BIODIVERSITY RESEARCH

Independent introductions and sequential founder events shape genetic differentiation and diversity of the invasive green anole (*Anolis carolinensis*) on Pacific Islands

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Abstract

Aim: Natural range expansions and human-mediated colonizations usually involve a small number of individuals that establish new populations in novel habitats. In both cases, founders carry only a fraction of the total genetic variation of the source populations. Here, we used native and non-native populations of the green anole, *Anolis carolinensis*, to compare the current distribution of genetic variation in populations shaped by natural range expansion and human-mediated colonization.

Location: North America, Hawaiian Islands, Western Pacific Islands.

Methods: We analysed 401 mtDNA haplotypes to infer the colonization history of *A. carolinensis* on nine islands in the Pacific Ocean. We then genotyped 576 individuals at seven microsatellite loci to assess the levels of genetic diversity and population genetic differentiation for both the native and non-native ranges.

Results: Our findings support two separate introductions to the Hawaiian Islands and several western Pacific islands, with subsequent colonizations within each region following a stepping-stone model. Genetic diversity at neutral markers was significantly lower in the non-native range because of founder effects, which also contributed to the increased population genetic differentiation among the non-native regions. In contrast, a steady reduction in genetic diversity with increasing distance from the ancestral population was observed in the native range following range expansion.

Main conclusions: Range expansions cause serial founder events that are the spatial analogue of genetic drift, producing a pattern of isolation-by-distance in the native range of the species. In human-mediated colonizations, after an initial loss of genetic diversity, founder effects appear to persist, resulting in overall high genetic differentiation among non-native regions but an absence of isolation-by-distance. Contrasting the processes influencing the amount and structuring of genetic variability during natural range expansion and human-mediated biological invasions can shed new light on the fate of natural populations exposed to novel and changing environments.

KEYWORDS
Anolis lizards, founder effects, human-mediated colonizations, islands, microsatellites, mtDNA, range expansion

1 | INTRODUCTION

The current range of a species reflects a combination of its dispersal ability and climatic tolerances as well as the influence of interspecific interactions (Gaston, 1996; Sexton, McIntyre, Angert, & Rice, 2009). Range expansions and colonization events occur throughout evolutionary history and over long time periods, for example, during the Pleistocene in post-glacial Europe (Hewitt, 1999). However, rapid range expansions (and shifts) have been documented in parallel with climate change in many taxonomic groups and geographical locations during the 20th century (Parmesan, 2006; Pecl et al., 2017), and via human-mediated introductions of organisms into new locations (Suarez & Tsutsui, 2008). In all instances, modifications of a species' distribution, population size and connectivity should be reflected in the amount and structuring of genetic diversity within and genetic differentiation among contemporary populations, largely because of the effects of natural selection, genetic drift and gene flow. Understanding how these mechanisms drive population, differentiation as well as generate diversity is a major aim in evolutionary biology. A key question is whether recent human-mediated changes in species ranges (i.e., non-native species introductions) result in different patterns of genetic diversity and differentiation in the non-native compared to the native ranges of a species.

Historical range expansions, through a series of colonization events, have resulted in a steady reduction of heterozygosity and increased between-population genetic differentiation with increasing geographic distance from the ancestral population (Slatkin & Excoffier, 2012). However, gene flow from nearby subpopulations can reduce the effects of genetic drift and potentially erode genetic differentiation among established populations. On the contemporary (and faster) side of the spectrum of species movements, human-mediated introductions often lead to a loss of genetic diversity because of founder effects (Uller & Leimu, 2011). Subsequent introductions from established populations in the non-native range should further reduce genetic diversity and increase genetic differentiation (i.e., serial founder scenario, Clegg et al., 2002). However, species introductions often involve complex routes with multiple introductions and admixture that counteract the severity of founder events (Kolbe, Larson, Losos, & de Queiroz, 2008; Kolbe et al., 2004; Michaelides et al., 2016). In both natural range expansions and human-mediated invasions, the specific details of the colonization process will determine the evolutionary trajectory of populations. Consequently, evaluating the mechanisms and processes influencing the amount and structure of genetic diversity in human-mediated colonizations and contrasting this with the patterns associated with natural range expansions could assist in planning better conservation practices (e.g., species translocations), predicting the evolutionary potential of organisms under climate change and preventing further spread of invasive species.

The green anole, *Anolis carolinensis*, provides an excellent opportunity to investigate whether natural range expansions and human-mediated colonizations unfold in a similar way. The species is the only anole native to North America and is a natural colonizer (arrived in Florida from Cuba: Glor, Losos, & Larson, 2005) widely distributed in the south-eastern United States. The phylogeographic structure of the species across its native range has received considerable attention regarding the initial colonization of the continent and subsequent range expansion. These studies support an origin of the species in southern Florida with northward range expansion accompanied by a latitudinal gradient in genetic diversity and niche expansion leading to increased genetic isolation between populations in different vs. similar thermal environments (Campbell-Staton, Edwards, & Losos, 2016; Campbell-Staton et al., 2012; Glor et al., 2005; Manthey, Tollis, Lemmon, Moriarty Lemmon, & Boissinot, 2016; Tollis, Ausubel, Ghimire, & Boissinot, 2012; Tollis & Boissinot, 2014). The species is also a successful invader, having been introduced to Europe (Spain), Caribbean islands (Anguilla, Bahamas) and many islands in the Pacific since the 1940s (Kraus, 2009; Lever, 2003). Historical records and observational data associate the occurrence of the green anole (and other non-native reptiles) in the Pacific region to shipment-cargo movements and military activities during and after World War II (Chapple, Miller, Kraus, & Thompson, 2013; Crombie & Pregill, 1999; Fritts & Rodda, 1998). The current distribution of the species in these regions probably conforms to a pattern of stepping-stone colonization, from one island to another, within and among archipelagos. However, testing and confirming these hypotheses require a combination of molecular markers and analytical tools to unravel a potentially complex introduction history (Estoup & Guillemaud, 2010).

In this study, we (1) inferred the colonization history of *A. carolinensis* in the Hawaiian Islands (Oahu, Hawaii, Maui and Lanai) and on other islands in the Western Pacific (Guam, Palau, Saipan, Yap and Rota) and (2) assessed the population genetic structure and levels of genetic diversity between and within the native and non-native ranges. To complement our sampling of the native range in the U.S. and non-native range on Pacific islands, we also used published microsatellite data for introducing *A. carolinensis* on three Japanese Islands (Chichijima, Hahajima and Anijima; Sugawara, Takahashi, & Hayashi, 2015) for our comparative analyses. We hypothesize that the genetic characteristics of the native range sampling locations will vary spatially in accordance with a historical range expansion model, whereas in the non-native range, these characteristics will be influenced by the specific details of recent colonizations. We predict that in the native range, (1) genetic diversity (i.e., heterozygosity and allelic richness) will show a steady reduction and (2) increased population differentiation (F_{ST} values) with increasing distance from the ancestral population(s) in southern Florida. In the non-native range, we predict (1) lower overall levels of genetic diversity in relation to the native range, (2) reduced genetic diversity in stepping-stone colonizations, (3) increased population differentiation from native range source(s) due to sequential founder events and (4) stronger population differentiation between rather than within archipelagos (i.e., isolation-by-colonization). We evaluate these predictions and discuss our findings in relation to historical and observational data and the mechanisms generating population genetic structure in the native and non-native ranges.

2 | METHODS

2.1 | Sampling, sequencing and genotyping

We used 590 lizards, 492 previously sampled by Campbell-Staton et al. (2012) and 98 new (including 59 museum specimens), from 27 locations (18 in the native range and nine in the non-native range). Tail tip or liver tissue preserved in 70%–90% ethanol was used to extract genomic DNA using Bioline DNA Isolate Kits (Bioline, USA). For the phylogenetic analysis, we amplified an approximately 1200 base-pair (bp) region of the mtDNA including the genes encoding $ND2$, $tRNA^{Trp}$ and $tRNA^{Ala}$ from 98 individuals from the non-native range with primer pair H5730 (5′-AGCGAATRGAAGCCCGCTGG-3′) (Glor et al., 2004) and L4437a (5′-AAGCTTTCGGGCCCATACC-3′) (Macey, Larson, Ananjeva, & Papenfuss, 1997). Amplifications were carried out in a total volume of 30 µl consisting of 15 µl of MyTaq HS Mix (Bioline), 1.2 µl (0.4 mM) of each primer, 10.6 µl PCR grade H_2O and 2 µl template DNA (20 ng). PCR conditions were as follows: an initial denaturation step at 95°C for 1 min, followed by 30 cycles at 95°C for 1 min, 53°C for 35 s and 72°C for 80 s and a final extension step at 72°C for 5 min. PCR products were purified using the QIAquick PCR Purification Kit (Qiagen) and sequencing reactions were performed on the ABI 3130xl genetic analyser at URI Genomics and Sequencing Centre.

Mitochondrial DNA sequences in both directions were corrected by eye and aligned to obtain a consensus sequence. Accepted sequences were then aligned using MAFFT (Katoh, Misawa, Kuma, & Miyata, 2002) implemented in GENEIOUS 8 (Kearse et al., 2012) and trimmed to a uniform length of 1172 bp. For 29 sequences, we amplified a length of 705 bp because of poor DNA quality. We translated the sequenced $ND2$ regions to amino acid sequences to verify that no premature stop codons disrupted the reading frame. Unique sequences were submitted to GenBank (accession numbers MG252703–MG252730). We also genotyped 576 individuals from 23 locations (four locations in the non-native range, Lanai, Maui, Rota and Yap were not genotyped because of limited sample size, $n < 4$) at seven polymorphic microsatellite loci (Table S1). Single locus and multiplexed PCRs were carried out in a total volume of 10 µl consisting of 5 µl of MyTaq HS Mix (Bioline), 0.25 µl (0.25 mM) of forward-labelled primer, 0.75 µl (0.75 mM) of forward primer and 1 µl (1 mM) of reverse primer, 1 µl PCR grade H_2O and 2 µl template DNA (20 ng). PCR conditions were as follows: an initial denaturation step at 95°C for 3 min, followed by 30 cycles at 95°C for 45 s, Ta (58–62°C anneal temperature modified for each locus) for 45 s and 72°C for 45 s and a final extension step at 72°C for 5 min. PCR products were co-plexed and run on a 3730 × L 96-Capillary Genetic Analyzer at the Yale DNA Analysis Facility.

2.2 | Phylogenetic analyses

We used the phylogenetic analysis to reconstruct relationships among haplotypes and to assign genetic origin of the introduced haplotypes. We combined our sequences with 371 sequences (of varying lengths) obtained from GenBank from across the native range of the species (see Table S2, Glor et al., 2005; Kolbe et al., 2007; Campbell-Staton et al., 2012; Tollis et al., 2012). We also included two sequences from the non-native populations in Ogasawara (Bonin) Islands, Japan (Hayashi, Shima, & Suzuki, 2009). Three sequences from *Anolis altitudinalis* (AY654023, Glor et al., 2004), *Anolis isolepis* (AY654022, Glor et al., 2004), *Anolis porcatus* (AY654025, Glor et al., 2004) were used as outgroup in the phylogenetic analysis using Bayesian inference (BI). We implemented BI analyses using the MRBAYES 3.2.6 (Huelsenbeck & Ronquist, 2001) plugin in GENEIOUS 8 (Kearse et al., 2012), under the HKY+G nucleotide substitution model as selected by the best-fit model applying the Akaike Information criterion (AICc) in MEGA 7 (Tamura et al., 2011). The BI analysis was run with four chains of 2,000,000 generations and sampling every 1,000 trees, with default priors (unconstrained branch lengths). We discarded (burn-in-length) the first 10% of trees after checking for convergence of the chains and the posterior probability branch support was estimated from the 50% majority-rule consensus tree.

To investigate the source location of the introduced haplotypes further, we calculated pairwise nucleotide distance (average number of nucleotide substitutions per site between populations, Dxy) between native and non-native populations in DNASP v.5.10 (Librado & Rozas, 2009) and constructed a neighbour-joining (NJ) phylogenetic tree in MEGA 7 (Tamura et al., 2011) with default parameters. We also constructed parsimonious phylogenetic networks using a median–joining algorithm in NETWORK v.4.6.12 (Bandelt, Forster, & Röhl, 1999). The method uses median vectors as a hypothetical ancestral sequence required to connect existing sequences in the network with maximum parsimony. For this analysis, we first used a subset of 286 sequences from the Gulf/Atlantic native range clade (see Results), which included all haplotypes from non-native populations. Haplotypes were trimmed to a uniform length of 705 bp. We also constructed a median-joining network with all the non-native range haplotypes and 27 haplotypes from possible source populations identified in the NJ phylogenetic tree analysis (see Results).

2.3 | Population genetic analyses

We calculated basic genetic diversity indices, observed and unbiased-expected heterozygosities (H_O, H_E) and private alleles (P_A) with GENALEX 6.5 (Peakall & Smouse, 2012), allelic richness (A_R) with FSTAT v.2.9.3 (Goudet, 2001) and nucleotide diversity (Pi) with DNASP v.5.10 (Librado & Rozas, 2009). We compared H_E and A_R in native vs. introduced populations with a Welch Two Sample t test in R (R Development Core Team, 2011). Populations for these comparisons and additional population-level analyses were defined as those locations with ten or more sampled individuals. We then inferred and contrasted the population genetic consequences of range expansion (in the native range) and human-mediated colonization (in the non-native range). First, we calculated the geographic distance of native populations from a reference population (since the diversification in *A. carolinensis* occurred northward from southern Florida, we considered the
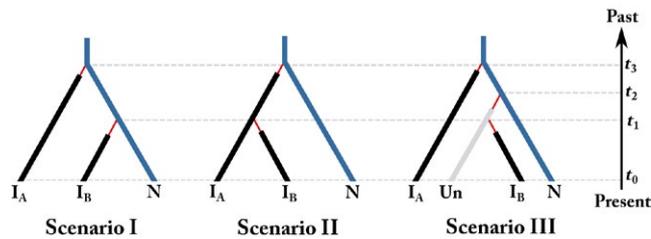
FIGURE 1 Graphical representation of possible colonization scenarios of *Anolis carolinensis* in the Pacific as tested using DIYABC. Scenario I assumes two independent colonization events (I_A and I_B) from a location in the native range (N). Scenario II assumes stepping-stone colonization from an established population in the non-native range (I_A). Scenario III assumes there was an unsampled location (Un) that served as a source for an introduced population. The thin red lines indicate reductions in the effective population size due to bottlenecks following the introductions and t1, t2 and t3 represent time

southern sampling location, SEFL, as the reference, Manthey et al., 2016) and then regressed levels of genetic diversity (H_E, A_R) and differentiation (linearized F_{ST}). We contrasted patterns in the non-native range by ordering populations from east (Hawaiian Islands) to west (Japanese Islands) and by age (oldest introduction) within each archipelago. For this comparison, we also used multilocus microsatellite genotypes (five loci) from five populations on three Japanese Islands (Chichijima, Hahajima and Anijima: Sugawara et al., 2015) and calculated A_R and H_E. We also tested whether genetic distance (linearized F_{ST}) was related to geographic distance, separately for non-native and native sampling locations, using Mantel tests implemented in the "VEGAN" package in R (Oksanen et al., 2016).

To evaluate the magnitude of founder events on population differentiation, we first calculated all pairwise F_{ST} values in ARLEQUIN v3.5.1.2 (Excoffier & Lischer, 2010). Then, using native populations belonging to the Gulf-Atlantic clade (see Results), we averaged their pairwise F_{ST} values with non-native populations on Oahu, Hawaii, Guam, Palau and Saipan. We then plotted these values along with pairwise F_{ST} values for comparisons among and within non-native regions (Hawaiian Islands, Western Pacific Islands and Japanese Islands). Also, as under a sequential colonization model, F_{ST} values should increase with the number of founder events from the source, we calculated the number of founder events from a particular source (based on colonization scenario, see below) and the F_{ST} values within each event. We also included averaged F_{ST} values from the three Japanese islands (combined sampling locations within each island).

We used two approaches to detect whether populations in both the native and non-native ranges had undergone genetic bottlenecks after range expansion and colonization, respectively. First, we calculated the degree of heterozygosity excess, which occurs because of the loss of rare alleles shortly after bottlenecks using BOTTLENECK (Piry, Luikart, & Cornuet, 1999). We used a two-phase mutation model, with 95% stepwise and 5% non-stepwise mutations. The significance of heterozygosity excess was then calculated using Wilcoxon tests. Second, we calculated Garza and Williamson's index (M), by dividing the number of alleles in a population (k) by the range in allele size (r) (Garza &

Williamson, 2001) in Arlequin (Excoffier & Lischer, 2010). This statistic is sensitive to population bottlenecks because the number of alleles is usually reduced more than the range of alleles by a recent reduction in population size, such that the distribution of allele length will show "vacant positions" (Excoffier & Lischer, 2010). Consequently, the M-index should be very small in populations that have been through a bottleneck and close to unity in stationary populations. We expected significantly lower values in non-native populations, because they are recent introductions, compared to populations in the native range, and tested this prediction with a Welch Two Sample t test.

We used two approaches to infer population structure in our sampling locations. First, we implemented a Bayesian clustering analysis in STRUCTURE v.2.3.4 (Pritchard, Stephens, & Donnelly, 2000), using the admixture model (Falush, Stephens, & Pritchard, 2003) with correlated allele frequencies. We ran simulations with a burn-in of 100,000 iterations and a run length of 10^6 iterations from $K = 2$ through 15. Runs for each K were replicated four times and the best supported K was determined according to the method described by Evanno, Regnaut, and Goudet (2005) in the online software STRUCTURE HARVESTER v.0.6.93 (Earl & vonHoldt, 2011). Multiple runs were combined with CLUMPP (Jakobsson & Rosenberg, 2007). We also ran simulations for each range separately. Second, we used a discriminant analysis of principal components (DAPC) implemented in the R package "ADEGENET" (Jombart & Ahmed, 2011; Jombart, Devillard, & Balloux, 2010). This approach, as opposed to STRUCTURE, uses coefficients of the alleles in linear combinations and seeks to maximize between-group variance and minimize within-group variance without the assumption of Hardy–Weinberg equilibrium (Jombart et al., 2010). Preliminary analysis revealed that locations in central to southern Florida and North Carolina differ considerably from the rest of the native range, which shows little population structure from the Atlantic coast to Texas. We therefore re-ran the analysis excluding sampling locations in North Carolina and southern Florida to increase resolution.

2.4 | Approximate Bayesian computation (ABC) analyses

We estimated the relative likelihood of alternative scenarios to explain the colonization route(s) of *A. carolinensis* to islands in the Pacific using approximate Bayesian computation (ABC, Beaumont, Zhang, & Balding, 2002) in the program DIYABC v.2.0.4 (Cornuet et al., 2014). We first pooled native populations of the Gulf-Atlantic mtDNA clade to create a native (N) pool of genotypes that could be evaluated against the genotypes of introduced populations. Considering the population on Oahu (oldest introduction) as an independent colonization, we tested plausible scenarios of colonization for the other four islands (HI-Hawaii, WP-Guam, WP-Palau and WP-Saipan). We followed a sequential-event approach, based on historical records of introduction dates (or first observed-documented), to create a pool of introduced genotypes (I_A) from populations preceding the next non-native population (I_B). Then, we tested for an introduction event directly from the native range (primary colonization, scenario I), an introduction from an already established non-native

population (I_A; stepping-stone colonization, scenario II) and an introduction from an unsampled location (unsampled source, scenario III) (Figure 1). For populations for which stepping-stone colonization was supported, we further tested scenarios to clarify their source (Figure S1a,b). We also used the microsatellite data set from three Japanese islands (Chichijima, Hahajima and Anijima) to infer their colonization patterns. To simplify the analyses and because more specific historical records were limited, we pooled individuals from all three locations on Chichijima and considered them as one independent colonization (oldest documented in the archipelago). We then tested whether populations on Hahajima (pooled into one population) and Anijima were established from Chichijima or from an unknown source (see Figure S1c for a graphical representation). The parameters defining each scenario (i.e., effective population sizes (N_E), effective number of founders (N_F), time of introduction (t) and duration of bottlenecks (db)) were considered random variables drawn from prior distributions (see Table S3 in supplementary material). The mutation model for microsatellite loci was assumed to be a generalized stepwise-mutation (GSM) model (Estoup, Jarne, & Cornuet, 2002) and default values were used (Cornuet et al., 2014). The coalescent-based algorithm simulates data sets for a number of predefined scenarios and compares the summary statistics of these with the summary statistics of the observed data. Summary statistics used in ABC were one-sample summary statistics including mean genetic diversity and mean number of alleles, two-sample summary statistics including mean genetic diversity, mean number of alleles and pairwise F_{ST} values. We first performed pre-evaluation

of scenarios and prior distributions (option implemented in DIYABC v.2.04) to check that at least one combination of scenarios and priors can produce simulated data sets that are close enough to the observed data set. We then simulated 10^6 data sets for each competing scenario and estimated posterior probabilities using the direct approach, the 500 simulated data sets closest to the observed, and a polychotomous logistic regression on 1% of simulated data sets closest to the observed data set. For this analysis, summary statistics were transformed by linear discriminant analysis (LDA) (Estoup et al., 2012). We also performed model checking following standard procedures in DIYABC.

3 | RESULTS

3.1 | Phylogenetic origin

We found 30 unique haplotypes in the non-native range, six from locations in the Hawaiian Islands (HI), 22 from locations in the Western Pacific Islands (WP) and two (previously published) from the Japanese Islands (JP); all were nested within the Gulf-Atlantic native range clade (Bayesian inference tree, supplementary Figure S2). One haplotype from Hawaii (HI-H4) was identical to a haplotype from Brownsville, Texas (TX-H24) and one haplotype from Guam (WP-H17) was identical to a haplotype from the Japanese Islands (JP-H1). There was further haplotype sharing among islands within each non-native region (Table 1). Also, after sequences were trimmed to a uniform length of 705 bp (for the network analyses, see below), more haplotypes in the

TABLE 1 Date of introduction (or first documented occurrence) and mtDNA haplotype information for the Pacific Islands, including the number of individuals sequenced (N) and number of haplotypes (Nh)

Region	Island	Date	N	Nh	Haplotype (frequency)[a]
Hawaiian Islands (HI)	Oahu	1950	16	5	**HI-H1(2)**, HI-H3(2), HI-H4, **HI-H5(10)**, HI-H6
	Maui	1964	2	1	**HI-H1**
	Hawaii	1978	4	2	HI-H2, **HI-H5(3)**
	Lanai	NA	3	1	**HI-H1**
Western Pacific Islands (WP)	Guam	1953	20	7	**WP-H15(4)**, WP-H17(5), WP-H18(5), WP-H19(3), WP-H20, WP-H21, WP-H22
	Palau	1960[b]	33	10	**WP-H1(9)**, **WP-H3(4)**, WP-H4, WP-H10, WP-H11(2), WP-H12, WP-H13(2), WP-H14(2), **WP-H15(9)**, WP-H16(2)
	Yap	1968	3	2	**WP-H2**, WP-H5(2)
	Saipan	1979	16	7	**WP-H1**, **WP-H2**, **WP-H3**, WP-H6, WP-H7, **WP-H8**, WP-H9(10)
	Rota	1988	1	1	**WP-H8**
Japanese Islands (JP)	Chichijima	1960	NA	2	**JP-H1**, JP-H2
	Hahajima	1980	NA	1	**JP-H1**
	Anijima	2013	NA	1	**JP-H1**

[a]Haplotypes highlighted in bold are shared between sampling locations.
[b]Approximate date based on Crombie and Pregill (1999).
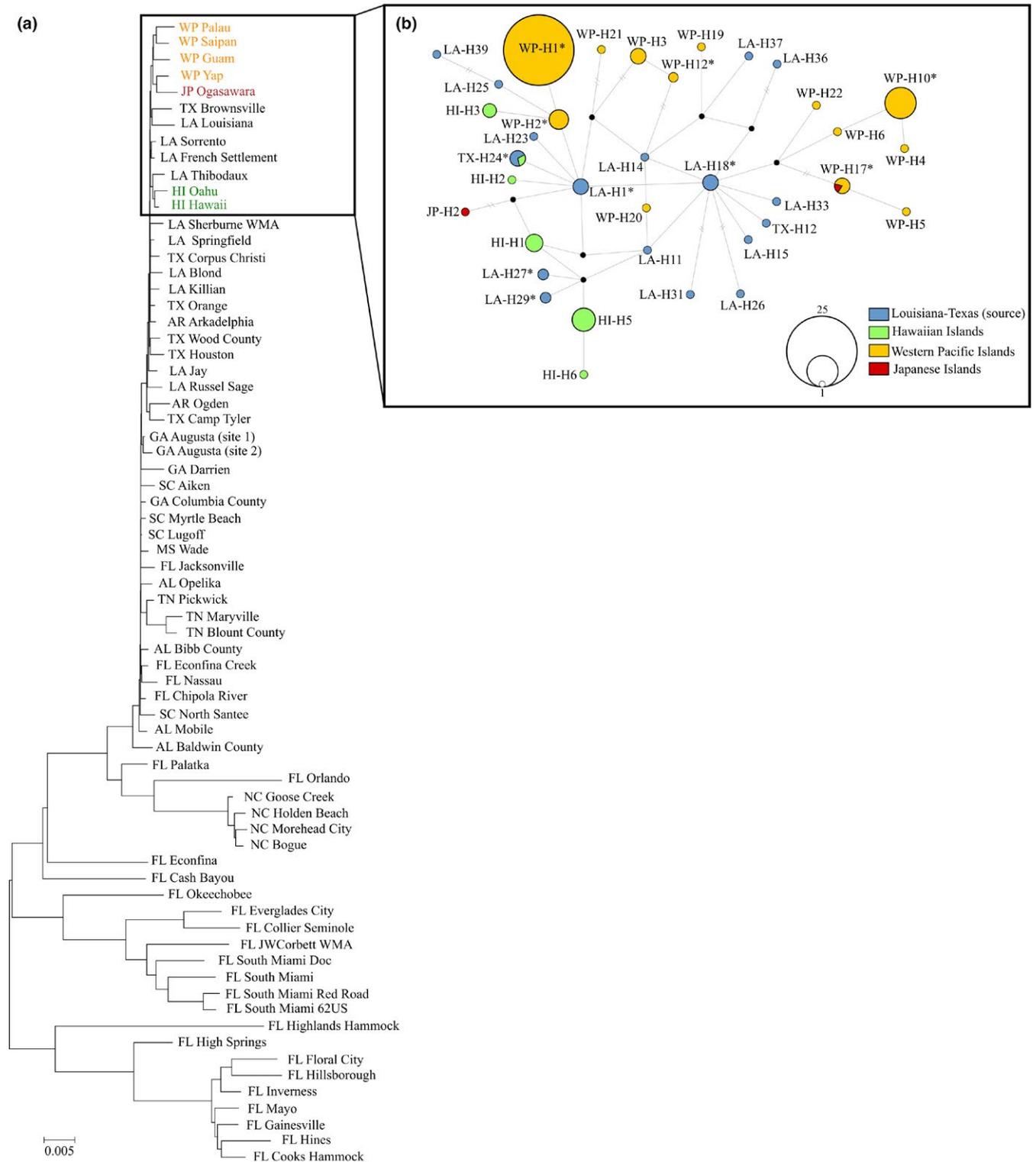
FIGURE 2 Results from phylogenetic analyses. (a) The evolutionary history of populations was inferred using the Neighbor-Joining method in MEGA7 from pairwise genetic distances (Dxy) calculated in DNASP. The optimal tree with the sum of branch length = 0.41584892 is shown. The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. Introduced populations are shown within the box and are colour coded to sampling regions. (b) Median-joining network of 125 mtDNA *ND2* haplotypes (trimmed to 705 bp) from introduced locations on Pacific Islands and five most probable native range source locations (in the box in panel a). Black dots represent median vectors and connections among haplotypes are single nucleotide mutations unless marked otherwise (slashes). The size of the pie (haplotype) corresponds to the number of individuals sharing the same haplotype. The asterisk denotes a list of identical haplotypes after trimming; WP-H1 (identical to WP-H8, 14, 15, 16), WP-H12 (WP-H13), WP-H2 (WP-H7, WP-H18), WP-H10 (WP-H9, H11), WP-H17 (JP-H1), LA-H27 (LA-H28), LA-H29 (LA-H30), LA-H1 (LA-H2, H3, H8), LA-H18 (LA-H21, TX-H13), TX-H24 (TX-H23, HI-H4)

Western Pacific islands became identical as did some haplotypes in the native range (e.g., LA-H18 identical to LA-H21 and TX-H13, see also Figure 2b).

The network analysis, conducted using sequences from the Gulf-Atlantic clade, grouped haplotypes roughly into two geographic

regions (one along the Gulf coast and one farther north, Figure S3). The location of haplotypes from non-native populations in the network indicates that the source region(s) is probably somewhere in Louisiana and Texas (see also Table S4). The NJ tree based on nucleotide distance also grouped all non-native populations with four southern Louisiana

TABLE 2 Summary statistics from population-level genetic analyses, with populations defined as those sampling locations with ten or more sampled individuals (see Methods). Number of genotyped individuals (N), observed (H_O) and expected heterozygosity (H_E), allelic richness (A_R), number of haplotypes (H), nucleotide diversity (Pi), Garza–Williamson's index (M) and Wilcoxon tests for heterozygosity excess (P_{HZe}). Values highlighted in bold are those indicative of a bottleneck ($p \leq .05$ for the Wilcoxon test)

Range	State/Region	PopID	N	H_O	H_E	A_R	H	Pi (%)	M	P_{HZe}
Native	Alabama	AL	33	0.65	0.73	6.01	3[a]	0.21	0.17	0.34
	Arkansas	AR	30	0.57	0.68	5.01	3[a]	0.18	0.14	0.53
	Florida	NFL	32	0.71	0.77	6.06	4[a]	0.55	0.20	0.77
	Florida	MFL	28	0.58	0.78	6.39	5[a]	3.71	0.20	0.99
	Florida	SEFL	32	0.67	0.82	7.44	5[a]	1.2	0.24	0.95
	Florida	SWFL	16	0.68	0.78	6.61	4[a]	1.5	0.17	0.41
	Florida	NWFL	32	0.72	0.75	5.78	3[a]	2.1	0.17	0.71
	Georgia	GA	26	0.75	0.81	6.69	3[a]	0.29	0.18	0.15
	Louisiana	SLA	20	0.76	0.78	6.16	3[a]	0.47	0.17	0.29
	Louisiana	NLA	21	0.69	0.74	6.05	4[a]	0.98	0.16	0.34
	Mississippi	MS	31	0.69	0.72	5.77	3[a]	0.21	0.16	0.71
	North Carolina	NC	33	0.74	0.77	6.34	4[a]	0.26	0.19	0.41
	South Carolina	SC	28	0.76	0.80	6.57	4[a]	0.30	0.20	0.85
	Tennessee	ETN	25	0.66	0.77	6.34	3[a]	0.55	0.19	0.97
	Tennessee	WTN	33	0.52	0.72	6.01	4[a]	0.60	0.17	0.81
	Texas	TYTX	18	0.61	0.71	5.64	4[a]	0.18	0.15	0.66
	Texas	CCTX	21	0.59	0.76	5.56	3[a]	0.3	0.16	0.53
	Texas	ORTX	32	0.69	0.70	5.53	3[a]	0.29	0.18	0.99
Non-native	Hawaiian Islands	HI-Oahu	12	0.65	0.66	4.87	5	0.42	0.11	1.00
	Hawaiian Islands	HI-Hawaii	17	0.55	0.62	4.47	2	0.3	0.13	0.96
	Western Pacific Islands	WP-Palau	19	0.6	0.66	4.41	10	0.58	0.11	0.34
	Western Pacific Islands	WP-Saipan	17	0.75	0.71	4.80	7	0.47	0.11	**0.01**
	Western Pacific Islands	WP-Guam	20	0.62	0.66	4.76	7	0.57	0.13	0.95
	Ogasawara Islands (Japan)	JP-Chi1[b]	25	0.64	0.59	4.48	2	0.88	0.35	0.41
	Ogasawara Islands (Japan)	JP-Chi2[b]	24	0.68	0.62	4.53	2	0.88	0.34	**0.03**
	Ogasawara Islands (Japan)	JP-Chi3[b]	22	0.76	0.65	5.00	2	0.88	0.36	**0.03**
	Ogasawara Islands (Japan)	JP-Haha1[b]	27	0.60	0.55	4.31	1	-	0.33	0.31
	Ogasawara Islands (Japan)	JP-Haha2[b]	25	0.50	0.55	4.50	1	-	0.35	0.92
	Ogasawara Islands (Japan)	JP-Ani1[b]	59	0.74	0.62	4.73	1	-	0.36	0.31

[a]Data from Campbell-Staton et al. (2012).
[b]Data from Sugawara et al. (2015); three sampling locations on Chichijima (Chi1–Chi3), two sampling locations on Hahajima (Haha1 and Haha2) and one location on Anijima (Ani1).

locations and the Brownsville, Texas population (Figure 2a) suggesting separate source regions for the Hawaiian Islands (Oahu, Hawaii) and the other Pacific populations (Guam, Palau, Saipan, Yap and the Japanese islands). Constructing the median-joining network including only introduced-range haplotypes and 27 haplotypes from these five potential sources (Figure 2b) also suggests two possible source regions based on the number of mutation steps between haplotypes (1–9 steps).

3.2 | Population genetic diversity, differentiation and structure

Expected heterozygosity was 0.57–0.82 in the native range and 0.62–0.71 in the non-native range, and allelic richness was 5.01–7.44 in the native and 4.12–4.87 in the non-native ranges (Table 2). Genetic diversity (H_E and A_R) was significantly lower in the non-native range (H_E; $t = -5.67$, $df = 7.68$, $p < .001$, A_R; $t = -9.48$, $df = 18.19$, $p < .001$) and the loss of genetic diversity was greater after secondary colonizations (with the exception of the population on Saipan, see Figure 3a). Private allele analyses showed few low-frequency alleles in non-native populations. There were two private alleles on Guam not sampled in the native range (Table S7). Population genetic differentiation in the native range was significantly related to geographic distance (Mantel test, $R = .48$, $p < .001$) suggesting a pattern of isolation-by-distance (IBD), but no pattern was detected in the non-native range (Figure S6). Allelic richness and heterozygosity were both reduced significantly in the native range compared to the ancestral population in southern Florida (H_E; $R^2 = .34$, $p < .05$, A_R; $R^2 = .49$, $p < .001$, Figure 3a), whereas

genetic differentiation increased over the same distance (linearized F_{ST}; $R^2 = .40$, $p < .05$, Figure 3b). The bottleneck index (M) was significantly lower in the non-native range (M; $t = -8.15$, $df = 14.74$, $p < .001$, Table 2, Figure 3b) and there was a bottleneck signal (i.e., heterozygosity excess test) in Saipan and Chichijima ($p < .05$, Table 2). Population differentiation, measured as F_{ST}, was low to moderate (0.02–0.10) between populations within the three introduced regions (HI-Islands, WP-Islands and JP-Islands), moderate to high (0.06–0.12) between native and introduced populations, and high (0.18–0.20) between populations when comparing across the two regions in the non-native range (Figure 3a, see also Table S6). Between successive founder events, F_{ST} values increase (with the exception of the colonization on Saipan, Figure 4b).

The Bayesian clustering analysis in Structure including all sampling locations revealed $K = 3$ as the most likely number of genetic clusters (Figure 5a). One cluster included populations in central and southern Florida, the second cluster included all non-native populations and native populations from the Gulf coast and the third cluster included mainly the northern native range populations. When looking at the plot for $K = 4$ (second highest Delta K, Figure S4a), non-native populations from the two regions (Hawaiian and Western Pacific Islands) form two separate clusters, which was also supported in the best-fit model when running the analysis including only non-native locations (Figure S4c). Three clusters ($K = 3$) were the most likely number of genetic clusters for native range locations (Figure S4b). Similarly, the discriminant analysis of principal components (DAPC) placed non-native populations with locations along the Gulf coast (Figure S5) and indicated different source(s) for the two regions (Hawaiian and Western Pacific Islands) in the non-native range.
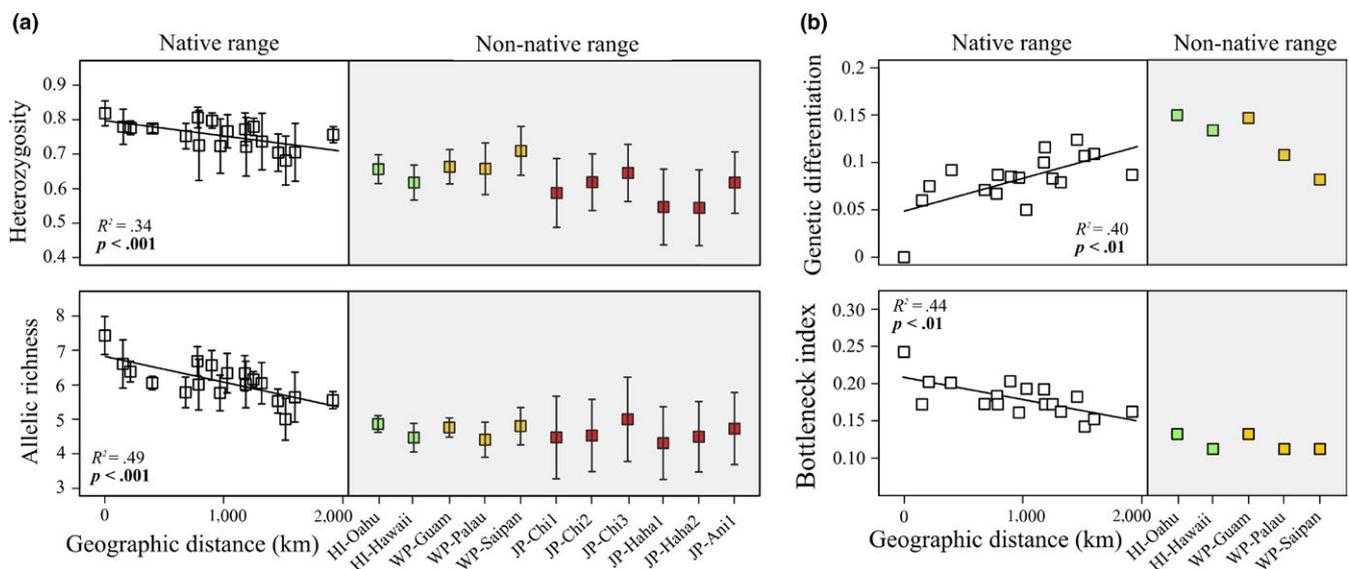
FIGURE 3 Contrasting genetic trends after range expansion in the native range and human-mediated colonizations in the non-native range. (a) Genetic diversity indices, expected heterozygosity (top) and allelic richness (bottom), in the native range in relation to geographic distance from south Florida (SEFL, as reference population, see text for further explanation) and ordered by island group from east to west (HI; Hawaiian, WP; Western Pacific and JP; Japanese) in the non-native range. (b) Top plot: population differentiation (linearized F_{ST} values) compared to geographic distance within the native range (SEFL as reference population) showing isolation-by-distance and averaged pairwise F_{ST} values between a non-native population and populations belonging to the Gulf-Atlantic clade; bottom plot: Garza–Williamson's bottleneck index in relation to geographic distance from south Florida. Populations on the three Japanese Islands were genotyped at a different set of microsatellite markers; therefore, comparisons with native and non-native populations should be interpreted with caution
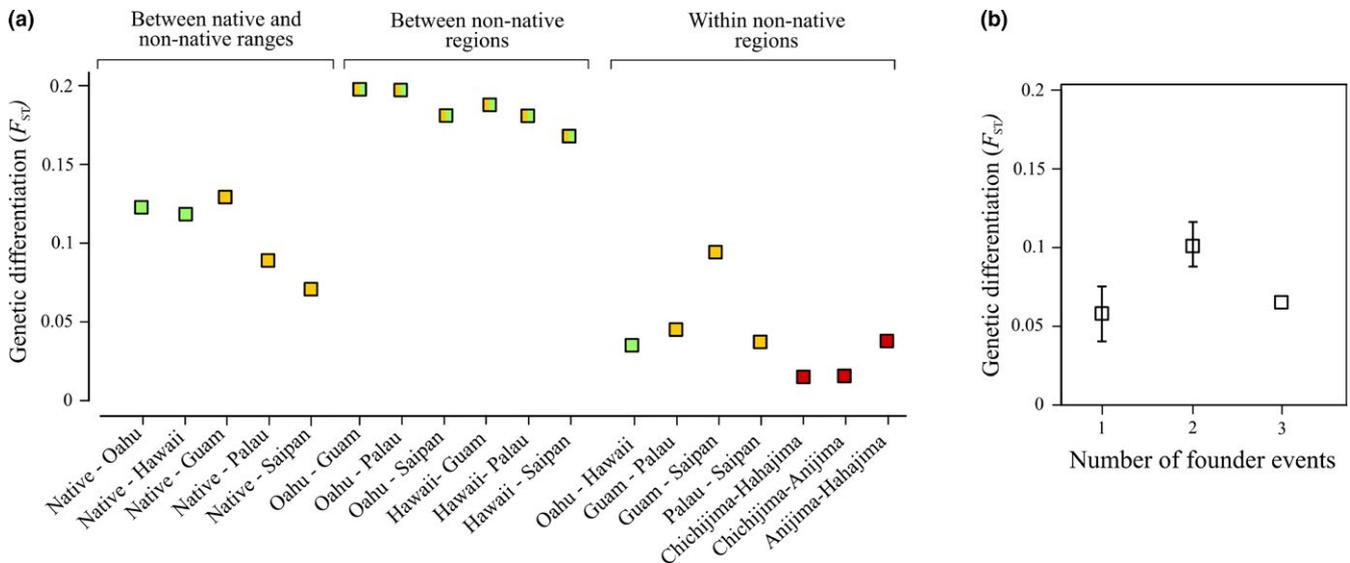
FIGURE 4 Founder effects in non-native populations. (a) Genetic differentiation (F_{ST} values) between ranges (native and non-native), between and within non-native regions; (b) the effect of number of founder events (sequential events, based on colonization scenario) on F_{ST} values (averaged pairwise values between a population and its source). Populations on the three Japanese Islands (sampling locations within each island were pooled together) were genotyped at a different set of microsatellite markers; therefore, comparisons with native and non-native populations should be interpreted with caution. Native range populations belonging to the Gulf-Atlantic clade were used for the between ranges analyses

## 3.3 | Approximate Bayesian computation (ABC) analyses

Pre-evaluation of scenarios and prior distributions showed that the summary statistics from the observed data produced eigenvectors that were within the margins of the sets of simulated data (data not shown). Considering the population on Oahu (oldest introduction) as an independent colonization from the native range, the analyses supported a second independent introduction to Guam (posterior probabilities of $p = .42$ and $p = .51$ based on logistic regression and direct approach, respectively) and a stepping-stone colonization scenario for Palau (from Guam), Saipan (from Palau) and Hawaii (from Oahu) (Table S5). Secondary colonizations were also supported for populations in the Japanese archipelago with introductions from Chichijima to Hahajima and Anijima (Table S5).

4 | DISCUSSION

We established the phylogenetic origin of introduced populations of *Anolis carolinensis* on islands in the Pacific and assessed the level of genetic diversity and population genetic differentiation in relation to populations in the native range of the south-eastern United States. Our findings support at least two introduction events, one on Oahu in the Hawaiian Islands and another on Guam in the Western Pacific Islands, and further colonizations within each of these non-native regions following a stepping-stone model. Based on the phylogenetic analysis and haplotype similarity, the likely geographic origin of these introductions was Louisiana or Texas in the native range. Genetic diversity at neutral markers was significantly lower

in the non-native range because of founder effects, which also contributed to increased population genetic differentiation among the introduced regions. In contrast to an isolation-by-colonization pattern observed in the non-native range, a steady reduction in genetic diversity and increased population differentiation with increasing distance from the ancestral population in southern Florida suggests a pattern of isolation-by-distance following natural range expansion in the native range.

Despite some earlier confusion concerning the identity of the species occurring in the Pacific (some authors have claimed that it is Cuban in origin, *Anolis porcatus* or *A. c. porcatus*; Crombie & Pregill, 1999; Lever, 2003; Kraus, 2009), we confirmed with genetic analyses that populations on Pacific islands are typical of *A. carolinensis* of the south-eastern United States. It is difficult to pinpoint the exact geographic origin(s) within the native range because of the relatively weak phylogeographic structure in the source region. However, mitochondrial DNA haplotypes from the non-native range cluster within the Gulf-Atlantic clade and are closely related to sequences from locations in Louisiana and Texas, with small differences (1–3 bp out of 1172 bp haplotypes). Louisiana has a major shipping port (New Orleans) and data from the mid-1990s suggest that nearly a million *A. carolinensis* a year are collected and sold commercially as pets (at least one major supplier is located in Louisiana, Losos, 2009). Certain populations in eastern Tennessee may have been established from escapees and/or released animals from Louisiana (Wade, Echternacht, & McCracken, 1983). At least two introductions via the pet trade have also been documented in Texas as well as seven other introductions (Kraus, 2009). Our mtDNA analyses showed that one haplotype from Brownsville, Texas was 99.9% similar (at 1,172 bp) to two haplotypes from Louisiana, suggesting an affinity between these locations. It is
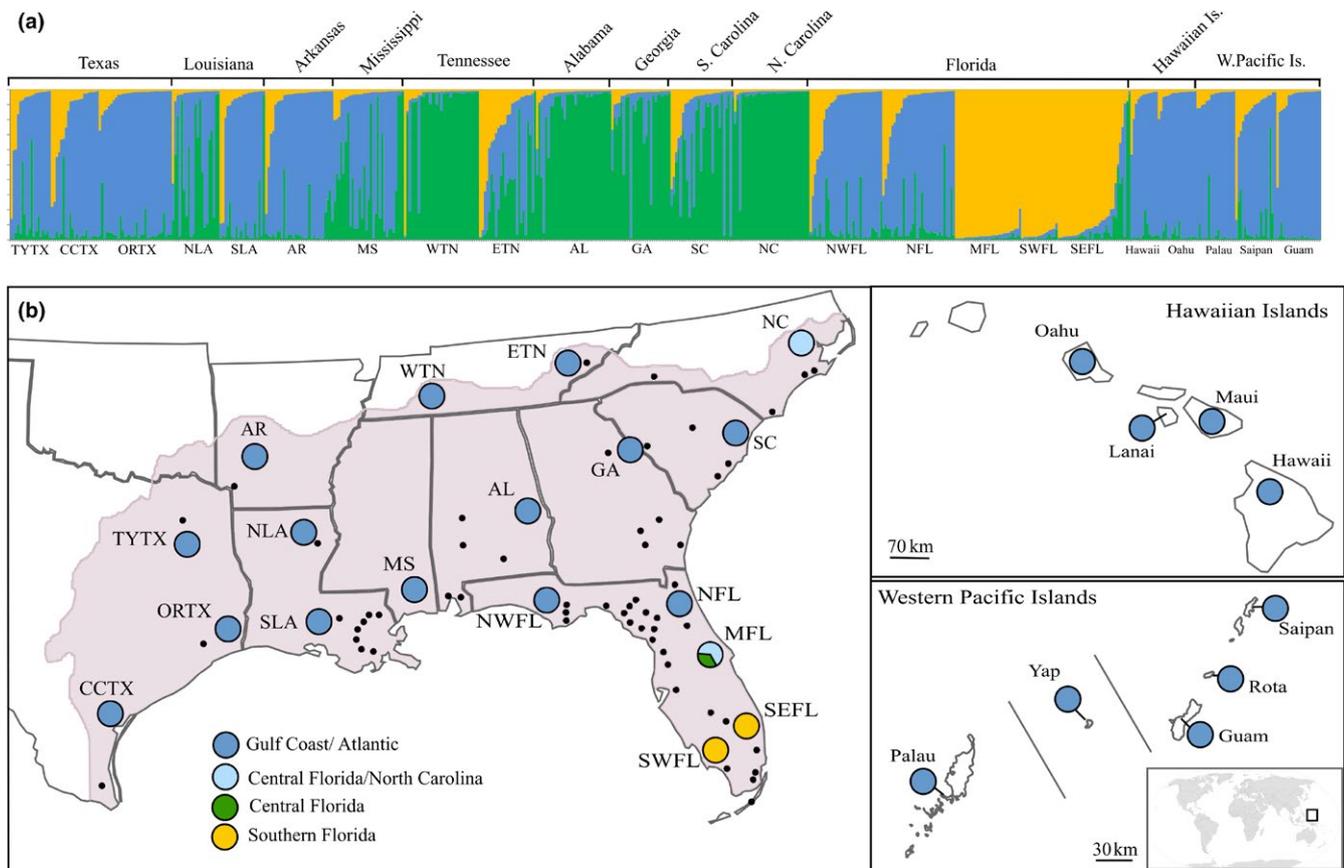
FIGURE 5 (a) Structure results for all individuals (*n* = 576) from native and non-native locations forming *K* = 3 genetic clusters. (b) Distribution of sampling locations in native and non-native ranges with pie charts indicating mtDNA clade assignment (populations on Japanese Islands, not shown here, are assigned to the Gulf coast-Atlantic mtDNA clade, see map in Sugawara et al., 2015). Black dots in the native range indicate sampling locations of mtDNA haplotypes obtained from GenBank and the shaded (light purple) region indicates the approximate native range of *Anolis carolinesis*

thus plausible that the geographic origin of all introduced populations in the Pacific was several locations around southern Louisiana.

Historical records and museum collections first documented the occurrence of green anoles on the Hawaiian Island of Oahu in 1950, the records believed to be based on the offspring of released or escaped pets (reviewed in Kraus, 2009). The species was subsequently introduced to Maui (1964), Hawaii (1978) and Kauai (1987). Shared haplotypes among islands in this region support a scenario of introductions from a common source. Specifically, for the population on Hawaii, our ABC analysis supported a stepping-stone colonization scenario from the previously established population on Oahu. Levels of genetic diversity were lower on Oahu than in the native range and the secondary introduction to Hawaii has resulted in further loss, a characteristic of serial founder events (Clegg et al., 2002).

The situation in the Western Pacific appears more complicated. Our analyses indicate a separate introduction to this region with no evidence of haplotypes shared with Hawaiian Island populations and high genetic differentiation between the two non-native regions. The introduction on Guam (1953) was probably an independent event from a source(s) in the native range. Our analysis showed that the population in Palau (1960) was established from Guam and this colonization scenario is supported with historical records (Lever, 2003).

The population on Saipan (1979) has probably established through stepping-stone colonization from Palau. Levels of genetic diversity on Saipan were higher than in Palau, which might indicate multiple introduction events (or a large propagule size). Indeed, a unique haplotype on Saipan, not found in other non-native populations, might indicate an undocumented, independent introduction. *Anolis carolinensis* was also introduced to the Ogasawara Islands in the late 1960s where it has expanded its range substantially (but see Suzuki-Ohno et al., 2017) and caused negative impacts on native species and the ecosystem (e.g., competing with and preying upon an endemic lizard, *Cryptoblepharus boutonii nigropunctatus*; Abe, Makino, & Okochi, 2010; Toda, Takahashi, Nakagawa, & Sukigara, 2010; Sugawara et al., 2015; Suzuki-Ohno et al., 2017). One mtDNA haplotype from Chichijima (Hayashi et al., 2009) was identical to one from Guam, indicating a common source (it has been suggested that islanders or American soldiers brought in several green anoles from Guam; Hasegawa, Kusano, & Miyashita, 1988). Two additional mtDNA haplotypes were found in Okinawa (Suzuki-Ohno et al., 2017), one of which was identical to Texas and Louisiana haplotypes. The colonization pattern within the Japanese archipelago also followed a stepping-stone model with decreasing levels of genetic diversity from island to island (Sugawara et al., 2015) mirroring the patterns observed in other Pacific populations. These introductions in

the region were associated with intentional and/or unintentional release of captive animals via the pet trade as well as post-World War II shipment-cargo movements. Green anoles may have been introduced as pets for American military personnel or came with supplies and construction material during the rebuilding of cities after the end of WWII (Crombie & Pregill, 1999; Fritts & Rodda, 1998; Kraus, 2009; Lever, 2003).

Over the last 100 years, Pacific islands have been the recipient of numerous non-native species, causing significant ecological impacts in many cases (e.g., Harper & Bunbury, 2015). Colonization varied from single events and a reduction in genetic diversity (e.g., brown tree boa, *Boiga irregularis*, Richmond, Wood, Stanford, & Fisher, 2015), to multiple waves of introductions (e.g., brown skink, *Carlia ailanpalai*; Austin et al., 2011) and genetic admixture in source population(s) leading to increased genetic diversity (e.g., house gecko, *Hemidactylus frenatus*; Tonione, Reeder, & Moritz, 2011). The stepping-stone colonizations in the non-native range of *A. carolinensis* are consistent with a process of isolation-by-colonization, whereby independent introductions have resulted in overall high genetic differentiation between the two non-native regions (Western Pacific and Hawaiian archipelagos). This results in no relationship between the pattern of neutral genetic differentiation and geographic distance. Within each archipelago, there is a small reduction in genetic variation but levels of population differentiation remain relatively low (with the exception of the population on Saipan). In recently introduced populations and experimental studies, it is unclear how long such founder effects will persist (Kolbe, Leal, Schoener, Spiller, & Losos, 2012). In populations that have been separated for longer periods of time, other forces (i.e., natural selection) may contribute additional differences to the apparent patterns of population genetic structure.

Differences in the amount and structuring of genetic variability during range expansions and biological invasions have implications for the fate of natural populations exposed to novel and changing environments. Human-mediated introductions of *A. carolinensis* in the Pacific have resulted in strong erosion of genetic diversity, which appeared lower than that in populations at the expansion front in the native range. Climate matching is considered the most important predictor of global establishment of non-native reptiles (Mahoney et al., 2015; Tingley, Thompson, Hartley, & Chapple, 2016); however, low levels of genetic diversity, small population sizes and isolation could restrict the survival and persistence of non-native populations. It remains to be tested whether these independent evolutionary units will have the capacity for rapid adaptation if faced with unfavourable conditions. On the other hand, our analyses support an isolation-by-distance pattern in the native range of *A. carolinensis* (but see Campbell-Staton et al., 2016) where natural range expansion has resulted in a steady reduction of genetic diversity at the leading edge of the expansion front. However, these larger and more connected populations may be better able to respond to novel conditions compared to the low diversity, isolated introduced populations in the Pacific. Indeed, green anole populations near their southern range limit in Texas showed an adaptive response to an extreme cold weather event by decreasing their low-temperature tolerance, and shifting gene expression and allele frequencies to be more similar to cold-adapted northern populations (Campbell-Staton et al., 2017). Whether lower diversity, more isolated introduced populations are capable of similar adaptive responses are unknown. Also, when rapid climate change is accompanied by habitat loss and fragmentation, a species' ability to respond to the combined effect could be significantly limited (see Henle et al., 2016). We will need to incorporate an eco-evolutionary framework to understand the complex effect of species range expansions and climate change on genetic diversity and adaptive potential (Bailey et al., 2014; Fronhofer & Altermatt, 2015).

DATA ACCESSIBILITY

Unique haplotypes generated in this study are deposited on GenBank (accession numbers MG252703–MG252730). A list of published sequences used in the phylogenetic analyses can be found in the supplementary information (Table S2).

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SUPPORTING INFORMATION

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