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Brief Communication

Phylogeography of the Endangered Lesser Antillean Iguana, *Iguana Delicatissima*: A Recent Diaspora in an Archipelago Known for Ancient Herpetological Endemism

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Abstract

Iguana delicatissima is an endangered endemic of the Lesser Antilles in the Caribbean. Phylogeographic analyses for many terrestrial vertebrate species in the Caribbean, particularly lizards, suggest ancient divergence times. Often, the closest relatives of species are found on the same island, indicating that colonization rates are so low that speciation on islands is often more likely to generate biodiversity than subsequent colonization events. Mitochondrial sequence analysis of the region spanning *ND4* was performed on *I. delicatissima* individuals from islands across the species' range to estimate genetic divergence among geographically isolated populations. Five unique haplotypes were recovered from 46 individuals. The majority of animals carry a single common haplotype. Two of the haplotypes were only present in individuals classified as hybrids from Îles des Saintes. The final 2 haplotypes, single nucleotide substitutions, were present in animals from Îlet Chancel of Martinique and Saint Barthélemy, respectively. Despite the great distances between islands and habitat heterogeneity within islands, this species is characterized by low haplotype diversity. The low mtDNA variation of *I. delicatissima* suggests a single colonization coupled with rapid range expansion, potentially hastened by human-mediated dispersal.

Subject areas: Population structure and phylogeography; Conservation genetics and biodiversity
Key words: Archipelago, Conservation, *Iguana delicatissima*, Mitochondrial DNA

A phylogeographic study of Lesser Antillean iguanas, *Iguana delicatissima*, was conducted to address key aspects of the region's herpetological biogeography and to provide an intraspecific phylogeny that could inform conservation management planning. As they are the most diverse terrestrial vertebrates on islands, reptiles and amphibians have played a central role in studies of island biogeography (Ota 1998; Ricklefs and Bermingham 2008). Molecular systematics coupled with a detailed understanding of species' geographic

distributions has offered insights into common modes of speciation and elucidates the timing and patterns of colonization on islands and in archipelagos (Vences et al. 2003; Camargo et al. 2010).

The herpetofauna of island systems is characterized by a high degree of endemism (Wallace 1881; Kier et al. 2009). The origins of species complexes can often be traced to 1 or 2 colonization events followed by adaptive radiation (Jackman et al. 1997). In many cases, species occupying different niches on the same island are more closely related to each other than species occupying similar niches on different yet geographically proximate islands (Calsbeek et al. 2006; Losos 2009). These findings have led biogeographers to infer that over-water dispersal by terrestrial vertebrates is rare, or rarely results in successful colonization. In fact, few species of endemic herpetofauna have broad distributions (Bisconti et al. 2001; Diesmos et al. 2002; Powell and Henderson 2012). Phylogeographic studies of reptiles across island chains also typically suggest low rates of over-water dispersal followed by high rates of diversification and speciation in isolation (Amer and Kumazawa 2008; Cox et al. 2010; Townsend et al. 2011; Linkem et al. 2013).

Iguana delicatissima resides in the Lesser Antilles, a geologically unique island chain between South America and the Greater Antilles (Figure 1) representing 2 volcanic arcs formed during the Cenozoic by the subduction of the Atlantic Plate beneath the Caribbean Plate, with the present period of volcanic activity beginning in the early Miocene (20 mya) (Macdonald et al. 2000). Based on what is known of the island chain's origin, none of these islands have ever been

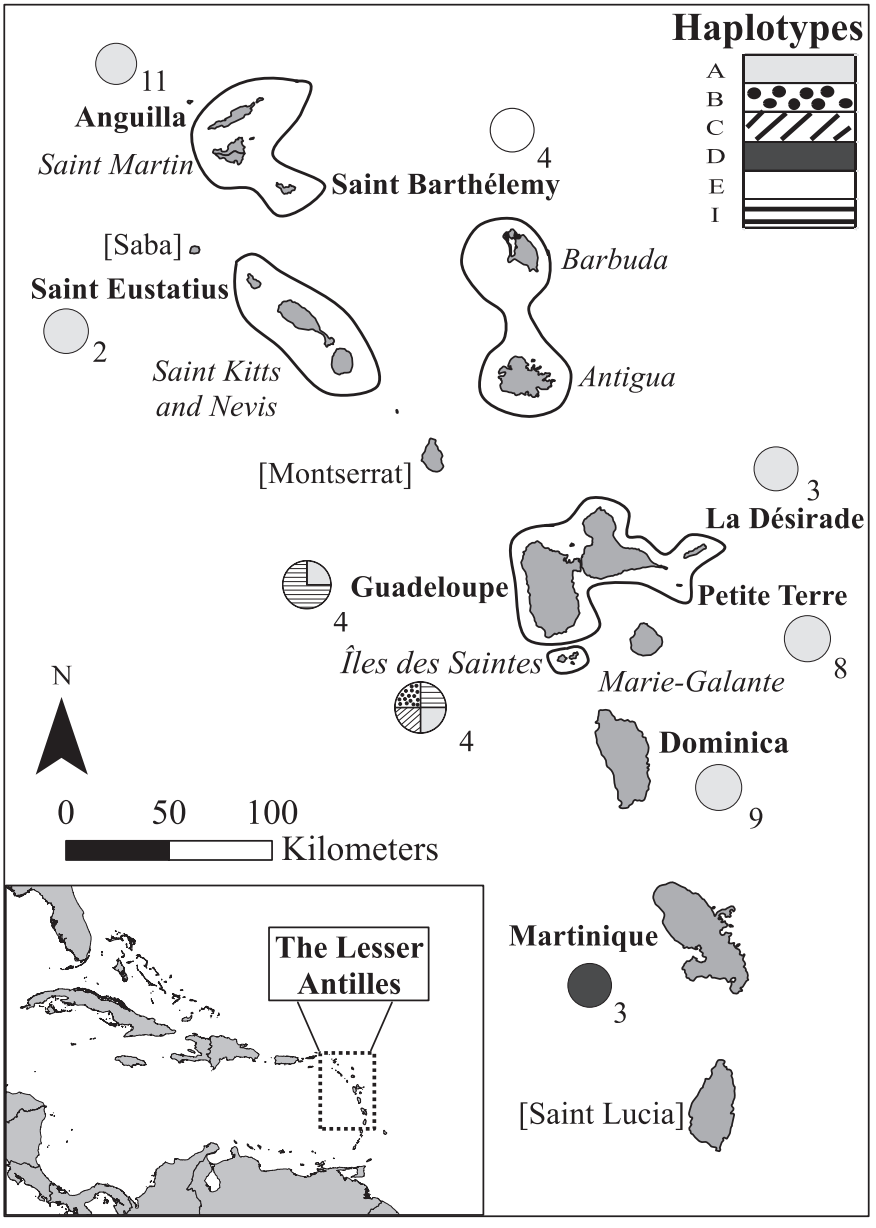

Figure 1. Map of the range of the Lesser Antillean iguana. Circled island groups represent islands that were likely connected during the last glacial maxima (Ricklefs and Bermingham 2008). Island names in bold represent islands currently inhabited by *I. delicatissima*, names in italics represent islands where the species is now extinct, and names in brackets are islands where the species is currently not known to have existed. The pie charts represent the percentage of individuals with a certain haplotype from the sampled islands, and the numbers below the pie charts indicate the sample size for the island (see Table 1 for more detailed information on sampling and haplotypes).

connected to a mainland, and most of these islands appear to have been isolated for their entire histories (Ricklefs and Bermingham 2008). Certain island groups, however, may have been connected due to temporary drops in sea level including that associated with the last glacial maximum (Figure 1; Ricklefs and Bermingham 2008).

The Lesser Antillean herpetofauna are also typified by very limited geographic ranges (Powell and Henderson 2012). Phylogenetic patterns of these taxa are also consistent with low colonization rates coupled with high rates of diversification in isolation (Hedges 1996). For example, studies of Lesser Antillean *Anolis* lizards have uncovered high haplotype diversity on multiple occasions, demonstrating variation both among islands and among populations on the same island (Stenson et al. 2004). Divergence in many Lesser Antillean reptiles reflects not only diversification in isolation following over-water dispersal, but also adaptation to unique ecological pressures within each island (Thorpe and Stenson 2003).

Despite the pervasive evidence that successful over-water dispersal is rare, some native reptiles and amphibians of the Lesser Antilles do have broader ranges. Notable among these taxa is the Lesser Antillean iguana, *I. delicatissima*, the largest terrestrial vertebrate native to these islands with a range spanning from Martinique in the south to Anguilla in the north (Figure 1; Lazell 1973; Knapp 2007). It seems unlikely that insular populations of *I. delicatissima* are highly divergent as the morphology of *I. delicatissima* is generally conserved across its range and no subspecies are recognized (Day et al. 2000). Further, a previous study utilizing a mitochondrial locus in *I. delicatissima* on 3 of the inhabited islands revealed no genetic variation among these islands (Malone et al. 2000; Stephen et al. 2013). However, in the absence of molecular data from across the species' range, the presence of cryptic species cannot be ruled out.

Understanding the evolutionary history of *I. delicatissima* is also now critical for informing conservation management planning. Since the original description of the species by Laurenti (1768), habitat fragmentation and hybridization concerns have contributed to *I. delicatissima*'s conservation status being increased to Endangered according to the criteria of the International Union for the Conservation of Nature (IUCN) Red List of Threatened Species (Breuil et al. 2010). Populations have been recently extirpated from the islands of Saint Kitts and Nevis, Antigua, Barbuda, Les Îles des Saintes, Marie-Galante, Grande-Terre of Guadeloupe, and Saint-Martin (Figure 1; Breuil et al. 2010). As the largest terrestrial vertebrate native to these islands, this species is not only iconic, but also likely plays an important ecological role through burrowing, repetitive plant cropping, and seed dispersal (Day et al. 2000). Using mitochondrial DNA sequence data from across the range of *I. delicatissima*, we hope to better understand the phylogeographic history of this species.

Materials and Methods

Sampling

Iguana delicatissima samples of tail tissue or blood were collected from the islands of Saint Barthélemy, Saint Eustatius, Anguilla, Îles de la Petite Terre (Terre-de-Bas and Terre-de-Haut), La Désirade, Guadeloupe (Basse-Terre), Îles des Saintes (Terre-de-Bas and Terre-de-Haut), Dominica, and Îlet Chancel of Martinique. When possible, tissues collected from different populations across each island were used to maximize the geographic range sampled. *I. iguana* tissue samples were collected from Îles des Saintes (Terre-de-Bas and Terre-de-Haut). Many samples from Îles des Saintes were identified as possible hybrids between *I. delicatissima* and *I. iguana* based on scale counts in the head region, including the presence of a subtympanic scale, and the number of gular spikes (Table 1). Samples of tail tissue collected by Bangor University in 1992 and 1993 were preserved in tubes with 95% ethanol and stored at 4 °C. Blood samples were collected from Dominica in the summer months of 2007 and 2008 and Anguilla in 1997. Blood samples were collected by venipuncture of the ventral coccygeal vein using a heparinized syringe, and the blood was stored in vacutainer tubes with 100 mM Tris, 100 mM Na_2 EDTA, 10 mM NaCl, and 1% SDS at a ratio of 1:2. These samples have been stored at either 4 °C or −80 °C.

Data Collection and Analysis

DNA was extracted from blood and tissue using an ABI PRISM™-6100 Nucleic Acid Prep Station (Applied Biosystems, Foster City, CA) or a Maxwell® 16 Nucleic Acid Extraction System with a Maxwell® 16 Tissue DNA Purification Kit (Promega™). A 903 bp region of the mitochondrial genome has been used for multiple studies involving *Cyclura* (Malone et al. 2000; Bryan et al.

Table 1. Sampling and haplotype information

Species	Island	Collector	*n*	Haplotype ID	GenBank Accession No.
Iguana delicatissima	Anguilla	Glenn Gerber	11	delicatissima_A	KJ561221; AF217783[c]
	Dominica	Charles Knapp	8	delicatissima_A	
		Bangor University	1	delicatissima_A	
	Guadeloupe	Bangor University	1	delicatissima_A	
	La Désirade	Bangor University	3	delicatissima_A	
	St. Eustatius	Bangor University	2	delicatissima_A	
	Petite Terre	Bangor University	8	delicatissima_A	
	Îles des Saintes	Bangor University	1[a]	delicatissima_A	
			1[a]	delicatissima_B	KJ561222
			1[a]	delicatissima_C	KJ561223
	Martinique	Bangor University	3	delicatissima_D	KJ561224
	St. Barthélemy	Bangor University	4	delicatissima_E	KJ561225
Iguana iguana	Guadeloupe	Bangor University	3[b]	iguana_I	KJ561226
	Îles des Saintes	Bangor University	1	iguana_I	

[a]Denotes individuals that were identified by morphology as hybrids of *Iguana delicatissima* and *Iguana iguana*

[b]Indicates individuals that were identified by morphology as *Iguana delicatissima* but shared an *Iguana iguana* haplotype.

[c]Indicates the sequence identified in *I. delicatissima* individuals from the studies of Malone et al. (2000) and Stephen et al. (2013) that aligns with haplotype A in this study. As the sequence from this study is shorter than the sequence identified by Malone et al. (2000), both GenBank Accession numbers are listed.

2007) and *Iguana* (Stephen et al. 2013) systematics, and these previous studies have used the ND4 and LEU primers of Arévalo et al. (1994) for amplification. Two primers, CcarND (5′ ACG GAA TCA TCC GAA TTA CC 3′) and CcarLeu (5′ TTA AAA GTG AGG GGT CTG AGG A 3′), were developed to amplify an 801 bp region within the original 903 bp region for ease of use in *Cyclura carinata* (Welch et al. in prep). We utilized these *carinata*-specific primers to amplify this mitochondrial sequence in *I. delicatissima* and *I. iguana*. The polymerase chain reaction (PCR) amplification protocol of mtDNA for samples with a high DNA concentration (>1 ng/μL) consisted of a touchdown cycle (Don et al. 1991) with a denaturing temperature of 94 °C for 5 min and then 10 cycles beginning with 30 s at 94 °C, 65 °C for annealing, and 45 s at 72 °C for elongation, with the annealing temperature decreasing by 1 °C each of the 10 cycles to 55 °C. This was followed by 25 cycles with an annealing temperature of 55 °C and a final elongation period of 7 min at 72 °C. Amplification of low mtDNA concentration samples (<1 ng/μL) was achieved using a denaturing temperature of 95 °C for 1 min followed by 35 cycles consisting of 15 s at 95 °C, 15 s at 50 °C for annealing, and 45 s at 72 °C for elongation, with a final elongation period of 7 min at 72 °C. Sequence amplification was verified on a 1% agarose gel. All extraction and amplification was performed at Mississippi State University, and PCR product was shipped for mtDNA sequencing at Arizona State University. The ND4 mitochondrial region was sequenced in both directions, and primer sequences were trimmed out before final sequence alignment. Sequencher® (Gene Codes Corp., Ann Arbor, MI) was utilized to align and edit individual mtDNA sequences and a simple network was constructed using parsimony to represent the mtDNA haplotypes in Network 4.6.1.1 (Fluxus Technology Ltd.). In fulfillment of data archiving guidelines (Baker 2013), we have deposited the primary data underlying our analyses with GenBank (Table 1). Several tests of neutral sequence evolution were employed. Tajima's *D* (Tajima 1989), Fu's F_S (Fu 1997), R_2 (Ramos-Onsins and Rozas 2002), and Fu and Li's *D** and *F** (Fu and Li 1993) were calculated using DnaSP Version 5 (Librado and Rozas 2009). Significance of these values was determined using 10 000 coalescent simulations in accordance with the recommended parameters for the software.

Results

After editing the 801 bp mtDNA sequence, 689 bp were utilized in the final analysis. A total of 47 individuals (including one *I. iguana* individual) from across *Iguana delicatissima's* range were sequenced at the mtDNA ND4 region (Table 1). With only a few polymorphisms distinguishing 5 *I. delicatissima* haplotypes, a simple network was constructed to depict their relationships (Figure 2). 76 polymorphic sites were identified that distinguish the single *I. iguana* haplotype identified in this study, Haplotype I, from the most common *I. delicatissima* haplotype, Haplotype A. These polymorphic sites include 11 transversions and 65 transitions. The mtDNA haplotypes associated with *I. delicatissima* exhibit less than 0.3% sequence divergence. One substitution distinguishes Haplotype E, found in all 4 individuals from Saint Barthélemy. Another single transition distinguishes Haplotype D, which was present in all 3 Martinique individuals. Finally, one unique transition was present in a morphologically hybrid individual from Terre-de-Bas des Saintes (Haplotype C), and 2 separate unique polymorphisms, both transitions, were found in a single morphologically hybrid individual from the same island (Haplotype B). Upon examining the sequences of Guadeloupe individuals, 3 of the 4 individuals identified by morphology as

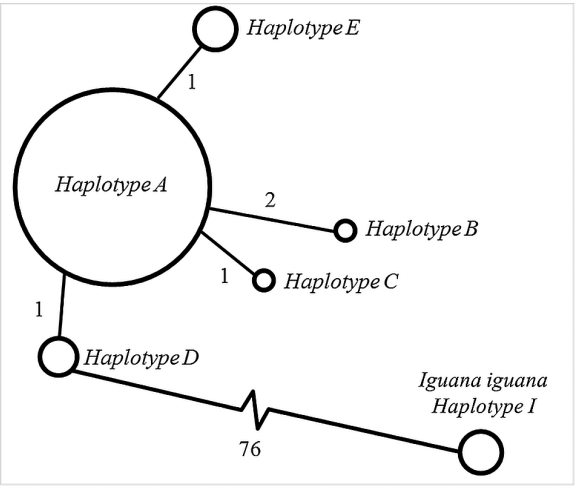

Figure 2. Parsimony network of haplotypes identified in this study. The numbers represent the single nucleotide polymorphisms that distinguish the haplotypes, and the haplotype names correspond to those found in Table 1 and Figure 1. The sizes of the circles are proportional to the number of individuals sampled with the identified haplotype.

I. delicatissima during sampling on Basse-Terre all shared an *I. iguana* haplotype. The remaining *I. delicatissima* individuals from Anguilla, Dominica, St. Eustatius, La Désirade, Guadeloupe, and Îles de la Petite Terre all shared one haplotype, Haplotype A. Tajima's *D* (–1.6187, $P = 0.037$) and Fu and Li's *F** (–1.9493, $P = 0.043$) were both negative and significant. No other tests revealed statistically significant departures from neutrality (Fu's $F_S = -2.696$, $P = 0.07$; $R_2 = 0.0728$, $P = 0.13$; Fu and Li's $D^* = -1.6913$, $P = 0.12$).

Discussion

The lack of unique haplotypes across the majority of the range of *I. delicatissima* suggests that populations of the species have not been isolated from each other for an extended period of time. Genetic similarities imply that the species may have spread via recent over-water dispersal rather than having been isolated following ancient dispersal events or a vicariance event of the proto-Antilles (Hedges 1996). This lack of genetic structure among *I. delicatissima* populations contrasts starkly with the high degree of island endemism observed for most terrestrial taxa of the Lesser Antillean herpetofauna. For example, a study concerning the phylogenetic relationships of the Lesser Antillean *Anolis bimaculatus* group used 2 mitochondrial DNA regions, cytochrome *b* (cyt*b*) and cytochrome oxidase I, and uncovered 396 polymorphisms in 1005 base pairs (Stenson et al. 2004). Haplotypes were unique both across islands and within island populations, suggesting diversification in isolation has been occurring within the genus for millions of years (Stenson et al. 2004). Additionally, a study of Lesser Antillean geckos, *Sphaerodactylus vincenti*, revealed 47 unique haplotypes in the cyt*b* mtDNA region in 53 individuals sampled from across the range of the species complex (Surget-Groba and Thorpe 2013). Both of these studies of Lesser Antillean reptile phylogeography are consistent with limited over-water dispersal. Further, the overall lack of haplotype diversity across the range of *I. delicatissima* is inconsistent with the general pattern observed for other iguanids of the Caribbean (Malone et al. 2000; Malone and Davis 2004). One possible explanation for the unique pattern of low haplotype heterogeneity observed in our study is a rapid range expansion in *I. delicatissima*. Significant negative

values of neutrality statistics suggest either population expansion or selection on the mitochondrial genome (Fahey et al. 2014). Tajima's *D* and Fu and Li's *F** were found to be both negative and significant. Fu and Li's *D** and Fu's F_S, though not significant, were also negative. These findings are consistent with either a recent population expansion or selection. Range expansion may have even been aided by human-mediated dispersal as Amerindians colonized these islands 6000 years ago (Ricklefs and Bermingham 2008). Alternatively, the lack of haplotypes may reflect mitochondrial mutation rate heterogeneity in *I. delicatissima* relative to its iguanid counterparts or a selective sweep of the mitochondrial genome.

The hypothesis that *I. delicatissima* recently underwent a rapid range expansion requires frequent over-water dispersal followed by successful colonization. While this is inconsistent with the biogeography of the Lesser Antillean herpetofauna, the ecology of *I. delicatissima* is distinct from other Lesser Antillean herpetofauna in ways that might enhance its likelihood of over water dispersal. The species is arboreal and typically associated with coastal habitats, which increases the incidence of iguanas being swept into the water during storms or beach erosion (Day et al. 2000). Additionally, iguanas have larger bodies than other native, terrestrial vertebrates and can inflate their body cavities (Iverson 1979). These traits should enhance salt tolerance and keep iguanas afloat longer, especially when compared to their amphibian counterparts. Further, these iguanas can rid their bodies of excess salts due to specialized glands, unlike other lizards that lack salt glands such as the Gekkonidae (Hazard 2004). Finally, the generalist herbivore diet and large clutch sizes could facilitate the colonization of the species following dispersal (Breuil et al. 2010). In fact, there are records of sweepstakes routes in iguanids, including accounts of *I. iguana* individuals blown off their respective islands by hurricanes and floating hundreds of kilometers to other islands (Censky et al. 1998). The potential frequency of these dispersal events suggests that individuals from various origins might regularly establish populations. If true, these frequent migration events would prevent genetic divergence between populations.

Human activities may have contributed to a rapid range expansion of *I. delicatissima*. Similar to *I. delicatissima*, there are a few examples of reptiles and amphibians in island chains that display a broader geographic range or low haplotype divergence. A common element uniting a majority of these cases is the role of human-mediated dispersal. A study of the cyt*b* mitochondrial locus in the 2 extant populations of a West Indian frog, *Leptodactylus fallax*, revealed no sequence divergence despite the 2 populations never having been connected by land (Hedges and Heinicke 2007). It was concluded that one or both of the extant populations are a result of intentional human introduction because this species is a known food source for Amerindians (Hedges and Heinicke 2007). Another Caribbean frog, *Eleutherodactylus johnstonei*, is widely distributed across the Eastern Caribbean and boasts the largest range of any herpetofauna in the Lesser Antilles despite an endemic status (Kaiser 1997). This range expansion has historically followed human expansion in the Lesser Antilles, as this species is known to adapt well to fragmented landscapes following the human-mediated destruction of native habitat (Kaiser 1997). *Iguana delicatissima* may have been moved by native peoples to provide a food source during their travels, and such movement could have caused a rapid range expansion across the Lesser Antilles. Translocation of animals by Amerindians would also serve to enhance gene flow among islands. Generalist species would be more likely to succeed in human-mediated colonization, as with *E. johnstonei* (Kaiser 1997). However, *I. delicatissima* is sensitive to habitat degradation and fragmentation. Much of the decline of the species has been attributed to the changing of landscapes by invasive species and human development (Malhotra et al. 2007), particularly the disturbance of coastal nesting habitat (Powell and Henderson 2005). For example, the presence of an oil storage facility on St. Eustatius has led to a decrease in available nesting habitat, which is likely contributing to the rapid decline of *I. delicatissima* on the island (Debrot et al. 2013). These aspects of *I. delicatissima*'s ecology suggest that although human-mediated dispersal may have contributed to the species' wide range and lack of haplotype diversity, it is unlikely that it is the only factor involved in the biogeographic history of the species.

Many taxa show mitochondrial mutation rate heterogeneity, and this may be possible in the genus *Iguana, with I. delicatissima* exhibiting reduced mitochondrial mutation rates when compared to other iguanid species. However, the ND4 region has been utilized in many studies of iguanid phylogeography, and the lack of haplotype diversity displayed in *I. delicatissima* is distinct from that observed in other iguanas of the West Indies. A study of the ND4 mtDNA region in *I. iguana* populations from the Lesser Antilles uncovered 2 haplotypes with 1.7% sequence divergence between the 2 haplotypes, which is much greater than the divergence observed in *I. delicatissima* (Stephen et al. 2013). As this species is sister to *I. delicatissima*, it is very unlikely that mitochondrial mutation rate heterogeneity explains the lack of haplotype diversity at the ND4 region in *I. delicatissima*. Alternatively, species with low mtDNA haplotype diversity may have experienced a mitochondrial selective sweep that fixes a single common haplotype across a large range (Maynard-Smith and Haigh 1974). Two excellent examples of species with a common mtDNA haplotype present over a large area are the Moorish gecko, *Tarentola mauritanica*, and the Mediterranean house gecko, *Hemidactylus turcicus* (Rato et al. 2010; Rato et al. 2011). In both cases, a comparison of nuclear and mitochondrial markers revealed that the mitochondrial genome was under strong selective pressure, which was contributing to low haplotype variation across a large portion of the species' range. The values for Tajima's *D* and Fu and Li's *F** were negative and significant in our study, which could suggest selection on the mitochondrial genome (Fahey et al. 2014). Fu and Li's *F** and *D** are particularly sensitive to background selection, and the significant value for Fu and Li's *F** could suggest selection rather than population expansion (Fu 1997). However, a previous study using 7 *I. delicatissima* individuals from 3 islands found no haplotype divergence at 3 nuclear loci (Stephen et al. 2013). The similarity between haplotype diversity in nuclear and mitochondrial markers of *I. delicatissima* suggests that the mtDNA is not under a stronger selective pressure and that a rapid population expansion is the more likely explanation for the lack of haplotype diversity.

From a conservation perspective, the greatest threats to *I. delicatissima* may come from the common Green Iguana, *I. iguana*. The unique haplotypes represented in the morphologically hybrid individuals sampled from Îles des Saintes and the presence of the *I. iguana* haplotype from Îles des Saintes in the 3 individuals identified as *I. delicatissima* in Guadeloupe are evidence for recent or historic hybridization between the 2 species. Although hybridization is occurring, based on mtDNA haplotypes the 2 species are quite distinct. The 2 hybridizing species have a much greater pair-wise divergence at mitochondrial and nuclear loci than other sister species of iguanids (*Conolophus* and *Cyclura*) and even nonsister *Sauromalus* (Stephen et al. 2013). It would be beneficial to investigate whether these species have historically coexisted in the Lesser Antilles. The presence of *I. iguana* haplotypes in *I. delicatissima* provides evidence

of gene flow between the species, suggesting that *I. delicatissima* may persist in spite of historic hybridization.

The lack of varying haplotypes suggests that *I. delicatissima* displays a unique phylogenetic history not shared by other Lesser Antillean reptiles. The mtDNA sequences suggest that morphological consistency across islands reflects a recent common ancestry, and that cryptic species or subspecies are not likely present. Translocation may be an effective way of restoring populations of the species on islands where they have been extirpated following the mitigation of pressures credited with the loss of original populations. The populations on Îlet Chancel of Martinique and Saint Barthélemy, however, carry unique haplotypes, and hence may deserve special concern when planning for the conservation of *I. delicatissima*. Further detailed analysis of individual populations using variable nuclear markers may reveal a different geographic pattern of relatedness, or it may confirm a recent common ancestry of *I. delicatissima* throughout its range as the mtDNA sequence variation suggests.

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