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ORIGINAL ARTICLE

Geographical structure and cryptic lineages within common green iguanas, *Iguana iguana*

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ABSTRACT

Aim Our aim was to investigate genetic structure in Neotropical populations of common green iguanas (*Iguana iguana*) and to compare that structure with past geological events and present barriers. Additionally, we compared levels of divergence between lineages within *Iguana* with those within closely related genera in the subfamily Iguaninae.

Location Neotropics.

Methods DNA sequence data were collected at four loci for up to 81 individuals from 35 localities in 21 countries. The four loci, one mitochondrial (*ND4*) and three nuclear (*PAC*, *NT3*, *c-mos*), were chosen for their differences in coalescent and mutation rates. Each locus was analysed separately to generate gene trees, and in combination in a species-level analysis.

Results The pairwise divergence between *Iguana delicatissima* and *I. iguana* was much greater than that between sister species of *Conolophus* and *Cyclura* and non-sister species of *Sauromalus*, at both mitochondrial (mean 10.5% vs. 1.5–4%, respectively) and nuclear loci (mean 1% vs. 0–0.18%, respectively). Furthermore, divergences within *I. iguana* were equal to or greater than those for interspecific comparisons within the outgroup genera. Phylogenetic analyses yielded four strongly supported, geographically defined mitochondrial clades (3.8–5% divergence) within *I. iguana*. Three of the four clades were found using *PAC* (0.18–1.65% divergence) and two using *NT3* (0.6% divergence) alone. The primary divergence, recovered in three polymorphic loci, was between individuals north and south of the Isthmus of Panama. The southern group was differentiated into clades comprising individuals on either side of the northern Andes, using both *PAC* and *ND4*.

Main conclusions Deep genetic divergences were found within *I. iguana* that are congruent with past and current geological barriers. These divisions are greater than sister species comparisons in other Iguaninae genera, indicating the possible presence of cryptic species. Geological changes from the mid-Miocene through the Plio-Pleistocene have shaped the pattern of divergence in *I. iguana*. The uplift of the northern Andes presented a barrier between South American *I. iguana* populations by 4 Ma. Populations north of the Isthmus of Panama form a clade that is distinct from those to the south, and may have expanded northwards following the closing of the Isthmus of Panama 2.5 Ma.

Keywords

Iguaninae, Isthmus of Panama, mtDNA, Neotropics, nuclear DNA, phylogeography, species tree.

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INTRODUCTION

The evolutionary cohesiveness of a wide-ranging species, however morphologically homogeneous, is often in doubt (Klatau *et al.*, 1999; Witt & Hebert, 2000; Zeh *et al.*, 2003). This is particularly true of Neotropical species, where the region has experienced dramatic geological changes as recently as the Plio-Pleistocene, including uplift, marine incursions, land-bridge formation and eustatic changes (Haq *et al.*, 1987; Coates, 1997; Haug *et al.*, 2001; Iturralde-Vinent, 2006; Retallack & Kirby, 2007; Antonelli *et al.*, 2009), providing ample opportunity for vicariance and/or habitat specialization within taxa (Prance, 1982; Cracraft & Prum, 1988; Patton & Smith, 1992; Bush, 1994; Burnham & Graham, 1999; Hasbun *et al.*, 2005; Brumfield & Edwards, 2007; Milá *et al.*, 2009).

In the last two decades, many genetic studies of species with extensive Neotropical ranges have been undertaken to ascertain whether they harbour cryptic taxa. Cryptic species have been identified in woodcreepers (Marks *et al.*, 2002), catfish (Martin & Bermingham, 2000), pseudoscorpions (Wilcox *et al.*, 1997), bushmasters (Zamudio & Greene, 1997), sigmodontine rodents (Peppers & Bradley, 2000), and spectacled caimans (Venegas-Anaya *et al.*, 2008). Their presence among wide-ranging species in other regions [Holarctic wrens (Toews & Irwin, 2008), North American amphipods (Witt & Hebert, 2000) and copepods (Colbourne & Hebert, 1996), Eurasian parasitoids (Heraty *et al.*, 2007), and circumtropical bonefish (Colborn *et al.*, 2001)] indicates their widespread taxonomic and geographical occurrence (Pfenninger & Schwenk, 2007). On the other hand, some wide-ranging species show little genetic structure, including a Neotropical beetle (Zeh *et al.*, 2003) and rattlesnake (Wüster *et al.*, 2005), a West Indian boa (Henderson & Hedges, 1995), and a North American microhylid frog (Makowsky *et al.*, 2009).

Common green iguanas, *Iguana iguana* (Linnaeus, 1758), have a broad Neotropical range, generally occurring below 875 m (Campbell, 1998), from Mexico through Central America, deep into South America and on several Lesser Antillean islands (Etheridge, 1982) (Fig. 1). Their sister taxon, *Iguana delicatissima* Laurenti, 1768, is restricted to a few islands in the Lesser Antilles (Etheridge, 1982). They gain much of their energy through hind-gut fermentation and can exploit a broad diet of leaves and fruit, allowing this species to occupy a variety of habitats at high densities, with little competition for food resources (Swanson, 1950; Rand *et al.*, 1990). They are characterized by a large subtympanic scale that is absent in *I. delicatissima*, and multiple osteological characters (Lazell, 1973; Conrad & Norell, 2010). Several characters show morphological variation within and/or among wild populations, including colour and pattern, shape and size of nasal tubercles, body size, and occurrence of sexual size dimorphism (Dunn, 1934; Swanson, 1950; Müller, 1968; Lazell, 1973; Bakhuis, 1982; Harris, 1982; Alvarado *et al.*, 1995). As this species occupies such variable habitats, it also has variable behaviours and life histories (Harris, 1982; Alvarado *et al.*, 1995). Given this species' preference for lower elevation, mountain ranges such as the Talamancas and the Andes could present significant barriers to gene flow. Additionally, data on home ranges and movement patterns, dispersal distance by juveniles and adult female nest philopatry, do not indicate high levels of interpopulation connectivity (Henderson, 1974; Dugan, 1980; Bock, 1984; Rodda, 1992). In fact, Bock & McCracken (1988) found that population structure could be detected 'over distances of only several km'. Thus, there is a possibility that common green iguanas are not as homogeneous as current taxonomy implies. Although subspecies of *I. iguana* have been recognized in the past (Hollingsworth, 2004), none are currently recognized.

In the present study we used mitochondrial DNA (mtDNA) and nuclear DNA (nDNA) sequence data to assess geographical structure within *I. iguana* across its range. We used loci with differing coalescent and mutation rates in order to infer the timing of various cladogenic events. We assessed the roles of past geological events in shaping phylogeographical structure within *I. iguana,* and whether distinct populations are characteristic of distinct historical and/or current geographical regions. Lastly, we explored the conservation management and taxonomic implications of our results.

MATERIALS AND METHODS

Sampling

Previous morphological and molecular studies support either *Cyclura* (de Quieroz, 1987; Wiens & Hollingsworth, 2000) or *Sauromalus* (Sites *et al.*, 1996; Petren & Case, 1997; Malone *et al.*, 2000) as sister taxa to *Iguana*. Sister species pairs from both genera, plus sister species from a more distantly related Iguaninae genus, *Conolophus*, were included in the analyses as outgroups to *Iguana*. Seven outgroup species were included: *Cyclura carinata*, *Cyclura ricordii*, *Conolophus subcristatus*, *Conolophus pallidus*, *Sauromalus ater* (= *australis*), *Sauromalus ater* (= *obesus*), and *Sauromalus klauberi*. A total of 73 *I. iguana* and seven *I. delicatissima* samples were collected in the field, from captive zoo specimens of known wild origin, or from museum specimens, representing 35 localities in 21 countries. Detailed sample information is reported in Appendix S1 in Supporting Information and localities for *Iguana* are mapped in Fig. 1.

Data collection

DNA sequence data were collected from one mtDNA and three nDNA loci for up to 87 individuals (Appendix S1). These four loci were chosen for their differences in coalescent and mutation rates. The mtDNA genome coalesces approximately four times faster than the nuclear loci, and generally evolves more rapidly than nuclear coding regions.

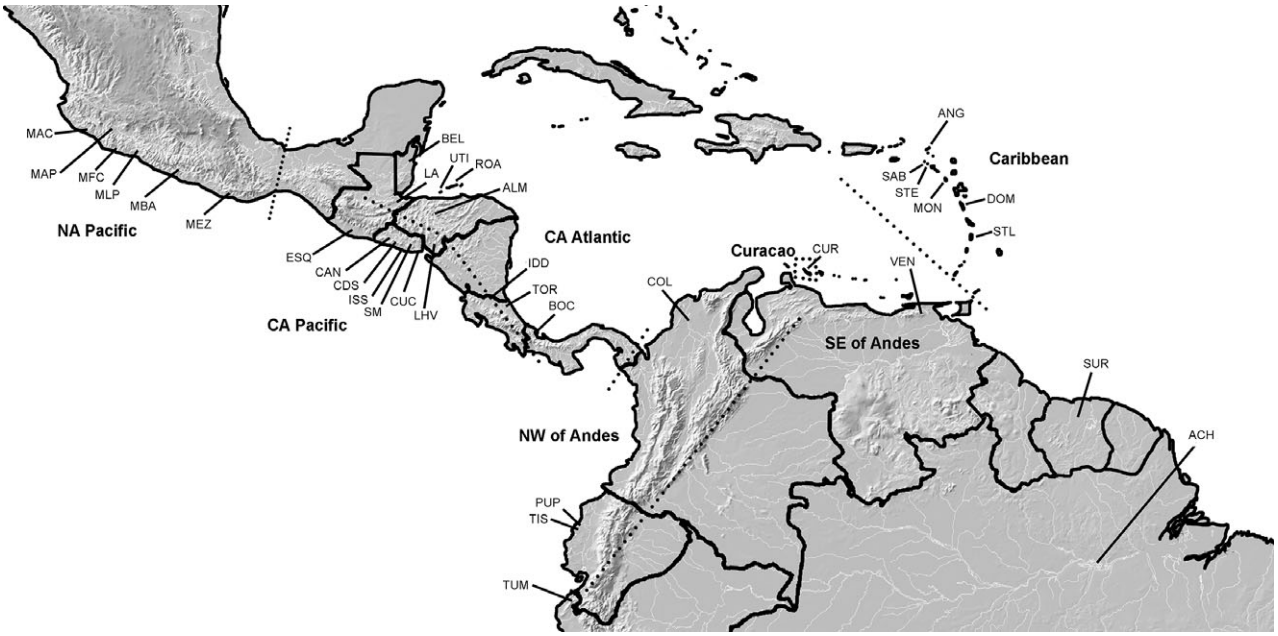

Figure 1 Collection localities of *Iguana iguana* and *I. delicatissima* in the Neotropics. The three-letter codes correspond to the information in Appendix S1. Sample localities fall into five generalized geographical regions as indicated on the map by dashed lines.

The 3′ untranslated region of the polymerase alpha catalytic subunit (*PAC*) locus recovered a high level of intraspecific polymorphism in other Iguaninae species (Pasachnik *et al.*, 2008) and the nuclear locus neurotrophin 3 (*NT3*) accumulates mutations more rapidly than the oocyte maturation factor (*c-mos*) (Noonan & Chippindale, 2006a).

Genomic DNA was isolated using Qiagen's QIAamp DNA extraction kit (Qiagen Inc., Valencia, CA, USA). A 825-base pair (bp) fragment of the mtDNA *ND4* locus, encompassing 607 bases of the 3′ end of the NADH dehydrogenase subunit 4 gene (*ND4*) and the tRNA genes histidine, serine and leucine (partial 5′ end), was amplified with the polymerase chain reaction (PCR) using the primers and PCR protocols in Malone *et al.* (2000). In a subset of individuals (representatives of different *ND4* haplotypes and geographical regions, see Appendix S1), a 563-bp region of the *PAC* locus, a 489-bp region of the *NT3* locus and a 375-bp region of the *c-mos* locus were amplified using published primer sequences (Noonan & Chippindale, 2006b; Pasachnik *et al.*, 2008). All PCR products were verified on a 1% agarose gel, purified using Qiagen's QIAquick kit (Qiagen Inc.) and cycle sequenced using BigDye Terminators (Applied Biosystems, Foster City, CA, USA) at Northwoods DNA, Inc. (Solway, MN, USA). Each gene was aligned separately in the program MAFFT 6.875b (Katoh *et al.*, 2005), using the G-INS-I algorithm. Indels were not polymorphic in size and the state (present or absent) of each indel region was monomorphic within a species. The phase of double heterozygotes (individuals that were heterozygous at two bases along the locus) was determined by the presence of homozygotes for alternative alleles. Unique sequences were deposited in GenBank (Appendix S1).

Data analysis

Gene tree topologies

The three nDNA and single mtDNA sequence data sets were analysed individually under maximum parsimony (MP) with each nucleotide site considered an unordered character with four possible states in PAUP* 4.02b (Swofford, 2000). Base substitutions were equally weighted and each gap was treated as a fifth state. Gene trees were generated using heuristic search routines employing 1000 random addition sequences and tree bisection–reconnection (TBR) branch swapping on multiple trees (MulTrees option). Because of their low numbers of mutations and lack of homoplasy, *NT3* and *c-mos* were only individually analysed using parsimony.

The *ND4* and nDNA *PAC* data sets were further analysed using maximum likelihood (ML) and Bayesian methods. An appropriate model for the Bayesian analysis was chosen by comparing the likelihoods of multiple models using PAUP* and MODELTEST 3.7 (Posada & Crandall, 1998) under the Bayesian information criterion (BIC) (an approximation of the Bayes factor; Kass & Raftery, 1995) for choosing among models available in MRBAYES 3.1.2 (Huelsenbeck & Ronquist, 2001). The optimal models were the HKY and HKY+G models for the *PAC* and *ND4* data sets, respectively.

In MRBAYES, we ran each gene independently with no partitions, running two replicate Markov chain Monte Carlo (MCMC) analyses with one cold chain and seven hot chains for 1×10^7 generations using default priors and sampling every 1000 generations. We used TRACER 1.4 (Rambaut & Drummond, 2007) to inspect likelihood scores from the replicates to estimate the burn-in period and to evaluate

convergence. The independent MrBayes runs for both genes reached a mean standard deviation split frequency < 0.01 and the likelihoods converged at the set burn-in of 2×10^4 for each gene. ML analyses were performed in RAxML (Stamatakis, 2006) using the GTR+G model by executing 400 searches, 200 starting from random trees and 200 starting from every fifth topology recovered in the bootstrap analysis.

Nodal support

Support for nodes was assessed using three methods. We executed Felsenstein's (1985) bootstrapping method with 3000 replicates under MP criteria for all loci in PAUP*. For ML, 1000 bootstraps were estimated for branch support for the *PAC* and *ND4* gene in RAxML. We also determined support for individual nodes of the *ND4* and *PAC* trees from the Bayesian posterior probabilities (PP) of the majority rule consensus tree.

Species-level tree

Two nDNA loci (*NT3* and *PAC*) and the mtDNA locus (*ND4*) were further used to estimate a 'species' tree in *BEAST 1.6.1 (Heled & Drummond, 2010). Only individuals sequenced for each locus were included in this analysis (see Appendix S1). The species-level analysis co-estimates the gene trees and has been shown to outperform concatenated analysis. The *BEAST model assumes that 'species' are groups that, after a period of divergence, have no history of breeding with individuals outside of the designated group. To determine groups for operational taxonomic units (OTUs) in *BEAST we used the model-based Bayesian clustering program STRUCTURE v.2.3.1 (Falush *et al.*, 2003) to estimate the number of natural genetic groups (K) using the admixture model. We ran STRUCTURE 20 times for each $K = 2$–20 for 10,000 MCMC replications after a burn-in of 100,000. STRUCTURE HARVESTER 0.56.4 (Earl & vonHoldt, 2012) was used to determine K following the method of Evanno *et al.* (2005). The program CLUMMP 1.1.2 (Jakobsson & Rosenberg, 2007) was used to summarize the assignment of each individual using the GREEDY OPTION 2 with 10,000 random input orders of the 20 runs for K.

For the reduced *BEAST data set we followed model selection methods detailed above. We tested each gene for the assumption of a molecular clock using a likelihood ratio test (LRT) (Felsenstein, 1981). The LRT failed to reject the hypothesis of a molecular clock for *NT3* ($P = 0.846$) and rejected the clock for *PAC* and *ND4* ($P = \leq 1 \times 10^{-6}$). Wertheim *et al.* (2010) showed no loss in phylogenetic accuracy using a relaxed clock for data simulated under a strict clock. However, this assumption has yet to be tested in *BEAST, so we used two different approaches for clock assignment. First, we implemented a strict clock for *NT3* and an uncorrelated lognormal relaxed clock for *PAC* and *ND4*. We set an uninformative uniform prior (0–100) for the ucld.mean for *PAC* and *ND4* and set the rate of *NT3* to 1. Second, we set an uncorrelated lognormal relaxed clock for all three genes with uninformative uniform prior (0–100) on the ucld.mean for each gene. Two *BEAST runs per clock strategy were started from random trees and run for 1×10^8 generations sampling every 5000 generations. An additional *BEAST analysis was performed without nucleotide data using only the set priors, in order to examine the influence of our priors on the resulting rates and topologies. Convergence of the two runs and effective sample size values of the two clock strategies were checked and burn-in was estimated using TRACER 1.4. The post-burn-in trees from each run per clock strategy were combined and a maximum clade credibility tree was estimated. The likelihoods from the post-burn-in distribution of both clock strategies were compared with Bayes factors (Kass & Raftery, 1995; Suchard *et al.*, 2001) in the program TRACER 1.4 (Rambaut & Drummond, 2007).

Pairwise comparisons

We compared relative divergences of congenerics of both outgroups and ingroups, and of conspecific clades within *I. iguana*. Because of the very low level of homoplasy in the data sets (see Results), as indicated by high consistency and retention indices (Sanderson & Donoghue, 1989), we did not use a corrected distance measure. Uncorrected distances were calculated for each locus separately. Mean values were used for comparisons between major *I. iguana* clades.

RESULTS

Haplotypes

Thirty-five haplotypes are new to this study and the remaining are identical to eight published previously in Sites *et al.* (1996) or Malone *et al.* (2000) (see Appendix S1). Data collected at the mtDNA *ND4* locus (825 nucleotides) yielded 25 haplotypes from 76 *Iguana* individuals sampled at 35 localities. Over all taxa, 261 sites were polymorphic at the *ND4* locus (171 transitions, ti; 90 transversions, tv), while 147 of these sites were polymorphic within *Iguana* (130 ti, 17 tv) (Table 1).

The three nuclear loci had slower evolutionary rates than the mtDNA locus, with *c-mos* being slowest and *PAC* fastest. Analysis of the *PAC* locus yielded 55 polymorphic nucleotides over the entire data set (36 ti/19 tv) and 14 *Iguana* haplotypes. Additionally, two indels distinguished *I. delicatissima* from *I. iguana*; a single-nucleotide indel and another spanning five nucleotides. Overall, 17 nucleotide sites of the *NT3* locus were polymorphic (14 ti, 3 tv), while seven were polymorphic within the ingroup (6 ti, 1 tv) (Table 1). The *c-mos* locus yielded only two *Iguana* haplotypes, one in each species. This locus had 16 polymorphic sites overall (10 ti, 6 tv), but only four sites were polymorphic within *Iguana* (all transitions, Table 1).

Table 1 Characteristics of nucleotide substitutions for each locus, pairwise comparisons of nucleotide changes between conspecifics and between *Iguana iguana* clades (as defined in Fig. 2), and parsimony analysis results with tree support indices.

	No. of variable characters		Nucleotide substitutions between or within species		Parsimony	
Locus (total bp)	All taxa	*Iguana*	Congenerics	Intraspecific	No. of trees, length	CI/RI
ND4 (825 bp)	171ti 90tv	130ti 17tv	*Iguana* (79–92) *Conolophus* (12) *Cyclura* (33) *Sauromalus* (12–15) *Iguana* (6–11) + 6 bp indel	*I. delicatissima* (0) *I. iguana* I, II, III (31–47)	54, 456 steps	0.719/0.890
PAC (563 bp)	36ti 19tv	15ti 3tv	*Conolophus* (0) *Cyclura* (0) *Sauromalus* (0) *Iguana* (4–7)	*I. delicatissima* (0) *I. iguana* I, II, III (1–9)	1, 58 steps	0.946/0.984
NT3 (489 bp)	14ti 3tv	6ti 1tv	*Conolophus* (0) *Cyclura* (0) *Sauromalus* (0) *Iguana* (4)	*I. delicatissima* (0) *I. iguana* I & II vs. III (3–4)	1, 17 steps	1/1
c-mos (375 bp)	10ti 6tv	4ti 0tv	*Conolophus* (0) *Cyclura* (0) *Sauromalus* (0)	*I. delicatissima* (0) *I. iguana* (0)	1, 18 steps	1/1

CI, consistency index; RI, retention index; ti, transitions; tv, transversions.

Tree topology and nodal support

Each of the four data sets had very different levels of polymorphism, reflected in their different degrees of phylogenetic resolution (Figs 2 & 3). Parsimony analysis of the *ND4* locus generated 54 trees of length 456 (Table 1). A low level of homoplasy and high level of retained homology is indicated by a consistency index (CI) of 0.719 and retention index (RI) of 0.890. These 54 trees differed in the relationships among terminal taxa within clades IIb and III (see Fig. 2) of *I. iguana*. The ML (–3372.423) topology (Fig. 2) differs from that generated by parsimony, with a more basal placement of the samples from the island of St Lucia. The Bayesian topology of the mtDNA tree was nearly identical to the ML tree and differed only in the relationships among Central American haplotypes of *I. iguana*. Heuristic searching of the *PAC* data set under parsimony criteria yielded one tree of 58 steps with a low level of homoplasy (CI = 0.946) and a high level of retained homology (RI = 0.984). The ML (–1105.251) and Bayesian topologies were identical to each other and the MP tree (Fig. 3a). Heuristic searching of the *NT3* data set under parsimony criteria generated one tree of 17 steps with no homoplasy (CI and RI = 1, Fig. 3b; Table 1). Likewise, the *c-mos* data set contained no homoplasy and analysis under parsimony yielded a single topology (16 steps, CI and RI = 1, Fig. 3c; Table 1).

There was strong support for the monophyly of *Iguana* using the *ND4* and *PAC* loci, whereas support from the other two loci was weak (*NT3*) to ambiguous (*c-mos*). Samples from the three island populations of *I. delicatissima* yielded a single haplotype at each of the four loci. Conversely, four well-defined *I. iguana* clades (Fig. 2) were resolved in the *ND4* topology: three from South America (I, IIa, IIb) and a single clade uniting Mexico and Central America (III). The Curacao mtDNA clade (I) was highly divergent from the other lineages sampled, and was basal in the ML and Bayesian analyses. Clades IIa and IIb were composed of South American individuals north-west (excluding Curacao) and south-east of the northern Andes, respectively. By contrast, clade III was composed of many shallow lineages with little geographical structure. While support for these four clades was very strong using the mtDNA locus, the relationships among them had only weak support. Three of the mtDNA clades (IIa, IIb, III) were also resolved with the *PAC* locus (Fig. 3a). These clades had strong support in one or more analyses, even with the limited number of substitutions separating the clades. Haplotypes recovered from Curacao individuals at the *PAC* locus did not form a single clade, nor were these shared with individuals from any other populations.

Two of the nuclear loci, *NT3* and *c-mos*, had very few base differences between or within genera. However, two well-supported, geographically defined *I. iguana* groups were resolved by the *NT3* locus; a single haplotype was present in samples from South America and the Lesser Antilles, while a second haplotype was recovered in all individuals included from Central America and Mexico.

Species-level tree

The largest ΔK was clearly identified at $K = 4$. Assignment probabilities of individuals to the four clusters were high, with all but two samples assigned to a single cluster with > 95% probability. The latter two samples were assigned with > 86% probability to a single cluster, with the majority of remaining genetic variation attributed to clusters with

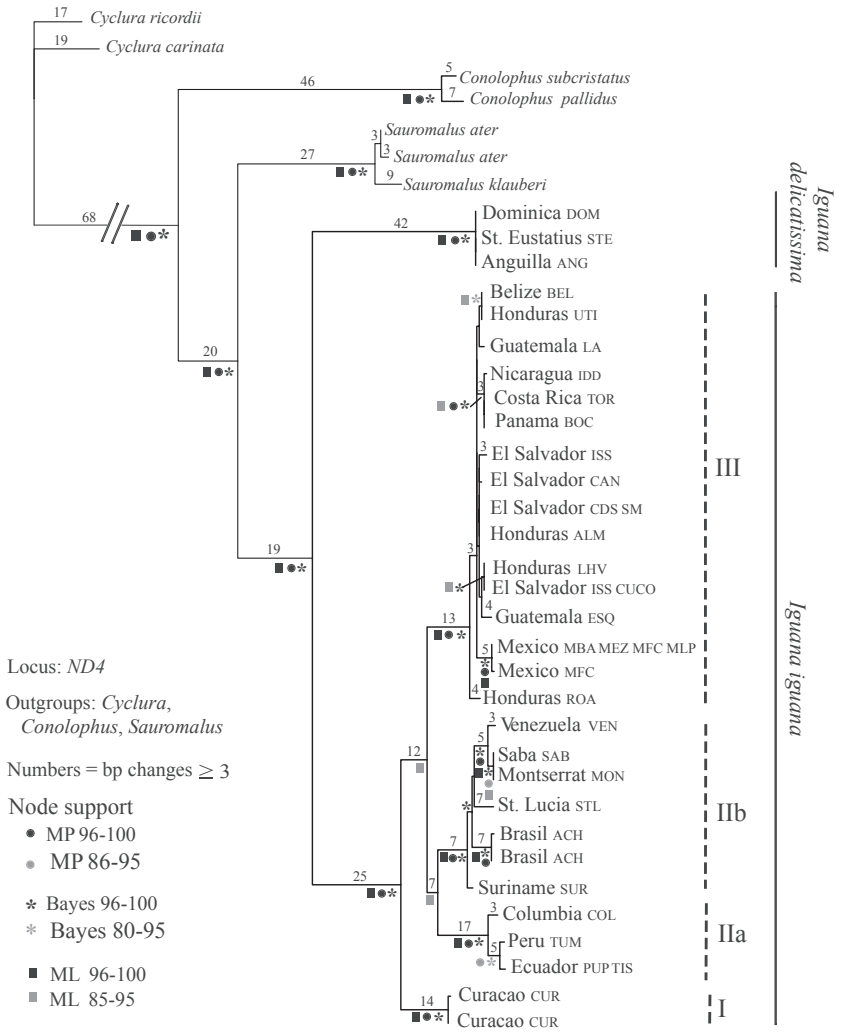

Figure 2 Maximum likelihood generated gene tree for the mitochondrial DNA *ND4* locus of *Iguana iguana* and *I. delicatissima* in the Neotropics, with nodal supported from various analyses indicated in the key (ML, maximum likelihood; MP, maximum parsimony; Bayes). Nucleotide changes greater than two are indicated along each branch. Major infraspecific clades of *I. iguana* are indicated by numerals I–IV. The three-letter codes correspond to the localities shown in Figure 1 and information in Appendix S1.

samples from adjacent geographical regions. The four genetic clusters correspond to the geographical distribution of the samples (cluster 1, North and Central America; cluster 2, north-west of the Andes; cluster 3, Curaçao; cluster 4, Lesser Antilles and south-east of the Andes). For both clock strategies the two independent runs converged and burn-in was set at 7.5×10^7. The post burn-in ESS values for each parameter were above 200. The results of our priors-only *BEAST run, compared with the runs including data, were very different, indicating that our data were the driving force of the resulting topology and rate estimation. Bayes factors indicated the clock strategies with all genes set to relaxed clocks were positive, but lacked support ($\log_{10}$ Bayes factor 0.017). Both clock strategies gave the same species tree topology with similar support for all nodes (Fig. 4a). The tree supports the monophyly of *I. iguana* (PP = 1) and provides little to no support for the relationships between genetic clusters. The *ND4*, *PAC* and *NT3* gene trees are represented within the species-level tree in Fig. 4b, with haplotypes coloured according to geographical region.

Pairwise comparisons

Comparisons of sequence divergence showed large divergences at all four loci within the *Iguana* genus and between *I. iguana* clades, relative to those found in other genera (Table 1). At the nuclear loci, the intrageneric comparisons of outgroup taxa (*Sauromalus*, *Cyclura*, and *Conolophus*) predominantly yielded little or no sequence divergence. This contrasts strongly with *Iguana*, in which the intrageneric comparison yielded an accumulated mean of 8.5 mutations (1.5%) and two indels at the *PAC* locus, 5.5 mutations (1%) at the *NT3* locus, and four mutations (1%) at the *c-mos* locus. Furthermore, within *I. iguana*, two clades (South America vs. Central America) defined by the *NT3* locus were separated by three mutations (0.6%).

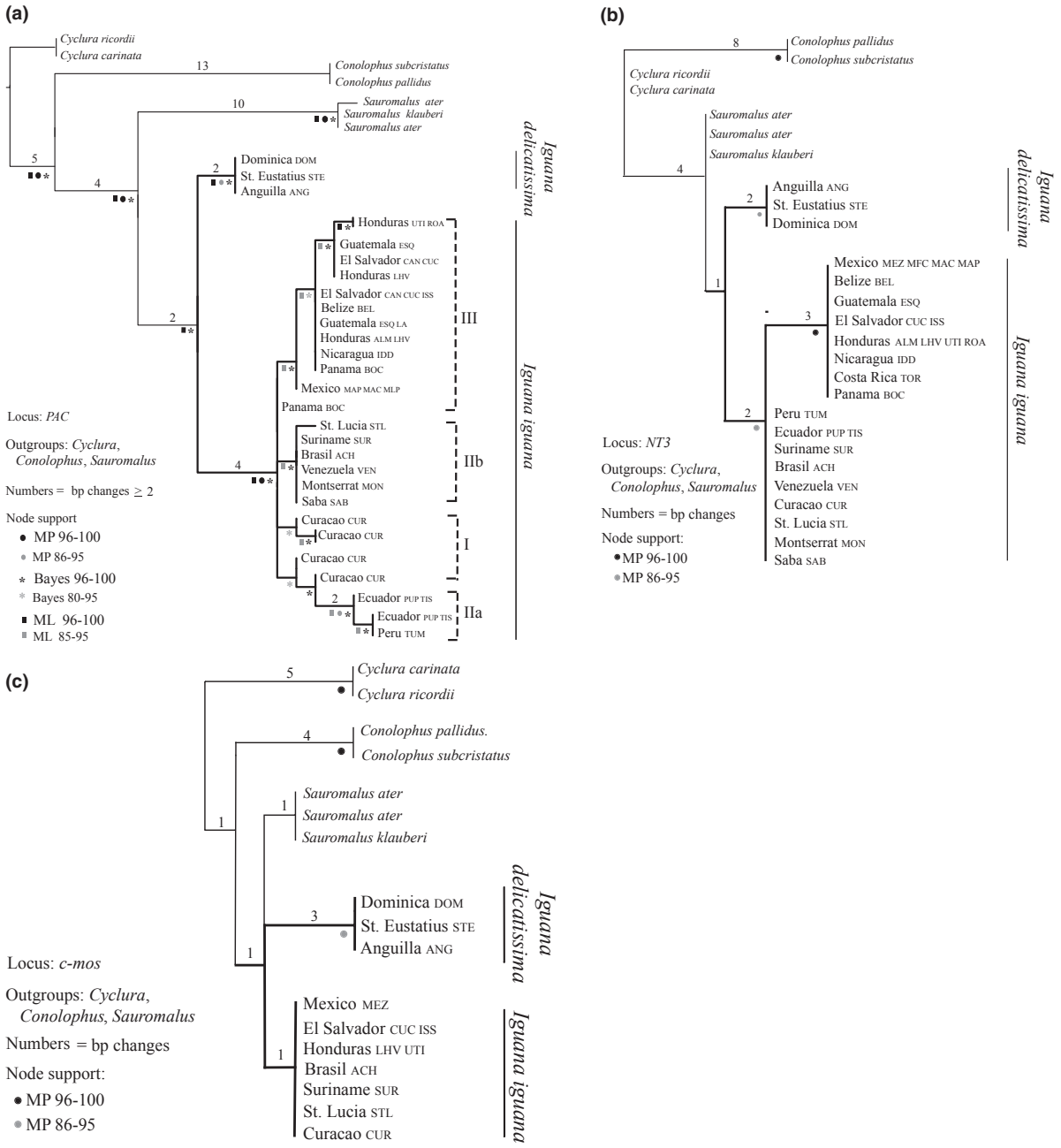

Figure 3 Maximum likelihood generated gene tree for each of three nuclear DNA loci of *Iguana iguana* and *I. delicatissima* in the Neotropics, with nodal supported from various analyses indicated in the key (ML, maximum likelihood; MP, maximum parsimony; Bayes): (a) *PAC*, (b) *NT3* and (c) *c-mos*. Nucleotide changes are indicated along each branch. Major infraspecific clades of *Iguana iguana* are indicated by numerals I–IV for the *PAC* locus. The three-letter codes correspond to the localities shown in Figure 1 and information in Appendix S1.

The mtDNA *ND4* locus showed higher sequence divergence than any of the nuclear loci for all pairwise comparisons, and again the intrageneric divergence of *Iguana* was much greater than the sister species comparisons within the other genera (mean 10.5% vs. 1.5–4%). Lastly, within *I. iguana,* three strongly supported mtDNA clades (I, II, III) had pairwise divergences (mean 3.8–5%) greater than or equal to any comparisons between congeneric outgroup species.

DISCUSSION

Both species of *Iguana* were sampled across much of their ranges; *I. delicatissima* on three Lesser Antillean islands, and *I. iguana* across many current and historical geographical boundaries. At all loci, the divergences within *Iguana* (inter- and intraspecific) are markedly deeper than those between species of the three outgroup genera (Table 1), which

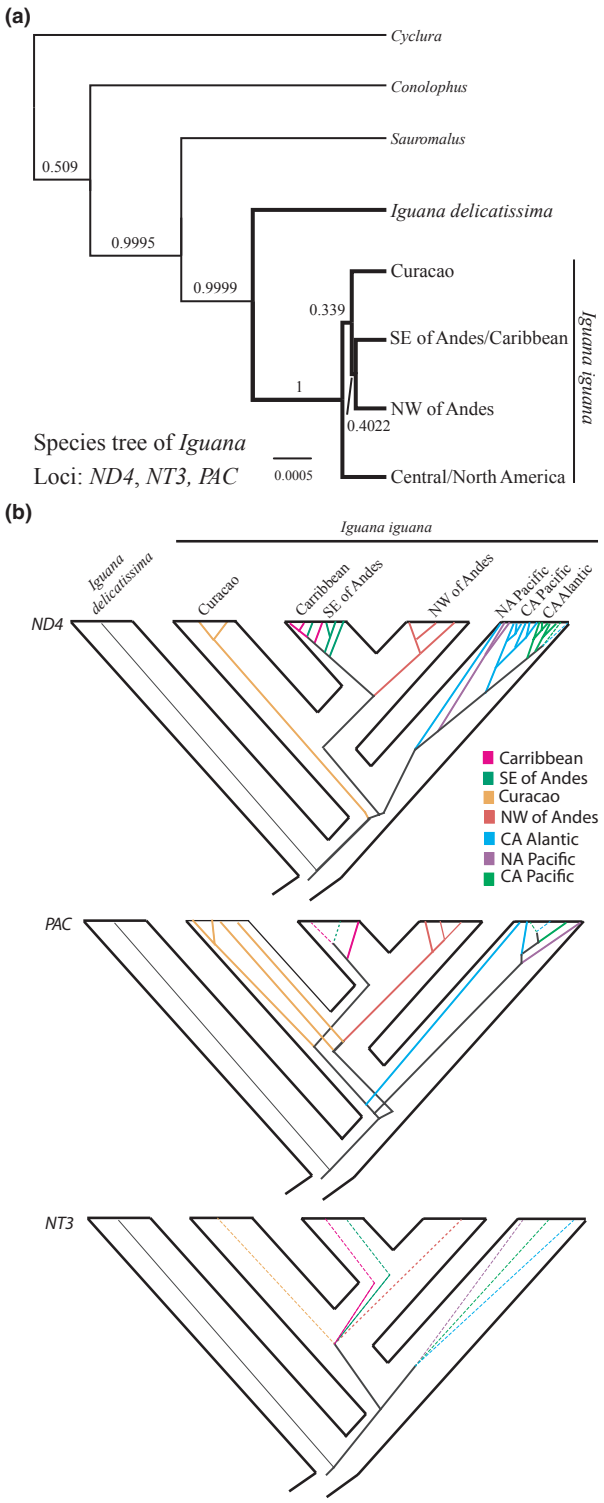

Figure 4 (a) Species tree for *Iguana* generated using three loci (*ND4*, *PAC* and *NT3*) in *BEAST with posterior probabilities indicated at strongly supported nodes, and (b) gene trees of each of the three loci drawn inside the species topology, where full lines denote a haplotype only found in a single geographical region (regions are defined in Fig. 1) and dashed lines indicate a single haplotype found in multiple regions. Geographical regions are colour coded as shown in the key.

include morphologically distinct sister species (Hollingsworth, 1998). At the three nuclear loci, *I. iguana* was clearly differentiated from *I. delicatissima*, while there was no differentiation between the congeneric species of the outgroup taxa. Furthermore, two of the nuclear loci are polymorphic within *I. iguana* and differentiate the Central and North American populations from those found in South America and the Lesser Antilles. The mtDNA locus yielded interspecific polymorphism in all genera and, congruent with the nuclear data, results in intraspecific divergences of *I. iguana* lineages greater than or equal to interspecific divergences within the outgroups.

Phylogeography

The geographical origin of the genus *Iguana* is unclear. The fossil stem group to *Iguana*, *Pumilia*, has been found in North American Pliocene sediments (Norell, 1989). Additionally, genetic data collected to date (Sites *et al.*, 1996; Malone *et al.*, 2000; C.L. Stephen & L.J. Buckley, unpublished data) identify *Sauromalus*, currently distributed throughout the North American Sonoran and Mojave deserts, as the sister genus to *Iguana*. Thus, *Iguana* could be reconstructed as North American in origin. However, *I. delicatissima* is limited in range to the Lesser Antilles, and our genetic data suggest that the oldest *Iguana* populations are located in South America. The most basal mitochondrial lineages recovered within *Iguana* occur in populations currently found in the Lesser Antilles (*I. delicatissima*) and Curaçao (*I. iguana*), a continental island (Bellizzia & Dengo, 1990). Additionally, the mtDNA *I. iguana* clade III, composed of populations north of the Isthmus of Panama, is phylogenetically shallow compared with divergences between the populations north-west and south-east of the Andes (clades IIa and IIb). This genetic distinction between regions south of the Isthmus of Panama is also resolved with the *PAC* locus. These data suggest a relatively recent northerly range expansion from a southern ancestor. Extensive sampling throughout Panama, the Maracaibo Delta and the Amazon Basin is needed to investigate the geographical origin of the genus *Iguana* further.

Genetic data indicate that other species have expanded their ranges across the suture zone post-closure, including the wood lizard *Enyalioides heterolepis*, the howler monkey *Alouatta palliata*, and the antshrike *Thamnophilus atrinucha* (Cortés-Ortiz *et al.*, 2003; Brumfield & Edwards, 2007; Torres-Carvajal & Queiroz, 2009). There has been no obvious geographical barrier to iguana dispersal between Central and South America since the closure of the isthmus at 2.5 Ma (Iturralde-Vinent, 2006), yet our data indicate a surprising lack of gene flow between iguana populations to the north and south of the region. The *NT3* locus yields two clearly defined groups within *I. iguana*; all individuals sampled north of the Isthmus of Panama (Clade III) share a single haplotype, and all those to the south share another (Clades I and II). The mtDNA data revealed more recent gene flow between populations on either

side of the East Andean Cordillera (Clades IIa and IIb) than between populations north and south of the isthmus. The *PAC* locus likewise separated all of the northern haplotypes from the southern haplotypes, and the population-level analysis strongly supported the assignment of all individuals to four distinct clusters corresponding to clades I, IIa, IIb and III. We recognize that our sampling is not fine scaled, and more extensive sampling from populations adjacent to the suture zone could reveal localized gene flow. However, this genetically deep north/south split has also been found in species that are not obviously separated by a barrier, like bay wren subspecies (*Thryothorus nigricapillus*; González *et al.*, 2003) and pseudoscorpions (*Cordylochernes scorpioides*; Zeh *et al.*, 2003).

The mtDNA haplotype found in Curaçao casts some doubt on the cohesiveness of the southern clade: the branch is similar in length to those of each regionally defined clade (Table 1) and does not group closely with either of the other clades. This is surprising given that the population is geographically intermediate between the two regional South American clades. Our results might simply reflect sparse sampling of South American lineages. Alternatively, the Curaçao population might retain an ancestral mtDNA lineage because it was colonized early in the history of *I. iguana*. One hypothesis to explore in future work is that the range of the ancestral *I. iguana* population included Curaçao, an island that would have been connected to the mainland during periods of low sea level. The fact that half (four of eight) of the *PAC* haplotypes from South America and the Lesser Antilles were recovered from individuals on Curaçao supports this hypothesis.

The preference of *Iguana* for lower elevations implies that the Andes currently constitute barriers to gene flow. By 4 Ma, during the final rapid uplift of the eastern cordillera of the Columbian Andes, the range had reached 40% of its current height (Gregory-Wodzicki, 2000) of 3000–2000 m. Our data suggest that common green iguanas ranged across this region prior to uplift as evidenced by the deep divergence of South American clades IIa and IIb. The uplift of the eastern cordillera of the northern Andes (6.8 Ma) has been implicated in the divergence of howler monkeys (*Alouatta*) into north-west and south-east clades (Cortés-Ortiz *et al.*, 2003), while the same geographical divergence within the woodcreeper *Glyphorynchus spirurus* is linked to the final phase of uplift 2.5 Ma (Milá *et al.*, 2009). In accordance with Malone & Davis (2004), our data support two dispersal events into the Lesser Antilles that resulted in *I. iguana* populations on St Lucia and the Saba Bank (Montserrat and Saba islands). While it is not impossible that a single dispersal event into the Lesser Antilles was followed by a back dispersal to the mainland, the prevailing easterly ocean and wind currents make this scenario less likely.

According to the topologies of the *ND4* and *PAC* loci, the initial divergence of the northern clade (III) occurred within the same time frame as the north-west/south-east Andean divergence (clades IIa and IIb). This could have resulted from lower sea levels between 5 and 4 Ma, yielding a brief overland dispersal opportunity or at least a well-formed island arc between the two continents (Haug *et al.*, 2001; Iturralde-Vinent, 2006); yet the long branch leading to this relatively shallow mtDNA clade (III) suggests a recent range expansion or selective sweep through Central and North America. One possibility is that an early divergence resulted in an isolated population that dispersed into Central America later, possibly with the final uplift of the Isthmus of Panama. Within the northern clade, there are few geographically defined groups based on few nucleotide changes. The Honduran highlands seem to present a barrier, as localities north and south of them (LVH, BEL and LA versus IDD, BOC and TOR) fall into distinct mtDNA groups. Branch lengths of the two Honduran island mtDNA haplotypes reflect the difference in age of the islands, with the longer branch on Roatan (at least 75 ka; R. Cox, University of Memphis, pers. comm.) and the shorter branch on Utila (less than 10 ka, R. Rogers, California State University, Stanislaus, pers. comm.). We do not see these phylogeographical patterns in the nuclear loci, reflecting their slower coalescent and mutation rates.

Taxonomy and conservation implications

Among many synonyms in the taxonomic checklist (Hollingsworth, 2004), two specific epithets have been used to describe individuals with enlarged tubercle scales on the snout: *Iguana tuberculata* Laurenti, 1768 and *Iguana rhinolophus* Wiegmann, 1834. These two species were recognized as equivalent by Boulenger (1885) and the names were synonymized by Van Denburgh (1898), but he retained *I. iguana rhinolopha*. Enlarged tubercles are found on individuals in various geographical areas: the Pacific and Atlantic coasts of Mexico; the Atlantic side of Costa Rica; the Canal Zone of Panama; St Lucia in the Lesser Antilles; Providencia, and San Andrés Island in the Caribbean; the Pacific coast of El Salvador; Sinaloa and Veracruz, Mexico; the Orinoco Delta in Venezuela (Fowler, 1913; Hallinan, 1920; Dunn, 1934; Rand, 1957; Valdivieso & Tamsitt, 1963; Henderson, 1974). The use of *I. i. rhinolopha* was supported by Dunn (1934), although he noted that within one population the snout scales can range from very pronounced to absent. In 1973, these names were synonymized with *I. iguana* in independent studies by Lazell (1973) and Hoogmoed (1973). Our results support the 1973 assessments. There is no congruence between our best supported tree and earlier specific or subspecific taxonomy. Individuals with enlarged tubercles are scattered throughout the genetically defined, and geographically distinct, clades.

We agree with Malthora & Thorpe (2004) that resolving and delineating cryptic species depends on obtaining an adequate genetic sampling throughout the range. As noted by others (e.g. DeSalle *et al.*, 2005; Starrett & Hedin, 2007), and clearly demonstrated by Leaché (2009), deep mitochondrial lineages are insufficient to define species. Absence of gene flow between groups must be established by other means, such as nDNA sequencing and/or morphological characters. Congruent recovery, using both mtDNA and nDNA

sequences, of a Central American clade distinct from South American lineages suggests the presence of cryptic species in *I. iguana* (using the definition of Bickford *et al.*, 2007). Additionally, the comparatively lower genetic divergence at these same loci between sister species of closely related Iguaninae genera, support this conclusion (Table 1). Further geographical sampling of genetic data is necessary for a rigorous analysis of gene flow between the north and south clades. Given the data at hand, recognition of the two groups as evolutionary significant units (ESUs; Moritz, 1994) for conservation and management purposes is justified.

In healthy habitat, green iguanas occur in high densities and play vital roles in their ecosystems as seed dispersers (Henderson, 1974; Benítez-Malvido *et al.*, 2003) and as a food resource for other taxa (Sexton, 1975; Greene *et al.*, 1978; Rivas *et al.*, 1998). In many countries within its range, this species is consumed by humans and some of its wild populations have been heavily depleted. This, coupled with the loss of suitable habitat to development and agriculture and a significant trade to supply international pet markets, has justified the listing of *I. iguana* under Appendix II of the Convention on International Trade in Endangered Species (CITES).

Despite this, widespread species like the green iguana are often the last to garner regional conservation attention. Active captive breeding of green iguanas for the pet trade occurs in many countries and breeding stocks for these facilities arrive from distant places (Stephen *et al.*, 2011). Some countries have released captive-bred iguanas in efforts to restore their depleted wild populations, without considering the possible impacts (Stephen *et al.*, 2011). Conservation and management decisions must take into account the presence of the deep genetic divergences between regional *I. iguana* populations.

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REFERENCES

Alvarado, J., Ibarra, L., Suazo, I., Rodríguez, G. & Zamora, R. (1995) Reproductive characteristics of a green iguana (*Iguana iguana*) population of the west coast of Mexico. *The Southwestern Naturalist*, **40**, 234–237.

Antonelli, A., Nylander, J.A., Persson, C. & Sanmartín, I. (2009) Tracing the impact of the Andean uplift on Neotropical plant evolution. *Proceedings of the National Academy of Sciences USA*, **106**, 9749–9754.

Bakhuis, W.L. (1982) Size and sexual differentiation in the lizard *Iguana iguana* on a semiarid island. *Journal of Herpetology*, **16**, 322–325.

Bellizzia, A. & Dengo, G. (1990) The Caribbean mountain system, northern South America; a summary. *The geology of North America: the Caribbean region*, Vol. H (ed. by G. Dengo and J.E. Case), pp. 167–175. Geological Society of America, Boulder, CO.

Benítez-Malvido, J., Tapia, E.M., Suazo, I., Villaseñor, E. & Alvarado, J. (2003) Germination and seed damage in tropical dry forest plants ingested by iguanas. *Journal of Herpetology*, **37**, 301–308.

Bickford, D., Lohman, D.J., Sodhi, N.S., Ng, P.K.L., Meier, R., Winker, K., Ingram, K. & Das, I. (2007) Cryptic species as a new window on diversity and conservation. *Trends in Ecology and Evolution*, **22**, 148–155.

Bock, B.C. (1984) *Movement patterns relative to nesting site locations in a population of green iguanas (*Iguana iguana*) in Panama*. PhD Thesis, University of Tennessee, Knoxville, TN.

Bock, B.C. & McCracken, G.F. (1988) Genetic structure and variability in the green iguana (*Iguana iguana*). *Journal of Herpetology*, **22**, 316–322.

Brumfield, R.T. & Edwards, S.V. (2007) Evolution into and out of the Andes: a Bayesian analysis of historical diversification of *Thamnophilus* antshrikes. *Evolution*, **39**, 346–367.

Burnham, R.J. & Graham, A. (1999) The history of Neotropical vegetation: new developments and status. *Annals of the Missouri Botanical Garden.*, **86**, 546–589.

Bush, M.B. (1994) Amazonian speciation: a necessarily complex model. *Journal of Biogeography*, **21**, 5–17.

Campbell, J. (1998) *Amphibians and reptiles of northern Guatemala, the Yucatan, and Belize*. University of Oklahoma Press, Norman, OK.

Coates, A.G. (1997) *Central America: a natural and cultural history*. Yale University Press, New Haven, CT.

Colborn, J., Crabree, R.E., Shalee, J.B., Pfeiler, E. & Bowen, B.W. (2001) The evolutionary enigma of bonefishes (*Albula* spp.): cryptic species and ancient separations in a globally distributed shorefish. *Evolution*, **55**, 807–820.

Colbourne, J.K. & Hebert, P.D.N. (1996) The systematics of North American *Daphnia* (Crustacea: Anomopoda): a molecular phylogenetic approach. *Philosophical Transactions of the Royal Society B: Biological Sciences*, **351**, 349–360.

Conrad, J.L. & Norell, M.A. (2010) Cranial autapomorphies in two species of iguana (Iguanidae: Squamata). *Journal of Herpetology*, **44**, 307–312.

Cortés-Ortiz, L., Bermingham, E., Rico, C., Rodriguez-Luna, E. & Sampaio, I. (2003) Molecular systematics and biogeography of the Neotropical monkey genus, *Alouatta*. *Molecular Phylogenetics and Evolution*, **26**, 64–81.

Cracraft, J. & Prum, R.O. (1988) Patterns and processes of diversification: speciation and historical congruence in some Neotropical birds. *Evolution*, **42**, 603–620.

DeSalle, R., Egan, M.G. & Siddall, M. (2005) The unholy trinity: taxonomy, species delimitation, and DNA barcoding. *Philosophical Transactions of the Royal Society B: Biological Sciences*, **360**, 1905–1916.

Dugan, B.A. (1980) *A field study of the social structure, mating system, and display behaviour of the green iguana*. PhD Thesis, University of Tennessee, Knoxville, TN.

Dunn, E.R. (1934) Notes on *Iguana*. *Copeia*, **1**, 1–4.

Earl, D.A. & vonHoldt, B.M. (2012) STRUCTURE HARVESTER: a website and program for visualizing STRUCTURE output and implementing the Evanno method. *Conservation Genetics Resources*, **4**, 359–361.

Etheridge, R. (1982) Checklist of the iguanine and Malagasy iguanid lizards. *Iguanas of the world: their behavior, ecology and conservation* (ed. by G.M. Burghardt and A.S. Rand), pp. 7–37. Noyes Publishing, Park Ridge, NJ.

Evanno, G., Regnaut, S. & Goudet, J. (2005) Detecting the number of clusters of individuals using the software STRUCTURE: a simulation study. *Molecular Ecology*, **14**, 2611–2620.

Falush, D., Stephens, M. & Pritchard, J.K. (2003) Inference of population structure: extensions to linked loci and correlated allele frequencies. *Genetics*, **164**, 1567–1587.

Felsenstein, J. (1981) Evolutionary trees from DNA sequences: a maximum likelihood approach. *Journal of Molecular Evolution*, **17**, 368–376.

Felsenstein, J. (1985) Confidence limits on phylogenies: an approach using the bootstrap. *Evolution*, **39**, 783–791.

Fowler, H.W. (1913) Amphibians and reptiles from Ecuador, Venezuela, and Yucatan. *Proceedings of the Academy of Natural Sciences of Philadelphia*, **65**, 153–176.

González, A.M., Brehm, A., Pérez, J.A., Maca-Meyer, N., Flores, C. & Cabrera, V.M. (2003) Mitochondrial DNA affinities at the Atlantic fringe of Europe. *American Journal of Physical Anthropology*, **120**, 391–404.

Greene, H., Burghardt, G.M., Dugan, B.A. & Rand, A.S. (1978) Predation and the defensive behavior of green iguanas (Reptilia, Lacertilia, Iguanidae). *Journal of Herpetology*, **12**, 169–176.

Gregory-Wodzicki, K. (2000) Uplift history of the central and northern Andes: a review. *Geological Society of America Bulletin*, **112**, 1091–1105.

Hallinan, T. (1920) Notes on the Canal Zone, Isthmus of Panama. *Copeia*, **83**, 45–49.

Haq, B.U., Hardenbol, J. & Vail, P.R. (1987) Chronology of fluctuating sea levels since the Triassic. *Science*, **235**, 1156–1167.

Harris, D.M. (1982) The phenology growth, and survival of the green iguana, *Iguana iguana*, in northern Colombia. *Iguanas of the world: their behavior, ecology and conservation* (ed. by G.M. Burghardt and A.S. Rand), pp. 150–161, Noyes Publishing, Park Ridge, NJ.

Hasbun, C.R., Gomez, A., Kohler, G. & Lunt, D.H. (2005) Mitochondrial DNA phylogeography of the Mesoamerican spiny-tailed lizards (*Ctenosaura quinquecarinata* complex): historical biogeography, species status and conservation. *Molecular Ecology*, **14**, 3095–3107.

Haug, G.H., Tiedemann, R., Zahn, R. & Ravelo, A.C. (2001) Role of Panama uplift on oceanic freshwater balance. *Geology*, **29**, 207–210.

Heled, J. & Drummond, A.J. (2010) Bayesian inference of species trees from multilocus data. *Molecular Biology and Evolution*, **27**, 570–580.

Henderson, R.W. (1974) Aspects of the ecology of the juvenile common iguana (*Iguana iguana*). *Herpetologica*, **30**, 327–332.

Henderson, R.W. & Hedges, S.B. (1995) Origin of West Indian populations of the geographically widespread boa *Corallus enydris* inferred from mitochondrial DNA sequences. *Molecular Phylogenetics and Evolution*, **4**, 88–92.

Heraty, J.M., Woolley, J.B., Hopper, K.R., Hawks, D.L., Kim, J. & Buffington, M. (2007) Molecular phylogenetics and reproductive incompatibility in a complex of cryptic species of aphid parasitoids. *Molecular Phylogenetics and Evolution*, **45**, 480–493.

Hollingsworth, B.D. (1998) The systematics of chuckwallas (*Sauromalus*) with a phylogenetic analysis of other iguanid lizards. *Herpetological Monographs*, **12**, 38–191.

Hollingsworth, B.D. (2004) The evolution of iguanas: an overview of relationships and a checklist of species. *Iguanas: biology and conservation* (ed. by A.C. Alberts, R.L. Carter, W.K. Hayes and E.P. Martins), pp. 19–44. University of California Press, Berkeley, CA.

Hoogmoed, M.S. (1973) Notes on the herpetofauna of Surinam. *Biogeographica*, **4**, 1–119.

Huelsenbeck, J.P. & Ronquist, F. (2001) MRBAYES: Bayesian inference of phylogenetic trees. *Bioinformatics*, **17**, 754–755.

Iturralde-Vinent, M.A. (2006) Meso-Cenozoic Caribbean paleogeography: implications for the historical biogeography of the region. *International Geology Review*, **48**, 791–827.

Jakobsson, M. & Rosenberg, N.A. (2007) CLUMPP: a cluster matching and permutation program for dealing with label switching and multimodality in analysis of population structure. *Bioinformatics*, **23**, 1801–1806.

Kass, R.E. & Raftery, A.E. (1995) Bayes factors. *Journal of the American Statistical Association*, **90**, 773–795.

Katoh, K., Kuma, K., Toh, H. & Miyata, T. (2005) MAFFT version 5: improvement in accuracy of multiple sequence alignment. *Nucleic Acids Research*, **33**, 511–518.

Klatau, M., Russo, C.A.M., Lazoski, C., Thorpe, J.P. & Sole-Cava, A.M. (1999) Does cosmopolitanism result from overconservative systematics? A case study using the marine sponge *Chondrilla nucula*. *Evolution*, **53**, 1414–1422.

Lazell, J.D., Jr (1973) The lizard genus *Iguana* in the Lesser Antilles. *Bulletin of the Museum of Comparative Zoology*, **145**, 1–28.

Leaché, A.D. (2009) Species tree discordance traces to phylogeographic clade boundaries in North American fence lizards (*Sceloporus*). *Systematic Biology*, **58**, 547–559.

Makowsky, R., Chesser, J. & Risler, L.J. (2009) A striking lack of genetic diversity across the wide-ranging amphibian *Gastrophryne carolinensis* (Anura: Microhylidae). *Genetica*, **135**, 169–183.

Malone, C.L. & Davis, S.K. (2004) Genetic contributions to Caribbean iguana conservation. *Iguanas: biology and conservation* (ed. by A.C. Alberts, R.L. Carter, W.K. Hayes and E.P. Martins), pp. 45–57. University of California Press, Berkeley, CA.

Malone, C.L., Wheeler, T., Taylor, J.T. & Davis, S.K. (2000) Phylogeography of the Caribbean rock iguana (*Cyclura*): implications for conservation and insights on the biogeographic history of the West Indies. *Molecular Phylogenetics and Evolution*, **17**, 269–279.

Malthora, A. & Thorpe, R.S. (2004) Maximizing information in systematic revisions: a combined molecular and morphological analysis of a cryptic green pitviper complex (*Trimeresurus stejnegeri*). *Biological Journal of the Linnean Society*, **82**, 219–235.

Marks, B.D., Hackett, S.J. & Capparella, A.P. (2002) Historical relationships among Neotropical lowland forest areas of endemism as determined by mitochondrial DNA sequence variation within the wedge-billed woodcreeper (Aves: Dendrocolaptidae: *Glyphorynchus spirurus*). *Molecular Phylogenetics and Evolution*, **24**, 153–167.

Martin, A.P. & Bermingham, E. (2000) Regional endemism and cryptic species revealed by molecular and morphological analysis of a widespread species of Neotropical catfish. *Proceedings of the Royal Society B: Biological Sciences*, **267**, 1135–1141.

Milá, B., Wayne, R.C., Fitze, P. & Smith, T.B. (2009) Divergence with gene flow and fine-scale phylogeographical structure in the wedge-billed woodcreeper, *Glyphorynchus spirurus*, a Neotropical rainforest bird. *Molecular Ecology*, **18**, 2979–2995.

Moritz, C. (1994) Defining 'evolutionarily significant units' for conservation. *Trends in Ecology and Evolution*, **9**, 373–375.

Müller, H. (1968) Untersuchungen über Wachstum und Altersverteilung einer Population des Grünen Leguans *Iguana iguana iguana* L. (Reptilia: Iguanidae). *Instituto Colombo-Alemán Investigacion Científica Punta de Betín*, **2**, 57–65.

Noonan, B.P. & Chippindale, P.T. (2006a) Dispersal and vicariance: the complex evolutionary history of boid snakes. *Molecular Phylogenetics and Evolution*, **40**, 347–358.

Noonan, B.P. & Chippindale P.T. (2006b) Vicariant origin of Malagasy reptiles supports Late Cretaceous Antarctic land bridge. *The American Naturalist*, **168**, 730–741.

Norell, M.A. (1989) *Late Tertiary fossil lizards of the Anza-Borrego Desert, California*. Contributions in Science, No. 414. Natural History Museum of Los Angeles County, Los Angeles, CA.

Pasachnik, S.A., Fitzpatrick, B.M., Near, T.J. & Echternacht, A.C. (2008) Gene flow between an endangered endemic iguana, and its wide spread relative, on the island of Utila, Honduras: when is hybridization a threat? *Conservation Genetics*, **10**, 1247–1254.

Patton, J.L. & Smith, M.F. (1992) MtDNA phylogeny of Andean mice: a test of diversification across ecological gradients. *Evolution*, **46**, 174–183.

Peppers, L.L. & Bradley, R.D. (2000) Cryptic speciation in *Sigmodon hispidus*: evidence from DNA sequences. *Journal of Mammalogy*, **81**, 332–343.

Petren, K. & Case, T.J. (1997) A phylogenetic analysis of body size evolution and biogeography in chuckwallas (*Sauromalus*) and other iguanines. *Evolution*, **51**, 206–219.

Pfenninger, M. & Schwenk, K. (2007) Cryptic animal species are homogeneously distributed among taxa and biogeographical regions. *BioMed Central Evolutionary Biology*, **7**, 1–6.

Posada, D. & Crandall, K.A. (1998) Modeltest: testing the model of DNA substitution. *Bioinformatics*, **14**, 817–818.

Prance, G.T. (1982) A review of the phytogeographic evidences for Pleistocene climate changes in the Neotropics. *Annals of the Missouri Botanical Garden*, **69**, 594–624.

de Quieroz, K. (1987) *Phylogenetic systematics of iguanine lizards: a comparative osteological study*. University of California Publications in Zoology 118. University of California Press, Berkeley.

Rambaut, A. & Drummond, A.J. (2007) Tracer version 1.4. Available at: http://beast.bio.ed.ac.uk/Tracer.

Rand, S.A. (1957) Notes on amphibians and reptiles from El Salvador. *Fieldiana Zoology*, **34**, 505–534.

Rand, S.A., Dugan, B.A., Monteza, H. & Vianda, D. (1990) The diet of a generalized folivore: *Iguana iguana* in Panama. *Journal of Herpetology*, **24**, 211–214.

Retallack, G.J. & Kirby, M.X. (2007) Middle Miocene global change and paleogeography of Panama. *Palaios*, **22**, 667–679.

Rivas, J.A., Molina, C.R. & Avila, T.M. (1998) *Iguana iguana* (green iguana). Juvenile predation. *Herpetological Review*, **29**, 238–239.

Rodda, G.H. (1992) *The mating behavior of* Iguana iguana. Smithsonian Contributions to Zoology No. 534. Smithsonian Institution, Washington, DC.

Sanderson, M.J. & Donoghue, M.J. (1989) Patterns of variation in levels of homoplasy. *Evolution*, **43**, 1781–1795.

Sexton, O.J. (1975) Black vultures feeding on iguana eggs in Panama. *American Midland Naturalist*, **93**, 463–468.

Sites, J.W., Jr, Davis, S.K., Guerra, T., Iverson, J.B. & Snell, H.L. (1996) Character congruence and phylogenetic signal in molecular and morphological data sets: a case study in the living iguanas (Squamata, Iguanidae). *Molecular Biology and Evolution*, **13**, 1087–1105.

Stamatakis, A. (2006) RAxML-VI-HPC: maximum likelihood-based phylogenetic analyses with thousands of taxa and mixed models. *Bioinformatics*, **22**, 2688–2690.

Starrett, J. & Hedin, M. (2007) Multilocus genealogies reveal multiple cryptic species and biogeographical complexity in the California turret spider *Antrodiaetus riversi* (Mygalomorphae, Antrodiatidae). *Molecular Ecology*, **16**, 583–604.

Stephen, C.L., Pasachnik, S., Reuter, A., Mosig, P., Ruyle, L. & Fitzgerald, L. (2011) *Survey of status, trade, and exploita-*

tion of Central American iguanas. United States Fish and Wildlife Services, Washington, DC.

Suchard, M.A., Weiss, R.E. & Sinsheimer, J.S. (2001) Bayesian selection of continuous-time Markov chain evolutionary models. *Molecular Biology and Evolution*, **18**, 1001–1013.

Swanson, P.L. (1950) The iguana *Iguana iguana iguana* (L). *Herpetologica*, **6**, 187–193.

Swofford, D.L. (2000) *PAUP*. Phylogenetic analysis using parsimony (*and other methods), version 4.0*. Sinauer Associates, Sunderland, MA.

Toews, D.P. & Irwin, D.E. (2008) Cryptic speciation in a Holarctic passerine revealed by genetic and bioacoustic analyses. *Molecular Ecology*, **23**, 2691–2705.

Torres-Carvajal, O. & de Queiroz, K. (2009) Phylogeny of hoplocercine lizards (Squamata: Iguania) with estimates of relative divergence times. *Molecular Phylogenetics and Evolution*, **50**, 31–43.

Valdivieso, D. & Tamsitt, J.R. (1963) A checklist and key to the amphibian and reptiles of Providencia and San Andres. *Caribbean Journal of Science*, **3**, 77–79.

Van Denburgh, J. (1898) Reptiles from Sonora, Sinaloa, and Jalisco, Mexico, with a description of a new species of *Sceloporus*. *Proceedings of the Academy of Natural Science of Philadelphia*, **1897**, 460–464.

Venegas-Anaya, M., Crawford, A.J., Galván, A.H.E., Sanjur, O.I., Densmore, L.D., III & Bermingham, E. (2008) Mitochondrial DNA phylogeography of *Caiman crocodilus* in Mesoamerica and South America. *Journal of Experimental Zoology*, **309A**, 614–627.

Wertheim, J.O., Sanderson, M.J., Worobey, M. & Bjork, A. (2010) Relaxed molecular clocks, the bias-variance trade-off, and the quality of phylogenetic inference. *Systematic Biology*, **59**, 1–8.

Wiens, J.J. & Hollingsworth, B.D. (2000) War of the iguanas: conflicting molecular and morphological phylogenies and long branch attraction in iguanid lizards. *Systematic Zoology*, **49**, 143–159.

Wilcox, T.P., Hugg, L., Zeh, J.A. & Zeh, D.W. (1997) Mitochondrial DNA sequencing reveals extreme genetic differentiation in a cryptic species complex of Neotropical pseudoscorpions. *Molecular Phylogenetics and Evolution*, **7**, 208–216.

Witt, J.D.S. & Hebert, P.D.N. (2000) Cryptic species diversity and evolution in the amphipod genus *Hyalella* within central glaciated North America: a molecular phylogenetic approach. *Canadian Journal of Fisheries and Aquatic Sciences*, **57**, 687–698.

Wüster, W., Ferguson, J.E., Quijada-Mascareñas, J.A., Pook, C.E., da Graça Salomão, M. & Thorpe, R.S. (2005) Tracing an invasion: landbridges, refugia, and the phylogeography of the Neotropical rattlesnake (Serpentes: Viperidae: *Crotalus durissus*). *Molecular Ecology*, **14**, 1095–1108.

Zamudio, K.R. & Greene, H.W. (1997) Phylogeography of the bushmaster (*Lachesis muta*: Viperidae): implications for Neotropical biogeography, systematics, and conservation. *Biological Journal of the Linnean Society*, **62**, 421–442.

Zeh, D.W., Zeh, J.A. & Bonilla, M.M. (2003) Phylogeography of the giant harlequin beetle (*Acrocinus longimanus*). *Journal of Biogeography*, **30**, 747–753.

SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

Appendix S1 Collection information for individuals in the subfamily Iguaninae included in the study and GenBank accession numbers for all haplotypes.

BIOSKETCH

Catherine L. Stephen is an Associate Professor of Biology at Utah Valley University. Her primary research focus is on the phylogenetic relationships between, and biogeographical history of, species within the subfamily Iguaninae. As an active member of the IUCN Iguana Specialist Group her research is conducted with broad collaboration and results are applied to relevant conservation issues.

Author contributions: C.L.S. conceived the study; C.L.S. collected the data with significant contribution from W.C.; C.L.S. and J.W.B. performed the analyses; C.L.S. led the writing with contributions from J.W.B. and C.R.H.; C.L.S., J.W.B. and V.H.R. constructed the figures; and all authors discussed the results and commented on the manuscript.

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