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RESEARCH ARTICLE

# Genetic structure of Rhinoceros Rock Iguanas, *Cyclura cornuta*, in the Dominican Republic, with insights into the impact of captive facilities and the taxonomic status of *Cyclura* on Mona Island

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**Abstract**
Hispaniola is the second largest island in the Caribbean and harbors an extensive amount of biodiversity. The geologic history and resulting complex topography of the island has led to significant differentiation across various taxonomic groups. Hispaniola is the only Caribbean Island with two species of Rock Iguanas, genus *Cyclura*. Rhinoceros Rock Iguanas (*C. cornuta*) are wide-ranging across Hispaniola, occurring in isolated pockets, primarily in low elevation xeric areas. To better understand the population structure of this species, we used a combination of mtDNA and nuclear markers to elucidate the genetic variation of wild populations across 13 sampling regions in the Dominican Republic (DR), as well as neighboring Mona Island, home to a *Cyclura* population of uncertain taxonomic status. Further, we evaluate the origin of iguanas in captive facilities throughout the DR. Our data reveal a high degree of genetic diversity across wild populations within the DR and shed light on the taxonomic status of the Mona island population. Further, novel genetic diversity is found in captive facilities, most likely resulting from interbreeding between individuals from genetically distinct populations within the captive facilities. Our results suggest that the captive facilities may pose a threat to wild populations and increased regulation of these facilities is needed.

**Keywords** Captive breeding · Dominican Republic · Endangered species · Iguanas · Population genetics

## Introduction

The continuous and detrimental influx of human activities on natural habitats demands conservation actions that integrate population genetics and evolutionary biology into management decisions (Ralls et al. 2017). To address population decline and fragmentation with effective management strategies, accurate information regarding population genetic structure, gene flow, geographic barriers, and time in isolation is required. However, this information is often lacking and predicting patterns of local adaptation, inbreeding depression, and/or outbreeding depression is increasingly difficult in long-lived organisms (Taylor et al. 2017), making the selection of appropriate conservation strategies daunting. Assessing intraspecific variation remaining in the wild and characterizing population differences should be among the primary goals for conservation geneticists interested in contributing to management decisions (Waples 1998; Avise 2005; Pasachnik et al. 2011).

Populations ranging over large areas are likely to present significant genetic and morphological variation. This differentiation frequently correlates with the presence of conspicuous barriers to gene flow and migration, such as mountains, waterways, or simply patches of unsuitable habitat. These patterns of differentiation are not only expected on continental mainland but also within large islands, especially when geographic barriers were historically present.

Hispaniola is the second largest island in the Greater Antilles and ranks second in Caribbean biodiversity (Ministerio de Medio Ambiente y Recursos Naturales 2014). The

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island was formed when two paleo-island blocks collided in the mid-Miocene, ca. 10 mya. Major orogenic events ceased during this time creating the stable island topography known today (Mann et al. 1991; Iturralde-Vinent and MacPhee 1999). Hispaniola has five major mountain ranges covering most of the island and creating an extensive system of natural barriers (Fig. 1, Nairn and Stehli 1975). Further, low elevation areas, most notably the Enriquillo Basin, at the north and south paleo-island boundary, continued to submerge multiple times until the late Pleistocene (Maurasse 1982; Graham 2003), serving as barriers to dispersal for terrestrial fauna (Gifford et al. 2004; Gifford and Larson 2008; Glor and Warren 2011; Brace et al. 2012). These geographic barriers have throughout time contributed to the distribution of genetic variation in reptiles, such as *Ameiva chrysolaema* (now *Pholidoscelis chrysolaema*) (Gifford and Larson 2008; Gifford et al. 2004), *Chilabothrus striatus* (Reynolds et al. in prep), and *Cyclura ricordii* (Carreras De León et al. 2019), as well as other vertebrates (Townsend et al. 2007; Sly et al. 2011; Brace et al. 2012).

*Cyclura cornuta* is one of two species of Rock Iguanas endemic to Hispaniola. This species has a widespread distribution across the island but is primarily found in low elevation tropical dry forest habitats, and never on high elevation mountains. Recent surveys indicate that this species may be much less stable than previously thought (Pasachnik and Carreras De León 2014), and an updated IUCN Red List Assessment supports its current Endangered status (Pasachnik and Carreras De León 2019). The primary threats to this species are habitat degradation, invasive species, and harvesting for human use (Rupp et al. 2009; Rupp and León 2009). More recently Pasachnik and Carreras-De León (2014) identified iguanarios, or captive facilities, as a novel potential threat to *C. cornuta* within the Dominican Republic (DR).

These captive facilities range from small (1–5 individuals) to large scale (> 100 individuals) and are distributed across the DR with greatest prevalence in the eastern portion of the country (Pasachnik and Carreras De León 2014). The first iguanario, Manati Park, was created in 1997 to help alleviate an overabundance of confiscated iguanas at the National Zoo in Santo Domingo, DR (ZooDom). In the 1990's it was also believed that iguanarios could serve as education centers and breeding source populations, in time supplementing wild populations (Powell et al. 2002). Augmenting declining populations with individuals bred in captive or semi-captive conditions is a conservation strategy that has been widely used (Grativol et al. 2001; Searcy et al. 2009; Frankham et al. 2010; Grant and Hudson 2015). However, in order for these strategies to be successful, detailed information concerning genetics and distribution must be used to create a long-term management plan (Frankham et al. 2010). In the case of iguanarios in the DR, little to no record keeping has occurred, animals are not given a unique identifier, the movement of individuals between facilities is not recorded, reproduction is not managed, and there is no

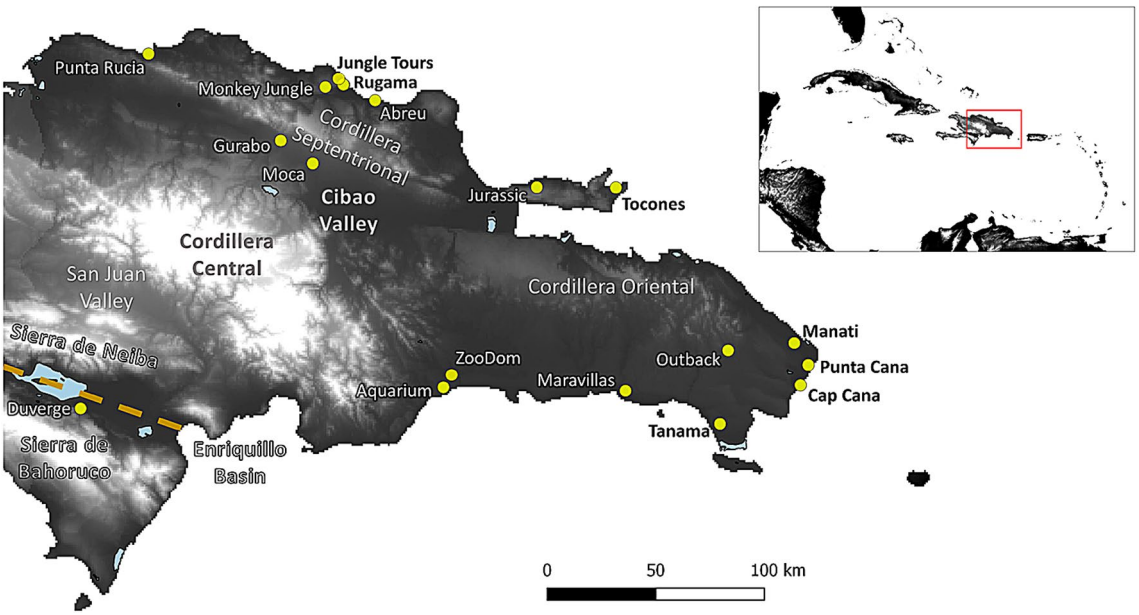


**Fig. 1** Notable geographic features (mountains and valleys) in the Dominican Republic. Topology is represented with lighter shades indicating higher elevation. Blue indicates water areas. The dashed brown line represents the historical collision site between the northern and southern paleo-islands, forming Hispaniola. The location of 18 sampled iguanarios within the Dominican Republic is represented with yellow dots

overarching plan by which the facilities function together. Breeding has been extremely successful in the larger facilities and most now have an overabundance of iguanas. Further, growing tourist interest in these sites has triggered the creation of additional iguanarios in the southeastern portion of the country, where tourism is most prominent. The condition in many of these facilities is very poor, due to overcrowding and a lack of proper husbandry knowledge. As a rapid solution, iguanas are often released haphazardly into the surrounding area or given away to create new facilities. In addition, animals sometimes escape, due to poor security measures (Pasachnik and Carreras De León 2014).

There is very little information available concerning the genetic variability of *C. cornuta* in the wild (Malone et al. 2000), and virtually no data on the individuals currently kept in iguanarios across the DR (Pasachnik and Carreras De León 2014). The potential of using iguanarios as an effective conservation strategy is not viable under the current conditions. Instead, the situation may now pose a threat to the genetic integrity and health of the wild populations through the potential loss of local adaptation and disease introduction (Pasachnik and Carreras De León 2014).

In this paper, we aim to elucidate the overall population genetic structure of wild *C. cornuta* iguanas across the DR and test the hypothesis that vicariant events may have contributed to local differentiation of the species in the wild. Specifically, we predict that high elevation mountain ranges as well as low lying areas with frequent sea inundation have contributed to population isolation and subsequent differentiation. We then use the information from the wild populations to reconstruct the possible origin of captive individuals and guide proper management strategies for iguanarios. Further, while elucidating the population genetic structure of *C. cornuta* in the Dominican Republic, we aim to address the long-standing issue of the taxonomic status of Rock Iguanas on Mona Island. Mona is a small island in the Mona Channel, residing approximately 40 km to the east of Hispaniola and 40 km to the west of Puerto Rico. The population of Rock Iguanas on Mona has a close phenotypic resemblance to *C. cornuta* on Hispaniola and has been considered both a subspecies of *C. cornuta* (e.g., Schwartz and Carey 1977) as well as a separate species, *Cyclura stejnegeri* (Barbour and Noble 1916; see ITWG 2016 for an overview).

## Material and methods

### Field collection

DNA samples were collected from wild and captive individuals across the DR from 2010 to 2016 (Figs. 1, 2, 3). Wild iguana samples were collected across 13 regions of the DR (Figs. 2, 3; Table 1). The exact locations are not presented herein due to the threatened status of this species. The authors, upon legitimate request, may provide additional information. Samples were collected from Mona Island by Néstor Pérez Buitrago and loaned to San Diego Zoo Global Institute for Conservation Research. Captive samples were collected from 18 iguanarios (Fig. 1). In larger facilities samples were collected from a random subset of individuals. At the time of captive sample collection, informal interviews were conducted with the facility managers

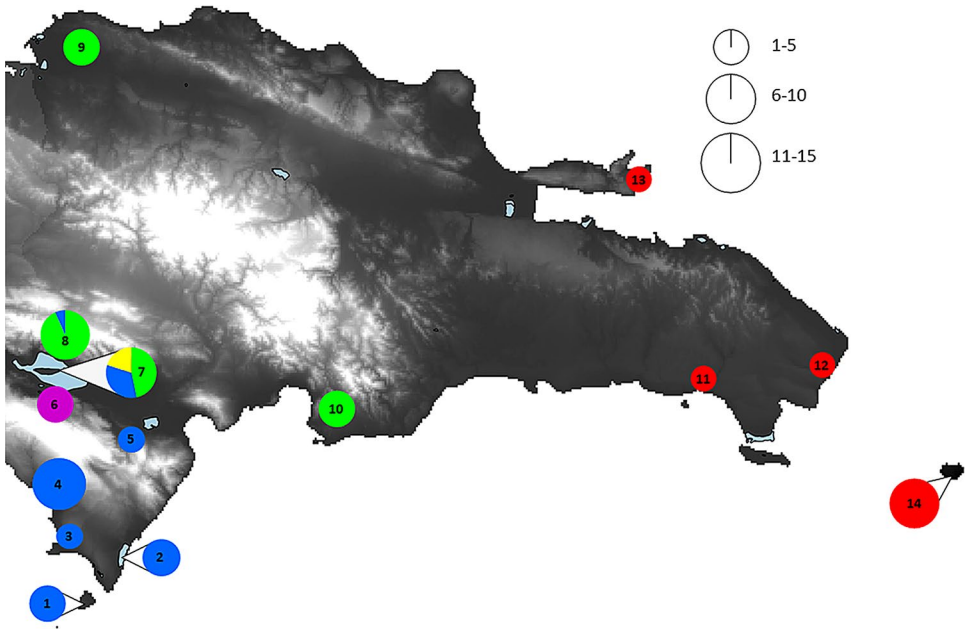


**Fig. 2** Mitochondrial DNA haplotype distribution in wild individuals from the Dominican Republic and Mona Island. The size of the circle represents the number of samples in a given location as shown in the figure key. Numbers within circles represent sampling area number. Topology is represented with lighter shades indicating higher elevation. Pale blue areas indicate water

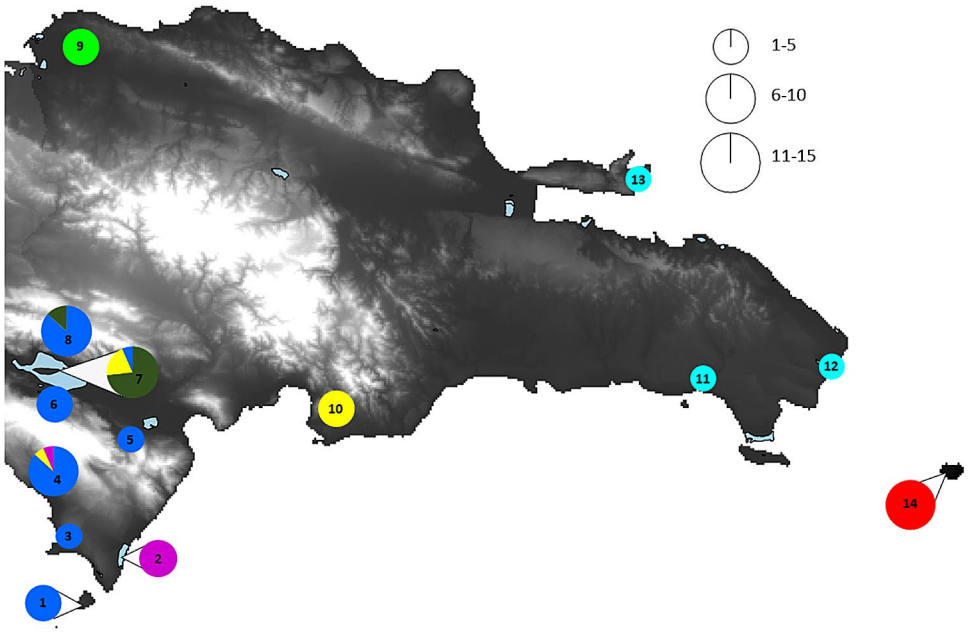


**Fig. 3** Distribution of nuclear DNA variability according to the DAPC analysis in wild individuals from the Dominican Republic and Mona Island. The size of the circle represents the number of samples in a given location as shown in the figure key. Numbers within circles represent sampling area number. Topology is represented with lighter shades indicating higher elevation. Pale blue areas indicate water

**Table 1** Summary of wild samples collected for this study

| Group ID | Sites ID | N | $A_r$ | $H_o$ | $H_e$ | $F_{IS}$ | p-val |
|---|---|---|---|---|---|---|---|
| Group1 | 1 | 10 | 2.854 | 0.603 | 0.628 | 0.088 | 0.155 |
| Group2 | 2 | 7 | 2.384 | 0.420 | 0.499 | 0.134 | 0.158 |
| Group3 | 3 and 4 | 29 | 3.663 | 0.648 | 0.693 | 0.101 | 0.000* |
| Group4 | 5 and 6 | 10 | 3.274 | 0.588 | 0.681 | 0.168 | 0.005* |
| Group5 | 7 | 15 | 2.753 | 0.584 | 0.591 | 0.014 | 0.427 |
| Group6 | 8 | 15 | 3.226 | 0.633 | 0.677 | 0.041 | 0.234 |
| Group7 | 9 | 8 | 1.967 | 0.446 | 0.470 | 0.088 | 0.256 |
| Group8 | 10 | 10 | 3.033 | 0.561 | 0.579 | − 0.010 | 0.624 |
| Group9 | 11 and 12 and 13 | 10 | 2.819 | 0.461 | 0.573 | 0.218 | 0.005* |
| Group10 | 14 | 30 | 2.009 | 0.384 | 0.457 | 0.041 | 0.237 |

Sampling area ID (see Figs. 1 and 2), number of individuals per population (N), observed heterozygosity ($H_o$), expected heterozygosity ($H_e$), fixation index ($F_{IS}$) and associated p-values. Significance is represented with an asterisk

to obtain information regarding the history of the facility, the source of their iguanas, and the movement of iguanas from their facility (see Pasachnik and Carreras De León 2014 for a summary of this information). Samples could not be obtained from Haiti due to permitting obstacles. We collected samples from a total of 144 wild and 114 captive individuals.

Upon capture of all wild and most captive individuals, snout-vent length, tail length, mass, and sex were recorded. To avoid re-sampling a passive integrated transponder (PIT) tag was implanted subcutaneously for permanent identification in all wild and some captive individuals. In addition, some individuals received bead tags (Rodda et al. 1988) for rapid identification. A 0.5 ml sample of blood was collected, from the caudal vein, from all live individuals. Blood was stored in EDTA buffer (Longmire et al. 1997). Tissue, collected from deceased individuals, was stored in 100% ETOH.

## DNA extraction and genotyping

Genomic DNA was extracted using the Qiagen DNeasy DNA extraction kit (QIAGEN®). To assess mitochondrial DNA variation the polymerase chain reaction (PCR) was used to amplify a 674 bp portion of NADH dehydrogenase subunit 4 (see Pasachnik et al. 2009 for detailed procedures and primers). PCR products were verified by gel electrophoresis and successful amplicons were purified

**Table 2** Name, number of alleles (calculated using all adult wild caught individuals), frequency of null alleles estimated over all adult wild caught individuals using FreeNA (Chapuis and Estoup 2007) and the EM algorithm (Dempster et al. 1977), NCBI accession number, and source reference of the 18 microsatellites used in this study

| Name | # Alleles | Null Freq | NCBI | Source |
|---|---|---|---|---|
| CIDK177 | 17 | 0.097 | HQ186269 | Welch et al. (2011) |
| CIDK113 | 17 | 0.107 | HQ186265 | Welch et al. (2011) |
| CIDK184 | 10 | 0.067 | HQ186270 | Welch et al. (2011) |
| CCYC09 | 9 | 0.084 | KF646798 | Colosimo et al. (2014) |
| 60HDZ106 | 4 | 0.169 | AY159682 | An et al. (2004) |
| 60HDZ09 | 10 | 0.104 | AY159675 | An et al. (2004) |
| CCSTE01 | 4 | 0.184 | EU040043 | Rosas et al. (2008) |
| CCSTE22 | 10 | 0.152 | EU040064 | Rosas et al. (2008) |
| D130 | 13 | 0.134 | FJ555512 | Lau et al. (2009) |
| D135 | 10 | 0.149 | FJ555514 | Lau et al. (2009) |
| CIDK135 | 13 | 0.096 | HQ186266 | Welch et al. (2011) |
| 60HDZ65 | 12 | 0.184 | AY159679 | An et al. (2004) |
| 60HDZ66 | 8 | 0.039 | AY159680 | An et al. (2004) |
| CCSTE07 | 9 | 0.043 | EU040049 | Rosas et al. (2008) |
| CIDK101 | 14 | 0.132 | HQ186263 | Welch et al. (2011) |
| 60HDZ780 | 8 | 0.170 | AY159694 | An et al. (2004) |
| CCSTE03 | 9 | 0.109 | EU040045 | Rosas et al. (2008) |
| CCSTE02 | 11 | 0.092 | EU040044 | Rosas et al. (2008) |

using exonuclease I/shrimp alkaline phosphate (ExoSAP). Sequencing reactions were preformed using original PCR primers by Eton Bioscience Inc. (San Diego, CA, USA).

Nuclear DNA variation was assessed using 18 microsatellites developed and used in previous studies involving *Cyclura* iguanas (see Table 2 for details). Double stranded DNA was quantified using Promega QuantiFluor® dsDNA System (Promega®) and the Thermo Fluoroskan Ascent Fluorometer (Thermo Scientific™). The extracted DNA was then normalized to 5 ng/µL. All forward primers were labeled with a fluorescent tag (6FAM, NED, VIC, or PET) attached to the 5′ end. Multiplexed PCR was run using five tagged primer sets per panel, the Qiagen Multiplex PCR mix (1X final concentration; QIAGEN®), and 20 ng of DNA. PCR cycling parameters included a 15-min hot start at 95 °C, followed by 40 cycles of 95 °C for 30 s, 60 °C annealing temperature for 45 s, and extension at 72 °C for 45 s. PCR products were diluted to an appropriate concentration determined by dilution tests. One microliter of diluted PCR product was added to 10 µl of HiDi Formamide with the 500 MW LIZ size standard (Applied Biosystems®) and 7µL of molecular grade water. Samples were analyzed on an ABI Prism 3730 DNA Analyzer (Applied Biosystems®) and data were called using GeneMarker® (Softgenetics®). Sample normalization, PCR, and preliminary analysis were performed at the University of Nevada, Nevada Genomics Center (https://www.ag.unr.edu/genomics/).

## Data analysis

### Wild genetic diversity and distribution

Sequences were trimmed and aligned using Sequencher® 4.6 (Gene Code Corporation, Ann Arbor, MI, USA). Ambiguous base calls were verified manually by examining the forward and reverse electropherogram reads. DnaSP v5 (Librado and Rozas 2009) was used to investigate the total number of haplotypes in our samples. Individual haplotypes were then grouped and visualized based on their geographic origin. Data were analyzed within the R statistical environment (R Development Core Team 2011) to test different hypothesis concerning geographic and genetic differentiation via a hierarchical Analysis of Molecular Variance (AMOVA). The R package *poppr* v2.5.0 (Kamvar et al. 2013) was used to run an AMOVA following Excoffier et al. (1992). The significance of our results was tested using a randomization test with 10,000 replicates in the R package *ade4* v1.7.10 (Dray and Dufour 2007). Finally, identified haplotypes were used to reconstruct a phylogenetic network using the median joining algorithm implemented in the program PopART (Bandelt et al. 1999; Leigh and Bryant 2015).

Homozygous genotypes can sometimes be overrepresented when the frequency of null alleles in the dataset is too high. To estimate the frequency of null alleles in our dataset we used FreeNA (Dempster et al. 1977; Chapuis and Estoup 2007), and we set the null allele cut-off frequency to 0.2 as suggested by Chapuis and Estoup (2007). For individuals sampled at each independent location we further estimated a fixation index, observed heterozygosity, and expected heterozygosity ($F_{IS}$, $H_o$, and $H_e$ respectively) using Arlequin v3.5 (Excoffier et al. 1992, 2010). Additionally, we used the R package *hierfstat* (Goudet 2005) to calculate allelic richness, an estimate of the number of alleles rarefied by the number of individuals analyzed. To investigate patterns of differentiation across sampled regions we used microsatellite alleles to build a pairwise comparison of differences expressed using the $F_{ST}$ index as calculated in Arlequin v3.5 (Excoffier et al. 2010). We then tested for significance across these comparisons with 1,000 permutations. Given the small sample sizes in some locations we grouped sites 3 and 4, 5 and 6, and 11–13, for these analyses based on geography and the STRUCTURE results (see below).

Microsatellite data are generally considered neutral to natural selection, but can hint to adaptive divergence when in tight linkage with regions of the genome directly under selection (Ellegren 2004; Edelist et al. 2006). To investigate the possibility of adaptive differences in wild populations of *C. cornuta* we used the microsatellite data in BAYESCAN v2.1 (Foll and Gaggiotti 2008). The software analyzes differences in allele frequencies across subpopulations following a model of isolation after splitting from an ancestral

population. It then uses a fully Bayesian approach to estimate the posterior probability that differences in overall $F_{ST}$ values between subpopulations could be caused by population specific effects (randomly evolving subpopulations) or by the interaction of locus and population specific effects (Foll and Gaggiotti 2008).

Bayesian based clustering algorithms are widely used in the analysis of population structure. To investigate the population genetic structure of wild *Cyclura cornuta* iguanas across the DR and Mona Island we used the software STRUCTURE *v.2.3.4* (Pritchard et al. 2000; Falush et al. 2007). This software groups individuals according to a model of genetic distribution. We were interested in understanding the number of assigned genetic clusters (K) most explicative of our samples. The STRUCTURE software is known to present some biases when sample sizes are uneven across sampling locations (Puechmaille 2016). To address this issue, we limited the sample size from each sampled site to a maximum of 15 randomly selected individuals. Specifically, in regions 4 and 14, we collected 24 and 30 individuals respectively (Table 1). Therefore, for these sites, we randomly selected 15 individuals and ran the STRUCTURE analysis with a total of 120 wild samples. The simulation was run using a flat prior for the admixture parameter and using correlated allele frequencies. We set the program to test values of K ranging from 1 to 13. For each value of K the program performed 20 replicates of the analysis (Gilbert et al. 2012). In each independent analysis, Structure ran $10^6$ MCMC while discarding the first $10^5$ as burn-in. The final results were used to calculate the second order of differences in the likelihood function of K [ΔK] as implemented in the online software Structure-Harvester (Evanno et al. 2005; Earl and VonHoldt 2012). We used a bar-plot to represent relative probability of membership to assigned clusters.

We also investigated the population genetic structure with a Discriminant Analysis of Principal Components (DAPC) (Jombart et al. 2010). This multivariate analysis complements Bayesian clustering algorithms, such as STRUCTURE, because it doesn't rely on any a priori model or assumption. The DAPC methodology partitions the overall genetic variance between- and within-groups, and looks for those variables maximizing the former while reducing the latter (Jombart et al. 2010).

To perform a DAPC we selected the same individuals used in the STRUCTURE analysis and used the R package *adegenet*, and its dependencies (Jombart 2008). We followed the procedure suggested by the author of the program and as implemented by others (Jombart 2008; Jombart et al. 2010; Vuillaume et al. 2015). Briefly, we first characterized the synthetic variables maximizing genetic variation in our samples (Principal Component Analysis). We then looked for the minimum number of clusters (K) best describing this variation using a K-means algorithm. We set the algorithm to investigate values of K ranging from 1 to 20, and we performed $1 \times 10^7$ iterations. Cluster membership was represented at the individual and population level using a bar-plot.

### Captive genetic diversity

Once a reference database of the wild genetic variability was built, individuals of unknown wild origin, i.e., those collected from iguanarios, were tested for genetic variability in their nuclear and mitochondrial DNA. Many of the iguanarios we sampled were established more than 15 years ago with individuals likely brought from different areas across the DR. Since then haphazard mating is known to have occurred, as was conveyed in our informal discussion with iguanario staff. It is therefore probable that samples in this study may not have a wild genetic makeup, but rather a combination of genes from different populations. Nuclear markers are subject to recombination and can, therefore, demonstrate a composite genetic makeup over generations of admixture between isolated lineages. As mitochondrial DNA is maternally inherited, it doesn't recombine, and can be helpful in understanding the origin of captive individuals given an existing pattern of mtDNA distribution in the wild (Gentile et al. 2013); however, it can also be misleading as only the maternal ancestry is represented. Therefore, we tried to infer the population of origin of the captive individuals by performing a population assignment test using a combination of mtDNA and nuclear markers and the Bayesian framework implemented in BAPS v6 (Corander et al. 2006, 2008). This approach has proven useful in other studies (Zarza et al. 2016). Briefly, we used the *trained clustering algorithm* implemented in the software, which allows the assignment of samples of unknown origin to predefined clusters. We combined nuclear and mtDNA data, with each mtDNA haplotype coded as diploid with a missing allele. We ran two independent analyses: first, we forced the software to assign the iguanas collected in captivity to any of the 14 wild sampled locations; second, we reran the analysis without any constraint, i.e., allowing the software to cluster the captive individuals in as many clusters as needed.

## Results

### Wild genetic diversity and distribution

We identified five unique mtDNA haplotypes in 120 wild individuals (Figs. 2, 4). No additional haplotypes were discovered in the 24 additional samples not included in further analysis. All individuals from the eastern region of the DR (sampling areas 11–13) and Mona Island (sampling area 14) shared the same unique haplotype (Hap. A, Figs. 2, 4). Individuals from the southwestern DR

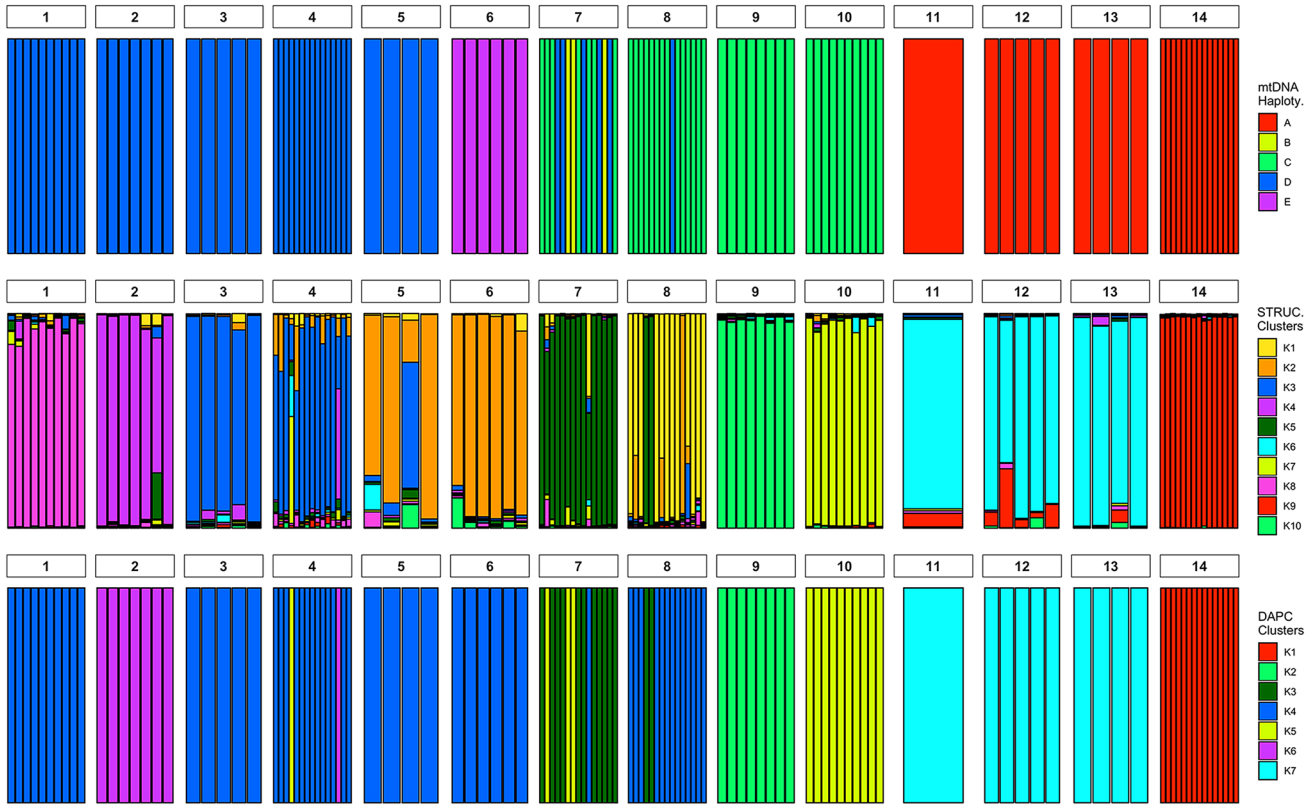


**Fig. 4** Distribution of genetic variation in mitochondrial and nuclear DNA markers from 120 wild iguanas across 14 sampling sites identified in Figs. 1 and 2. Each bar represents an individual. Top: distribution of the 5 different mtDNA haplotypes identified in this study. Middle: results of the STRUCTURE algorithm on microsatellite data. Bottom: results of the DAPC clustering algorithm on microsatellite data

(sampling areas 1–5) were monomorphic for haplotype D (Figs. 2, 4). Samples collected from the region south of Enriquillo Lake (sampling area 6) all showed a distinct haplotype (Hap. E, Figs. 2, 4). Individuals from sampling areas 8–10 were monomorphic for haplotype C with the exception of one individual with haplotype D from sampling area 8 (Figs. 2, 4). Samples collected from Cabritos Island (sampling area 7) were characterized by the presence of haplotypes C and D, as well as haplotype B, not found elsewhere in the DR (Figs. 2, 4). We ran an AMOVA separating individuals into an eastern (sampling areas 11–14) and western (sampling areas 1–10) clade. This analysis was significant at all hierarchical levels investigated (Table 3). In particular, partitioning individuals into an eastern versus a western clade was sufficient to explain more than half of the genetic variance found in our samples ($\varphi_{CT} = 0.520$, Table 3). Haplotype D was the most divergent from all other haplotypes found in this study (Fig. 5) and its distribution corresponds closely to what was the southern paleo-island. Therefore, we ran an additional AMOVA clustering individuals from sampling areas 1 through 4, all fixed for haplotype D, in a separate group. This AMOVA was able to explain almost 70% of the genetic variance found on the island ($\varphi_{CT} = 0.687$, Table 3).

**Table 3** Significance test results for AMOVA using mtDNA. Individuals are partitioned in 2 ways: 2 groups—a western (sampling areas 1–10, haplotype diversity = 0.571) and an eastern (sampling areas 11–14, haplotype diversity = 0.000) region of the DR; 3 groups—a southern Paleo-Island (sampling areas 1–4, haplotype diversity = 0.000), a northern Paleo-Island (sampling areas 5–10, haplotype diversity = 0.514) and a western region (sampling areas 11–14, haplotype diversity = 0.000) of the Dominican Republic

| Test | AMOVA | φ | p value |
|---|---|---|---|
| 2 Groups | Variation between regions ($\varphi_{CT}$) | 0.520 | < 0.001 |
| 3 Groups | Variation between regions ($\varphi_{CT}$) | 0.687 | < 0.001 |

None of the 18 microsatellite loci selected for our analysis showed evidence of null alleles with a frequency higher than 0.2 (Table 2). We therefore retained all markers for downstream analyses. Individuals collected from sampling areas three and four showed a slight but significant excess of homozygosity (Table 1). Pairwise $F_{ST}$ comparisons

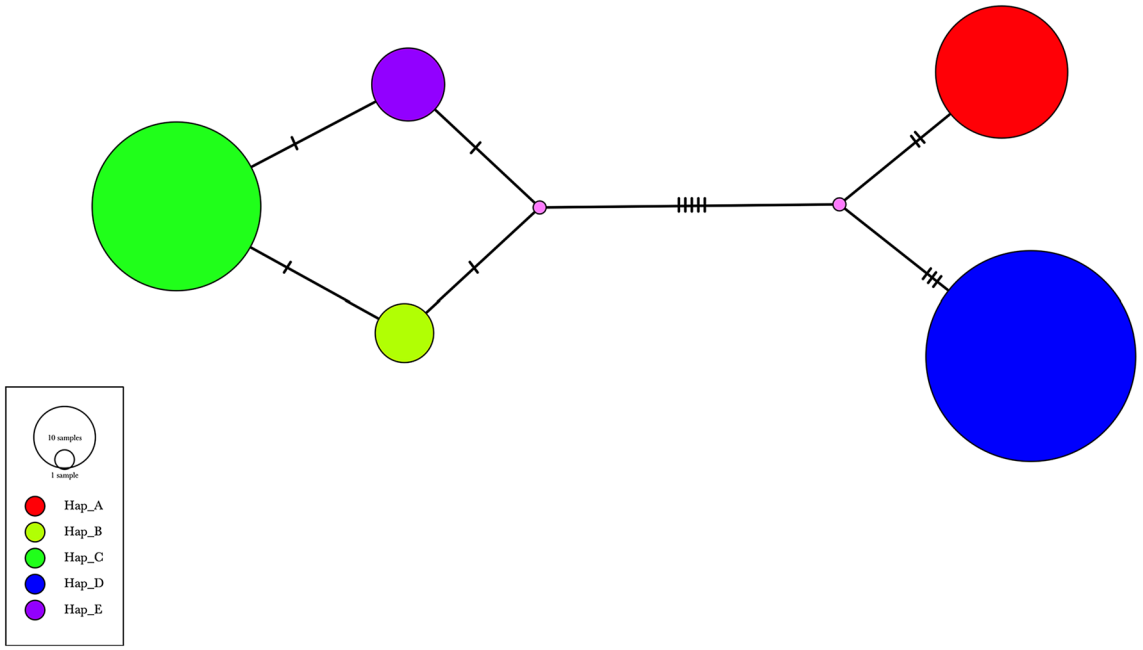


**Fig. 5** Median Joining Network built with the five different haplotypes identified in this study. Colors coding is identical to Figs. 2 and 3. Pink circles represent haplotypes inferred by the median joining algorithm. Bars across the branches connecting the various haplotypes represent the number of nucleotide differences between any of the two considered haplotypes. The size of each circle is proportional to the haplotype's frequency

between groups ranged from 0.079 (Group 1 VS Group 4) to 0.526 (Group 7 VS Group 10; Fig. 6; see SM Tables 1, 2 for all $F_{ST}$ pairwise comparison values and their statistical significance). None of the analyzed loci showed evidence of significant departure from a model of neutral evolution (SM Fig. 1). Although we feel confident in our conclusions, we recognize the potential bias of interpreting $F_{ST}$ values obtained by pairwise comparisons of sites with uneven and small sample size. Unfortunately, sampling

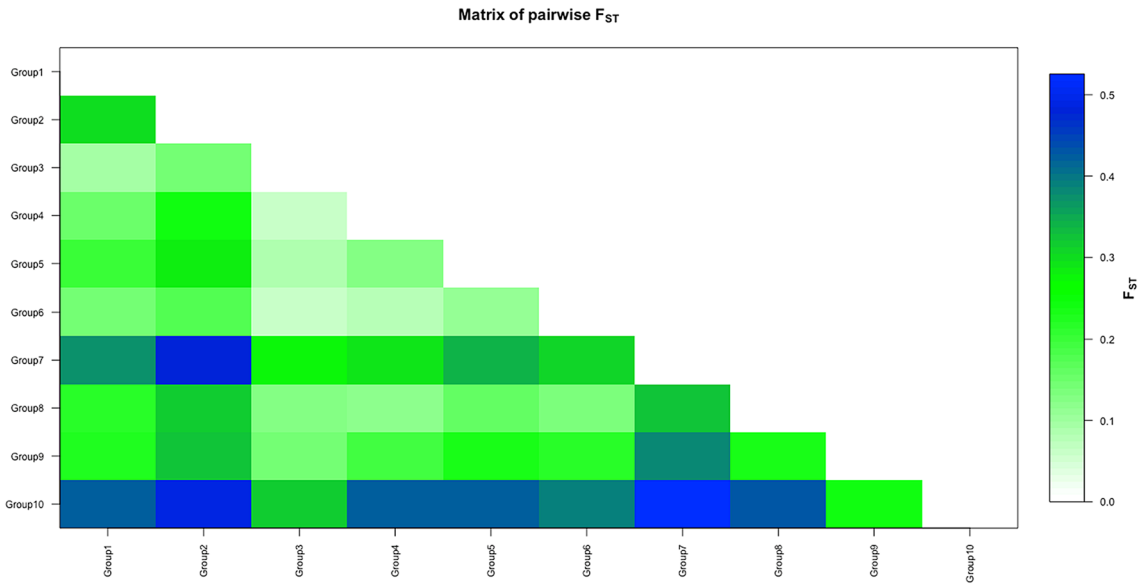


**Fig. 6** Heat map of pairwise $F_{ST}$ estimated using microsatellite data between all groups of wild sampled sites for *Cyclura cornuta* in the Dominican Republic. All pairwise comparisons are significant at 0.05

wild *C. cornuta* in the DR can be extremely difficult given their rarity and elusive nature in some regions.

The STRUCTURE analysis partitioned wild individuals in 10 different genetic clusters (Fig. 4, see also SM Figs. 2 and 3). Individuals from sampling area 14 clustered together, in a separate group. The individuals from the eastern DR (sampling areas 11–13) clustered together (Fig. 4). The majority of the other individuals were clustered in different groups corresponding to their sampling areas. We ran two separate AMOVAs, first partitioning individuals into western and eastern clades, where 16.8% of the genetic variance was explained by the eastern versus western separation ($\phi_{CT}=0.168$, Table 4). The second AMOVA partitioned the regions in the southern paleo-Island, northern paleo-Island, and western region of the DR, and was able to explain 11.4% of the genetic variance found ($\phi_{CT}=0.114$, Table 4).

**Table 4** Significance test results for AMOVA using microsatellites. Individuals have been partitioned in 2 different ways: 2 groups—a western (sampling areas 1–10, n = 104, $A_r=8.171$) and an eastern (sampling areas 11–14, n = 40, $A_r=5.500$) region of the DR; 3 groups—a southern Paleo-Island (sampling areas 1–4, n = 46, $A_r=7.471$), a northern Paleo-Island (sampling areas 5–10, n = 58, $A_r=7.434$) and a western region (sampling areas 11–14, n = 40, $A_r=5.500$) of the Dominican Republic

| Test | AMOVA | F | p value |
|---|---|---|---|
| 2 Groups | Variations within samples ($F_{IT}$) | 0.374 | << 0.001 |
| | Variations between samples ($F_{SC}$) | 0.190 | << 0.001 |
| | Variation between regions ($F_{CT}$) | 0.168 | << 0.001 |
| 3 Groups | Variations within samples ($F_{IT}$) | 0.330 | << 0.001 |
| | Variations between samples ($F_{SC}$) | 0.187 | << 0.001 |
| | Variation between regions ($F_{CT}$) | 0.114 | << 0.001 |

The DAPC output produced a very similar pattern to that of STRUCTURE, but identified only seven clusters. Mona Island and the sampling areas from the eastern DR (areas 11–13) were again recognized as separate groups (Figs. 3, 4). Almost all individuals from sampling areas 1, 3–6, and 8 formed a single genotypic group. Samples from sites 2, 7, 9, and 10 were assigned to their own separate clusters, with the exception of a few individuals from sampling area seven who showed alternative group assignments (Figs. 3, 4).

## Captive genetic diversity

No novel mtDNA haplotypes were discovered in the 114 captive samples analyzed (Fig. 7). Despite the large number of iguanarios located in the eastern DR, only three individuals had the wild haplotype from that area (i.e. haplotype A, Figs. 1, 2, 7), which is consistent with information gained

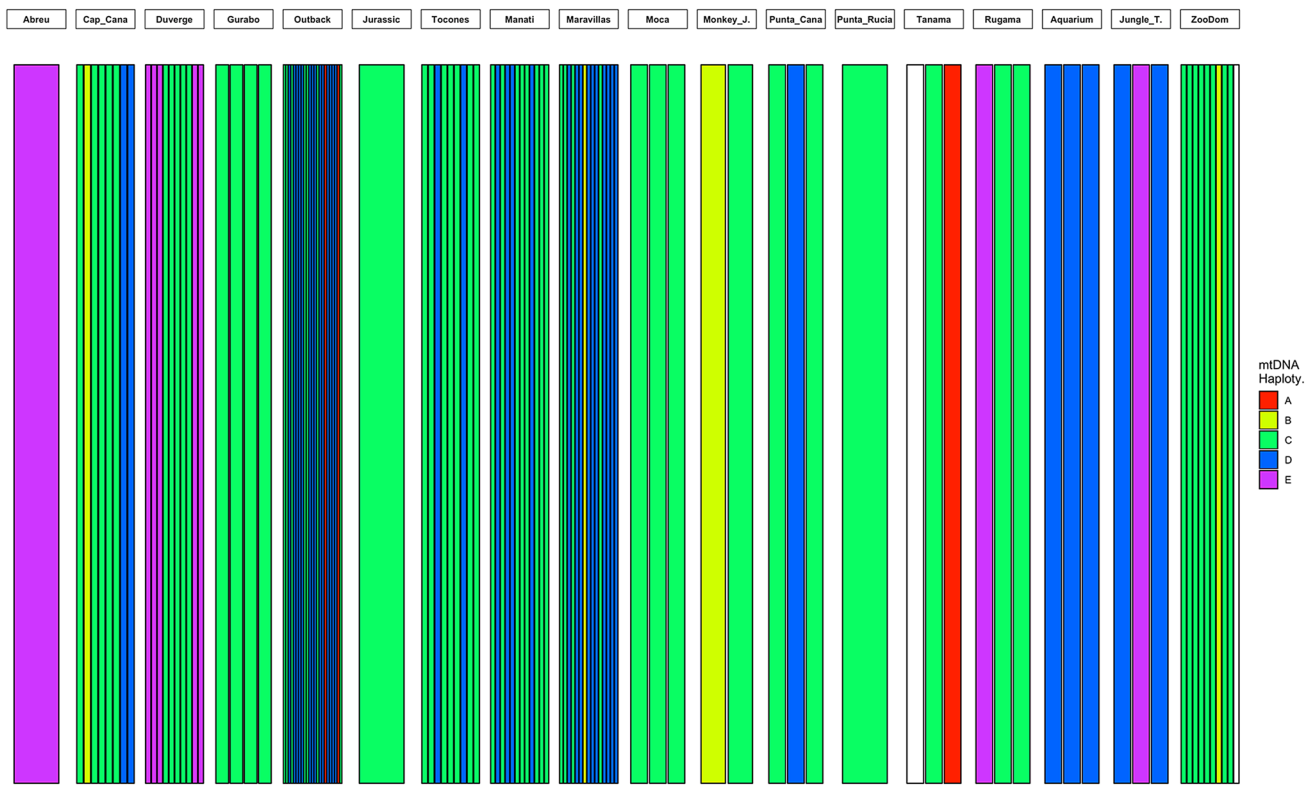


**Fig. 7** Distribution of genetic variation in mtDNA from 114 iguanas sampled across different iguanarios (see Fig. 1 for location of various iguanarios). Each bar represents an individual. White bars indicate that no mtDNA sequence was obtained from those individuals

from discussions with iguanario staff suggesting that most captive individuals originally came from the western DR, where hunting is most common. The analysis with BAPS shows that individuals sampled from captive facilities are likely the result of intraspecific hybridization events of parents coming from different areas across the country. Results from the BAPS constrained analysis grouped the majority of individuals with the wild individuals collected from sampling area three (SM Tab. 3). A few individuals were clustered with samples collected from area 5. A single captive sample from iguanario "Jurassic Park" was clustered with the only wild individual sampled from area 11, while the only three captive individuals with haplotype A were clustered with the wild individuals from sampling area 12, in the eastern region of the island (SM Tab. 3). When we ran the same analysis without constraining the captive individuals within any of the wild populations the results changed dramatically (SM Tab. 3), and all individuals were grouped in clusters inferred by the algorithm, suggesting that extensive recombination is occurring within the iguanarios.

## Discussion

Understanding the population genetic structure of threatened species is vital to developing appropriate management strategies, particularly when a metapopulation approach may be necessary due to a disjunct distribution (Akçakaya et al. 2007). We investigated the population genetic structure of *Cyclura cornuta* iguanas from most known regions of occurrence, fragmented across the Dominican Republic. Unfortunately, our data do not encompass the overall genetic diversity of the species, as we were unable to collect samples from Haiti. Further, due to the rare and elusive nature of this species in some areas of its range in the DR our sample sizes are sometimes low. Nonetheless, our results speak to the conservation of this species as we were able to: (1) identify patterns of significant population structure across the DR; (2) elucidate the possible detrimental effect that unmanaged captive facilities could have on wild iguana populations; and (3) shed light on the taxonomic dispute of *Cyclura* iguanas on Mona Island.

The collision of the north and south paleo-islands, resulting in the creation of Hispaniola, in the mid-Miocene, as well as subsequent sea inundations submerging the Enriquillo Basin until the mid-Pleistocene have impacted the genetic structure of many species (Glor et al. 2004; Townsend et al. 2007; Gifford and Larson 2008; Sly et al. 2011; Brace et al. 2012; Grigorev et al. 2018) (Fig. 1). A single *C. cornuta* mtDNA haplotype was found in the region corresponding to the southern paleo-island, or the area south of the Enriquillo Basin (haplotype D; Figs. 2, 4). The pattern found in the nucDNA is less clear, but a southern signature also exists (Fig. 3). This north–south pattern accounts for almost 70% of the total genetic variance found in the mitochondrial DNA ($\phi_{CT} = 0.687$, Table 3) and 11.4% of the nuclear genetic variance ($F_{CT} = 0.114$, Table 4). The southwest portion of the DR is the only sampled area in this study that occurs on the southern paleo-island, south of the Enriquillo Basin. Additional sampling along the Tiburon peninsula in Haiti will further elucidate the impact of the Enriquillo Basin in shaping the genetic structure of this species. Understanding which events, paleo-island collision or various sea inundations, caused the observed pattern is a more complicated issue as it depends upon an accurate divergence time estimate. Malone et al. (2017) estimated that the *Cyclura* clade dates to approximately 20 mya. Further, Reynolds (in prep.) dates the divergence of *C. cornuta* at approximately 2.5 mya based on a 50 PIC Bayesian BEAST analysis of *Cyclura*. This timing would reject the hypothesis that paleo-island collision and orogenic activity in the Miocene caused the divergences among populations of *C. cornuta*. Instead, a more likely scenario is that ancestral populations of *C. cornuta* colonized Hispaniola during sea level recession at the Pliocene/Pleistocene boundary, dispersing throughout the low-lying areas, and then becoming fragmented by sea inundations during Pleistocene interglacial periods (1–1.5 mya) (Raymo et al. 2006). More recent habitat degradation and hunting pressures have also likely impacted the current distribution, but on a finer scale. Further, the potential that human mediated movements have impacted the observed genetic structure must be considered. One such case, undertaken by the DR Ministry of Environment, is known (Secretaria de Estado de Medio Ambiente y Recursos Naturales 2001; Secretaria de Estado de Medio Ambiente y Recursos Naturales 2002); however, the relocation was not far from the point of origin. Another such case has been mentioned for Cabritos Island; however, there are no written accounts of this movement. In general, human mediated movements are rare, usually involving few individuals, and short distances from the origin. Thus, the impact of these movements on the observed genetic structure is likely minimal.

Both mitochondrial and nuclear genetic data reveal an elevated level of genetic variation within the Enriquillo Basin. Haplotype D is found within all individuals sampled from the area south of the Enriquillo Basin, as well as within the Enriquillo Basin (Figs. 2, 4). Likewise, individuals sampled from the south-central and northwestern populations in the DR (areas 9 and 10) share the same mtDNA (haplotype C; Figs. 2, 4), which can also be found in two of the sampling areas within the Enriquillo Basin. Additionally, two unique mtDNA haplotypes (B and E) were discovered within two sampling regions (6 and 7) in the Enriquillo Basin (Figs. 2, 4). DAPC results also indicate a common genetic signature across the southern sampling locations, which extends into the Enriquillo Basin, and a shared genetic

signature between sampling area 10, in the south-central region, and the Enriquillo Basin (Figs. 3, 4). Further, there is novel variation found within the basin (Figs. 3, 4). The STRUCTURE results exhibit a similar pattern but further divide the areas in the western DR, such as separating sampling areas 5 and 6 in the southern portion of the Enriquillo Basin (Fig. 4). This is likely indicative of sea inundation, followed by secondary colonization into this region following sea level recession in the Pleistocene. This increased genetic variation demonstrates that this region is of great conservation importance when considering the protection of diversity within this species.

The Dominican Republic has a complex system of mountains, holding the record for the highest mountain peak among all Caribbean islands. These mountains, which stabilized in the mid-Miocene, continue to inhibit migration for species preferring xeric lowlands. Pico Duarte, reaching 3098 m a.s.l., is found within the Cordillera Central, or the central mountain range, of Hispaniola (Fig. 1; Nairn and Stehli 1975). This mountain range represents a natural barrier dividing the island from the south-central region of the DR to the northwest of Haiti. Wild *Cyclura cornuta* samples collected east of this mountain range all presented a distinctive and unique mtDNA haplotype (Hap. A), and nuclear genotypes separate them from iguanas sampled throughout the rest of the DR (Figs. 2, 3, 4). This east–west pattern accounts for over 50% of the total genetic variance found at the mitochondrial DNA level ($\phi_{CT} = 0.520$, p value $< 0.001$), and 15% of the variance found at the nuclear DNA level ($F_{CT} = 0.154$, p value $< < 0.001$). Individuals collected from Mona Island fall within this eastern group based on their mitochondrial DNA (Figs. 2, 4). However, nuclear genetic variation further partitions the eastern group, with individuals from Mona Island recognized as a separate cluster (Figs. 3, 4; see below for a more detailed discussion).

Although areas 9 (group 7) and 10 (group 8) share a mtDNA haplotype, the nuclear data differentiates these areas ($F_{ST} = 0.325$, p value $< < 0.000$; Figs. 3, 4, 6). Further, area 9 (group 7) is one of the most genetically distinct locations within the DR ($F_{ST}$ values ranging from 0.290 to 0.479) (Fig. 6; see SM Tabs. 1, 2). This is consistent with the Cordillera Central mountain region, considerable geographic distance separating these two locations, and sea inundations during interglacial periods likely isolating these regions from all other locations (Schubert 1984; Iturralde-Vinent and MacPhee 1999). Lastly, there is a novel nuclear genetic signature in sampling area 2, which is consistent with this location being a small cay (Figs. 3, 4). The multifaceted geologic history of Hispaniola, coupled with prominent geographic barriers, have contributed to the complex pattern of genetic differentiation seen today.

The genetic variability and distribution in *C. cornuta* present important issues related to the conservation and management of this species. In our analysis, we only recovered five mtDNA haplotypes from over 250 sequenced individuals, with some of them restricted to small geographic areas. Although there may be additional variation in Haiti, our sampling encompasses most known areas in the DR where wild populations of *C. cornuta* are known to persist (Glor et al. 2000). The total surface area of the DR is over 48,000 $km^2$. The distribution of Rhinoceros Rock Iguanas is much smaller, estimated is to be approximately 10,000 $km^2$ (Pasachnik and Carreras-De León 2019). Finding only five mitochondrial haplotypes in a species with a wide-spread distribution is unexpected. For comparison, the Critically Endangered *C. carinata* in the Turks and Caicos Islands, is known to persist on a total surface area no larger than 13 $km^2$ (Gerber 2004). A recent study on the genetic structure of this species identified six different mtDNA haplotypes, using only a similar section of the ND4 region (Welch et al. 2017). Low levels of genetic variability at the mitochondrial level have been associated with selective sweep events, fixing one or few haplotypes (Ballard and Whitlock 2004), or with a dramatic reduction in population size. Based on the analysis of microsatellites, we did not find any evidence of significant departure from a model of neutral evolution at any site (SM Fig. 1). Thus, the latter hypothesis seems more plausible here, although selective sweep events may have occurred and should be evaluated with additional genetic markers. Pasachnik and Carreras De León (2014) report that recent surveys in areas with previously documented iguana populations have resulted in no sightings or very scarce signs of iguanas. This suggests that the conservation status of *C. cornuta* is more grave than previously thought, and an updated IUCN Red List Assessment now considers the species Endangered (Pasachnik and Carreras De León 2019). Future work using morphological characters and genetic data with higher resolution should focus on elucidating adaptive differences between populations across its patchy distribution.

Information collected from wild iguanas provides a baseline population structure that can be used to compare captive individuals and determine their origin. Mitochondrial DNA data can, at times, be sufficient to determine the general source location of an individual of uncertain origin (Gentile et al. 2013), especially if captured from the wild. The inclusion of nuclear DNA will; however, aid in these efforts, elucidating a more thorough genetic history of the individuals in question (Zarza et al. 2016). In this study, the analysis of mtDNA sequences from captive individuals did not reveal any novel haplotypes; however, our microsatellite data demonstrated that individuals did not genetically resemble those found in the wild. In particular, our results show that many of the captive facilities harbor individuals whose genetic composition is likely the result of interbreeding between geographically and genetically

separated groups. Predicting the long-term evolutionary impacts of these breeding events between naturally segregated lineages is difficult. However, genetic data as well as information from discussions with staff suggests that the majority of individuals entering into captive facilities from the wild come from areas in the southwest. The majority of large-scale iguanarios occur in the southeast, thus the potential to negatively impact the wild populations in the southeast, which are genetically distinct, is probable. Given that iguanas have been shown to successfully mate with members of differentiated genetic clusters, species, and even other genera, these releases could have long-term negative impacts to the local populations (Pasachnik et al. 2009, 2011; Zarza et al. 2011, 2016) if local adaptations have evolved. Moreover, there are other threats to consider. The conditions within many of the captive facilities are poor, and diseases may be prevalent (Pasachnik and Carreras De León 2014). Therefore, as a cautionary method, the release of individuals from iguanarios into the wild should immediately cease and reproduction should be prevented at this time.

Resolving the taxonomic status of the Mona Island *Cyclura* population will require additional morphological data as well as more in-depth analyses of genetics data. Although originally described as a species, *Cyclura stejnegeri* (Barbour and Noble 1916), most authors have followed Schwartz and Carey (1977) and recognized the *Cyclura* population on Mona Island and the extinct *C. onchiopsis* from Navassa Island as subspecies of *Cyclura cornuta* prior to 2000. Schwartz and Carey (1977) did; however, discuss a number of morphologically unique characters distinguishing the Mona Island population from *C. cornuta* found in the DR, and other authors have more recently recommended recognizing all three taxa as separate species (Glor et al. 2000; Powell 2000; Powell and Glor 2000; Henderson and Powel 2009). Although the shared mtDNA haplotype between Mona and the eastern DR demonstrates common ancestry between these populations, pairwise $F_{ST}$ comparisons using microsatellite data clearly indicate that the iguana population on Mona Island has been isolated for a significant amount of time (SM Tabs. 1 and 2). The geology of this area demonstrates that Mona Island was likely never connected to any other islands in its vicinity (Kaye 1959), suggesting that its flora and fauna are likely the result of various colonization events since its emergence in the Pliocene (Kaye 1959). Although it is difficult to be certain of the exact timing, it is clear that these populations have been reproductively isolated from one another for a substantial amount of time, gene flow is not occurring, and the Mona Island population is on its own evolutionary trajectory. We find there is potential for this population to be considered a distinct species, endemic to Mona Island; however, additional data are necessary to reach a robust conclusion.

Although additional genetic and morphological data are needed to elucidate adaptive differences across the known populations of *Cyclura cornuta* within the DR, our data demonstrate unique genetic signatures across the country, as well as shed light on the specific status of *Cyclura* iguanas on Mona Island. These results should be used to guide conservation actions and management decisions that preserve the greatest amount of genetic diversity across this region. Most importantly populations from the southwestern, northwestern, and eastern regions of the DR should be managed independently, and special attention should be given to the populations within the Enriquillo Basin as they harbor increased genetic diversity within a small area (see also $A_r$ and haplotype diversity values from Tables 1, 3, 4). Further, unregulated reproduction within captive facilities should be terminated immediately to avoid further genetic admixture through breeding of individuals with different origins. Although wild *C. cornuta* populations are diminishing and becoming more threatened, iguanarios throughout the DR cannot be considered for conservation breeding and release at this time. The use of breeding and release for conservation should only be considered when an individual's wild origin is known, no individuals in the program are the progeny of unregulated captive breeding, and a proper marking system and studbook for breeding can be created and managed. Plans are currently underway with a local non-governmental organization, Grupo Jaragua, and DR Ministry of the Environment to improve these facilities such that they can be considered for conservation and management purposes in the future.

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## Compliance with ethical standards

**Conflict of interest** The authors declare that they have no conflict of interest.

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