Evolutionary relationships of atyid shrimps imply both ancient Caribbean radiations and common marine dispersals

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Abstract. The evolutionary relationships of the surface genera of shrimps of the family Atyidae from the Caribbean were inferred using mitochondrial 16S ribosomal DNA and cytochrome oxidase I gene sequences. The genetic divergence among the 4 Caribbean genera (*Atya*, *Jonga*, *Micratya*, *Potimirim*) is extensive and dates from between the Eocene and Miocene. This result suggests a vicariant origin or the ancient dispersal of some taxa. Most intrageneric divergences date to the late Miocene–Pliocene and, thus, are probably the result of dispersal. Some species show low levels of intraspecific genetic divergence between distant islands, and thus, present-day or geologically recent gene flow is likely. This gene flow is probably a consequence of the amphidromous life histories of most Caribbean freshwater shrimps. Despite the ancient divergences between the genera, the Caribbean surface atyids form a single evolutionary lineage when compared with atyid shrimp from throughout the world, and this result implies an ancient evolutionary radiation in the Caribbean. The sister group to the Caribbean atyids are the large-bodied and robust *Atya*-like shrimps of the Indo-Pacific, which share a similar size and shape with Caribbean *Atya*. Thus, the common ancestor probably was also large and robust. In contrast, the other Caribbean atyids are much smaller, and *Jonga* has a distinct morphology that is associated with a switch from lotic to lentic environments. This radiation may have been the result of the absence from the Caribbean of other small shrimps that are common in the Indo-Pacific.

Key words: biogeography, Caribbean, phylogenetics, evolutionary radiation, Neotropics, islands, dispersal, vicariance, amphidromy, Atyidae.

The Caribbean interests zoologists and biogeographers because of its complex geology and well-documented evolutionary radiations, such as those of the *Anolis* lizards (Calsbeek and Smith 2003), land crabs (Schubart et al. 1998), and beetles (Buskirk 1985). The Caribbean has a varied geography, with archipelagos of large islands, and small high-volcanic and low-uplifted islands (Bass 2003). It is bounded by the Atlantic to the east, and the coasts of North, Central,

and South America on other sides. It shares many freshwater species with all surrounding land masses (Bănărescu 1995). Thus, it is a biotic crossroads (Fièvet 1998), similar to the islands of Wallacea between the zoological provinces of Australia and Asia.

Early theories on the formation of the biota of the Caribbean were based on dispersalist theories (Darlington 1938) or on the presence of an Oligocene land bridge linking the Greater Antilles and South America (Chace and Hobbs 1969, Peck 1999). The wide acceptance of plate tectonics led to vicariant theories for the Caribbean (Rosen 1976). These theories suggest that the proto-Antillean islands themselves dispersed eastward, away from North America and South America, and carried their constituent biota with them

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as they moved. Thus, the Antillean biota might not represent that of oceanic islands, but rather, the remnants of an ancient continental fauna.

Understanding freshwater taxa, in particular, has been important for understanding the origin of Caribbean biodiversity. In theory, freshwater-restricted species should be faithful to their areas of origin because of a supposed inability to disperse across either the marine or terrestrial environments (Fièvet 1998). Rosen (1976) used the distributions of many freshwater fishes, among other taxa (including Atyidae), to advance his vicariant theories. Stock (1986) also used the distribution of Caribbean atyid shrimp, but found that a dispersalist explanation of their distribution was more likely than a vicariant explanation. Stock (1986) pointed out that the geological history of the area is by no means certain, despite a long and distinguished history of study (Darlington 1938, Rosen 1976, Buskirk 1985).

A potential weakness of using freshwater species to understand Caribbean biodiversity is the underlying assumption that they are truly confined to freshwater. Caribbean fish and shrimps in freshwaters are often sympatric and have similar amphidromous life histories (Fièvet et al. 2001). Amphidromy entails active or passive movement downstream to estuarine and marine areas for larvae to grow before moving back upstream as larger juveniles or adults (McDowall 2007). Thus, amphidromous organisms are not solely freshwater species. The freshwater fish (Fièvet et al. 2001, Echelle et al. 2006) and shrimp fauna of the Caribbean (Chace and Hobbs 1969, Bass 2003) and Pacific (Page et al. 2007a) often are found in estuaries or close to the coast, as well as in upriver habitats. The ability to use marine habitats is likely to facilitate dispersal between isolated freshwater systems (Fièvet 1998), as has been found in studies of shrimp in the Pacific (Page et al. 2007a). Thus, these groups might be better characterized as littoral faunas (Fièvet 1998) of oceanic islands than as ancient continental remnants (Darlington 1938, Bănărescu 1995, Peck 1999).

A proper understanding of the history of Caribbean taxa requires distributional data and knowledge of evolutionary relationships. Recent studies have applied modern molecular phylogenetics to Caribbean freshwater fish and highlight the heterogeneous nature of the freshwater fauna. Some studies support vicariance (Chakrabarty 2006), some support old dispersals (Perdices et al. 2005), and others support more recent dispersals (Echelle et al. 2006).

Neither molecular nor morphological cladistic techniques have been applied to the common and diverse shrimp of the family Atyidae found throughout the Caribbean. Atyids are mostly small (<35 mm), except

for *Atya* and *Atya*-like shrimp (~60–100 mm; Hobbs and Hart 1982). Caribbean atyids often are found in high-gradient freshwater streams and riffles, where they passively filter and scrape detritus with a fan of hair-like setae on their chelipeds (claws) (Fryer 1977). They are often abundant in tropical freshwater systems and their small size belies their key ecological role in these systems (March and Pringle 2003, Yam and Dudgeon 2006). Their presence (or absence) can affect entire systems by mediating the overall level of litter biomass (March et al. 2001, Yam and Dudgeon 2005) and inorganic sedimentation (Crowl 2001, Moulton et al. 2004) on the substrate.

The Atyidae of the Caribbean are particularly diverse, with 4 surface genera (*Atya*, *Jonga*, *Micratya*, *Potimirim*) and 1 subterranean genus (*Typhlatya*) (Chace and Hobbs 1969). *Jonga* and *Micratya* are endemic to the region, whereas *Potimirim* and *Atya* are also found in South America (Fryer 1977). *Atya* species are the largest bodied, most robust, most speciose, and widest distributed of the Caribbean genera, and are found as far away as West Africa and the Cape Verde Islands (Hobbs and Hart 1982). Certain atyid species, such as *Potimirim americana*, *Atya lanipes*, and the cave species *Atya brachyrhinus*, which was known from a single cave in Barbados (Fryer 1977, Hobbs and Hart 1982) but might be now extinct or very rare (A. Karge, personal communication), are restricted to the Antilles.

Current distributions can be overlaid on a framework of phylogenetic relationships of the Caribbean subterranean (Webb 2003, Hunter et al. in press) and surface atyids (this study), once the framework is available. The shrimp phylogeny can then be compared with freshwater fish phylogenies already available for the area, so that theories, such as the influence of geological history (Covich 1988), and key life-history traits, such as amphidromy (Fièvet 1998), that account for freshwater biodiversity in the Caribbean can be assessed. The goal of our study is to understand and unravel the biogeography and evolution of the Caribbean atyids in the light of 2 apparently competing factors: 1) the ancient age and isolation of Caribbean islands, which should foster deep divergence in freshwater taxa, and 2) the predilection of Caribbean freshwater taxa for amphidromy, which should allow some dispersal between the ancient land masses and, thus, foster genetic homogenization.

Methods

Specimen sampling

Multiple species of the 4 genera of atyid shrimp present in Caribbean surface freshwater systems (*Atya*, *Jonga*, *Micratya*, *Potimirim*) were included in our study

TABLE 1. Atyid specimens and sequences included in Caribbean combined analyses on the basis of the mitochondrial gene sequences for the 16S fragment of ribosomal DNA (16S) and cytochrome oxidase I (COI). Specimen vouchers are deposited at Griffith University (GU) and Humboldt University (HU). Geographic areas: GA = Greater Antilles, LA = Lesser Antilles, CAC = Central America Caribbean, CAP = Central America Pacific, SAA = South America Atlantic, WA = West Africa. Specimen sources are indicated by footnotes.

Species	Sample site (geographic area)	GenBank accessions		Voucher number
		16S	COI	
<i>Atya gabonensis</i>	Pet shop in USA (origin in WA or SAA) ^a	EF489989	EF489961	GU-898
<i>Atya gabonensis</i>	Pet shop in Germany (origin in WA) ^b	EF489989	EF489962	HU-437
<i>Atya innocous</i>	Río Espíritu Santo, Puerto Rico (GA) ^c	EF489987	EF489968	GU-EL1
<i>Atya innocous</i>	Courland River, Tobago (LA) ^a	EF489988	EF489969	GU-1004
<i>Atya lanipes</i>	Río Guayanés, Puerto Rico (GA) ^c	EF489990	EF489970	GU-859
<i>Atya lanipes</i>	Río Mameyes, Puerto Rico (GA) ^c	EF489990	EF489970	GU-876
<i>Atya margaritacea</i>	Pet shop in Germany (origin in CAP) ^b	EF489983	EF489963	HU-433
<i>Atya ortmannioides</i>	Río Horcones, Mexico (CAP) ^a	EF489993	EF489971	GU-905
<i>Atya scabra</i>	Río Guayanes, Puerto Rico (GA) ^c	EF489984	EF489966	GU-860
<i>Atya scabra</i>	Río Guarumo, Panamá (CAC) ^b	EF489985	EF489964	HU-510
<i>Atya scabra</i>	Quebrada Botija, Panamá (CAC) ^a	EF489986	EF489967	GU-897
<i>Atya scabra</i>	Courland River, Tobago (LA) ^a	EF489983	EF489965	GU-1113
<i>Jonga serrei</i>	Courland River, Tobago (LA) ^a	EF490003	EF489972	GU-1002
<i>Micratya poeyi</i>	Río Espíritu Santo, Puerto Rico (GA) ^d	EF489994	EF489981	GU-1130
<i>Micratya poeyi</i>	Río Guanajibo, Puerto Rico (GA) ^c	EF489994	EF489981	GU-858
<i>Micratya poeyi</i>	Oropuche River, Trinidad (LA) ^a	EF489994	EF489982	GU-1003
<i>Micratya</i> sp. PR	Río Guayanés, Puerto Rico (GA) ^d	EF489991	EF489979	GU-1118
<i>Micratya</i> sp. PR	Río Guayanés, Puerto Rico (GA) ^d	EF489992	EF489980	GU-1119
<i>Potimirim glabra</i>	Cuffie River, Tobago (LA) ^a	EF489997	EF489978	GU-1005
<i>Potimirim glabra</i>	Main Ridge, Tobago (LA) ^e	EF489997	EF489978	GU-819
<i>Potimirim glabra</i>	Paria River, Trinidad (LA) ^e	EF489998	EF489978	GU-820
<i>Potimirim glabra</i>	Rio 2 de Abraao, Rio de Janeiro, Brazil (SAA) ^f	EF489999	EF489977	GU-348
<i>Potimirim glabra</i>	Rio Perenque, Sao Paulo, Brazil (SAA) ^f	EF489999	EF489977	GU-PO1
<i>Potimirim potimirim</i>	Rio Guarumo, Panamá (CAC) ^b	EF490004	EF489975	HU-431
<i>Potimirim potimirim</i>	Oropuche River, Trinidad (LA) ^a	EF490001	EF489974	GU-1006
<i>Potimirim potimirim</i>	Petshop in Germany (possible origin in SAA) ^b	EF490002	EF489973	HU-425
<i>Potimirim</i> sp.	Punta Arena, Río Conde, Costa Rica (CAC) ^a	EF490000	EF489976	GU-900, GU-1117
Outgroup				
<i>Typhlatya mitchelli</i>	Yucatán Peninsula, Mexico ^g	AF513538	AF513523	
<i>Typhlatya pearsei</i>	Yucatán Peninsula, Mexico ^g	AY115539	AY115534	

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^g Sequence from Webb (2003)

(Table 1). A Caribbean subterranean atyid genus, *Typhlatya* (from Webb 2003), the only extant representative of the non-Atyinae (sensu Page et al. 2007b) in the Caribbean, served as an outgroup. For our purposes, the Caribbean area includes the islands of the Greater and Lesser Antilles and the eastern slope of Central America (Table 2). Our specimens are from the Greater (Puerto Rico) and Lesser (Trinidad, Tobago) Antilles, the Caribbean (Costa Rica, Panamá) and Pacific coasts of Central America (México), and the Atlantic coast of South America (Brazil) (Table 1, Fig. 1). Some specimens were obtained from pet shops and

came from these areas or from West Africa (e.g., *Atya gabonensis*).

Atyid species from throughout their global distribution also were included in separate analyses to place the Caribbean taxa into a larger geographic and phylogenetic context. These species represent all 4 subfamilies of the Atyidae (sensu Holthuis 1993) and include the putative sister group of *Atya*, the *Atya*-like shrimp of the Indo-Pacific (Chace 1983); *Caridina* and *Caridina*-like shrimps of the Indo-Pacific; and other more-divergent atyids (non-Atyinae) from Europe, the Indo-Pacific, and the Caribbean (*Typhlatya*) (Table 3,

TABLE 2. Distributions of species of Atyinae in the Americas. Geographic areas: GA = Greater Antilles, LA = Lesser Antilles, CAC = Central America Caribbean, CAP = Central America Pacific, SAA = South America Atlantic, SAP = South America Pacific, CI = Cocos Islands, CV = Cape Verde Islands, WA = West Africa. X = present, x = present, but uncommon.

Species	Species distribution ^d								
	Caribbean			CAP	CI	SAA	SAP	CV	WA
	GA	LA	CAC						
<i>Archaeatya chacei</i>				x	X				
<i>Atya abelei</i>				X					
<i>Atya africana</i>									X
<i>Atya brachyrhinus</i>		X							
<i>Atya crassa</i>			X	X			X		
<i>Atya dressleri</i>				X					
<i>Atya gabonensis</i> ^a						X			X
<i>Atya innocous</i> ^a	X	X	X	X					
<i>Atya intermedia</i>									X
<i>Atya lanipes</i> ^a	X								
<i>Atya margaritacea</i> ^a				X			X		
<i>Atya ortmannioides</i> ^a				X					
<i>Atya scabra</i> ^a	X	X	X	X		X		X	X
<i>Jonga serrei</i> ^a	X	X	x			x			
<i>Micratya poeyi</i> ^a	X	X	x						
<i>Micratya</i> sp. PR ^a	X								
<i>Potimirim americana</i>	X	X							
<i>Potimirim glabra</i> ^{a,b}		X	X	X		X	X		
<i>Potimirim mexicana</i>	X		X						
<i>Potimirim potimirim</i> ^a		X	X			X			
<i>Potimirim</i> sp. ^{a,c}			X						

^a Species included in this study

^b Considered here as a synonym of *P. brasiliensis* (Lima et al. 2006)

^c Might be *Potimirim mexicana*

^d References: Villalobos (1959), Hart (1961), Chace and Hobbs (1969), Abele and Blum (1977), Fryer (1977), Hunte (1978), Hobbs and Hart (1982), Felgenhauer and Martin (1983), Hickmann and Zimmerman (2000), W. Klotz (personal communication)

Fig. 2). These analyses also included the related Caribbean freshwater shrimp family Xiphocarididae (Holthuis 1993), which previously was considered a basal atyid (Fryer 1977, Christoffersen 1986), and Caribbean and non-Caribbean outgroup taxa (families Palaemonidae and Penaeidae). Specimens were kindly provided by many colleagues (Tables 1, 3).

Genetic analyses

DNA extraction and sequencing.—Genomic DNA was extracted using a modified version of a cetyltrimethylammonium bromide–phenol/chloroform extraction (Doyle and Doyle 1987). Approximately 50 mg of dissected muscle tissue was used per specimen, except for some museum specimens from which only a single leg was taken. Fragments of the mitochondrial 16S ribosomal DNA (16S) and cytochrome oxidase subunit I (COI) genes were amplified with polymerase chain reaction and sequenced (primer information is in Table 4). Cycling conditions for all 16S reactions were: 3 min at 94°C; 40 cycles of 30 s at 94°C, 30 s at 50°C, 30 s at

72°C; 7 min at 72°C; and then held at 4°C. Cycling conditions for all COI reactions were: 3 min at 94°C; 15 cycles of 30 s at 94°C, 30 s at 40°C, 60 s at 72°C; then 25 cycles of 30 s at 94°C, 30 s at 55°C, 60 s at 72°C; 7 min at 72°C, and then held at 4°C. All individuals were sequenced in both directions. BigDye v.3.1 Terminator (Applied Biosystems, Foster City, California) was used for the sequencing reaction, and sequences were produced on an Applied Biosystems 3130xl Genetic Analyser at the DNA Sequencing Facility at Griffith University or at Humboldt University Berlin.

Data set construction.—Two separate data sets were assembled. One data set (Caribbean combined) consisted of the relatively conserved 16S fragment and the more-variable COI gene sequences from all Caribbean specimens (Table 1). A 2nd data set (Worldwide 16S) included 16S gene sequences from every worldwide atyid genus available to us (19 in total), either from our own collections or from GenBank (accessed March 2007) (Table 3). The 16S fragment is applicable to deeper phylogenetic analyses at the level of differentiating genera within a family, whereas COI can be inaccurate

FIG. 1. Sampling locations for atyid shrimp in the Caribbean combined data set consisting of mitochondrial gene sequences for the 16S fragment of ribosomal DNA (16S) and cytochrome oxidase I (COI).

at this level because multiple mutations at the same sequence sites over evolutionary time (saturation) can introduce so much noise into the data set that accurate relationships are difficult to recover (Lefébure et al. 2006). Moreover, in GenBank, the 16S fragment is the most taxonomically widely represented gene region.

Twenty-eight new 16S and COI sequences from Caribbean genera (GenBank accession numbers are given in Table 1) were generated for the Caribbean combined data set. These sequences were added to 4 existing sequences from *Typhlatya* species (from Webb 2003). 16S sequences were aligned using ClustalX version 1.81 (Thompson et al. 1997) at default settings, resulting in an alignment of 478 base pairs (bp), corresponding to positions 11430 to 11901 of the atyid *Halocaridina rubra* Holthius, 1963 mitochondrial genome (GenBank accession number NC008413; Ivey and Santos 2007). Gblocks version 0.91b (Castresana 2000) was used to identify poorly aligned sites, which were excluded from analyses (1.9% of sites; parameters: minimum number for conserved position = $\frac{2}{3}$ of sequences, minimum number for flanking position = $\frac{2}{3}$ of sequences, maximum number contiguous non-conserved positions = 8, minimum length of block = 8, allowed gap positions = with half). The COI data set was aligned to 536 bp, corresponding to positions 761 to 1296 of the *H. rubra* genome. The 2 genes were combined in this data set after a partition homogeneity test (PAUP*; Swofford 2002) showed that phylogenetic signal did not differ between the 2 genes (partition homogeneity test, $p = 0.440$).

Fourteen new 16S sequences from various genera (including specimens of *Xiphocaris* from the Dominican Republic and Puerto Rico and *Macrobrachium* from Puerto Rico) were generated for the Worldwide 16S

data set. These sequences were added to the 16S sequences from the Caribbean combined data set and 20 16S sequences obtained from GenBank (Webb 2003, Quan et al. 2004, Page et al. 2005, 2007a, b, Zakšek et al. 2007; Table 3). The Worldwide 16S data set was aligned to 495 bp in the manner described above with ClustalX and Gblocks.

Phylogenetic methods.—Both data sets were analyzed with maximum likelihood (PHYML version 2.4.4; Guindon and Gascuel 2003) and Bayesian (MrBayes version 3.1.2; Huelsenbeck and Ronquist 2001) methods of phylogenetic inference. Modeltest version 3.06 (Posada and Crandall 1998) was used to identify the best-fit model of evolution (Akaike information criterion) for each data set (Caribbean 16S portion, Caribbean COI portion, Caribbean combined, Worldwide 16S). For maximum likelihood analyses, a single model of evolution was selected for each main data set (Caribbean combined, Worldwide 16S) and each was bootstrapped 1000 times. For MrBayes analyses, the parameters were 2 million generations, trees sampled every 100 cycles, data set partitioned by gene for the combined data set, 25% burn in, and 2 runs of 4 chains heated to 0.2.

A molecular clock was applied to the Caribbean 16S sequence divergences (all positions) to give a rough estimate of the geological age in which the Caribbean genus-level taxa and selected species might have diverged from each other. A likelihood ratio test (PAUP*) was used to test for nonclocklike molecular evolution. Net divergence times between clades were calculated using a correction for within-clade polymorphism. The two 16S sequence divergence rates used were 0.65% (Schubart et al. 1998) and 0.9% (Sturmbauer et al. 1996) per 10^6 years.

TABLE 3. Atyid specimens and sequences included in the Worldwide analysis on the basis of the mitochondrial gene sequences for the 16S fragment of ribosomal DNA. Specimen sources are indicated by alphabetical footnotes. Published sources of sequences are indicated by superscripted numbers next to GenBank accessions (1 = Page et al. 2007b, 2 = Page et al. 2005, 3 = Page et al. 2007a, 4 = Zakšek et al. 2007, 5 = Webb 2003, 6 = Quan et al. 2004).

Species	Sample site	GenBank accessions
<i>Atya</i> -like shrimps of the Indo-Pacific		
<i>Atyoida bisulcata</i>	Waiau Stream, Hawaii ^a	DQ681278 ¹
<i>Atyoida bisulcata</i>	Oahu, Hawaii ^b	EF489995
<i>Atyoida pilipes</i>	Putoa Falls, Moorea, French Polynesia ^c	DQ681279 ¹
<i>Atyoida pilipes</i>	Yela River, Kosrae, Micronesia ^d	DQ681276 ¹
<i>Atyoida pilipes</i>	Mele, Efate Island, Vanuatu ^b	DQ681277 ¹
<i>Atyopsis moluccensis</i>	Pet shop in Germany (origin in the Indo-Pacific) ^b	DQ681281 ¹
<i>Atyopsis moluccensis</i>	Pet shop in USA (origin in the Indo-Pacific) ^e	DQ681280
<i>Atyopsis spinipes</i>	Tchino Spring, East Timor ^f	EF489996
<i>Atyopsis spinipes</i>	Matepono River, Guadalcanal, Solomon Islands ^g	DQ681282 ¹
<i>Australatya striolata</i>	Johnstons Creek, Manning River, Australia ^b	AY795035 ²
<i>Caridina</i> and <i>Caridina</i> -like shrimps of the Indo-Pacific		
<i>Caridina gracilirostris</i>	North Johnstone River, Australia ^d	DQ478452 ³
<i>Caridina nilotica</i>	Malagarassi River, Lake Tanganyika, Tanzania ^h	DQ681275
<i>Caridina serratirostris</i>	Pouembout, New Caledonia ⁱ	DQ478516 ³
<i>Caridina spinula</i>	McIlwraith Range, Lockhart, Australia ^j	DQ478527 ³
<i>Caridina trifasciata</i>	Task Yue Wu, New Territories, Hong Kong ^k	DQ478557 ³
<i>Caridinides wilkinsi</i>	Harmer River, Queensland, Australia ^l	DQ681272
<i>Neocaridina denticulata</i>	Manoa Stream, Oahu, Hawaii ^l	EF490006
<i>Neocaridina denticulata</i>	Oahu, Hawaii ^b	EF490005
<i>Parisia unguis</i>	Cutta Cutta Caves, Katherine, Australia ^m	DQ681288 ¹
<i>Pycnisia raptor</i>	Grants Cave, Katherine, Australia ^m	DQ681271 ¹
Other atyid shrimp (non-Atyinae)		
<i>Atyaephyra desmarestii</i>	Rio Douro, Portugal ⁿ	DQ681284
<i>Dugastella valentina</i>	Baldovi, Valencia, Spain ^o	DQ641600 ⁴
<i>Halocaridina rubra</i>	Oahu, Hawaii ^b	EF490008
<i>Paratya australiensis</i>	Brisbane River, Queensland, Australia ^p	EF490007
<i>Spelaecaris pretneri</i>	Ljelješnica, Dabarsko polje, Bosnia ^o	DQ641590 ⁴
<i>Troglocaris hercegovinensis</i>	Obodska pećina, Rijeka Crnojevića, Croatia ^o	DQ641596 ⁴
<i>Troglocaris inermis</i>	Sauve, Montpellier, France ^o	DQ641597 ⁴
<i>Typhlatya mitchelli</i>	Yucatán Peninsula, Mexico ^q	AF513538 ⁵
<i>Typhlatya pearsei</i>	Yucatán Peninsula, Mexico ^q	AY115539 ⁵
Xiphocarididae		
<i>Xiphocaris elongata</i>	Río Yasica, Dominican Republic ^r	EF490010
<i>Xiphocaris elongata</i>	Río Guayanilla, Puerto Rico ^s	EF490011
Outgroups		
<i>Macrobrachium australe</i>	Laloki River, Papua New Guinea ^t	DQ681290 ¹
<i>Macrobrachium faustinum</i>	Río Guayanés, Puerto Rico ^b	EF490009
<i>Metapenaeus affinis</i>	Xiamen, East China Sea, China ^u	AY264904 ⁶
<i>Metapenaeus</i> sp.	Brunswick River, New South Wales, Australia ^b	DQ681283 ¹

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^u Sequence from Quan et al. (2004)

FIG. 2. Sampling locations of *Atya*-like shrimp from the Indo-Pacific and Caribbean-Atlantic included in the Worldwide data set consisting of mitochondrial gene sequences for the 16S fragment of ribosomal DNA (16S). The dashed rectangle shows the sampling area in Fig. 1. Is. = island, Fr. = French.

Results

Molecular models and sequence variation

Modeltest selected the following models of evolution: Caribbean 16S portion (transversional model with equal base frequencies [TVM] + $I + \Gamma$, where proportion of invariable sites [I] = 0.368, Γ distribution shape = 0.417), Caribbean COI portion (Hasegawa–Kishino–Yano model [HKY] + $I + \Gamma$, I = 0.534, Γ = 0.547), Caribbean combined (TVM + $I + \Gamma$, I = 0.460, Γ = 0.462), and Worldwide 16S (HKY + $I + \Gamma$, I = 0.348, Γ = 0.498). In the Caribbean data set, 29.6% of base positions in the ingroup sequences of the 16S portion were variable (after the removal of the Gblocks positions), whereas 34.5% of base positions in the ingroup sequences of the COI portion were variable (11% in the 1st codon position, 1% in 2nd, 88% in 3rd). In the Worldwide 16S data set, 49.6% of positions in the Atyidae sequences were variable (after the removal of the Gblocks positions). Base frequencies did not differ across ingroup taxa in any data set (χ^2 tests of homogeneity, $p > 0.999$).

Tree scores and molecular-clock estimates

Maximum likelihood and Bayesian analyses recovered 1 tree each for the Caribbean combined data set

(maximum likelihood log score = –6134.525, Bayes factor harmonic mean = –6065.48; Fig. 3). Maximum likelihood and Bayesian analyses recovered 1 tree each for the Worldwide 16S data set (maximum likelihood log score = –6185.055, Bayes factor harmonic mean = –6145.42; Fig. 4).

A hypothesis of clocklike molecular evolution could not be rejected for the Caribbean 16S data set (likelihood ratio test, $p = 0.169$). Pairwise molecular-clock divergence estimates between all Caribbean genus-level taxa (e.g., *Atya* [without *A. ortmannioides*], *Atya ortmannioides*, *Jonga*, *Micratya*, *Potimirim*) ranged from 47.1 million years ago (mya) to 16.9 mya (full range using both rates $\pm$ SE). The oldest estimated divergence was Eocene–Oligocene (*Atya* vs *Jonga*, 47.1–33.3 mya). Four divergences were Oligocene (*Atya* vs *Micratya*, *Atya* vs *Potimirim*, *A. ortmannioides* vs *Jonga*, *Jonga* vs *Micratya*), 4 were Oligocene–Miocene (*Atya* vs *A. ortmannioides*, *A. ortmannioides* vs *Micratya*, *Potimirim* vs *A. ortmannioides*, *Potimirim* vs *Jonga*), and the youngest was Miocene (*Micratya* vs *Potimirim*, 24.3–16.9 mya). Species divergences within genera were mostly late Miocene or Pliocene (see below).

Tree topologies

Caribbean genera.—Both forms of analyses (maximum likelihood and Bayesian) agreed strongly on the

TABLE 4. Details for primers used to sequence mitochondrial genes 16S ribosomal DNA (16S) and cytochrome oxidase I (COI).

Primer name	Direction	Primer sequence	Primer reference
16S-F-Car	Forward	TGCCTGTTTATCAAAAACATGTC	von Rintelen et al. 2007
16S-R-Car	Reverse	AGATAGAAACCAACCTGGCTC	von Rintelen et al. 2007
16S-R-Car1	Reverse	GAAAGATAGAACTAACCTGGCT	von Rintelen et al. 2007
COI-F-Car	Forward	GCTGCTAATTTTATATCTACAG	von Rintelen et al. 2007
CDC0.La	Forward	CCNGGGTTYGGRATAATTTCTC	Page et al. (2005)
COI.f	Forward	CCTGCAGGAGGAGGAGAYCC	Palumbi et al. (1991)
COIa.H	Reverse	AGTATAAGCGTCTGGGTAGTC	Palumbi et al. (1991)
COI-R-Macro	Reverse	TGTGTAGGCATCTGGGTAGTC	This study

FIG. 3. Maximum likelihood (ML) phylogram for atyid shrimp in the Caribbean combined data set consisting of mitochondrial gene sequences for the 16S fragment of ribosomal DNA (16S) and cytochrome oxidase I (COI). The phylogram shows specimen geographic origin. ML bootstrap values are shown above nodes, with Bayesian posterior probabilities below nodes. Nodes with <60% for both values have been collapsed. Shrimp are shown in correct relative sizes (drawings adapted from Fryer 1977).

topology of the Caribbean genera (Fig. 3), but the deeper intergeneric relationships are unresolved. Within *Atya*, *Atya scabra* and *Atya margaritacea* are very close, a result that agrees with the morphological studies of Hobbs and Hart (1982). However, the identification of our specimen of *A. margaritacea* is uncertain (W. Klotz, personal communication). These taxa might represent either slightly differing conspecific populations on the Atlantic and Pacific slopes of the Americas, respectively, or an example of a recent speciation. *Atya scabra/margaritacea*, *A. lanipes*, and *Atya*

innocuous (all Caribbean) diverged from each other in the late Miocene–Pliocene (7.8–4.9 mya) and form a sister clade to *A. gabonensis* (likely of West African origin), with a divergence estimate in the Miocene (10.4–6.8 mya). Together, these *Atya* taxa form a strong clade that excludes *A. ortmannioides* (western México), which Hobbs and Hart (1982) consider primitive. *Atya ortmannioides* has not been studied extensively (Hobbs and Hart 1982), and is no more closely related to other *Atya* than to other genera from the region.

Three of 4 species of *Potimirim* (Lima et al. 2006)

FIG. 4. Maximum likelihood (ML) phylogram of *Atya*-like shrimp from the Indo-Pacific and Caribbean-Atlantic included in the Worldwide data set consisting of mitochondrial gene sequences for the 16S fragment of ribosomal DNA (16S). ML bootstrap values are shown above nodes, with Bayesian posterior probabilities below nodes. Nodes with <50% for both values have been collapsed. Shrimp shown in correct relative sizes (drawings adapted from Fryer 1977, Williams 1980, Chace 1983, Richard and Clark 2005).

were included in our study, and 1 species from Costa Rica was identified to genus level only (possibly *Potimirim mexicana*). Morphological identification of these species is not straightforward because the keys often are based only on adult males. The interrelationship of the *Potimirim* species is somewhat enigmatic (Chace and Hobbs 1969). The 3 *Potimirim* species appear to have diverged from each other in the late Miocene–Pliocene (7.0–2.7 mya). Together, they form a strong clade. The deepest divergence within a *Potimirim* species is within *Potimirim glabra*, between specimens from Trinidad/Tobago and Brazil (Pliocene, 3.1–2.0 mya). This divergence might represent different species. *Potimirim glabra* has a wide distribution and is found in a wide variety of stream types (Abele and Blum 1977).

Micratya forms a strong and especially deep clade (Miocene, 17.3–12.5 mya), between *Micratya poeyi* and an undescribed *Micratya* species from Puerto Rico. They both share dorsal teeth on their rostrums, a character that is diagnostic for *Micratya* among Caribbean atyids (Chace and Hobbs 1969). Last, the genus *Jonga* is monotypic and well differentiated from the other genera.

Caribbean atyid taxa in a global context.—Topologies are also mostly congruent between analyses at the larger geographic and phylogenetic scales of the Worldwide 16S data set (Fig. 4), but support values tend to be considerably higher in the Bayesian than the maximum likelihood analyses. The Caribbean genera form a clade, and this result implies that they have a common ancestor relative to the Atyidae elsewhere and that the Caribbean clade might represent a radiation of genera and species in the Caribbean area. Holthuis (1993) placed *Micratya* in a different subfamily (Caridellinae) from the other Caribbean genera (Atyinae), but our results imply that *Micratya* also belongs in Atyinae.

Furthermore, the sister group to the Caribbean genera is recovered as the larger-bodied *Atya*-like shrimp of the Pacific (*Atyoida*, *Atyopsis*, *Australatya*; Chace 1983). The sister to the lineage comprising Caribbean and Indo-Pacific *Atya* and *Atya*-like shrimps is the smaller-bodied, widespread *Caridina* and *Caridina*-like shrimp of the Indo-Pacific. The position of the sole representative of Xiphocarididae is unclear. If it is related to the Atyidae, the relationship must be distant and might be too divergent to place accurately here. This larger-scale analysis is highly suggestive of a number of interesting relationships. However, the addition of more highly conserved nuclear genes will be required to resolve the deeper nodes fully.

Discussion

Vicariance in the Caribbean and Americas

The molecular divergence of Caribbean atyid genera is extensive and dates mostly to the Oligocene/Miocene. Thus, older divergences could potentially be consistent with Rosen's (1976) vicariant theories for the Caribbean, which entail ancient continental taxa being carried east on proto-Antillean islands by continental drift rather than active biotic dispersal. Molecular clocks are imprecise by their very nature, but they do give a general and relative idea of timescales. Regardless of the calibration rate, the great differentiation among and within genera in our study indicates long stretches of evolutionary time and has implications for Caribbean biogeography because it shows that not all taxa are recently arrived or derived, despite life-history traits that enhance dispersal ability.

One of the most interesting facets of the *Atya* story is the presence of *Atya* in the Americas and in West Africa, a distribution that Hobbs and Hart (1982) interpret as a consequence of the ancient Gondwanan vicariant split between South America and Africa. Two species (*A. gabonensis*, *A. scabra*) are shared across the Atlantic, and 2 more are closely related (*A. innocuous*, Americas; *Atya intermedia*, islands off the African coast; Hobbs and Hart 1982). Hobbs and Hart (1982) found little morphological difference between populations of *A. gabonensis* and *A. scabra* on separate continents and interpreted this similarity as an indication of a slow rate of morphological change, rather than transoceanic dispersal (which seemed unlikely to Hobbs and Hart [1982]). Our molecular estimate of 10.4 to 6.8 mya as the time of divergence between Caribbean (*A. scabra/margaritacea*, *A. lanipes*, *A. innocuous*) and West African (*A. gabonensis*) *Atya* is considerably after the split between the continents (>100 mya; McLoughlin 2001), and thus, implies dispersal. However, our estimate is hampered by an imprecise knowledge of the collection locations of our *A. gabonensis* specimens (West Africa is the most likely collection location; W. Klotz, personal communication).

A vicariant explanation for the presence of *Atya* in the Americas and in West Africa is highly unlikely for several reasons unrelated to our molecular data or the fact of the amphidromous life histories of *Atya*. First, morphological divergence between the conspecific populations on the different continents is slight, yet other *Atya* taxa, such as *A. scabra* and *A. margaritacea* on opposite sides of the isthmus of Panamá, appear to have diverged morphologically over much shorter time spans and geographic distances (Hobbs and Hart 1982, W. Klotz, personal communication). Second, *A.*

scabra is found not only in the Americas and Africa, but also on the oceanic Cape Verde Islands (Hobbs and Hart 1982), which are between the 2 continents in the Atlantic (600 km off the African coast; Fig. 2) and must have been reached by dispersal. Third, the Pacific genera, *Atyoida* and *Atyopsis*, are similar enough to *Atya* in size, morphology, and ecology to have been considered congeneric until fairly recently (Hobbs and Hart 1982, Chace 1983). These genera are spread throughout the Indo-Pacific (*Atyoida*: Madagascar to Hawaii, 17,000 km; *Atyopsis*: Sri Lanka to Samoa 12,000 km; Chace 1983), in areas so disparate that no vicariant theory could link them. Thus, dispersal in the Pacific must occur through ocean currents. Furthermore, closely related species of the freshwater shrimp genus *Macrobrachium* also are found in both South America and West Africa (Fièvet 1998), and their molecular divergences also imply long-distance dispersal rather than an ancient vicariant split (Murphy and Austin 2005). Last, for vicariance to explain the presence of *Atya* in South America and Africa, the genus *Atya* and both *A. scabra* and *A. gabonensis* would have to predate the ancient split, events that would be surprising because both species are among the most derived *Atya* (Hobbs and Hart 1982).

Rosen (1976) suggested that vicariance was responsible for the diversity of freshwater taxa within the Caribbean. This suggestion was supported by molecular data for Greater Antilles freshwater cichlid fish (Chakrabarty 2006), although Chakrabarty (2006) did not consider an explanation on the basis of ancient marine dispersal, which could have produced a similar pattern (Cook and Crisp 2005). Perdices et al. (2005) preferred ancient dispersal as an explanation for molecular divergences among Cuban freshwater swamp eels. The very old divergences among Caribbean atyid fauna could conceivably support any one (or all) of the biogeographic theories.

One way to identify vicariance is to focus on taxa presumed least likely to be capable of marine dispersal. Many atyid surface taxa have been found in estuarine habitats because of their amphidromous life history. Therefore, the distribution of subterranean shrimps might be more likely to be the result of vicariance than that of surface taxa, especially because groundwaters are more isolated than surface waters and should persist for a very long time (Stock 1986). Nevertheless, genetic (Hunter et al., in press) and geological (Stock 1986, Alvarez et al. 2005) data suggest that the distribution of subterranean troglotic genus *Typhlatya* throughout the Caribbean is probably the result of dispersal, both ancient and recent.

Amphidromy and dispersal in the Caribbean and the Americas

Our samples included conspecific specimens from distant parts of the Caribbean region, (e.g., *A. scabra* [Greater Antilles, Lesser Antilles, Central America], *A. innocous* and *Micratya poeyi* [Greater Antilles, Lesser Antilles]), but only very shallow genetic differences were found within each species. This result implies geologically recent gene flow, such as during Pleistocene episodes of lower sea levels, or ongoing gene flow throughout the Caribbean region, as was inferred for Caribbean freshwater pupfish (Echelle et al. 2006). Divergence estimates between Caribbean atyid species within each genus date to the late Miocene–Pliocene (see above). These divergences are relatively recent in geological terms. Therefore, they are likely to be the result of dispersal, which appears to be the dominant process in atyid biogeography in the Caribbean and in the Pacific (Page et al. 2007a).

Darlington (1938) noted presciently that, although dispersal might appear haphazard at first glance (Rosen 1976), the patterns derived are decidedly nonrandom (Cook and Crisp 2005). Darlington's (1938) fairly simple formula to calculate the chance of dispersal included distance between islands as an important factor. Other important geographical/ecological factors are island area/elevation/age (Abele and Blum 1977, Bass 2003), ocean currents (Darlington 1938), number of freshwater habitats (Fryer 1977), freshwater flow rate and temperature (Hunte 1978), human land use at estuaries (Fièvet et al. 2001), nutrient availability (Covich 1988), and many others (see Covich 1988, 2006). One or more of these factors, in their current state or as they have been through geological time (Covich 2006), can influence present-day distributions and degree of isolation in complex ways.

Darlington (1938) also noted the importance of the "nature of the constituent organisms". In other words, some innate capacities in an animal's biology could predispose it to successful dispersal over (or through) water. The inorganic factors listed above can be viewed as barriers/filters (sensu Poff 1997, Covich 2006) through which an organism must pass to disperse and colonize successfully. The particular suite of traits possessed by an animal might allow it to pass through filters at various geographical scales (Poff 1997, Covich 2006, Page and Hughes 2007). The important trait for Caribbean atyid shrimp and freshwater fish is an amphidromous life cycle with a requirement for high salinity during larval growth (McDowall 2007). Freshwater communities on widely separated tropical islands in the Pacific and Caribbean

are similar because of the related life-history traits of their constituent taxa (Bass 2003, Covich 2006), rather than because of linked geological histories. This conclusion is evident in the frequent sympatry of fellow travellers, such as the Atyidae and Palaemonidae, throughout the tropics.

Caribbean atyid and palaemonid larvae require brackish water to develop (Hunte 1978). Among crustaceans, egg size reflects life history, in that species with many small eggs (i.e., all Caribbean surface atyids; Hobbs and Hart 1982, Fièvet 1998, Anger et al. 2002) have many larval stages and are lowland/estuarine, whereas species with few, large eggs have direct development and usually are fully freshwater (Anger et al. 2002). Reproductive strategy can be correlated with dispersal ability (Page and Hughes 2007) because r-selected species, such as Caribbean atyids, often are able to disperse near and far (Fièvet 1998, McDowall 2007).

This reproductive and dispersal strategy is useful in the variable environment of the Caribbean, where hurricanes continuously disrupt community equilibrium (Bass 2003) and provide opportunities for more r-selected species to recolonize (Covich 1988, Fièvet 1998, McDowall 2007). Hurricanes might even foster dispersal (Calsebeek and Smith 2003) because high flow associated with hurricanes can flush freshwater and its denizens out to sea in extensive freshwater plumes that can extend from northern South America to Puerto Rico (Fièvet 1998).

The marine environment provides a continuous medium through which aquatic animals can move between catchments, islands, and continents, as long as an individual can tolerate salinity in varying amounts (Fièvet 1998). Atyids in the Caribbean migrate within a catchment, up waterfalls, and possibly, through subsurface passages (Fièvet and Eppe 2002). Caribbean atyids also move between adjacent catchments (Hobbs and Hart 1982, Fièvet and Eppe 2002, Cook et al., in press), between islands, and between continents (see above). Darlington (1938) said, "Even if there is only one chance in a million that any given individual of a species will cross a given water gap, out of many million individuals some may be sure to cross...". Dispersal over several geographical and temporal scales has shaped much of the freshwater and terrestrial biodiversity of the Caribbean (Fryer 1977).

Evolutionary radiations in the Caribbean

Inclusion of many species and genera from outside the Caribbean in the Worldwide 16S data set allowed us to see how the Caribbean taxa relate to taxa

elsewhere and provided insights into the evolution of the Caribbean taxa. The main conclusion from the Worldwide 16S analyses was that all surface genera of the Caribbean atyids (*Atya*, *Jonga*, *Micratya*, *Potimirim*) form a clade relative to the rest of the world, with the large-bodied *Atya*-like shrimp of the Indo-Pacific (*Atyoida*, *Atyopsis*, *Australatya*) as their sister group. Thus, the immediate ancestor of the *Atya*-like shrimp of the Indo-Pacific and of the surface genera of the Caribbean probably was large and robust. This morphology is similar to that of all *Atya*-like shrimp of the Indo-Pacific and *Atya*, but not to that of the other Caribbean genera, which are smaller (all) or more gracile (*Jonga* only).

The sister-group to all *Atya*-like shrimp of the Indo-Pacific and Caribbean is the group of widespread, speciose, and smaller-bodied *Caridina* and *Caridina*-like shrimps of the Indo-Pacific (Indo-Pacific smaller atyids; Fig. 4). All other atyids are smaller bodied. Thus, the inference is that the ancestor of the *Caridina*-like and *Atya*-like shrimps was small, and the *Atya*-like lineage must have evolved to a larger size before spreading through the Caribbean and Indo-Pacific. This inferred relationship between all of the Caribbean surface atyids and the large-bodied *Atya*-like shrimp of the Indo-Pacific (described above) is not intuitively obvious because only *Atya* spp. are morphologically and ecologically similar. On the other hand, *Jonga* would seem more likely to be related to *Caridina* than to the *Atya*-like shrimp (see Chace and Hobbs 1969) because they share similar size, morphology, and affinity for slackwater environments (Fryer 1977).

Micratya is essentially an *Atya* in miniature (Fryer 1977). *Potimirim* is similar to *Micratya* and resembles juvenile *Atya* (Chace and Hobbs 1969), although *Potimirim* is slightly more primitive than *Atya* (i.e., uses its setae more for sweeping and less for passive filtering; Fryer 1977). *Jonga* is also small, although it is morphologically distinct. The smaller Caribbean atyids must have reverted from a larger to a smaller body size, more akin to that of all other atyids, because the Caribbean surface atyids form a clade and their immediate ancestor was probably larger bodied in the *Atya* mold. This reversion might have been a response to competition with bigger *Atya* (Fryer 1977) or a smaller size might have provided access to deep subsurface water in interstitial spaces (Abele and Blum 1977). More simply, an ecological vacuum might have existed in the Caribbean (Chace and Hobbs 1969, Sturmbauer et al. 1996), with the niches of smaller shrimp not yet filled.

Micratya is smaller than *Atya*, but it also inhabits high-gradient, O₂-rich streams similar to those inhabited by some *Potimirim* (see Hunte 1978). However,

other *Potimirim* species, and *Jonga*, in particular, inhabit warmer, slower lowland streams (Hunte 1978). *Jonga* has changed the most from the *Atya* morphology and has converged on a *Caridina*-like morphology (less-robust body and longer rostrum), which is suited to slackwaters (Chace and Hobbs 1969, Fryer 1977). Like *Caridina*, *Jonga* is found in vegetation and feeds by scraping rather than filtering (Fryer 1977). It has independently evolved to a similar morphology given a similar environment.

Caribbean players' role on the world stage

The relationship between Caribbean and Pacific *Atya*-like shrimp should not be surprising because the Isthmus of Panamá is a relatively new barrier (~3 mya; Knowlton and Weigt 1998). Thus, as Knowlton and Weigt (1998) and Sturmbauer et al. (1996) note for many other Caribbean and Pacific taxa, the Isthmus of Panamá cannot be the cause of the ancient splits within the atyids of Caribbean area. The huge, and largely empty, eastern Pacific is a barrier to many other taxa (Sturmbauer et al. 1996) and would seem to be a likely barrier between the Caribbean and Pacific for the proto-*Atya*-like shrimp. The *Atya*-like and *Caridina*-like shrimps are found mostly in the central and western Pacific, rather than in the eastern Pacific. The most easterly edge of the Pacific has been partially colonized by American Atyinae (*Archeatya* in Isla de Cocos and the Galapagos Islands; Villalobos 1959, Hickmann and Zimmerman 2000) and non-Atyinae (*Typhlatya* in the Galapagos; Alvarez et al. 2005) (Fig. 2). Between these isolated eastern-Pacific areas and the species-rich western Pacific is a mostly empty zone that may have been crossed successfully only once, although in which direction is unclear.

Concluding remarks

Many interesting evolutionary questions become apparent only after phylogenies are overlaid with geography. For example, one might ask why Caribbean *Atya*-like shrimp evolved small-bodied lineages, whereas Indo-Pacific *Atya*-like shrimp did not. Perhaps the Caribbean *Atya*-like shrimp experienced an ecological release in the Caribbean. Any smaller-bodied *Atya*-like shrimp in the Indo-Pacific would have been in competition with the small-bodied and widespread *Caridina* already existing there, whereas small-bodied *Atya*-like shrimp might have had no competitors in the Caribbean. However, if *Atya*-like shrimps were able to cross the eastern Pacific, then why did the *Caridina*-like shrimps not also do so? Perhaps by chance only *Atya*-like shrimp were successful. Perhaps *Caridina* did cross the eastern

Pacific, but only in the wake of the *Atya*-like shrimps, which already had occupied the niche of smaller shrimps in the Caribbean.

Given the prevalence of dispersal in the Caribbean, one might ask why deep lineages exist there rather than complete homogenization and panmixia. Dispersal must have played a role in atyid speciation, but some form of allopatric differentiation (Fièvet 1998) that might have included vicariance also must have been operating. Perhaps geographical dispersal barriers of which we are currently unaware existed in the past. Not all stages of atyid larvae go to sea, and multiple stages of atyid larvae tend to stay in the estuary (Hunte 1978, Benstead et al. 2000). Thus, marine dispersal might be only occasional for some species. Lima et al. (2006) and Chace and Hobbs (1969) point out that different species of *Potimirim* and *Atya*, respectively, occupy different longitudinal sections of rivers, and thus, have differing likelihoods of being swept out to sea or of surviving such an event. Perhaps certain species are prone to regular dispersal (Page and Hughes 2007), and thus, are unlikely to found a lineage of many species. Other species that disperse less readily and less often might remain isolated long enough for speciation to occur after a rare dispersal event, such as for Australian atyid shrimp (Cook et al. 2006).

We hope that the evolutionary relationships inferred in our study will aid future morphological work in a total evidence approach to taxonomy (Page et al. 2005). Furthermore, we hope the combined use of molecular and morphological data will provide more discrete and accurate taxonomic units that can be used in formal methods of historical biogeography that require clarity of units, both taxonomic and geographic (Ball 1990). The use of molecular and morphological phylogenies makes it possible to ask the sorts of questions called for by Covich (1988, 2006) in light of the evolutionary relationships that underpin the historical element of Caribbean freshwater biodiversity and biogeography.

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