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Invasion Histories Differ Markedly Among the Four *Anolis* Lizard Species Introduced to Bermuda

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Abstract.—Islands are the recipients of numerous invasive amphibians and reptiles, often resulting in negative impacts on native biodiversity. In the Caribbean, *Anolis* lizards, a well-studied system for their adaptive radiation, are also commonly introduced outside of their native range, which has resulted in reshaping species-area and species-isolation relationships. We used mitochondrial DNA (mtDNA) sequence data in a phylogeographic framework to reconstruct and compare invasion histories of four non-native *Anolis* species (*A. grahami*, *A. extremus*, *A. leachii*, and *A. sagrei*) established in Bermuda over the past 120 years. Our findings support different invasion histories for the four species, including the number of native-range sources, genetic diversity, secondary introductions and opportunities for intraspecific hybridization between previously isolated lineages. The extent of population genetic structure in the native range and the mode of introduction (i.e., intentional vs. unintentional) may influence patterns of the invasion history and genetic diversity for introduced *Anolis* lizards in Bermuda. These results suggest that human-mediated introductions can create substantial variation in invasion dynamics, which in turn may influence fates of species in new environments.

Human-mediated introductions have reshaped species-area and species-isolation relationships across many groups (Sax et al., 2002; Helmus et al., 2014; Capinha et al., 2015) while posing major threats to biodiversity. For reptiles, and lizards in particular, unintentional transport through cargo shipments or the nursery trade as well as intentional transport via the pet trade have been predominant introduction pathways (Lever, 2003; Kraus, 2009). Unlike some other species (e.g., *Rhinella marina*; Shanmuganathan et al., 2010), reptiles are less common in deliberate introductions, such as biological control agents (but see e.g., *Leiocephalus carinatus* in Florida; Krysko et al., 2011). In many instances, little is known about the history of introductions, such as pathways, dates, number of events, or propagule sizes, because these events are often undocumented, poorly reported, or information is widely scattered in obscure sources (Kraus, 2009; Mahoney et al., 2015). Information on invasion history is often necessary for testing hypotheses about genetic and phenotypic divergence during invasions, allowing for appropriate comparisons between native-range sources and introduced populations (Estoup and Guillemaud, 2010). Such studies can provide important insights into ecological and evolutionary causes and consequences of biological invasions.

Application of molecular tools has significantly advanced our ability to verify documented introduction histories, detect cryptic non-native species, identify the likely geographic origin or origins of non-native populations, and elucidate complex invasion pathways (Estoup and Guillemaud, 2010; Fitzpatrick et al., 2012). Mitochondrial DNA (mtDNA) sequences are excellent markers for inferring certain aspects of invasion histories due to their high mutation rate and lack of frequent recombination. For example, mtDNA can help determine whether an invasion resulted from a single introduction or multiple introductions from different source populations (Kolbe et al., 2004; Rollins et al., 2011; Michaelides et al., 2013). Depending on the amount and structure of genetic variability in the native range, low mtDNA genetic diversity in the introduced populations may also indicate a founder event (i.e., small propagule size), while high diversity may suggest multiple events (from the same source, different sources, or both) or a single event from a diverse source in the native range (Taylor and Keller, 2007). Since mtDNA has been widely used in phylogeographic studies involving reptiles, published mtDNA sequences from diverse populations in their native ranges can complement new sampling, thus enabling reconstruction of invasion histories and identification of source regions with greater accuracy.

Lizards of the genus *Anolis* are known for their remarkable adaptive radiation (Losos, 2009) and high endemism in the Caribbean due to their limited overwater dispersal ability, which has resulted in only rare long-distance dispersal events (Williams, 1969; Glor et al., 2003; 2004; Reynolds et al., 2020). However, recent human activities, that are usually unintentional, are responsible for the occurrence of *Anolis* lizards beyond their native islands being introduced throughout the Caribbean and elsewhere (Williams, 1969; Kraus, 2009; Helmus et al., 2014). More than 20 *Anolis* species have been introduced and have become established in over 80 populations worldwide, the majority by human assistance through transport of agricultural and ornamental plants as well as the pet trade (Lever, 2003; Kraus, 2009; Krysko et al., 2011). Strong phylogeographic structure in the native ranges of most *Anolis* species (i.e., distinct genetic lineages found in specific geographic regions) has made it possible for genetic analyses to trace the sources of many introduced anole species (e.g., Kolbe et al., 2004, 2007; Eales et al., 2010; Michaelides et al., 2018, 2019). Thus, introduced *Anolis* species are typically good candidates for invasion history reconstruction.

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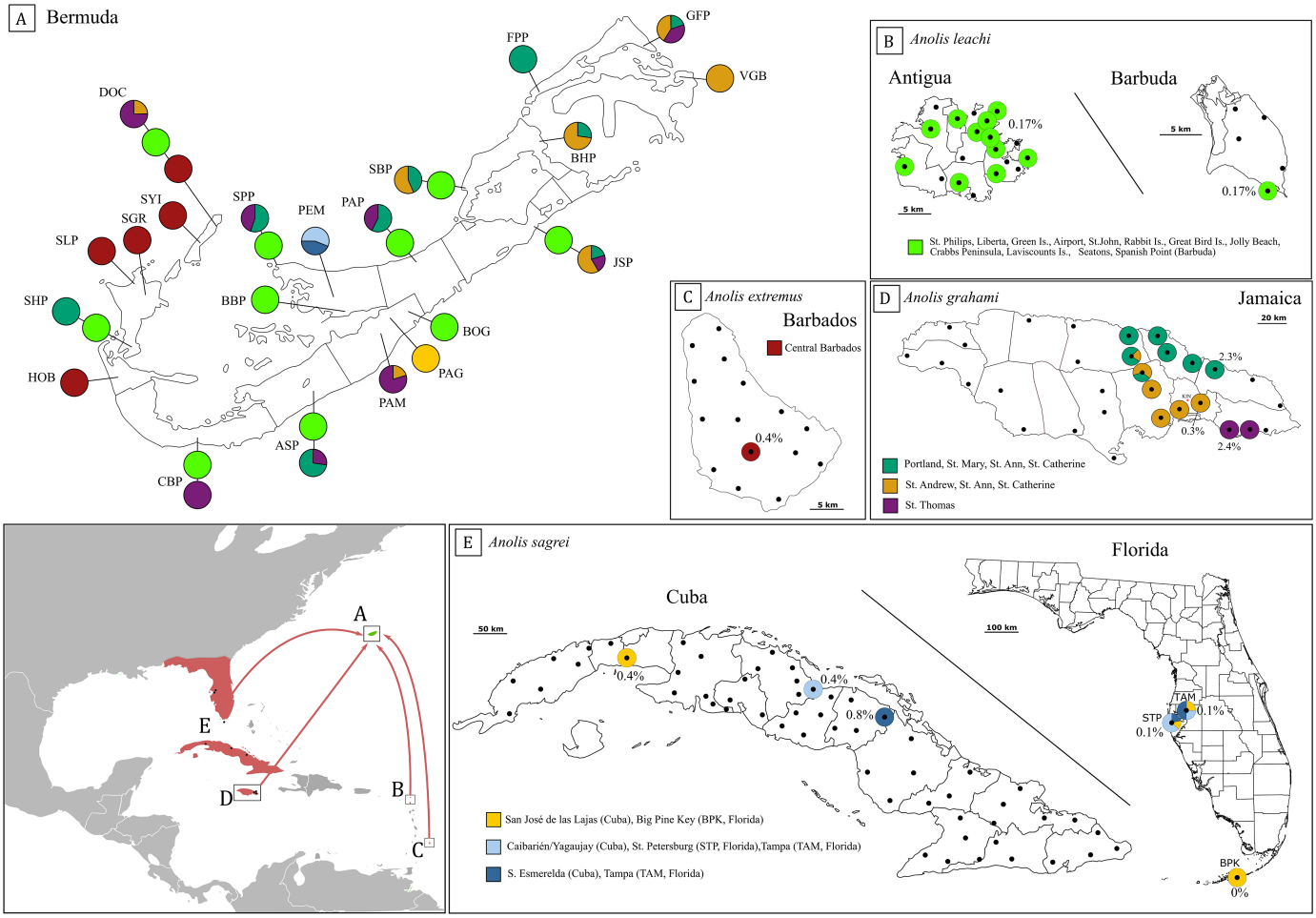

FIG. 1. Sampling locations for introduced *Anolis* species in (A) Bermuda and their respective native ranges (black dots) of (B) Antigua and Barbuda for *A. leachii*, (C) Barbados for *A. extremus*, (D) Jamaica for *A. grahami*, and (E) Cuba and Florida (non-native) for *A. sagrei*. Pie charts indicate haplotype frequency in introduced-range populations and are color-matched to putative native-range source localities (i.e., those native-range locations with the least divergent haplotypes compared to haplotypes sampled in Bermuda). Percentages above these native-range sources localities indicate minimum pairwise sequence divergence between haplotypes from introduced populations and that native-range population. Within each panel, the legend shows colors associated with native-range haplotypic variation for each introduced *Anolis* species, locations in the native range with the most similar haplotypes to those sampled in Bermuda. The inset map shows the location of Bermuda (A) and all possible native and non-native (for *A. sagrei*) range source areas in the region (B–E) with red arrows showing introduction events.

Bermuda is a small, oceanic archipelago of about 5,500 ha located in the western North Atlantic (Fig. 1) with only one endemic terrestrial vertebrate, the Critically Endangered Bermuda Skink, *Plestiodon* (syn. *Eumeces*) *longirostris* (Bacon et al., 2006). As with other islands and mainland coastal regions that are hotspots of established non-native species (Dawson et al., 2017), during the first half of the twentieth century *Anolis* species were introduced to the archipelago. First, in 1905, *A. grahami* was purposefully introduced from Jamaica as a biological control agent of the fruit fly *Ceratitis capitata* and have since spread over most of the archipelago (Wingate, 1965; Losos, 1996). Wingate (1965) reported that the propagule consisted of 71 individuals collected in the Kingston area, which is on the south coast of eastern Jamaica. In the 1950s, or perhaps as early as the 1940s (Wingate, 1965), two additional *Anolis* species were introduced, albeit this time unintentionally. *Anolis leachii*, from Antigua and Barbuda, first observed in west-central Bermuda and now found throughout the main island, and *A. extremus*, from Barbados, first recorded from Sandy's Parish in northwest Bermuda and still geographically restricted to this area (Losos, 1996; Macedonia et al., 2016). Another species, *A. sagrei*, was introduced to central Bermuda around 2011 (Stroud et al., 2017, 2019), originally from the Bahamas and Cuba but now widely established outside of its native range, including Florida (Kolbe et al., 2004). *Anolis sagrei* is now established at two sites in Bermuda, one of which is a plant nursery (Stroud et al., 2017). A fifth species, *A. carolinensis*, has been recently recorded due to a single individual being found in a cargo dock and is therefore not considered established (Stroud et al., 2016).

In this study, we used phylogeographic analyses of mtDNA haplotypic variation to reconstruct invasion histories of four species of *Anolis* lizards introduced to Bermuda over the past 120 years. We focused on several aspects of the invasion histories of the four *Anolis* species: (1) identifying the number of native-range source populations; (2) delimiting the geographic area in the native range from which introduced populations are derived; (3) assessing whether multiple, previously isolated mtDNA lineages are detected in the introduced populations; (4) comparing genetic variation in native and introduced populations; and (5) determining whether secondary introductions from previously established non-native populations occurred in the case of *A. sagrei*. Our findings support a wide range of invasion histories

among the four introduced *Anolis* species, including introductions derived from one to several native-range sources, variable levels of mtDNA haplotype variation within introduced populations, and secondary introductions from Florida for *A. sagrei*. Different modes of introduction and the extent of population genetic structure in the native range appear to influence patterns of invasion history for introduced *Anolis* lizards in Bermuda.

Materials and Methods

Sampling, DNA Extraction, and PCR Amplification.—We sampled a total of 155 lizards from 21 locations in Bermuda (Fig. 1, Table S1), comprising four non-native *Anolis* species: *A. extremus* ($n = 25$), *A. grahami* ($n = 64$), *A. leachii* ($n = 48$), and *A. sagrei* ($n = 18$). To identify patterns of invasion history, we also used samples collected from the native range for *A. leachii* in Antigua and Barbuda (51 individuals from 24 locations) and *A. grahami* in Jamaica (74 individuals from 28 locations). For *A. extremus* and *A. sagrei*, we used published data from their native ranges in Barbados for *A. extremus* (67 individuals from 15 locations; Thorpe et al. 2005, 2008) and the Bahamas, Belize, Caymans, and Cuba as well as introduced populations in Grand Cayman, Grenada, Jamaica, Taiwan, and the United States (i.e., Florida, Hawaii, Louisiana, and Texas) for *A. sagrei* (773 individuals from 133 locations; Kolbe et al., 2004, 2007; see also the *Phylogenetic and Population Genetic Analyses* section). We extracted DNA from liver or tail tissue preserved in 70–90% ethanol using DNA extraction kits from Viogene and Bioline.

Different mtDNA genes have been used in past phylogenetic, biogeographic, and taxonomic studies of *Anolis* species (e.g., Poe et al., 2017). To this end, we chose to sequence a specific mtDNA gene for each of the four introduced *Anolis* species taking into consideration available primer information and published sequences for comparative analyses. For *A. leachii*, *A. grahami*, and *A. sagrei*, we sequenced an approximately 1,200 base pair (bp) region of the mitochondrial NADH dehydrogenase 2 (*ND2*) gene using the primer pair H5730 (5′-AGCGAATRGAAGCCCGCTGG-3′; Glor et al., 2004) and L4437a (5′-AAGCTTTCGGGCCCATACC-3′; Macey et al., 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, 72 °C for 80 s, and a final extension step at 72 °C for 5 min. For *A. extremus*, we amplified an 1139 bp region of the mitochondrial cytochrome b (*cytB*) gene with the following primers Mt-A (5′-CTCCCAGCCCCATCCAACATC TCAGCATGATGAAACTTCG-3′; Lenk and Wink, 1997) and Mt-F (5′-AGGGTGGAGTCTTCTGTTTTTGGTT TACAAGACCAATG-3′; Wink, 1995; Malhotra and Thorpe, 2000; Thorpe and Stenson, 2003). PCR was carried out in 50 μl volumes containing 20 ng template DNA, 20 mM Tris-HCl, 50 mM KCl, 2 mM $MgCl_2$, 0.2 mM each dNTP, 0.4 mM each primer, and 2 units of Taq DNA polymerase (Invitrogen). PCR cycling parameters were 94 °C for 3 min, 30 cycles of 93 °C for 1 min, 48 °C for 2 min, 72 °C for 2 min, and a final extension step at 72 °C for 3 min. PCR products were purified using the QIAquick PCR Purification Kit (Qiagen) and sequencing was performed on the AB 3130xl genetic analyzer.

Mitochondrial DNA sequences from both directions (forward and reverse) were first aligned together to obtain a consensus sequence. Accepted sequences were then aligned using the MAFFT program (Katoh et al., 2002) implemented in GENEIOUS 8 (Kearse et al., 2012) and trimmed to a uniform length of either 1172 bp for *ND2* or 1044 bp for *cytB*. We translated sequenced *ND2* and *cytB* regions to amino acid sequences to verify that no premature stop codons disrupted the reading frame. Unique sequences were submitted to GenBank under the accession numbers PV460752-PV460753 and PV494569-PV494682 (Table S3).

Phylogenetic and Population Genetic Analyses.—We used phylogenetic analysis to reconstruct relationships among haplotypes and assign genetic origin of introduced haplotypes from Bermuda. Final alignments for *A. leachii* and *A. grahami* contained 36 and 81 haplotypes, respectively, including outgroup sequences belonging to the closely related species *A. wattsi* (MH375645; Michaelides et al., 2019), *A. pogus* (AY296193; Harmon et al., 2003), and *A. garmani* (Kolbe, unpubl. data). For *A. extremus*, the final alignment contained 51 haplotypes from introduced (this study) and native ranges (Thorpe et al., 2005) including two outgroup sequences belonging to the closely related species of *A. aeneus* and *A. oculatus* (KP677043, KP677210; Thorpe et al., 2015). For *A. sagrei*, the final alignment contained 510 haplotypes including three outgroup sequences of the closely related species *A. homolechis, A. bremeri*, and *A. quadriocellifer* (Kolbe et al., 2004). Because *A. sagrei* has been widely introduced outside of their native range, we included all published non-native haplotypes (Kolbe et al., 2004, 2007) to test for secondary introduction events from already established non-native populations.

We ran Bayesian inference (BI) analyses in MRBAYES (Huelsenbeck and Ronquist, 2001) by implementing a plugin in GENEIOUS 8 (Kearse et al., 2012). The best-fit model for each species (HKY+G, for *A. extremus* and *A. leachii*, GTR+G for *A. grahami*, and HKY+G+I for *A. sagrei*) was identified by applying the Akaike Information Criterion (*AICc*) using MEGA 7 (Kumar et al., 2016). 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 the first 10% (burn-in-length) of trees after checking for convergence of the chains, and the posterior probability branch support was estimated from the 50% majority-rule consensus tree. When distinct mtDNA lineages, clades, or subclades from the consensus tree were also associated with specific geographic regions or locations in the native range, we considered the species to exhibit strong phylogeographic structure. In contrast, when lineages or clades were not strongly associated with specific native-range locations, we inferred weak (or no) phylogeographic structure. Well-supported clades (>50% consensus support, including ancestral and all descendant sequences) and native-range phylogeographic structure were used to infer whether divergent clades coexisting in the same location in the introduced range created opportunities for intraspecific admixture.

To further investigate mtDNA divergence between introduced and native haplotypes and identify the likely source location or locations, we constructed phylogenetic networks for each species using a median-joining algorithm in PopArt (Leigh and Bryant, 2015). To construct the phylogenetic networks, we used median vectors as hypothetical ancestral sequences required to connect existing sequences within the network with maximum parsimony. We used a subset of mtDNA sequences for each species that included the introduced-range haplotypes from Bermuda and the native-range sequences from phylogenetically

closely related clades or subclades identified from the phylogenetic tree analyses above. Haplotype similarity based on the distance in mutation steps of an introduced-range haplotype in Bermuda from the nearest native-range haplotype on the median-joining network was used to infer the likely native-range geographic origin. A strong phylogeographic structure in the native range increases accuracy and confidence in the geographic source location of introduced haplotypes.

We calculated nucleotide diversity (Pi) for both native and introduced-range populations in DNAsp 6 (Rozas et al., 2017). Pairwise sequence divergence (D) was calculated among native and introduced haplotypes in MEGA 7 (Kumar et al., 2016).

Results

In *A. grahami*, the first anole species introduced to Bermuda, our phylogenetic analysis revealed 14 mtDNA haplotypes in Bermuda (Table S1), which were nested within the east-central Jamaica clade (Fig. S1). There were four unresolved subclades (i.e., a four-edge polytomy) within east-central Jamaica (Figs. 2B, S1). One group (EC1) contained 64% of haplotypes from Bermuda and native-range haplotypes from sampling locations around Kingston, the second group (EC2) contained haplotypes geographically north of Kingston, the third group (EC3) contained the remaining introduced haplotypes from Bermuda solely, and the fourth group (EC4) contained haplotypes that were sampled southeast of Kingston (Fig. 1D). Minimum sequence divergence between haplotypes sampled in introduced locations and the most similar haplotypes from the native range varied from 0.3% (east-central group 1), 2.3% (east-central group 2), and 2.4% (east-central group 4, Tables 1, S2; Figs. 1, 2). Nine of 13, almost 70%, sampling locations in Bermuda contained haplotypes from more than one of these east-central subgroups (Fig. 1). The most likely geographic origin of introduced haplotypes was either multiple locations in the east-central part of Jamaica, near Kingston (Figs. 1D, 2), or a single location in the native range where divergent haplotypes from multiple subgroups

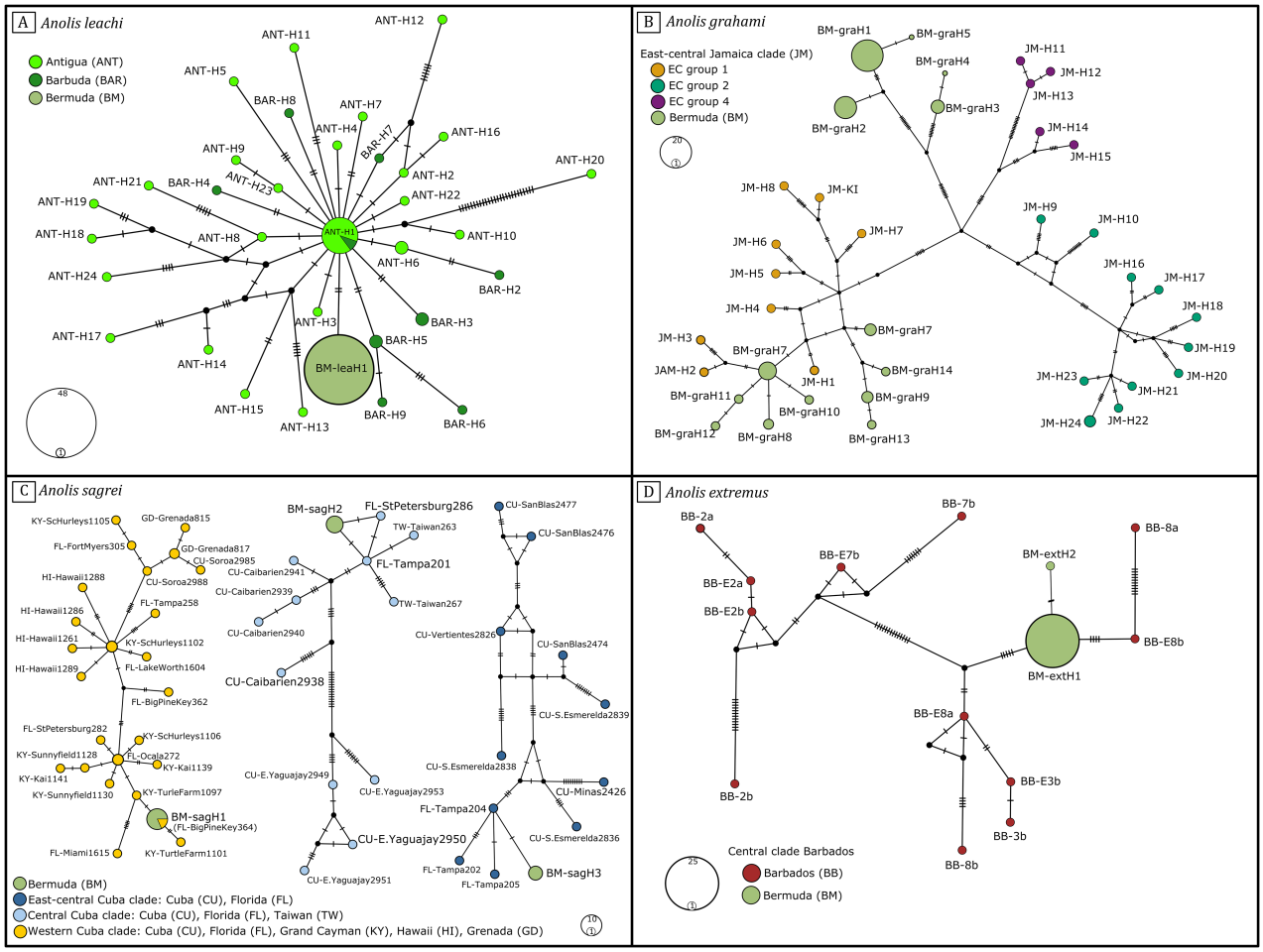

Fig. 2. Median-joining networks for each introduced *Anolis* species in Bermuda. (A) For *A. leachii*, the network was derived from 81 ND2 sequences. All individuals in Bermuda share the same haplotype (BM-leaH1). One haplotype from Barbuda, BAR-H1, was identical to a haplotype sampled on Antigua, ANT-H1. (B) For *A. grahami*, the network was derived from 88 ND2 sequences; introduced haplotypes in Bermuda (BM-gra) cluster within east-central haplogroups (EC1, EC2, EC4) from the native range in Jamaica. (C) For *A. sagrei*, three independent networks were constructed, each using the subset of ND2 sequences corresponding to the native-range clade with which the three haplotypes on Bermuda clustered. BM-sagH1 from Bermuda was identical to FL-BigPine364 from Florida (originally derived from the Western Cuba lineage). (D) For *A. extremus*, the network was derived from 37 cytB sequences. Only haplotypes belonging to the Central lineage in Barbados were used along with the two haplotypes found in Bermuda. The size of circles corresponds to the number of sampled individuals sharing that haplotype. All haplotypes from Bermuda are shown in the same olive-green color across networks, and different colors are used for the closely related native-range haplotypes. Lines represent mutation steps.

TABLE 1. Introduction information and summary of mtDNA analyses for four *Anolis* species. Number of introduced-range sampling locations (N_{ISL}), introduced-range samples analyzed (N_I), introduced-range haplotypes (H_I), and mean within introduced-population nucleotide diversity (Pi_{INT}) are shown for the introduced range in Bermuda. For the native ranges, we show the number of native-range sampling locations (N_{NL}), native-range samples analyzed (N_{NS}), native-range haplotypes (H_N), mean within native-range population nucleotide diversity (Pi_{NAT}), and pairwise haplotype divergence (D) between introduced and native-range haplotype(s). Note that for *A. sagrei*, one haplotype (BM-sagH1) is identical (0% divergence) to a haplotype from the non-native range, Big Pine Key, Florida (see also Fig. 2, Table S2).

Species	Ecomorph	Native range	Date of introduction	Mode of introduction	N_{ISL}	N_I	H_I	Pi_{INT} (%)	N_{NL}	N_{NS}	H_N	Pi_{NAT} (%)	D (%)
A. grahami	Trunk-crown	Jamaica	1905	Biological control	13	64	14	1.04	28	74	65	1.24	0.3–2.5
A. extremus	Intermediate Lesser Antilles	Barbados	1940–50s	Cargo stowaway	5	25	2	0.03	15	67	47	1.43	0.4–0.5
A. leachii	Large Lesser Antilles	Antigua/Barbuda	1940–50s	Unknown	10	48	1	0	24	51	33	0.40	0.17
A. sagrei	Trunk-ground	Cuba/Bahamas	2011–2015	Unknown, nursery trade	2	18	3	4.3	133	773	504	1.78	0.4–0.8

were present. Within populations, mean pairwise nucleotide diversity was similar for introduced and native ranges (Table 1).

We found two haplotypes for *A. extremus* in Bermuda that are clustered within the central clade of native haplotypes from Barbados (Figs. 2, S2). A single location in Bermuda (SGR) contained both haplotypes, whereas all other locations shared the same haplotype (Fig. 1; Table S1). The geographic origin of these introduced-range haplotypes is consistent with a single origin from central Barbados where these introduced-population sequences were 0.4–0.5% divergent from native-range haplotypes (Tables 1, S2; Fig. 1). Mean pairwise nucleotide diversity was much lower in introduced populations compared to native ones (Table 1).

All *A. leachii* individuals in Bermuda shared the same haplotype, which was 0.17% divergent from the most common native-range haplotype (ANT-H1) found throughout Antigua and in southern Barbuda (Figs. 1, 2). There was poor resolution in the phylogenetic tree for this species (Fig. S3), indicative of low divergence among native-range haplotypes (Fig. 2A) and low nucleotide diversity within populations of *A. leachii* (Table 1). Because many of the native-range populations shared the same haplotype (ANT-H1), the lack of phylogeographic structure for *A. leachii* precludes inference of a specific source location.

Anolis sagrei has a strong phylogeographic structure in its native range of Cuba and the Bahamas with numerous well-supported clades (Fig. S4; Kolbe et al., 2004). We found three mtDNA haplotypes in the two introduced locations in Bermuda that clustered with three separate geographically distinct native-range clades (Fig. S4). The Paget Parish (PAG) location was monomorphic with a haplotype derived from the western Cuba clade, whereas Pembroke Parish (PEM) had two haplotypes belonging to the east-central and eastern Cuba clades (Fig. 1). Investigating the relationship of these haplotypes with a neighbor-joining network revealed that the western Cuba haplotype (BM-sagH1) was identical to a haplotype found in an introduced population in Big Pine Key, Florida (Figs. 1E, 2C). The other two haplotypes were very closely related (i.e., 0.1% sequence divergence) to haplotypes found in introduced locations in Tampa and St. Petersburg, Florida (Fig. 2C), rather than any other haplotypes sampled from previously identified native-range source locations in Cuba or the Bahamas (Kolbe et al., 2004). It should also be noted that both Tampa and St. Petersburg populations harbor haplotypes from all three native-range mtDNA clades (i.e., western, east-central, and eastern Cuba). Our results suggest that Bermuda populations are most likely secondary introductions from established non-native populations in Florida as opposed to independent introductions of the same or similar haplotypes directly from the native range and that the mixture of haplotypes observed in the Bermuda population likely occurred in Florida. On average, within-population nucleotide diversity in Bermuda was much greater than in the native range (Table 1), but lower than in Tampa and St. Petersburg, the putative sources for secondary introductions to Bermuda.

Discussion

Our phylogeographic and population genetic analyses of mtDNA haplotypes revealed that the invasion histories for the four *Anolis* species introduced to Bermuda differed markedly. The pathway of introduction (primary vs. secondary) and the extent of phylogeographic structure in the native range appear to influence invasion patterns as well as haplotypic and genetic diversity. Variation in native-range population structure had a strong influence on the ability to detect the number and specific geographic location of source populations. Co-occurrence of divergent haplotypes in some introduced populations appears to result from a secondary introduction from Florida for *A. sagrei*, whereas the larger propagule size associated with the biocontrol introduction of *A. grahami* may explain their higher haplotype diversity. Our findings highlight the diverse outcomes of species invasions and contribute to our understanding of how human activities can reshape the distribution of genetic and phenotypic diversity of species.

Unintentional introductions, such as those resulting from species hitchhiking in shipping cargo or via the nursery trade, typically involve a small number of individuals from a restricted geographic area and single introduction events. Consequently, such introductions are often associated with lower haplotypic and genetic diversity due to founder effects (Uller and Leimu, 2011). However, *A. sagrei* in Bermuda, likely introduced via nursery plants, exhibit high nucleotide diversity despite a low number of haplotypes. The observed high nucleotide diversity likely results from a secondary introduction from genetically variable, admixed, non-native populations in Florida (Kolbe et al., 2004, 2008; Bock et al., 2021). Such genetic diversity within non-native populations of *A. sagrei* in Florida may enhance adaptability to new environments, increasing establishment success (Kolbe et al., 2004), and likelihood of serving as a source for future introductions. Secondary introductions within the non–native

range, both locally and globally, are common in species invasions (Kolbe et al., 2004; Floerl et al., 2009; Lombaert et al., 2010). For example, Green Anoles, *A. carolinensis* (native to southeastern USA), have established several populations in Pacific islands from earlier introductions within the region (Michaelides et al., 2018). In Bermuda, *A. sagrei* currently occur in two locations (Stroud et al., 2017). Given their history of secondary introductions and high genetic variation due to admixture (Kolbe et al., 2004; Bock et al., 2021), we might expect that *A. sagrei* could be more effective dispersers than other species of *Anolis*. To this end, substantial ecological overlap in diet and habitat between introduced *A. sagrei* and the native skinks on Bermuda suggests a serious conservation threat if the two species come into contact (Stroud et al., 2017).

The intentional introduction of *A. grahami* to Bermuda for biocontrol differed from the unintentional introduction of the other three species in several respects. *Anolis grahami* is found throughout the archipelago and exhibits substantially more haplotypic variation (i.e., 14 versus 1–3 haplotypes) compared to the other introduced species. Intentional efforts associated with biocontrol and the pet trade, for example, may deliberately sample more individuals from multiple locations to increase genetic or phenotypic diversity and favor characteristics that predispose them toward successful establishment (e.g., "introduction biases," Street et al., 2023). Similarly, Kolbe et al. (2013) hypothesized that genetic diversity among Italian Wall Lizard (*Podarcis siculus*) populations was the result of introductions from multiple native-range sources and driven by desire for variety in the pet trade. Biocontrol efforts may include large propagules and repeated introduction events to establish the biocontrol agent. The documented source of the original *A. grahami* propagule was near Kingston (Wingate, 1965), where some Bermuda haplotypes are most closely related (the east-central Jamaica group 1; Figs. 1, 2, S1). However, additional haplotypes detected in Bermuda, belonging to the four-edge polytomy of the east-central Jamaica clade (Figs. 2, S2), show higher divergence and are more closely related to native-range locations northeast and southeast of Kingston (Fig. 1, Table S2). The additional haplotypes may have been part of the original propagule, and it is possible that Kingston, as a hub of traffic and human activity, facilitated transport of lizards from various parts of east-central Jamaica. Presence of multiple native-range haplotypes within introduced populations in Bermuda may represent secondary contact and opportunities for intraspecific hybridization (i.e., admixture). However, levels of within-population diversity are similar between introduced and native-range populations in Jamaica. Whether *A. grahami* lizards originated from multiple divergent populations or a single admixed population in their native range remains to be tested. Additionally, an undocumented introduction from a different location or locations in east-central Jamaica sometime after the original biocontrol release is possible.

Moderate to strong population structure is a nearly ubiquitous feature of *Anolis* species (Glor et al., 2003, 2004; Thorpe et al., 2005, 2008, among others) facilitating the identification of native-range sources and admixture in introduced populations (e.g., Kolbe et al., 2004). For example, strong native-range population structure for *A. extremus* increases our confidence in the interpretation of a single introduction from central Barbados. In *A. leachii*, however, the single haplotype detected in Bermuda, along with generally low haplotypic diversity and lack of population structure in the native range, precludes the ability to identify sources or use haplotype variation as a proxy for propagule size (i.e., the minimum number of introduced females). Whether a large or small number of individuals was introduced cannot be deduced when the native range lacks geographically structured genetic variation (Taylor and Keller, 2007).

In conclusion, the four *Anolis* lizard species introduced to Bermuda span a range of invasion histories, including number and geographic location of sources, genetic diversity, secondary introductions, and presence of multiple divergent mtDNA haplotypes in introduced populations, creating opportunities for admixture. *Anolis extremus* were derived from a single geographically and genetically distinct native-range source, whereas the lack of population structure in the native range of *A. leachii* precludes assigning them to a source area. Haplotypic variation in *A. sagrei* is ultimately derived from three distinct areas in Cuba, but evidence points to secondary introductions from Florida. Haplotypes found in Bermuda for *A. grahami* could represent multiple sources or a single variable native-range source in east-central Jamaica. Some species, due to coexistence of divergent haplotypes, have opportunities for admixture (i.e., *A. sagrei* and to some extent *A. grahami*) and others no opportunity (i.e., *A. extremus* and *A. leachii*). Nucleotide diversity was low in these single source species, whereas diversity between introduced and native populations was similar for *A. grahami* but substantially higher in introduced *A. sagrei* populations. Genetic analyses of mtDNA data may also provide insight into the minimum number of individuals involved in species introductions, ranging from a single female for *A. leachii*, to three females for *Anolis sagrei*. *Anolis grahami* had the highest haplotypic variation (14 haplotypes), likely reflecting native-range phylogeographic structure and larger propagule size. Taken together, our findings suggest that human-mediated introductions can create substantial variation in invasion dynamics, which may influence future spread of species in new environments.

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Literature cited

Bock, Dan G., Simon Baeckens, Jessica N. Pita-Aquino, Zachary A. Chejanovski, Sozos N. Michaelides, Pavitra Muralidhar, Oriol Lapiedra, Sungdae Park, Douglas B. Menke, Anthony J. Geneva, Jonathan B. Losos, and Jason J. Kolbe. 2021. "Changes in Selection Pressure Can Facilitate Hybridization During Biological Invasion in a Cuban Lizard." *Proceedings of the National Academy of Sciences of the United States of America* 118 (42): e2108638118. https://doi.org/10.1073/pnas.2108638118.

Capinha, César, Franz Essl, Hanno Seebens, Dietmar Moser, and Henrique Miguel Pereira. 2015. "Biogeography. The Dispersal of Alien Species Redefines Biogeography in the Anthropocene." *Science* 348 (6240): 1248–51. https://doi.org/10.1126/science.aaa8913.

Dawson, Wayne, Dietmar Moser, Mark van Kleunen, Holger Kreft, Jan Pergl, Petr Pyšek, Patrick Weigelt, Marten Winter,

Bernd Lenzner, Tim M. Blackburn, Ellie E. Dyer, Phillip Cassey, Sally L. Scrivens, Evan P. Economo, Benoit Guénard, César Capinha, Hanno Seebens, Pablo García-Díaz, Wolfgang Nentwig, Emili García-Berthou, Christine Casal, Nicholas E. Mandrak, Pam Fuller, Carsten Meyer, and Franz Essl. 2017. "Global Hotspots and Correlates of Alien Species Richness Across Taxonomic Groups." *Nature Ecology & Evolution* 1 (7): 0186. https://doi.org/10.1038/s41559-017-0186.

Eales, Jacqualyn, Roger S. Thorpe, and Anita Malhotra. 2010. "Colonization History and Genetic Diversity: Adaptive Potential in Early Stage Invasions." *Molecular Ecology* 19 (14): 2858–69. https://doi.org/10.1111/j.1365-294X.2010.04710.x.

Estoup, Arnaud, and Thomas Guillemaud. 2010. "Reconstructing Routes of Invasion Using Genetic Data: Why, How and so What?" *Molecular Ecology* 19 (19): 4113–30. https://doi.org/10.1111/j.1365-294X.2010.04773.x.

Fitzpatrick, Benjamin M., James A. Fordyce, Matthew L. Niemiller, and R. Graham Reynolds. 2012. "What Can DNA Tell Us About Biological Invasions?" *Biological Invasions* 14 (2): 245–53. https://doi.org/10.1007/s10530-011-0064-1.

Floerl, O., G. J. Inglis, K. Dey, and A. Smith. 2009. "The Importance of Transport Hubs in Stepping-Stone Invasions." *Journal of Applied Ecology* 46 (1): 37–45. https://doi.org/10.1111/j.1365-2664.2008.01540.x.

Glor, Richard E., Jason J. Kolbe, Robert Powell, Allan Larson, and Jonathan B. Losos. 2003. "Phylogenetic Analysis of Ecological and Morphological Diversification in Hispaniolan Trunk-Ground Anoles (*Anolis cybotes* Group)." *Evolution; International Journal of Organic Evolution* 57 (10): 2383–97. https://doi.org/10.1111/j.0014-3820.2003.tb00250.x.

Glor, Richard E., Matthew E. Gifford, Allan Larson, Jonathan B. Losos, Lourdes Rodríguez Schettino, Ada R. Chamizo Lara, and Todd R. Jackman. 2004. "Partial Island Submergence and Speciation in an Adaptive Radiation: A Multilocus Analysis of the Cuban Green Anoles." *Proceedings. Biological Sciences* 271 (1554): 2257–65. https://doi.org/10.1098/rspb.2004.2819.

Harmon, Luke J., James A. Schulte, Allan Larson, and Jonathan B. Losos. 2003. "Tempo and Mode of Evolutionary Radiation in Iguanian Lizards." *Science* 301 (5635): 961–4. https://doi.org/10.1126/science.1084786.

Helmus, Matthew R., D. Luke Mahler, and Jonathan B. Losos. 2014. "Island Biogeography of the Anthropocene." *Nature* 513 (7519): 543–6. https://doi.org/10.1038/nature13739.

Huelsenbeck, John P., and Fredrik Ronquist. 2001. "MRBAYES: Bayesian Inference of Phylogenetic Trees." *Bioinformatics* 17 (8): 754–5. https://doi.org/10.1093/bioinformatics/17.8.754.

Katoh, Kazutaka, Kazuharu Misawa, Kei-Ichi Kuma, and Takashi Miyata. 2002. "MAFFT: A Novel Method for Rapid Multiple Sequence Alignment Based on Fast Fourier Transform." *Nucleic Acids Research* 30 (14): 3059–66. https://doi.org/10.1093/nar/gkf436.

Kearse, Matthew, Richard Moir, Amy Wilson, Steven Stones-Havas, Matthew Cheung, Shane Sturrock, Simon Buxton, Alex Cooper, Sidney Markowitz, Chris Duran, Tobias Thierer, Bruce Ashton, Peter Meintjes, and Alexei Drummond. 2012. "Geneious Basic: An Integrated and Extendable Desktop Software Platform for the Organization and Analysis of Sequence Data." *Bioinformatics* 28 (12): 1647–9. https://doi.org/10.1093/bioinformatics/bts199.

Kitson, Lisa, Jennifer Gray, and Jamie Bacon. 2006. "Status and Conservation of the Reptiles and Amphibians of the Bermuda Islands." *Applied Herpetology* 3 (4): 323–44. https://doi.org/10.1163/157075406778905063.

Kolbe, Jason J., Richard E. Glor, Lourdes Rodríguez Schettino, Ada Chamizo Lara, Allan Larson, and Jonathan B. Losos. 2004. "Genetic Variation Increases During Biological Invasion by a Cuban Lizard." *Nature* 431 (7005): 177–81. https://doi.org/10.1038/nature02807.

Kolbe, Jason J., Richard E. Glor, Lourdes Rodríguez Schettino, Ada Chamizo Lara, Allan Larson, and Jonathan B. Losos. 2007. "Multiple Sources, Admixture, and Genetic Variation in Introduced *Anolis* Lizard Populations." *Conservation Biology* 21 (6): 1612–25. https://doi.org/10.1111/j.1523-1739.2007.00826.x.

Kolbe, Jason J., Allan Larson, Jonathan B. Losos, and Kevin de Queiroz. 2008. "Admixture Determines Genetic Diversity and Population Differentiation in the Biological Invasion of a Lizard Species." *Biology Letters* 4 (4): 434–7. https://doi.org/10.1098/rsbl.2008.0205.

Kolbe, Jason J., Brian R. Lavin, Russell L. Burke, Lorenzo Rugiero, Massimo Capula, and Luca Luiselli. 2013. "The Desire for Variety: Italian Wall Lizard (*Podarcis siculus*) Populations Introduced to the United States via the Pet Trade Are Derived from Multiple Native-Range Sources." *Biological Invasions* 15 (4): 775–83. https://doi.org/10.1007/s10530-012-0325-7.

Kraus, Fred. 2009. *Alien Reptiles and Amphibians: A Scientific Compendium and Analysis*. Springer.

Krysko, Kenneth L., Joseph P. Burgess, Michael R. Rochford, Christopher R. Gillette, Daniel Cueva, Kevin M. Enge, Louis A. Somma, Jennifer L. Stabile, Dustin C. Smith, Joseph A. Wasilewski, Guy N. Kieckhefer III, Michael C. Granatosky, and Stuart V. Nielsen. 2011. "Verified Non-indigenous Amphibians and Reptiles in Florida from 1863 Through 2010: Outlining the Invasion Process and Identifying Invasion Pathways and Stages." *Zootaxa* 3028 (1): 1–64. https://doi.org/10.11646/zootaxa.3028.1.1.

Kumar, Sudhir, Glen Stecher, and Koichiro Tamura. 2016. "MEGA7: Molecular Evolutionary Genetics Analysis Version 7.0 for Bigger Datasets." *Molecular Biology Evolution* 33 (7): 1870–4. https://doi.org/10.1093/molbev/msw054.

Leigh, Jessica W., and David Bryant. 2015. "Popart: Full-Feature Software for Haplotype Network Construction." *Methods in Ecology & Evolution* 6 (9): 1110–6. https://doi.org/10.1111/2041-210X.12410.

Lenk, P., and M. Wink. 1997. "A RNA/RNA Heteroduplex Cleavage Analysis to Detect Rare Mutations in Populations." *Molecular Ecology* 6 (7): 687–90. https://doi.org/10.1046/j.1365-294x.1997.00233.x.

Lever, Christopher. 2003. *Naturalized Reptiles and Amphibians of the World*. Oxford University Press. https://doi.org/10.1093/oso/9780198507710.001.0001.

Lombaert, Eric, Thomas Guillemaud, Jean-Marie Cornuet, Thibaut Malausa, Benoît Facon, and Arnaud Estoup. 2010. "Bridgehead Effect in the Worldwide Invasion of the Biocontrol Harlequin Ladybird." *PLOS One* 5 (3): e9743. https://doi.org/10.1371/journal.pone.0009743.

Losos, Jonathan B. 1996. "Dynamics of Range Expansion by Three Introduced Species of *Anolis* Lizards on Bermuda." *Journal of Herpetology* 30 (2): 204–10. https://doi.org/10.2307/1565511.

Losos, Jonathan B. 2009. *Lizards in an Evolutionary Tree: Ecology and Adaptive Radiation of Anoles. University of California Press*

Macedonia, Joseph, David Clark, and Andrew McIntosh. 2016. Differential Range Expansion and Habitat Use Among the Naturalized Anolis Lizards of Bermuda. *Herpetological Review 47*: 529–35.

Macey, J. Robert, Allan Larson, Natalia B. Ananjeva, and Theodore J. Papenfuss. 1997. "Evolutionary Shifts in Three Major Structural Features of the Mitochondrial Genome Among Iguanian Lizards." *Journal of Molecular Evolution* 44 (6): 660–74. https://doi.org/10.1007/pl00006190.

Mahoney, Peter J., Karen H. Beard, Andrew M. Durso, Aimee G. Tallian, A. Lexine Long, Ryan J. Kindermann, Nicole E. Nolan, Daniel Kinka, and Harrison E. Mohn. 2015. "Introduction Effort, Climate Matching and Species Traits as Predictors of Global Establishment Success in Non-native Reptiles." *Diversity & Distributions* 21 (1): 64–74. https://doi.org/10.1111/ddi.12240.

Malhotra, Anita, and Roger S. Thorpe. 2000. "The Dynamics of Natural Selection and Vicariance in the Dominican Anole: Patterns of Within-Island Molecular and Morphological Divergence." *Evolution; International Journal of Organic Evolution* 54 (1): 245–58. https://doi.org/10.1111/j.0014-3820.2000.tb00025.x.

Michaelides, Sozos, Geoffrey M. While, Celia Bell, and Tobias Uller. 2013. "Human Introductions Create Opportunities for Intraspecific Hybridization in an Alien Lizard." *Biological Invasions* 15 (5): 1101–12. https://doi.org/10.1007/s10530-012-0353-3.

Michaelides, Sozos N., Rachel M. Goodman, Ronald I. Crombie, and Jason J. Kolbe. 2018. "Independent Introductions and Sequential Founder Events Shape Genetic Differentiation and Diversity of the Invasive Green Anole (*Anolis carolinensis*) on Pacific Islands." *Diversity & Distributions* 24 (5): 666–79. https://doi.org/10.1111/ddi.12704.

Michaelides, Sozos N., Noah Gilbert, Brian E. Smith, Graham L. White, Adrian Hailey, and Jason J. Kolbe. 2019. "Genetic Reconstruction of the Invasion History of Anolis Wattsi in Trinidad with a Comment on the Importance of Ecological Similarity to Invasion Success." *Herpetological Journal (Volume 29, Number 3)*: 131–7. https://doi.org/10.33256/hj29.3.131137

Poe, Steven, Adrián Nieto-Montes de Oca, Omar Torres-Carvajal, Kevin De Queiroz, Julián A. Velasco, Brad Truett, Levi N. Gray, Mason J. Ryan, Gunther Köhler, Fernando Ayala-Varela, and Ian Latella. 2017. "A Phylogenetic, Biogeographic, and Taxonomic Study of All Extant Species of Anolis (Squamata; Iguanidae)." *Systematic Biology* 66 (5): 663–97. https://doi.org/10.1093/sysbio/syx029.

Reynolds, Robert Graham, Jason J. Kolbe, Richard E. Glor, Marta López-Darias, C. Verónica Gómez Pourroy, Alexis S. Harrison, Kevin de Queiroz, Liam J. Revell, and Jonathan B. Losos. 2020. "Phylogeographic and Phenotypic Outcomes of Brown Anole Colonization Across the Caribbean Provide Insight into the Beginning Stages of an Adaptive Radiation." *Journal of Evolutionary Biology* 33 (4): 468–94. https://doi.org/10.1111/jeb.13581.

Rollins, Lee Ann, Andrew P. Woolnough, Ron Sinclair, Nick J. Mooney, and William B. Sherwin. 2011. "Mitochondrial DNA Offers Unique Insights into Invasion History of the Common Starling." *Molecular Ecology* 20 (11): 2307–17. https://doi.org/10.1111/j.1365-294X.2011.05101.x.

Rozas, Julio, Albert Ferrer-Mata, Juan Carlos Sánchez-DelBarrio, Sara Guirao-Rico, Pablo Librado, Sebastián E. Ramos-Onsins, and Alejandro Sánchez-Gracia. 2017. "DnaSP 6: DNA Sequence Polymorphism Analysis of Large Data Sets." *Molecular Biology Evolution* 34 (12): 3299–302. https://doi.org/10.1093/molbev/msx248.

Sax, Dov F., Steven D. Gaines, and James H. Brown. 2002. "Species Invasions Exceed Extinctions on Islands Worldwide: A Comparative Study of Plants and Birds." *American Naturalist* 160 (6): 766–83. https://doi.org/10.1086/343877.

Shanmuganathan, T., J. Pallister, S. Doody, H. McCallum, T. Robinson, A. Sheppard, C. Hardy, D. Halliday, D. Venables, R. Voysey, T. Strive, L. Hinds, and A. Hyatt. 2010. "Biological Control of the Cane Toad in Australia: A Review." *Animal Conservation 13 (s1)*: 16–23. https://doi.org/10.1111/j.1469-1795.2009.00319.x.

Street, Sally E., Jorge S. Gutiérrez, William L. Allen, and Isabella Capellini. 2023. "Human Activities Favour Prolific Life Histories in Both Traded and Introduced Vertebrates." *Nature Communications* 14 (1): 262. https://doi.org/10.1038/s41467-022-35765-6.

Stroud, James T., Mark E. Outerbridge, and Sean T. Giery. 2016. "First Specimen of an American Green Anole (*Anolis carolinensis*) on the Oceanic Island of Bermuda, with a Review of the Species' Current Global Distribution." *Reptiles & Amphibians* 23 (3): 188–90. https://doi.org/10.17161/randa.v23i3.14134.

Stroud, James T., Sean T. Giery, and Mark E. Outerbridge. 2017. "Establishment of *Anolis sagrei* on Bermuda Represents a Novel Ecological Threat to Critically Endangered Bermuda Skinks (*Plestiodon longirostris*)." *Biological Invasions* 19 (6): 1723–31. https://doi.org/10.1007/s10530-017-1389-1.

Stroud, James T., Sean T. Giery, Mark E. Outerbridge, and K. J. Feeley. 2019. "Ecological Character Displacement Alters the Outcome of Priority Effects During Community Assembly." *Ecology* 100 (8): e02727. https://doi.org/10.1002/ecy.2727.

Taylor, Douglas R., and Stephen R. Keller. 2007. "Historical Range Expansion Determines the Phylogenetic Diversity Introduced During Contemporary Species Invasion." *Evolution; International Journal of Organic Evolution* 61 (2): 334–45. https://doi.org/10.1111/j.1558-5646.2007.00037.x.

Thorpe, Roger S., and Andrew G. Stenson. 2003. "Phylogeny, Paraphyly and Ecological Adaptation of the Colour and Pattern in the *Anolis roquet* Complex on Martinique." *Molecular Ecology* 12 (1): 117–32. https://doi.org/10.1046/j.1365-294x.2003.01704.x.

Thorpe, Roger S., D. L. Leadbeater, and C. E. Pook. 2005. "Molecular Clocks and Geological Dates: Cytochrome b of *Anolis extremus* Substantially Contradicts Dating of Barbados Emergence." *Molecular Ecology* 14 (7): 2087–96. https://doi.org/10.1111/j.1365-294X.2005.02574.x.

Thorpe, Roger S., Yann Surget-Groba, and Helena Johansson. 2008. "The Relative Importance of Ecology and Geographic Isolation for Speciation in Anoles." *Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences* 363 (1506): 3071–81. https://doi.org/10.1098/rstb.2008.0077.

Thorpe, Roger S., Axel Barlow, Anita Malhotra, and Yann Surget-Groba. 2015. "Widespread Parallel Population Adaptation to Climate Variation Across a Radiation: Implications for Adaptation to Climate Change." *Molecular Ecology* 24 (5): 1019–30. https://doi.org/10.1111/mec.13093.

Uller, Tobias, and Roosa Leimu. 2011. "Founder Events Predict Changes in Genetic Diversity During Human-Mediated Range Expansions." *Global Change Biology* 17 (11): 3478–85. https://doi.org/10.1111/j.1365-2486.2011.02509.x.

Williams, Ernest E. 1969. "The Ecology of Colonization as Seen in the Zoogeography of Anoline Lizards on Small Islands." *Quarterly Review of Biology* 44 (4): 345–89. https://doi.org/10.1086/406245.

Wingate, D. 1965. "Terrestrial Herpetofauna of Bermuda." *Herpetologica* 21: 199–219.

Wink, Michael 1995. "Phylogeny of Old and New World Vultures (Aves: Accipitridae and Cathartidae) Inferred from Nucleotide Sequences of the Mitochondrial Cytochrome b Gene." *Zeitschrift Fur Naturforschung. C, Journal of Biosciences 50 (11–12)*: 868–82. https://doi.org/10.1515/znc-1995-11-1220.

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Supplementary Data

Supplementary data associated with this article can be found online alongside the manuscript.