Phylogeography of the Caribbean Rock Iguana (*Cyclura*): Implications for Conservation and Insights on the Biogeographic History of the West Indies[1]

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The Caribbean rock iguana, *Cyclura,* has had an unstable intrageneric taxonomy and an unclear phylogenetic position within the family Iguanidae. We use mtDNA sequence data to address these issues and explore the phylogeographic history of the genus. ND4 to leucine tRNA sequence data were collected from multiple individuals of each of the eight species of *Cyclura* (including 15 of 16 subspecies) and from four localities of *Iguana iguana* (representative of this species' broad geographic range). This data set was combined with sequence data from Sites *et al.* (1996, *Mol. Biol. Evol.* 13, 1087–1105) and analyzed under maximum-parsimony and maximum-likelihood optimization criteria. The ND4 region provided good resolution for the majority of nodes, as indicated by high bootstrap support. In agreement with several recent molecular studies, *Cyclura* is recovered as monophyletic and is not closely related to any other genus, whereas *Iguana* is strongly supported as the sister taxon to *Sauromalus.* This result is statistically more likely than other published hypotheses of Iguanid relationships. *Cyclura* shows a southeast to northwest speciation sequence in the Caribbean, with the most ancient lineage on the Puerto Rican Bank. The amount of interspecific sequence divergence within *Cyclura* (maximum 11.4%) is very high in comparison to data from other iguanid taxa at this locus, suggesting that this group either has been in the Caribbean for a very long time or has gone through a very rapid rate of evolution at this locus. Using dates from other published studies, we calculate a molecular clock that suggests that *Cyclura* colonized the Caribbean between 15 and 35 mya. Several questions regarding subspecific taxonomy are raised in the analysis and await further investigation using a more rapidly evolving marker such as nuclear microsatellites. © 2000 Academic Press

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INTRODUCTION

The Caribbean rock iguanas (family Iguanidae, genus *Cyclura*) are one of the largest and rarest members of the native vertebrate fauna of the Greater Antilles. *Cyclura* are characterized by a high degree of endemism and are generally limited to a single species or subspecies per island (Fig. 3). This results in the species being particularly vulnerable to human disturbance and many populations face the real possibility of extinction (Carey, 1975; Iverson, 1978; Alberts, 2000). All extant species of *Cyclura* are considered endangered or vulnerable by the IUCN (IUCN, 1996) and all are listed as CITES appendix 1. Conservation resources are limited and their allocation should be based on biological factors such as the uniqueness of a lineage, the immediacy of the threat of extinction, and species distributions (Vane-Wright *et al.,* 1991; Crozier, 1997; Moritz, 1994). Despite the critical conservation status of many populations of *Cyclura,* data on the phylogenetic distinctiveness of the different island races are lacking. Without genetic data addressing species boundaries and distinctiveness of named subspecies, management decisions remain on a precarious footing. The single effort to reconstruct the evolutionary relationships within *Cyclura* was made by Schwartz and Carey (1977). Using a variety of morphological characters, they postulated a weakly supported phylogeny in which *C. carinata* and *C. ricordi* were identified as basal taxa to a second lineage consisting of *C. nubila, C. cornuta, C. collei,* and *C. rileyi.* The phylogenetic position of *C. pinguis* remained wholly enigmatic. In 1982 Burghart and Rand wrote, "this island group's taxonomy is unstable, and it may never be resolved as extinction closes in on these remarkable animals."

Not only are the relationships within *Cyclura* un-

clear, the evolutionary relationships within the Iguanidae (Frost and Etheridge, 1989) are problematic and there is controversy surrounding the taxonomic placement of *Cyclura* (de Quieroz, 1987; Lazell, 1992; Norell and de Quieroz, 1991; Petren and Case, 1997; Sites *et al.,* 1996). Studies using morphological characters (de Quieroz, 1987; Norell and de Quieroz, 1991) and mitochondrial 12s and 16s ribosomal sequence data (Rassman, 1997) have placed *Cyclura* as the sister clade to *Iguana,* although this relationship was weakly supported in those studies. A morphological study by Lazell (1973) led to his assertion that *Cyclura, Brachylophus,* and *Iguana* should be lumped into one genus. Molecular studies using mitochondrial sequence data from cytochrome *b* (Petren and Case, 1997) and ND4, both with and without the inclusion of morphological characters (Sites *et al.,* 1996), have established somewhat stronger support for *Cyclura* as a monophyletic lineage without close taxonomic affinity to any other iguanid genus. All of these studies have used limited taxonomic sampling of *Cyclura* and only a few individuals of *Iguana iguana.* Sites *et al.* (1996) and Weins and Hollingsworth (2000) have pointed out that this increases the potential for erroneous phylogenetic hypotheses due to long-branch attraction (Felsenstein, 1978).

Whereas the determination of systematic relationships is crucial for making conservation-oriented decisions, these data can also be used to explore phylogeographic history. A major question regarding the radiation of *Cyclura* is whether or not the group has had an intimate association with the geologic formation of the current West Indian islands. Unfortunately, the geological history of this area is unclear and the origin of its biota is a subject of intense debate (Crother and Guyer, 1996; Hedges, 1996a; Hedges *et al.,* 1992, 1994; Kluge, 1988; Williams, 1989). The controversy centers on the relative importance of vicariance versus dispersal in shaping the distribution of the founding Caribbean biota. The vicariance scenario maintains that the major portion of Caribbean taxa colonized the Great Arc as it passed eastward between the North and South American continents (Crother and Guyer, 1996; Guyer and Savage, 1986; Rosen, 1975, 1985). In contrast, the dispersalists view the Caribbean biota as more recent, originating through waif dispersal.

The classic approach to testing a theory of vicariance involves the use of multiple taxonomic cladograms and contemporary distributions of disparate taxa in combination with geologic models to detect congruent patterns of phylogenetic history (Donnelly, 1990; Rosen, 1985). Ideally, these studies would focus on highly endemic taxa with well-supported phylogenetic relationships. This method has proven ineffective for Caribbean biogeographers because of a paucity of fossils in the West Indies (which provide an upper time boundary for estimating colonization events), lack of well-supported phylogenies for Caribbean biota, and unresolved questions surrounding the geological history of this area (Hass *et al.,* 1993; Kluge, 1988).

The purpose of this study is primarily to undertake a comprehensive examination of the intrageneric relationships of *Cyclura.* The insight gained from our best-supported phylogeny is synthesized with current geologic information to examine the possible roles that vicariance and/or dispersal mechanisms have had in shaping the current distribution of *Cyclura.* We discuss the implications of our results in focusing conservation goals and for prioritizing future studies of *Cyclura.* As a secondary issue, we reanalyzed the intrafamilial relationships of Iguanidae to determine whether the results of the Sites *et al.* (1996) study are affected by breaking the long branches within *Cyclura* and *Iguana,* as hypothesized by Wiens and Hollingsworth (2000). We then test the fit of our data set with alternative published hypotheses.

METHODS

Collection

Blood was collected from wild and captive individuals of all eight species of *Cyclura,* including 15 of 16 subspecies (*C. carinata bartschi* was unavailable) to explore the phylogenetic relationships within the genus. At least two individuals were sampled from each subspecies. Individuals of *I. iguana* were sampled from disparate localities for use in the broader taxonomic investigation. Blood was stored in storage buffer (100 mM Tris, 100 mM Na$_2$EDTA, 10 mM NaCl, 1% SDS) at a ratio of 1 part blood to 2 parts buffer. Table 1 summarizes the collection information of all individuals.

Laboratory Procedures

Blood ($\sim$100 μg in 200 μl storage buffer) was digested in a 2-h incubation with 250 μg proteinase K at 55°C followed by phenol extractions and ethanol precipitation (Sambrook *et al.,* 1989). The resulting DNA pellet was dried, resuspended in 100–300 μl water, and quantified by comparison to a standard of known concentration on a 1% agarose gel.

The mitochondrial DNA target sequence was a 903-bp region including 709 bases of the 3′ end of the ND4 subunit of the nicotinamide adenine dinucleotide dehydrogenase gene and the tRNA genes histidine, serine, and leucine (partial). This sequence was amplified by the polymerase chain reaction (PCR) with the primers ND4 and LEU, originating from Arévalo *et al.* (1994). The PCR parameters were set to the following: 96°C denaturation (30 s), 50°C annealing (15 s), a 4 min ramp to 72°C, and 72°C extension (1 min) for 35 cycles. Double-stranded PCR products were purified using Qiagen's QIAquick Spin PCR Purification Kit (Qiagen Inc., Chatsworth, CA) to remove any excess

TABLE 1

Collection and Haplotype Information of Each Species Sampled for This Study

Species	Collector	Locality	n	Haplotype ID No.	GenBank Accession No.
Cyclura pinguis	Glenn Gerber	Anegada Island	3	pinguis_1	AF217772
Cyclura cornuta stejnegeri	Miguel A. Garcia	Mona Island	2	stejnegeri_1	AF217769
	Timothy Reichard		1	stejnegeri_2	AF217770
	Peter Tolson				
Cyclura cornuta cornuta	Jose Ottenwalder	Isla Cabritos, Dominican Republic	2	cornuta_1	AF217771
Cyclura ricordi	Bill Christie	Isla Cabritos, Dominican Republic	1	ricordi_1	AF217767
			1	ricordi_2	AF217768
Cyclura carinata carinata	Glenn Gerber	Turks and Caicos Islands	1	carinata_1	AF217778
			1	carinata_2	AF217779
Cyclura collei	Rick Hudson	Jamaica	3	collei_1	AF217773
Cyclura rileyi rileyi	Bill Hayes	San Salvador Islands	3	rileyi_1	AF217777
Cyclura rileyi cristata	Bill Hayes	San Salvador Islands	2	*	*
Cyclura rileyi nuchalis	Bill Hayes	San Salvador Islands	2	*	*
Cyclura cychlura cychlura	Chuck Knapp	Andros Island, Bahamas	1	cychlura_1	AF217780
			1	cychlura_2	AF217781
Cyclura cychlura figginsi	Chuck Knapp	Exuma Cays, Bahamas	3	figginsi_1	AF217775
			2	figginsi_2	AF217776
Cyclura cychlura inornata	Chuck Knapp	Exuma Cays, Bahamas	2	inornata_1	AF217774
Cyclura nubila nubila	Allison Alberts	Guantanamo Bay, Cuba	2	nubila_1	AF217765
			1	nubila_2	AF217766
Cyclura nubila lewisi	Fred Burton	Grand Cayman	1	lewisi_1	AF217763
	Glenn Gerber		3	lewisi_2	AF217764
Cyclura nubila caymanensis	Ed Lewis	Little Cayman	2	caymanensis_1	AF217761
			1	caymanensis_2	AF217762
Iguana iguana					
Caribbean	James Gilardi	St. Lucia	2	iguanaCar_1	AF217782
N. America	Allison Alberts	Belize	2	iguanaNA_1	AF217784
S. America	Brian Leysner	Curacao	2	iguanaSA_1	AF217785
Central America	Roberto Hasbun	El Salvador	2	iguanaCA_1	AF217786
Iguana delicatissima					
Caribbean	Steve Reichling	Dominica	4	delicatissima_1	AF217781

Note. Information for remaining species can be found in Sites *et al.* (1996).

* All three subspecies of *Cyclura rileyi* have identical haplotypes.

primers, nucleotides, and polymerases. Five microliters of the purified PCR products were checked on a 1.0% agarose gel with 5 μl of pGEM(r)-3Zf(+) double-stranded DNA Control Template (0.2 g/L; Applied Biosystems, Perkin–Elmer, Foster City, CA) to approximate concentrations.

Amplified products were sequenced using Applied Biosystems (Perkin–Elmer) ABI Prism DYE Termina-

tor Cycle Sequencing Reaction Kit in a Perkin–Elmer 9700 thermal cycler. The target sequence was read in both directions using each of the PCR primers and six internal primers developed for this study (Table 2). Cycle sequencing products were filtered using a Sigma G-50 Sephadex matrix (conc. 0.0625 μg/L) in Centri-Sep spin columns (Princeton Separations, Inc., Adelphia, NJ) to remove excess primers, dye-labeled termi-

TABLE 2

Primer Sequences Used in This Study to Sequence the Mitochondrial DNA Region ND4 to Leucine

	5′–3′ Sequence	Genus	Source
ND4	cacctatgactaccaaaagctcatgtagaagc	*Sceloporous*	Arévalo *et al.* (1994)
LEU	cattactttttacttggatttgcacca	*Sceloporous*	Arévalo *et al.* (1994)
CYC-CCND4	tcgttcatagttrgtrttggc	*Cyclura*	This study
CYC-ND4REV	ctatgatgagrtgctctcg	*Cyclura*	This study
CYCEM	acaggactaggaaccctaatyacagc	*Cyclura*	This study
IGND4REV	cgagaacacctaatcatgrcc	*Iguana*	This study
IGF1	gcaatcatctcrctacacatatt	*Iguana*	This study
IgND4 No. 2	gaggcatcgtaataaccagc	*Iguana*	This study

nators, nucleotides, and polymerases. Samples were dried under vacuum and stored at −80°C until analyzed on an Applied Biosystems 377XL Automated DNA Sequencer. Sequences were aligned manually, using iguanid sequences from Benabib *et al.* (1997) and Sites *et al.* (1996) as a guide. Sequence data collected for this study were pooled with a subset of the Sites *et al.* (1996) dataset. GenBank accession numbers for new haplotypes are given in Table 1.

Phylogenetic Analyses

Phylogenetic analyses utilized PAUP version 4.0b2a (D. L. Swofford, pers. comm.). Default search parameters were used unless otherwise specified. *Oplurus cuvieri* and *Enyaliodes laticeps* were used as paraphyletic outgroups, as was found appropriate in the Sites *et al.* (1996) study. Plots were constructed to examine patterns of divergence at each codon position separately for both transitions and transversions. Pairwise distances, estimated via maximum-likelihood (ML; uncorrected), were plotted against the number of base substitutions at each codon position. These were examined for evidence of nonlinearity under the assumption that change (as measured by sequence divergence) is proportional to time.

The data set was analyzed under maximum-parsimony (MP) criteria with and without third codon position transitions. Parsimony trees were generated using heuristic search routines with 1000 random-addition sequences and TBR branch swapping. Each nucleotide site was considered an unordered character with four possible states. Support for the resulting nodes was assessed using Felsenstein's (1985) bootstrapping method with 3000 replicates.

Maximum-likelihood analysis was run under the GTR model of evolution (Yang, 1994) using a successive approximation approach. The model parameters (substitution rate matrix, gamma distribution approximation with four rate classes, and empirical nucleotide frequencies) were estimated initially from the starting trees generated by the equally weighted parsimony analysis. These estimates were used in a ML analysis to produce a tree from which the parameters were then reestimated. In an iterative fashion, these steps were repeated until the ML score converged to its maximum value (Anderson, 2000; Frati *et al.,* 1997; Sullivan *et al.,* 1997; Swofford *et al.,* 1996). The process was repeated with alternative starting topologies (those published by Norell and de Quieroz, 1991 and Rassman, 1997) to determine whether the ML outcome was dependent upon the starting topology.

Hypothesis Testing

The phylogenetic hypothesis for the family Iguanidae resulting from this study was compared to the alternative published hypotheses of Rassman (1997) and Norell and de Quieroz (1991). This was accom-
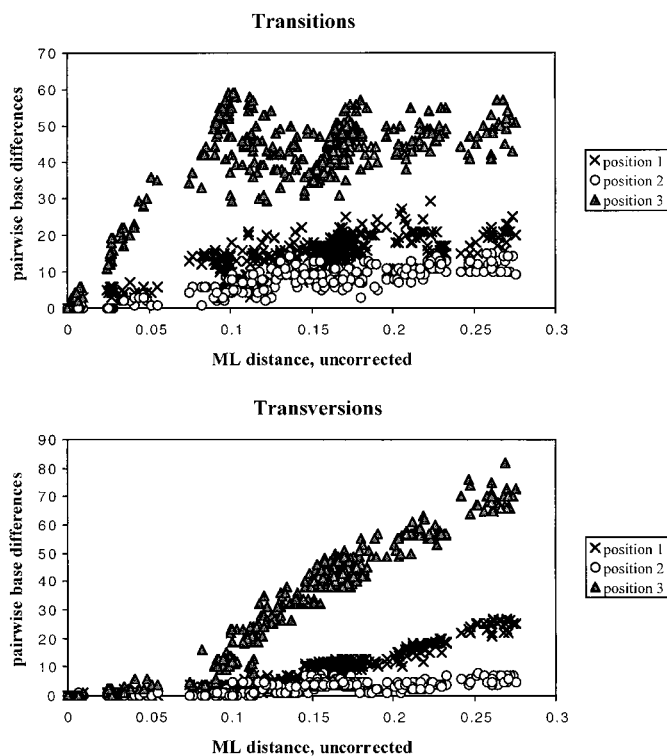

FIG. 1. Saturation plots for transitions and transversions. In both panels pairwise distances are estimated via a simple maximum-likelihood model and plotted against the number of base substitutions at each codon position.

plished by constraining the heuristic search algorithm of PAUP so that only those trees conforming to an *a priori* topology were retained. The differences in topologies were tested for statistical significance using the Templeton-Wilcoxon (1983) signed-rank test under maximum-parsimony.

RESULTS

Sequence data were collected for 52 individuals (26 haplotypes) representing all species of *Cyclura* and the broad geographic range of *I. iguana* (North America, Central America, Lesser Antilles, and South America). The combination of our data set with that of Sites *et al.* (1996) yields sequence data from all iguanine genera and appropriate outgroups. Of the 457 variable characters, 335 were parsimony informative. The number of base substitutions plotted against pairwise distance (Fig. 1) indicates that saturation in the Nd4 gene exists for transitions at the 3rd codon position at greater than 10% sequence divergence. Based on pairwise sequence divergence, most intrageneric comparisons are not affected by saturation, whereas all intergeneric comparisons are potentially affected by saturation of 3rd position transitions.

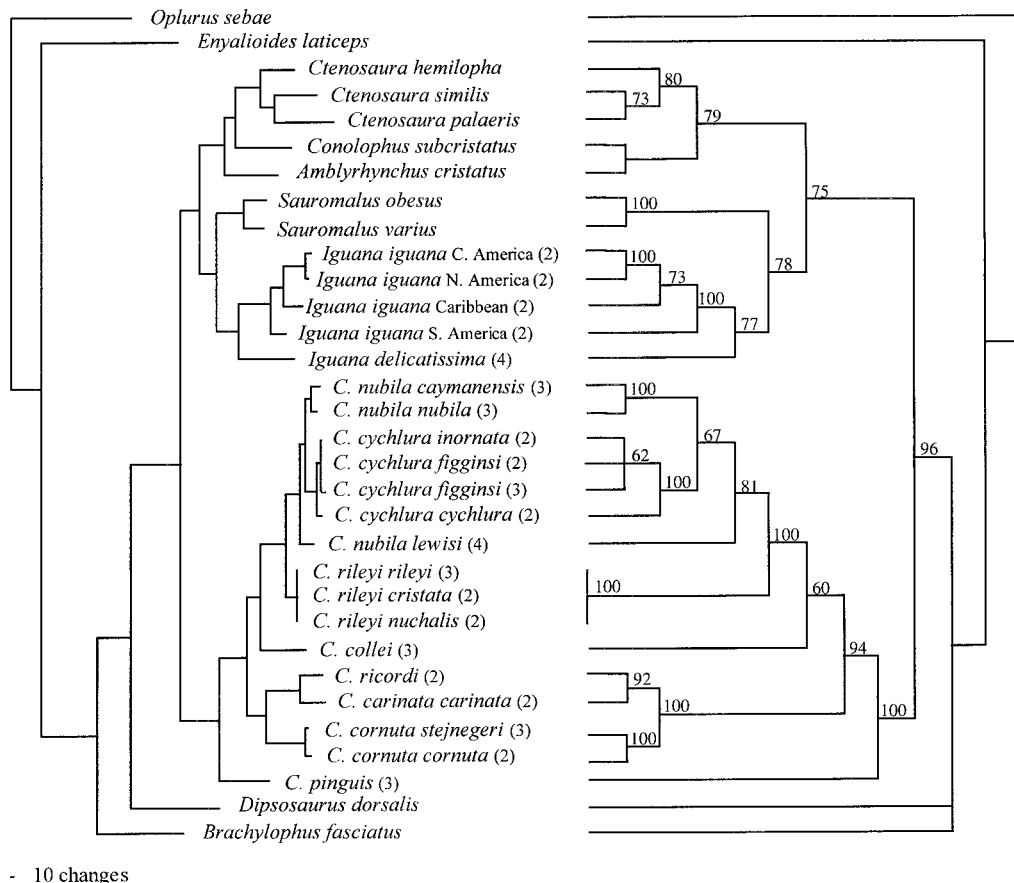Maximum-parsimony analysis resulted in four most-

FIG. 2. Results of phylogenetic analysis. The maximum-likelihood estimate represented as a phylogram (left) mirrored with the strict consensus of four equally parsimonious topologies obtained from the maximum parsimony analysis (right). Numbers in parentheses represent the number of individuals sequenced. Numbers above each node show the level of support from 3000 bootstrap replicates. Nodes without a value indicate less than 50% bootstrap support.

parsimonious trees of length 1420 steps; RI = 0.704, CI = 0.496. These trees differ in the subspecific relationships of three haplotypes within *Cyclura cychlura* and the basal member of the Iguanidae, alternating between *Dipsosaurus* and *Brachylophus*. A strict consensus of four trees, with bootstrap support indicated on each node, is shown in Fig. 2. Intergeneric relationships are generally supported with high bootstrap values with the exception of the Galapagos clade (*Amblyrhynchus* and *Conolophus*) and the basal position within the family. Only two nodes within *Cyclura* have low support (less than 70% bootstrap value), whereas strong support above and below these branches reflect the potential for the data to resolve relationships at this level. When 3rd position transitions were excluded from the maximum-parsimony analysis, there was loss of resolution at the subspecific level within *C. cychlura* and *C. cornuta,* whereas the remaining relationships remained well supported (data not shown).

The phylogeny recovered by the ML analysis (-ln likelihood = 7529.847) was similar to that recovered by the MP analysis and is illustrated as a phylogram in

Fig. 2. From all starting topologies the ML analysis eventually converged to the same topology. Parameter estimates for this topology were proportion of invariant sites = 0.357 and gamma shape parameters ($\propto$) = 0.727; the substitution rates were A–G = 9.257, A–C = 2.171, A–T = 1.007, C–T = 10.42, C–G = 0.342, and G–T = 1. One intergeneric relationship differs between the ML result and the MP result: *Amblyrhynchus* resolves as the sister to the group *Ctenosaur + Conolophus* in the ML analysis, although the MP topology (a monophyletic Galapagos group) is not rejected by a Kishino–Hasegawa (Kishino and Hasegawa, 1989) test under the ML model (P = 0.6633). The ML analyses indicate that *Brachylophus* is the sister taxon to the rest of the family Iguanidae. The relationships within *Cyclura* and between *Iguana, Sauromalus,* and *Cyclura* are constant in all analyses.

The addition of multiple localities within the *I. iguana* lineage and the addition of more species of *Cyclura* to the Sites *et al.* (1996) data set supported their conclusions and was effective in gaining more support (as measured by bootstrap proportions) for
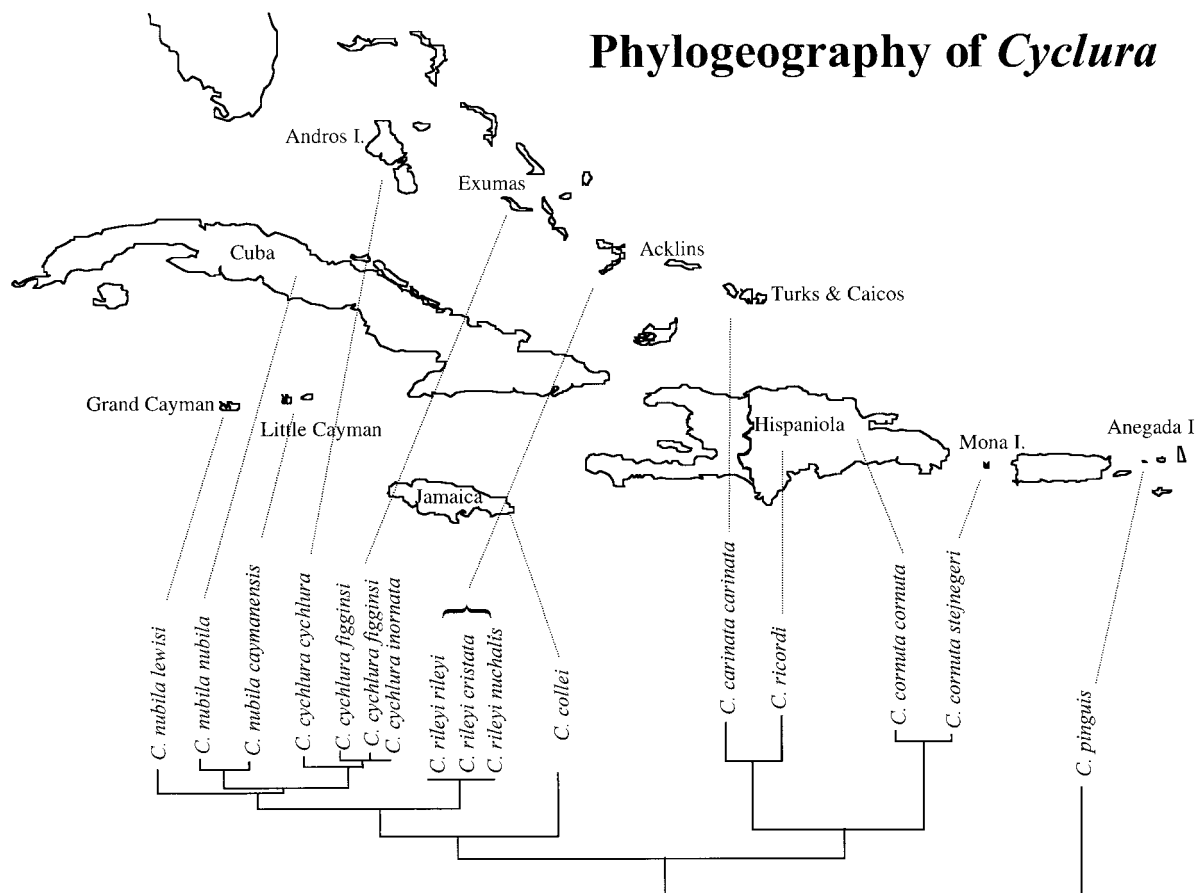
FIG. 3. Phylogeographic distribution of the genus *Cyclura*. The maximum-likelihood estimate of historical relationships within *Cyclura* is superimposed onto a map of the Greater Antilles.

most nodes and an increase in the retention and consistency indices, from 0.403 and 0.462 to 0.704 and 0.496, respectively (see Sites *et al.,* 1996, Fig. 2a). The intrafamilial relationships resulting from our analyses (Fig. 2) are consistent with those of Petren and Case (1997) and Sites *et al.* (1996). *Cyclura* is a distinct lineage, not closely related to any other iguanine. The genus *Iguana* resolves as the sister taxa to the chuckwallas, *Sauromalus,* which together define the sister group to the clade of *Ctenosaura* plus the Galapagos iguanas, *Conolophus,* and *Amblyrhynchus.* As in Sites *et al.* (1996) and Rassman (1997), we found only weak support for the monophyly of the Galapagos clade. Finally, the desert and banded iguanas, *Dipsosaurus* and *Brachylophus,* represent the most ancient extant groups. Our phylogenetic hypothesis was found to be statistically more parsimonious compared to the alternative hypothesis of Iguanid relationships given by Norell and de Quieroz (1991) ($P = 0.0007$, $z = -3.5496$) but not compared to that of Rassman (1997) ($P = .0817$, $z = -1.9419$) using the Templeton–Wilcoxon signed-rank test.

The interspecific relationships of the rock iguana

lineage are resolved with a high level of support using the ND4 region of the mtDNA. The phylogeographic distribution of the genus is shown in Fig. 3. *C. pinguis,* endemic to the Puerto Rican bank, is quite distinct (7.7% sequence divergence from the nearest clade) and represents the most basal lineage. Our topology is in disagreement with that of Schwartz and Carey (1977) with regard to their basal placement of the (*ricordi + carinata*) group and their grouping of *cornuta* with *rileyi, collei, nubila,* and *cychlura.* Under the assumption that cladogenetic events are coincident with island colonization, our best-supported phylogeny suggests multiple independent colonizations into the Bahamas: the earliest onto the southern Bahaman bank (*C. rileyi*) followed by an invasion of the Turks and Caicos islands (*C. carinata*) and, most recently, onto the northern Bahaman bank (*C. cychlura*). There appears to have been two independent radiations onto the Cayman islands: an older *C. nubila lewisi* colonization of Grand Cayman and, more recently, colonization of Little Cayman and Cayman Brac. In summary, our sequence data do not support the existing subspecific taxonomy: the three subspecies of *C. rileyi* share an

identical haplotype, we find paraphyly within *C. cychlura* (between *C. c. inornata* and *C. c. figginsi*), and *C. nubila* is not supported as monophyletic (although the recovered topology is not statistically more likely than monophyly).

DISCUSSION

Systematics of the Iguanidae

The addition of our data set to that of Sites *et al.* (1996) breaks some long branches within the *Cyclura* and *Iguana* clades, which had been of concern in the previous study, but does not alter relationships between these taxa (see phylogram in Fig. 2). In concurrence with recent molecular studies (Sites *et al.,* 1996; Rassman, 1997; Petren and Case, 1997), the monophyly of the Iguanidae is strongly supported. Our results contradict Lazell's (1973) assertion of the "trivial nature of the putative distinctions between *"Cyclura, Brachylophus,"* and *"Iguana"* and support the results of recent authors (Norell and de Quieroz, 1991; Petren and Case, 1997; Rassman, 1997; Sites *et al.,* 1996) that *Brachylophus* is among the most ancient iguanid lineages. Our analyses support the hypothesis of Sites *et al.* (1996) and Petren and Case (1997) that *Cyclura* is a monophyletic lineage, equally related to the iguanids *Ctenosaur, Conolophus, Amblyrhynchus, Sauromalus,* and *Iguana.* Our result that the sister group to *Iguana* is *Sauromalus,* rather than *Cyclura,* contradicts the recent conclusion by Wiens and Hollingsworth (2000), that previous studies using molecular data were led to erroneous conclusions due to long-branch attraction.

Phylogenetics and Conservation of Cyclura

Endemism in *Cyclura* is extreme in that each distinct lineage is restricted to one island (or one small island group); Hispaniola is the only island that contains two lineages. The general pattern of the *Cyclura* radiation, illustrated by superimposing the phylogeny onto a map of the Caribbean (Fig. 3), shows a southeast to northwest directionality. The oldest extant lineage, *C. pinguis,* had an historical range consisting of much of the Puerto Rican Bank but is now restricted to Anegada island and a nearby cay. *C. ricordi* and *C. cornuta* occur in sympatry on Hispaniola, a unique occurrence within *Cyclura* but paralleling a pattern seen in other highly endemic Caribbean taxa, such as snakes of the genus *Alsophis* and *Epicrates* (Schwartz and Henderson, 1991). The current southern peninsula of Hispaniola existed as a separate island until colliding with the main island during the mid to late Miocene (Donnelly, 1990). This pattern of sympatry could be the result of independent colonization and subsequent speciation on each island fragment followed by reciprocal exchange after their collision. An alternative explanation is that the submergence of the Valle de Neiba

during interglacial periods resulted in extended isolation of the southern peninsula (based on sea level data from Haq *et al.,* 1987) and, hence, the same vicariant pattern. The sister group to *C. ricordi, C. carinata,* is found on the Turks and Caicos islands, suggesting that *C. ricordi* originated on the main Island and *C. cornuta* on the southern peninsula. Probably once abundant, *ricordi* is now restricted to a few small populations protected by reserves within the Dominican Republic. Recently, paleontologists have discovered a fossil *Cyclura* on Grand Turk island which is roughly one meter in length, exceeding the known size of *carinata* by almost a foot (Dr. Richard Franz, pers. comm.). Dating these fossil beds would provide a minimum date for the dispersal of *Cyclura* to these islands.

The Bahamas contain two species, *C. cychlura* and *C. rileyi. C. cychlura* was one contiguous population on the exposed Bahama Bank during the Pleistocene glacial maximum (approximately 18,000 years bp), subsequently becoming fragmented as sea level rose. Whereas the Andros Island population (*C. c. cychlura*) is phylogenetically distinct from the Exuma Cay island populations (subspecies *C. c. figginsi* and *C. c. inornata*), the relationships between the Exuma Cay populations are not in agreement with their current nomenclature (Fig. 2). Conversely, *C. rileyi* colonized the Bahamas prior to the speciation between *C. nubila* and *C. cychlura,* yet no mtDNA differentiation exists among the three designated subspecies. This indicates that *C. rileyi* has only very recently been separated into the three discreet populations and raises the question of whether or not the subspecific status of each population is justifiable. We feel that this issue would be best addressed using more rapidly evolving loci to discern whether there are any diagnosable characters for each subspecies.

Our hypothesis suggests two independent colonizations from Cuba into the Caymans—an older dispersal event onto Grand Cayman (*C. n. lewisi*) and a more recent radiation onto Little Cayman (*C. n. caymanensis*): 3% vs 1% sequence divergence, respectively. Interestingly, *C. n. lewisi* is as genetically different from the other *C. nubila* subspecies as is their sister taxon, *C. cychlura.* The fact that nodes above and below this node are completely resolved, with high bootstrap support, points to the ability of the data to resolve issues at this taxonomic level and, hence, indicates a rapid radiation of these taxa. Due to the decline of *C. n. lewisi* populations, its status has become critical to management decisions. Clearly, *C. n. lewisi* is genetically distinct, but decisions regarding species status will require analyses which include multiple localities within Cuba and/or corroborating evidence from other sources of data (e.g., nuclear markers or morphological data).

Using our molecular data we can quantify diversity in the context of phylogenetic relationships as a means of prioritizing conservation efforts (Crozier *et al.,* 1997;

TABLE 3

Conservation Priorities

	% Biodiversity loss	IUCN assessment
Cyclura pinguis	16.356 ± 0.577	Critically endangered
Cyclura collei	12.396 ± 0.603	Critically endangered
Cyclura cornuta	12.238 ± 0.630	Vulnerable
Cyclura carinata	6.825 ± 0.567	Critically endangered
Cyclura nubila	4.657 ± 0.559	Vulnerable
Cyclura ricordi	4.607 ± 0.578	Critically endangered
Cyclura cychlura	4.424 ± 0.555	Vulnerable
Cyclura rileyi	3.507 ± 0.566	Endangered
Cyclura nubila lewisi	3.367 ± 0.564	Critically endangered

Note. Species are ranked by the amount of impact that their extinction would have on the biodiversity of the genus using Conserve 3.2.1. Confidence limits of $P = 0.05$ are based on 100 bootstrap replicates. The 1996 IUCN assessment of each species (and *Cyclura nubila lewisi,* see text) is included.

Krajewski, 1994; Vane-Wright, 1991). We utilize the method outlined in Crozier (1992), which combines the merits of both a topological- and a divergence-based approach (Conserve 3.2.1; http://www.bio.ic.ac.uk/evolve/software/conserve; Crozier *et al.,* 1999). As explained in Crozier *et al.* (1997), to estimate the amount of current diversity being retained, branch lengths approximated by a difference method is appropriate, whereas the topology should reflect evolutionary relationships. We used a Jukes–Cantor distance measure and the neighbor-joining algorithm in PHYLIP (Felsenstein, 1993) to build a tree for the prioritization analysis (this topology was identical to that of our maximum-likelihood-generated hypothesis (Fig. 2)) and confidence intervals were based on 100 bootstrap replicates using SEQBOOT in PHYLIP. Species of *Cyclura* are ranked by the degree of impact that their extinction would have on the diversity of the entire genus. Rojas (1992) points out that this type of analysis is most clear when operational taxonomic units (OTU's) define the same taxonomic level. We adhere to this suggestion except for the case of *C. n. lewisi,* which we treat as an OTU separate from that of its conspecifics because of the molecular evidence given above for its distinctiveness. These ranks, along with the most current IUCN assessment of each taxon, are summarized in Table 3. Two of the highest ranking species (*C. pinguis* and *C. collei*) are also in the most peril, due primarily to predation by feral animals and habitat loss (Alberts, 2000). The extinction of *C. pinguis,* which would have a drastic impact on genetic diversity (16% loss), is currently being averted through intensive conservation efforts, primarily headstarting and public education (Hudson, 1999a). Future plans include control programs for invasive species, particularly feral cats (Allison Alberts, pers. comm.). *C. collei* was believed extinct from the 1940's until 1990 (Vogel, 1995) when its discovery catalyzed an ongoing conservation

effort by a consortium of United States zoos and the Hope Zoo of Kingston. They have undertaken a program of headstarting (with prerelease health screening), mongoose control, and managing a captive population (Hudson, 1999b). Conversely, the rhinoceros iguana, *C. cornuta,* contributes a substantial amount of diversity to the genus (12%) and is not in an immediate threat of extinction (Table 3). In fact, this species is a good candidate for preventative conservation efforts because it has multiple populations of various sizes, under varying degrees of disturbance. The distinctiveness of *C. nubila lewisi* is made readily apparent by this analysis, contributing as much diversity to the genus as the species *C. rileyi* and *C. cychlura.* We concur with much of the conservation community (e.g., Legge *et al.,* 1996; Moritz, 1994; Sites and Crandall, 1997; Metrick and Weitzman, 1998), that the setting of conservation priorities must also take into account factors independent of phylogenetic uniqueness, such as ecological significance, geographical uniqueness, economics, and the threat of extinction. Integration of our results with other considerations will provide much needed guidelines in the preservation of this group.

Historical Phylogeography of the Caribbean

Many biogeographers have tested the vicariance hypothesis by looking for congruence between current phylogeographic patterns and the historical island group relationships (presented by Buskirk, 1985). This synthesis of geologic data is summarized as follows: Cuba, North Hispaniola, and Puerto Rico passed between North and South America ~80 mya as an island arc on the edge of the Caribbean plate. They stayed in close proximity until the Miocene when Cuba split off from the group followed by divergence of the other two islands in the late Miocene. A recent study by Crother and Guyer (1996) used Brooks Parsimony Analysis (Brooks and McLennan, 1991) to infer a common phylogenetic pattern across 11 disparate taxa and claimed congruency with the above synthesis of geologic data. Unfortunately, newly emerging geologic evidence does not support this model and suggests two alternative models for the breakup of the islands after they separate from the mainland: (1) the island arc remained in close proximity until Puerto Rico broke away in the middle Oligocene, while Cuba and Hispaniola stayed clustered for several million more years (Burke, 1988; Lewis and Draper, 1990; Pindell and Barrett, 1990), or (2) Cuba, North Hispaniola, and Puerto Rico broke apart at approximately the same time in the early/middle Miocene (Pindell and Draper, 1991). If we explore the pattern of intrageneric relationships of *Cyclura* in view of current geologic evidence (Fig. 3), we find that the phylogeographic pattern is most congruent with geologic model 1.

Another approach to testing the vicariance theory is presented by Hass *et al.* (1993) whereby "the direct

test of these two alternative explanations rests on the ability to determine the time that sister taxa in the Caribbean and on the mainland last shared a common ancestor." Abandoning the use of cladistic biogeography, Hedges *et al.* (1992) and Hass *et al.* (1993) focused on estimating the time of divergence between numerous island and mainland taxa to test the vicariance theory. Hedges *et al.* (1992) dated the *Cyclura* and *Iguana* split to 12 mya using serum albumin data and the "standard" ID calibration of 1 ID unit = 0.6 million years. The study encompassed a variety of vertebrate taxa, including the frog genus, *Eleuthrodactlyus.* They concluded that the majority of vertebrate taxa arrived in the West Indies by overwater dispersal but that *Eleuthrodactlyus* may have been present on the proto-Antillean arc. Critiques of this methodology, focusing mainly on the absence of complete phylogenetic information, have been published by Page and Lydeard (1994) and Crother and Guyer (1996) and responded to by Hedges *et al.* (1994) and Hedges (1996b), respectively.

Based on 12s and 16s sequence data, Rassman (1997) estimated that the Galapagos iguanas, *Conolophus* and *Amblyrhynchus,* diverged approximately 10.5 mya. The distance to the point of divergence between these taxa at the ND4 to leucine locus is 7.77% sequence differentiation. Therefore, this translates to an approximate molecular clock of 1.29 million years for every 1% sequence divergence at the ND4 to leucine locus. Our data show 15.3–18.4% sequence divergence of *Cyclura* since the group's origin. Extrapolating from above, this is equivalent to between 19 and 23 mya. If these dates are within a ±40 million year error range, then the ancestral *Cyclura* could not have ridden on the proto-Antillean island arc. Conversely, our phylogenetic hypothesis is consistent with waif dispersal; wind, hurricane, and ocean currents all travel a general path from the South American mainland, up the Lesser Antilles, and along the Greater Antilles. Waif dispersal up the Lesser Antilles, as far as Anguilla, has occurred once by *I. delicatissima* and twice by *I. iguana.* The recent dispersal event of *I. iguana* to Anguilla in October 1995, following hurricanes Luis and Marilyn, demonstrate that this is an ongoing process (Censky *et al.,* 1998). The more recent invasions by *I. iguana* (and human-mediated introductions) result in the extirpation of *I. delicatissima* by competitive exclusion and/or introgression whenever they come into contact (Day *et al.,* 2000). The absence of *Cyclura* from the Lesser Antilles could be a result of at least three factors: dispersal up the Aves Ridge, a currently submerged chain of islands which extended from South America to Puerto Rico between 15 and 35 mya (Donnelly, 1990; Holcombe and Edgar, 1996), competitive exclusion by *Iguana,* or eradication due to island submergence following a rise in sea level. Unless proto-

Cyclura fossils are discovered in the Lesser Antilles, this part of the puzzle cannot be solved.

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