Biological Journal of the Linnean Society, 2020, **129**, 114–127. With 5 figures.

Historical allopatry and secondary contact or primary intergradation in the Puerto Rican crested anole, *Anolis cristatellus*, on Vieques Island in the Caribbean

QUYNH N. QUACH[1,*,†], R. GRAHAM REYNOLDS[2] and LIAM J. REVELL[1,3,*]

[1]*Department of Biology, University of Massachusetts Boston, Boston, MA 02125, USA*
[2]*Department of Biology, University of North Carolina Asheville, Asheville, NC 28804, USA*
[3]*Departamento de Ecología, Universidad Católica de la Santísima Concepción, Concepción, Chile*

Received 17 August 2019; revised 23 September 2019; accepted for publication 24 September 2019

Recent work has revealed surprisingly deep mitochondrial genetic divergence in the lizard *Anolis cristatellus* among samples obtained from the small Caribbean island of Vieques. Here we sought to determine whether this had resulted from natural or anthropogenic causes, and (if the former) whether divergence occurred in a biogeographical context of allopatry followed by secondary contact, or via isolation-by-distance across the species' historical range. We first estimated a mitochondrial gene tree for 379 samples and then genotyped 3407 single nucleotide polymorphic sites from 48 individuals using a modified genotyping-by-sequencing approach. We found that *A. cristatellus* samples from Vieques belong to two highly divergent mitochondrial subclades, but the geographical distribution of these haplogroups indicates that this pattern is probably natural in origin. Analysis of our single nucleotide polymorphic dataset revealed differentiation that is consistent with isolation-by-distance between the western and eastern ends of Vieques, suggesting that the overall pattern of divergence probably reflects primary intergradation with a mitochondrial break on the historical Puerto Rico Bank palaeo-island that happened to coincide with the present-day location of Vieques. Our findings help to underline the growing consensus that results from a single genetic marker can prove highly misleading in studies of historical population genetic structure.

ADDITIONAL KEYWORDS: gene flow – genomic – isolation-by-distance – mitochondrial DNA – phylogeography.

INTRODUCTION

In recent decades, mitochondrial DNA (mtDNA) has been widely used for reconstructing phylogenies, measuring population genetic processes and inferring patterns of historical biogeography (Paulo *et al.*, 2001; Kolbe *et al.*, 2007; Campbell-Staton *et al.*, 2012). With a high evolutionary rate, a lower effective population size than the nuclear genome, and minimal recombination, mtDNA can sometimes permit the reconstruction of relatively recent historical events useful in phylogeographical studies in areas with a complex geological history (Galtier *et al.*, 2009). Deep mitochondrial divergence within widespread *Anolis* lizard species (anoles) in the Greater and Lesser Antilles has been well documented (e.g. Glor *et al.*, 2003; Kolbe *et al.*, 2004; Thorpe *et al.*, 2005; Rodriguez-Robles *et al.*, 2007). In some cases, mitochondrial divergence between populations of a widespread anole can be equal to or greater than genetic differentiation of sister species in other groups (e.g. Kolbe *et al.*, 2004). Deep mitochondrial divergence within species might be a symptom of a very large effective population size (Smith & Klicka, 2013) or of ancient historical allopatry even if divergence has been succeeded by secondary contact and gene flow between once-isolated reaches of a species' range (Schneider, 1996). Occasionally, these divergence patterns manifest in lineages that otherwise show no apparent geographical or ecological separation, a phenomenon that might result from the above historical scenarios, or via a third: anthropogenic introduction. For example, Kolbe *et al.* (2004, 2007) have shown that multiple mtDNA lineages of an invasive anole species (*Anolis sagrei*) can coexist in small areas in Florida – a consequence of multiple source populations during the introduction.

*Corresponding authors. E-mail: qquach@tulane.edu; liam.revell@phytools.org
†Current address: Department of Ecology and Evolutionary Biology, Tulane University, New Orleans, LA 70118, USA.

Several years ago, as part of a related project, we discovered that samples of the Puerto Rican crested anole (*Anolis cristatellus*) mtDNA gene NADH dehydrogenase 2 (*ND2*) from the island of Vieques originated from at least two deeply divergent mitochondrial clades (Reynolds *et al.*, 2017). One of these clades was nested within *A. cristatellus* of the Puerto Rican Bank Virgin Islands; however, the other was more closely allied with samples that we had obtained from the metropolitan area of San Juan on the main island of Puerto Rico to the west of Vieques. Because *A. cristatellus* from Vieques have long been recognized as being affiliated with Virgin Islands populations to the east (owing to similarities in dewlap colour), this discovery opened the intriguing possibility that novel genetic material from the main island of Puerto Rico had been anthropogenically introduced to the island of Vieques via trade, vehicular or boat traffic, or through intentional transport of animals. This inspired us to undertake the most thorough genetic sampling effort to date of *A. cristatellus* on the island of Vieques (Fig. 1) – an effort that forms the basis for the research presented herein.

Anolis cristatellus is a medium-sized 'trunk-ground' anole (so-called because of its ecomorphological specialization on this microhabitat component; Losos, 2009). It is widespread throughout the main island of Puerto Rico, as well as the Spanish (Vieques and Culebra), British and United States Virgin Islands (excluding Saint Croix), and most other islands of the Puerto Rico Bank (Brandley & de Queiroz, 2004). *Anolis cristatellus* is separated into two subspecies based mostly on differences in dewlap colour: *A. c. cristatellus* on Puerto Rico and *A. c. wileyae* on the Virgin Islands, including Vieques (Grant, 1931; Heatwole, 1976). Nevertheless, *A. cristatellus* shows considerable phenotypic and genetic variation across its range (Heatwole, 1976; Hahn & Köhler, 2010). Dewlap colour is variable across populations (Leal & Fleishman, 2004) and in our experience can occasionally be insufficient to predict subspecific assignment (this study). In previous research, *A. cristatellus* populations on the Virgin Islands and Puerto Rico have been found to form deeply divergent mtDNA clades, and even populations on the main island of Puerto Rico show strong phylogeographical structure in the absence of known barriers to gene flow (Rodriguez-Robles *et al.*, 2007; Reynolds *et al.*, 2017).

Vieques is a Spanish Virgin Island (~135 km^2) that lies ~10 km south-east of the main island of Puerto Rico (Fig. 1). The Spanish, US and British Virgin Islands (hereafter the Virgin Islands) emerged in the late Eocene and have been connected via a land bridge to Puerto Rico at various times throughout prehistory (excluding Saint Croix, which is on its own bank), including during a long intraglacial period that lasted from ~119 000 to 15 000 years before the present day (Heatwole & MacKenzie, 1967; Thomas & Joglar, 1996; Severinghaus & Brook, 1999; Trejo-Torres & Ackerman, 2001; Siddall *et al.*, 2003; Brandley & de Queiroz, 2004). Note that the island of Vieques was the last of the Virgin Islands to become isolated from the main island of Puerto Rico and may have been connected by a land bridge for as long as 2000 years after the separation of the remainder of the archipelago (Heatwole & MacKenzie, 1967).

A recent mtDNA study of *A. cristatellus* throughout the Virgin Islands (Reynolds *et al.*, 2017) revealed that individuals sampled from Vieques belonged to two distinct clades, referred to in that study as 'Puerto Rico East' and 'Virgin Islands' based on their geographical affinities. The former clade (Puerto Rico East) is most closely allied with individuals sampled from the metropolitan area of San Juan and from eastern Puerto Rico, whereas the latter (Virgin Islands) is more closely related to samples obtained from other eastward Virgin Islands. These two clades are deeply divergent genetically at the mtDNA locus *ND2* (estimated 7–7.5% nucleotide sequence divergence to a common ancestor), which led to our aforementioned preliminary hypothesis that the Puerto Rico East genetic material, closely related to samples obtained near densely populated areas on the main island, may have spread to Vieques via anthropogenic activities.

While a number of reptiles on Vieques are thought to share recent evolutionary affinity with the main island of Puerto Rico (Heatwole & McKenzie, 1967; Perry & Gerber, 2006; Mayer, 2012), other studies of Puerto Rican fauna and flora (e.g. on anurans and orchids) have shown greater overlap between Vieques and the other Virgin Islands than with the main island (Hedges, 1999; Trejo-Torres & Ackerman, 2001; Barker *et al.*, 2012). Anthropogenic introduction of novel genetic material from the main island of Puerto Rico to Vieques also seemed reasonable given the history of the two islands. Human settlement on Vieques dates back at least 7000 years (Burney, 1994; Sara *et al.*, 2003). Under Spanish rule, thousands of migrants came to Vieques to work on sugarcane plantations in the 19th century, an activity that utilized the majority of the island's arable lands. During this time, many invasive species such as feral cats, dogs, cattle and horses, along with the Indian mongoose (*Herpestes javanicus*), were also introduced. Ceded to the USA in 1898, the US Navy owned two-thirds of Vieques Island which it used as a practice and training ground from 1941 to 2003. This major Navy presence resulted in substantial air and boat traffic to and from the main island, particularly between Vieques and the now decommissioned US Navy base Roosevelt Roads in the main island

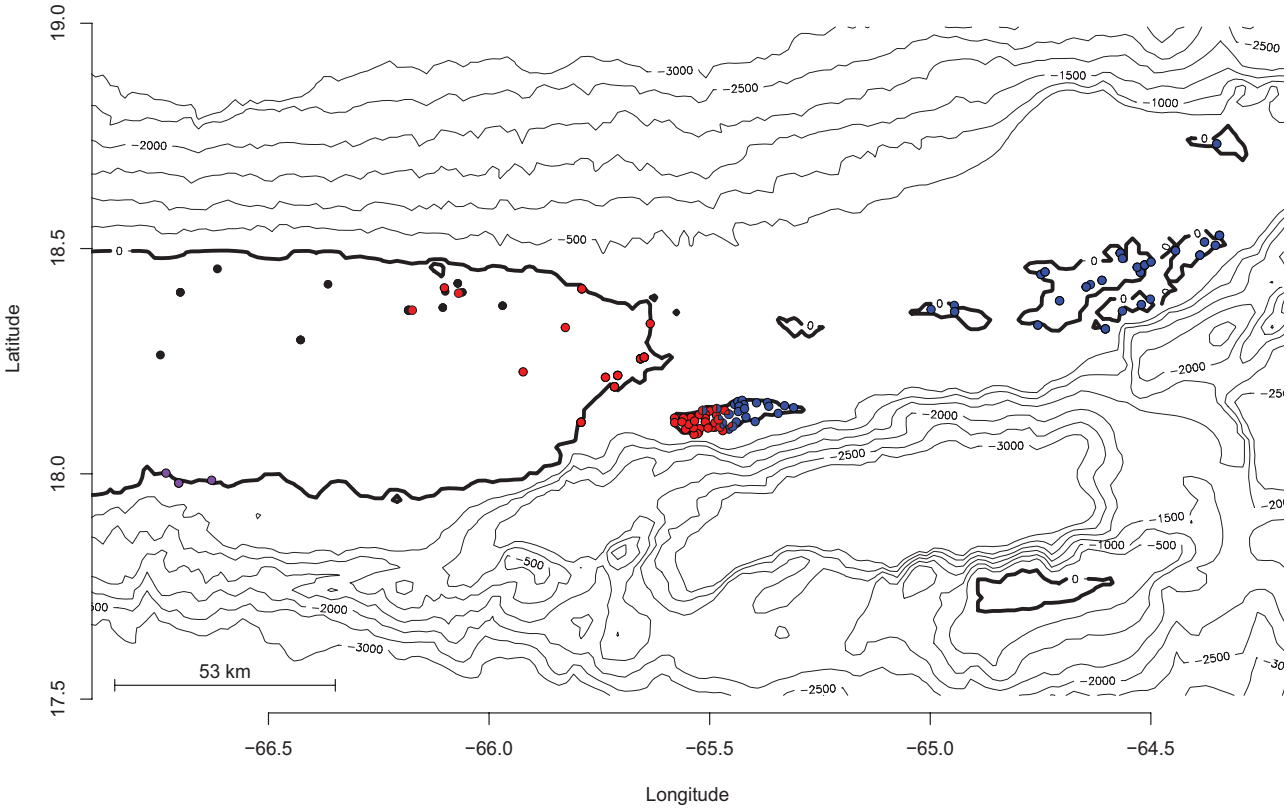

Figure 1. Sampling locations of *Anolis cristatellus* with axes denoting longitude and latitude. Locations are colour-coded according to the major clades in the mitochondrial phylogeny in Figure 2: Northern Puerto Rico (PR; black), Southern PR (purple), Eastern PR and West Vieques (red), East Vieques and Virgin Islands (blue). Samples are from our own collection as well as GenBank. Thin solid lines indicate present-day bathymetric depth, whereas thick lines demarcate the approximate limits of currently emergent landmasses.

municipality of Ceiba (McCaffrey, 2006). Since the US Navy ceased activities on the island in 2003, Vieques' tourism industry has increased steadily, adding a new plausible mechanism of anthropogenic dispersal of *A. cristatellus* within and between islands. Currently there are numerous flights, both commercial and charter, that travel daily between at least three airports in Puerto Rico and Vieques. Pedestrian and car ferries frequent the islands up to four times daily, along with numerous private vessels which travel between Puerto Rican main island ports and Vieques. This extensive historical and contemporary traffic between the two islands has undoubtedly provided abundant opportunity for *A. cristatellus* to be transported. Finally, other evidence suggests that *A. cristatellus* is a good anthropogenic disperser. For instance, Perry *et al.* (2006) reported observing *A. cristatellus* on potted plants being shipped from Florida to Guana Island, and *A. cristatellus* has been introduced and established several times outside of its native area, including in Florida, Dominica, the Dominican Republic and Costa Rica (Hahn & Köhler, 2010).

This pattern – the relatively high colonization potential of *A. cristatellus*, the frequent and long-term commercial, military, and public boat and air traffic between Vieques and the Puerto Rican main island, and the genetic pattern in which one of two mitochondrial clades initially discovered by Reynolds *et al.* (2017) is closely allied to haplotypes obtained from the metropolitan area of San Juan and other densely populated areas of Puerto Rico – led us to a preliminary hypothesis that the mtDNA allied haplotypes from Vieques Island may have been anthropogenically introduced to the island. (An alternative anthropogenic scenario, but one that we considered less likely, was that the Puerto Rico East mitochondrial clade is native to Vieques Island and the Virgin Islands clade is introduced. Although this is inconsistent with recent and contemporary human activity, our sampling protocol nonetheless considers this possibility.)

Herein, we have consequently undertaken to clarify: (1) whether evidence exists suggesting that one or more genetic lineages presently found on the island of Vieques arrived there owing to anthropogenic dispersal; and (2) to what extent current populations on the island are structured by isolation-by-distance (IBD) or historical allopatry succeeded by secondary contact. To explore these questions we collected the most geographically extensive sample of Vieques Island *A. cristatellus* (to our knowledge; Fig. 1), for which we sequenced both an mtDNA locus as well as over 3400 single nucleotide polymorphisms (SNPs) using a modified genotyping-by-sequencing (GBS) protocol, also presented herein.

MATERIALS AND METHODS

Sampling

We sampled 300 individuals of *A. cristatellus* from 79 sites as evenly distributed as possible across the island of Vieques. We collected from sites that were accessible on foot or by vehicle. Most areas of western and central Vieques are relatively easy to access; however, much of eastern Vieques is subject to tightly controlled access owing to the continued presence of unexploded ordnance as a legacy of six decades of naval exercises in the region. We were granted rare access to parts of eastern Vieques, under the guidance and close supervision of the US Fish & Wildlife Service personnel who presently manage the area. Collection sites varied from urban areas, to isolated beaches, to dense woodlands. At each site, we captured between one and six male *A. cristatellus* and obtained the following from each individual: (1) a small portion of the tail for genetic analysis; (2) body length measured as snout–vent length (SVL); (3) total hind limb length from the inguinal region at the proximal end of the thigh to the length of the longest toe; and (4) total body mass. For each site we also recorded latitude, longitude and elevation using a Garmin eTrex 20 GPS (WGS 84).

DNA extraction and sequencing

We extracted DNA from the collected tail tips using the Promega Wizard SV Genomic DNA Purification System (Promega Corp., Madison, WI, USA). We used PCR to amplify the mitochondrial gene *ND2* and sequenced purified products using an automated sequencer (ABI 3730XL) at the Massachusetts General Hospital DNA Core Facility (Cambridge, MA, USA).

Mitochondrial phylogenetic analyses

We manually cleaned, aligned, and analysed all raw *ND2* sequences using Geneious 7.1.2 (Biomatters Ltd, Auckland, New Zealand). We combined cleaned *ND2* sequences of *A. cristatellus* from Puerto Rico and the Virgin Islands from GenBank (data from Kolbe *et al.*, 2007; Rodríguez-Robles *et al.*, 2007; Reynolds *et al.*, 2017) with our Vieques samples. We also included available sequences for *Anolis scriptus*, *A. monensis*, *A. cooki*, *A. desechensis* and *A. ernestwilliamsi* as out- and in-groups (the latter being defined as nominally distinct 'taxa' that turn out to be nested within *A. cristatellus*, see Results). We used JMODELTEST 2.1.10 (Darriba *et al.*, 2012) to identify TIM2+G as the best-fit model based on the Bayesian information criterion (BIC) for the dataset. We used MRBAYES 3.2 (Ronquist *et al.*, 2012) to estimate phylogenetic relationships among our sequences of *A. cristatellus* with GTR+G as the substitution model, because the TIM2+G model is not implemented in MRBAYES. We conducted a run with 50 million generations with a sampling frequency of 1000, and we assessed convergence to the posterior distribution using the R packages 'coda' and 'phangorn' (Plummer *et al.*, 2006; Schliep, 2011; R Core Team, 2016). We visualized the consensus tree using 'phytools' (Revell, 2012). We also performed maximum-likelihood (ML) phylogeny inference using the software RAXML (Stamatakis, 2014), called from within R using the package 'ips' (Heibl, 2014), with 1000 bootstrap resamples using the GTR+GAMMA model (as the TIM2+G model is not available in RAXML). We used R 3.2.2 (R Core Team, 2016) in RSTUDIO 0.98.1091 (RStudio Team, 2015) and the R packages 'maps' (Brownrigg, 2016) and 'maptools' (Bivand *et al.*, 2016) to map the distribution of mitochondrial *ND2* lineages from our samples. Based on the phylogeny inferred with ML methods, we estimated sequence divergence as the mean patristic distances between major clades using MEGA 7.0.14 (Kumar *et al.*, 2016). The 'phangorn', 'phytools' and 'ips' packages all depend on the R core phylogenetics package 'ape' (Paradis *et al.*, 2004).

GBS library preparation and sequencing

We used the GBS approach (Elshire *et al.*, 2011) to further examine genetic divergence and diversity within a representative subset of 48 *A. cristatellus* samples. We intentionally selected samples to maximize the geographical dispersion across our Vieques localities, as well as to include samples from eastern Puerto Rico and the Virgin Islands. The GBS method is described elsewhere (e.g. Elshire *et al.*, 2011; Peterson *et al.*, 2012; Martin & Feinstein, 2014), although we used a novel, modified protocol that is presented in detail in the Supporting Information (Appendix S1). Briefly, we initially optimized our library preparation by selecting a restriction enzyme (*Sbf*I) based on *in-silico* digests of the congener *A. carolinensis* (Davey *et al.*,

2011; Gamble & Zarkower, 2014) and designing 48 barcoded adaptors complementary to the cut site of the (infrequent cutting, non-methylation sensitive) 8-base cutter *Sbf*I using the Deena Bioinformatics GBS Barcode Generator. We ordered HPSF-purified adaptors from Eurofins [www.operon.com (accessed 19 October 2019)], with barcodes ranging from 4 to 8 bp and differing by at least 3 bases. We annealed barcoded and common adaptors together and diluted adaptor concentrations to 0.6 ng/μL. Before starting, we assessed and normalized concentrations of genomic DNA using a Qubit 2.0 Fluorometer (Life Technologies, Carlsbad, CA, USA) with standard assay reagents. We individually digested the samples and adaptors with high-fidelity *Sbf*I, then ligated adaptors to the 'sticky' ends of the DNA fragments left by the enzyme. We next size selected for fragments of 200–500 bp using a 1:1 ratio of AMPure XP beads (Agencourt Bioscience Corp., Beverly, MA, USA) and then pooled all 48 individuals into a multiplexed library. We used PCR to amplify the restriction fragment library and to attach Illumina (Illumina, Inc., San Diego, CA, USA) adaptors. We conducted library-amplification and PCR primer annealing using PCR in 50-μL reactions with Phusion high-fidelity DNA polymerase master mix (Thermo Fisher Scientific, Inc., Waltham, MA, USA) in a Mastercycler Pro at 98 °C for 30 s, followed by 18 cycles of 98 °C for 30 s, 65 °C for 30 s, 72 °C for 30 s and a final extension step of 72 °C for 4 min. We then purified libraries with AMPure XP beads as above with a final elution volume of 30 μL. Before sequencing, we conducted an adaptor titration experiment (Elshire *et al.*, 2011) to determine the appropriate concentration of adaptors relative to our DNA samples. We tested eight concentrations of adaptors (6–24 μL of adaptor mixes), carrying the experiment through to the PCR purification stage. We then analysed these eight libraries on an Agilent TapeStation 2200 at the Harvard FAS Center for Systems Biology (Cambridge, MA, USA) to find an optimum concentration of adaptors (12 μL of adaptor mix) that minimized the formation of adaptor dimers, which we used in subsequent sequencing experiments. We checked final libraries by running 1 μL of sample on the TapeStation as above. We then sequenced the 48-plex library on an Illumina HighSeq 2500 machine using single-end 100-bp chemistry at the University of Massachusetts Boston Center for Personalized Cancer Therapy Genomics Core. As noted above, our full protocol is detailed in Appendix S1.

SNP DATA PROCESSING

We processed all raw sequence data following the DDOCENT (Puritz *et al.*, 2014) pipeline for *de novo* assembly, read mapping and variant calling. We used a clustering threshold of 90% similarity (which yielded the highest number of loci) and minimum depth of coverage of 6. We note that this depth of coverage is on the low side, although we found that we were able to call more SNPs with less drop-out using this coverage depth, and we note that our downstream methods are somewhat more robust to lower coverage (e.g. Andrews *et al.*, 2016). We filtered raw SNPs using VCFTOOLS (Danecek *et al.*, 2011) to select for SNPs present in at least 90% of individuals, with non-reference allele frequencies between 0.001 and 0.9999, a minimum minor allele count of 1, and a minimum Phred score of 30, meaning there is a 1:1000 chance that a base is called incorrectly. We called only one SNP per locus.

GENETIC DIVERSITY AND POPULATION STRUCTURE

We used STRUCTURE 2.3.4 (Pritchard *et al.*, 2000) to infer the number of genetic clusters in the SNP dataset. STRUCTURE uses a Bayesian Markov chain Monte Carlo approach to characterize a known (or unknown) number of discrete populations (K) based on a set of allele frequencies at each locus. All individuals are then assigned probabilistically to populations based on their genotypes. The model also assumes Hardy–Weinberg equilibrium (HWE) and that loci are unlinked and at linkage equilibrium within populations. We evaluated possible K values ranging from 1 to 4 (ten runs per K value) with and without population priors using the admixture model with a Markov chain of 1 million replicates and an initial burn-in of 250 000 replicates per run. We then visualized STRUCTURE results with the web-based program STRUCTURE HARVESTER 0.6.94 (Earl & vonHoldt, 2012) which uses the Evanno *et al.* (2005) ΔK method to identify the value of K that best fits the data.

In addition, we conducted discriminant analysis of principal components (DAPC) on the SNP data using the R package 'adegenet' (Jombart *et al.*, 2010) to cross-validate our findings with STRUCTURE. DAPC is a multivariate analysis which uses K-means clustering of principal components to define groups of individuals. Unlike STRUCTURE, DAPC does not assume that populations are in HWE, which may help it to better identify other genetic spatial structure in the data such as IBD or genetic clines. We first performed a principal component analysis (PCA) which ensures that variables subsequently used as input for our discriminant analysis (DA) are not correlated. Then, the clusters and their likelihood are compared using the Bayesian information criterion (BIC) for the best supported number of clusters. We used both STRUCTURE and DAPC to analyse all 48 individuals' polymorphic SNP data (3407 SNPs) as well as a hierarchical subset which included only SNPs that were polymorphic among the subsample

of 37 individuals from Vieques (2433 SNPs). Based on population assignment from STRUCTURE and DAPC, we used the R package 'hierfstat' (Goudet & Jombart, 2015) to compute pairwise F_{ST} between inferred clusters from the Vieques dataset alone. We did not compute F_{ST} between Vieques and Puerto Rico or Virgin Islands samples, because the latter were very few (only 11 in total), and were intentionally distributed across multiple sites (and thus probably representing multiple differentiated populations) on the main island of Puerto Rico and in the Virgin Islands, respectively.

Tests for isolation-by-distance

We tested for IBD by first computing genetic distances using Provesti's distances for the SNP data and geographical distances from our collected GPS points. In the latter case, we converted GPS coordinates to Universal Transverse Mercator (UTM) coordinates, a 2-D Cartesian coordinate system, to calculate geographical distances using R package 'PBSmapping' (Schnute *et al.*, 2013). We then performed a matrix correlation analysis (Mantel test; Mantel, 1967) using the R package 'adegenet' (Jombart & Ahmed, 2011).

Under a scenario of historical allopatry, we might expect that mitochondrial clade membership would significantly explain variation in genomic distance between samples controlling for the effect of geography. To test this hypothesis, we conducted a partial Mantel test implemented in 'phytools' (Revell, 2012) in which the dependent matrix was pairwise genomic (SNP) distance between individuals, and the independent matrices were pairwise geographical distance and mtDNA *ND2* distance. The expectation here is that if deep mitochondrial divergence is the product of historical allopatry, then numerous SNPs should have also fixed for alternative states during this allopatric period, and a significant portion of the variance in genomic distance (even controlling for geographical distance) should be explained by mitochondrial clade membership. On the other hand, if mitochondrial divergence instead reflects deep coalescence combined with IBD, then we might expect genomic distance to increase monotonically with geographical distance, and that little to no variation among individuals in genomic divergence would be explained by mitochondrial clade membership. For this test we calculated SNP data and geographical distance by the same methods used in testing for IBD, as above. We calculated mtDNA distances using the package 'ape' (Paradis *et al.*, 2014).

We additionally undertook similar partial Mantel tests using the morphological trait data for all samples, as well as for subsamples generated to include only individuals with mtDNA sequence and only individuals with GBS data. The methods and results for these analyses are fully described in the Supporting Information, Appendix S2.

RESULTS

Mitochondrial sequencing

We obtained 1191 bp (partial coding sequence) of the mtDNA locus *ND2* for a selected subset of 155 out of the 300 individuals sampled for morphology. We selected individuals for sequencing based on their collection sites to achieve as uniform a geographical representation as possible in our sample. After combining these sequences with others from across the Puerto Rico Bank (Reynolds *et al.*, 2017) and those available from GenBank, we aligned a maximum of 1191 bp of *ND2* sequence data from a total of 374 *A. cristatellus* samples (177 from Vieques, 93 from the main island of Puerto Rico, and 104 from the US and British Virgin Islands). Combined with our in- and out-group samples, our final alignment contained 379 sequences in total. Average sequence length of the dataset was 1098 bp, encompassing the majority of the *ND2* coding region (alignment available in Dryad).

mtDNA phylogeography of *A. cristatellus* on Vieques

Both our estimated ML and Bayesian phylogenies are broadly consistent with topologies from other analyses (e.g. Reynolds *et al.*, 2017), resulting in *A. scriptus*, *A. monensis* and *A. cooki* as sister taxa and *A. ernestwilliamsi* well-nested within the Virgin Island clades (Fig. 2, Supporting Information, Fig. S1). We found that our samples from Vieques fall into two major, and deeply divergent, groups. The first is a clade ('Puerto Rico East') for which our Vieques samples are rendered paraphyletic by samples from eastern Puerto Rico (obtained from the eastern municipalities of Ceiba, Fajardo, Naguabo, Humacao, San Juan, Rio Grande, Maunabo and Juncos; Fig. 2; Fig. S1). The second Vieques Island clade is a monophyletic group that is nested within all other samples from the Virgin Islands ('Virgin Islands'). In the ML phylogenetic inference, all major clades are well supported with bootstrap support values of 70–100% (Fig. 2; Fig. S1). Our Bayesian analysis resulted in a consensus tree that was largely congruent with our ML phylogeny (Fig. S2).

On Vieques, the geographical distribution of the two major mtDNA lineages was highly structured. In general, samples with *ND2* sequences that were closely related to the eastern Puerto Rican clade were collected in western Vieques, while samples allied

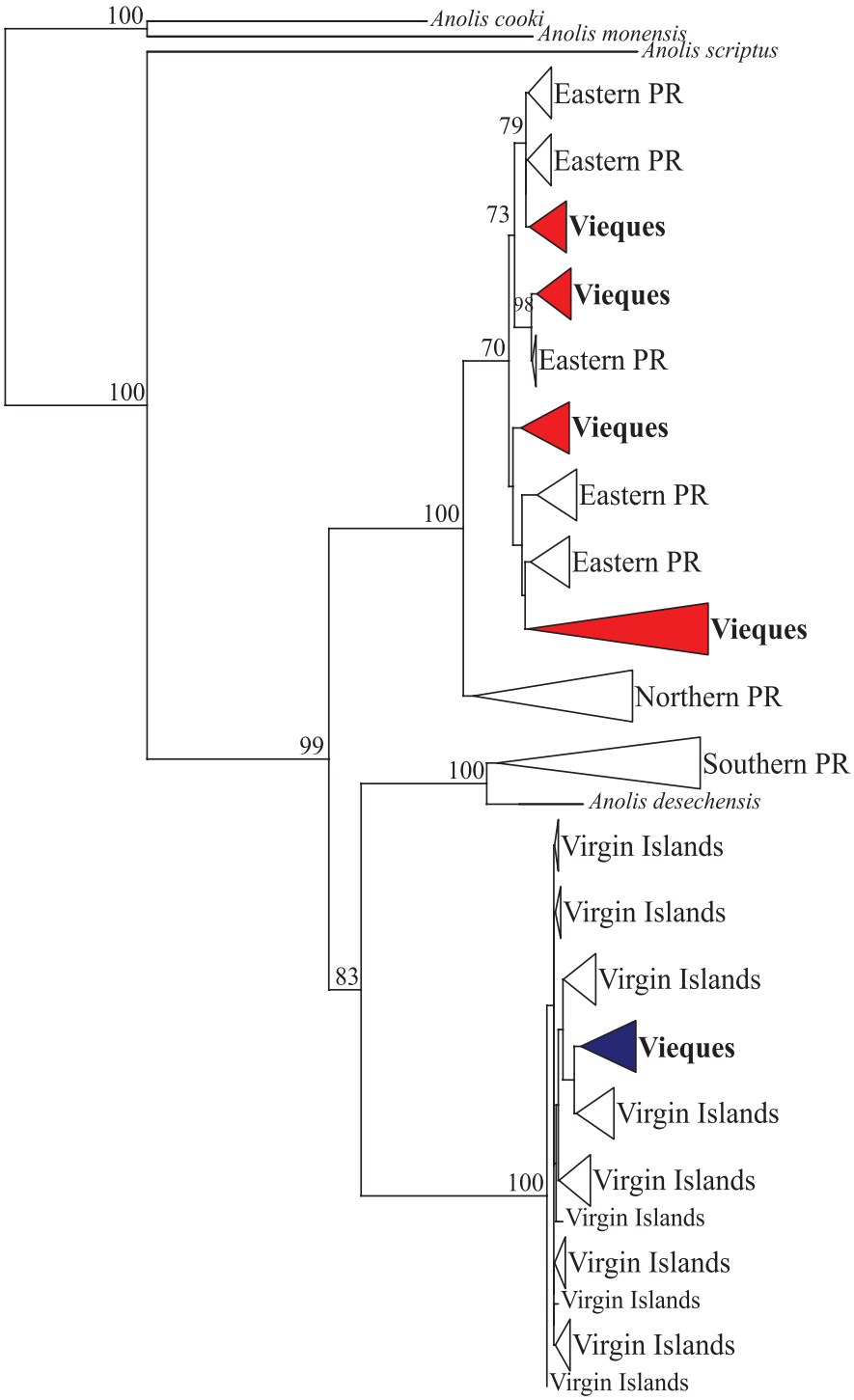

Figure 2. Mitochondrial *ND2* gene phylogeny from 379 *Anolis cristatellus* samples inferred by maximum likelihood, with clades collapsed and Vieques clades colour-coded by mtDNA haplotype membership. Red Vieques clades are allied with Puerto Rico East (PR) haplotypes and blue Vieques clades are allied with Virgin Islands haplotypes. Numbers at nodes represent bootstrap support; only nodes with bootstrap support >70% are labelled. *Anolis ernestwilliamsi* falls within the first Virgin Islands clade. The full tree is presented in Supporting Information, Figure S1.

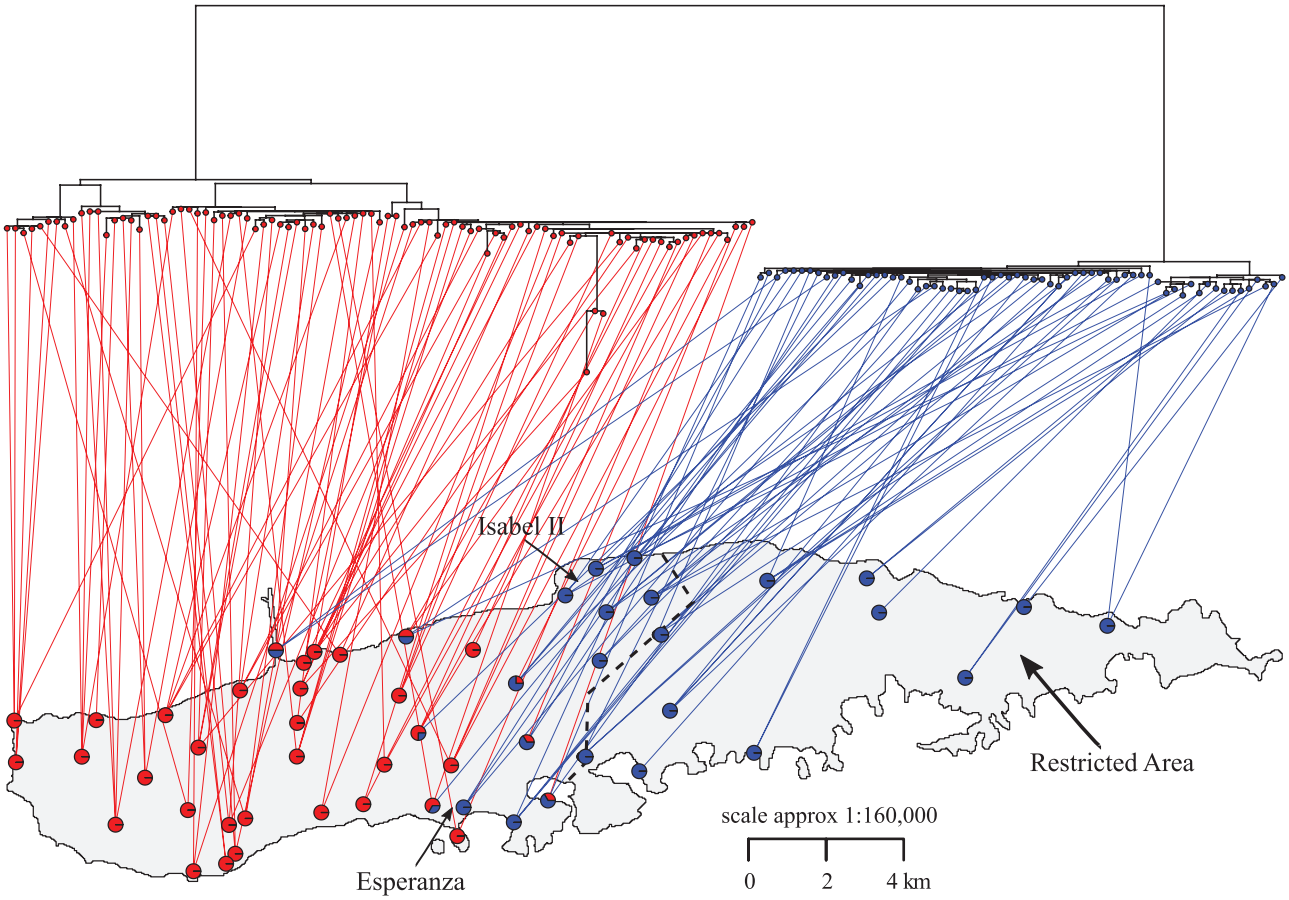

Figure 3. Projection of the subset of Vieques samples (N = 177) from the inferred maximum likelihood tree (Fig. 1; Supporting Information, Fig. S1) to their geographical locations on the island of Vieques. Samples are colour-coded by clade membership in Figure 2. The main settlements of Isabel II and Esperanza, where major travel ports are located, are indicated on the map. The dashed line represents the boundary of the restricted US Fish and Wildlife Refuge that encompasses the eastern part of Vieques.

with the Puerto Rico Bank Virgin Islands tended to have been obtained from the eastern part of the island (Fig. 3).

Overall, the average Tamura–Nei corrected total divergence between western and eastern Vieques Island samples for mtDNA was ~11.8% (Supporting Information, Table S1). This degree of divergence is equal or comparable to the greatest degree of mtDNA sequence divergence measured between any pair of populations of this widespread anole (Kolbe *et al.*, 2007). Most sampling locations only contained haplotypes from one clade or the other, although some sites in the longitudinal centre of the island were found to have both haplogroups present (Fig. 3).

Genomic SNP data

Two lanes of sequencing enabled us to generate over 310 million raw single-end reads from 48 individuals, including 37 from Vieques, six from eastern Puerto Rico and five from the Virgin Islands. The average number of raw reads per individual was 6 019 315 ± 4 617 963, ranging from a low of 607 080 reads to a high of 28 850 642 reads. The average percentage of reads we trimmed in DDOCENT was 0.32% per individual, and we called a total of 16 808 SNPs. After filtering, we retained 3407 loci for further analysis.

Anolis cristatellus population structure and genomic diversity

For these analyses, we first pooled all 48 individuals sampled from main island of Puerto Rico, the island of Vieques, and the British and US Virgin Islands. Next, we subsampled our data to include only the 37 individuals from the island of Vieques and collected specifically for this study. The purpose of this two-stage analysis was to first affirm that our genomic-scale SNP data

captured the obvious genetic break between main island and Virgin Island samples (if one exists), and, next, to investigate possible genetic structure among sampling localities within the relatively small island of Vieques.

For our full dataset we analysed a total of 3407 loci with less than 5% missing data using the programs STRUCTURE and DAPC. We used the ΔK method of Evanno *et al.* (2005) to determine that $K = 2$ best fitted the data for our analyses both with and without a location prior. We also investigated $K = 3$ given the known bias towards $K = 2$, especially when hierarchical structure is present (as we might expect to find in our data; Janes *et al.*, 2017). Our DAPC analysis also identified two clusters based on the BIC after comparing different K values (Supporting Information, Fig. S3). DAPC analysis showed that individuals from Vieques and Puerto Rico form one cluster while individuals from the Virgin Islands comprised a different, separate cluster. STRUCTURE analysis did not discriminate between these groupings at $K = 2$, but did at $K = 3$ (Fig. S4). Divergence between Virgin Islands and Puerto Rico/Vieques clusters was 4.1% (Tamura–Nei corrected; Table S2). Due to our widely divergent sample sizes between Vieques Island, the Virgin Islands and Puerto Rico, and particularly because our samples from the main island and the Virgin Islands do not come from one geographical locality but several, we did not feel it was sensible to compute F_{ST} for Virgin Islands and Puerto Rico populations.

For the second part of our analysis, we removed samples from the Virgin Islands and East Puerto Rico from the SNP dataset, resulting in some monomorphic loci. We thus retained 2433 polymorphic loci from the 37 Vieques Island samples. We analysed these data first using STRUCTURE, as above, and then DAPC. A ΔK analysis performed with STRUCTURE revealed $K = 2$ for the dataset. Posterior probabilities for group membership, however, were not well aligned with geography (Fig. 4D). By contrast, DAPC identified two slightly overlapping clusters that generally correspond with the eastern and western halves of the island (and thus crudely with the mtDNA haplogroups; Fig. 4A–C). Nonetheless, concordance between mtDNA and SNP clustering was imperfect as five individuals with eastern Vieques mtDNA haplotypes were assigned to the western Vieques SNP cluster with highest probability. Divergence between the DAPC inferred clusters within the Vieques SNP dataset was 0.5% (Tamura–Nei corrected distances). F_{ST} between the west and east Vieques clusters (as defined by DAPC) was 0.02.

Tests for isolation-by-distance

Our test for IBD in the Vieques dataset revealed a significant relationship between calculated Provesti's genetic distances and UTM-transformed geographical distances (Mantel test; $r = 0.36$, $P = 0.001$; Fig. 5). This shows that individuals sampled closer together on the island tend to be more genetically similar, and thus suggests that gene flow may be reduced between geographically distant individuals on Vieques Island.

In our partial Mantel analysis to test for a marginal effect of mitochondrial clade membership on genomic distance, we found no evidence to support a significant correlation between mitochondrial distance and SNP distance when controlling for geography (partial regression coefficient, $\beta_2 = 0.0007$, $t = 0.126$, $P = 0.927$ from Mantel permutations; Supporting Information, Fig. S6). We also failed to find evidence of a significant correlation between morphological dissimilarity for the attributes measured in this study and geographical or genetic distance within Vieques Island (Appendix S2).

DISCUSSION

We undertook this project after a broad-scale study of phylogeography across the Puerto Rico Bank revealed an intriguing pattern of two deeply divergent mtDNA haplogroups from a modest number of representative samples of *A. cristatellus* obtained on the relatively small island of Vieques. One of these two haplogroups was quite closely allied to samples from eastern Puerto Rico including the metropolitan area of San Juan and the former US Naval Base at Roosevelt Roads (Reynolds *et al.*, 2017). This observation, coupled with the knowledge that Vieques has been subjected to very a high frequency and duration of military, commercial, and public boat and air traffic over the past 60–100 years or more, suggested to us the possibility that members of this haplogroup might have been anthropogenically introduced to Vieques within the last century or so. Furthermore, *A. cristatellus* is a reasonably successful invader in other areas, having been introduced and become successfully established in several localities outside its native range (Hahn & Kohler, 2010).

To investigate this hypothesis we undertook the most thorough geographical sampling of *A. cristatellus* to date on Vieques. We sequenced 1191 bp of mtDNA from 155 samples collected across Vieques, and aligned these samples with 224 previously accessioned sequences from prior studies of *A. cristatellus* and other closely related taxa. In addition, we used GBS to generate SNP data for an additional 3407 polymorphic nuclear loci for 48 individuals: 37 from Vieques, and the remainder from Puerto Rico (six samples) and from the Puerto Rico Bank Virgin Islands (five samples). We analysed these data using gene tree inference by Bayesian and ML methods (mtDNA), and using population genetic methods based on an explicit model

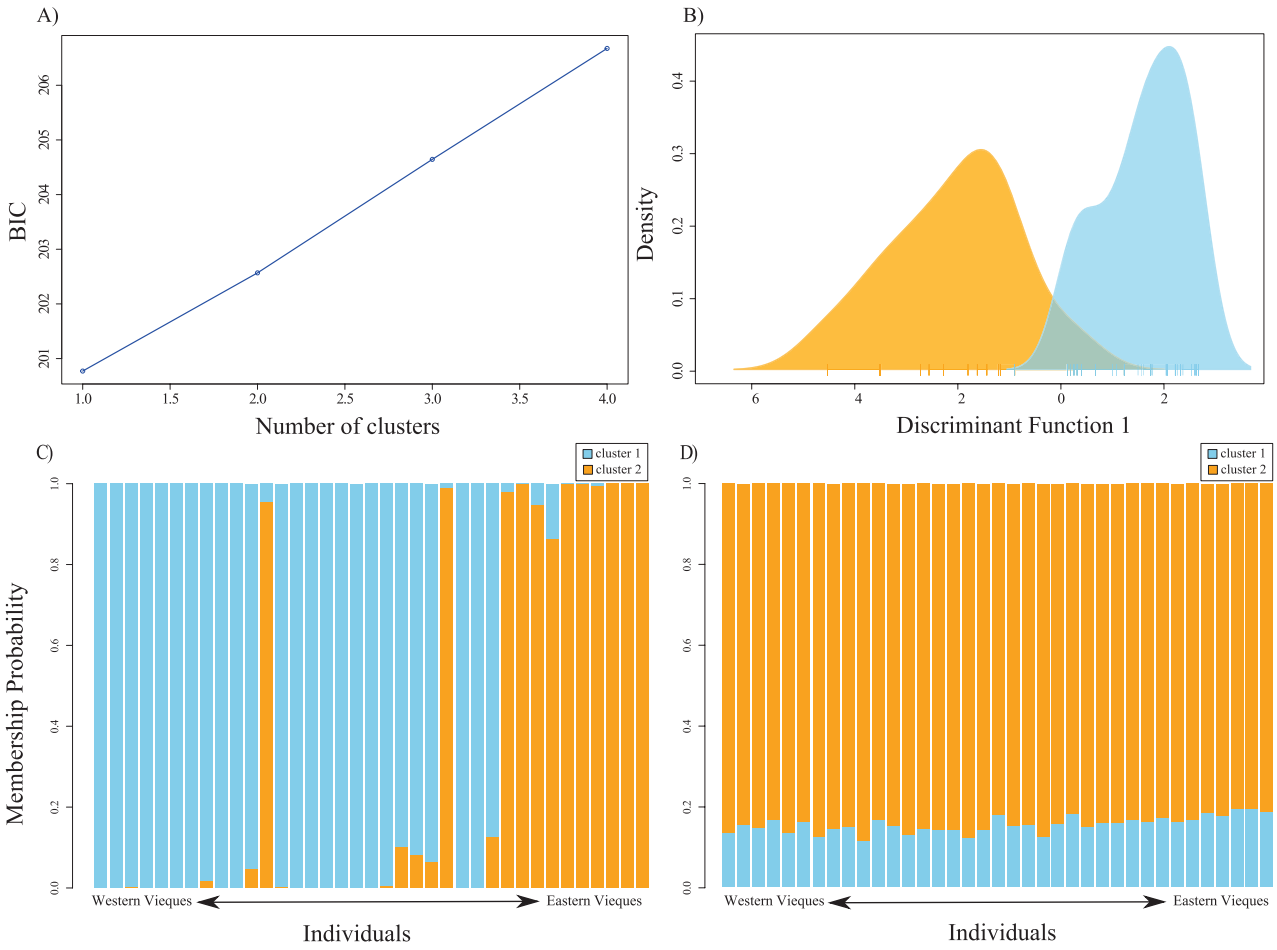

Figure 4. Results from clustering analyses without a location prior for the Vieques SNP dataset. A, BIC vs. number of clusters plot from DAPC analysis. B, DAPC clustering showing two separate clusters from the all-SNP dataset. Membership probability plots from (C) DAPC and (D) STRUCTURE $K = 2$ showing a lack of geographical discrimination between clusters. Each bar represents the genotype of one of the 37 samples from Vieques. The colour fraction of each bar represents the probability of the individual belonging to each of the two clusters. Individuals are ordered left-to-right by values of one canonical axis extracted from a multidimensional scaling analysis; however, this corresponds roughly to their west to east locations on the island.

(STRUCTURE) and on no particular model (DAPC) of among-population divergence.

In general, our results did not support our a priori hypothesis that the mitochondrial haplotypes found in Vieques that are most closely allied to Puerto Rico East haplotypes result from an anthropogenic introduction of main island genetic material into Vieques. Rather, this pattern seems to have resulted from an historical mitochondrial break on the Puerto Rico Bank palaeo-island, inclusive of the Virgin Islands. This is supported by the high genetic diversity within each mitochondrial haplogroup for both nuclear and mitochondrial DNA, and by the strong pattern of genetic structure among the *ND2* haplotypes (Fig. 3; Supporting Information, Fig. S1). If the presence of individuals with Puerto Rico East clade haplotypes on the island was the product of recent dispersal, we would expect low diversity and low geographical structure, merely owing to the stochastic sampling of individual migrants and the shortage of time during which to establish a genetically structured geographical distribution in the introduced area (Fitzpatrick *et al.*, 2012). Furthermore, we might expect to see introduced haplotypes co-occurring with native haplotypes, a pattern that is generally not observed (Fig. 3). Instead, we see a consistent tendency for mitochondrial haplotypes allied with main island lineages to be found in the western part of Vieques, while haplotypes closely related to Virgin Island clades are circumscribed to the east (Fig. 3). This pattern is wholly consistent with the geographical configuration of the present and past (palaeo-)island as well as with a contemporary ecological gradient on the island,

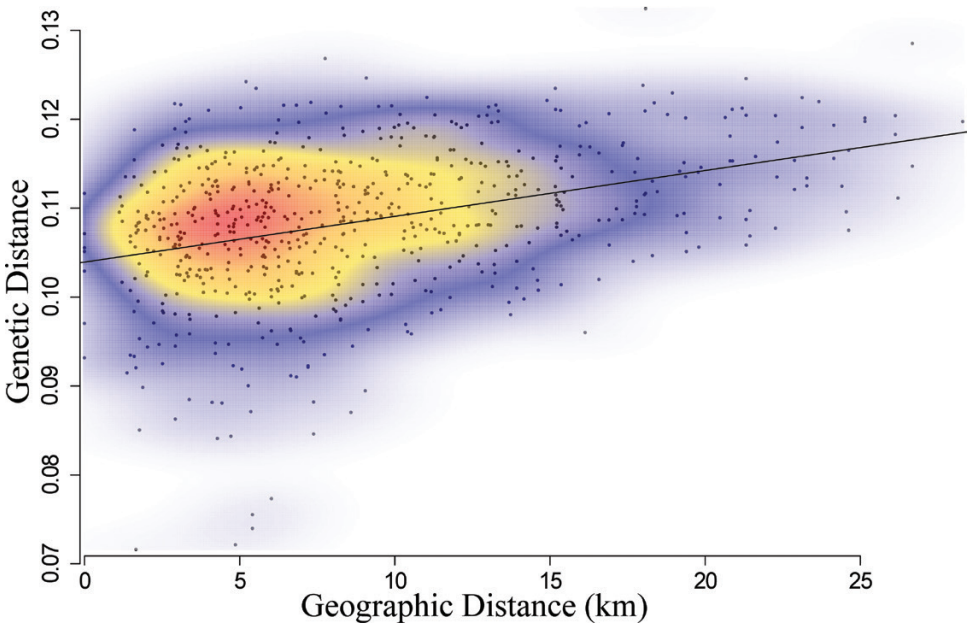

Figure 5. Isolation-by-distance plot for the Vieques SNP dataset from 37 individuals with a total of 2433 loci. Mantel's test for correlation was significant (r = 0.36; P = 0.001). Genetic distance is calculated with Nei's distance method and geographical distance is calculated from UTM coordinates converted from GPS coordinates. Colours represent the density of data points with red to blue as most to least dense.

whereby eastern Vieques consists of subtropical dry forest with <80 mm of rainfall annually and west Vieques consists of subtropical mesic forest with 80–150 mm of annual rainfall (Ewel & Whitmore, 1973; Brandeis *et al.*, 2009). Additionally, in our broad and dense geographical sampling on the island we found no tendency for either haplogroup to be consistently associated with either ports or densely inhabited areas. Finally, analysis of 3407 nuclear SNPs showed similar, but imperfectly matched, population genetic structure in nuclear markers compared to mtDNA, probably reflecting natural geographical genetic differentiation rather than the introduction of foreign genetic material to the island.

A second question that arises is whether the apparent genetic break we have documented for mtDNA represents historical allopatry (on the Puerto Rico Bank palaeo-island) followed by secondary contact and introgression, or primary intergradation. To answer this question, we used data for 2433 SNPs sampled from 37 individuals dispersed across the island of Vieques including mtDNA sequences from both of the two major *ND2* haplogroups. STRUCTURE analysis of the data showed no evidence for discrete populations on Vieques (Supporting Information, Fig. S5). Analysis using DAPC, which may be more sensitive to genetic clines, showed more evidence for population genetic structure; however, we found no support for a significant effect of mtDNA haplogroup membership on SNP divergence. This suggests that the lack of correlation between SNP and mtDNA divergence may simply be due to IBD in the former and deep coalescence with geographical structure in the latter, rather than an alternative of correlation owing to concerted evolutionary histories of SNP and mtDNA in lineages evolving under a demographic scenario such as allopatry (see Reynolds *et al.*, 2017). At the time of writing, a new analytical model had just been published to characterize genetic clines in continuously distributed populations (Bradburd *et al.*, 2018). Although we have not applied it to our data herein, this may represent an intriguing avenue of analysis for other similarly motivated studies in the future.

Overall, we found little support for a hypothesis of historical allopatry and thus propose that the pattern we have observed is most probably the result of primary intergradation combined with IBD. Unfortunately, historical allopatry can never be entirely ruled out in this case, because with sufficient gene flow its signal would eventually disappear. In this context, it seems reasonable to acknowledge that IBD and deep coalescence for mtDNA is effectively our null hypothesis, and a non-significant result reflects a failure to reject the null, not definitive evidence that the null is correct. Nevertheless, we show that an unusual pattern of deep mitochondrial divergence across a relatively small island with no

apparent geographical or ecological separation is not particularly well explained by historical allopatry; instead, it may be the product of more nuanced evolutionary and geological factors that can only be understood via analysis of both mitochondrial and nuclear genomes.

Our study underlines the importance of using multiple genetic markers when assessing population structure in a species. In this case results from mtDNA alone may have caused us to erroneously infer either the introduction of foreign genetic material to the island or ancient allopatry followed by recent secondary contact. When combined with data from over 3400 SNPs, the picture changes quite dramatically and we find relatively little support for either hypothesis. Our results suggest instead a process of IBD in which we happen to have sampled across a deep coalescence event in geographically structured mtDNA.

ACKNOWLEDGEMENTS

We are grateful to the Puerto Rico Departamento de Recursos Naturales y Ambientales (DRNA) for permits and assistance. All samples were collected under DRNA permit 2014-IC-044. We are also grateful to the US Fish and Wildlife Service on Vieques for allowing our stay at their field station, and in particular to M. Barandiaran with the USFWS Vieques National Wildlife Refuge. We thank A. R. Puente-Rolón (Universidad Interamericana de Puerto Rico Arecibo and Universidad de Puerto Rico Mayagüez) for assistance with permits and, along with A. Balderas LaRosa, for considerable help in the field. We also thank T. R. Strickland and K. M. Winchell for contributing sequence data. We are grateful to the University of Massachusetts Boston and the Nancy Goranson Endowment Fund for funding and support for our project. We furthermore gratefully acknowledge support from the University of North Carolina Asheville and the Bauer Core Facility at Harvard University. Considerable gratitude is owed to J. Allen, G. Mayer and two anonymous reviewers for their patient and helpful comments during the review process. Finally, we thank members of the Revell Lab at UMass Boston for comments and advice on this project. This research was funded in part by grants from the National Science Foundation (DEB 1354044 and DEB 1350474). The title of this article was chosen as an intentional homage to Schneider (1996).

AUTHOR CONTRIBUTIONS

Q.N.Q., L.J.R. and R.G.R. conceived of the project. Q.N.Q. designed the study and conducted most of the field sampling, data acquisition and analyses, and drafted the manuscript. R.G.R. designed the GBS protocol. R.G.R. and L.J.R. provided logistical and intellectual input for data analyses and interpretation. All authors contributed to the writing and editing of the manuscript.

REFERENCES

Andrews KR, **Good JM**, **Miller MR**, **Luikart G**, **Hohenlohe PA. 2016.** Harnessing the power of RADseq for ecological and evolutionary genomics. *Nature Reviews Genetics* **17:** 81–92.

Barker BS, **Rodríguez-Robles JA**, **Aran VS**, **Montoya A**, **Waide RB**, **Cook JA. 2012.** Sea level, topography and island diversity: phylogeography of the Puerto Rican red-eyed coquí, *Eleutherodactylus antillensis*. *Molecular Ecology* **21:** 6033–6052.

Bivand R, **Lewin-Koh N. 2016.** *maptools: tools for reading and handling spatial objects*. Available at: https://CRAN.R-project.org/package=maptools (accessed 19 October 2019).

Bradburd GS, **Coop GM**, **Ralh PL. 2018.** Inferring continuous and discrete population genetic structure across space. *Genetics* **210:** 33–52.

Brandeis TJ, **Helmer EH**, **Marcano-Vega H**, **Lugo AE. 2009.** Climate shapes the novel plant communities that form after deforestation in Puerto Rico and the US Virgin Islands. *Forest Ecology and Management* **258:** 1704–1718.

Brandley MC, **de Queiroz K. 2004.** Phylogeny, ecomorphological evolution, and historical biogeography of the *Anolis cristatellus* series. *Herpetological Monographs* **18:** 90–126.

Brownrigg R. 2016. *maps: draw geographical maps*. Available at: https://CRAN.R-project.org/package=maps (accessed 19 October 2019)..

Burney DA, **Burney LP**, **MacPhee RDE. 1994.** Holocene charcoal stratigraphy from Laguna Tortuguero, Puerto Rico, and the timing of human arrival on the island. *Journal of Archaeological Science* **21:** 273–281.

Campbell-Staton SC, **Goodman RM**, **Backstrom N**, **Edwards SV**, **Losos JB**, **Kolbe JJ. 2012.** Out of Florida: mtDNA reveals patterns of migration and Pleistocene range expansion of the Green Anole lizard (*Anolis carolinensis*). *Ecology and Evolution* **2:** 2274–2284.

Danecek P, **Auton A**, **Abecasis G**, **Albers CA**, **Banks E**, **DePristo MA**, **Handsaker RE**, **Lunter G**, **Marth GT**, **Sherry ST**, **McVean G**, **Durbin R**, **1000 Genomes Project Analysis Group**. **2011.** The variant call format and VCFtools. *Bioinformatics* **27:** 2156–2158.

Darriba D, **Taboada GL**, **Doallo R**, **Posada D. 2012.** jModelTest 2: more models, new heuristics and parallel computing. *Nature Methods* **9:** 772.

Davey JW, **Hohenlohe PA**, **Etter PD**, **Boone JQ**, **Catchen JM**, **Blaxter ML. 2011.** Genome-wide genetic marker discovery and genotyping using next-generation sequencing. *Nature Review Genetics* **12:** 499–510.

Earl DA, **vonHoldt BM. 2012.** STRUCTURE HARVESTER: a website and program for visualizing STRUCTURE output and implementing the Evanno method. *Conservation Genetics Resources* **4:** 359–361.

Elshire RJ, **Glaubitz JC**, **Sun Q**, **Poland JA**, **Kawamoto K**, **Buckler ES**, **Mitchell SE. 2011.** A robust, simple genotyping-by-sequencing (GBS) approach for high diversity species. *PLoS ONE* **6:** e19379.

Evanno G, **Regnaut S**, **Goudet J. 2005.** Detecting the number of clusters of individuals using the software STRUCTURE: a simulation study. *Molecular Ecology* **14:** 2611–2620.

Ewel JJ, **Whitmore JL. 1973.** The ecological life zones of Puerto Rico and the US Virgin Islands. *USDA Forest Service, Institute of Tropical Forestry, Research Paper ITF-018*, 18.

Fitzpatrick BM, **Fordyce JA**, **Niemiller ML**, **Reynolds RG. 2012.** What can DNA tell us about biological invasions? *Biological Invasions* **14:** 245–253.

Galtier N, **Nabholz B**, **Glemin S**, **Hurst GDD. 2009.** Mitochondrial DNA as a marker of molecular diversity: a reappraisal. *Molecular Ecology* **18:** 4541–4550.

Gamble T, **Zarkower D. 2014.** Identification of sex-specific molecular markers using restriction site-associated DNA sequencing. *Molecular Biology Resources* **14:** 902–913.

Glor RE, **Kolbe JJ**, **Powell R**, **Larson A**, **Losos JB. 2003.** Phylogenetic analysis of ecological and morphological diversification in Hispaniolan trunk-ground anoles (*Anolis cybotes* group). *Evolution* **57:** 2383–2397.

Goudet J, **Jombart T. 2015.** *hierfstat: estimation and tests of hierarchical F statistics*. Available at: https://CRAN.R-project.org/package=hierfstat (accessed 19 October 2019).

Grant C. 1931. A new species and two new sub-species of the genus *Anolis*. *Journal of the Department of Agriculture of Puerto Rico* **15:** 219–222.

Hahn M, **Köhler G. 2010.** Morphological variation in *Anolis cristatellus*. *Salamandra* **46:** 117–120.

Heatwole H. 1976. Herpetogeography of Puerto Rico. VII. Geographic variation in the *Anolis cristatellus* complex in Puerto Rico and the Virgin Islands. *Occasional Papers of the Museum of Natural History University of Kansas* **46:** 1–18.

Heatwole H, **MacKenzie F. 1967.** Herpetogeography of Puerto Rico. IV. Paleogeography, faunal similarity and endemism. *Evolution* **21:** 429–438.

Hedges SB. 1999. Distribution patterns of amphibians in the West Indies. In: Duellman WE, ed. *Regional patterns of amphibian distribution: a global perspective.* Baltimore: John Hopkins University Press, 211–254.

Heibl C. 2014. *ips: interfaces to phylogenetic software in R*. Available at: https://CRAN.R-project.org/package=ips (accessed 19 October 2019).

Janes JK, **Miller JM**, **Dupuis JR**, **Malenfant RM**, **Gorrell JC**, **Cullingham CI**, **Andrew RL. 2017.** The K=2 conundrum. *Molecular Ecology*, **26:** 3594–3602.

Jombart T, **Ahmed I. 2011.** adegenet 1.3-1: new tools for the analysis of genome-wide SNP data. *Bioinformatics* **27:** 3070–3071.

Jombart T, **Devillard S**, **Balloux F. 2010.** Discriminant analysis of principal components: a new method for the analysis of genetically structured populations. *BMC Genetics* **11:** 94–108.

Kolbe JJ, **Glor RE**, **Schettino LR**, **Lara AC**, **Larson A**, **Losos JB. 2004.** Genetic variation increases during biological invasion by a Cuban lizard. *Nature* **431:** 177–181.

Kolbe JJ, **Glor RE**, **Schettino LR**, **Lara AC**, **Larson A**, **Losos JB. 2007.** Multiple sources, admixture, and genetic variation in introduced *Anolis* lizard populations. *Conservation Biology* **21:** 1612–1625.

Kumar S, **Stecher G**, **Tamura K. 2016.** MEGA7: molecular evolutionary genetics analysis version 7.0 for bigger datasets. *Molecular Biology and Evolution* **33:** 1870–1874.

Leal M, **Fleishman LJ. 2004.** Differences in visual signal design and detectability between allopatric populations of *Anolis* lizards. *The American Naturalist* **163:** 26–39.

Losos JB. 2009. *Lizards in an evolutionary tree: ecology and adaptive radiation of anoles.* Berkeley: University of California Press.

Mantel N. 1967. The detection of disease clustering and a generalized regression approach. *Cancer Research* **27:** 209–220.

Martin CH, **Feinstein LC. 2014.** Novel trophic niches drive variable progress toward ecological speciation within an adaptive radiation of pupfishes. *Molecular Ecology* **23:** 1846–1862.

Mayer GC. 2012. Puerto Rico and the Virgin Islands. In: Powell R, Henderson RW, eds. *Island lists of West Indian amphibians and reptiles*. *Bulletin of the Florida Museum of Natural History* **51:** 85–166.

McCaffrey KT. 2006. The battle for Vieques' future. *Centro Journal* **XVIII:** 124–147.

Paradis E, Claude J, Strimmer K. 2004. APE: Analyses of phylogenetics and evolution in R language. *Bioinformatics* **20:** 289–290.

Paulo OS, **Dias C**, **Bruford MW**, **Jordan WC**, **Nichols RA. 2001.** The persistence of Pliocene populations through the Pleistocene climatic cycles: evidence from the phylogeography of an Iberian lizard. *Proceedings of the Royal Society B: Biological Sciences* **268:** 1625–1630.

Perry G, **Gerber GP. 2006.** Conservation of amphibians and reptiles in the British Virgin Islands: status and patterns. *Applied Herpetology* **3:** 237–256.

Perry G, **Powell R**, **Watson H. 2006.** Keeping invasive species off Guana island, British Virgin Islands. *Iguana* **13:** 273–277.

Peterson BK, **Weber JN**, **Kay EH**, **Fisher HS**, **Hoekstra HE. 2012.** Double digest RADseq: an inexpensive method for *de novo* SNP discovery and genotyping in model and non-model species. *PLoS One* **7:** e37135.

Plummer M, Best N, Cowles K, Vines K. 2006. CODA: Convergence Diagnosis and Output Analysis for MCMC. *R News* **6:** 7–11.

Pritchard JK, **Stephens M**, **Donnelly P. 2000.** Inference of population structure using multilocus genotype data. *Genetics* **155:** 945–959.

Puritz JB, **Hollenbeck CM**, **Gold JR. 2014.** *dDocent*: a RADseq, variant-calling pipeline designed for population genomics of non-model organisms. *PeerJ* **2:** e431.

Quach QN, Reynolds RG, Revell LJ. 2019. Historical allopatry and secondary contact or primary intergradation in the Puerto Rican Crested Anole, *Anolis cristatellus*, on Vieques Island in the Caribbean, v2, Dryad, Dataset, https://doi.org/10.5061/dryad.80gb5mkn4.

R Core Team. **2016.** *R: a language and environment for statistical computing.* Vienna: R Foundation for Statistical Computing. Available at: https://www.R-project.org (accessed 19 October 2019).

Revell LJ. 2012. phytools: an R package for phylogenetic comparative biology (and other things). *Methods in Ecology and Evolution* **3:** 217–223.

Reynolds RG, **Strickland TR**, **Kolbe JJ**, **Falk BG**, **Perry G**, **Revell LJ**, **Losos JB. 2017.** Archipelagic genetics in a widespread Caribbean anole. *Journal of Biogeography* **44:** 2631–2647.

Rodríguez-Robles JA, **Jezkova T**, **García MA. 2007.** Evolutionary relationships and historical biogeography of *Anolis desechensis* and *Anolis monensis*, two lizards endemic to small islands in the eastern Caribbean Sea. *Journal of Biogeography* **34:** 1546–1558.

Ronquist F, **Teslenko M**, **van der Mark P**, **Ayres DL**, **Darling A**, **Hohna S**, **Larget B**, **Liu L**, **Suchard MA**, **Huelsenbeck JP. 2012.** MrBayes 3.2: efficient Bayesian phylogenetic inference and model choice across a large model space. *Systematic Biology* **61:** 539–542.

RStudio Team. **2015.** *RStudio: Integrated Development Environment for R*. Boston: R Studio. Available at: http://www.rstudio.com (accessed 19 October 2019).

Sara TR, **Ortiz Aguilu JJ**, **Newsom LA**, **Parrish NA**, **Jones JG**, **Pantel AG. 2003.** Paleoenvironmental investigation of Navy lands of Vieques, Puerto Rico. *Miscellaneous Reports of Investigations* **280:** 1–134.

Schliep K. 2011. Phylogenetic analysis in R. *Bioinformatics* **27:** 592–593.

Schneider CJ. 1996. Distinguishing between primary and secondary intergradation among morphologically differentiated populations of *Anolis marmoratus*. *Molecular Ecology* **5:** 239–249.

Schnute JT, **Couture-Beil A**, **Haigh R**, **Kronlund AR. 2013.** PBSmodelling 2.65: user's guide. *Canadian Technical Report of Fisheries and Aquatic Sciences* **2674:** viii–194.

Severinghaus JP, **Brook EJ. 1999.** Abrupt climate change at the end of the last glacial period inferred from trapped air in polar ice. *Science* **286:** 930–934.

Siddall M, **Rohling EJ**, **Almogi–Labin A**, **Hemleben C**, **Meischner D**, **Schmelzer I**, **Smeed DA. 2003.** Sea-level fluctuations during the last glacial cycle. *Nature* **423:** 854–858.

Smith BT, **Klicka J. 2013.** Examining the role of effective population size on mitochondrial and multilocus divergence time discordance in a songbird. *PLoS One* **8:** e55161.

Stamatakis A. 2014. RAxML version 8 a tool for phylogenetic analysis and post-analysis of large phylogenies. *Bioinformatics* **30:** 1312–1313.

Thomas R, **Joglar R. 1996.** The herpetology of Puerto Rico: past, present, and future. In: Figueroa Colon JC, ed. *The scientific survey of Puerto Rico and the Virgin Islands: an eighty-year reassessment of the islands' natural history*. New York: The New York Academy of Sciences, 181–120.

Thorpe RS, **Leadbeater DL**, **Pook CE. 2005.** Molecular clocks and geological dates: cytochrome b of *Anolis extremus* substantially contradicts dating of Barbados emergence. *Molecular Ecology* **14:** 2087–2096.

Trejo-Torres JC, **Ackerman JD. 2001.** Biogeography of the Antilles based on a parsimony analysis of orchid distributions. *Journal of Biogeography* **28:** 775–794.

SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article at the publisher's website.

Appendix S1. Supplementary methods: laboratory protocol for GBS library preparation.
Appendix S2. Supplementary methods and results for morphological data.
Appendix S3. Supplementary figures.
Appendix S4. Supplementary tables.

SHARED DATA

All data from this study are archived on Dryad (doi:10.5061/dryad.80gb5mkn4, Quach *et al.*, 2019).