

Origins and Genetic Diversity of Introduced Populations of the Puerto Rican Red-Eyed Coquí, *Eleutherodactylus antillensis*, in Saint Croix (U.S. Virgin Islands) and Panamá

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The Red-eyed Coquí, *Eleutherodactylus antillensis*, is a terrestrial frog endemic to the Puerto Rican Bank (Puerto Rico and numerous islands and cays off its eastern coast), in the eastern Caribbean Sea. The species was likely introduced in Saint Croix, an island c. 100 km southeast of Puerto Rico, in the late 1930s, and in Panamá City, Panamá, in the late 1950s or early 1960s, but the source(s) of these introductions are unknown. We analyzed sequence data from one mtDNA locus and four nuDNA introns to infer the origin(s) of the Saint Croix and Panamá City populations and quantify their genetic diversity. Saint Croix and Panamanian populations do not share any haplotypes, and they cluster with different native populations, suggesting that they are derived from separate sources in the Puerto Rican Bank. Patterns of population structure trace the probable sources of *E. antillensis* in Saint Croix to islands off Puerto Rico's eastern coast, which include Vieques, Culebra, Saint Thomas, Saint John, Tortola, and Virgin Gorda, and possibly to eastern Puerto Rico as well. In contrast, Panamá City *E. antillensis* probably originated from either western or eastern Puerto Rico. Genetic diversity in the introduced populations is similar to or lower than in populations in the species' native range, indicating that genetic diversity has not increased in the alien frogs. Our findings may facilitate the development of preventive measures to minimize introductions of non-native amphibians in the Caribbean and Central America.

INVASIVE species are considered to be one of the main drivers of biodiversity loss in the Caribbean (Kairo et al., 2003; Waugh, 2009), one of the world's centers of biodiversity and endemism (Myers et al., 2000). A large number of amphibian and non-avian reptile introductions, both deliberate and accidental, have occurred in the Caribbean during the past 100 years, primarily due to the nursery trade and cargo shipping (Kraus, 2003). The high number of introduced herpetofauna in the Caribbean, combined with the small sizes of remaining native habitats, place endemic island species at particularly high risk (Li et al., 2016). Invasive herpetofauna can disrupt food webs and nutrient cycles, transmit disease, and lead to decreases in population density or extinction of endemic species. Accordingly, alien taxa can simplify, homogenize, and impact the evolution of native communities (Kraus, 2015).

Identifying the origin(s) and genetic characteristics of introduced, well-established amphibians and non-avian reptiles is central to understanding the circumstances that may facilitate their spread. Elucidating the origin(s) of introduced species provides insight into their dispersal pathways, information that is crucial for scientists and managers who aim to minimize or prevent future movements of non-native organisms (Wilson et al., 2009; Estoup and Guillemaud, 2010). Knowledge of levels of genetic diversity of introduced species may also be important, because elevated genetic diversity in alien populations can potentially increase their adaptive potential (Kolbe et al., 2004; Dlugosch and Parker, 2008; Dlugosch et al., 2015). A handful of genetic studies has pinpointed the sources of introduced Caribbean herpetofauna and clarified the origins of species whose status (i.e., native or introduced) in certain islands was unknown due to an absence of historical records (Hedges and Heinicke, 2007; Kolbe et al., 2007; Eales and

Thorpe, 2010; Heinicke et al., 2011). However, the origins and genetic diversity of most introduced amphibians and reptiles in the Caribbean remain unclear.

Eleutherodactylus antillensis, the Red-eyed Coquí (Anura: Eleutherodactylidae), is a small, terrestrial frog native to the Puerto Rican Bank, an archipelago in the eastern Caribbean Sea that comprises Puerto Rico and the Eastern Islands, which include Vieques, Culebra, the Virgin Islands (Saint Thomas, Saint John, Tortola, Virgin Gorda, Anegada), and more than 180 associated small cays (Fig. 1). The species also occurs on Saint Croix, a c. 215 km² island c. 100 km southeast of Puerto Rico (Fig. 1E) that is jurisdictionally part of the U.S. Virgin Islands, but that has never had a direct land connection to the Puerto Rican Bank, and probably is a separate biogeographical unit (Heatwole and MacKenzie, 1967). *Eleutherodactylus antillensis* was doubtfully reported from Saint Croix at the beginning of the 20th century (Stejneger, 1904; Barbour, 1914). The frog's presence on the island was confirmed in the late 1930s, but its occurrence presumably was the result of a deliberate release of an unknown number of individuals (Grant and Beatty, 1944; Powell et al., 2011). However, another report suggested that the species may be native to Saint Croix (Hedges, 1999). A previous molecular analysis of *E. antillensis* did not detect a single unique haplotype in Saint Croix (Barker et al., 2012), a result consistent with a relatively recent human-mediated introduction (or introductions). Nevertheless, the latter study was based on a small number of individuals from Saint Croix, and sparse sampling can bias conclusions about population genetic patterns (Muirhead et al., 2008; Geller et al., 2010). Thus, the question of whether populations of *E. antillensis* in Saint Croix are native or introduced is still unresolved.

An undocumented number of *E. antillensis* was introduced to the Federico Boyd Avenue and El Cangrejo neighborhood

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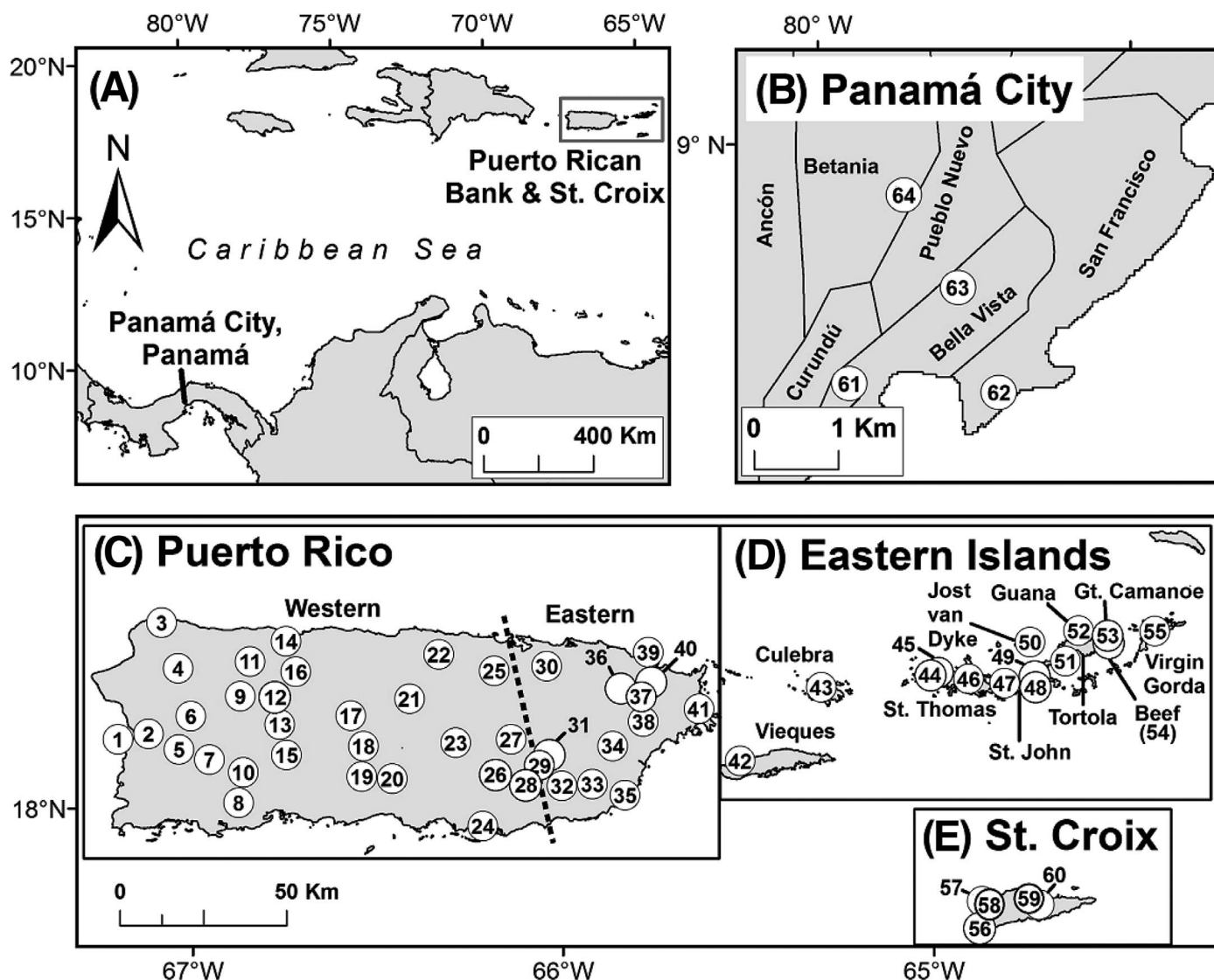


Fig. 1. Maps of (A) the Puerto Rican Bank, in the eastern Caribbean Sea, (A, B) Panamá City, Panamá, (C) Puerto Rico, (D) the Eastern Islands (specifically Vieques, Culebra, Saint Thomas, Saint John, Jost van Dyke, Tortola, Guana, Great Camanoe, Beef, Virgin Gorda), and (E) Saint Croix. Circles indicate the approximate geographic locations of the sampling localities of *Eleutherodactylus antillensis* (note that localities 57 and 58 were depicted as a single locality in Fig. 1 of Barker et al. [2012]). The lines within (B) Panamá City depict the borders of districts. The dotted line in Puerto Rico (C) delineates a phylogeographic break in *E. antillensis* (Barker et al., 2012) that we used to define two groups for genetic analyses (western Puerto Rico; eastern Puerto Rico).

of Panamá City, Panamá, in the late 1950s or early 1960s (de Sousa et al., 1989; Ibáñez et al., 1999, 2001). Frogs may have been deliberately (de Sousa et al., 1989) or accidentally introduced via imported ornamental plants (Roberto Ibáñez, pers. comm.). Since then, the frog has spread into suburban and rural gardens, abandoned lands, and pastures in the metropolitan area (de Sousa et al., 1989; Ibáñez et al., 1999). No additional introductions of *E. antillensis* in Panamá City have been reported.

We relied on an expanded DNA sequence dataset to infer the putative source(s) of populations of *E. antillensis* in Saint Croix and Panamá City, and to compare their levels of genetic diversity to those from the Puerto Rican Bank. We tested two hypotheses. First, Saint Croix populations originated via human-mediated introduction(s) from the Puerto Rican Bank, because of their low divergence from Puerto Rican Bank populations (Barker et al., 2012). Second, the Panamanian population originated from a source in Puerto Rico, because a local family visited the island before they

putatively released individuals of *E. antillensis* into their yard in the Bella Vista district of Panamá City (de Sousa et al., 1989). To test these hypotheses, we relied on an enhanced, multi-locus DNA sequence dataset that included samples of *E. antillensis* from across the species' range in the Puerto Rican Bank and Saint Croix (Barker et al., 2012). For the current study, we increased our sampling in Saint Croix and collected individuals from Panamá City. Additionally, we assessed levels of genetic diversity in *E. antillensis* from the Puerto Rican Bank, Saint Croix, and Panamá City, to determine whether genetic diversity has increased in the introduced frogs as a result of admixture between invaders from differentiated populations.

MATERIALS AND METHODS

Our sampling of *E. antillensis* encompassed its entire known range in the Puerto Rican Bank, Saint Croix, and Panamá City, Panamá (Fig. 1). For this study we added a fifth

Table 1. Geographical distribution of haplotypes of the mtDNA control region (CR) and four nuDNA intron loci (CRYBA, MYH, RH1, and RPL9int4) of *Eleutherodactylus antillensis* shared between the Puerto Rican Bank (western Puerto Rico, eastern Puerto Rico, Eastern Islands) and Saint Croix, U.S. Virgin Islands, and/or Panamá City, Panamá. Numbers represent the number of individuals with a particular haplotype.

Haplotypes	Western Puerto Rico	Eastern Puerto Rico	Eastern Islands	Saint Croix	Panamá City
CR					
H14	—	11	7	2	—
H35	2	3	6	1	—
H57	2	—	—	—	15
H78	—	—	4	30	—
H79	—	—	2	1	—
H99	—	—	—	3	—
CRYBA					
H1	153	61	74	56	20
MYH					
H1	158	45	37	8	20
H2	3	6	29	44	—
H10	—	—	1	4	—
RH1					
H1	75	53	47	37	20
H3	25	14	31	11	—
H8	—	—	3	8	—
RPL9int4					
H1	4	7	—	—	4
H2	19	8	1	—	6
H3	117	4	—	—	4
H4	12	9	3	5	—
H10	—	—	16	29	—
H11	1	4	56	22	—
H12	6	4	—	—	6

sampling locality in Saint Croix (Table S1, Supplemental information, see Data Accessibility), increased the total number of specimens from this island from 15 to 37, and added 15 individuals from Panamá City. The published dataset included mitochondrial (mtDNA) control region (CR; 552 bp) sequences of 285 individuals from 55 localities ($\bar{x} = 4.91$) in the Puerto Rican Bank and Saint Croix, and nuclear DNA (nuDNA) sequences of four intron-spanning loci (β -crystallin [intron 1; CRYBA; 192 bp], myosin heavy chain [putative flanking intron of exon 36; MYH; 463 bp], rhodopsin [intron 1; RH1; 596 bp], and ribosomal protein L9 [intron 4; RPL9int4; 382 bp]) of two to three ($\bar{x} = 2.98$) randomly chosen individuals per locality (Barker et al., 2012). Published sequences are available in GenBank under accession numbers JN385299–JN385511 (CR), JN385586–JN385931 (CRYBA), JN385932–JN386277 (MYH), JN386278–JN386623 (RH1), and JN386628–JN386969 (RPL9int4; Table S1, Supplemental information). We generated sequence data for these five loci for the newly acquired samples. The final dataset for Saint Croix included CR sequences of 37 frogs from five localities (range = 6–9; $\bar{x} = 7.4$), and nuDNA sequences of 28 individuals from these locations (range = 4–7; $\bar{x} = 5.6$). The dataset for Panamá City included CR sequences of 15 frogs from four localities (localities 61–64; Fig. 1B; $\bar{x} = 3.8$), and nuDNA from a subset of these individuals ($\bar{x} = 2.5$) from each location. Because of low sample size and close proximity (<1.5 km), we pooled

samples from localities 62–64 in Panamá City (Fig. 1B) for genetic analyses. Voucher specimens associated with this study are deposited at the Museum of Southwestern Biology (MSB), University of New Mexico, Albuquerque, or the Museum of Vertebrate Zoology (MVZ), University of California, Berkeley.

We isolated DNA using a modified cetyl trimethylammonium bromide/polyvinylpyrrolidone (CTAB/PVP)–chloroform/isoamyl alcohol DNA extraction technique (Stewart and Via, 1993). Information on primers and PCR amplification conditions are reported elsewhere (Barker et al., 2012). We edited the new sequences using SEQUENCHER 4.5 (Gene Codes Corporation, Ann Arbor, MI; <http://www.genecodes.com>) and deposited them in GenBank (accession numbers KY636451–KY636719; Table S1, Supplemental information). We aligned sequences in MAFFT 6 (Katoh et al., 2002). For heterozygote individuals polymorphic at more than one position, the gametic phase was inferred using PHASE 2.1 (Stephens et al., 2001; Stephens and Donnelly, 2003). For the RPL9int4 locus, PHASE analyses included known haplotypes that were reconstructed via cloning (Barker et al., 2012), and multi-base insertion-deletions (indels) were collapsed into single-base polymorphisms. Three independent PHASE runs from different starting seeds were run for 1,000 iterations, with a single thinning interval and 100 burn-in iterations. Phase posterior probabilities of ≥ 0.85 were obtained at each position across all individuals for each locus.

We explored the geographic distribution of haplotypes and quantified population structure of *E. antillensis* to infer the source(s) of the Saint Croix and Panamá City populations of this frog. Accuracy of assignment of source populations depends on the degree of genetic differentiation among them (Muirhead et al., 2008). We expected the accuracy of assignment of source population(s) to be at the scale of western Puerto Rico (localities 1–28; Fig. 1C), eastern Puerto Rico (localities 29–41; Fig. 1C), and the Eastern Islands (localities 42–55; Fig. 1D), because *E. antillensis* exhibits genetic differentiation among these regions (Barker et al., 2012, 2015). To identify the native-range geographic distribution of CR haplotypes and explore relationships between introduced and native populations, we generated a maximum parsimony network for CR haplotypes with NETWORK 4.2 (Polzin and Daneschmand, 2003; <http://www.fluxus-technology.com>). We used our PHASE output to elucidate the native-range geographic distribution of nuDNA haplotypes in Saint Croix and Panamá City. We visualized population structure with a principal coordinates analysis (PCoA) of a haplotype dataset that included 202 individuals that had both CR and nuDNA sequence data. The PCoA was performed with the ADEGENET 1.4 and ADE4 packages (Dray and Dufour, 2007; Jombart, 2008; Jombart and Ahmed, 2011) in R 3.2.3 (R Development Core Team, 2015).

We compared levels of genetic diversity in populations in Saint Croix and Panamá City to those in the Puerto Rican Bank, to assess whether genetic diversity has increased in the introduced frogs, perhaps because of admixture between colonists from various source regions (western Puerto Rico, eastern Puerto Rico, and/or the Eastern Islands). We removed from these analyses a single population from the island of Great Camanoe (locality 53; Fig. 1D) due to its low sample size ($n = 2$). We estimated nucleotide diversity (π) across all populations in western Puerto Rico, eastern Puerto Rico, the Eastern Islands, Saint Croix, and Panamá City with ARLEQUIN 3.5 (Excoffier and Lischer, 2010), and calculated the average π for each region. We estimated haplotype richness

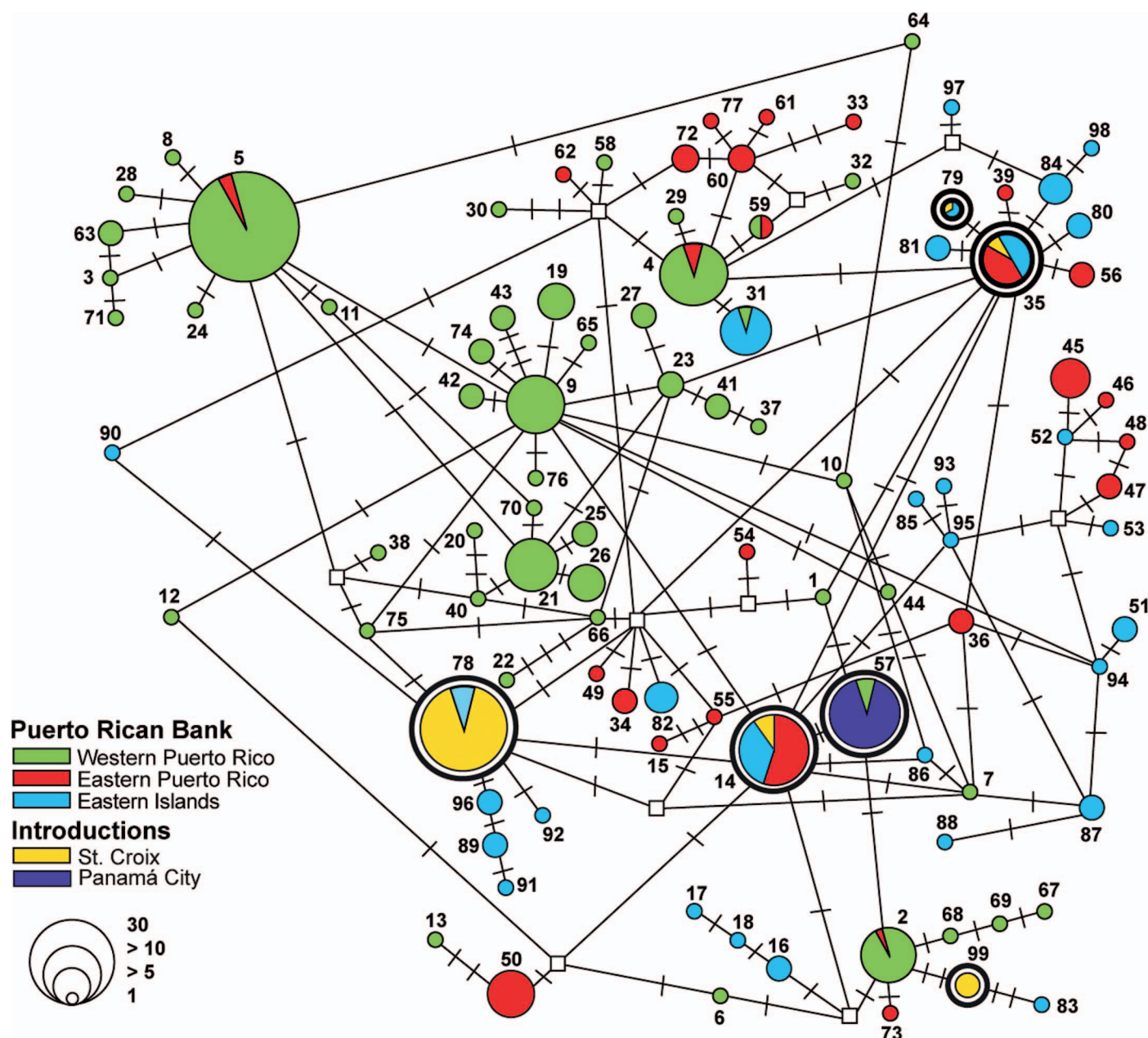


Fig. 2. Maximum parsimony network representing the relationships among haplotypes of the mtDNA control region of 323 *Eleutherodactylus antillensis* from their native ranges in the Puerto Rican Bank (western Puerto Rico [green], eastern Puerto Rico [red], Eastern Islands [light blue]), and their introduced ranges in Saint Croix (yellow) and Panamá City, Panamá (violet). Indels were included in this analysis. Haplotypes detected in the introduced ranges are indicated with a thick, black line. Circles represent unique haplotypes; hatch marks depict single mutations; empty squares indicate missing (i.e., unsampled or extinct) haplotypes. Circle size is proportional to haplotype frequency. (Modified from Barker et al., 2012.)

(H_R) across all populations in ADZE v. 1.0 (Szpiech et al., 2008), which uses rarefaction to correct for unequal sample sizes, because H_R can be heavily influenced by variation in sample size (Leberg, 2002). We calculated average H_R for each region after randomly sampling four individuals for the CR dataset and three individuals for the nuDNA datasets from each population, to match the smallest population sample size.

RESULTS

Our analyses revealed differences in the haplotypes of *E. antillensis* present in Saint Croix and Panamá City, as well as in the population genetic structure of the frog at these two locations. Five CR haplotypes, one CRYBA haplotype, two

MYH haplotypes, three RH1 haplotypes, and three RPL9int4 haplotypes were detected in Saint Croix (Fig. 2; Tables 1, 2). Four (H14, H35, H78, H79) of the five CR haplotypes were distributed in the Eastern Islands, and two (H14, H35) of these were also found in Puerto Rico. A fifth CR haplotype (H99) was only detected in Saint Croix. Seven nuDNA haplotypes in Saint Croix were recovered from at least two of the three regions of the Puerto Rican Bank (western Puerto Rico, eastern Puerto Rico, Eastern Islands). Single haplotypes each of MYH, RH1, and RPL9int4 (H10, H8, H10, respectively) were found only in the Eastern Islands (Table 1). The PCoA analysis indicated that individuals in Saint Croix clustered with those in the Eastern Islands, although the 95% confidence ellipses of individuals from Saint Croix and eastern Puerto Rico overlapped slightly (Fig. 3).

Table 2. Total number of haplotypes (H), average haplotype richness corrected for unequal sample size (H_R), and nucleotide diversity (π) for populations of *Eleutherodactylus antillensis* in their native range (western Puerto Rico, eastern Puerto Rico, the Eastern Islands) and their introduced ranges in Saint Croix, U.S. Virgin Islands, and Panamá City, Panamá, estimated from sequences of the mitochondrial DNA control region (CR) and four nuclear DNA loci (CRYBA, MYH, RH1, and RPL9int4).

Locus	Total H	Western Puerto Rico			Eastern Puerto Rico		
		(28 populations)			(13 populations)		
		H	H_R	π	H	H_R	π
CR	99	50	2.71 (0.15)	0.0407 (0.0044)	26	2.42 (0.28)	0.0394 (0.0064)
CRYBA	6	4	1.19 (0.07)	0.0017 (0.0006)	4	1.60 (0.11)	0.003 (0.0009)
MYH	10	5	1.16 (0.06)	0.0003 (0.0001)	6	1.87 (0.12)	0.0016 (0.0003)
RH1	8	4	1.75 (0.07)	0.0017 (0.0002)	6	1.66 (0.12)	0.0009 (0.0002)
RPL9int4	39	13	1.65 (0.11)	0.003 (0.0007)	29	2.57 (0.11)	0.0094 (0.0013)

One haplotype each of CR, CRYBA, MYH, and RH1, and four RPL9int4 haplotypes were detected in Panamá City (Fig. 2; Tables 1, 2). The single CR haplotype (H57) in Panamá City also occurred in western Puerto Rico, whereas the CRYBA,

MYH, and RH1 haplotypes detected in Panamá City were recovered from all three regions of the Puerto Rican Bank. Three (H1, H3, H12) of the four RPL9int4 haplotypes in Panamá City were detected in western and eastern Puerto Rico (Table 1). Panamanian individuals clustered with those in western and eastern Puerto Rico (Fig. 3).

Estimates of genetic diversity in populations of *E. antillensis* in Saint Croix and Panamá City obtained from the five assayed loci were similar to or lower than those of populations in the species' native range (Table 2). Saint Croix populations exhibited variation at four of the five loci (CRYBA being the exception), whereas the Panamá City populations only displayed variation at RPL9int4. Estimates of average haplotype richness (H_R) and nucleotide diversity (π) for CR were lower in populations in Saint Croix and Panamá City, compared to those in the frog's native range. Estimates of average H_R and π of CRYBA, MYH, RH1, and RPL9int4 in Saint Croix populations were similar to those found in *E. antillensis* from the Puerto Rican Bank, whereas those in Panamanian populations were substantially lower, due to lack of variation at most loci.

DISCUSSION

We relied on genetic data to infer the origin(s) and quantify levels of genetic diversity of populations of *E. antillensis* in Saint Croix and Panamá City, because this knowledge can help elucidate the factors responsible for biological invasions and inform management recommendations for minimizing introductions of herpetofauna in the Caribbean. Our analyses support the hypothesis that *E. antillensis* arrived on Saint Croix via a human-mediated introduction (or introductions; Stejneger, 1904; Barbour, 1914; Grant and Beatty, 1944). Four of the five mtDNA haplotypes (the exception being H99), and all ten nuDNA haplotypes in Saint Croix were also detected in the Puerto Rican Bank (Fig. 2; Table 1), a scenario consistent with a relatively recent colonization event (or events) in Saint Croix. Many mtDNA (CR) haplotypes in the Puerto Rican Bank were detected at a single locality (Barker et al., 2012), which suggests that H99 may exist in an unsampled or undersampled source population. Patterns of population structure trace the probable source(s) of the Saint Croix populations to the Eastern Islands, and possibly to eastern Puerto Rico as well (Fig. 3), regions geographically close to Saint Croix.

A natural, recent arrival of *E. antillensis* to Saint Croix is possible, but less likely than a human-mediated introduction event (or events). To colonize Saint Croix naturally, *E. antillensis* would have had to disperse to this island via

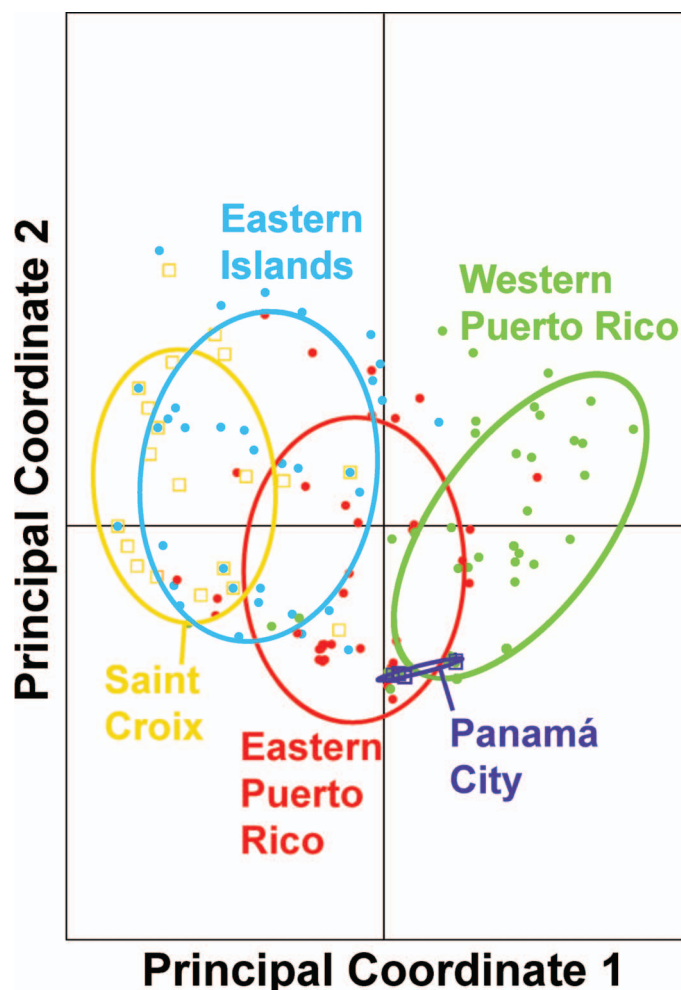


Fig. 3. Principal coordinates analysis based on haplotypes of one mtDNA locus (CR) and four nuDNA loci (CRYBA, MYH, RH1, RPL9int4) from 202 *Eleutherodactylus antillensis* from their native ranges in the Puerto Rican Bank (western Puerto Rico [green, $n = 84$], eastern Puerto Rico [red, $n = 39$], Eastern Islands [light blue, $n = 41$]) and their introduced ranges in Saint Croix (yellow, $n = 28$) and Panamá City, Panamá (violet, $n = 10$). Ovals are 95% confidence ellipses representing regional groups. Individuals from the Puerto Rican Bank are depicted by circles, whereas individuals from Saint Croix and Panamá City are represented by empty squares.

Table 2. Extended.

Eastern Islands			Saint Croix			Panamá City		
(13 populations)			(5 populations)			(2 populations)		
H	H_R	π	H	H_R	π	H	H_R	π
30	2.44 (0.28)	0.0357 (0.0072)	5	1.56 (0.28)	0.0015 (0.0004)	1	1.0 (0.0)	0.0 (0.0)
3	1.08 (0.05)	0.003 (0.0002)	1	1.0 (0.0)	0.0 (0.0)	1	1.0 (0.0)	0.0 (0.0)
5	1.92 (0.14)	0.0019 (0.0003)	3	1.36 (0.16)	0.0009 (0.0004)	1	1.0 (0.0)	0.0 (0.0)
4	1.62 (0.09)	0.0008 (0.0002)	3	1.67 (0.15)	0.0008 (0.0002)	1	1.0 (0.0)	0.0 (0.0)
9	1.75 (0.16)	0.0038 (0.0008)	3	1.73 (0.09)	0.0041 (0.0006)	4	2.38 (0.06)	0.0072 (0.001)

rafting, because Saint Croix has never had a direct land connection with the Puerto Rican Bank (Heatwole and MacKenzie, 1967). The overall absence of differentiation among Saint Croix and Puerto Rican Bank populations (Figs. 2, 3) implies that an over-water dispersal event would have occurred recently. Over-water dispersal of *E. antillensis* among islands has probably been limited, because anurans are intolerant of saltwater (Balinsky, 1981; Duellman and Trueb, 1994). Indeed, successful over-water dispersal via rafting has rarely occurred in the history of eleutherodactyline frogs (Heinicke et al., 2007, 2011), and survivorship of organisms floating on flotsam in the Puerto Rican Bank is low (Heatwole and Levins, 1972). Further, prevailing surface currents in the eastern Caribbean Sea travel in a northwestern direction, which makes transport of flotsam from the Puerto Rican Bank to Saint Croix exceedingly difficult (Heatwole and MacKenzie, 1967). Whereas several studies have documented human-mediated transport of *Eleutherodactylus* frogs among Caribbean islands (Platenberg, 2007; Powell et al., 2011) and to mainland countries (Crawford et al., 2011; Heinicke et al., 2011; Cedeño-Vázquez et al., 2014) in the past 100–200 years, none has reported evidence of colonization of islands by these anurans via rafting over the same time period. For instance, *E. coqui* (Common Coqui), a co-distributed relative of *E. antillensis*, was introduced to Saint Croix, Saint John, and Saint Thomas (Schwartz and Thomas, 1975; Schwartz and Henderson, 1991), most likely as a result of horticultural and landscaping imports from Puerto Rico (Platenberg, 2007). Additionally, *E. lentus* (Mute Frog) was likely introduced to Jost Van Dyke in the British Virgin Islands (Perry, 2009), and *E. johnstonei* (Johnstone's Whistling Frog) was introduced to Tortola and Jost Van Dyke in the British Virgin Islands (Powell et al., 2011). The nearly ubiquitous presence of *E. antillensis* in residential gardens and plant nurseries in Puerto Rico (Joglar, 1998), frequent transport of horticultural and landscaping materials across the Puerto Rican Bank and Saint Croix, and a long history of species introductions in Saint Croix (Platenberg, 2007) point to accidental or intentional transport of individuals and/or eggs of *E. antillensis* to this island. Nevertheless, genetic evidence suggests that some anuran species have crossed salt-water barriers via rafting (Vences et al., 2003; Heinicke et al., 2007; Bell et al., 2015; Duryea et al., 2015), and therefore we cannot entirely discount the possibility that *E. antillensis* dispersed to Saint Croix naturally.

Our findings support the hypothesis that Panamá City populations of *E. antillensis* originated from Puerto Rico (de Sousa et al., 1989). The single mtDNA haplotype and three of the seven nuDNA haplotypes in Panamá City were only

detected in Puerto Rico (Fig. 2; Table 1), and Panamanian individuals clustered with those from western and eastern Puerto Rico (Fig. 3). Populations in Panamá City did not share any haplotypes with those in Saint Croix, and clustered with different native populations, suggesting that the Panamanian and Saint Croix populations are derived from separate sources in the Puerto Rican Bank.

Identifying the precise source(s) and number of introductions was not possible with our dataset, because *E. antillensis* exhibits little genetic structure at small spatial scales in the Puerto Rican Bank (Barker et al., 2012, 2015). Additionally, our sampling in the native range included five or fewer individuals per locality, which raises the possibility that we did not detect all haplotypes present in those populations. Accurate inferences of the precise location and number of sources often require dense sampling across the species' native range, and these conclusions may still only be possible when the species has significant genetic structure at fine spatial scales (Muirhead et al., 2008; Geller et al., 2010). Therefore, resolving the exact source(s) of populations of *E. antillensis* in Saint Croix and Panamá City and determining the precise number of introductions to each region likely requires additional sampling across the Puerto Rican Bank, as well as the analysis of hundreds or thousands of genome-wide single nucleotide polymorphisms (SNPs) and/or of rapidly evolving markers, such as microsatellites. Nevertheless, estimates of genetic diversity suggest two different scenarios for the origins of Saint Croix and Panamanian *E. antillensis*. First, it is improbable that this frog arrived in Saint Croix from various localities in different regions of the Puerto Rican Bank, because this would have likely resulted in Saint Croix populations having higher levels of genetic diversity than native populations (e.g., Kolbe et al., 2004, 2007), which is not the case. Second, populations in Panamá City lacked variation in mtDNA and in three of the four nuDNA loci assayed, which is consistent with the documented single introduction event there (de Sousa et al., 1989). However, we cannot rule out the possibility of multiple introductions to Panamá City from the same source in the Puerto Rican Bank.

Admixture between colonists from different source regions may increase genetic diversity of introduced populations, resulting in the evolution of new invasive genotypes (Kolbe et al., 2004, 2007; Keller et al., 2014; Dlugosch et al., 2015). Indeed, an increase in genetic variation has been implicated in the successful invasion of various amphibian species (Kraus, 2015). Nevertheless, we documented levels of genetic diversity in populations of *E. antillensis* in Saint Croix and Panamá City that were similar to or lower than those found in the Puerto Rican Bank, a result consistent with studies of

anuran species that have become successful invaders in the absence of elevated genetic diversity. At least two independent introductions established *E. coqui* in the Hawaiian Islands, and this species has thrived despite a loss of genetic diversity in those non-native populations (Peacock et al., 2009). Other successful invasions by populations with low genetic diversity have been documented for *Rhinella marina* (Cane Toad) in Australia (Slade and Moritz, 1998; Leblois et al., 2000; Lillie et al., 2014), *E. planirostris* (Greenhouse Frog) in Florida (Heinicke et al., 2011), *Lithobates catesbeianus* (American Bullfrog) in Europe (Ficetola et al., 2008) and China (Bai et al., 2012), *Xenopus laevis* (African Clawed Frog) in Sicily (Lillo et al., 2013) and Chile (Lobos et al., 2014), and *Litoria dentata* (Bleating Tree Frog) in Lord Howe Island, off Australia's southeastern coast (Plenderleith et al., 2015). As we did, all these studies measured genetic diversity at putatively neutral markers, but the progress of an invasion may be more strongly influenced by the types of genetic variation introduced, rather than the magnitude of that variation (Dlugosch et al., 2015). Additionally, changes in gene expression may allow invasive amphibians to adapt to novel environments in situations where genetic diversity is low (Rollins et al., 2015).

Our documentation of the sources of introduced populations of *E. antillensis* may contribute to the development of preventive measures to minimize introductions of non-native anurans in the Caribbean, and to the creation of management plans for this species in Saint Croix and Panamá. Identifying the origin(s) of introduced species can provide valuable information, such as modes of dispersal of non-native species and common dispersal pathways, knowledge that managers can use to create specific monitoring and quarantine measures to minimize or prevent future, unintentional transport of organisms (Kairo et al., 2003; Waugh, 2009). Saint Croix has a high proportion of endemic species that are threatened with extinction mediated by habitat loss and negative interactions with non-native herpetofauna, including direct predation, competition for limited resources (e.g., breeding sites, prey), and disease transmission (Platenberg, 2007; Treglia et al., 2013). The determination that *E. antillensis* is probably not native to this island is relevant. The frog is presently common across the western portion of Saint Croix (Hedges, 2017), but its potential impact on endemic species is yet undocumented. In Panamá City, populations of *E. antillensis* are patchily distributed and have spread slowly since their introduction (de Sousa et al., 1989; Roberto Ibáñez, pers. comm.). However, the possibility that the frog colonizes the extensively cultivated and pasture lands outside Panamá City is concerning, because *E. antillensis* reaches high densities in similar habitat types in Puerto Rico (Herrera-Montes and Brokaw, 2010). *Eleutherodactylus antillensis* has been diagnosed with the chytrid fungus *Batrachochytrium dendrobatidis* in certain regions of Puerto Rico (Burrowes et al., 2008), which raises the prospect that it could transmit this pathogen to Bd-sensitive native amphibians in Central America. Further, invasive *Eleutherodactylus* frogs may outcompete native species, threaten native invertebrates, alter ecosystem processes, and reduce property values by creating noise pollution (Platenberg, 2007; Beard et al., 2009; Rödder, 2009). Accordingly, we recommend that additional research be conducted to evaluate possible ecological impacts of introductions of *E. antillensis*, and determine whether control measures are necessary. Chemical (e.g., citric acid) and mechanical controls (e.g., traps and hand capture) have been effective for minimizing the spread

of *E. coqui* in Hawaii (Beard and Pitt, 2012), and may provide a mechanism for controlling introduced populations of *E. antillensis*. Minimizing the spread of non-native species in the Caribbean and Central America is critical for reducing their negative impacts on native taxa, ecosystems, and local economies in these regions (Kairo et al., 2003; Waugh, 2009).

DATA ACCESSIBILITY

Supplemental material is available at <http://www.copeiajournal.org/cg-16-501>.

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