ORIGINAL ARTICLE

Testing for biogeographic mechanisms promoting divergence in Caribbean crickets (genus *Amphiacusta*)

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ABSTRACT

Aim This work examines whether the history of diversification of *Amphiacusta* (Orthoptera, Gryllidae) in the Caribbean corresponds to a vicariant or a dispersalist model.

Location The Greater Antillean islands of the Caribbean region.

Methods The phylogenetic relationships among species were estimated using a procedure that directly estimates the underlying species tree from independent loci (in this case, one mitochondrial and one nuclear locus). This tree was then used to test for topological congruence with a vicariant model, and to estimate divergence times.

Results The analyses based on the expected pattern of species divergence (i.e. species-tree topology) support a vicariant model. With the notable exception of a dispersal event marking the colonization of Jamaica, the timing of the events are generally consistent with a vicariant scenario, given the current taxon sampling and potential errors with dating the divergence events.

Main conclusions The tendency of species to co-segregate by island suggests that intra-island diversification is common. Despite their flightlessness, species of *Amphiacusta* are apparently capable of long-distance dispersal, such as colonization from the Puerto Rican/Virgin Island bank to Jamaica. The topology of the species tree is consistent with a vicariant model of divergence, and the dates of divergence between island groups are generally consistent with an island–island vicariance model. A strict island–island vicariance scenario can, however, be rejected because of inferred dispersal events such as the colonization of Jamaica. Nevertheless, the biogeographic tests suggest that most of the diversity was generated under a combination of intra-island diversification and island–island vicariance. Additional sampling of taxa will be needed to verify this hypothesized scenario. Our findings indicate that *Amphiacusta* presents an ideal opportunity for examining the role of sexual selection in promoting diversification, which would complement the large number of studies focused on adaptive divergence of Caribbean taxa.

Keywords
Amphiacusta, Caribbean, dispersal, diversification, Greater Antilles, historical biogeography, island–island vicariance, sexual selection.

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INTRODUCTION

The Caribbean is characterized by a rich and diverse fauna that exhibits high levels of endemism. Numerous attempts have been made over the past several decades to elucidate the means by which this unique fauna emerged (Barbour, 1914; Darlington, 1938; Simpson, 1956; Rosen, 1975; Pregill, 1981; Iturralde-Vinent & MacPhee, 1999; Dávalos, 2007), but the region's complex geological history has made it difficult to draw general conclusions about the mechanisms responsible

(Donnelly, 1989a). Both vicariance (Rosen, 1975; Iturralde-Vinent & MacPhee, 1999) and dispersal (Hedges *et al.*, 1992; Hedges, 1996; Heinicke *et al.*, 2007) have been proposed as the predominant forces behind the emergence and diversification of Caribbean taxa. There has also been debate over possible connections between continental mainlands and the Caribbean islands, possibly through emergent islands between North and South America (Donnelly, 1989b, 1990; Pindell & Barrett, 1990), or a South American connection via a subaerial landspan centred on the Aves Ridge ('GAARlandia') (Iturralde-Vinent & MacPhee, 1999). Debate also surrounds the timing of emergence and connections between the Greater Antillean islands (Perfit & Williams, 1989), as well as the role of inter-island diversification in generating the diverse Caribbean fauna – the focus of this study.

Continual tectonic and volcanic activity in the Caribbean basin has resulted in a geological history that is dynamic and complex (Pindell, 1994; Iturralde-Vinent & MacPhee, 1999), making it difficult to distinguish between dispersal- and vicariance-induced species diversification from geological evidence (Hedges, 2001). Nevertheless, various aspects of the geological history generate explicit predictions about geographic patterns of taxonomic relationships, as well as the timing of diversification. The Lesser Antilles evolved independently from the Greater Antilles, and is composed of two arc systems, one of older islands originating in the late Eocene to Oligocene, and another of younger islands emerging in the late Miocene (MacDonald *et al.*, 2000). The landforms comprising the current islands of Cuba, Hispaniola and Puerto Rico did not begin to emerge until the late Eocene (Pindell, 1994; Robinson, 1994; Iturralde-Vinent & MacPhee, 1999). Cuba and Hispaniola are both composites of three separate land-blocks, and eastern Cuba was connected with northern Hispaniola until the early to mid-Miocene (25–20 Ma) (Pindell & Barrett, 1990), while the connection between southern and northern Hispaniola was established in the mid-Miocene (Pindell & Barrett, 1990). The connection between Hispaniola and Puerto Rico was severed *c.* 16–11 Ma with the formation of Mona Canyon (Iturralde-Vinent & MacPhee, 1999). During this time Jamaica experienced successive periods of emergence followed by submergence, becoming fully emergent only in the middle Miocene (*c.* 12 Ma; Robinson, 1994). Changes in sea level have also profoundly affected the connections between some islands – climatic cycles during the Pliocene and Pleistocene isolated the Virgin Islands from Puerto Rico and resulted in the repeated submergence/emergence of the Bahamas between *c.* 3 Ma and 118 ka (Hearty & Neumann, 2001; Reijmer *et al.*, 2002). To the extent that the geological history and associated vicariance events contributed to the species diversity of Caribbean faunas, the signature of this history will be evident in the biogeographic pattern and timing of species divergence (see review by Knowles, 2009).

The vicariance model of Iturralde-Vinent & MacPhee (1999) proposes that subsequent to the submergence of putative continental connections, formerly widespread taxa diverged allopatrically as islands moved apart during the Oligocene and Miocene. This model predicts that the history of species divergence in the Greater Antilles will reflect the history of island separation. This vicariance model is distinct from that of Rosen (1975), which proposed that current species distributions are the result of vicariant fragmentation of island connections during the Cretaceous – a hypothesis that is refuted by molecular clock estimates of divergence times between Caribbean and mainland taxa (Hedges *et al.*, 1992; Heinicke *et al.*, 2007), and by phylogenies of Caribbean fauna and flora (Malone *et al.*, 2000; Fritsch, 2003; Heinicke *et al.*, 2007).

In contrast, the emergence and diversification of Caribbean taxa may be the result of overwater dispersal from South America, followed by inter-island dispersal and divergence (Hedges *et al.*, 1992; Hedges, 1996). The extent to which island–island vicariance and inter-island dispersal are responsible for shaping the evolutionary history and modern distributions of island taxa remains uncertain (Dávalos, 2004). Although it is clear that dispersal is the only mechanism by which Jamaican taxa could arise (because of its constant isolation from other islands), dispersal among the other islands might also play a role in speciation. However, because such dispersal events would not be constrained to any particular geological period (i.e. the dispersal scenario does not make explicit predictions about the timing and pattern of diversification), the focus of this study is on whether the phylogeny of a group of flightless crickets (genus *Amphiacusta*) supports a vicariance model of diversification. Specifically, this study tests the timing and pattern of divergence among island groups predicted by island–island vicariance (Fig. 1) using an estimate of the underlying species tree (e.g. Maddison, 1997; Carstens & Knowles, 2007a; Edwards *et al.*, 2007; Knowles & Chan, 2008; Kubatko *et al.*, 2009; Liu, 2009; McCormack *et al.*, 2009) derived from the mitochondrial locus cytochrome *c* oxidase
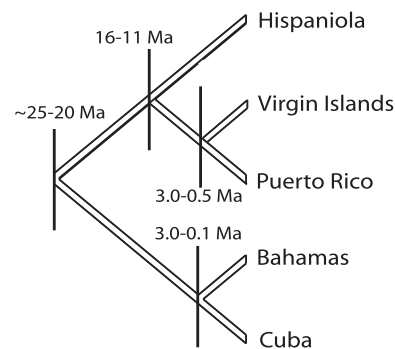

Figure 1 Topology of species relationships of Caribbean *Amphiacusta* predicted by the island–island vicariance model, with predicted dates of divergence (Iturralde-Vinent & MacPhee, 1999; MacPhee *et al.*, 2003). The model predicts only the relationships among Cuban, Hispaniolan, Puerto Rican and Virgin Island taxa. Because Jamaica was emergent only by the mid-Miocene (*c.* 12 Ma) (Robinson, 1994) its fauna must have arisen by dispersal, and is therefore not included in the model.

subunit I (COI) and the nuclear locus elongation factor 1-alpha (EF1-α).

Amphiacusta (Saussure, 1874) is a genus of ground crickets restricted to the Caribbean and constitutes the largest portion of Neotropical diversity in the Phalangopsinae (Orthoptera, Gryllidae) – a subfamily that is distributed world-wide. There are more than 80 described species of *Amphiacusta*, most of which are found in the Greater Antilles and are endemic to one island (Desutter-Grandcolas & Otte, 1997; Otte & Perez-Gelabert, 2009). The species are nocturnal and occur in a variety of habitats, including sandy beaches, wet and dry tropical forest, and caves. All species in this family are flightless, a feature which may hinder inter-island colonization by dispersal and make inter-island vicariance and intra-island speciation more likely (e.g. Mendelson & Shaw, 2005; Carstens & Knowles, 2007b). This reduced potential for dispersal coupled with the impressive diversity of *Amphiacusta* means that the genus may provide insights into how the complex geological history may have contributed to species diversification in this biodiversity hotspot.

MATERIALS AND METHODS

Sampling

Twenty-eight species in the genus *Amphiacusta* and a co-distributed outgroup in the same family, *Yoyuteris phacodes*, were sampled (Table 1, Fig. 2). This sampling constitutes *c.* 35% of described species, with sampling particularly dense in Hispaniola, Puerto Rico and the Virgin Islands (i.e. the targeted study region). We consider the implications of our sampling in the Discussion. All specimens are located in the Knowles Lab at the University of Michigan Museum of Zoology, Ann Arbor, MI, USA, or the Academy of Natural Sciences, Philadelphia, PA, USA. The specimen awaiting formal description (i.e. species 1) exhibits significant differen-

Table 1 Locality information for studied specimens of the genus *Amphiacusta* and a co-distributed outgroup in the same family, *Yoyuteris phacodes*. The undescribed species from Puerto Rico is referred to as *Amphiacusta* species 1.

Species	Locality	Island	ID no.
Amphiacusta henrymorgani[1]	Henry Morgan's Cave, Andros Town	Bahamas	UMMZ E174
Amphiacusta ruizi[2]	Los Morenos, Santiago Prov.	Cuba	ANS 3183
Amphiacusta sincerus[3]	Holquin Prov.	Cuba	UMMZ E18
Amphiacusta mythica[4]	Ebano Verde, La Vega Prov.	NE Hispaniola (Dom. Rep.)	UMMZ E175
Amphiacusta hespera[4]	Loma Guaconejo, Sanchez Prov.	NE Hispaniola (Dom. Rep.)	ANS 2603
Amphiacusta annulipes[4]	Ebano Verde, La Vega Prov.	NE Hispaniola (Dom. Rep.)	ANS RD-021
Amphiacusta apoplanos[5]	La Ciénaga, Barahona Prov.	SW Hispaniola (Dom. Rep.)	UMMZ E169
Amphiacusta dapanera[4]	Sierra de Neiba, Barahona Prov.	SW Hispaniola (Dom. Rep.)	ANS 2608
Amphiacusta dimalea[6]	Parque Nacional Jaragua, Pedernales Prov.	SW Hispaniola (Dom. Rep.)	ANS RD-156
Amphiacusta domingensis[4]	Sierra de Neiba, Barahona Prov.	SW Hispaniola (Dom. Rep.)	UMMZ E164
Amphiacusta eritheles[6]	Padre Las Cases, Azua Prov.	SW Hispaniola (Dom. Rep.)	ANS RD-122
Amphiacusta exacerbans[6]	Sierra de Neiba, Barahona Prov.	SW Hispaniola (Dom. Rep.)	ANS RD-110
Amphiacusta expansa[4]	Sierra Martin Garcia, Azua Prov.	SW Hispaniola (Dom. Rep.)	ANS 2575
Amphiacusta extenta[4]	Parq. Nac. Armando Bermúdez, La Vega Prov.	SW Hispaniola (Dom. Rep.)	ANS RD-49
Amphiacusta ominosa[4]	Monte Rio, Azua Prov.	SW Hispaniola (Dom. Rep.)	UMMZ E171
Amphiacusta pomeriense[4]	La Ciénaga, Barahona Prov.	SW Hispaniola (Dom. Rep.)	UMMZ E170
Amphiacusta sterna[4]	Rojo-Aceitillar, Pedernales Prov.	SW Hispaniola (Dom. Rep.)	UMMZ E173
Yoyuteris phacodes[4]	Sierra Martin Garcia, Azua Prov.	SW Hispaniola (Dom. Rep.)	UMMZ E22
Amphiacusta aetheria[7]	Mano Matuey, San Cristobal Prov.	SE Hispaniola (Dom. Rep.)	ANS RD-002
Amphiacusta obstipa[5]	Puerto Escondido, Altagracia Prov.	SE Hispaniola (Dom. Rep.)	UMMZ E172
Amphiacusta opsia[4]	Matadero, Peravia Prov.	SE Hispaniola (Dom. Rep.)	ANS RD-053
Amphiacusta nauta[8]	Mandeville, Cockpit Country	Jamaica	UMMZ J5-6-1
Amphiacusta pronauta[9]	El Yunque	Puerto Rico	UMMZ PR1-5-2
Amphiacusta lares[8]	Lares	Puerto Rico	UMMZ PR1-6-1
Amphiacusta viequesense[8]	Viequez Island	Puerto Rico	UMMZ PR7-6-1
Amphiacusta species 1[9]	Cerro Las Piñas	Puerto Rico	UMMZ PR1-5-4
Amphiacusta tijicohniae[8]	Guanica	Puerto Rico	UMMZ PR3-6-1
Amphiacusta hyperphobos[6]	Guana Island	Virgin Islands	UMMZ E157
Amphiacusta sanctaecrucis	Rain forest, Rte. 76, Saint Croix	Virgin Islands (US)	UMMZ STC5-2

NE, north-eastern; SE, south-eastern; UMMZ, University of Michigan Museum of Zoology; ANS, Academy of Natural Sciences, Philadelphia.
[1]Collected by D. Otte. [2]Collected by A. Infante. [3]Collected by A. E. Ruiz and D. Otte. [4]Collected by D. Perez-Gelabert, R. Bastardo and B. Hierro. [5]Collected by D. Perez-Gelabert and R. Bastardo. [6]Collected by D. Otte and D. Perez-Gelabert. [7]Collected by D. Otte, D. Perez-Gelabert and R. Bastardo. [8]Collected by D. Otte, L. L. Knowles, E. Oneal and H. Huang. [9]Collected by D. Otte, L. L. Knowles and E. Oneal.
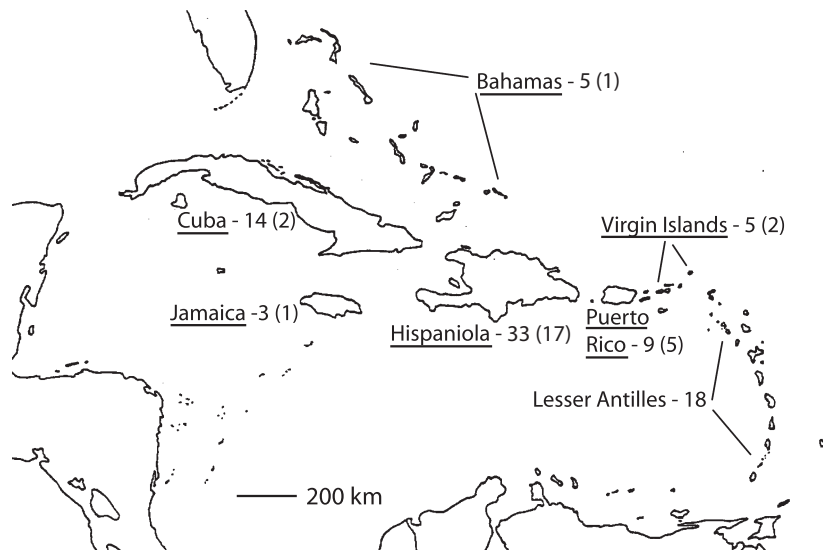
Figure 2 Map of the Caribbean with the number of endemic *Amphiacusta* taxa (first number) for each of the largest islands and the smaller island chains. The names of the islands included in the study are underlined and the numbers of species sampled are given in parentheses.

tiation in the morphology of its genitalia, the primary distinguishing feature among species in this genus.

Genomic DNA was extracted from the femur of each individual using a Qiagen DNeasy kit (Qiagen, Valencia, CA, USA). A 1185-base pair (bp) fragment of the mitochondrial locus COI and a 900-bp fragment of the nuclear EF1-α were sequenced (see Table 2 for primers and Knowles, 2000, for the PCR protocol). Sequences can be found in GenBank under accession numbers EU939163–EU939220; the standard genetic code for degenerate sites was used for heterozygous sites in EF1-α (18 sites in 12 species). Both strands of each product were sequenced on an ABI Model 3730 sequencer (Applied Biosystems, Foster City, CA, USA). All sequences were aligned using Sequencher 3.1 (Gene Codes, Ann Arbor, MI, USA) and Clustal; EF1-α sequences were readily aligned by eye as well.

Phylogenetic analysis

Maximum likelihood (ML) in paup* 4.0 (Swofford, 2002), as well as a Bayesian analysis using MrBayes 3.0 (Ronquist &

Table 2 A list of primers used for cytochrome *c* oxidase subunit I (COI) and elongation factor 1-alpha (EF1-α). All primers are written 5′–3′. PCR protocols are found in Knowles (2000).

Gene	Primer	Sequence
COI	C1-J-1718-2	GGA-GGA-TTY-GGA-AAT-TGA-TTA-GTW-CC
	L2-N-3014	TCC-ATT-GCA-CTA-ATC-TGC-CAT-ATT-A
	C1-J-2183	CAA-CAT-TTA-TTT-TGA-TTT-TTT-GG
	C1-N-2191	CCC-GGT-AAA-ATT-AAA-ATA-TAA-ACT-TC
EF1-α	EF1-F1	AGA-TGG-GYA-ARG-GTT-CCT-TCA-A
	EF1-R1	GAA-CAC-CAG-TCT-CCA-CAC-GA
	EF1-AF4	AGA-TGG-GYA-ARG-GTT-CCM-TCM-A
	EF1-BF2	TCG-TTT-CGA-GGA-AAT-MAA-GAA
	EF1-BR3	TGG-CAC-TGT-TCC-AAT-ACC-AC

Huelsenbeck, 2003), was used to estimate a gene tree for each locus, and to determine whether concatenation of these two loci was appropriate. For the ML analyses, 25 random addition replicates and tree bisection–reconnection (TBR) branch swapping were used for each data set with a maximum of 100 trees saved; branch support was evaluated with nonparametric ML bootstrapping, with 100 random addition replicates limited to 10 trees each. Four Markov chains of 5.5 million generations were used in the Bayesian analyses, with trees sampled every 1000 generations; posterior branch probabilities were calculated after excluding the first 0.5 million generations. Convergence was assessed by confirming the consistency of the results across two independent runs, as well as plotting the log likelihood of the trees against the number of generations to determine that the log likelihood of trees had plateaued. A HKY+I+G model of sequence evolution for COI (Hasegawa *et al.*, 1985) (base frequencies A = 0.3610, C = 0.1966, G = 0.0611, T = 0.3818, transition/transversion ratio = 8.09, α = 0.81, and proportion invariant sites = 0.58), and a Tamura–Nei+G model of evolution for EF1-α (Tamura & Nei, 1993) (base frequencies A = 0.2931, C = 0.1670, G = 0.2309, T = 0.3090, rate matrix rAC = 1, rAG = 2.17, rAT = 1, rCG = 2.17, rCT = 3.81, rGT = 1 and α = 0.30) were used in the phylogenetic analyses (gaps were not included in the phylogenetic analyses); these models were identified by the program DT ModSel (Minin *et al.*, 2003) based on a comparison of likelihood scores among possible models of evolution.

Recent studies have identified potential problems with concatenation of multiple loci for phylogenetic analysis (e.g. concatenated data may not allow accurate inferences of the species history; Kubatko & Degnan, 2007). For any particular history of divergence, there is also no way of knowing whether the estimated tree from concatenated data matches the underlying species history (Maddison & Knowles, 2006). Moreover, the approximately unbiased (AU) test (Shimodaira,

2002) identified significant incongruence between the estimated gene trees, confirming that concatenation would be inappropriate. This approach involves the comparison of *P*-values for individual gene tree estimates that are calculated based on a site-wise log-likelihood value. Therefore, we chose to estimate the species tree directly using the program BEST (Bayesian estimation of species trees; Liu, 2009), which jointly estimates the gene trees and species trees under a Bayesian framework. Priors for the gene trees were based on the models of evolution selected by DT ModSel. Convergence was assessed by plotting the gene tree length against the number of generations for each locus, and the log likelihood against the number of generations for the estimated species tree. Consistency of the results was confirmed across two independent runs, with 50 million Markov chain Monte Carlo (MCMC) generations per run.

Test of biogeographic hypotheses

Since inter-island vicariance makes predictions about the relationships among island groups, but not within islands, only the branching sequence among islands was considered in this test [i.e., the following relationships: (Cuba, Bahamas), (Hispaniola (Puerto Rico, Virgin Islands))]. Although fossils would ideally be used to calibrate the divergence times estimated from phylogenies (Smith & Peterson, 2002), such data are not available. In addition to the scarcity of orthopteran fossils (Poinar, 1993, 1999), controversy surrounds the age of insect fossils collected in the region (Grimaldi, 1995). For example, dates range from 15–20 Ma (Iturralde-Vinent & MacPhee, 1996) to 25–40 Ma (Poinar, 1993) for fossils from amber in the Dominican Republic, depending on the method used and the source of amber. Estimates of the timing of divergence were derived from the estimated species tree. Branch lengths in species trees produced in BEST are expressed as the number of substitutions per site or μT, where μ is the mutation rate and T is the number of generations (Liu, 2009); as locus-specific estimated models of nucleotide substitution were used in these analyses, species tree branch lengths inherently take into account differences in the substitution models of COI and EF1-α. To estimate an absolute divergence time for nodes of interest (e.g. the timing of divergence between Hispaniolan and Cuban taxa) the species tree branch lengths were divided by the mutation rate of the arthropod mitochondrial genome (1.1×10^{-8}) as derived by Brower (1994), which is commonly used for dating divergence events in insects (e.g. Carisio *et al.*, 2004; Kölsch & Pedersen, 2008; Carletto *et al.*, 2009). A range of divergence times was calculated, which corresponds to two to three generations per year (based on field observations, as well as generation times in the laboratory).

RESULTS

The average length of EF1-α sequence was 841 bp, of which 234 were variable. Of 1185 bp of the COI sequence, 470 were variable. Absolute sequence divergence within the ingroup ranged from 0% to 9.1% for EF1-α, and 0.09% to 21.4% for COI. The divergence between the ingroup and outgroup taxa (*Yoyuteris phacodes*) ranged from 16.2% to 20.4% for EF1-α and 18.7% to 20.7% for COI.

The primary incongruence between the COI and EF1-α gene trees, in terms of topological differences relevant to the biogeographic hypotheses of interests, is the placement of the Cuban and Bahaman taxa (Figs 3 & 4). Nevertheless, the gene trees estimated for COI and EF1-α also exhibit some broad similarities, most of which are reflected in the species tree estimated by BEST (Fig. 5). In the species tree, taxa from Puerto Rico, the Virgin Islands and Jamaica form a clade with *Amphiacusta tijicohniae* and *Amphiacusta* species 1 basal to the rest of the more recently derived taxa. Species also tend to co-segregate by island, although only Hispaniolan taxa formed a well-supported monophyletic clade. The Cuban species (*Amphiacusta ruizi* and *Amphiacusta sincerus*) and the Bahaman species *Amphiacusta henrymorgani* formed a clade that was basal to all other taxa, although with weak nodal support. The Jamaican species *Amphiacusta nauta* derived from a clade comprising Puerto Rican and Virgin Island taxa. The topology of the species tree was consistent with a vicariant history of diversification.

The divergence times derived from the species tree place the divergence of the Cuban–Bahaman group from all other taxa at *c.* 16.3–10.9 Ma, while the split between the Hispaniolan clade and the Puerto Rican–Virgin Island taxa occurs very soon afterwards, around 15.6–0.4 Ma. The split between the Bahaman species *A. henrymorgani* and the Cuban species *A. ruizi* and *A. sincerus* corresponds to a divergence around 3.0–2.0 Ma. *Amphiacusta* species 1, which is basal to the Puerto Rican taxa, split off around 11.4–7.6 Ma. The speciation events within the group of Virgin Island, Puerto Rican and Jamaican taxa that are sister to *Amphiacusta tijicohniae* all appear to have occurred recently and rapidly. For example, the divergence of *Amphiacusta sanctaecrucis* from *Amphiacusta hyperphobos* is very recent, and occurred around 123–82 ka, while the dispersal event that led to the Jamaican species *A. nauta* occurred *c.* 478–319 ka.

DISCUSSION

Emerging evidence suggests roles for both vicariance and dispersal as mechanisms promoting diversification of Caribbean taxa. The importance of overwater dispersal has been demonstrated in a range of taxa, including reptiles (Hedges *et al.*, 1992; Malone *et al.*, 2000), amphibians (Hedges *et al.*, 1992; Heinicke *et al.*, 2007), mammals (Dávalos, 2007) and plants (McDowell & Bremer, 1998; Negrón-Ortiz & Watson, 2002; Lavin *et al.*, 2003; McDowell *et al.*, 2003). Nevertheless, it is likely that vicariance has also played a role, and in some cases support for both processes has been found (Lavin *et al.*, 2003; McDowell *et al.*, 2003; Brandley & de Quieroz, 2004). Similarly, interplay between these two mechanisms is evidenced by the phylogenetic relationships within *Amphiacusta*,
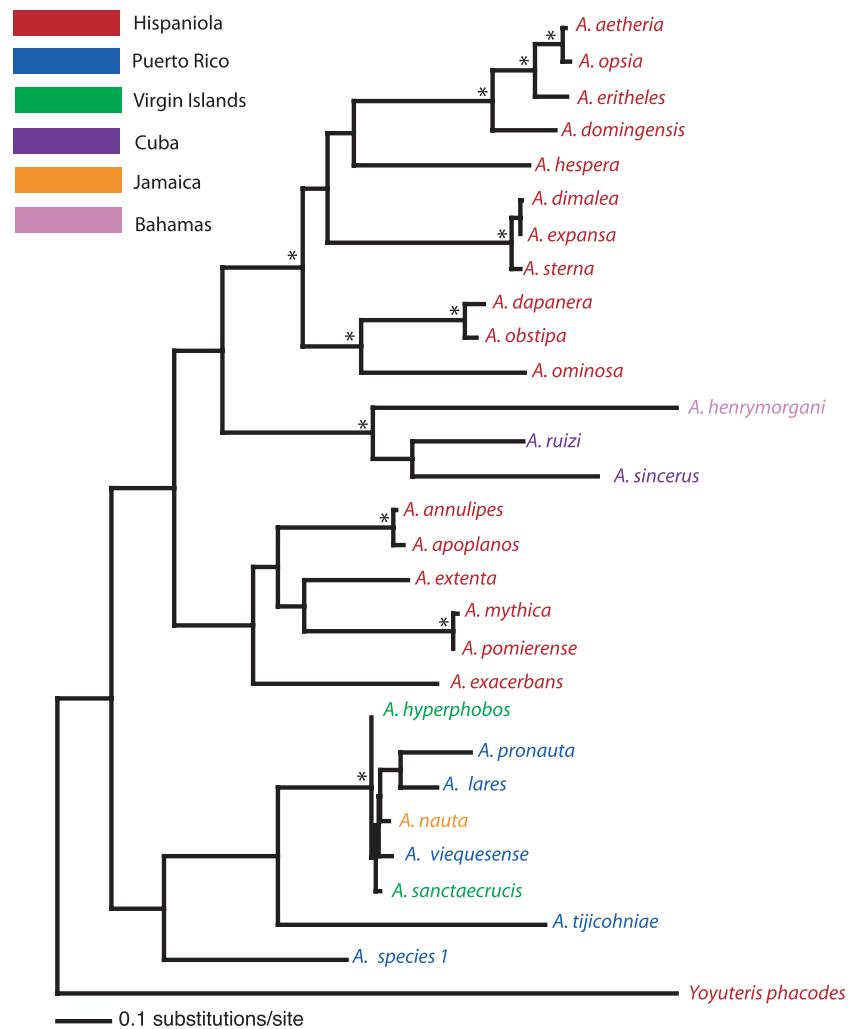
Figure 3 Estimated gene tree for Caribbean *Amphiacusta* from a maximum likelihood analysis of cytochrome *c* oxidase subunit I (COI) under an HKY+I+G with base frequencies A = 0.3610, C = 0.1966, G = 0.0611, T = 0.3818, transition/transversion ratio = 8.09, α = 0.81 and proportion of invariant sites = 0.58. *Bayesian posterior probabilities $\geq$ 95%.

but dispersal does not appear to play a major role. However, the predominance of intra-island diversification and island–island vicariance needs confirmation with additional sampling of taxa.

The timing of divergences between Puerto Rican and Virgin Island taxa within the last million years suggests a history of vicariance resulting from the rise in sea level during the Pleistocene, severing populations that then diverged in allopatry, and is consistent with the recency of separation among those islands (Heatwole & MacKenzie, 1967). At the same time, the derivation of the Jamaican species *A. nauta* from Puerto Rico is clear evidence of long-distance dispersal. Despite their flightlessness, the ocean does not constitute a complete barrier to dispersal for these crickets. While such dispersal events over long distances may seem counterintuitive, release and recapture experiments with buoys have demonstrated that flotsam can drift across large distances in the Caribbean and may take a meandering course affected by prevailing currents and storm conditions (Molinari *et al.*, 1979). Furthermore, observations for a range of taxa, including insects, indicate that rafting on floating vegetation (Holzapfel & Harrell, 1968; Censky *et al.*, 1998) may result in successful

colonization events. Nevertheless, this one example involving the colonization of Jamaica by *Amphiacusta* suggests that successful colonization by dispersal may indeed be quite rare for these crickets.

While the tests for topological congruence allow for the possibility of vicariance among the islands of Hispaniola and Puerto Rico, there is some ambiguity in the data. The placement of the Cuban–Bahaman group as basal to all the other taxa supports the inter-island vicariance model; however, this node does not show strong support (Fig. 5). Furthermore, the timing of divergence is not entirely consistent with predictions of island–island vicariance. For example, the split of the Cuban–Bahaman group from the rest of the islands dates to 16.3–10.9 Ma, which is more recent than the predicted divergence of 25–20 Ma from the model (Fig. 1). However, this discrepancy is not so extreme as to suggest that it is outside the margin of error associated with estimating branch lengths in the species tree, especially given that estimates of older divergences may be prone to greater errors (Edwards *et al.*, 2007). The timing of divergence of Hispaniolan taxa from the Puerto Rican–Virgin Island group at 15.6–10.4 Ma, however, is consistent with vicariance. Interestingly, the
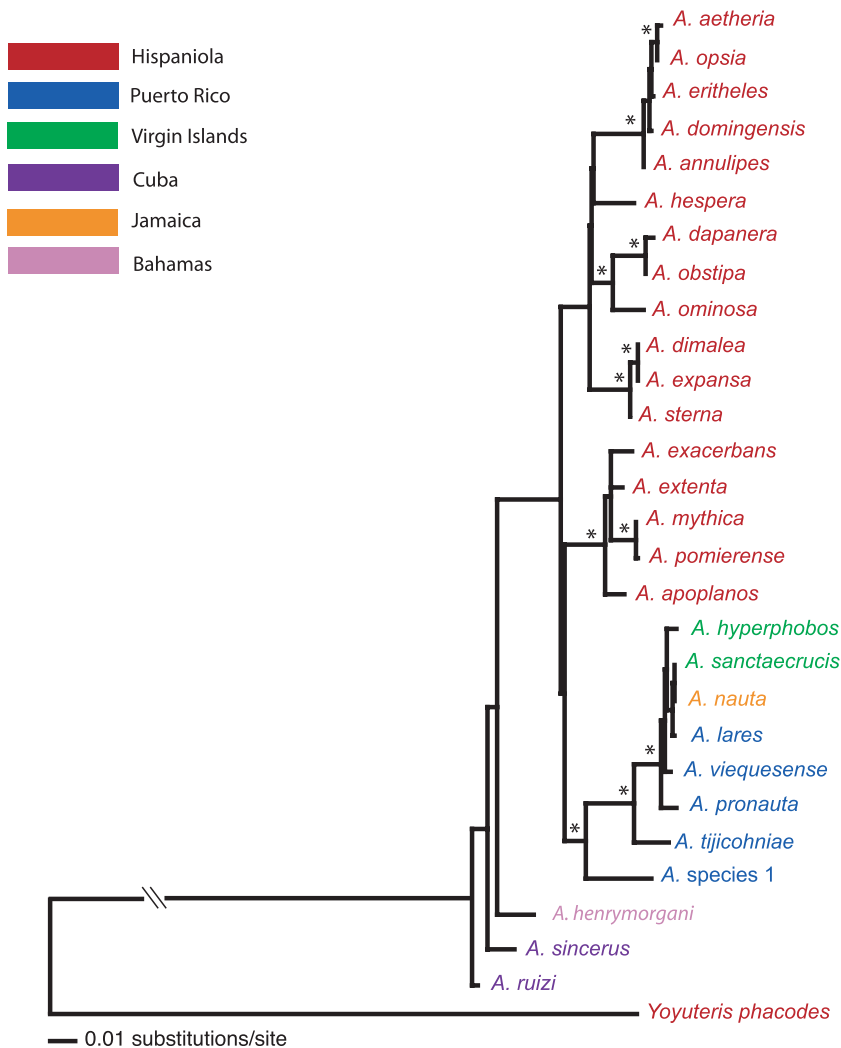
Figure 4 Estimated gene tree for Caribbean *Amphiacusta* from a maximum likelihood analysis of elongation factor 1-alpha (EF1-α), under a Timura–Nei+G model with base frequencies A = 0.2931, C = 0.1670, G = 0.2309, T = 0.3090, rate matrix rAC = 1, rAG = 2.17, rAT = 1, rCG = 2.17, rCT = 3.81, rGT = 1 and α = 0.30. *Bayesian posterior probabilities ≥ 95%.

finding that *A. henrymorgani* diverged from *A. ruizi* and *A. sincerus*, a split of a Bahaman taxon from Cuban taxa *c.* 3.0–2.0 Ma, is much older than expected if Pleistocene fluctuations of sea level drove speciation in that area (Hearty & Neumann, 2001). However, other researchers have found evidence for similarly deep divergences between Bahaman and Cuban taxa (Glor *et al.*, 2005), which suggest the possibility of refugia among the Bahaman islands (Glor *et al.*, 2005). Our result may also reflect errors in the estimated branch lengths of the species trees (e.g. variance in the estimate of the population-mutation parameter, θ, would affect the branch lengths of the species tree; Kubatko *et al.*, 2009; Liu, 2009).

In addition to a basal relationship between Cuban and other Greater Antillean taxa, a close affinity between eastern Cuban and northern and central Hispaniolan taxa has been taken as evidence for vicariance (McDowell *et al.*, 2003) because of the historical connections between these landmasses; however, we do not see this pattern in our data. The Hispaniolan clade sister to the Cuban/Bahaman clade is composed of species occurring in each of the north-eastern, south-western and south-eastern parts of Hispaniola (see Table 1). *Amphiacusta*

from these regions do not co-segregate, suggesting that intra-island diversification on Hispaniola may have occurred following the collision in the mid-Miocene (*c.* 15 Ma) between the landmasses that now comprise the southern and northern portions of the island (Graham, 2003). It must be noted that limited sampling of Cuban taxa makes it difficult to draw definitive conclusions about vicariance versus dispersal in this case. Cuba is the largest island and probably hosts a diversity of *Amphiacusta* species. The two Cuban species sampled are representatives from two out of the five different species groups defined by Otte & Perez-Gelabert (2009) on the basis of morphological, and specifically genitalic, differences. Therefore, while only a small proportion of the Cuban species were sampled, the sampling does at least encompass some of the breadth of species diversity found on the island. Nevertheless, additional sampling will be key to confirming the biogeographic scenario supporting vicariance in the estimated species tree (Fig. 5). Similarly, while the pattern of species splitting is consistent with vicariance (Fig. 1), with the limited sampling (both taxa and loci) the timing of divergence estimated from the species tree makes it difficult to rule out a possible role for
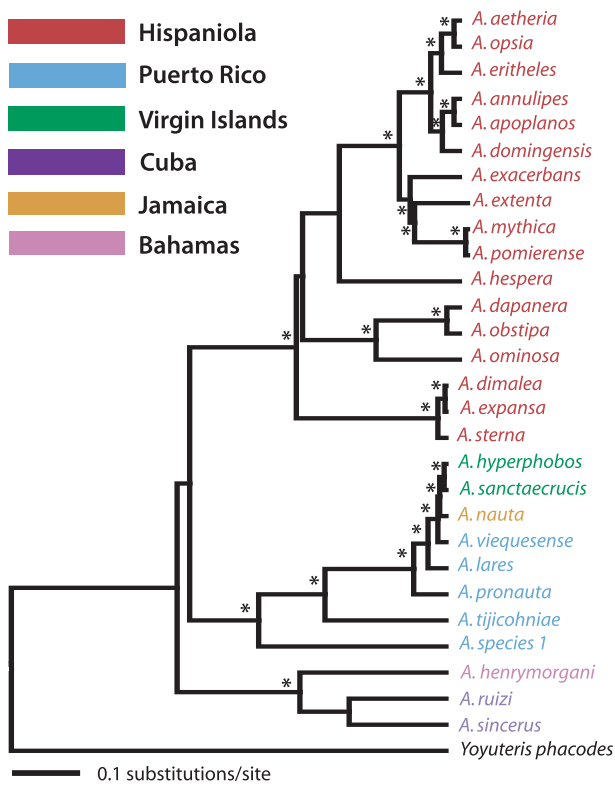
Figure 5 Species tree for Caribbean *Amphiacusta* estimated from the Bayesian program BEST (Liu, 2009). *Bayesian posterior probabilities ≥ 95%.

dispersal (i.e. while the divergence times are more recent than expected, errors for these deeper divergence dates are possible, as discussed above).

This is one of a few studies on the historical biogeography of terrestrial invertebrates in the Caribbean (see also Davies & Bermingham, 2002; Wilder & Hollocher, 2003; Brisson *et al.*, 2006; Velez & Feder, 2006). Most work on the biogeography of Caribbean fauna has focused on vertebrates (Ricklefs & Bermingham, 2004; Glor *et al.*, 2005; Perdices *et al.*, 2005; Dávalos, 2007; Heinicke *et al.*, 2007). Furthermore, several studies have investigated the ecological mechanisms and evolutionary dynamics driving the diversification of Caribbean fauna (Warheit *et al.*, 1999; Wilder & Hollocher, 2003; Langerhans *et al.*, 2006). Given that the analyses support a history of vicariance, with a limited role for dispersal, as well as a preponderance of intra-island diversification (in the case of Hispaniola and Puerto Rico), it is worthwhile to investigate what factors other than historical biogeography may have resulted in the great diversity of *Amphiacusta*. The substantial differences in male genital morphology among species raises the possibility that sexual selection on male genitalia, often suggested to promote the evolution of species diversity (Eberhard, 1985; Arnqvist, 1998), may play a role in diversification in this group. For example, the divergences between *A. hyperphobos* and *A. sanctaecrucis* and between Virgin Island and Puerto Rican species *Amphiacusta viequesense* all appear to be very recent, occurring within the last 478,000–

319,000 years. Yet, the species in this clade differ significantly in shape of the male genitalia at both the species and population level (E. Oneal, unpublished data), suggesting that selection has played a role in the diversification of the crickets (e.g. Masta & Maddison, 2002; Marquez & Knowles, 2007). With its island distribution and history of intra-island diversification, *Amphiacusta* presents an opportunity to examine the role of sexual selection in promoting diversification that would complement the large number of studies focused on the adaptive divergence of Caribbean taxa.

CONCLUSIONS

Tests of the biogeographic history of *Amphiacusta* support a predominant role for vicariance among islands and intra-island diversification, paralleling the movements of the Greater Antilles islands since the Oligocene. Specifically, an estimated species tree based on analyses of two independent molecular markers suggests a history of long-distance dispersal in the colonization of Jamaica that led to the formation of the Jamaican species *A. nauta*, with both the timing and pattern of divergence supporting a possible variance event separating the Hispaniolan and Puerto Rican clades, accompanied by *in situ* diversification within Hispaniola and Puerto Rico, as well as vicariant separation between Virgin Island and Puerto Rican taxa resulting from Pleistocene changes in sea level.

There are several limitations to our analysis. Our species tree is based on only two genes, and a lack of fossil evidence means that we must rely on previously published mutation rates (Brower, 1994). Moreover, while the estimation of the species tree accounts for variance between gene trees in the estimation of branch lengths, there remains some intrinsic variance in the estimation of branch lengths for more ancestral taxa (Edwards *et al.*, 2007). This, coupled with the limited sampling of taxa from Cuba (because of limited access), makes it difficult to rule out the possibility that the taxon sampled from Cuba might represent a secondary colonization from Hispaniola, while other Cuban species actually fit a vicariance model. However, as a clearer picture about the geographic and geological context of species diversification emerges with additional sampling of species (and perhaps loci), Caribbean crickets will provide a unique opportunity to investigate the role of sexual selection in promoting diversification, as the primary patterns of divergence in *Amphiacusta* are found in sexual characters and not in ecological traits, as is commonly found for other Caribbean taxa.

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