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Phylogenomics and historical biogeography of West Indian Rock Iguanas (genus *Cyclura*)

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ABSTRACT

The genus *Cyclura* includes nine extant species and six subspecies of West Indian Rock Iguanas and is one of the most imperiled genera of squamate reptiles globally. An understanding of species diversity, evolutionary relationships, diversification, and historical biogeography in this group is crucial for implementing sound long-term conservation strategies. We collected DNA samples from 1 to 10 individuals per taxon from all *Cyclura* taxa (n = 70 ingroup individuals), focusing where possible on incorporating individuals from different populations of each species. We also collected 1–2 individuals from each of seven outgroup species of iguanas (*Iguana delicatissima*; five *Ctenosaura* species) and *Anolis sagrei* (n = 12 total outgroup individuals). We used targeted genomic sequence capture to isolate and to sequence 1,872 loci comprising of 687,308 base pairs (bp) from each of the 82 individuals from across the nuclear genome. We extracted mitochondrial reads and assembled and annotated mitogenomes for all *Cyclura* taxa plus outgroup species. We present well-supported phylogenomic gene tree/species tree analyses for all extant species of *Cyclura* using ASTRAL-III, SVDQuartets, and StarBEAST2 methods, and discuss the taxonomic, biogeographic, and conservation implications of these data. We find a most recent common ancestor of the genus 9.91 million years ago. The earliest divergence within *Cyclura* separates *C. pinguis* from a clade comprising all other *Cyclura*. Within the latter group, a clade comprising *C. carinata* from the southern Lucayan Islands and *C. ricordii* from Hispaniola is the sister taxon to a clade comprising the other *Cyclura*. Among the other *Cyclura*, the species *C. cornuta* and *C. stejnegeri* (from Hispaniola and Isla Mona) form the sister taxon to a clade of species from Jamaica (*C. collei*), Cuba and Cayman Islands (*C. nubila* and *C. lewisi*), and the eastern (*C. rileyi*) and western (*C. cychlura*) Lucayan Islands. *Cyclura cychlura* and *C. rileyi* form a clade whose sister taxa are *C. nubila* and *C. lewisi*. *Cyclura collei* is the sister taxon to these four species combined.

1. Introduction

Rock iguanas of the genus *Cyclura* are among the most threatened of all squamate reptile genera (IUCN 2021; Fig. S1). Despite their peril, a broad consensus regarding the evolutionary history of the genus has not been achieved. The Iguana Taxonomy Working Group (ITWG 2016) of the International Union for the Conservation of Nature (IUCN) Species Survival Commission (SSC) Iguana Specialist Group acknowledged ten extant species, eight extant and geographically isolated subspecies, and one extinct species, similar to Lemm and Alberts (2011). However, it has also been proposed that several of these subspecies should be considered species (e.g., Hedges et al. 2019). Hence, a phylogenetically-informed taxonomic synthesis for the West Indian Rock Iguanas is essential to resolve this dialogue (Alberts 2016). To date, the best assessment of

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genus-wide phylogeny employing genetic data in *Cyclura* is based on a 900 base pair (bp) region of the mitochondrial genome (mtDNA; Malone et al. 2000), which has guided conservation efforts in this genus for twenty years. Given recent advances in genetic sequencing technology, we revisited the relationships in this genus using genomic data on a scale 3–4 orders of magnitude greater than employed twenty years ago.

An understanding of the colonization history, evolution, and diversification of the genus *Cyclura* requires a phylogenetic hypothesis incorporating signals from both the mitochondrial (mtDNA) and nuclear (nucDNA) genomes (Malone and Davis 2004; Alberts 2016; Malone et al. 2017). Mitochondrial phylogenies (Malone et al. 2000; Shaney et al. 2020) had low support for some nodes, several polytomies, differing divergence-time estimates, and were unable to resolve relationships among some subspecies, underscoring the need for genome-wide sampling. Contemporary approaches allow inference of divergence times from multiple genetic markers and can provide improved understanding of evolutionary relationships and historical biogeography among squamates by leveraging large amounts of sequence data (e.g., Zheng and Wiens 2015). The approach used here involved ultraconserved elements (UCEs; Faircloth et al. 2012). Use of UCEs has resolved evolutionary relationships on both recent and ancient timescales (Smith et al. 2014; Meiklejohn et al. 2016), among squamate reptiles generally (Leaché et al. 2014; Grismer et al. 2016) and iguanians specifically (Streicher et al. 2016), thus making these markers a good choice for investigating both population-level and higher-level divergences. Because UCEs are conserved for vast periods of evolutionary time, they provide conserved priming sites throughout the tetrapod genome. Sequencing in both directions from these conserved regions of the genome captures variability in the flanking regions (Faircloth et al. 2012). This variability provides an excellent source of phylogenetic information, while the conserved regions at their core establish homology and alignment.

Twenty years after the publication of a taxon-complete molecular phylogeny for the genus, we apply a phylogenomic approach to further our understanding of the evolutionary relationships of the imperiled genus *Cyclura*. We leverage millions of base pairs of DNA, phylogenomic species-tree reconstruction, and divergence-time estimates to characterize the relationships within the genus. In so doing we provide a robust framework for examining the evolution and historical biogeography of these species.

2. Materials and methods

2.1. Sample collection and DNA extraction

We collected blood and/or tissue samples from wild populations of all extant *Cyclura* taxa (Fig. 1) over the course of several decades of fieldwork by the authors and others. We attempted to secure multiple individuals, from multiple populations, of each *Cyclura* taxon. We also obtained 1–3 samples of several species of the outgroups *Ctenosaura* and *Iguana* (Pyron et al. 2013; Malone et al. 2017), as well as one individual of the distant outgroup *Anolis sagrei* (Table S1). Our full dataset consisted of 70 ingroup (*Cyclura*) samples and 12 outgroup samples (Table S1). Blood samples consisted of heparinized whole blood preserved in buffer, while tissues consisted of toe clips preserved in ethanol. We extracted whole genomic DNA from blood and tissue samples obtained both recently and up to two decades ago using the Wizard SV® Kit (Promega, Madison, WI) and subsequently stored extracts at −20 °C.

2.2. Sequencing and bioinformatics

We examined the quality of each extraction using an electrophoresis gel check of fragment lengths, followed by fluorometric quantitation of each extraction using a Qubit 3.0® (Thermo Fisher Scientific, Waltham, MA) with a dsDNA BR Assay Kit. We considered sample concentrations above 2.5 ng/ml acceptable and rejected extractions below this concentration. We then further confirmed extraction quality on a random sample of 12 extractions for PCR amplification and sequencing using a mitochondrial ND2 primer set for lizards (Reynolds et al. 2017). We sent normalized extraction aliquots to RAPiD GENOMICS® LLC (Gainesville, FL) for UCE sequence capture and sequencing following the original UCE protocol (Faircloth et al. 2012). We selected the myBaits® UCE Tetrapods 5kv1 primer set for sequence capture on all samples, followed by 150 base-pair (bp) paired-end sequencing on a single-lane Illumina® HiSeq 2500 run. Libraries were pooled following barcode adapter ligation and then the pooled libraries were sequenced.

We performed an initial quality control visualization on raw Illumina® fastq sequence reads using FastQC 0.10.1 (Andrews 2014) and visualized relevant data using R (R Core Team 2021, Vienna, Austria). We then batched raw fastq reads into the Linux *phyluce* pipeline— a

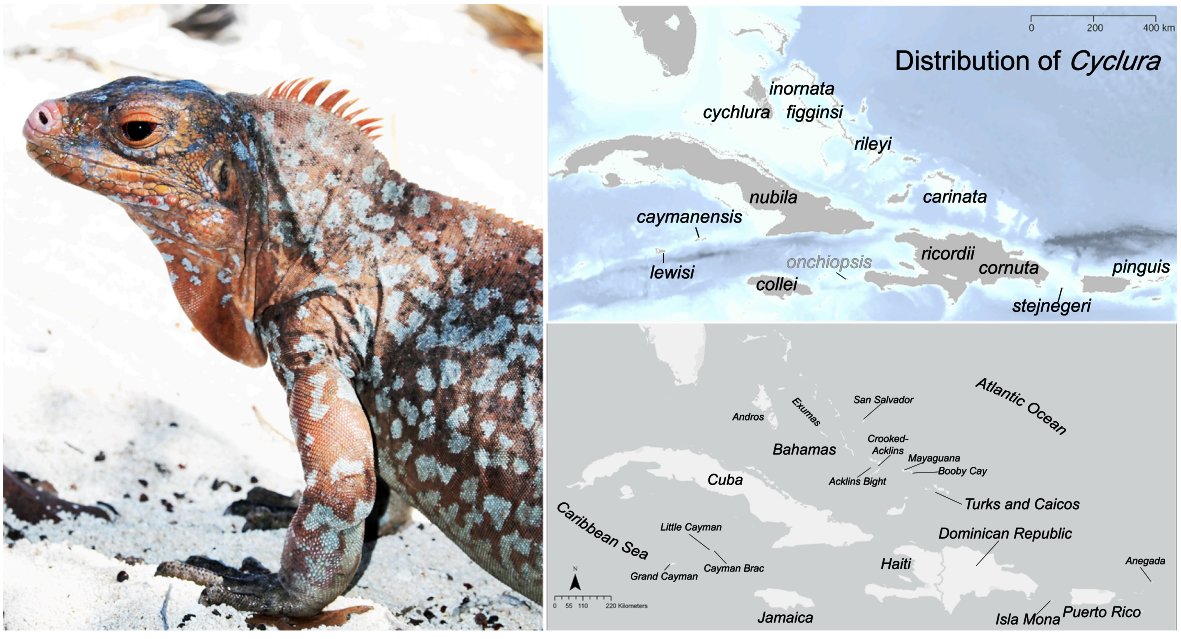

Fig. 1. Distribution of Rock Iguanas (genus *Cyclura*) in the West Indies. *Cyclura rileyi* from the Crooked-Acklins Bank, Bahamas, is shown to the left (photo by RGR). Names given are taxon names (species or subspecies). Note that *Cyclura onchiopsis* (in gray) from Navassa Island is extinct. See ITWG (2016) for detailed range maps.

toolkit comprising conda-packaged Python scripts to facilitate UCE identification and extraction (Faircloth 2016). We used the Illumiprocessor Python script (Faircloth 2014), which calls Trimmomatic (Bolger et al. 2014) to trim adapters and low-quality sequences reads. We then assembled contigs from the cleaned reads using the Velvet algorithm (Zerbino and Birney 2008) with *kmer* = 51. We then extracted UCE loci from the contigs file by matching contigs to the UCE Tetrapods 5kv1 probe set (available at https://github.com/faircloth-lab/uce-probe-sets/blob/master/uce-5k-probe-set/uce-5k-probes.fasta). We aligned UCE loci using the MAFFT algorithm (Katoh and Standley 2013) with edge trimming, then removed prepended locus names from the alignments. We created a 50% complete data matrix, whereby we retained aligned UCE loci with at least 50% of the taxa represented for every retained locus. This supermatrix dataset consisted of 82 taxa, 1,872 loci, and 687,308 bp. More stringent matrices (i.e., 75%, 95%) have significantly lower phylogenetic resolution and were therefore not used. We conducted all UCE bioinformatics on a Dell® (Dell Inc., Round Rock, TX) PowerEdge© Server (64c, 128gb RAM) running Ubuntu 16.04.5 as well as the *CHILABOTHRUS* Dell® PowerEdge© Server (16c, 128gb RAM) running Ubuntu 20.04, both at the University of North Carolina Asheville.

2.3. UCE phylogenomic analyses

Using our concatenated 50% UCE supermatrix (82 taxa, 1,872 loci, 687,308 bp) we estimated a maximum-likelihood tree with the RAxML algorithm implemented in the RAxML-HPC (Stamatakis 2006; Stamatakis et al. 2008) executable distributed with the *phyluce* package for Linux (Faircloth 2016). We used the GTRGAMMA model, first running a search for the best ML tree, then matching the best tree with 1000 bootstrap repetitions. We conservatively consider BS values above 70% to indicate well-supported clades (Felsenstein 2004; Taylor and Piel 2004).

Given that concatenated ML methods can converge on incorrect species trees (Roch and Steel 2015), we opted to use the coalescent species-tree estimation tool SVDquartets (Chifman and Kubatko 2014) in PAUP* v4.0a169 (Swofford 2002). SVDquartets accounts for differences in genealogical histories from individual loci to infer the species tree by calculating SVD scores for each possible quartet of taxa in the dataset, then uses the quartet tree agglomeration method Quartet FM (Reaz et al. 2014) to combine quartets into a species tree (Chou et al. 2015). We used as input the 50% multi-locus sequence dataset (1,872 loci, n = 81, one *C. similis* outgroup sample was excluded), including in the data file a partition to assign tips to their respective taxa. We evaluated all possible quartets using an optimal tree search and assessed support via 1,000 bootstrap replicates (Reaz et al. 2014), generating a bootstrapped 50% majority-rule consensus tree.

The method implemented in SVDquartets can sometimes underperform other coalescent gene tree/species tree methods (Chou et al. 2015), so we opted also to use the robust method implemented in ASTRAL-III (Zhang et al. 2018). We first used ParGenes v1.2.0 (Morel et al. 2019) to determine best fit models and to estimate individual gene trees for each of the 1,872 loci in the 50% dataset (n = 82 taxa). ParGenes takes as input multiple sequence alignments, selects best-fit models of evolution using ModelTest-NG (https://github.com/ddarriba/modeltest), and infers ML trees with bootstrap supports using RAxML-NG (Kozlov 2018). Because model selection and gene-tree inference are parallelized, the runs were computationally efficient on our CHILABOTHRUS server. ASTRAL-III takes as input all the individual ML gene trees to infer an unrooted species tree with measures of branch support called the local posterior probability, which is the probability of branch topology under the multispecies coalescent model. We ran ASTRAL-III v5.6.3 (Rabiee et al. 2018) on our server with the flag *-a* to provide a mapping file to assign tips to taxa, yielding a species-tree output that we visualized with FigTree v1.4.4 (Rambaut 2018).

To improve computational efficiency for subsequent analyses, we pared down our UCE dataset to a more modest number of loci, with the realization that we would be sacrificing information relevant to building the species tree. To attempt to reduce this information loss while still keeping a reasonable computation time, we calculated the number of parsimony informative sites (PIS) for each UCE locus from the 50% dataset using R scripts from Alexander (2018), then extracted the 32 most informative loci, which we used for subsequent analyses (*32PIS* dataset). We then used StarBEAST2 (Ogilvie et al. 2017) implemented in BEAST2 (Bouckaert et al. 2019) to infer a time-calibrated gene tree/species tree. This method jointly estimates species-tree topology, divergence times, and effective population sizes from multiple embedded gene trees under the multispecies coalescent model, which assumes that incongruence among gene trees is owing to incomplete lineage sorting in lieu of gene flow. We unlinked site and tree models but used a linked clock model for all partitions in BEAUti v2.6.6 (Bouckaert et al. 2019) and used the HKY substitution model for all loci. We used a strict clock model owing to the expectation of mutations that have not yet reached fixation (Ho et al. 2005; Peterson and Masel 2009), and we used a birth–death process speciation prior for the branching rates.

Some relevant divergence time estimates from fossil-calibrated studies include 8.9 MY for the root of *Cyclura* (Malone et al. 2017) and 18.9 MY for the most recent common ancestor (MRCA) of *Iguana* and *Ctenosaura* (Malone et al. 2017). For the MRCA of *Cyclura* and *Iguana* + *Ctenosaura* Malone et al. (2017) estimated 20.1 MY while MacLeod et al. (2015) estimated ~15.5 MY. We calibrated the MRCA of *Iguana* and *Ctenosaura* in BEAUti v2.6.6 (Bouckaert et al. 2019) using a log normal distribution of divergence times with a median of 18.9 MY (mean of the log-transformed distribution = 2.94) and a standard deviation of the log-transformed distribution of 0.085 based on a previous fossil-calibrated analysis of the iguanas (Malone et al. 2017). The intention was not to re-estimate the timing of the origin of *Cyclura* per se, which would likely be improved by a deeper family-level iguanine phylogeny, but rather to gain insight into the relative divergence times of ingroup *Cyclura* given a known calibrated node in the Miocene.

We ran the Markov Chain Monte Carlo (MCMC) simulations for 50 million generations and three independent replications, implementing the Beagle library v3.0.1 (Ayres et al. 2011) to speed up computations. We repeated the analyses three times with different starting numbers, sampling every 10^4 generations and discarding the first 10% of trees as burn-in following examination of log likelihood plots. We assessed convergence of the independent runs by a comparison of likelihood scores and model parameter estimates in Tracer v1.7.2 (Rambaut et al. 2018), calculating the effective sample size (ESS) values for each model parameter with the expectation that ESS values greater than 200 indicate adequate sampling of the posterior distribution. We combined the results from the three analyses using Logcombiner v2.6.6 (Rambaut and Drummond 2021) and generated a maximum clade credibility (MCC) tree using TreeAnotator v2.6.6 (Rambaut and Drummond 2021). We visualized resulting trees in DensiTree 2 v.2.2.7 (Bouckaert and Heled 2014) and FigTree v1.4.4 (Rambaut 2018).

2.4. Mitogenomes

We gathered cleaned contigs generated from Velvet alignments, organized by individual, and mapped *Cyclura* contigs to a reference mitogenome of *C. pinguis* (GenBank ID LN835346; Gan et al., unpubl.) in Geneious 10.2.5 (Biomatters® Auckland, New Zealand) using the Geneious mapper with medium sensitivity. For outgroups, we used the reference sequence *Anolis carolinensis* (EU747728) for our *A. sagrei* sample and *Iguana iguana* (AJ278511) for our *Iguana delicatissima* and *Ctenosaura* samples (Table 1). For each taxon, we mapped all available contigs from all available specimens, and then deleted the reference genome and extracted the consensus sequence. Thus, each consensus mitogenome sequence is chimeric, in that it is a consensus of nucleotides from multiple individuals of the same taxon to improve overall mitogenome coverage. We retained ambiguity codes in the consensus

Table 1
Mitogenome summary statistics for taxa in this study. The mitogenome for *I. delicatissima* is described elsewhere (Miller et al. 2019). Length is the total number of bases called for each sequence, while % coverage is the proportion of bases called relative to the reference alignment. % PC coverage is the number of bases called in each sequence for the protein-coding alignment relative to the reference alignment. The raw alignment is available on Dryad (https://doi.org/10.5061/dryad.9w0vt4bhq).

Taxon	Length (bp)	% Coverage	GC%	PC Length	% PC Coverage	Reference
C. carinata	16,620	99.9	43.8	11,349	100	LN835346
C. collei	10,730	64.9	44.3	7,602	67.0	LN835346
C. cornuta	16,610	100	42.5	11,349	100	LN835346
C. c. cychlura	15,859	95.5	43.4	10,977	96.7	LN835346
C. c. figginsi	16,222	97.7	43.1	11,082	97.6	LN835346
C. c. inornata	14,086	84.9	43.4	10,397	91.6	LN835346
C. lewisi	9,041	54.7	42.7	5,996	52.8	LN835346
C. n. caymanensis	16,138	97.1	43.1	11,135	98.1	LN835346
C. n. nubila	13,579	81.7	42.9	9,326	82.2	LN835346
C. pinguis	15,781	94.9	42.9	10,784	95.0	LN835346
C. ricordii	15,848	95.4	44.3	11,157	98.3	LN835346
C. r. cristata	15,397	92.7	42.9	10,629	93.7	LN835346
C. r. nuchalis	16,427	98.9	42.7	11,280	99.4	LN835346
C. r. rileyi	16,616	100	42.8	11,349	100	LN835346
C. stejnegeri	16,612	100	42.5	11,349	100	LN835346
Ct. bakeri	16,626	100	43.5	11,349	100	AJ278511
Ct. melanosterna	15,764	95.1	43.0	10,741	94.6	AJ278511
Ct. oedirhina	16,620	100	43.5	11,349	100	AJ278511
Ct. palearis	16,171	97.3	43.6	10,969	96.7	AJ278511
Ct. similis	16,588	99.8	42.6	11,317	99.7	AJ278511
I. delicatissima	16,614	100	44.5	11,349	100	AJ278511

sequence, then aligned all consensus sequences together to create a mitogenome alignment for all our *Cyclura* taxa as well as outgroups. We then annotated these mitogenomes using the MITOS2 webserver (https://mitos2.bioinf.uni-leipzig.de/index.py; Bernt et al. 2013) and verified correct annotation via alignment with the reference mitogenomes. We subsequently manually verified the positions of start and stop codons and indels in protein-coding regions of all alignments.

We used the MAFFT algorithm (Katoh and Standley 2013) to generate an alignment of only protein-coding loci from the mitogenomes, consisting of an average of 10,611 bp of sequence data (range 5996–11,349 bp per taxon; Table 1). We then conducted maximum likelihood (ML) analysis on this alignment using the RAxML algorithm (Stamatakis 2006) implemented in the RAxML plugin in Geneious. We used the GTRGAMMA model and the rapid bootstrapping procedure with 1000 bootstrap (BS) replicates followed by the thorough ML search option with 100 independent searches. To estimate divergence times across the mitochondrial gene tree, we inferred a time-calibrated coalescent tree in Beast2 (Bouckaert et al. 2019) with Beagle v3.0.1 (Ayres et al. 2011). We used a strict clock model with a yule prior and calibrated the MRCA of *Iguana* and *Ctenosaura* as above, then ran the MCMC as above with 100 million generations per run. We visualized resulting trees in FigTree v1.4.4 (Rambaut 2018).

3. Results

3.1. Sequencing and bioinformatics

We obtained 150-bp raw sequence reads for all 82 individuals of ingroup and outgroup taxa, with a range of 5.0×10^5–2.7×10^6 reads per individual (approx. 7.5×10^7–3.9×10^8 bp per individual; Table S1). After cleaning and adapter trimming, we retained on average 96.9% of the sequence reads (range across individuals 95.7% – 98.1%; Fig. S2)—with only one individual (*Cyclura nubila nubila* from Cuba) retaining fewer (45%) of the sequence reads, likely owing to DNA degradation in this older sample (Table S1). Summary statistics of UCE loci from these reads are described below.

3.2. Mitogenome phylogenomic analyses

We obtained partial to near-complete mitogenomes for all taxa represented by our sample set, including all 15 ingroup *Cyclura* taxa (10 species and five subspecies) and seven outgroup species (Table 1). We fully characterized the *I. delicatissima* genome as proof-of-concept for our UCE bycatch approach, which is described elsewhere (Miller et al. 2019). Mitogenome sequences ranged from 9,041–16,626 bp in length (mean = 15,426; Table 1), with % GC content from 42.5 to 44.5 (mean = 43.2) (Table 1), comparable to fully annotated genomes for *C. pinguis* (16,631 bp; Gan et al., unpub), *I. delicatissima* (16,614 bp, % GC = 44.5; Miller et al. 2019), and *I. iguana* (16,633 bp; Janke et al. 2001). We did not detect long control region repeats, as are found in the Galápagos iguanas *Conolophus* and *Amblyrhynchus* (MacLeod et al. 2016). All annotated mitogenomes are available on Dryad (https://doi.org/10.5061/dryad.9w0vt4bhq).

Both our Maximum Likelihood and time-calibrated Bayesian phylogeny using the mitogenome data (Figs. S3, S4) reveal well-supported and identical topologies concordant with previous studies using mitogenomes with fewer taxa (e.g., MacLeod et al. 2016) or fewer samples and a single mtDNA locus (e.g., Malone et al. 2000).

3.3. UCE phylogenomic analyses

Our bioinformatics pipeline recovered a total of 4,055 independent UCE loci across 82 samples with a mean of 2,131 UCE loci per sample (Table S1; Fig. S5). These loci ranged from 35 to 1982 bp in length, with a mean per-locus length of 463 bp (Fig. S6). Zero UCE loci had all 82 samples represented, and hence we reduced our dataset to the 50% complete matrix that contained 1,872 loci and 687,308 bp. Clades in our ML tree corresponded to recognized taxonomic units in *Cyclura*, and major clades were well supported (Fig. 2). Our ASTRAL-III species tree showed strong local posterior probability for most currently recognized species divisions but weak support for some subspecies divisions (Fig. 3). Our SVDquartets species tree similarly showed strong bootstrap support for species and was nearly identical to our ASTRAL tree in topology, with the exception of a rearrangement of (and stronger support for) sister lineages in *C. rileyi* (Fig. 3).

For our *32PIS* dataset, our measure of parsimony-informative sites in each of these 32 loci ranged from 44 to 93 (mean = 55.1), with an average locus length of 597.5 bp (range 227–1208 bp). Loci with more parsimony-informative sites also tended to be longer (Fig. S7), while 367 of 4055 loci had zero parsimony-informative sites. Thus, our *32PIS* dataset included a maximum of 19,120 bp of sequence data per taxon. Our StarBEAST2 analysis produced a moderately resolved species tree

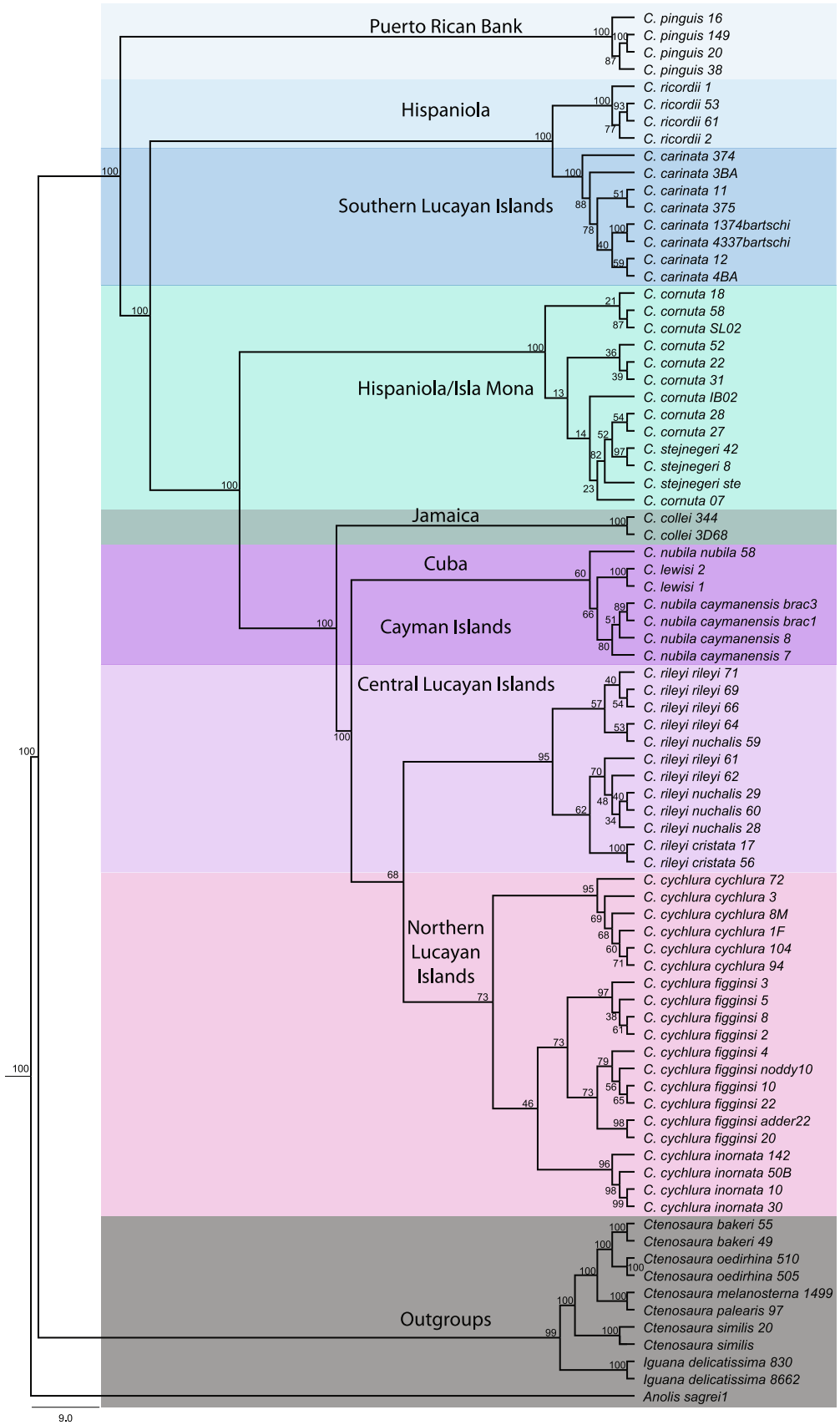

Fig. 2. Maximum likelihood phylogeny for all sampled taxa constructed from the 50% UCE dataset (n = 81, 1,872 loci, 687,308 bp). Numbers at nodes represent bootstrap support from our RAxML-HPC analyses.

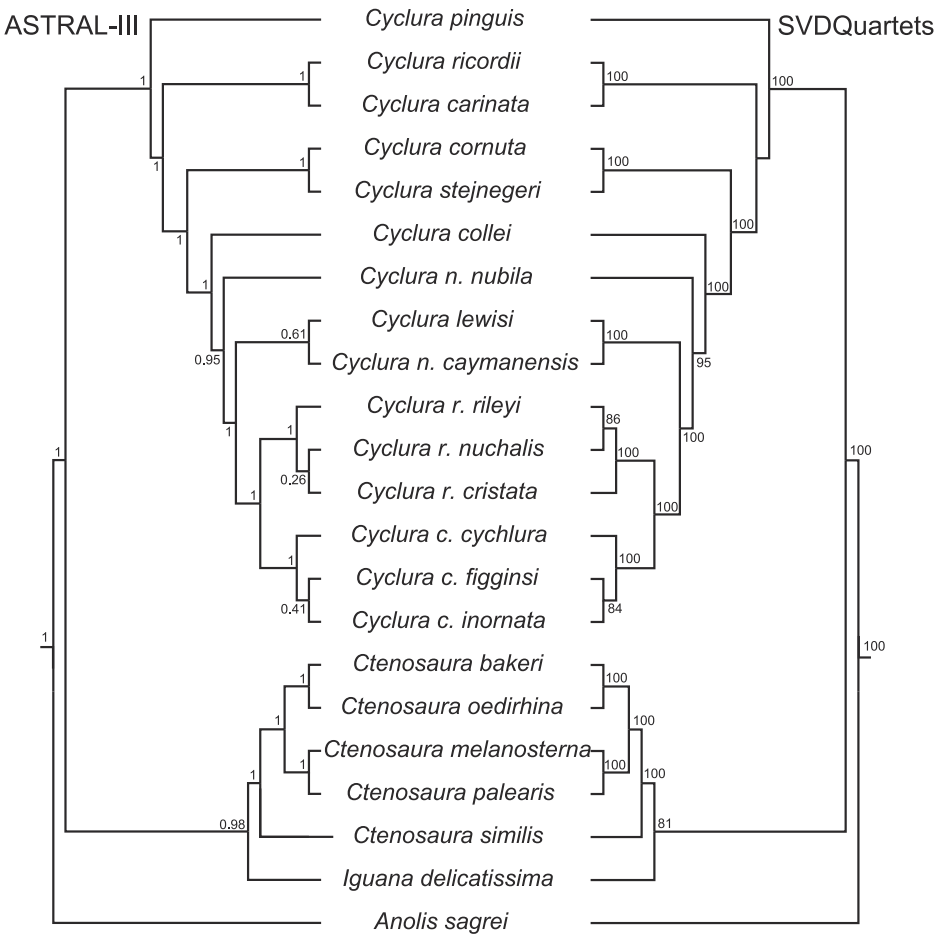

Fig. 3. Gene tree/species tree reconstructions using ASTRAL-III (left) and SVDQuartets (right) on the 50% UCE dataset (n = 81, 1,872 loci, 687,308 bp). Numbers at nodes represent local posterior probabilities (ASTRAL) and bootstrap support (SVDQuartets).

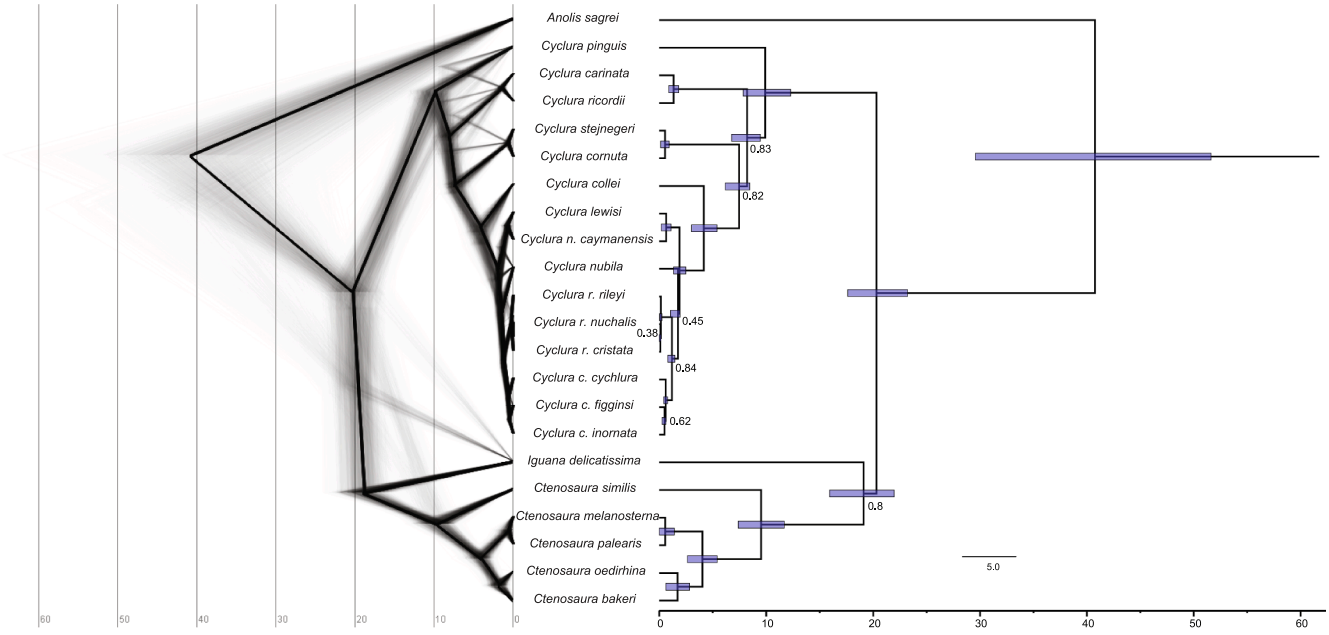

Fig. 4. Left: DensiTree phylogram from post-burn-in trees resulting from multi-locus gene tree/species tree analyses in StarBEAST2, with the maximum-clade credibility tree overlaid. Right: the maximum-clade credibility tree from the same analyses, with blue bars indicating the 95% Highest Posterior Density estimates for node ages. These trees were generated from a maximum of 19,120 bp from the 32 most informative UCE loci. Numbers at nodes represent posterior probabilities (PP) <1.0. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

(Fig. 4), owing in part to our inability to yield ESS values greater than 100 for the posterior parameter despite altering run lengths and priors in initial runs. Hence, we prefer to discuss cladogenesis with our SVDQuartets and ASTRAL topologies (given that they contain two orders of magnitude more loci) and divergence times with our StarBEAST2 tree.

All these UCE analyses revealed a topology that was discordant with our (and previous) mtDNA topologies. Mitogenomes and UCE analyses both show a clade grouping a Hispaniolan (*C. ricordii*) and a southern Lucayan species (*C. carinata*), and another clade grouping *C. cornuta* and *C. stejnegeri* of Hispaniola/Isla Mona. These clades are sister taxa in the mitogenomic analysis, whereas the UCE analysis places *C. carinata* and *C. ricordii* as the sister taxon to all other *Cyclura* outside of *C. pinguis* (Figs. 3, 4, S3, S4). Among our outgroups, mitogenomes show *Ctenosaura similis* as the sister taxon to all other *Ctenosaura*. Our UCE data confirm placement of *C. similis* as the sister taxon to a clade comprising the other *Ctenosaura*, with the Honduran Bay Island *C. bakeri* + *C. oedirhina* forming the sister taxon to the mainland *C. melanosterna* + *C. palearis* (Figs. 3, 4).

Among the UCE topologies, we found moderate support for monophyly of each of the three Lucayan subspecies *C. cychlura cychlura*, *C cychlura inornata*, and *C cychlura figginsi* (BS = 46–100%; Figs. 2, 3). We

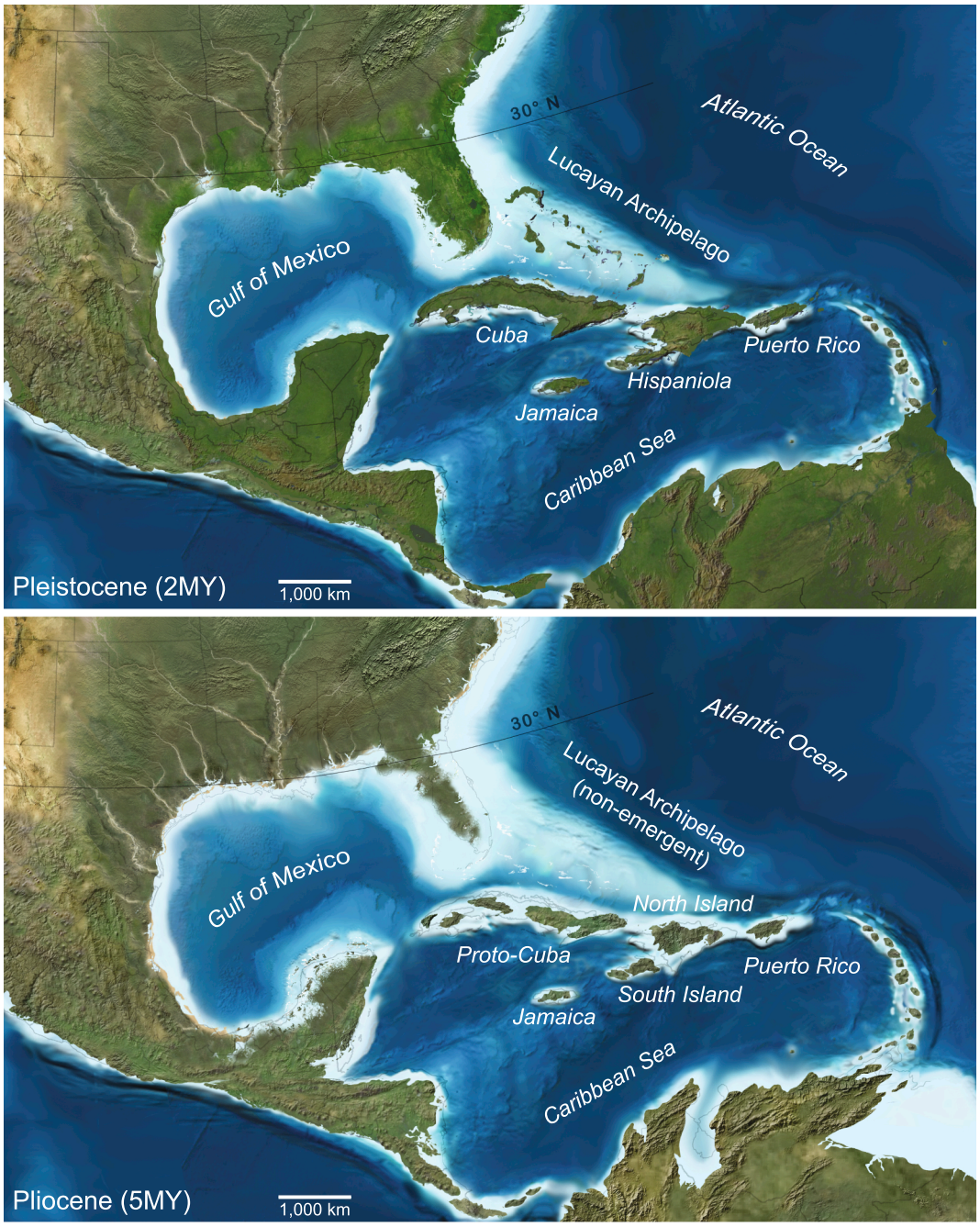

Fig. 5. Reconstructions of the paleogeography of the Greater Antilles during the Pliocene (5 MY ago) and the Pleistocene (2MY), when the genus *Cyclura* underwent significant cladogenesis. The outlines of the current islands are shown as thin black lines. Note that during these time periods most of the major Greater Antillean islands were emergent, although only Puerto Rico and Jamaica were not fragmented into smaller paleoislands. Hispaniola existed as yet-unjoined north and south paleoislands, and Cuba as an archipelago of islands that eventually coalesced. The Lucayan Archipelago did not emerge until the start of the Pleistocene. Maps used with permission, © 2022 Colorado Plateau Geosystems Inc.

found no support for distinction of the three Lucayan *C. rileyi* subspecies in our ML analysis (Fig. 2), but some inconsistent support for their separation in our gene tree/species tree analyses (Figs. 3, 4). The northwest Caribbean species *C. n. nubila*, *C. lewisi*, and *C. n. caymanensis* were similarly only moderately supported (BS = 60–100%; Figs. 2, 3). We further found that Isla de Mona *C. stejnegeri* was non-monophyletic and nested within Hispaniolan *C. cornuta* (Fig. 2; but see below), although species-tree analyses suggested strong support for a sister-group relationship between the two (Figs. 3, 4). Our divergence-time analysis suggested an MRCA of extant *Cyclura* at 9.91 MY (95% Highest Posterior Density [HPD] interval = 12.28–7.85) at the node subtending the sister-group relationship between *C. pinguis* and all other *Cyclura* (Fig. 4), with early cladogenesis occurring in the paleo Hispaniolan and Puerto Rican islands (see Fig. 5). Most cladogenesis among extant lineages in the genus occurred during the Pleistocene (<2.5 MY).

The Caymanian lineages *C. n. caymanensis* and *C. lewisi* were recently diverged (0.64 MY, 95% HPD = 1.09–0.17), which is entirely contrary to the mtDNA results (Fig. S4). Jamaican *C. collei*, among the most imperiled species in the genus, was the sister species to Lucayan-Cuban-Caymanian lineages, with an MRCA at 4.15 MY (95% HPD = 5.39–3.01), which is well after the re-emergence of Jamaica (possibly in the mid-Miocene; Robinson 1994). Cuban lineages formed the sister group to Lucayan and Caymanian lineages, with an MRCA of 1.45 MY (95% HPD 1.93–1.03), which is far more recent than reported by Shaney et al. (2020) at 8.2 MY. Northern Lucayan lineages share an MRCA at 1.16 MY (95% HPD = 1.45–0.78), while the southern Lucayan *C. carinata* split from Hispaniolan *C. ricordii* 1.32 MY (95% HPD = 1.81–0.88).

Among the outgroups, the Honduran Bay Island lineages (*Ctenosaura bakeri* and *Ct. oedirhina*) are 1.71 MY divergent (95% HPD = 2.83–0.60). The Mesoamerican mainland lineages (*Ct. melanosterna* and *Ct. palearis*) are 0.55 MY divergent (95% HPD interval 1.4–0.00), and they share a MRCA 4.03 MY ago (95% HPD interval 5.41–2.62) with the Bay Islands species. The cladogenesis of extant *Ctenosaura* dates to 9.53 MY (95% HPD interval 11.69–7.37).

4. Discussion

It is clear that the biodiversity of islands around the world is in peril (Fernández-Palacios et al. 2021). Addressing the impending loss of biodiversity requires an understanding of biodiversity, and such an approach might include working to resolve a Linnean shortfall (unknown or uncharacterized species) as well as a Darwinian shortfall (unknown evolutionary relationships among species; Brown and Lomolino 1998). Such is the motivation for our desire to leverage modern genomic tools to understand the radiation of *Cyclura* iguanas in the Caribbean. This entire genus is highly imperiled, and one species has already gone extinct in historical times (Fig. S1). Addressing biodiversity shortfalls means that we must have a holistic understanding of both species diversity as well as species relationships, as the knowledge from this will be directly relevant to making crucial conservation decisions. Below we discuss our results in the context of the biogeography of the genus *Cyclura*, illustrating how our novel genomic data boost our understanding of the origins and relationships of these species.

The genus *Cyclura* almost certainly arose in the Miocene when Greater Antillean islands were mostly emergent and beginning to coalesce. Shaney et al. (2020) used a calibration of 14.5 MY for the root of *Cyclura*, based in part on MacLeod et al. (2015), although the latter authors found a divergence time about 1 MY prior to that date. Shaney et al. (2020) found much earlier divergence times for some species pairs, such as *C. carinata/C. ricordii* (6.1 MY) and *C. nubila* + *C. cychlura* (8.2 MY). Indeed, they estimate as much as 6.8 MY of divergence among lineages of *C. n. nubila* on Cuba. With a calibrated MRCA of 18.9 MY for the *Iguana-Ctenosaura* clade (Malone et al. 2017), our mitogenome dataset yielded a much deeper set of divergence times, with an MRCA of extant *Cyclura* at 18.82 MY (95% HPD 22.17–15.60, PP = 1.0; Fig. S4). Our gene tree/species tree analyses, using the same calibration as our mitogenome analyses, suggested that extant *Cyclura* share a MRCA about 9.91 MY ago (95% HPD 12.28–7.85, PP = 1.0; Fig. S4). This is not the time when *Cyclura* arose, but the time of the first cladogenesis of the extant taxa (Figs. 3, 4). This corresponds to a time when Puerto Rico and the Hispaniolan north and south paleoioslands were emergent and in relatively close proximity (Fig. 5). *Cyclura* exclusive of *C. pinguis* began cladogenesis in the late Miocene ~ 8.11 MY (PP = 1, BS = 100%, 95% HPD = 9.45–6.77 MY) to produce (1) a lineage ancestral to species currently occupying Hispaniola and the southern Lucayan islands, (2) a lineage ancestral to species on Hispaniola and Isla de Mona, and (3) a third lineage, ancestral to species currently occupying Cuba, Jamaica, Cayman Islands, and The Bahamas. The Hispaniola–Mona divergence of *C. stejnegeri* and *C. cornuta* was very recent, in the Pleistocene (PP = 1, BS = 100%, 0.53 MY, 95% HPD = 0.91–0.12 MY), while most other sister species diverged between ~3.0–0.78 MY (Fig. 4). Of note, our mitogenome analyses suggested reciprocal monophyly among the Hispaniola-southern Lucayan species (Figs. S3, S4), which was not supported in our UCE analyses. Within the Bahamas-Cuba-Cayman clade (PP = 1, BS = 100%, 1.89 MY, 95% HPD = 2.48–1.33 MY), our ASTRAL and SVDQuartets analyses found that the Caymanian lineage is the sister taxon to the cental and northern Lucayan lineages (Fig. 3), while our StarBEAST2 analyses grouped the Caymanian *C. n. caymanensis* and *C. lewisi* as the sister groups to the remaining Lucayan and Cuban lineages. This is likely owing to our inability to resolve the complexity of Cuban-Caymanian lineages (see below and Shaney et al. 2020). We discuss cladogenesis, biogeography, conservation, and taxonomy in the context of the UCE trees and divergence estimates below.

4.1. Southern Lucayan Islands and Hispaniola

The geologic history of Hispaniola is complex and included two large Miocene paleoislands (Fig. 5) that merged in the Pliocene/Pleistocene, followed by repeated periods of isolation due to sea-level changes during the Plio-Pleistocene (Iturralde-Vinent 2006). Hence, determining whether ancestral *Cyclura* evolved on the north or south paleoisland in the Miocene is difficult. Nevertheless, Hispaniola likely served as an early cladogenic region for *Cyclura* during the Miocene (Fig. 5), with three major lineages comprising all extant species except for *C. pinguis* tracing ancestry to that region (Figs. 3, 4). Early cladogenesis in the Miocene led to all extant *Cyclura*, including two ancestral lineages leading to the two extant Hispaniolan species *C. ricordii* and *C. cornuta* (Figs. 3, 4). Each of these ancestral lineages in turn gave rise to additional species; the former yielding *C. carinata* in the southern Lucayan islands (PP = 1, 1.32 MY, 95% HPD = 1.81–0.88 MY) and the later yielding *C. stejnegeri* on Isla de Mona in the mid-Pleistocene (PP = 1, 0.53 MY, 95% HPD = 0.91–0.12 MY; Fig. 4). A third lineage, *C. onchiopsis* from Navassa Island 74 km west of Hispaniola, has recently gone extinct and was thought to be a product of dispersal by ancestral *C. cornuta* (Powell 2000). Ancient DNA might resolve this, although we attempted to sequence DNA from preserved specimens and were not able to obtain useful sequence reads (Myers, Miller, Reynolds, and de Queiroz, unpubl. data).

Cyclura stejnegeri is endemic to Isla de Mona, likely having colonized this area from Hispaniola during the mid-Pleistocene (Fig. 4; see additional discussion below). The MRCA of *Cyclura cornuta* + *C. stejnegeri* diverged from other *Cyclura* 7.39 MY (PP = 1, 95% HPD = 6.17–8.46 MY). It is thought that the historic range of *C. cornuta* could have spanned most of the xeric habitat on Hispaniola (Schwartz and Carey 1977), although modern *C. cornuta* populations are localized and fragmented (Schwartz and Henderson 1991; Ottenwalder 2000; Powell et al. 2000, Pasachnik et al. 2020).

Two of the species derived from the Hispaniolan diaspora are restricted to small island banks. *Cyclura carinata* was widespread across the Caicos and Turks banks prior to human activity (Lemm and Alberts 2011; ITWG 2016; Welch et al. 2017; Gerber et al. 2020), a region that would have been relatively easily colonized from northern Hispaniola

when the (now submerged) Navidad, Silver, and Mouchoir banks were emergent. Despite the inferred avenue for dispersal when banks were emergent in the late Pleistocene, *C. carinata* appears to be considerably older than the most recent emergence of these banks (1.32 MY divergence between *C. carinata* and *C. ricordii*; Fig. 5), although this is consistent in age with other terrestrial squamates in the Lucayan Archipelago (e.g., Reynolds et al. 2016). It is also worth noting that Europeans might have played a role in establishing the Mayaguana (i.e., Booby Cay) population of *C. carinata*, which is not at all divergent from Turks and Caicos Islands populations (Bryan et al. 2007; Welch et al. 2017; Fig. 2), and that the Turks versus Caicos Island banks do not delimit population genetic structure (Welch et al. 2017).

4.2. Lucayan Archipelago, Cayman Islands, Cuba

Mitochondrial DNA from our study and previous studies (Malone et al., 2000, 2003; Figs. S3, S4) suggested that Cuban and Cayman *Cyclura* are nested within Lucayan lineages, and further that *C. rileyi* is the sister lineage to the remainder of this Cuba-Lucayan-Caymans clade. The biogeographic implications are that the Lucayan Islands served as the source population from which *Cyclura* colonized Cuba and the Cayman Islands during the Pleistocene, a dubious scenario considering that the Lucayan Islands did not emerge until the Pleistocene (e.g., Godefroid et al. 2018; Fig. 5). On the contrary, our analyses of genomic UCE loci suggest a different and more plausible scenario in which Cuban *Cyclura* served as the ancestral population from which the Lucayan Islands were colonized, followed by speciation there in allopatry (Figs. 3, 4). Such a scenario is much more congruent with other squamate historical biogeographic reconstructions concerning Lucayan, Cayman, and Cuban lineages (e.g., Reynolds et al. 2020). Under this scenario, the northern Lucayan ancestral lineage (ancestral *C. rileyi* and *C. cychlura*) diverged from Cuban ancestors in the Pleistocene (1.45 MY, 95% HPD 1.93–1.03 MY; also see Schwartz and Carey 1977), likely coincident with the formation of the modern Lucayan Banks (Fig. 5). This finding, that northern Lucayan species are nearly as ancient as the Caymanian species, was previously suggested by Schwartz and Carey (1977). Lucayan colonization and speciation events around 1.16 MY are consistent with numerous other phylogeographic reconstructions of Lucayan squamate evolution that lend weight to the growing understanding that some islands were present in the region throughout at least the Pleistocene (Schwartz 1968; Reynolds et al. 2013, 2016, 2020; Tucker et al. 2017). We are less confident in our reconstruction of Caymanian lineages (*C. lewisi* and *C. n. caymanensis*) owing to our limited sampling of Cuban *C. n. nubila*. Recent mitochondrial and microsatellite analyses with more sampling from Cuba tell a more complex story, suggesting multiple colonization of the Cayman Islands from divergent Cuban lineages (see discussion below; Shaney et al. 2020).

4.3. Taxonomic implications for West Indian Rock iguanas

4.3.1. Isla de Mona, Navassa, and Hispaniola

Recent work (Pasachnik et al. 2020) did not support reciprocal monophyly of *C. stejnegeri* and *C. cornuta* across genetic markers. Those authors found that the two species share mtDNA haplotypes, but that more rapidly-evolving microsatellite markers demonstrate a pattern of long-standing isolation on Isla de Mona. Our mitogenome data supported reciprocal monophyly (Figs. S3, S4), but our UCE data showed that individual samples from Isla de Mona and the Dominican Republic were not reciprocally monophyletic (Fig. 2). The population genetic analysis of Pasachnik et al. (2020) indicated that incomplete lineage sorting among relatively slowly-evolving loci might account for this pattern. Nevertheless, we find strong support for these two lineages in our gene tree/species tree analyses (Figs. 3, 4), suggesting that individual gene trees are recording a history of isolation of these lineages and that collectively these gene trees support the distinction of the two.

Although we have previously attempted to obtain DNA from specimens of *C. onchiopsis* (Myers, Miller, de Queiroz, and Reynolds, unpubl. data), the effort was unsuccessful and hence we only have morphological data for this species. *Cyclura onchiopsis* is likely closely related to *C. cornuta* and was elevated to species status based on presumed reproductive isolation prior to its extinction, as well as some morphological traits (Schwartz and Carey 1977; ITWG 2016 and references therein).

4.3.2. Cuban and Cayman Cyclura

A stark difference exists for inferred biogeographic histories of these taxa based on mitochondrial versus nuclear data. A previous study (Malone et al. 2000) as well as our mitogenome dataset (Figs. S3, S4) suggested that Cayman *C. lewisi* is the sister taxon to a clade consisting of *Cyclura* from Cuba, the Cayman Islands, and the northern Lucayan Islands – a result that contradicted a previous biogeographic hypothesis by Schwartz and Carey (1977) and was challenging to interpret geographically. These data were also used to justify significant conservation interventions such as the recognition of *C. lewisi* instead of *C. nubila lewisi* (Burton 2004; Stephen 2012), even though a subsequent study could not reproduce this result with additional Cuban sampling (Starostová et al. 2009). Previous authors (Echternacht et al. 2011; Hedges et al. 2019) suggested that each of the lineages *C. n. nubila*, *C. n. caymanensis*, and *C. lewisi* should be recognized as separate allopatric species. Our UCE dataset revealed that the mitochondrial phylogeny is not consistent regarding evolutionary relationships and rather supports the prior biogeographic hypothesis of Schwartz and Carey (1977) (Figs. 2–4). Our species tree analyses showed that ancestral *Cyclura* probably dispersed from Cuba to the Cayman Islands and the Bahamas separately (Fig. 3). The ancestral Caymanian lineage separated from the ancestral northern Lucayan lineage around 1.0–2.65 MY following cladogenesis from a MRCA with Cuban *C. n. nubila* (Figs. 3, 4). However, our analysis included only a single sample of *C. n. nubila* from all of Cuba, an island that was extensively fragmented from the Pliocene to the late Pleistocene (e.g., Fig. 11 in Iturralde-Vinent 2006). A recent study using island-wide sampling of Cuban *C. n. nubila* revealed that Lucayan and Cayman lineages are derived from structured populations of *C. n. nubila* on Cuba (Shaney et al. 2020), as was also recently found in the lizard *Anolis sagrei* (Reynolds et al. 2020). Further, there is deep east–west phylogenetic structure on Cuba (Starostová et al. 2009; Shaney et al. 2020), and it is worth noting that many Cuban terrestrial vertebrates show a distinct east–west divergence on the island (Weiss and Hedges 2007; Rodríguez-Schettino et al. 2010), without always leading to their recognition as separate species (e.g., Reynolds et al. 2020). This study suggested that *C. lewisi* was the sister lineage to the western phylogroup of *C. n. nubila*, and that *C. n. caymanensis* is derived from multiple lineages of the eastern phylogroup of *C. n. nubila*. Given the results of our collective studies, there was likely more than one colonization of the Cayman Islands from Cuba, and it is not yet clear whether *C. lewisi* and *C. n. caymanensis* should be recognized as separate species from *C. nubila*.

4.3.3. Northern Lucayan Cyclura

Our analyses suggest that ancestral *Cyclura* on Cuba colonized the Lucayan Islands by dispersal around 1.45 MY (PP = 1; 95% HPD = 1.93–1.03 MY; but PP = 0.45 in Fig. 4), followed by the divergence of the central (*C. rileyi*) and northwestern Lucayan Islands populations (*C. cychlura*), beginning around 1.16 MY (PP = 0.84; 95% HPD = 1.45–0.78 MY).

The subspecies of *C. rileyi* were initially diagnosed based on minor differences in color and modal scale character counts (Schwartz and Carey 1977). *Cyclura r. cristata* from Sandy Cay, Exumas, was distinguished by dark gray dorsal background color and enlarged post-sacral dorsal crest scales, whereas *C. r. rileyi* from San Salvador and *C. r. nuchalis* from Crooked-Acklins were distinguished by slightly differing dorsal color and minor differences in head scale characters (Schwartz and Carey 1977). Carter and Hayes (2004) identified apparent

differences among the subspecies in body sizes, femoral pore counts, and some head scalation characters (the latter with small sample sizes), characters that might be reasonably expected to be plastic or environmentally-induced rather than a reflection of divergent evolution and the process of speciation (Fox et al. 1961; Wegener et al. 2014; Baeckens et al. 2015; Ortega et al. 2019). Malone et al. (2000) found no variable positions (=segregating sites) among these subspecies using a single mtDNA gene, and the subspecies were described as "poorly-defined" by Lemm and Alberts (2011). Carter and Hayes (2004) stated that "final resolution of taxonomic status of *C. rileyi* should be determined by analysis of more rapidly diverging nuclear markers." To the latter point, if these subspecies were indeed evolutionarily distinct enough to warrant taxonomic recognition it is not clear why more rapidly evolving markers would be necessary, but it is the case that analysis of genetic structure (partitioning of intraspecific diversity and divergence) could be assessed using a genotyping approach.

Our genetic datasets and analyses suggested that these populations are not very genetically (evolutionarily) divergent or reciprocally monophyletic (e.g., Figs. 2–4, S4). Further, field observations of these populations suggest that coloration and scalation are variable in this species, and that there is little morphologically to distinguish among these populations (Iverson et al. 2016). For example, populations of *C. rileyi nuchalis* also have enlarged post-sacral dorsal crest scales (Fig. S8), a trait supposedly diagnostic only of *C. r. cristata* (Schwartz and Carey 1977). Despite the obvious conservation concerns (all populations are small and vulnerable), we find no support for recognizing the subspecies *C. rileyi nuchalis* and *C. rileyi cristata* and recommend their synonymy under *C. rileyi* which has taxonomic precedent.

Further, our results suggest the possibility that the population in the Exumas (*C. r. cristata*) and those in the Crooked-Acklins (*C. r. nuchalis*) could be the result of historic human-mediated dispersal. For example, *C. r. cristata* is known only from Sandy Cay in the southern Exumas, which lies on the Great Bahama Bank (including at least Andros, the Exumas, and New Providence), otherwise inhabited by *C. cychlura* (ITWG 2016), and both geographically and zoogeographically remote from San Salvador. Similarly, *C. r. nuchalis* is only known today from an introduced population in the northern Exumas (Iverson et al. 2016) and two small islands on the western edge of the Acklins Bight (also zoogeographically remote), adjacent to Long Cay (with historic records of *C. r. nuchalis*: Schwartz and Carey 1977, and believed by local residents still to exist on the cay). Long Cay was formerly called Fortune Island, because it was the site of intensive development based on sponge and salt production and it served as a major trading port as early as the 1700s; thus, colonists almost certainly interacted with this population. The lack of genetic divergence among these remote populations suggests that *C. r. cristata* and *C. r. nuchalis* represent recent introductions prior to or following European settlement (e.g., LeFebvre et al. 2019), a hypothesis that could be examined with future fine-scale sampling and historical demographic reconstruction.

Whereas our mitochondrial data suggested that northern Lucayan *Cyclura* (*C. c. cychlura*, *C. c. inornata*, *C. c. figginsi*) are not the sister taxa to *C. rileyi*, our phylogenomic analyses showed that they are reciprocally monophyletic (Figs. 3, 4), and that they derived from a (likely) Cuban/Cayman ancestor (Fig. 3). We found strong support for cladogenesis between *C. c. cychlura* and the lineage *C. c. figginsi* + *C. c. inornata*, but low support for reciprocal monophyly of the latter two (Figs. 3, 4). Interestingly, differentiation between the Exuman taxa, *C. c. figginsi* and *C. c. inornata*, is supported via microsatellite analysis (Colosimo 2016). That study also revealed a lack of distinction between *C. c. inornata* and *C. c. figginsi*, and Aplasca et al. (2016) found little divergence between presumably natural populations of *C. c. inornata*. Our 50% UCE data set (Fig. 2) supported the reciprocal monophyly of the central Exuman populations from southern Exuman populations, which suggests some genetic structure in that taxon. This finding has been further supported by Colosimo et al. (2021), who found that both genetic data and the genetic structure of parasites support at least two lineages of *C. c. figginsi*. It should be noted that although the estimated divergences in the *C. cychlura* complex exceed 0.5 million years, the three included taxa all occupied the same Great Bahama Bank (Knapp et al. 2011) during the last glaciation (<20 KA). Finally, it is also worth noting that *C. c. cychlura* exhibits considerable genetic variation across the fragmented island and cays of Andros, possibly owing to allopatry induced by the archipelago-like structure of the Androsian Islands (Colosimo et al. 2014, 2021), which merits future attention.

4.4. Conclusions

Our results clarify the species and subspecies-level taxonomy within the genus *Cyclura*, which is essential for prioritizing limited conservation resources. We note with interest that several biogeographic and evolutionary scenarios proposed by Schwartz and Carey (1977), based solely on morphology, were not supported by mtDNA data analysis (e.g., Malone et al. 2000; Figs. S3, S4), but were recognized as having been prescient based on our phylogenomic analysis (Figs. 3, 4). For example, we find support for an origin of Lucayan-Cuban-Cayman iguanas on Cuba and a single colonization of the central/northern Lucayan Islands from Cuba. Despite better resolution of their evolutionary relationships, it is not yet clear whether the two Caymanian lineages (*C. lewisi* and *C. n. caymanensis*) should be considered distinct species, and other studies (e.g., Shaney et al. 2020) suggested that their recognition would render *C. n. nubila* on Cuba paraphyletic. The subspecies of the northern Lucayan *C. rileyi* are not evolutionarily distinct, but we did find evidence for deeper structure in *C. c. figginsi*. Thus, we recommend recognition of ten extant species in line with recommendations from the ITWG (2016) and eight subspecies, but additional work could be done with better population-level sampling than we achieved to investigate the recent evolutionary history of the northern Lucayan taxa, the Cuban and Caymanian taxa, and the taxon on Isla Mona.

CRediT authorship contribution statement

R. Graham Reynolds: Conceptualization, Writing – original draft, Methodology, Writing – original draft. **Aryeh H. Miller:** Methodology, Writing – original draft. **Stesha A. Pasachnik:** Writing – review & editing. **Charles R. Knapp:** Writing – review & editing. **Mark E. Welch:** Writing – review & editing. **Giuliano Colosimo:** Writing – review & editing. **Glenn P. Gerber:** Writing – review & editing. **Brian Drawert:** . **John B. Iverson:** Conceptualization, Writing – original draft.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Islands (TCI), the British Virgin Islands (BVI), and the Cayman Islands (CI) were collected with permission of the TCI Department of Environment and Coastal Resources, the BVI National Parks Trust, and the CI Department of Environment, respectively. Samples from Isla de Mona were collected by Nester Perez. All *Ctenosaura* samples were collected under permits to Stesha Pasachnik by the Honduran and Guatemalan governments. All animal capture, handling, and sampling was performed following the American Society of Ichthyologists and Herpetologists (ASIH) guidelines for use of reptiles and amphibians in research and all methods were approved under the authors' IACUC permits. We are grateful to Allan Larson and Jim McGuire for excellent comments and suggestions on previous versions of this manuscript.

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Data accessibility

Additional supplemental data, tables, and figures follow this manuscript. Annotated alignments and trees are available on Dryad (https://doi.org/10.5061/dryad.9w0vt4bhq), and mitochondrial sequences are available on GenBank (ON322935-ON322953).

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ympev.2022.107548.

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