restricted and ecologically and morphologically differentiated from other *Atya* species. Using patterns of nucleotide variation at the COI mtDNA gene in five species of freshwater shrimp (*A. lanipes*, *A. scabra*, *A. innocuous*; Xiphocarididae: *Xiphocaris elongata*; Palaemonidae: *Macrobrachium faustinum*) from Puerto Rico, we expected to detect a signature of sequential colonization in these shrimp, consistent with the concept of taxon cycling, and expected that *A. lanipes* would be at a different taxon stage (i.e. an early stage species) to all other species. We also examined patterns of genetic population structure in each species expected with poor, intermediate and well-developed abilities for among-river dispersal. Population expansions were detected in all species, although the relative timing of the expansions varied among them. Assuming that population expansions followed colonization of Puerto Rico by freshwater shrimp, results bear the hallmarks of sequential colonization and taxon cycling in this fauna. *A. lanipes* had a star phylogeny, low mean pairwise nucleotide differences and recent (Holocene) estimates for an *in situ* population expansion in Puerto Rico, and it was inferred as an early stage species in the taxon cycle undergoing a secondary phase of expansion. All other species were inferred as late stage species undergoing regional population expansions, as their mean pairwise nucleotide differences were relatively high and phylogenetic patterns were more complex than *A. lanipes*. High rates of gene flow without isolation by distance among rivers were detected in all species, although results should be treated cautiously as some populations are unlikely to be in mutation–drift equilibrium. Nested clade analysis produced inconsistent results among species that all have high rates of gene flow and expanding populations.

Keywords: amphidromy, nested clade analysis, population expansion, Puerto Rico

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Introduction

Oceanic islands have served as templates for studying biogeographic phenomena, such as area- and isolation-diversity relationships, and strategies for colonization and biotic exchange (MacArthur & Wilson 1967). The concepts of taxon cycling and taxon pulses are central in island biogeography (Darlington 1943; Wilson 1959, 1961; Erwin 1981; Liebherr & Hajek 1990), although they are not universally accepted processes (e.g. Pregill & Olsen 1981). The former proposes that taxa undergo sequential (although not unidirectional) phases of expansion and contraction, whether in distribution or in ecological relationships (Ricklefs & Bermingham 2002), whereas the latter is a unidirectional model of distributional or ecological

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shift that facilitates speciation (Liebherr & Hajek 1990). Taxon cycles and taxon pulses are thus mechanisms of diversification over ecological and evolutionary timescales, respectively (Liebherr & Hajek 1990). Traditional approaches used to infer taxon cycles involved establishing relationships between distributional patterns and phenotypic or ecological differentiation (Wilson 1959, 1961). Thus, in the presence of some degree of phenotypic and/or ecological differentiation, an expanding species (i.e. a 'late stage' species in the taxon cycle) would have a widespread and continuous distribution, whilst a contracting species (i.e. an 'early stage' species) would have a restricted or fragmented distribution (Ricklefs & Bermingham 2002). Contracting species (i.e. early stage species) often undergo ecological shifts into marginal habitats affected by competition from colonizing late stage species and may initiate secondary phases of expansion after adapting to new habitat types or experiencing ecological release from predators or disease (Wilson 1961; Erwin 1981; Ricklefs & Bermingham 2002).

As taxon cycles are historical processes, the phylogenetic information contained in mitochondrial DNA (mtDNA) sequences makes molecular phylogeography an additional and highly informative approach to studying taxon cycles in island biota (Ricklefs & Bermingham 1999; Emerson 2002). Comparative phylogeographic studies have often focused on among-island patterns to infer taxon cycles (e.g. Seutin *et al*. 1994; Lovette *et al*. 1998). However, the characteristic signatures left by colonization and population expansion events in contemporary patterns of genetic variation (Templeton *et al*. 1995; Gübitz *et al*. 2005) mean that within-island phylogeographic patterns can be used to elucidate differences in the timing of population expansion among codistributed taxa and to develop putative scenarios for the chronology of colonization and temporal changes in assemblage structure (Brown & Pestano 1998; Vandergast *et al*. 2004; Gübitz *et al*. 2005). For example, star-like phylogenetic patterns, low pairwise nucleotide differences in DNA sequence data and molecular clock estimates for recent expansions would be indicative of a recent and perhaps localized expansion. This genetic pattern could reflect an early stage species that is undergoing a secondary expansion if it is ecologically divergent and has a restricted or fragmented distribution. In contrast, complex phylogenetic patterns, larger pairwise nucleotide differences in DNA sequence data and molecular clock estimates for older population expansions likely reflect older and/or multiple expansions from multiple places, characteristic of late stage species. These phylogeographic expectations for expanding or recently expanding populations provide testable hypotheses that can be reconciled with distributional and ecological data to explore prospects for taxon cycling in island biota.

The freshwater fauna of the Caribbean archipelago is dominated by migratory species of caridean shrimp (e.g. genera *Atya*, *Xiphocaris*, *Macrobrachium*). These species are all amphidromous, meaning that their larvae are released into fresh water, passively drift to estuarine or marine habitats and migrate upstream as postlarvae to headwater adult habitats (McDowall 2007). However, the extent to which amphidromy facilitates dispersal among rivers within an island (or at other spatial scales, e.g. among islands) and the length of time larvae remain in marine littoral areas is unknown (Holmquist *et al*. 1998). Despite this, the dependency of these shrimp on marine habitats as larvae has facilitated mostly widespread and sympatric distributions throughout the Caribbean archipelago, with the exception of *Atya lanipes* which is restricted to the Greater Antilles and Virgin Islands (Hobbs & Hart 1982; Fièvet 1998). Furthermore, different genera of these shrimps occupy semidiscrete ecological niches (i.e. *Atya,* filter feeders and scrapers; *Xiphocaris*, shredders; *Macrobrachium*, predatory and omnivorous; Crowl *et al*. 2001) and within the genus *Atya*, different species have distinct habitat preferences (e.g. *A. lanipes*, slow flowing (pool) habitats; *A. scabra*, fast-flowing (riffle) habitats; Chace & Hobbs 1969). Interestingly, the filter-feeding niche of *Atya* is most effective in riffle (fast-flowing) habitats (Fryer 1977), suggesting that slow-flowing pools inhabited by *A. lanipes* may be marginal habitat. Furthermore, *A. lanipes* is the most morphologically distinct *Atya* (Hobbs & Hart 1982) and it is not a recently derived taxon in the molecular phylogeny of the genus (Page *et al*. in press).

The varying patterns of distribution in these shrimp, their well-developed abilities for range expansion over evolutionary timescales and various levels of ecological differentiation lead us to hypothesize that taxon cycling characterizes their biogeographic history. Using cytochrome *c* oxidase I (COI) mtDNA variation in five species of amphidromous shrimp (Atyidae: *Atya lanipes*, *A. scabra*, *A. innocuous*; Xiphocarididae: *Xiphocaris elongata*; Palaemonidae: *Macrobrachium faustinum*) from Puerto Rico, we tested this hypothesis and expected to detect a distinct chronology in timing of population expansions in each species. In particular, we expected that *A. lanipes* would bear the molecular signatures of an early stage species, perhaps undergoing a secondary expansion, and that the other species would likely have molecular signatures expected for late stage species in the taxon cycle. Finally, as some Puerto Rican populations of caridean shrimp have declined or been locally extirpated in response to anthropogenic disturbance (e.g. dam construction, water abstraction and harvest-related poisoning) (Benstead *et al*. 1999; Greathouse *et al*. 2005), and they are known to have important functions in Caribbean island stream ecology (Pringle *et al*. 1993, 1999; Crowl *et al*. 2001), knowledge of within-island (among-river) dispersal in these shrimp may be important for river management (Holmquist *et al*. 1998). We predicted that if these shrimps could disperse effectively through marine

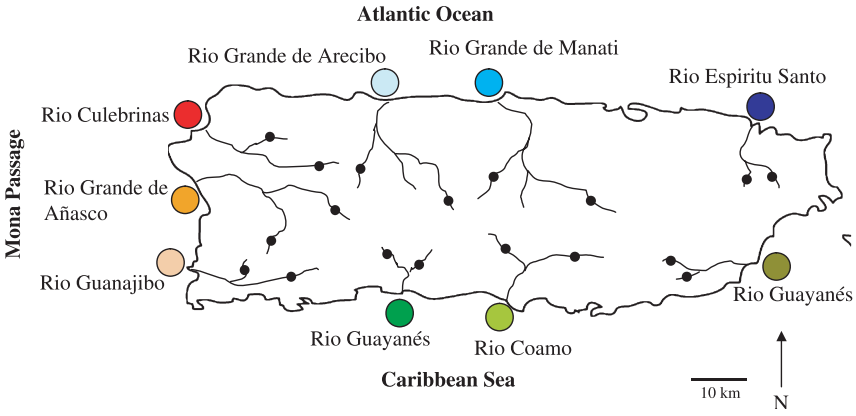

Fig. 1 Map of Puerto Rico showing the sites (black dots), rivers and regions sampled. Each river is colour coded and these colours correspond to those used in the haplotype networks (Fig. 2). Similarly, each region is colour coded: Atlantic Ocean, blues; Mona Passage, reds and oranges; Caribbean Sea, greens.

Table 1 Sample and fragment information and measures of molecular diversity

Species	*N*	*n*	bp	*h*	π	*k*	# hap	GenBank accession numbers
Atya lanipes	185	8	773	0.352 ± 0.003	0.0005 ± 0.0005	0.421 ± 0.385	32	EU005053–EU005083
A. scabra	225	9	596	0.986 ± 0.004	0.013 ± 0.007	7.714 ± 3.608	129	EU005084–EU005224
A. innocous	48	4	777	0.964 ± 0.012	0.010 ± 0.005	7.850 ± 3.717	31	EU005036–EU005052
Xiphocaris elongata	66	5	768	0.997 ± 0.003	0.011 ± 0.006	8.109 ± 3.811	59	EU004940–EU005000
Macrobrachium faustinum	71	7	708	0.731 ± 0.060	0.002 ± 0.001	1.356 ± 0.853	34	EU005001–EU005035

N, total number of individuals; *n*, number of sample sites (rivers); bp, number of COI bases used in analyses; *h*, haplotype diversity; π, nucleotide diversity; *k*, average number of pairwise differences; # hap, number of unique haplotypes.

habitats (at the island scale), we would detect no genetic substructure among populations from different rivers or marine regions in Puerto Rico and reveal no signatures of isolation by distance (IBD) in the genetic data. In contrast, if these shrimp had poor or intermediate abilities to disperse via marine habitats, we would find significant genetic subdivision among rivers or among marine regions, respectively, and detect significant patterns of IBD in the genetic data.

Methods

Sampling design and laboratory techniques

A hierarchical design was implemented to test the above predictions: three regions (i.e. Atlantic Ocean, Mona Passage, Caribbean Sea), each with three rivers (Fig. 1). Sample sizes are shown in Table 1. Genomic DNA was extracted from each individual using a standard phenol-chloroform procedure, and a fragment of the COI mtDNA gene was amplified via polymerase chain reaction (PCR) using specifically developed primers: CR-COI-F, CWACM AAYCATAAGAYATTGG; CR-COI-R, GCRGANGTRAARTA RGCTCG. PCR reactions contained approximately 40 ng of template DNA, 0.4 μM of each primer, 0.2 mM dNTP (Astral Scentific), 2 mM $MgCl_2$, 1.25 μL of 10x polymerase reaction buffer and 0.25 unit of *Taq* polymerase (Fisher Biotech), adjusted to a final volume of 12.5 μL with ddH_2O. The thermal-cycling profile followed: 5 min at 94 °C; 35 cycles of 30 s at 94 °C, 30 s at 55 °C and 30 s at 72 °C; an additional extension phase of 5 min at 72 °C; and a final hold stage at 4 °C. PCR product was purified with the exonucleoase I-shrimp alkaline phosphatase method, using 2.5 μL PCR product, 2.0 μL shrimp alkaline phosphatase (Promega) and 0.5 μL exonucleoase I (Fermenta), and a two-step thermal-cycling profile: 35 min at 37 °C, 20 min at 80 °C. Sequencing reactions contained 0.5 μL purified product, 0.32 μL forward primer, 2 μL BigDye v1.1 (Applied Biosystems) and 2 μL 5x sequencing buffer (Applied Biosystems), and the following thermal cycling conditions were used: 1 minute at 96 °C, 30 cycles of 10 s at 96 °C, 5 s at 50 °C, 4 min at 60 °C and a hold period of 4 °C. Sequencing was conducted on a 3130xl Capillary Electrophoresis Genetic Analyser (Applied Biosystems) and sequences were aligned and edited using SEQUENCHER version 4.1.2 (Gene Codes). An exemplar of each haplotype was sequenced in the reverse direction to verify bases at polymorphic sites.

Data analysis

Haplotype (*h*) and nucleotide (π) diversity and mean pairwise nucleotide differences (*k*) were calculated for each species

Table 2 Population demographic parameters. *P* values are in parenthesis and significant values are in bold

Species	D	Fs	R_2	τ	θ_0	θ_1
Atya lanipes	**–2.683 (<0.001)**	**–65.712 (<0.001)**	**0.012 (<0.001)**	0.714	0.000	0.771
A. scabra	**–2.005 (<0.001)**	**–201.469 (<0.001)**	**0.032 (0.003)**	2.130	7.059	4026.250
A. innocous	–0.811 (0.222)	**–11.990 (0.005)**	0.081 (0.227)	8.936	0.000	87.930
Xiphocaris elongata	**–2.139 (0.003)**	**–73.964 (<0.001)**	**0.036 (<0.001)**	8.502	0.003	175.938
Macrobrachium faustinum	**–2.487 (<0.001)**	**–45.119 (<0.001)**	**0.021 (<0.001)**	1.571	0.000	7.245

D, Tajima's (1989) D; Fs, Fu's (1997) Fs; R_2, Ramos-Onsins & Rozas' (2002) R_2; τ, tau, which is an index of time since the expansion expressed in units of mutational time; θ_0 & θ_1, pre- and post-expansion values for the mutation parameter (i.e. 2 Nμ, where N is the effective female population size and μ is the mutation rate per gene per generation).

in ARLEQUIN (Schneider *et al*. 2000) to obtain measures of molecular diversity in each species. The parameters D (Tajima 1989), Fs (Fu 1997) and R_2 (Ramos-Onsins & Rozas 2002) were calculated in DNASP (Rozas *et al*. 2003) to examine signatures of population expansions in the mtDNA data. Their significance was assessed using 10 000 coalescent simulations, given the observed number of segregating sites. Estimates for tau(τ) (Rogers & Harpending 1992), including lower- and upper- bound estimates, were also calculated for each species in ARLEQUIN and used to determine the number of generations since the last population expansion, using the formula of Rogers & Harpending (1992) and assuming a sequence evolution rate of 1.4% per Myr (Knowlton & Weigt 1998). Fragment lengths for each species are presented in Table 1, and a generation time of two years was assumed for each species as their growth rates are slow in comparison with caridean shrimp in other parts of the world (cf. Yam & Dudgeon 2005) and protandry is reported in some species of *Atya* (Carpenter 1978). AMOVA (Excoffier *et al*. 1992) was then implemented using ARLEQUIN to examine spatial mtDNA variation in each species according to the hierarchal design, and the relationship between mtDNA divergence among populations and geographical distance was tested using Mantel tests (Mantel 1967) in PRIMER version 5.2.8 (Clarke & Gorley 2001). Finally, haplotype networks were constructed using TCS version 1.18 (Clement *et al*. 2000) and cladistic analysis of the nested haplotypes (NCA, Templeton *et al*. 1995) was implemented using GEODIS version 2.4 (Posada *et al*. 2000). The November 2005 inference key was used in NCA (available at: Darwin.uvigo.ed/download/geodisKey_11Nov05.pdf).

Results

Genetic diversity and mean pairwise nucleotide differences were much lower in *Atya lanipes* than all other species (Table 1). Tajima's D and Fu's Fs were negative in all species (Table 2), with Fs being significant for all taxa and D being significant for all except *A. innocuous*. R_2 indicated significant signatures of population expansions in all amphidromous shrimp except *A. innocuous*. The number of generations that elapsed since the last population expansion differs substantially among some of the taxa, although the variance around these estimates was large (Fig. 2). The relative timing of population expansions for various species-pair combinations were nonoverlapping (Fig. 2), although they were indistinguishable among some of the species (i.e. no differences between *A. lanipes*, *A. scabra* and *Macrobrachium faustinum*). Most noteworthy are the nonoverlapping estimates for *A. lanipes* and *A. innocuous*, as they are congeners—*A. innoucus* had an upper bound limit for a population expansion that coincided with the late Pleistocene (approximately 50 000 years BP), whilst *A. lanipes* had an upper bound limit for a late Holocene population expansion (approximately 10 000 years BP; Fig. 2). AMOVA revealed that genetic variation was not significantly partitioned either among regions or among rivers within regions for the amphidromous shrimp (Φ_{CT}, Φ_{SC} and Φ_{ST} were nonsignificant for all taxa), with the exception of *M. faustinum*, which had significant genetic differentiation among rivers within regions (Φ_{SC} = 0.040, *P* = 0.027).

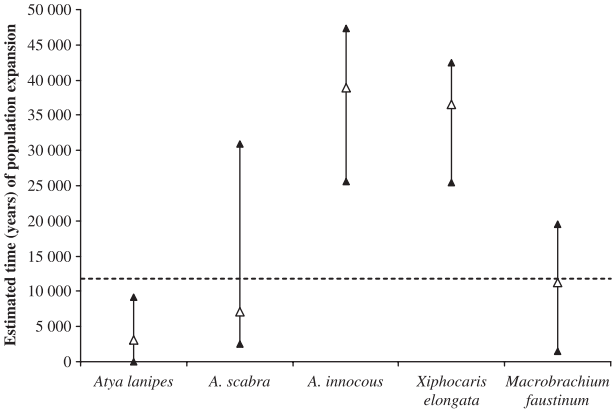

Fig. 2 Estimates for time (in years before present) since last population expansion in each taxon at the 0.05 confidence level, showing estimated year of expansion plus lower and upper bounds. The dotted line demarks the transition from the Pleistocene (above line) to the Holocene (below line).

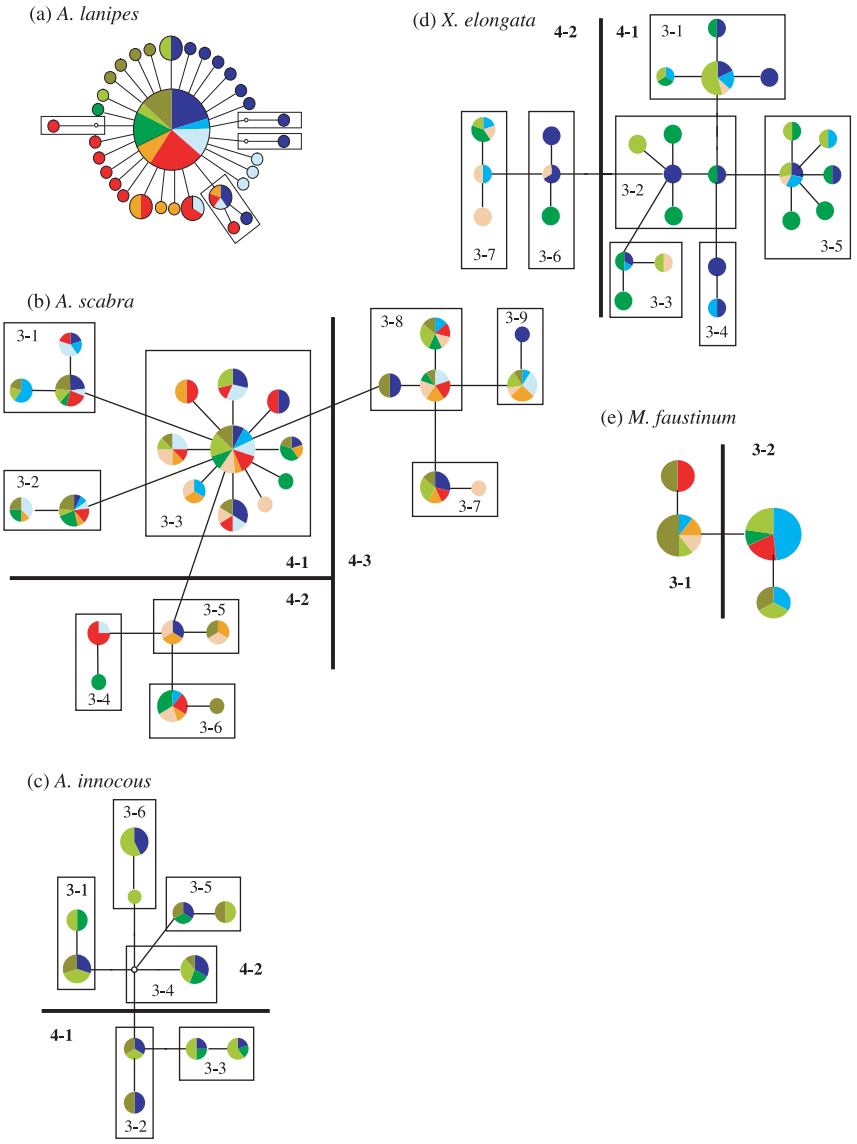

Fig. 3 Haplotype networks showing nesting levels used for NCA for (a) *Atya lanipes*, (b) *A. scabra*, (c) *A. innocuous*, (d) *Xiphocaris elongata*, and (e) *Macrobrachium faustinum*. Two-step and higher clades only are shown for b–e.

Pairwise analyses of Φ_{ST} among rivers for the amphidromous shrimp found no significant differences after the Bonferroni correction for multiple tests was applied (data not shown). There were no significant patterns of IBD in any of the amphidromous shrimp either for shortest coastline distance among river mouths (*A. lanipes*, $\rho = 0.298$, $P = 0.096$; *A. scabra*, $\rho = 0.072$, $P = 0.335$; *A. innocuous*, $\rho = 0.500$, $P = 0.498$; *Xiphocaris elongata*, $\rho = -0.943$, $P = 1.000$; *M. faustinum*, $\rho = 0.073$, $P = 0.409$) or for straight line geographical distance among river mouths (*A. lanipes*, $\rho = -0.049$, $P = 0.597$; *A. scabra*, $\rho = 0.076$, $P = 0.291$; *A. innocous*, $\rho = -0.500$, $P = 0.834$; *X. elongata*, $\rho = 0.464$, $P = 0.206$; *M. faustinum*, $\rho = 0.497$, $P = 0.600$). The genealogical patterns revealed in Puerto Rican freshwater shrimp varied considerably among species, including among congeners of *Atya* (Fig. 3). NCA revealed contiguous range expansion in some taxa and restricted gene flow with IBD in other species (Table 3).

Discussion

Sequential colonization and taxon cycling

We expected to detect a distinct chronology in the timing of population expansions in amphidromous shrimp in Puerto Rico. Our results indicate that population expansions in *Atya innocuous* and *Xiphocaris elongata* preceded those in *Macrobrachium faustinum* and *A. lanipes*, suggesting that the freshwater shrimp assemblage structure has changed over the recent past, assuming that expansions were associated with a founder event. Differential colonization of islands is reported to facilitate the development of a temporally dynamic assemblage structure in birds, rodents and lizards (Roughgarden & Pacala 1989; Ricklefs & Bermingham 1999; MacFarlane & Lundberg 2002), and our data supports the idea that time is an important

Table 3 Inferred evolutionary processes from NCA*

Species	Clade level	Inference Chain	Inferred evolutionary process
Atya lanipes	Total cladogram	1-2-11-12: No	Contiguous range expansion
A. scabra	Clade 2-13	1-2-11-17-4: No	Restricted gene flow with IBD
	Clade 3-1	1-2-3-4: No	Restricted gene flow with IBD
	Clade 3-3	1-2-3-5-6: too few clades	Range expansion or restricted gene flow
	Clade 3-8	1-2-3-4: No	Restricted gene flow with IBD
Xiphocaris elongata	Clade 4-2	1-2-3-4: No	Restricted gene flow with IBD
Macrobrachium faustinum	Clade 1-9	1-2-11-17:No	Inconclusive
	Clade 3-1	1-2-11-12: No	Contiguous range expansion
	Total cladogram	1-2-11-12: No	Contiguous range expansion

*NCA revealed no significant associations between geography and genealogy in *A. innocuous*.

predictor of species richness on islands (Parent & Crespi 2006). The chronology of colonization in Puerto Rican amphidromous shrimp, coupled with demographically growing populations, also supports the concept of taxon cycling and that island biotic assemblages and population sizes are changing in response to ecological and/or evolutionary drivers.

In attempting to characterize the taxon cycle for Puerto Rican shrimp, we predicted that *A. lanipes* would be an early stage species on account of its restricted distribution and morphological and ecological distinctiveness (Fryer 1977; Hobbs & Hart 1982). This prediction was supported by the genetic data, as the haplotype network reflected a star phylogeny, mean pairwise nucleotide diversity was low, molecular clock estimates for the expansion were recent and NCA revealed contiguous, and most likely *in situ*, range expansion. On their own, these genetic patterns could also have suggested that *A. lanipes* is a late stage taxon at the very start of an expansion phase. However, the known distinctiveness of *A. lanipes* in terms of ecology, morphology and phylogenetic relationships with other *Atya* suggest that it is not a recently derived taxon; thus, a secondary expansion phase is a more likely interpretation. Furthermore, all Caribbean amphidromous shrimp are described as 'littoral' species on the basis of their well-developed abilities for dispersal and range expansion (Fièvet 1998), suggesting that distributional differences between *A. lanipes* and other shrimp are due to taxon cycling stages rather than differences in intrinsic dispersal abilities. Multiple colonization events among islands are shown to produce stronger founder effects (i.e. genetic impoverishment) than a single colonization from the original (mainland) population (Clegg *et al*. 2002). Very low levels of genetic diversity in *A. lanipes* suggest that its recent colonization of Puerto Rico may be due to sequential colonization events among islands, and that colonization of Puerto Rico by *A. lanipes* was affected by dispersal from an already genetically impoverished population undergoing a secondary expansion. This idea would need formal examination by a broader phylogeographic study throughout the Greater Antilles and Virgin Islands.

Our prediction that *A. lanipes* is an early stage species in the taxon cycle is further supported by genetic data for the other shrimp species, which suggest that they are late stage species (i.e. more complex haplotype networks with higher mean pairwise nucleotide diversity). However, as there was a chronology of colonization events, even among these late stage species (cf. *A. innocuous* & *X. elongata* vs. *M. faustinum*), results also indicate a continuum between early stage and late stage species in taxon cycles. The complex haplotype networks of the widely distributed shrimp (i.e. *A. scabra*, *A. innocuous*, *X. elongata* and *M. faustinum*), indicated that each species had several divergent clades, with each clade containing several to many closely related haplotypes. This suggests the possibility for multiple expansions, most of which likely occurred outside of Puerto Rico. These species are thus likely to be experiencing regional population growth. NCA indicated continuous range expansion in *M. faustinum*, which is consistent with an expansion phase in the taxon cycle, whilst NCA indicated restricted gene flow with IBD for *A. scabra* and *X. elongata*. This result is not expected for expanding populations and contrasts with results of the Mantel tests and population demographic analyses, as IBD usually reflects limited dispersal in a population at gene flow–drift equilibrium (Slatkin 1985). Other studies have shown that NCA can infer restricted gene flow with IBD under nonequilibrium conditions (Alexandrino *et al*. 2002; Kotl'k & Berrebi 2007), and our study indicates inconsistent results for NCA among species that all have high rates of gene flow and expanding populations in drift–mutation disequilibrium.

Another interesting result is the distinct period of habitation of Puerto Rico by the congeners *A. lanipes* and *A. innocuous*, indicating a period of over 15 000 years of allopatry on the island. Most species are thought to arise in allopatry (Templeton 1980), and historical allopatry is reported to facilitate diversification in other caridean shrimp, with subsequent dispersal accounting for present-day

sympatric distributions (Cook *et al*. 2006). The concept of taxon pulses is similar to taxon cycles, although rather than reflecting distributional or ecological shifts over ecological time, taxon pulses result in speciation over evolutionary timescales (Liebherr & Hajek 1990). The molecular evidence for recent (i.e. late Pleistocene–Holocene) taxon cycles in Caribbean amphidromous shrimp may provide clues about longer-term processes of distributional change, periods of allopatry and diversification via taxon pulses in these shrimp further back in time (cf. Miocene–Pliocene origin of most Caribbean atyid species; Page *et al*., in press). Various marine biogeographical breaks are reported throughout the Caribbean, including Mona Passage (Taylor & Hellberg 2006), which may also represent barriers to dispersal and range expansion in other species that utilize marine environments, such as amphidromous shrimp. Marine biogeographical breaks could have facilitated differential historical distributions in shrimp, thereby catalyzing taxon pulses and allopatric speciation. Interestingly, taxon pulses are often accompanied by character displacement, with body size being a commonly displaced character among species of *Anolis* lizards (Losos 1992). The sister group to Caribbean atyid shrimps are large-bodied Indo-Pacific species, suggesting that speciation via taxon pulses in Caribbean atyids was also accompanied by size displacement, as evidenced by the radiation of small-bodied atyids in the region (Page *et al*., in press).

Identifying the drivers of assemblage structure change and taxon cycles on oceanic islands is a challenge and remains speculative (Ricklefs & Bermingham 2002). Using palaeontological data for Caribbean vertebrates, Pregill & Olsen (1981) invoked Pleistocene aridity throughout the Caribbean and associated shifts from xeric to mesic habitats and changing patterns of terrestrial island connectivity to explain changes in assemblage structure and distribution in vertebrate fauna. Indeed, they concluded that cyclic changes in the distribution of Caribbean vertebrates did not characterize their biogeographical history and went as far as to reject the taxon cycle as a nonexistent phenomenon. However, more recently, competition and host-disease resistance were invoked as putative drivers of taxon cycles in Caribbean birds (Ricklefs & Bermingham 1999, 2002), taxon cycles were shown for Antillean rodents (MacFarlane & Lundberg 2002) and competition across habitat gradients was shown to produce temporally dynamic changes in distribution in Caribbean geckos (MacLean & Holt 1979). Thus, taxon cycling can certainly not be rejected for Caribbean vertebrates. Although Pleistocene aridity in the Caribbean region may have facilitated evolution of adaptations to pool habitats in *A. lanipes*, as aridity would have reduced the number of fast-flowing habitats in Caribbean island rivers, competition is also likely to have been a contributor to habitat specialization, as *A. scabra* has adaptations suited to riffles where filter feeding is more effective (Fryer 1977). Furthermore, Caribbean shrimp occupy semidiscrete feeding guilds (Crowl *et al*. 2001), suggesting ecological segregation over trophic niches. Thus, although low flow conditions greatly reduce shrimp abundance (Covich *et al*. 2003), and this would have been exacerbated during historical periods of aridity, competition across ecological gradients would also have had a role in changing distribution and taxon cycling in these shrimp. If changing climatic conditions was the sole factor facilitating population expansions in our study species, we would have expected to detect a strong degree of congruence in the relative timing of their population expansions associated with a pronounced climatic event. This may have also indicated population expansions in pre-established populations and led to the rejection of our prediction for sequential colonization. However, population expansions were often non-contemporaneous and the absence of large historical climatic change in the Caribbean region throughout the Holocene (Metcalfe *et al*. 2000) make it unlikely that these shrimp expanded from small pre-existing populations in response to a profound historical event. Taxon cycling in Caribbean birds is also reported to occur independently of historical climatic changes and occurs independently among species (Ricklefs & Bermingham 2002). Disturbances (e.g. hurricanes and volcanoes) play an important role in determining Caribbean shrimp abundance and dynamic processes of species accumulation and turnover (Covich *et al*. 1991; Covich 2006). Thus, it is very likely that taxon cycling has been a central process in the biogeographic history of Caribbean shrimp, driven by both ecological and environmental factors.

Although our data support taxon cycling in amphidromous Caribbean shrimp, factors other than stage in the taxon cycle may also explain their patterns of nucleotide variation, including selective sweeps, genetic hitchhiking and background selection. For example, *A. lanipes* may be in the early stages of recovering from a selective sweep, whereby a gene tightly linked with the COI mtDNA gene underwent intense selection, resulting in the population losing variation at the selected and linked genes. Similarly, the shrimp inferred as late stage species could have undergone selective sweeps further in the past, and their present-day high levels of genetic diversity could suggest that their populations have been recovering for longer. However, as noted earlier, there is strong ecological and biogeographical evidence for taxon cycling in Caribbean amphidromous shrimp, which explains patterns of nucleotide variation better than selection, genetic hitchhiking or background selection.

Contemporary population genetic structure

The amphidromous life history of Puerto Rican freshwater shrimp appears to facilitate effective genetic exchange

among rivers, suggesting that larval dispersal via the marine environment will likely lead to recolonization of defaunated habitats if barriers to upstream migration are removed. This also suggests that the ecological functions of the shrimp assemblage (Pringle *et al*. 1993, 1999; Crowl *et al*. 2001) will be reinstated following changed river management practices. Interestingly, Mona Passage represents a marine biogeographic break for various coral reef fishes between Puerto Rico and other islands of the Greater Antilles (Taylor & Hellberg 2006), although this water body appears not to restrict marine littoral dispersal of shrimp larvae among Puerto Rican rivers. In contrast, populations of volcano shrimp (*Halocaridina rubra*) on the island of Hawaii are reported to be genetically isolated among anchialine habitats (Santos 2006). This suggests that this species does not disperse via the ocean despite inhabiting mixohaline environments. Evidence for population expansions in all Puerto Rican shrimp species may mean that they are not in drift–mutation equilibrium and that the magnitude of gene flow could be overestimated, although larval dependency on marine littoral habitats suggests that very high gene flow is a biologically feasible interpretation. The significant Φ_{SC} (i.e. 'among rivers within regions') for *M. faustinum* suggests that any one river (or pool in a river) may not reflect the overall genetic composition of the total population in this species, which is a pattern found in widespread fishes (Allendorf & Phelps 1981; Waples 1998) and widely dispersing stream insects (Bunn & Hughes 1997). Degradation of riverine spawning habitat used by anadromous species (i.e. marine species that migrate to freshwater for reproduction, such as many salmonids) is reported to have serious consequences for the viability of populations as site fidelity greatly reduces opportunities for recolonization among rivers (Policansky & Magnuson 1998; Jonsson *et al*. 1999; Prowles *et al*. 2000). In contrast, degradation of riverine habitat may have less serious consequences for amphidromous species, as they appear to have effective capabilities for among-river recolonization (McDowall 2003; Covich 2006; this study). However, multiple larval stages of Caribbean shrimp have been reported to stay in estuaries rather than drifting to coastal marine waters (Benstead *et al*. 2000). This suggests that gene flow among rivers may be effected by a subset of larval recruits, perhaps by recruits from reproducing adults in reaches closer to estuaries, or that it occurs sporadically in association with hurricanes (Covich 2006). High rates of genetic exchange in several migratory riverine species have been shown to overestimate abilities for among-river dispersal (Campana & Thorrold 2001). Our results that show high genetic connectivity among rivers for Puerto Rican amphidromous shrimp should thus be treated with caution and river management should foster strategies that maximize within- and among-river recruitment.

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