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RESEARCH PAPER

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Phylogeographic and phenotypic outcomes of brown anole colonization across the Caribbean provide insight into the beginning stages of an adaptive radiation

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Abstract

Some of the most important insights into the ecological and evolutionary processes of diversification and speciation have come from studies of island adaptive radiations, yet relatively little research has examined how these radiations initiate. We suggest that *Anolis sagrei* is a candidate for understanding the origins of the Caribbean *Anolis* adaptive radiation and how a colonizing anole species begins to undergo allopatric diversification, phenotypic divergence and, potentially, speciation. We undertook a genomic and morphological analysis of representative populations across the entire native range of *A. sagrei*, finding that the species originated in the early Pliocene, with the deepest divergence occurring between western and eastern Cuba. Lineages from these two regions subsequently colonized the northern Caribbean. We find that at the broadest scale, populations colonizing areas with fewer closely related competitors tend to evolve larger body size and more lamellae on their toepads. This trend follows expectations for post-colonization divergence from progenitors and convergence in allopatry, whereby populations freed from competition with close relatives evolve towards common morphological and ecological optima. Taken together, our results show a complex history of ancient and recent Cuban diaspora with populations on competitor-poor islands evolving away from their ancestral Cuban

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populations regardless of their phylogenetic relationships, thus providing insight into the original diversification of colonist anoles at the beginning of the radiation. Our research also supplies an evolutionary framework for the many studies of this increasingly important species in ecological and evolutionary research.

KEYWORDS

adaptive radiation, convergent evolution, ecological release, morphometrics, next-generation sequencing, phylogeography, population genomics

1 | INTRODUCTION

Dedication: This manuscript is dedicated to the memory of Lourdes Rodríguez-Schettino, a remarkable scientist and major contributor to the systematics and ecology of Cuban anoles, including A. sagrei. *Without her, the present work (and so much else) would not have been possible.*

Interest in adaptive radiation has increased greatly over the last two decades (Glor, 2010; Losos & Mahler, 2010; Parent & Crespi, 2009; Stroud & Losos, 2016; Yoder et al., 2010). Over a slightly longer period, the study of geographic variation, also known as phylogeography, has similarly expanded (Avise, 2009; Knowles, 2009). In many respects, these two areas of investigation are complementary. Research on adaptive radiation focuses on speciation and ecological diversification (Glor, 2010; Losos, 2011a; Schluter, 2000; Yoder et al., 2010), whereas phylogeographic investigation focuses on intraspecific differentiation through geographic space (Avise, 2000). Most phylogeographic work focuses on patterns of genetic divergence among populations, though the adaptive basis of phenotypic differentiation is also occasionally studied (e.g. Deagle, Jones, Absher, Kingsley, & Reimchen, 2013; Thorpe, Barlow, Malhotra, & Surget-Groba, 2015; Thorpe, 2017). Relatively few projects unite these two perspectives; however, it is reasonable to suppose that in many cases adaptive radiations are initiated by the geographic divergence among populations. A phylogeographic perspective should thus provide important insights into the origins of adaptive radiation.

Understanding the early stages of adaptive radiation is often difficult, especially for old and diverse radiations for which little information regarding ancestral taxa survives. Especially on islands, a species in an adaptive radiation might bud off colonizing populations that occupy areas allopatric to other members of the radiation (Carlquist, 1974). In this context, studying such populations might serve as a good analogue to the ancestral species that first colonized the islands, thus providing important insight into the earliest stages of adaptation and speciation that ultimately led to the radiation of the group (Grant, 1998). The ecological release or ecological opportunity hypotheses also suggest that populations colonizing areas with fewer competitors should exhibit greater morphological trait variance than populations of the same species found in multispecies communities (Bolnick, Svanback, Araujo, & Persson, 2007; Nosil & Reimchen, 2005; Yoder et al., 2010). Alternatively, populations might evolve directionally away from ancestral populations, owing to either idiosyncratic local selective pressures producing trait divergence among populations (i.e. Price, Phillimore, Awodey, & Hudson, 2010), or general 'relaxation' towards a phenotypic optimum that is favoured in the absence of competition resulting in similar phenotypic outcomes among populations (Lister, 1976b).

Caribbean *Anolis* lizards are well-known for their replicated adaptive radiations on the islands of the Greater Antilles (reviewed in Williams, 1969, Williams, 1983; Schluter, 2000; Losos, 2009), in which anole lineages have diversified ecologically and morphologically by specializing to use different microhabitats (Jackman, Losos, Larson, & Queiroz, 1997; Losos, Jackman, Larson, Queiroz, & Rodríguez-Schettino, 1998; Mahler, Ingram, Revell, & Losos, 2013). A key feature of these replicated radiations is that almost all species are each confined to a single island. That is to say, the radiations are independent because there has been almost no recent exchange of species between islands (at least prior to human activity (Helmus, Mahler, & Losos, 2014)). These radiations, however, are quite old. The most recent molecular divergence-time estimates suggest that anoles may have begun diversifying around 50 million years ago (Poe et al., 2017; Román-Palacios, Tavera, & Castañeda, 2018). Given that the oldest known fossil anoles date to about twenty million years before the present (Sherratt et al., 2015), we have little direct evidence about the early stages of the Greater Antillean anole radiations, shrouded as they have been by the depths of time (Stroud and Losos, 2016). Phylogenetic analysis makes clear that anoles are descended from an ancestral mainland taxon, some of whose descendants later diversified on the islands of the Greater Antilles. But the ecomorphology of that colonizing species is hard to infer owing to the enormous quantity of time that has elapsed and to the evolutionary lability of traits associated with anole ecomorphs, leading in turn to low confidence in deep-in-time ancestral reconstructions (Losos, 2011b; Losos & de Queiroz, 1997; Poe, Goheen, & Hulebak, 2007).

The evolution of multispecies communities is not a one-way street: some species that evolved in multispecies anole communities in the Greater Antilles have subsequently colonized smaller islands where very few or no other anole species occurred (Losos, Irschick, & Schoener, 1994; Losos & de Queiroz, 1997; Poe et al., 2007). Half a century ago, Ernest Williams noted that, in contrast to the insular endemism that characterizes anole replicated radiations,

two species complexes, '*A. carolinensis*' (more accurately *A. porcatus* given that Cuba appears to be the ancestral area for the complex) and *A. sagrei*, are widespread and relatively adept at colonizing over water (Figure 1a; Williams, 1969). Williams (1969) further suggested that much could be learned about anole evolutionary diversification via study of these two taxa. If co-occurrence with close relatives leads species to occupy and adapt to specialized microhabitats, then subsequent colonization of depauperate communities might be expected to reverse this trend.

The most thorough test of Williams' hypothesis remains Lister's (1976a,b) examination of seven populations of *A. sagrei* across its range. Lister found that on islands with few or no other anole species, *A. sagrei* perched higher off the ground than the other semi-terrestrial 'trunk-ground' habitat specialists of the Greater

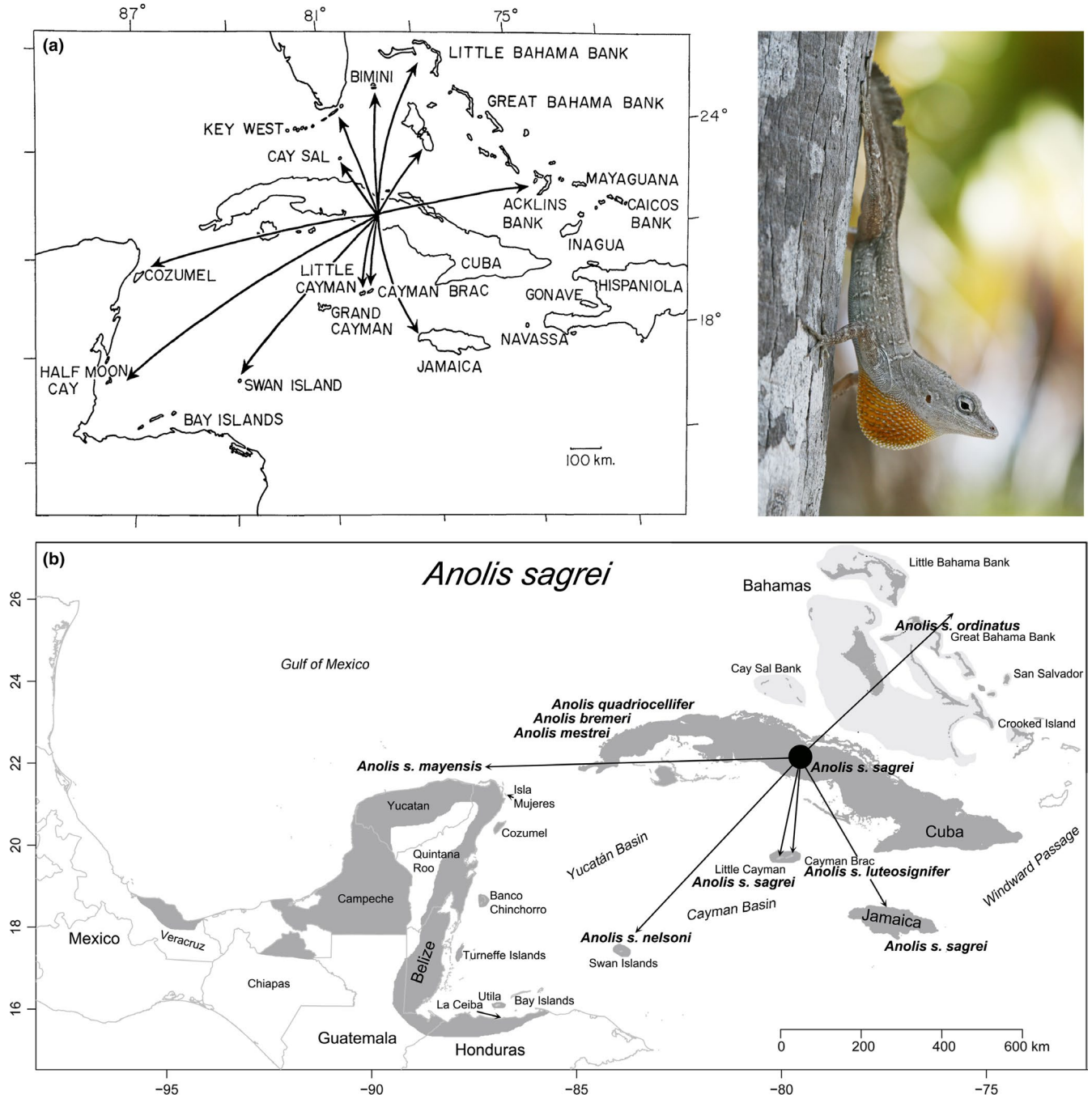


FIGURE 1 (a) Hypothesized spread of *Anolis sagrei* diaspora from an ancestral range in Cuba from Williams (1969), reprinted with permission. (b) Our interpretation of the native range of *A. sagrei* across the Caribbean Basin (in dark grey). Note that it has been considered unclear whether populations on Jamaica and in Mesoamerica are native or anthropogenically introduced. Proposed subspecies are shown (after Schwartz & Thomas, 1975), and the other species within the *sagrei* species group are shown in western Cuba. Arrows show hypothetical dispersal patterns in the *sagrei* group are overlaid on the map. Geographic points mentioned in the text are labelled. The photograph, taken by the first author, is of a male *A. sagrei* from Conception Island, Bahamas

Antilles, including Cuban *A. sagrei*. Correspondingly, these populations evolved better-developed toepads (more lamellae), a trait that is linked to greater arboreality in anoles (Irschick, Herrel, & Vanhooydonck, 2006). Despite the enormous amount of research devoted to anoles in the subsequent 40 years (Losos, 2009), only a few studies have been focused on the diaspora of either of these two prolifically colonizing taxa (Glor, Losos, & Larson, 2005; Kolbe et al., 2004), as well on whether or not colonizing lineages of either taxon might have evolved under a scenario of ecological release (increased phenotypic variance) or directionally (divergent or convergent selection in different populations) when freed from competitive interaction with congeners (or trophically similar species; Wright, 1981).

We aim to rectify this deficiency, focusing on *A. sagrei*, the most widely distributed anole species in the Caribbean (Figure 1b). Although *A. sagrei* is a workhorse in both ecological and evolutionary research programmes (e.g. Cox & Calsbeek, 2015; Driessens, Huyghe, Vanhooydonck, & Damme, 2015; Bonneaud et al., 2016; Delaney & Warner, 2016; Fleishman et al., 2016; Logan, Duryea, Molnar, Kessler, & Calsbeek, 2016; Schoener, Kolbe, Leal, Losos, & Spiller, 2017; Stroud, Giery, & Outerbridge, 2017; Kamath & Losos, 2018; Lapiedra, Schoener, Leal, Losos, & Kolbe, 2018), we have heretofore lacked an understanding of the magnitude and geographic distribution of genetic and morphological diversity within this widespread species. From its ancestral origins in Cuba, *A. sagrei* has colonized the Bahamas Archipelago to the north and east, as well as the Cayman Islands, Jamaica (although human assistance in this case is debated), the Swan Islands (nearly 500 km south of the western tip of Cuba) and the Atlantic versant of Honduras, Mexico and Belize (Figures 1b and 2a; not to mention several farther-flung inarguably anthropogenic introductions). Almost everywhere that it occurs naturally (with the possible exception of marginal habitats in Cuba), *A. sagrei* is the most common anole, with densities in some places as high as one lizard per square metre (Schoener & Schoener, 1980).

In their ecology and morphology, members of this species are classic 'trunk-ground' anoles (Williams, 1969), whose major habitat includes broad structures low to the ground, such as tree trunks and boulders. In Cuba, where the species evolved as part of the Cuban anole radiation (Kolbe et al., 2004; Williams, 1969) currently comprising at least 64 extant species (Uetz and Hošek, 2016), *A. sagrei* occurs in complex communities that can include as many as ten other anole species adapted to different structural and microclimatic niches (Rodríguez-Schettino, 1999; Rodríguez-Schettino et al., 2010; Williams, 1969). By contrast, throughout much of the rest of its non-Cuban range, *A. sagrei* co-occurs with few or no other anole species. In the Bahamas, for example, it can often be found on single-species islets that can be as small as 10 m^2 (Schoener & Spiller, 2010), as well as on larger islands with 1–3 other anole species (Buckner, Franz, & Reynolds, 2012).

This geographic dispersion of *A. sagrei* suggests several interesting questions, most generally: How many times have islands been colonized directly from Cuba? Williams' (1969) figure (reproduced here as Figure 1a, perhaps not meant to be taken literally in its original presentation) suggested as many as 12 dispersal events from Cuba to other regions in the Caribbean, thus raising the possibility that historical contingencies of the colonization process might lead to varying evolutionary outcomes across these islands.

This extraordinary colonizing ability, combined with its great abundance and ability to thrive in many different habitats, suggests that *A. sagrei* might be a good model for the ancestor of the anole adaptive radiations (Losos, 2009; Williams, 1969). In this situation, as populations in the *A. sagrei* diaspora are released from intense interspecific competition, we might expect them to experience ecological release (Lister, 1976a; Losos & de Queiroz, 1997; Nosil & Reimchen, 2005; Wellborn & Langerhans, 2015; Yoder et al., 2010). Nevertheless, more nuanced outcomes are also conceivable. Based on previous hypotheses related to evolution in West Indian anoles, we make four predictions of potential outcomes for populations descended from ancestors that occurred in multispecies communities, but which now find themselves in a relatively species-poor guild (Williams, 1969):

1. 'Trait Variance Expansion'. Island-colonizing populations might have evolved greater morphological variance, owing to selection favouring increased niche breadth related to ecological release/opportunity (e.g. Nosil & Reimchen, 2005; Bolnick et al., 2007; but this is not seen in Lesser Antillean anoles (Eaton, Larimer, Howard, Powell, & Parmerlee, 2002; Losos & de Queiroz, 1997)).
2. 'Intraspecific Divergence'. Island-colonizing populations might have evolved away from their immediate ancestral morphology. Specifically, intraspecific phenotypic divergence increases through time because individual populations tend to experience idiosyncratic directional selective pressures owing to environmental differences among colonized islands (e.g. Lister, 1976a; Price et al., 2010; Yoder et al., 2010).
3. 'Convergence'. Island-colonizing populations might evolve directionally towards a phenotypic optimum in the absence of competitors that differs from that of the progenitor populations existing in environments with congeneric competitors (Lister, 1976b; Losos et al., 1998).
4. 'Evolutionary Contingency'. Island-colonizing populations might show no clear patterns of morphological diversification owing to historically contingent differences among populations, such as differences among ancestral populations or in the genetic constitution of the individuals in founder populations (e.g. Price, Lovette, Bermingham, Gibbs, & Richman, 2000).

Here, we examine these not mutually exclusive scenarios in *A. sagrei* across the complete native range of the species.

2 | MATERIALS AND METHODS

2.1 | Phylogeography: Genetic sample collection and data

New genetic samples in this study represent collections spanning the last two decades by the authors and others. Our sampling of 298 individuals covered the range of *A. sagrei*, with between one

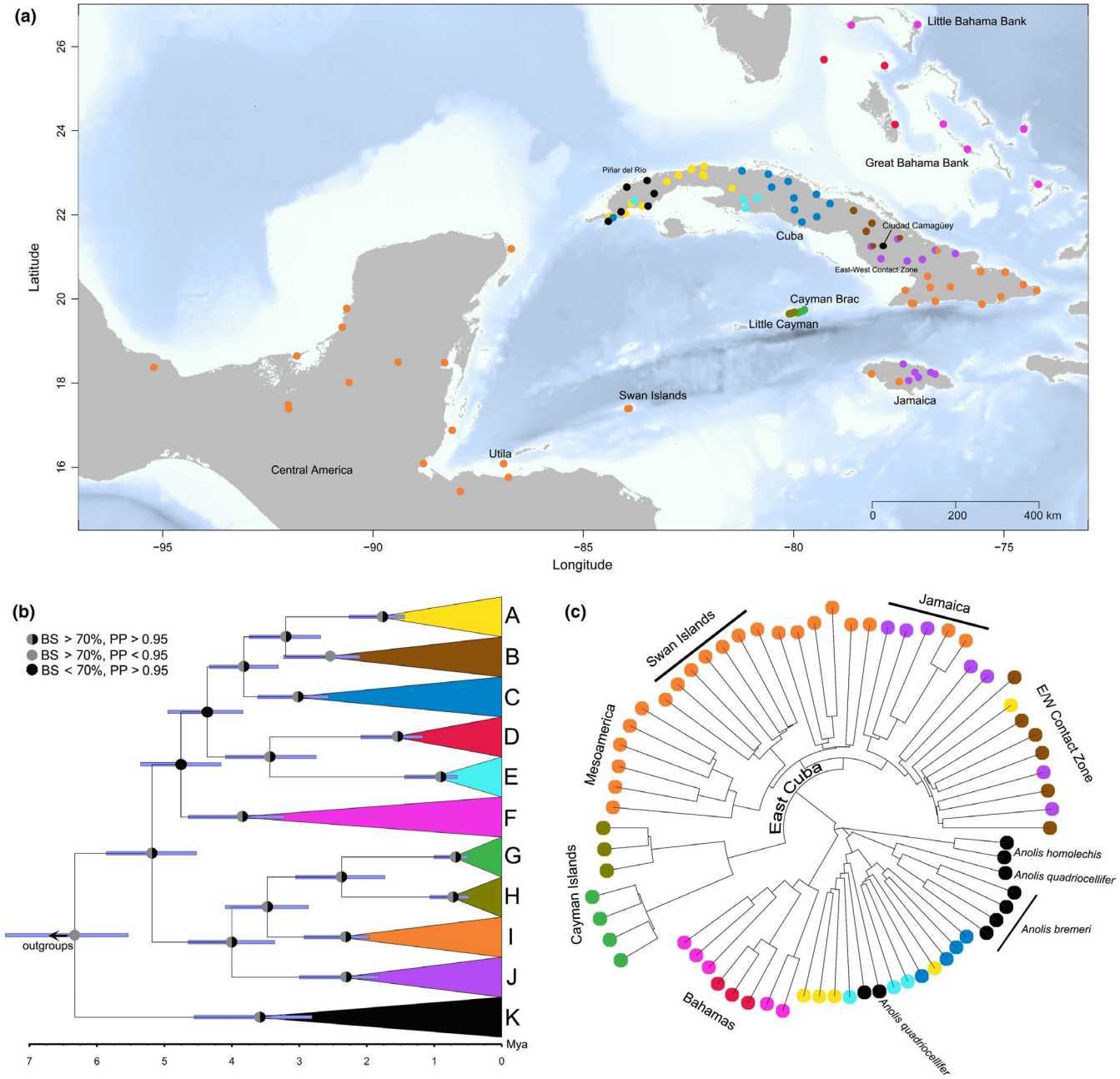


FIGURE 2 (a) Map of sampling locations in this study, colour-coded by the mtDNA clade to which they belong. (b) Time-calibrated mtDNA gene tree for *Anolis sagrei* from BEAST with nodal support shown. (c) Neighbour-joining SNP tree for a subset of samples from across the range of *Anolis sagrei*. Tips are colour-coded by mtDNA clade to show correspondence between mtDNA and nucDNA phylogenetic inferences. Map rendered in the R package *marmap* (Pante & Simon-Bouhet, 2013) from NOAA bathymetric data at a resolution of 1-arc minute

and five genetic samples from each of 95 localities across the northern Caribbean basin, including the Greater Antillean islands of Cuba (53 localities) and Jamaica (8 localities); the Great and Little Bahama Banks (9 localities); Cayman Islands (4 localities); Swan Islands ('Islas de Cisne'); and 15 localities on mainland Mesoamerica (Mexico, Belize and Honduras) (Figure 2a; Table S1). We also sampled other species closely related to *A. sagrei*, including *A. bremeri*, *A. quadriocellifer* and *A. homolechis* (all of which are members of the *A. sagrei* series; Cádiz et al., 2013; also referred to as the *Trachypilus* clade by Poe et al., 2017).

To generate sequences for our samples we extracted whole genomic DNA (gDNA) from tissue samples using the Wizard SV® Kit (Promega) and subsequently stored extracts at −20°C. We used the polymerase chain reaction (PCR) to amplify a fragment of the mitochondrial genome (NADH subunit 2 [*ND2*]; primers from Macey, Larson, Ananjeva, & Papenfuss, 1997; conditions in Revell, Harmon, Langerhans, & Kolbe, 2007). We sequenced PCR products in both directions on an automated sequencer (ABI 3730XL) at the Massachusetts General Hospital DNA Core Facility. We assembled

contigs and visually verified ambiguous base calls using GENEIOUS® 7.1.2 (Biomatters). We then generated an alignment including additional out-groups using the CLUSTALW 2.1 (Larkin et al., 2007) algorithm implemented in GENEIOUS using reference sequences and default parameters. We deposited this alignment in Dryad (accession # pending).

We analysed the complete mtDNA data set using both maximum-likelihood (ML) and Bayesian methods. We selected the best-fit model of molecular evolution for the *ND2* locus (*TrN + G*) using the Bayesian information criterion (BIC) in JMODELTEST2 (Darriba, Taboada, Doallo, & Posada, 2012; Guindon & Gascuel, 2003). We conducted ML analysis using RAxML (Stamatakis, 2006) via the RAxML plugin for GENEIOUS 7.1.2. We used the GTRGAMMA model, as the TrN model is not implemented in the software and the use of the invariant sites parameter (I) has been recommended against in RAxML (since RAxML, like all phylogeny inference software, implements a discretized Γ model, invariant sites are usually confounded with one of the Γ rate categories; Stamatakis, 2006). We used the rapid bootstrapping algorithm with 10^3 bootstrap (BS) replicates followed by the thorough ML search option with 100 independent searches. We consider BS values above 95% to indicate well-supported clades and values >70% but < 95% to indicate moderately supported clades (Felsenstein, 2004; Taylor & Piel, 2004). To generate rough estimates of divergence times across the mitochondrial gene tree, we inferred a time-calibrated *ND2* tree in the program BEAST v2.1.2 (Bouckaert et al., 2014). We estimated this tree using a relaxed molecular clock model and a rate of molecular evolution (1.3% pairwise divergence per million years) estimated via geologic events in related agamid lizards (Macey et al., 1998) that has been widely used in other studies of anoles (e.g. Campbell-Staton et al., 2013; Creer, Queiroz, Jackman, Losos, & Larson, 2001; Gartner, Gamble, Jaffe, Harrison, & Losos, 2013; Glor et al., 2004; Glor, Kolbe, Powell, Larson, & Losos, 2003; Glor et al., 2005; Jackman, Irschick, Queiroz, Losos, & Larson, 2002; Tollis, Ausubel, Ghimire, & Boissinot, 2012). We relied on this estimated rate of pairwise divergence because few pre-Pleistocene fossil records exist for *Anolis*, and the small number of specimens of amber-preserved anoles is either taxonomically ambiguous, making their placement on a phylogeny problematic (Castañeda, d. R., Sherratt, E., & Losos, J. B., 2014), or is not very closely related to *A. sagrei* (Sherratt et al., 2015). We ran BEAST for 100 million generations using the *TrN + G* substitution model, a Yule speciation prior and an uncorrelated lognormal (UCLN) relaxed molecular clock model. We repeated the analyses three times sampling every 10^4 generations and discarding the first 25% of generations as burn-in following analysis of likelihood trace files. All computations were conducted on the Odyssey cluster supported by the Harvard FAS Research Computing Group (odyssey.fas.harvard.edu). We assured adequate mixing of the chains by calculating the effective sample size (ESS) values for each model parameter, with ESS values greater than 200 taken to indicate adequate sampling of the posterior distribution. We assessed convergence of the independent runs by a comparison of likelihood scores and model parameter estimates in TRACER v1.5 (Rambaut, Suchard, Xie, & Drummond, 2013). We combined the results from the three analyses using LOGCOMBINER and generated a maximum clade credibility (MCC) tree using TREEANOTATOR. We estimated genetic distances (Tamura-Nei [TrN] distances) between major clades using MEGA 6.0 (Tamura, Stecher, Peterson, Filipski, & Kumar, 2013).

To examine nuclear diversity and divergence in the *A. sagrei* series, we selected a subset of 61 *A. sagrei* individuals representing each of the 11 main mtDNA clades identified in our analyses above and spanning the entire geographic range of the species. We selected a further eight individuals of closely related taxa (*A. bremeri n* = 4, *A. quadriocellifer n* = 2, *A. homolechis n* = 2) that were also represented in our mtDNA data set. We conducted GBS (genotyping by sequencing) using the nextRAD (Nextera®-tagmented reductively amplified DNA) GBS approach as implemented by SNPsaurus (Institute of Molecular Biology) followed by single nucleotide polymorphism (SNP) genotyping for these 69 samples from 58 localities. Briefly, nextRAD uses selective primers to amplify fragments across the genome, as opposed to using restriction enzymes followed by size selection (Baird et al., 2008; Etter & Johnson, 2012). These fragments are initially generated using Illumina® Nextera® tagmentation, followed by selective PCR amplification and ligation of sequencing adapters and barcoded indices (Russello, Waterhouse, Etter, & Johnson, 2015). Prior to library preparation, we quantitated each of our gDNA samples using a Qubit® 2.0 fluorometer system. Genomic DNA was fragmented using the Nextera reagent (Illumina®, Inc.), which also ligates short adapter sequences to the ends of each fragment. This reaction calls for 15ng of gDNA per sample, though 22.5ng of gDNA was included to compensate for the degradation of some of our older samples. We then amplified these fragments using a selective primer complementary to the adapter sequences, which also includes a nine base-pair (bp) selective sequence (GTGTAGAGC). We conducted amplification using PCR at 73°C for 26 cycles, during which only fragments of gDNA that were able to hybridize with the selective sequence are amplified. We performed sequencing on an Illumina® NextSeq 500 at the Institute of Molecular Biology, Eugene, OR, using a high-output run to generate ~ 400 million single-end sequencing reads 75bp in length. We initially quality-filtered our sequencing reads using Trimmomatic (Bolger, Lohse, & Usadel, 2014) to remove adapters and low-quality reads (with a Phred score of < 20), resulting in a mean of 4,178,671 reads per individual. We called SNPs using the SNPsaurus nextRAD pipeline. This pipeline consists of custom scripts (SNPsaurus, LLC) that create a de novo reference from abundant reads. All sequencing reads are then mapped to the reference with an alignment identity threshold of 93% (BBMAP, http://sourceforge.net/projects/bbmap/). We called genotypes using SAMTOOLS and BCFTOOLS using the following flags: (samtools mpileup –gu –Q 10 –t DP, DPR –f ref.fasta –b anolis.align_samples | bcftools call –cv -> anolis.vcf). We converted the resulting variant-call format (VCF) genotype file to subsequent input files using PDGSPIDER (Lischer & Excoffier, 2012) after excluding loci with only missing data and nonpolymorphic loci, as well as SNPs with Phred scores less than 10. This resulted in 31,702 polymorphic SNPs across 12,415 loci

(1–21 SNPs per locus), with an average of 7.62% missing data across 69 genotypes of *A. sagrei* and out-groups.

We used the package VCFR (Knaus & Grünwald, 2017) in RStudio v1.0.136 (RStudio Team, 2016) running R v3.2.4 (R Development Team, 2016) to import and convert our VCF file within the R environment. We first assessed the quality of the SNP data set by examining the distribution of missing data and minor alleles, as well as the distribution of SNPs across the 75bp reads using the R package ADEGENET (Jombart, 2008; Jombart & Ahmed, 2011). We then calculated per-locus statistics including observed and expected heterozygosity (H_o and H_e) and F_{ST} to test for deviations from Hardy–Weinberg equilibrium and to characterize genetic differentiation of populations.

As a first pass at understanding the relationships between the populations represented in our SNP data set, we constructed a neighbour-joining (NJ) tree using the R package APE (Paradis et al., 2016; Paradis, Claude, & Strimmer, 2004) after parsing the data with the package PEGAS (Paradis, 2010). We visualized the tree using the package ADEGENET (Jombart, 2008; Jombart & Ahmed, 2011). We do not suppose that the relationships between all of the different populations in our study are genuinely entirely 'tree-like' and as such we also constructed a network using the neighbour-net algorithm implemented in SPLITSTREE v.4.13.1 (Huson & Bryant, 2006) and assessed support among the major groups using 10^3 nonparametric bootstrap replicates.

Another approach to defining geographic evolutionary 'groupings' is to detect the degree to which individual island populations, lineages within islands or island groups form natural clusters of evolutionarily related populations. To do this, we used two methods to examine clustering of individual SNP genotypes across *A. sagrei* sampling locations. First, we used a discriminant analysis of principal components (DAPC; Jombart, Devillard, & Balloux, 2010) implemented in the R package ADEGENET (Jombart, 2008). This method attempts to maximize genetic differentiation between groups and minimize variation within groups by clustering individual genotypes using a principal component transformation of the genetic data prior to discriminant analysis. We used a BIC approach to obtain the predicted number of clusters between $K = 1$ and $K = 20$ after retaining n-1 PCs in optimizing the assignment of individuals to groups. To perform the DAPCs, we selected the optimal number of PCs to generate the discriminant functions using *optim.a.score()* in ADEGENET with 10^3 replications. We used a hierarchical set of analyses to examine clustering across our data set. Our initial analysis included all 69 genotypes (including nominally different species within the *A. sagrei* series). We followed this with independent substructure analyses until we no longer detected multiple clusters. We examined loading plots to ensure that a small number of loci were not contributing to discriminant function loading.

A more explicit population genetic (allele frequency-based) approach to clustering is the Bayesian clustering algorithm STRUCTURE (Pritchard, Stephens, & Donnelly, 2000), which uses an MCMC approach to cluster K groups based on individual allele frequencies. We trimmed our SNP data set to include only the first SNP from each of the first 1,000 loci to retain only presumably independent loci for clustering analyses (Pritchard et al., 2000). We used the admixture model and 10 replications of $K = 1$ to $K = 10$, with each replication consisting of a run length of 10^6 generations and a burn-in of 25% following evaluation of convergence. We selected values of K using the ΔK method of Evanno, Regnaut, and Goudet (2005) implemented in STRUCTURE HARVESTER (Earl & vonHoldt, 2012), and we visualized output using STRUCTURE PLOT (Ramasamy, Ramasamy, Bindroo, & Naik, 2014) after using the CLUMPP 1.1.2 (Jakobsson & Rosenberg, 2007) algorithm to combine results across independent runs. As with DAPC, we used a hierarchical set of analyses to examine natural evolutionary clusters, or groups, across our data set. Our initial run consisted of the full 61 genotype SNP data set representing *A. sagrei* individuals from each of the mtDNA clades A–K (excluding the eight individuals representing other *A. sagrei* series species). Following this preliminary clustering, we subsequently ran nested substructure analyses as above.

We calculated Cavalli-Sforza and Edwards Chord distance (*Dch*; Takezaki & Nei, 1996), and Nei's distance (*Ds*; Nei, 1972) for each of the genomic groupings delimited in the above DAPC clustering analyses using the R package HEIRFSTAT (Goudet, 2005). We tested for isolation by distance (IBD) in the *A. sagrei* SNP data set (61 genotypes) by calculating pairwise *Dch* genetic distances between samples grouped by sampling locations in HEIRFSTAT. We converted sampling locations from latitude/longitude coordinates (decimal degrees) to log-transformed Euclidean distance measures using the GEOGRAPHIC DISTANCE MATRIX GENERATOR v1.2.3 (Ersts, 2017). We then used ADEGENET (Jombart, 2008; Jombart & Ahmed, 2011) to test the hypothesis of IBD by computing matrix correlations (Mantel test; Mantel, 1967) between the matrices using the *mantel.rtest()* function from the R package ADE4 (Dray & Dufour, 2007) with alpha = 0.05 and 10^4 repetitions. We also conducted IBD analyses on the mtDNA data from the same individuals used to generate the SNP data set. We reduced the mtDNA alignment to these individuals and calculated genetic distances among them in HEIRFSTAT using the *Ds* (Nei distance) model as a measure of minimum distances. We then conducted IBD analysis as above with the same geographic distance data set.

To investigate whether a correlation exists between mitochondrial genetic distance and nuclear genomic distance, as might be expected given a strong phylogeographic signal (implicating allopatric diversification), we conducted a multiple matrix regression (MMR) analysis (partial Mantel test) controlling for geographic distance. We used the function *mantel.partial()* in the R package VEGAN (Legendre & Legendre, 2012; Oksanen et al., 2014) to model the SNP distance data set as the dependent matrix, and the geographic and mtDNA distance data sets as the independent matrices using a Pearson coefficient and 10^3 permutations. We calculated genetic distances and geographic distances as above, and simultaneously visualized the matrices using the *surf3D()* function in the R package PLOT3D (Soetaert, 2013).

2.2 | Phenotype: Quantitative trait diversification

Our scenarios for trait evolution in diasporic *A. sagrei* posit that we might see one or more of the following patterns: an expansion of

trait variance within populations, a shift in trait values among populations (with populations evolving idiosyncratically or convergently or both) or no apparent pattern of trait evolution owing to evolutionary contingency. To assess the evolution of presumably ecologically relevant quantitative morphological traits (QTs) in *A. sagrei*, we examined 558 museum specimens representing 40 sampling locations across the range of the species for which we also had genetic data. We measured intact and well-prepared individuals (e.g. no missing limbs and body proportions undistorted by preservation) in the collection of the Harvard Museum of Comparative Zoology (MCZ), supplemented by specimens from the Museum of Vertebrate Zoology at Berkeley (MVZ), the University of Kansas Biodiversity Institute (KU), the Natural History Museum of Los Angeles (NHMLA) and the Florida Museum of Natural History (FLMNH) (Table S2). To reduce potential complications owing to sexual dimorphism, we selected only adult males, ascertaining this condition by the presence of two or more of the following characteristics: an enlarged dewlap, a relatively wide tail base, everted hemipenes, enlarged post-anal scales and lack of a typical female dorsal colour pattern. We obtained a radiograph of each individual using a Thermo Kevex cabinet X-ray system (Model PX510-16W) at the MCZ, with a setting of 30μa and 30kV and a standard metric ruler on the detector plate. We taped each specimen to the detector plate to ensure that we obtained a consistent plane of focus, and we conducted image acquisition with Varian Image Viewing and Acquisition (v2.0, Varian Paxscan Medical Systems). In addition, we scanned the toepads of each individual using a standard flatbed scanner (Epson v500) at a resolution of 1200–2600 dpi.

We analysed both radiographs and scans in the program ImageJ 1.43 (Rasband, W.S., ImageJ, U. S. National Institutes of Health, Bethesda, Maryland, USA). A single observer obtained the following nine linear measurements for each radiograph (Figure S1): snout–vent length (SVL), head length from tip of rostrum to posterior of occipital (HL), head width behind the eyes (HW), snout width (SW), snout length (SL), upper jaw length (JL, average of left and right sides), femur length (FL, average of left and right sides), tibia length (TL, average of left and right sides) and metatarsus IV length (MTL, average of left and right sides). We conducted symmetry and repeatability measurements on a subset of individuals to ensure reliability of the measurements, with the average for left- and right-side measurements being used after excluding specimens scored as asymmetric or unrepeatable (details in Gómez Pourroy, 2014). We counted lamellae on scanned images of the fourth hind toe using the 'multipoint' function in ImageJ. We followed the methodology outlined in Köhler (2014), with the slight modification of counting lamellar scales from the distal to the proximal ends of phalanges III to V (numbered distal to proximal) of the fourth hind toe, beyond the plane of the basal joint where the fourth toe meets the third toe, to the last easily identifiable enlarged scale.

We log-transformed quantitative trait data to reduce skew and obtained residuals from a regression of each of the eight sets of linear measurements against SVL to control for overall size. We also size-corrected lamella counts, as body size and lamella number are potentially correlated among species in *Anolis* (Glossip & Losos, 1997). We checked residuals for outliers and normality and used the residuals for subsequent analyses, taking the mean residuals for each sampling site. We performed these and all ensuing analyses in R, with a complete data set consisting of the residuals of the nine linear measurements and counts.

We initially surveyed the data set for broad-scale clustering in a multivariate framework using both *K*-means and model-based BIC clustering analyses which we implemented in the R package mclust (Fraley & Raftery, 2002; Fraley, Raftery, Murphy, & Scrucca, 2012). We next used principal component analysis (PCA) to determine whether morphological variation in our nine-character QT data set is partitioned among Cuban anoles and their diaspora. We used population mean residuals from size correction and examined loadings, biplots and proportion of explained variance to examine our ability to discriminate between these two groups. To test whether Cuban populations differed significantly from diaspora populations, we conducted MANOVA on all PC axes and then on residuals from a regression of all QTs on SVL, followed by tests for mean trait shifts using independent *t* tests (Cuba vs. diaspora) on each of the nine QTs with Benjamini–Hochberg correction (Benjamini & Hochberg, 1995) for multiple tests. We calculated mean and variance of the size-corrected residuals for each trait grouped by Cuba or diaspora (Cuba n = 140; diaspora n = 420). Despite these groupings consisting of divergent evolutionary lineages (see below), we are interested in whether a reduction in the number of sympatric anole species yields a detectable shift in morphological traits. We then used Levene's test to test for trait variance expansion between Cuban and diaspora populations followed by visualizing the data using density plots in ggplot2 (Wickham, 2009).

Next, we assessed QT divergence within and among a series of a priori groupings to determine whether relationships exist between morphological variation and geographic/genetic/taxonomic grouping, or whether morphological variation is independent of prior grouping. To do this, we first repeated the above PCA with points colour-coded by broad geographic region (Bahamas, Cayman Islands, Cuba, Jamaica, Mesoamerica and Swan Islands), to visualize among-group separation in morphometric space. We then assessed whether QTs were different among regional groupings using an ANOVA followed by *post hoc* Tukey tests. To then explicitly test our ability to use morphometric data to discriminate among a priori groups, we conducted a linear discriminant function analysis using the *lda()* function in the R package MASS (Venables & Ripley, 2002). This multivariate method maximizes variance among groups specified a priori, rather than variance across the data set (as in PCA). Our intention was to assess our ability to distinguish among groups defined by geographic region, genetic group (as identified in both the SNP and mtDNA data sets) and named subspecies. We fit models to various a priori groupings (*Cuba/diaspora*, *Region*, *mtDNA* group, *DAPC* group, *Structure* group and *Subspecies*) against population means of size-corrected residuals from nine morphological measurements and assessed the intragroup variance represented by each discriminant function. We plotted the first two LD axes to

visualize group discrimination. We then estimated our ability to use the discriminant axes to predict group membership for individual populations. To do so, we used the *predict()* function in the MASS package to generate predicted population assignments based on the LD axes. We then determined the proportion of times in which that population was correctly assigned to its prior grouping (proportion of correct assignments = PoCA). We followed this by using a one-tailed exact binomial test to determine whether we were more successful than expected by chance in our ability to correctly reassign a population to a group based on LDA of QTs.

At such a broad geographic scale, morphological variation could simply be related to geographic distance ('morphological isolation by distance'). To investigate whether morphological divergence is correlated with geographic distance, we performed independent matrix correlation analyses (Mantel tests) between geographic distances and each of the first three PCs (which encompassed the majority of the variance) from the morphological data set. We converted the latitude/longitude coordinates (decimal degrees) associated with the museum specimens to UTM coordinates using the *convUL()* function in the R package PBSMAPPING (Schnute, Couture-Beil, Haigh, & Kronlund, 2013). We then calculated pairwise Euclidean distance matrices from each of the orthogonalized PC axes and the log-transformed geographic distances. We conducted Mantel tests as above using the *mantel.randtest()* function in the R package ADE4 (Dray & Dufour, 2007) with 10^3 permutations.

We tested phylogenetic signal in our QT data set by pruning our ultrametric Bayesian mtDNA tree to lineages for which we could obtain QT means for each tip. We then reconstructed continuously valued QT lineage means along the phylogenetic history of *A. sagrei* using the function *contMap()* in the R package PHYTOOLS (Revell, 2012, 2013). We obtained residuals for each QT by fitting a phylogenetic regression against body size (SVL) using *phyl.resid()* in PHYTOOLS (Revell, 2009). We tested for phylogenetic signal in each of the continuous traits by calculating Pagel's (1997, 1999) λ using *phylosig()* in PHYTOOLS to examine whether these QTs are evolving in concert with the phylogeny (λ close to zero implies that the trait is poorly correlated with the phylogeny and thus may be homoplastic). We conducted 10^3 Brownian motion simulations of the null hypothesis of homoplasy for each QT to generate *P*-values for tests of phylogenetic signal.

3 | RESULTS

3.1 | Phylogeography: Genetic sample collection and data

We generated an alignment of 1,101 bases for the mitochondrial *ND2* sequence data (full coding sequence plus 3' *tRNA-TRP*) from 298 *A. sagrei* series haplotypes, as well as individuals from the outgroups *A. homolechis*, *A. mestrei* and *A. ophiolepis* which we obtained through both direct sequencing and from online sequence repositories (Table 1). Both Bayesian and ML analyses resulted in similar estimated topologies for the *ND2* gene tree (Dryad # forthcoming) that was well-supported (Figure 2b). We collapsed the tree into 11 nonoverlapping clades representing biogeographic breaks (Figure 2b). As in previous studies (Cádiz et al., 2013; Kolbe et al., 2004; Kolbe, Larson, Losos, & Queiroz, 2008), a core clade of *A. sagrei* haplotypes (clades A–J) is distributed across the range of the species (Figure 2a,b), as well as a sister clade (clade K) containing a mixture of *A. sagrei*, *A. quadriocellifer* and *A. bremeri* haplotypes from western Cuba (Pinar del Río Province) with a tentative coalescent time (given caveats, see Methods) in the late Miocene or early Pliocene 6.4 Ma (BS = 87; PP = 0.42; 95% highest posterior density interval [HPD] 7.4–5.5 Ma). We hereafter refer to the inclusive clade composed of clades A–K as *A. sagrei* (sensu *lato*), whereas clades A–J represent *A. sagrei* (sensu *stricto*). The node subtending *A. sagrei* sensu *lato* and the out-group *A. homolechis* had a coalescent time of 6.8 Ma (95% HPD 8.5–5.4 Ma). There is coalescence between eastern (I and J) and western (A, B, C and E) Cuban clades in the early Pliocene 5.2 Ma (BS = 96; PP = 0.99; 95% HPD 5.9–4.5 Ma), with a contact zone east of Camagüey, Cuba.

Based on our tree topology, we inferred that subsequent dispersals from Cuba have given rise to other Caribbean populations (Figure 2b,c). The Bahamas contain two nonsister mtDNA clades (D and F), possibly resulting from independent colonization events from western Cuba (Figure 2b). These clades are not partitioned by island bank: instead, clade D is represented on the western Great Bahama Bank (BS = 86; PP = 1; 95% HPD 4.1–2.8 Ma), whereas clade *F* (BS = 51; PP = 0.99; 95% HPD 5.4–4.2 Ma) is distributed on the eastern Great Bahama Bank, the Little Bahama Bank and the southern Bahamas banks (San Salvador Bank and Crooked/Acklins Bank; Figure 2b,c). We found evidence for a single dispersal to the Cayman Islands from East Cuba (BS = 92; PP = 1; 95% HPD 4.1–2.9 Ma), with Cayman Brac (clade G) and Little Cayman (clade H) populations being reciprocally monophyletic and moderately divergent sister clades (BS = 100; PP = 1; 95% HPD 3.1–1.7 Ma).

Our Bayesian and ML analyses of the mtDNA data identified two distinct clades present in Mesoamerica, both of which are nested within the south Cuban clade (clade I; Figure 2b,c; Figure S2). The first clade contains haplotypes from Mesoamerica as well as Little Swan Island and is divergent from Cuban members of clade I with a coalescent time of 1.6 Ma (BS = 80; PP = 0.9; 95% HPD 1.8–1.3 Ma; Figure S2). The Little Swan Island population is sister to a haplotype from Campeche, Mexico (BS = 97; PP = 1; 95% HPD 0.8–0.3 Ma), which together are distinct from a sister Mesoamerican clade (BS = 99; PP = 1; 95% HPD 1.4–0.8 Ma) consisting of 18 Mesoamerican haplotypes from Veracruz south-east to Honduras (including four other Campeche, MX haplotypes). The second Mesoamerican clade consists of only three haplotypes from Belize and Honduras, which are sister (BS = 82; PP = 1) to haplotypes from South Cuba (and western Jamaica). Great Swan Island is not sister to Little Swan Island (Figure S2); instead, it is represented by a separate clade nested within the South Cuba clade (I), albeit with low nodal support (BS = 49; PP = 1; 95% HPD 1.0–0.6 Ma).

Jamaica has haplotypes representing two divergent mtDNA clades (clades I and J), with the south Cuba clade I represented in

TABLE 1 Mitochondrial DNA summary statistics for *Anolis sagrei* clades

Clade	*N*	*n*	*S*	*h*	π	Mean coalescent time (Mya)	95% HPD (Mya)	Posterior probability	Bootstrap %
A	35	31	44	0.99 ± 0.01	0.006 ± 0.003	3.2	3.7, 2.7	1	93
B	6	6	13	1.00 ± 0.09	0.005 ± 0.003	3.2	3.7, 2.7	1	93
C	7	5	15	0.90 ± 0.10	0.005 ± 0.003	3.8	4.3, 3.3	1	99
D	23	8	9	0.82 ± 0.06	0.002 ± 0.001	3.4	4.1, 2.8	1	86
E	33	18	26	0.91 ± 0.03	0.004 ± 0.002	3.4	4.1, 2.8	1	86
F	28	25	53	0.99 ± 0.01	0.009 ± 0.005	4.8	5.4, 4.2	0.99	51
G	8	4	10	0.75 ± 0.14	0.004 ± 0.003	2.4	3.1, 1.7	1	100
H	4	2	1	0.67 ± 0.20	0.001 ± 0.001	2.4	3.1, 1.7	1	100
I	11	5	9	0.71 ± 0.14	0.002 ± 0.001	3.5	4.1, 2.9	0.96	92
J	7	4	7	0.86 ± 0.10	0.002 ± 0.001	4.0	4.7, 3.4	1	100
K	7	6	13	0.95 ± 0.09	0.004 ± 0.003	6.4	7.4, 5.5	0.42	87

Note: N = number of individuals, *n* = number of haplotypes, *S* = number of segregating sites, *h* = haplotype diversity ± *SD*, π = nucleotide diversity ± *SD*. Coalescent times, highest posterior density (HPD) and posterior probabilities are from a molecular clock-calibrated analysis in BEAST, whereas bootstrap percentages are from a maximum-likelihood (ML) analysis in RaXML.

TABLE 2 Results from Mantel tests for correlation between geographic, genetic and morphological distance pairings. Genetic distances are calculated as the Cavalli-Sforza chord distance (SNPs) or Nei's pairwise distance (mtDNA). Multiple matrix regression is a partial Mantel test for correlation between two matrices when controlling for a third

Data set and method	↓Dependent | Independent→	Geographic Distance	mtDNA Distance
Genetic data			
Mantel test	mtDNA distance	r = .15, p = .016	–
Mantel test	SNP distance	r = .41, p < .001*	r = .53, p = .001*
Multiple matrix regression	SNP distance	–	r = .52, p = .001*
Morphometric Data			
Mantel test	PC1	r = .18, p = .001*	–
Mantel test	PC2	r = −0.06, p = .85	–
Mantel test	PC3	r = .02, p = .33	–

*Significant at p < .05.

the west and the east-central Cuba clade J represented in the east (Figures S2 and S3). Western Jamaican (clade I) haplotypes are interdigitated with, and minimally divergent from, Cuban haplotypes from the vicinity of Portillo (Figure S2). Eastern Jamaican (clade J) haplotypes on the other hand form a monophyletic group (BS = 99; PP = 1) sister to two of four haplotypes from Vertientes, Cuba, with a coalescent time of 0.5 Ma (95% HPD 0.7–0.3 Ma; Figure S3).

Genetic distances (Tamura-Nei [TrN] distances) between major mtDNA clades ranged from 6.1% pairwise divergence (Cayman Brac Clade G–Little Cayman Clade H) to 15.3% (Western Cuba Clade K–Western Bahamas Clade D; Table S3). There is a significant pattern of isolation-by-distance in the mtDNA data set, although the correlation between genetic and geographic distance explained little of the variation (r = .17, p = .001; Table 2, Figure 3a).

We used 31,702 polymorphic SNPs (12,415 loci) among 69 individuals to better characterize genetic structure of *A. sagrei* across the northern Caribbean. These SNPs were evenly distributed across the 75bp sequence reads (Figure S4; average depth of sequencing 20×), and whereas some loci might be under selection, there is no evidence for bias attributable to a minority of loci (Figure S5). Missing data and minor alleles were distributed evenly across the matrix of individuals and SNPs (Figure S6), with an average of 6.5% missing data per individual (mode = 2.6%) except for the two individuals of the out-group *A. homolechis* (50% and 49% missing data, probably owing to a combination of sample degradation and lower orthology relative to filtered in-group SNPs). Many alleles are nearly fixed across individuals, although a large number occur at intermediate allele frequencies (Figure S4). Observed heterozygosity (H_o) varied among loci (Figure S7) and a significant difference (Bartlett test of homogeneity of variances K^2 = 91.22, p < .001) existed between expected heterozygosity (H_e) and H_o on a per-locus basis (Figure S8), just as we might expect due to population subdivision.

As in the mtDNA data set, the deepest split in the nuclear genomic data set occurred between East Cuba and West Cuba, as visualized by both an NJ tree (Figure 2c) and a phylogenetic network (Figure 4). We also found phylogenetic groupings concordant with the mtDNA data set for other geographic regions, with

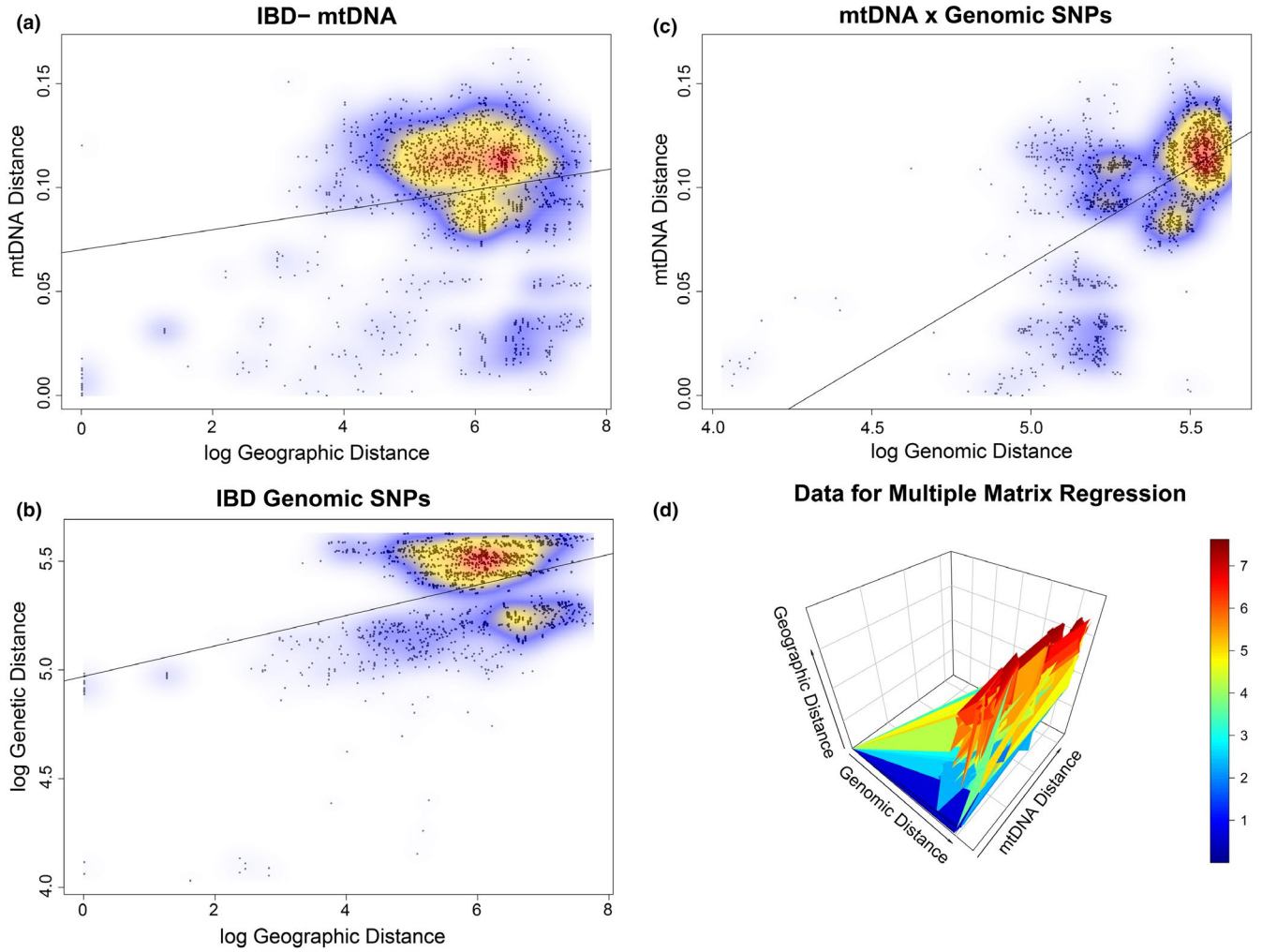


FIGURE 3 Relationships between genetic and geographic distance in *Anolis sagrei* across the Caribbean Basin, showing (a) an isolation-by-distance (IBD) plot for the mtDNA data set, (b) IBD for the nuclear data set, (c) a regression with the relationship between the dependent matrix of SNP distance on the independent matrix of mtDNA distance and (d) a visualization of the matrices from the multiple matrix regression (partial mantel test) showing the relationship between SNP and mtDNA distances when controlling for geographic distance. Note that mtDNA distance (axis *z*) is less dependent on geographic distance (axis *y*), whereas SNP distance (axis *x*) has a greater correlation with geographic distance (axis *y*)

the Cayman Islands representing a highly divergent split from the rest of *A. sagrei* (minimum *Ds* = 0.16; Table S4). Contrary to the results from mtDNA, Bahamian lineages represent a single clade separated from those in western Cuba (minimum *Ds* = 0.18; Table S4). Western Cuba has a series of clades that are discordant with the mtDNA phylogeographic breaks. One individual identified as *A. quadriocellifer* in our mtDNA tree (USNM 515920) belongs to the West Cuba lineage of *A. sagrei* (Figures 2 and 4). The Mesoamerican and Swan Island populations are sister to each other, and unlike in our mtDNA tree, are reciprocally monophyletic. As in the case with the mtDNA tree, Mesoamerican and Swan Island populations are nested with the clade containing all of the South Cuba samples (Figure 2c).

Our ΔK analysis on STRUCTURE runs of 1,000 SNP loci among 61 *A. sagrei* genotypes (Table 3) resulted in an initial partitioning of East Cuba (plus the Cayman Islands, Swan Islands and Mesoamerica) and West Cuba (plus the Bahamas) at $K = 2$, followed by the next likely clustering of $K = 4$ separating East Cuba, West Cuba, the Cayman Islands and the Bahamas (Figure 5a; Figure S9). Within the West Cuba group (20 genotypes), there are two clusters, separating the Bahamas from Cuba (Figure 5a; Figure S9). We also found two clusters in the East Cuba group (34 genotypes), separating South Cuba, the Swan Islands and Mesoamerica from east-central Cuba and Jamaica (Figure 5a), though most of these populations show some representation (assignment uncertainty possibly due to admixture) from both genetic clusters (Figure S9). The next likely clustering for the East Cuba cluster is $K = 5$, which separates geographic regions into clusters—the Swan Islands, South Cuba (admixed), Mesoamerica, Jamaica (admixed) and east-central Cuba (two samples admixed). In both cluster sets, Jamaica appears to be admixed between east-central Cuban lineages (Figure S9), as predicted by mtDNA, and some admixture between these lineages is apparent on Cuba as well, suggesting gene flow between these regions.

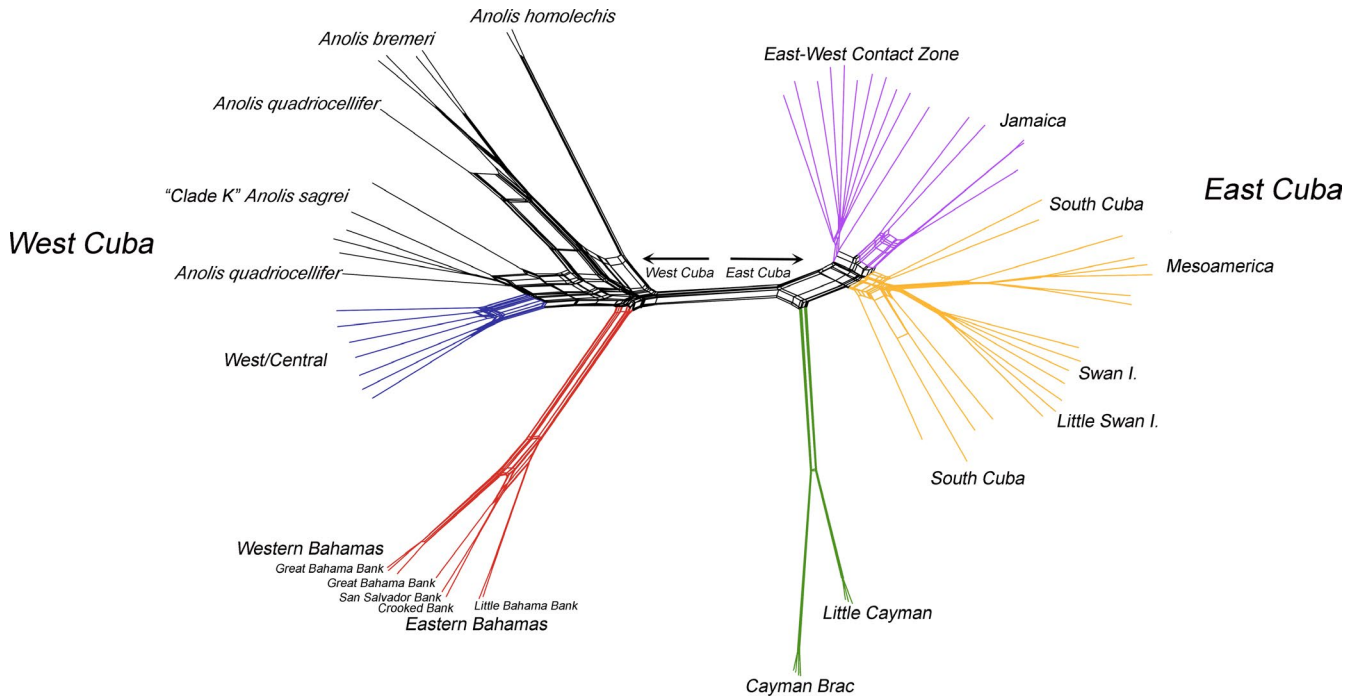


FIGURE 4 Phylogenetic network of 12,415 SNP loci among 69 representative samples from across the range of *Anolis sagrei*. Major nodes and nodes of interest with > 70% bootstrap support are labelled. Branches are coloured according to their genetic cluster assignment determined using STRUCTURE, with other members of the *Anolis sagrei* superspecies (*A. bremeri* and *A. quadriocellifer*) and the *A. sagrei* series (*A. homolechis*) coloured in black

TABLE 3 Clustering analyses for the SNP data sets. Also, see Figures 4 and 5. Data sets refer to inclusive (= 'all', including out-groups) and exclusive (*Anolis sagrei* sensu stricto) SNP genotype data sets

Method	Data set	*n*	*K*	PCs	Clusters
DAPC	All	69	5	5	Bahamas, West Cuba, East Cuba, Caymans, Other Species
	Anolis sagrei	61	3	2	West Cuba, East Cuba, Caymans
	West Cuba	20	4	3	West Cuba, West/Central Cuba, West Bahamas, East Bahamas
	East Cuba	34	3	2	East Cuba/Swan I., Mesoamerica, East Cuba/Jamaica
STRUCTURE	All	–	–	–	–
	A. sagrei	61	2	–	ECuba, WCuba
			4	–	ECuba, WCuba, Caymans, Bahamas
	West Cuba	20	2	–	WCuba, Bahamas
	East Cuba	34	2	–	E/C Cuba/Swan Islands/Central America, E/S Cuba/Jamaica
			5	–	Swan Islands, E/C Cuba, Central America, Jamaica, E/S Cuba

Discriminant analyses of 12,415 SNP loci implemented in DAPC were largely congruent with STRUCTURE clustering (Table 3; Figure 5; Figures S10 and S11). For the initial analysis including both *A. sagrei* and other members of the *A. sagrei* series (*A. bremeri*, *A. quadriocellifer*, *A. homolechis*), we obtained $K = 5$ (retaining 5 PCs) composed of Bahamas, West Cuba, East Cuba, Cayman Islands and other *A. sagrei* series species (Figure 5b; Figures S10 and S11). One of the samples of *A. quadriocellifer* was found to cluster with the Western Cuba group, and individuals from the East Cuba–West Cuba contact zone grouped with the East Cuba cluster. For the next analysis, we included only *A. sagrei* sensu *stricto* samples, obtaining $K = 3$ (retaining 2 PCs) composed of West Cuba, East Cuba (including contact zone individuals) and the Cayman Islands. For the West Cuba group, we found $K = 4$ (retaining 3 PCs), discriminating West Bahamas, north-east Bahamas, West Cuba and west-central Cuba. Within the East Cuba group, $K = 3$ (retaining 2 PCs) distinguishes east-central Cuba plus Jamaica (no inferred admixture in Jamaica), South Cuba (plus the Swan Islands) and Mesoamerica. We did not find evidence for discriminant functions being obviously driven by loadings of a small number of loci (Figure S12).

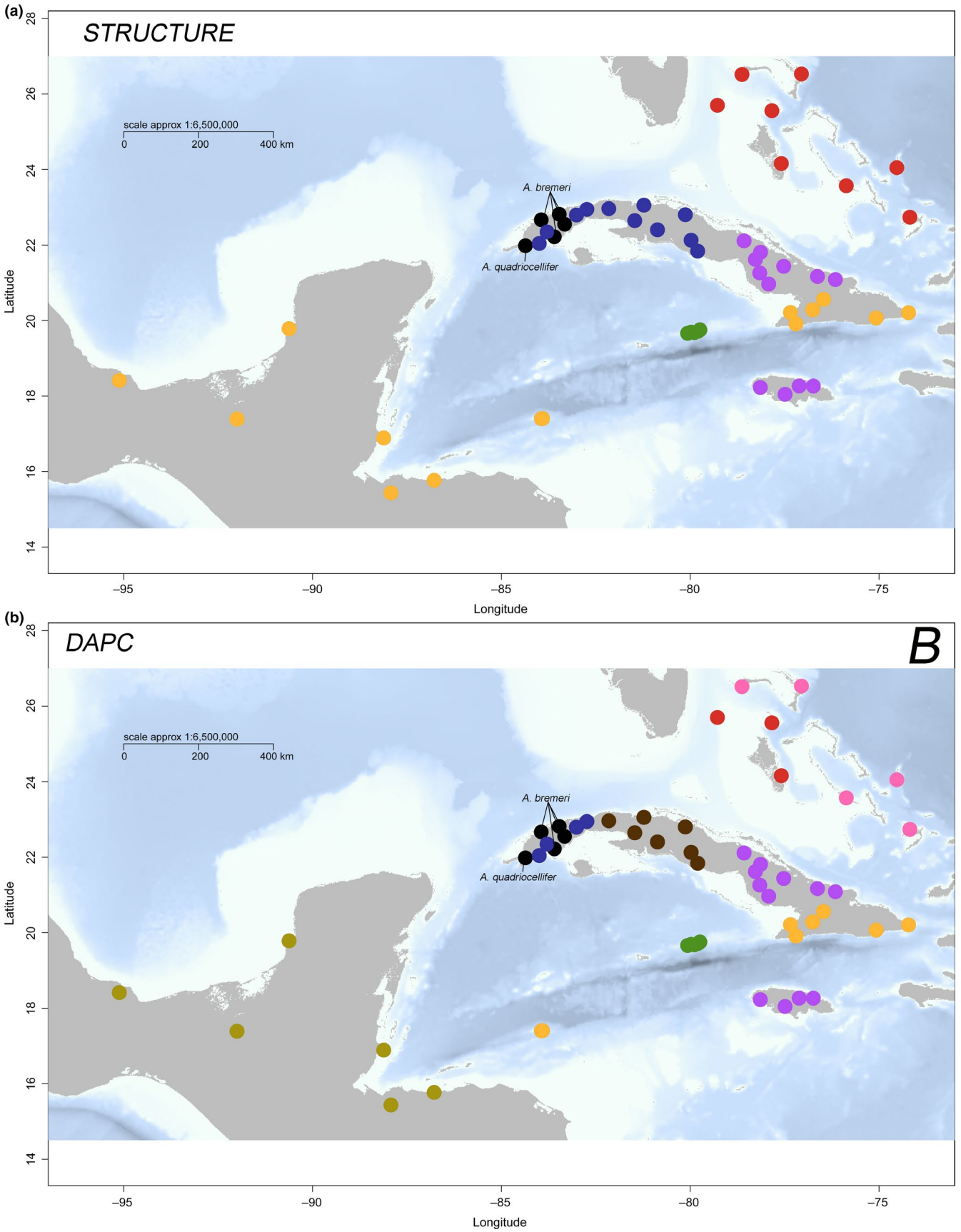


FIGURE 5 Map showing genetic cluster assignments from (a) 1,000 SNP loci using the Bayesian clustering algorithm STRUCTURE and (b) 12,415 SNP loci using discriminant analysis of principal components

Genetic distances (Cavalli-Sforza Chord and Nei's D) ranged from 0.05 (South Cuba–Swan Islands–east-central Cuba) to 0.34–0.36 (Cayman Islands–West Bahamas) among major genetic clusters of *A. sagrei* (Table S4). Other *A. sagrei* series species (*A. homolechis*, *A. quadriocellifer* and *A. bremeri*) are all highly divergent from *A. sagrei* sensu *stricto*, although they exhibit lower divergence from western Cuba *A. sagrei* samples. *Anolis quadriocellifer* had relatively smaller genetic distances to western Cuba *A. sagrei*, and at least one of the individuals (USNM 515920) appears to have *A. quadriocellifer* mtDNA haplotype but a nuclear genome most similar to western Cuba *A. sagrei*. This observation suggests an explanation for lower inferred genetic distance if gene flow (hybridization) between these species has occurred. There is a significant pattern of isolation by distance in the SNP data set (r = .38, p = .001; Table 2; Figure 3b). This strong effect of geographic distance on genomic variation is in contrast with the weaker geographic effect on mtDNA distances (r = .17, p = .001; Table 2; Figure 3a). Geographically neighbouring mtDNA haplotypes are not necessarily characterized by low mtDNA divergences across the range of *A. sagrei* (Figure 3d). Multiple matrix regression indicated a significant effect of mtDNA distance on genomic distance, controlling for geographic distance (r = .58, p = .001, Table 2).

3.2 | Phenotype: Quantitative trait diversification

We obtained complete QT data (consisting of SVL plus nine residuals from linear measurements regressed against SVL) from 558 individuals representing each of the major genetic groups found in this study (per-group mean n = 51.2; range n = 17–134), an unprecedented morphological data set for this group. We did not detect any outliers, and the data were multivariate normally distributed after log transformation. Analyses of mean and variance on a trait-by-trait basis for Cuban populations and diaspora populations showed an increase in mean SVL and lamellae count, as well as an increase in trait variance for SVL, head width and femur length in diasporic populations (Table 4; Figure 6).

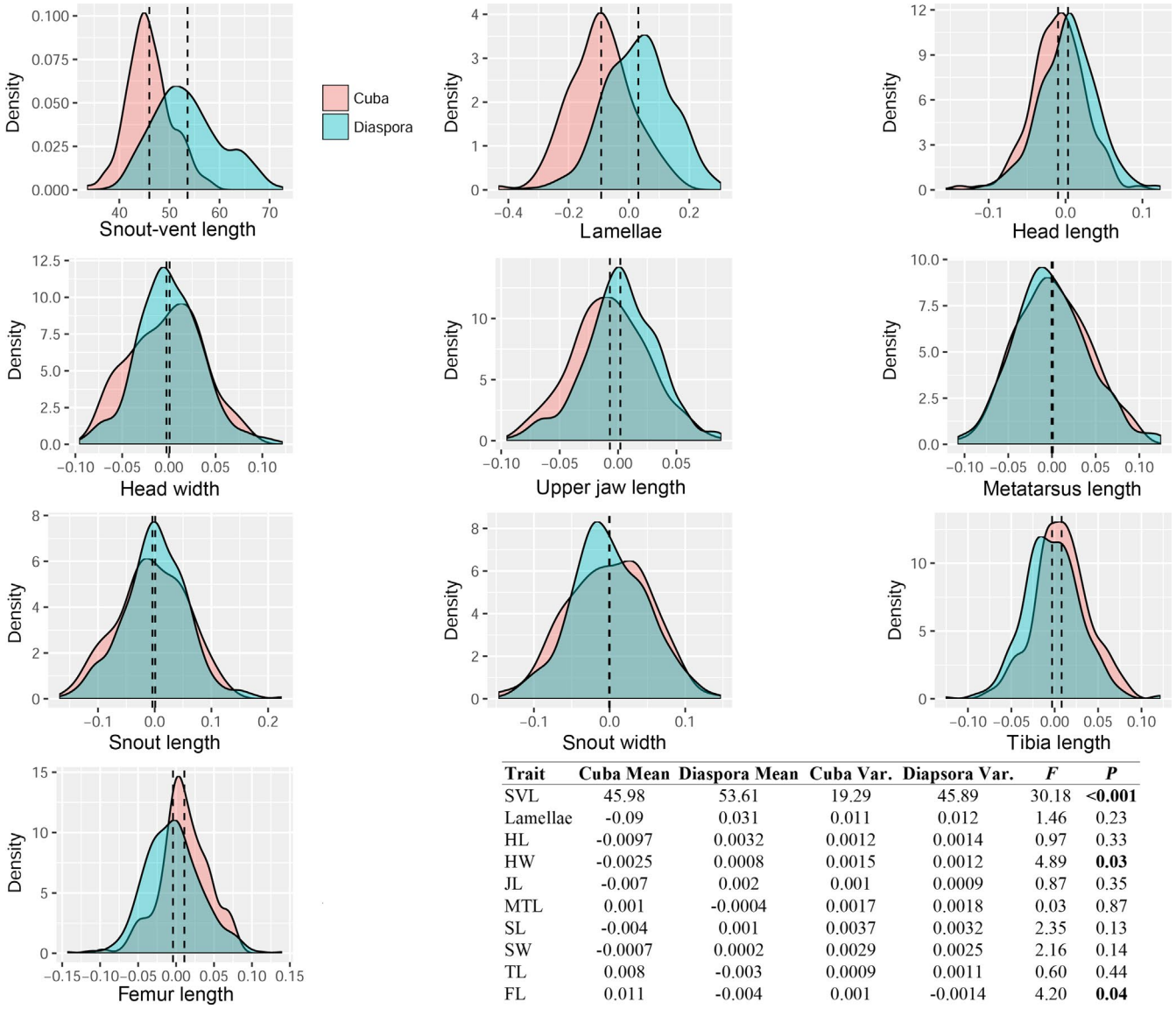
There are between 2 and 4 nondifferentiated 'clusters' across the entire data set by examining the within-group sum of squares from a *K*-means clustering analysis (Figure S13), and two clusters from our BIC clustering analysis ('VVE' model; Figure S14). These clusters overlapped extensively in morphospace. Visualization of the clusters in the space defined by the first two PC axes (75.6% of the variation) from our model-based BIC clustering analysis indicated a lack of clustering by genetic or geographic grouping in PC space (Figures S13 and S14). In PCA morphospace generated from our nine QTs, diaspora populations tended to disassociate from Cuban populations on PC axis 1 (Figure 7a), a dimension associated with size-corrected lamella number. Principal component axes 2 and 3, on which loaded snout size and leg length, respectively, showed little differentiation between Cuban and diaspora populations (Table S5). Our MANOVA suggested differentiation between Cuban and diaspora groupings based on all nine PC axes, and our individual t tests showed that this is largely driven by differences in SVL (t = 6.24, p < .001; Table 4) and size-corrected lamella number (t = −6.53, p < .001; Table 4). When we further examined QTs, we found that they differed significantly among groups (Cuba, Bahamas, Caymans, Mesoamerica, Swan Islands) in an ANOVA analysis (Table 4) and that all diaspora groups were significantly different from Cuban populations in at least two QTs (Figure S15), with diaspora groups evolving larger body size and more lamellae (Figure 8). In addition, most diaspora groups were different with respect to each other in a number of different QTs (Figure S15).

TABLE 4 Analyses of quantitative traits for *Anolis sagrei* from Cuban and diaspora groupings

		Wilks	*F*	*p*	
MANOVA	All PCs	0.26	9.5	<.001	
	QT residuals	0.62	37.0	<.001	
	Trait	***t***	***df***	***p***	**Padj**
t Tests	FL	2.06	37.9	.05*	0.12
	HL	−1.67	28.3	.11	0.22
	HW	−0.86	20.1	.40	0.57
	JL	−1.39	20.7	.18	0.30
	Lamellae	−6.53	36.5	<.001*	<0.001*
	MTL	0.10	27.9	.91	0.79
	SL	0.29	19.1	.78	0.79
	SW	0.39	19.8	.70	0.79
	TL	2.46	31.4	.02*	0.06
	SVL	6.24	37.1	<.001*	<0.001*
	Trait	***df***	**SS**	***F***	***p***
ANOVA	FL	5	0.10	17.6	<.001*
	HL	5	0.04	5.8	<.001*
	HW	5	0.06	10.1	<.001*
	JL	5	0.03	5.5	<.001*
	Lamellae	5	3.00	65.9	<.001*
	MTL	5	0.04	4.4	<.001*
	SL	5	0.13	8.1	<.001*
	SW	5	0.87	7.1	<.001*
	TL	5	0.03	5.2	<.001*
	SVL	5	16,433	157.1	<.001*

Note: Results from a MANOVA analysis based on orthogonalized axes from a PCA analysis, as well as raw residuals from QT regression on SVL, indicate differences between Cuban and diaspora groupings. Individual Welch's two-sample *t* tests performed on size-corrected residuals of population means indicate that diaspora populations have significantly more lamellae and larger body sizes than Cuban populations after a Benjamini–Hochberg correction (Padj). ANOVA of traits (SVL and size-corrected lamellae) from regional groupings (Cuba, Bahamas, Caymans, Mesoamerica, Swan Islands) show significant differences among groups. Post hoc Tukey analyses show that all diaspora regional groupings differ significantly from Cuba populations for both traits (Figure S15).

*Significant at $p \leq .05$.



Trait	Cuba Mean	Diaspora Mean	Cuba Var.	Diapsora Var.	*F*	*P*
SVL	45.98	53.61	19.29	45.89	30.18	**<0.001**
Lamellae	-0.09	0.031	0.011	0.012	1.46	0.23
HL	-0.0097	0.0032	0.0012	0.0014	0.97	0.33
HW	-0.0025	0.0008	0.0015	0.0012	4.89	**0.03**
JL	-0.007	0.002	0.001	0.0009	0.87	0.35
MTL	0.001	-0.0004	0.0017	0.0018	0.03	0.87
SL	-0.004	0.001	0.0037	0.0032	2.35	0.13
SW	-0.0007	0.0002	0.0029	0.0025	2.16	0.14
TL	0.008	-0.003	0.0009	0.0011	0.60	0.44
FL	0.011	-0.004	0.001	-0.0014	4.20	**0.04**

FIGURE 6 Distributions of snout–vent length and residuals from size-corrected quantitative trait mean and variance in Cuban and diaspora groupings of *Anolis sagrei*. Each plot shows density plots of residual trait mean and variance in Cuban (red) and diaspora (blue) populations. Dotted lines are the mean for each group. The table lists values for mean and variance for each trait as well as results from Levene's test of trait variance. Significant values are in bold

Our LDA approach also readily distinguished Cuban from diaspora lizards on a single LD axis (Figure 7b). Group prediction based on LD axes suggested a 93% probability of correct group reassignment for either population based on LD1 ($p < .001$; Table 5). When we overlaid geographic regions (Cuba, Bahamas, Cayman Islands, Mesoamerica, Swan Islands) onto the PCA morphospace, we saw that some regional diaspora groupings were especially well-differentiated from Cuban populations and to some extent each other, owing largely to differences in lamella number, which loads heavily on PC1 (Figure 9a). Our ANOVA analyses showed significant differences among some geographic groups for all QTs (Figure S15). LDA generated some additional predictive assignment ability based on QTs. Our LDA indicated that most traits contribute to discrimination along LD axis 1 and/or LD axis 2 (Table S6), though leg length (TL, FL) is strongly predictive of a priori geographic groupings among analyses. Our LD axes further demonstrated strong ability to discriminate among a priori geographic groupings (Figure 9b; Table 5), with an 88% probability of correct reassignment (PoCA), far higher than expected by chance ($p < .001$). We also found this method correctly assigns individuals to their groups for all other a priori groupings (*Subspecies* = 90%, *DAPC* = 80%, *STRUCTURE* = 85%, *mtDNA* = 85%) with much higher probabilities than expected by chance (Table 5).

There is a low, yet significant, correlation between geographic distance and morphological distance, as represented by the first orthogonal axis from our PCA analysis (Table 2; Figure S16). We found evidence of phylogenetic signal ($\lambda > 0$) only for SVL ($\lambda = 0.42$, $p = .008$), suggesting that other morphological traits, such as more

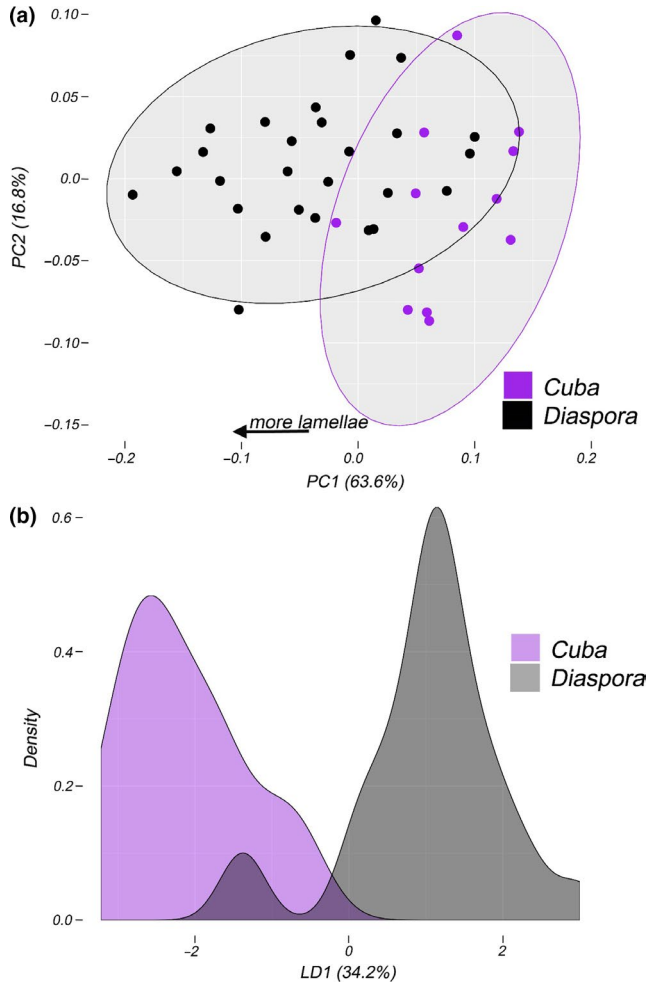


FIGURE 7 (a) PCA of Cuba versus diaspora populations (including Jamaican populations as diaspora). (b) Discriminant function analysis indicates good ability to diagnose Cuban and diaspora populations based on quantitative traits. All points represent population means

lamellae in diasporic populations, are largely homoplastic and do not reflect shared evolutionary history (Table S7).

4 | DISCUSSION

Interspecific studies of Caribbean lizards have advanced our understanding of macroscale evolutionary processes such as adaptive radiation (Losos, 2009; Poe et al., 2017), whereas intraspecific studies of Caribbean *Anolis* biogeography can provide insight into what might have unfolded during the early stages of diversification. In particular, careful investigation of intraspecific genetic and morphological variation within widespread anole species can reveal a great deal about the pattern and process of evolutionary divergence (Glor et al., 2003; Rodríguez-Robles, Jezkova, & García, 2007; Thorpe, Surget-Groba, & Johansson, 2008; Ng & Glor, 2011; Glor & Laport, 2012; Muñoz et al., 2013; Wollenberg, Wang, Glor, & Losos, 2013). Species that have colonized previously anole-free islands thus may serve as a model for early stages of adaptive radiation, analogous to the original species (singular or plural) of anoles that reached the Caribbean. In this research, we tested four possible, though not mutually exclusive, scenarios for how colonizing populations of *A. sagrei* have evolved morphologically following expansion from Cuba.

4.1 | Phenotype of a colonizing species

4.1.1 | Trait variance expansion scenario

The underlying rationale for a prediction of ecological release is that populations occurring largely without the competition of close relatives should expand their resource use to exploit those resources used by other species in multispecies communities (i.e. 'ecological opportunity' (Stroud & Losos, 2016; Wellborn & Langerhans, 2015; Yoder et al., 2010)) and that subsequently populations should evolve to be more morphologically variable because morphologically different individuals will use different components of the resource spectrum. Our data provide some evidence for increased morphological variance in SVL, head width and femur length (Figure 6), suggesting that increased ecological niche breadth in *A. sagrei* (Lister, 1976a; reviewed for anoles in Losos, 2009) is potentially accompanied by increased morphological variability (though we emphasize that our sample sizes are relatively modest for estimates of variance). An alternative mechanism for niche expansion involves the evolution of increased morphological and ecological sexual dimorphism (e.g. Bolnick & Doebeli, 2003). This is an intriguing dimension for future research, but it is one that we cannot address with our data.

4.1.2 | Intraspecific divergence scenario

Morphological evolution in diasporic populations might proceed under the suggested ecological release scenario (Lister's 1976a,b; Schoener, 1969), whereby anoles colonizing islands with few other anole species experience relaxed competition with closely related taxa and are thus subject to different selective regimes. This scenario might result in increased trait variance within populations as above but could also produce divergence among populations as populations adapt to different conditions on different islands (e.g. Price et al., 2010). For example, Bahamian lineages are relatively morphologically diverse—occupying the majority of the range of all *A. sagrei* specimens for LD1 (Figure 9b), and individual Bahamian island populations are known to exhibit a large degree of morphological differentiation (Driessens et al., 2015; Losos et al., 1994; Marnocha, Pollinger, & Smith, 2011; Vanhooydonck, Herrel, Meyers, & Irschick, 2009). Further, some diasporic populations are characterized by 'extreme' traits (relative to other *A. sagrei*) along some morphological axes (body size, lamella number, dewlap colour; Figure 8).

Whereas most diaspora are evolving directionally in PC morphospace relative to Cuban populations (Figures 7–9), our ability to detect differences among populations suggests that traits are evolving

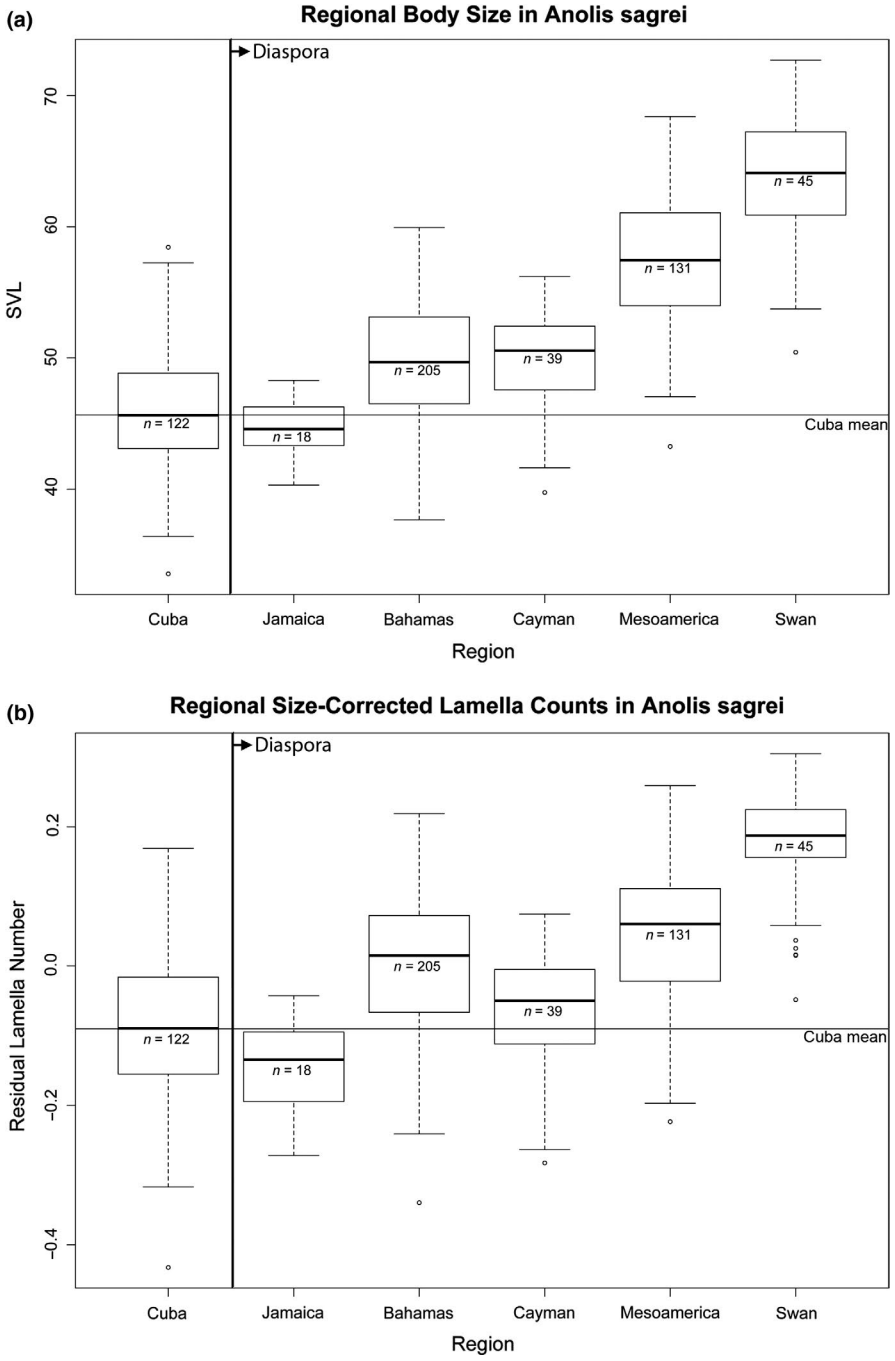


FIGURE 8 Box plots showing (a) body size (SVL) on top and (b) size-corrected lamella number on bottom for regional groupings of *Anolis sagrei* in order of mean body size of each regional population. Note that Jamaican *A. sagrei* are recent diaspora (including anthropogenic introductions from Cuba) and that Jamaica has six other native species of anoles

in at least slightly different ways in different Caribbean basin populations, possibly owing to environmental variation (e.g. Stuart et al., 2017). This ability to discriminate among populations suggests that there are some idiosyncratic evolutionary outcomes in diasporic populations. Furthermore, our ANOVA results and post hoc tests (Table 4; Figure S15) demonstrate that population pairs have evolved along independent trajectories for some QTs, but not others. Across the northern Caribbean, there is a weak (but significant) correlation between morphological distance and geographic distance. In other words, morphological distances might be explained, at least in part, by geographic distance, as neighbouring populations are more morphologically similar than distant ones. Taken together, these observations suggest that, whereas we see some concerted shifts in morphospace among diasporic populations (see next paragraph), we also are able to detect idiosyncratic morphological evolution and general support for divergence among populations owing to colonization of different islands.

4.1.3 | Convergence scenario

Lister (1976a,b) found that on islands with diasporic *A. sagrei* but few or no other anole species, *A. sagrei* perched higher and evolved more lamellae. This suggests that colonizing lineages might be convergent

TABLE 5 Results of discriminate function analysis for the morphometric data set, followed by using LDA axes to predict group membership yielding the proportion of correct assignments (PoCA)

Grouping	No. groups	LD1	LD2	PoCA	*p*-value
Subspecies	5	39.3	33.2	0.90	<.001*
Region	7	34.2	28.3	0.88	<.001*
mtDNA	9	31.9	28.1	0.85	<.001*
Structure	6	39.3	23	0.85	<.001*
DAPC	7	37.1	21.6	0.80	<.001*
Cuba/Diaspora	2	100	–	0.93	<.001*

Note: Groupings are a priori groupings, and LD1 and LD2 are the intragroup variance explained by each of the first two LDA axes. The data are population means, not individual animal data. P-values represent results from a 1-sided exact binomial test, where the alternative hypothesis is that the PoCA observed is higher than expected by chance.
*Significant at $p < .05$.

with regard to these traits owing to common selective pressures. We greatly expanded upon Lister's sampling and find results that generally support his conclusions. *Anolis sagrei* populations on outlying islands—the result of 4–5 evolutionary dispersals (see below)—have consistently evolved in the same direction (in terms of larger body size and more lamellae) relative to their Cuban counterparts that remain in the ancestral range (Figures 6–9), and Cuban *A. sagrei* are morphologically distinguishable in a LDA analysis from those of the diaspora (LD1 = 100%; Figure 7; Table 5). Prior work has shown that some ecologically important morphological characteristics can evolve rapidly in anoles (Stuart et al., 2014; Winchell, Reynolds, Prado-Irwin, Puente-Rolón, & Revell, 2016). Furthermore, these traits—increased body size and increased number of lamellae—are associated with increased arboreality in West Indian anoles (Irschick et al., 2006).

It is worth noting that on the islands of the Lesser Antilles, a number of species—members of the *bimaculatus* and *roquet* species groups—occur alone and that the evolutionary trends seen here for *A. sagrei* are similar to these species in having larger body sizes (Losos & de Queiroz, 1997; Poe et al., 2007) and more toepad lamellae (Knox, Losos, & Schneider, 2001; Losos & de Queiroz, 1997), traits associated with arboreality in Caribbean anoles (Irschick et al., 2006; Williams, 1983). This repeated pattern suggests that solitary occurrence (i.e. a lack of congeners) might favour particular morphological traits. On the other hand, our samples from Jamaica, which represent a diasporic population, do not differ morphologically from Cuban samples (Figure 9). This is further evidence that the absence of other anoles species (*A. sagrei* occurs with six other species on Jamaica), and not other factors, has allowed several diasporic populations to evolve away from the condition of ancestral populations on Cuba.

4.1.4 | Evolutionary contingency scenario

If morphological divergence is only weakly related to geographic distance, as our data show, another hypothesis is that morphological

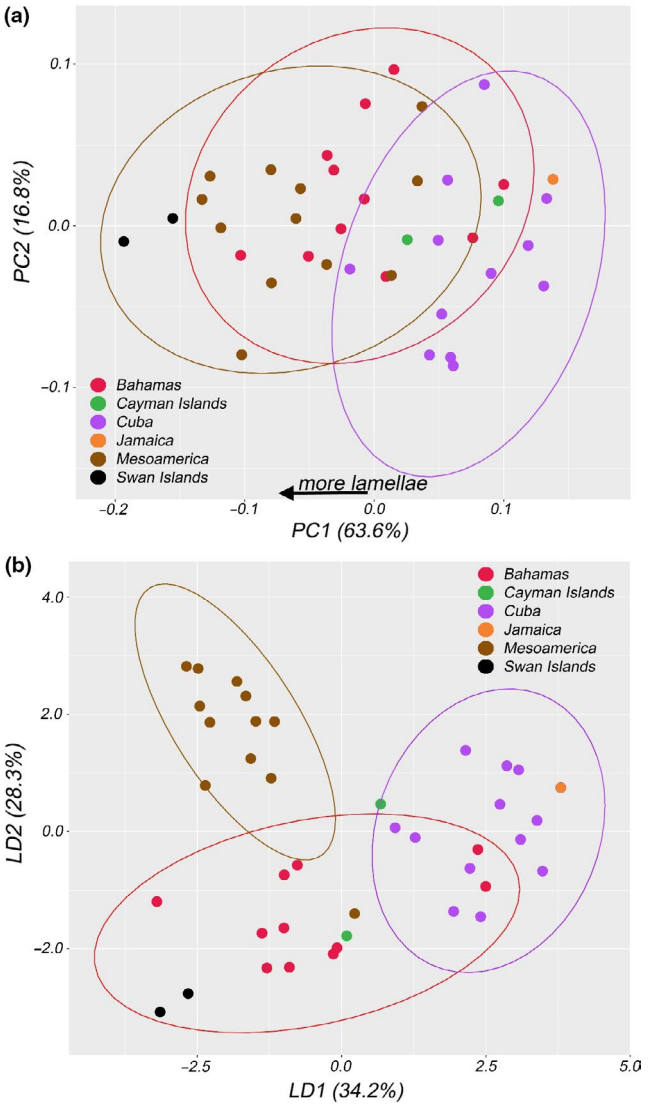


FIGURE 9 (a) PCA by region. (b) Discriminant function analysis by region. Populations from the Bahamas (red) that cluster with Cuba (purple) are from the Little Bahama Bank, where individuals tend to be smaller in body size than other Bahamian populations. All points represent population means, and 95% confidence ellipses are shown for groups with more than two populations

differences among diasporic populations might represent differences among their ancestors. Our data show precisely the opposite: phylogeographic relationships based on information from mtDNA genomes indicate that the evolution of phenotypic similarity among diasporic lineages has no relationship to the reconstructed evolutionary history of the different populations in this study (i.e. we found no indication of phylogenetic signal for nine QTs, and only limited signal for SVL; Table S7). That diasporic populations—all with different source populations and thus evolutionary backgrounds—would be convergent along some phenotypic axes (e.g. SVL in the Cayman Islands, Bahamas and Swan Islands populations) suggests that this situation likely owes to common selective pressures for these traits on anole-depauperate islands (Lister, 1976b). Thus, natural selection appears to be driving similar phenotypic outcomes

in populations with different evolutionary histories. Nevertheless, multivariate analyses can detect some population idiosyncrasies among subgroupings (Table 5).

An important caveat to this result is that phylogenetic inference methods impose a tree structure on the genetic data, even if the true populational history of Caribbean basin is not, in fact, tree-like. Consequently, an alternative explanation for our finding that most phenotypic traits show low phylogenetic correlation is that the tree is an ineffective representation of the relationships between the populations of our study. As our taxon is one of relatively low vagility and our populations tend to be found on different islands, we contend that a bi- or multifurcating tree is a reasonable approximation of the relationships among lineages in this study; however, we nonetheless feel that the assumption is one worth noting.

4.2 | Evolutionary history: Colonization and phylogeography

Our data suggest between four and five dispersal events from Cuba, leading to the populations presently found on the Bahamas Islands, Cayman Islands, Jamaica and Mesoamerica. We address each below.

4.2.1 | Origins and evolution of Cuban *Anolis sagrei*

Our genetic data suggest two important patterns in *A. sagrei sensu stricto* on the island of Cuba. First, the mtDNA shows a Miocene split between the eastern and western portions of the island (HPD 5.9–4.5 Ma), with a 'contact' zone east of Camagüey (Figure 2a). We note that our estimates of inferred coalescent time must naturally predate (and thus overestimate) the actual time of lineage separation (Degnan & Rosenberg, 2009); hence, this timing of coalescence is consistent with the suggested separation of Cuba into a western palaeoisland and central + eastern archipelago in the Miocene (Graham, 2003; Iturralde-Vinent, 2003, 2006; Iturralde-Vinent & MacPhee, 1999). This finding is congruent with evolutionary reconstructions of other Cuban taxa demonstrating an east–west split (5.0 Ma, Glor et al., 2004; 5.8 Ma, Alonso, Crawford, & Bermingham, 2012), and is also a common phylogenetic feature in many other intraspecific studies of Cuban terrestrial fauna (e.g. Weiss & Hedges, 2007; Rodríguez, Vences, Nevado, Machordom, & Verheyen, 2010; Matos-Maraví et al., 2014). Phylogeographic structure in the form of deep mtDNA coalescence is an increasingly common finding in anole species (Cádiz et al., 2013; Geneva, Hilton, Noll, & Glor, 2015; Glor et al., 2004; Knouft, Losos, Glor, & Kolbe, 2006; Malhotra & Thorpe, 2000), possibly owing to local adaptive differentiation in some cases (Irwin, 2012; Thorpe et al., 2015).

Analysis of our genomic SNP data set reveals many of the same phylogeographic patterns in Cuba suggested by mtDNA, although we find that the east–west contact zone shifts to the north-west by about 150 km to the vicinity of Sancti Spiritus province. That this deep east–west split within *A. sagrei* is evinced by both mtDNA and nuclear genomes indicates that it likely represents a historical break. Such a pattern might, for example, owe to ancestral populations evolving in allopatry for a period of time on Cuban palaeoislands. The latter would suggest a separation subsequent to the inundation of central Cuba following the formation of the Havana–Matanzas channel (Iturralde-Vinent, 2003; Iturralde-Vinent & MacPhee, 1999). This seems unlikely given that the reconnection of east and west Cuba is thought to have occurred in the Miocene and thus would pre-date the age of the inferred split in *A. sagrei*. Focused study of central Cuba and the evolutionary history of the *A. sagrei* series (*A. sagrei*, *A. homolechis* and *A. mestrei*) might clarify this question.

Other *A. sagrei* clades on Cuba are likely derived from palaeogeographic processes operating throughout the evolutionary history of the species. For example, Clade E is of more recent derivation, consistent with the later emergence of the Zapata Peninsula in the Pleistocene (Iturralde-Vinent, 2003), close to where this clade was found (although we lack samples from the peninsula itself). Undoubtedly, contact zones between lineages are dynamic through time, though geographically coherent groups such as Clade E (Figure 2b) are consistent across some other taxonomic groups (e.g. Alonso et al., 2012).

4.2.2 | Origins of Jamaican *Anolis sagrei*

Anolis sagrei is commonly thought to be anthropogenically introduced to Jamaica and some islands off of Mesoamerica (Helmus et al., 2014; Nicholson, Crother, Guyer, & Savage, 2012; Williams, 1969). Based on historical records, it seems that *A. sagrei* was present in localized areas on Jamaica prior to 1850 (Gosse, 1850 ['*Draconura catenata*']; Lynn & Grant, 1940; Underwood & Williams, 1959; Crombie, 1999). Kolbe et al. (2004) demonstrated that Jamaican *A. sagrei* most likely represents two independent colonizations from eastern Cuba, at least one of which might have been human-assisted. Our analyses found a similar pattern, with two distinct mtDNA clades represented on the island. Eastern Jamaica has east-central Cuba haplotypes (mtDNA clade J), whereas western Jamaica has South Cuba haplotypes (clade I). We find that eastern Jamaican haplotypes (east-central Cuban affinities) form a divergent clade (Figure S3), with an mtDNA coalescent time of 0.5 Ma (95% HPD 0.3–0.7 Ma) with Cuban clade J, suggesting that this population may be the result of a geologically recent natural colonization event. In contrast, western Jamaican haplotypes are interdigitated with haplotypes extant in the vicinity of Portillo, Cuba (Figure S2), and these populations are thus likely to be of recent, anthropogenic origin. Our sampling is not dense enough to determine an exact contact zone between these two lineages on Jamaica, although such a zone appears to be in either Manchester or Clarendon Parish in the south. Our genomic data also suggest two introductions to the island from these Cuban source populations, though no distinct contact zone is discernible at our level of sampling, and Jamaican individuals are likely admixed in the nuclear genome (based on STRUCTURE results; Figure S9),

suggesting interbreeding on the island. Such a situation is unsurprising given apparent admixture of these lineages in the native Cuban range (Figure S9) and the propensity of introduced *A. sagrei* lineages to interbreed (Kolbe et al., 2008, 2017). Given this finding, we suggest that *A. sagrei* on Jamaica likely resulted from the combination of an initial natural dispersal from east-central Cuba and a more recent human-facilitated introduction from south Cuba. Our nuclear genetic data suggest that these two lineages are probably interbreeding and expanding their ranges, and hence are not likely to remain separate over the long term. This anthropogenically facilitated secondary contact between a divergent peripatric population and the source range could be a very interesting subject for further study.

4.2.3 | Origins of Caymanian and Bahamian *Anolis sagrei*

The Cayman Islands have two morphologically divergent populations of *A. sagrei* (Kolbe et al., 2017). The population on Cayman Brac, the easternmost island in the Cayman Islands, has long been recognized as either a subspecies (*A. s. luteosignfer*; Ruibal, 1964) or a distinct species (*A. luteosignifer*; Garman, 1888; Nicholson et al., 2012; Uetz & Hošek, 2016), and is thought to be of ancient origin (e.g. Henderson & Powell, 2009; Poe, 2004). Individuals on Cayman Brac have relatively small dewlaps that range from rusty yellow to pale orange to red, either with or without a light margin. In contrast, *A. sagrei* on Little Cayman (7.5 km west of Cayman Brac) have very large red dewlaps with a light margin and hence have always been referred to as *A. s. sagrei*. Our genetic data show that these Caymanian lineages (excluding Grand Cayman, which harbours an introduced population; Kolbe et al., 2017) are highly divergent from other populations of *A. sagrei* (3.5 Ma minimum coalescent time; minimum Ds = 0.18; Table S4; Figures 2, 3, 4, 5). In addition, Cayman Brac and Little Cayman haplotypes form sister clades with an estimated mitochondrial coalescent time of 2.4 Ma (95% HPD 3.1–1.7 Ma), suggesting that these lineages have been separated since the early Pleistocene. Genomic data also support a single origin for Little Cayman and Cayman Brac populations (Figure 1; Figures S7–S9).

Populations in the Bahamas are currently recognized as *A. s. ordinatus*, and our results are mixed with regard to the origins of this group, demonstrating the importance of deploying nuclear and organellar genetic data. Our mtDNA tree strongly suggests two colonizations of the Bahamas from western Cuba, one of the eastern and southern Bahamas 4.8 Ma and a second of the western Bahamas approximately 3.4 Ma (Figure 2b). Deep mtDNA branches indicate that populations on isolated banks (San Salvador, Crooked Island) have persisted for some time, in spite of some prior suggestions to the contrary (Schwartz, 1968). Our nuclear genomic data, by contrast, suggest a single colonization from western Cuba though with long periods of isolation (*Ds* = 0.08; Table S4; Figures 2c and 4). These results are striking, as the Great Bahamas Bank was recently a single, fully emergent island at the height of the last glaciation (~15,000 years ago), and a number of previous studies have assumed that populations on the Great Bahama Bank constitute a recently panmictic genetic lineage (Calsbeek & Smith, 2003; Calsbeek, Smith, & Bardeleben, 2007). In our results, both nuclear and mtDNA markers suggest east–west divergence across the Bahamas. The presence of geographically structured mtDNA lineages could reflect separate colonizations of the Bahamas from Cuba, though the nuclear data do not support this (Figure 2c).

4.2.4 | Origins of Mesoamerican and Swan Island *Anolis sagrei*

Mesoamerican *A. sagrei* are presently recognized as a subspecies (*A. s. mayensis*; Smith & Burger, 1949), implying a natural origin (Schwartz & Thomas, 1975; Williams, 1969), though evidence of both natural and introduced populations exists for the Bay Islands off the coast of Honduras (Harrison, 2014; McCranie & Köhler, 2015; McCranie, Wilson, & Köhler, 2005; this study). The type specimen of this subspecies is from Campeche, Mexico (Cochran, 1961; Smith & Burger, 1949); hence, many authors treat Mexican and Belizean populations as *A. s. mayensis*, whereas Honduran populations are of unknown affiliation (e.g. Sexton & Brown, 1977; Lee, 2000; Kraus, 2009). Mesoamerican populations are generally characterized by larger body size (Lee, 1992; Figure 8) and represent natural occurrences of the species on the mainland derived from one or more overwater colonization events from south Cuba sometime in the mid- to late Pleistocene (Figure 2b; Figure S2). One of these colonization events likely also seeded Little Swan Island (Figure S2). Our finding of one Belizean and two Honduran mainland haplotypes nested within a separate clade of South Cuban, Jamaican and Big Swan haplotypes may suggest a second, more recent dispersal to Mesoamerica (Williams, 1969; Figure S2) from Cuba.

The Swan Islands, located between Honduras and the Cayman Islands, contain populations of *A. sagrei* that are highly distinctive from others in having a dark dewlap, unique dorsal coloration and body sizes far exceeding that of any other *A. sagrei* population (Harrison, 2014; Lee, 1992; Lister, 1976b; McCranie & Köhler, 2015; Figure 8). These populations have long been considered by some to be a separate species, *A. nelsoni* (Barbour, 1914; McCranie & Köhler, 2015; Nicholson et al., 2012), yet little was known regarding their phylogenetic affiliation with other *A. sagrei* populations. Our data show that Swan Island anoles are not deeply divergent from South Cuba animals (Ds = 0.05; Table S4), and we were minimally successful in finding natural clusters for Mesoamerican and Swan Island individuals based on allele frequency data (Figures S7–S9). Genomic data suggest a single colonization, possibly from Mesoamerica. Nevertheless, Mesoamerica and the Swan Islands display unique allele frequencies, suggesting sufficient time since colonization for selection and drift, which might be strong given the small size of the Swan Islands, to act upon both the genomes and the phenotypes of the anoles of this site.

Surprisingly, Little Swan Island and Big Swan Island haplogroups are not sister in the mtDNA analyses (Figure S2). It is unclear what

to make of this, given that the two islands are separated by a breaking shoal less than 250 m wide and 10 m deep. Our genomic data set suggests that Mesoamerican and Swan Island individuals form reciprocally monophyletic sister lineages (Figures 2c and 4), which point to a single colonization of these regions from Cuba and support relationships inferred in previous studies using morphological data (Lee, 1992). A likely explanation for the origin of the Swan Island organellar and nuclear inconsistencies might be lineage sorting, owing to sampling and founder effects generating consistent yet divergent haplotypes on the two islands, naturally subsampled from the diverse southern Cuba clade via overwater dispersal.

4.3 | An evolutionary background for a model species

For such an important species in studies of ecology, behaviour and evolutionary biology as *A. sagrei*, it is surprising that little was known regarding its range-wide variation in genetics and morphology. Here, we have provided a comprehensive reconstruction of the evolutionary history of *A. sagrei*, as well as the breadth of morphological diversity in this species.

Comparative studies of *A. sagrei* populations have addressed a wide variety of questions, including how populations respond to colonization, how populations conform to predictions from island biogeographic theory, the evolutionary and ecological outcomes of founder effect, how geography influences dewlap colour, and the relationship between behaviour and natural selection (Kolbe, Leal, Schoener, Spiller, & Losos, 2012; Lapiedra et al., 2018; Losos, Warheitt, & Schoener, 1997; Schoener & Schoener, 1983; Vanhooydonck et al., 2009). However, our finding of deep intraspecific genetic structure within the species highlights the importance of investigating phylogeographic relationships within widespread taxa. Particularly striking in this regard is our finding that *A. sagrei* of the Bahamas are composed of two distinct and divergent evolutionary lineages. Most comparative studies of Bahamian populations (e.g. Losos et al., 1994) have not incorporated phylogeographic information (as none was available for these populations). The findings of these prior studies may warrant re-examination in the light of our newfound knowledge.

For example, by examining microsatellite allele frequencies across island populations, Calsbeek and Smith (2003) and Calsbeek et al. (2007) suggested that hurricanes mediate adaptive diversification by influencing directional gene flow across the Bahamian Tongue of the Ocean. This has been influential in our understanding of the effect of gene flow on intraspecific adaptive diversification not only in lizards (e.g. de Queiroz, 2005; Glor et al., 2005), but also in other vertebrate taxa (e.g. Stankiewicz, Thiart, Masters, & Wit, 2006; Yoder & Nowak, 2006), as well as in biogeography more generally (e.g. Dayan & Simberloff, 2005; Garant, Forde, & Hendry, 2007; Riddle et al., 2008). We now know (as the authors of these studies could not have) that the populations compared in these studies come from two divergent clades that appear to coalesce 4 Ma before the present. Given the antiquity of the clades and issues of homoplasy with microsatellites (Anmarkrud, Kleven, Bachmann, & Lifjeld, 2008), one might question whether seemingly identical alleles in the different clades are in fact homologous. Re-examination of the genetic relationships with other markers, analysed in the context of phylogenetic relationships, would be desirable.

5 | CONCLUSIONS

In the context of intraspecific diversification, our data show a pattern of morphological evolution consistent with the predictions of an ecological release scenario replicated across several colonization events. Across the broad range of *A. sagrei*, we see a taxon that leaves a species-rich ecological context and then repeatedly undergoes morphological evolution on species-poor islands. Our analyses suggest that diaspora undergo morphological shifts relative to Cuban progenitor populations, leading in some cases to similar morphological outcomes (increased lamella number, large body size) as well as a general expansion of the morphospace of the species. This occurs despite a wide range of evolutionary histories for these diasporas, originating from one or the other of two deeply divergent progenitor lineages (East Cuba vs. West Cuba) and with millions of years of differences between colonization events. Thus, we find support for three of our four predicted scenarios: colonizing populations of *A. sagrei* evolve increased trait variance and experience directional and convergent evolution for some traits (SVL, lamellae) relative to Cuban and Jamaican populations in multispecies communities. We argue that *A. sagrei* represents a potential example of how a colonizing *Anolis* species might seed Caribbean islands to begin the process of adaptive radiation.

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DATA AVAILABILITY STATEMENT

We have archived phylogenetic trees and other associated data in Dryad https ://doi.org/10.5061/ dryad.dr7sq v9vb

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section. We have archived phylogenetic trees and other associated data in Dryad (https://doi.org/10.5061/dryad.dr7sqv9vb).

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