*Zootaxa* 5748 (3): 387–399
https://www.mapress.com/zt/


**Article**

ISSN 1175-5326 (print edition)
**ZOOTAXA**
ISSN 1175-5334 (online edition)

https://doi.org/10.11646/zootaxa.5748.3.4
http://zoobank.org/urn:lsid:zoobank.org:pub:2787F0EC-26A8-4668-BAD5-2965EB10DF60

# Consideration of range-wide variation is critical when splitting widely distributed species: the case of the proposed *Iguana melanoderma*

MATTHIJS P. VAN DEN BURG[1,2,*], KEVIN DE QUEIROZ[3], MARINA VENTAYOL[4], ADOLPHE O. DEBROT[5] & CATHERINE L. MALONE[1]

[1]*IUCN SSC Iguana Specialist Group, Gland, Switzerland*
*catherine.malone.isg@gmail.com; https://orcid.org/0009-0005-0931-5070*
[2]*Burg Biologica, The Hague, The Netherlands*
[3]*Department of Vertebrate Zoology, National Museum of Natural History, Smithsonian Institution, Washington, DC, USA*
*dequeirozk@si.edu; https://orcid.org/0000-0001-9165-3522*
[4]*Naturalis Biodiversity Center, Leiden, The Netherlands*
*ventayol.marina@gmail.com; https://orcid.org/0000-0002-8036-8475*
[5]*Wageningen Marine Research, Wageningen Research, Den Helder, The Netherlands*
*dolfi.debrot@wur.nl; https://orcid.org/0000-0001-9406-8150*
[*]*Corresponding author: thijs.burg@gmail.com; https://orcid.org/0000-0001-8276-0713*

## Abstract

Among Iguaninae species, *Iguana iguana* (Linnaeus) has the largest geographic range, spanning most of the Neotropical mainland and numerous islands including several in the Lesser Antilles. Genetic data indicated the presence of cryptic diversity and at least four major, mtDNA clades. However, rather than assessing the taxonomic status of these four major clades, recent taxonomic work has focused more narrowly on populations in the Lesser Antilles nested deeply within one major clade. In one such case, melanistic populations on Saba, Montserrat, and in northern Venezuela have been proposed as *Iguana melanoderma* Breuil *et al.* Here we re-evaluate the taxonomic status of *I. melanoderma* within the broader context of the *I. iguana* species complex. We generated museomic data from 10 specimens collected as early as 1929 from 10 new localities across northern Venezuela, and two samples from Trinidad, resulting in eight previously unknown ND4 haplotypes. We conducted divergence comparisons and phylogenetic analyses using mtDNA sequences. Our results indicate that the previously reported genetic distinctiveness of *I. melanoderma* was over-estimated due to limited geographic sampling. Instead, genetic data from northern South America reveal an eroded distinction and shallow divergences among sampled populations of the *I. iguana* species complex (Clade IV), including the proposed *Iguana melanoderma*. Numerous sampling gaps remain in this region of northern South America, which hamper taxonomic interpretations of current genetic data. Beyond genetics, the morphological dataset underlying the description of *I. melanoderma* was geographically limited and lacked data from crucial populations. We argue that, for the present, *I. melanoderma* should not be recognized but instead should be considered part of *I. iguana* (Clade IV), and we highlight the importance of broader sampling efforts in future taxonomic research on this species complex as well as the necessity of considering range-wide variation in taxonomic studies of wide-ranging taxa more generally.

**Key words:** Caribbean, Melanism, Museum specimens, Next Generation Sequencing, Sampling strategy, South America

## Introduction

Studies on "intraspecific" variation have demonstrated that what were previously thought to be wide-ranging species often represent complexes of several (cryptic) species (e.g., Nakahara *et al.* 2018; Jaramillo *et al*. 2020). Among iguanas (= Iguaninae sensu ITWG 2016), the species *Iguana iguana* (Linnaeus) (sensu Lazell 1973, ITWG 2016, 2022) has the largest geographic range, spanning most of the Neotropics (Bock *et al.* 2022): ~1,000k km$^2$ of South America and >700k km$^2$ from Panama to its northern limit in Mexico (Bock *et al*. 2022). So perhaps unsurprisingly, multiple, divergent genetic lineages, whose boundaries correspond to historical and current geographic barriers, have been identified within that broadly distributed taxon (Stephen *et al*. 2013), hereafter referred to as the *I. iguana*

*Accepted by L.J. Avila: 21 Nov. 2025; published: 14 Jan. 2026*

species complex. Recently, Breuil *et al*. (2020) proposed the species *Iguana melanoderma* for populations from Saba, Montserrat, and an undefined mainland range. However, the analyses and interpretation behind that proposal were made by focusing narrowly on populations in the Lesser Antilles and a few localities in northern South America. Herein, we demonstrate that the absence of a broader context biased the results and their interpretation to suggest that the populations in question are more unique and distinct than they are.

## Taxonomic Background

*Iguana iguana* sensu Lazell (1973) is a highly variable species, both phenotypically (Lazell 1973) and genetically (Stephen *et al*. 2013) that has yet to be studied using a geographically broad and dense dataset. Although numerous species have been proposed within this complex (see list of synonyms in Lazell 1973 and de Queiroz 1995), little has been published on this topic after Lazell (1973) reviewed morphological variation in *I. iguana* and recognized a single species, rejecting the subspecies *I. iguana iguana* and *I. i. rhinolopha,* which had previously been recognized by Dunn (1934). Those subspecies were resurrected by Breuil (2013) and subsequently recognized as species, without providing new data, in a study proposing two additional subspecies, *I. iguana insularis* and *I. i. sanctaluciae* (Breuil *et al.* 2019). Most recently, Breuil *et al.* (2020) proposed a new species, *I. melanoderma*, and treated the previously recognized subspecies *I. iguana insularis* and *I. iguana sanctaluciae* as subspecies of a separate species, *Iguana insularis*. While these proposed taxonomic changes have stimulated discussion in the broader community, only the subspecies *I. ig. rhinolopha*, *I. ig. iguana*, *I. ig. insularis* and *I. ig. sanctaluciae* have since been adopted by the IUCN-ISG Taxonomy Working Group (ITWG 2022).

Breuil *et al*. (2020) asserted support for their proposal of *I. melanoderma,* stating "Morphological studies (Breuil 2013, 2016) ... have shown that the Saba population has unique characteristics." The primary focus of Breuil (2013, English translation 2016), was on the characterization of morphological differences between *Iguana delicatissima* Laurenti, *I. iguana*, and their hybrids, not on morphological variation within these taxa. In fact, the 2013 dataset permits only a very limited understanding of range-wide morphological variation in the *I. iguana* species complex. Breuil (2013) divided the morphological dataset into four groups: two representing geographically broad taxa (*I. i. iguana* and *I. i. rhinolopha*) and two representing geographically narrow ones (the populations of Saba and St. Lucia). However, morphological data used to characterize *I. i. iguana* were only collected for individuals from French Guiana (85k $km^2$); a small fraction (ca. 0.0085%) of its South American range. Additionally, multiple morphological characters used in the 2013 dataset were not clearly defined (e.g., "abundant and prominent" nuchal spines; "very high" dorsal spines), and critical information was not reported, such as the specimens used and sample sizes underlying reported morphological data, resulting in a morphological dataset that is inadequate for supporting proposals to recognize additional species within the *I. iguana* species complex. On the other hand, these same data provided important insights and has been critical for the conservation of insular iguana populations in the Lesser Antilles, especially for the critically endangered *I. delicatissima*.

Stephen *et al*. (2013) generated a geographically broad mtDNA dataset for the *I. iguana* species complex. They inferred four major phylogeographic clades, although sampling density remained too sparse to delineate their geographic boundaries precisely. Lacking congruent, independent datasets, the authors refrained from proposing taxonomic changes. The three localities that Breuil *et al*. (2020) included from the presumed native range of the proposed species *I. melanoderma* (the islands of Saba and Montserrat, and mainland Cumaná, Venezuela) were also included in Stephen *et al*. (2013), where they formed a shallow clade within one of the four major haplotype clades of the *I. iguana* species complex. Although Breuil *et al*. (2020) generated additional mtDNA data (from Saba, Montserrat, and French Guiana), no mainland sampling localities for the proposed *I. melanoderma* were added to better define its geographic range, as those from French Guiana were assigned to *I. iguana*.

Our objective here is to evaluate the impact that increased geographic sampling has on the genetic distinctness of the proposed species *I. melanoderma* in the broader context of the *I. iguana* species complex. We collected mitochondrial DNA data from historical museum and modern *Iguana* specimens, representing new localities in northern South America, and combined these with all other relevant published data in a phylogenetic analysis. We discuss the appropriateness of the proposed taxonomic change considering our results and a re-examination of purported support for this proposed species by other datatypes (morphological and microsatellite).

## Materials and Methods

We sampled muscle tissue for genetic analysis from historical specimens of *I. iguana* collected in the northern coastal region of South America, including specimens from insular and both coastal and inland mainland localities (Fig. 1, Appendix 1). These include ten specimens collected in the 1930's (Hummelinck 1944) and housed at the Naturalis Biodiversity Center collection, Leiden, The Netherlands; and four from the Venezuelan mainland housed at the National Museum of Natural History, Smithsonian Institution (Appendix 1). Tissue samples, blood (live) or muscle (roadkill), were also collected from individuals between 2008 and 2013, including two *I. iguana* from Trinidad and six *Sauromalus* from both wild and captive sources representing multiple species and geographic regions.

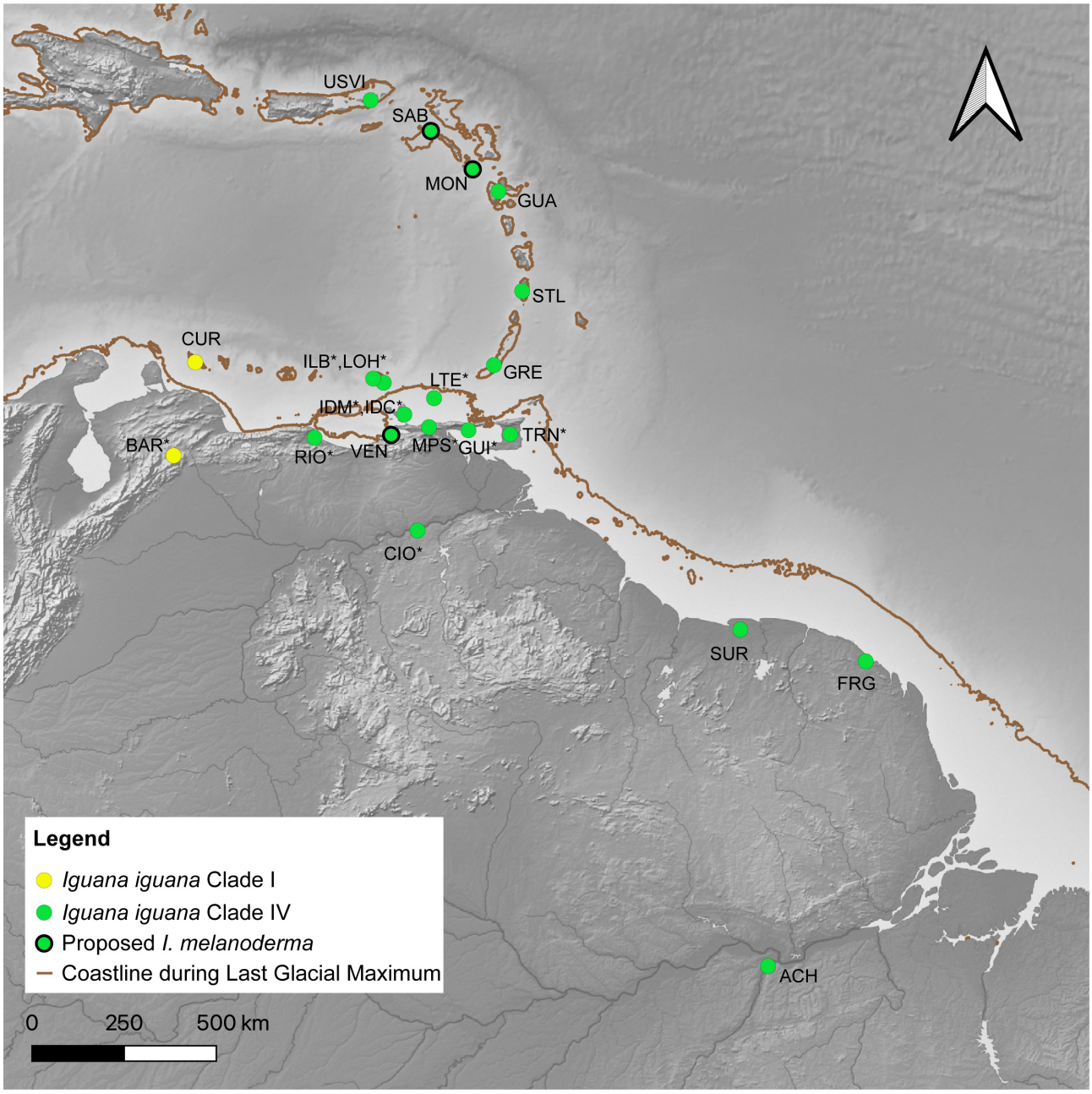


**FIGURE 1.** Map of northern South America and the Lesser Antilles showing sample locations, including those for the proposed *Iguana melanoderma*, and their assignment to major mtDNA clades in the *I. iguana* species complex (see Fig. 2). Locality codes are defined in Appendices 1 and 2; codes with an asterisk are new to this study. The sample locality RIO was not included in the phylogenetic analysis (see Results for details).

We extracted DNA from museum specimens using a formalin-fixed tissue preparation protocol (Hahn *et al*. 2022), followed by tissue digestion (Campos & Gilbert 2012). After measuring genomic DNA concentration, libraries were prepared with NEBNext® Ultra II DNA Library Prep Kit for Illumina® following the manufacturer's manual but downscaling to ⅛ of the instructed volumes. Bead clean-ups were performed at 1.2X ratios while size selection was avoided, followed by PCR amplification with 10uM IDT xGen UDI 10nt primers and the following conditions: adaptor ligated DNA fragments (5.25µl), NEBNext Ultra II Q5 Master mix 2X (6.25µl), and i5 and i7 indexed primers (0.5µl). The number of cycles varied between 10 and 17 depending on the input DNA. Follow-up PCR reaction cleanup was performed with a 1.2X bead ratio and eluted in 12µl. Libraries were pooled equimolarly before sequencing on an Illumina NovaSeq 6000 System, aiming for 5Gb of output per sample. We then performed adapter trimming and PhiX filtering, and thereafter retained shorter reads (<50 bp) given short insert size. Quality filtered reads were mapped against the currently available mitogenomes (GenBank NC_002793.1 and OQ076335.1; Janke *et al.* 2001, van den Burg *et al*. 2023a) in Geneious Prime (v2024.0.5, Kearse *et al*. 2012); we then retrieved all reads that mapped to the mtDNA ND4-tRNA Leu fragment (ND4). A consensus sequence was generated for each specimen and quality-checked by eye.

We isolated DNA from the eight post-2000 collected tissue samples using Qiagen's QIAamp DNA extraction kit (Qiagen Inc., Valencia, CA, USA). Genetic data at the ND4 locus were collected from these samples using the Sanger sequencing method, with primers and protocols as outlined in Malone *et al.* (2000).

Our resulting ND4 sequences were aligned with each other and then to those previously published for the *I. iguana* species complex and outgroups *I. delicatissima* and *Sauromalus*. To include relatively shorter published haplotypes from localities relevant to the taxonomic proposal in question, base positions from the 5' and 3' ends of the locus were trimmed to minimize excessive missing data in the phylogenetic analysis.

Phylogenetic relationships among haplotypes of *I. iguana* were estimated in PAUP* (v. 4.0a169, Swofford 2003) under the maximum likelihood (ML) optimality criterion in a successive approximation approach (Sullivan *et al*. 2005), using the model chosen by all metrics in ModelTest, implemented in PAUP*: Three substitution-rate classes (Tamura and Nei 1993), a gamma-distributed rate variation among sites, and a proportion of invariant sites (TN93 +G + I). These same model parameters were affirmed as optimal in a ModelTest analysis run with RAxML (v8; Stamatakis 2014) using raxmlGUI 2.0 (Edler *et al*. 2021), which was subsequently used to assess node support with 10,000 bootstrap pseudo-replicates. Nine outgroup haplotypes (one *Iguana delicatissima* and eight *Sauromalus*) were included in all analyses, and trees were rooted with a monophyletic *Sauromalus*. Results were visualized using Treeviewer v2.20 (Bianchi & Sánchez-Baracaldo 2024). Additionally, p-distances were calculated manually for all comparisons within Clade IV, excluding samples of *I. ig. insularis*, *I. ig. sanctaluciae*, the LOH locality (due to a high numbers of missing base pairs), and the non-native population of Guadeloupe.

## Results

We generated mtDNA sequence data at the ND4 locus from nine of fourteen formalin-fixed, historical specimens of *I. iguana* distributed across five Venezuelan islands and four mainland localities (Fig. 1; locality codes explained in Appendix 1). Seven of nine sequences are 816 bases long, while RMNH.RENA.48961_LOH is 556 bases and USNM216916_BAR is 796 bases. Of these, seven are novel haplotypes, since IDM and MPS had the same haplotype, and that from Isla la Blanquilla (ILB) is identical to a previously published haplotype (HM352505) obtained from iguanas on Saba (SAB), Montserrat (MON), and the US Virgin Islands (USVI) (Stephen *et al*. 2013; De Jesús Villanueva *et al*. 2021; van den Burg *et al*. 2023a,b). The two sequences collected from individuals on Trinidad were identical to each other and unique with respect to other known ND4 haplotypes.

Our trimmed data matrix for analysis consisted of 49 unique haplotypes (40 of which are from the *I. iguana* species complex), 726 bases in length, gapless, and beginning within the ND4 gene coding region at nucleotide 10905 of the *I. iguana* mitogenome (GenBank ID: OQ076335.1). Missing data remained in the matrix for eight previously published haplotypes and one new to this study, amounting to 8.7% of their combined data and 1.6% of the overall dataset.

Maximum likelihood analysis yielded four well-supported major mtDNA clades within the *I. iguana* species complex (labeled I–IV in Fig. 2), but the hypothesized phylogenetic relationships among them (I (III (II + IV))) are only weakly supported. Seven of the eight newly generated *Iguana* ND4 haplotypes were placed within Clade IV (Fig. 2), while the haplotype from the Venezuelan state of Lara (BAR) fell within Clade I. Haplotypes from six

Venezuelan localities, five insular (ILB, LOH, LTE, IDM and IDC), and the mainland locality of El Morro de Puerto Santo (MPS), formed a well-supported, shallow, and basally unresolved clade with localities previously assigned to *I. melanoderma* (VEN, SAB, MON; Breuil *et al*. 2020) (Fig. 2, Subclade A). This clade, along with haplotypes from two other mainland Venezuela localities (GUI, CIO), Trinidad (TRN), and an unknown locality (unk) form a larger clade (Fig. 2, Subclade B) united by a single nucleotide substitution.

Within Clade IV (excluding the above-mentioned localities), p-distances across all comparisons ranged between 0.14 and 2.28 (Table 1). For Subclades A, B, and the FRG-ACH clade, within-group distances were 0.14–0.69, 0.14–2.20, and 0.14–0.71, respectively. For the same respective subclades, distances for comparisons with non-clade members were 0.55–2.28, 0.83–2.28, and 0.96–2.28. Whether considering Subclade A or B as the proposed *I. melanoderma*, some pairwise distances within *I. melanoderma* (e.g., $D_{SAB\text{-}VEN} = 0.69$, e.g., $D_{SAB\text{-}unk} = 2.20$) are larger than those between *I. melanoderma* and non-*melanoderma* (e.g., $D_{MPS\text{-}CIO} = 0.55$, $D_{TRN\text{-}SUR} = 0.83$; $D_{TRN\text{-}ACH} = 0.96$; several others) (Table 1).

**TABLE 1.** P-distances for comparisons between non-identical haplotypes of Clade IV members, excluding *I. ig. insularis*, *I. ig. sanctaluciae*, the LOH locality, and the non-native population of Guadeloupe (GUA). Haplotype order follows that of Figure 2, which also contains associated GenBank accession numbers. Labels reduced to the first locality code. Subclade A = MPS through IDC, Subclade B = MPS through CIO. Largest p-distances within Subclades A and B are in bold, and smallest p-distances between those subclades and non-Subclade A/B members are underlined.

| | | Subclade B | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Subclade A | | | | | | | | | | | | | |
| | SUR | MPS | VEN | LTE | MON | SAB | IDC | unk | GUI | TRN | CIO | FRG1 | ACH1 | ACH2 | FRG2 |
| SUR | | | | | | | | | | | | | | | |
| MPS | 1.24 | | | | | | | | | | | | | | |
| VEN | 1.24 | 0.28 | | | | | | | | | | | | | |
| LTE | 1.52 | 0.28 | 0.55 | | | | | | | | | | | | |
| MON | 1.52 | 0.28 | 0.55 | 0.28 | | | | | | | | | | | |
| SAB | 1.65 | 0.41 | **0.69** | 0.41 | 0.14 | | | | | | | | | | |
| IDC | 1.38 | 0.14 | 0.41 | 0.41 | 0.41 | 0.55 | | | | | | | | | |
| unk | 1.93 | 1.79 | 2.07 | 2.07 | 2.07 | **2.20** | 1.93 | | | | | | | | |
| GUI | 1.10 | 1.38 | 1.65 | 1.65 | 1.65 | 1.79 | 1.52 | 1.52 | | | | | | | |
| TRN | <u>0.83</u> | 0.96 | 1.24 | 1.24 | 1.24 | 1.38 | 1.10 | 1.10 | 0.41 | | | | | | |
| CIO | 0.96 | <u>0.55</u> | 0.83 | 0.83 | 0.83 | 0.96 | 0.69 | 1.24 | 0.83 | 0.41 | | | | | |
| FRG1 | 1.54 | 1.69 | 1.97 | 1.97 | 1.97 | 2.11 | 1.83 | 1.83 | 1.54 | 1.26 | 1.12 | | | | |
| ACH1 | 1.24 | 1.65 | 1.93 | 1.93 | 1.93 | 2.07 | 1.79 | 1.79 | 1.38 | <u>0.96</u> | 1.10 | 0.28 | | | |
| ACH2 | 1.38 | 1.52 | 1.79 | 1.79 | 1.79 | 1.93 | 1.65 | 1.65 | 1.52 | 1.10 | <u>0.96</u> | 0.14 | 0.14 | | |
| FRG2 | 1.87 | 1.72 | 2.01 | 2.01 | 2.01 | 2.15 | 1.87 | 2.15 | 1.87 | 1.58 | 1.15 | 0.57 | 0.57 | 0.43 | |
| FRG3 | 1.99 | 1.85 | 2.13 | 2.13 | 2.13 | 2.28 | 1.99 | 2.28 | 1.99 | 1.71 | 1.28 | 0.71 | 0.71 | 0.57 | 0.43 |

Very few to zero quality-filtered reads were generated from five museum specimens (USNM 27827, RMNH.RENA.48959, RMNH.RENA.48977, RMNH.RENA.48979, and RMNH.RENA.48981). However, a 79-base read from USNM 27827 (mainland Venezuela, Río Chico; RIO) mapped to the posterior part of the mtDNA ND4L gene, a region anterior to ND4. This enabled a visual comparison of this region between USNM 27827 (RIO), seven other of our newly sequenced specimens (CIO, GUI, IDM, IDC, MPS, LOH, and ILB), the published *I. iguana* mtDNA genomes (NC_002793, OQ076335.1), all from Clade IV, and one specimen from each of the other three major mtDNA clades (represented by samples from Mexico (Clade III), Aruba (Clade I), and Panama (Clade II); van den Burg *et al*. unpublished data). Except for a single SNP with a unique base in the Isla Margarita individual (IDM), this 79bp region is identical across individuals that, in the analysis of the larger dataset of the ND4 locus, form part of the smallest clade encompassing all the samples previously included in *I. melanoderma* (Fig. 2: Subclade A). A single diagnostic SNP separates these individuals from all other assessed sequences.

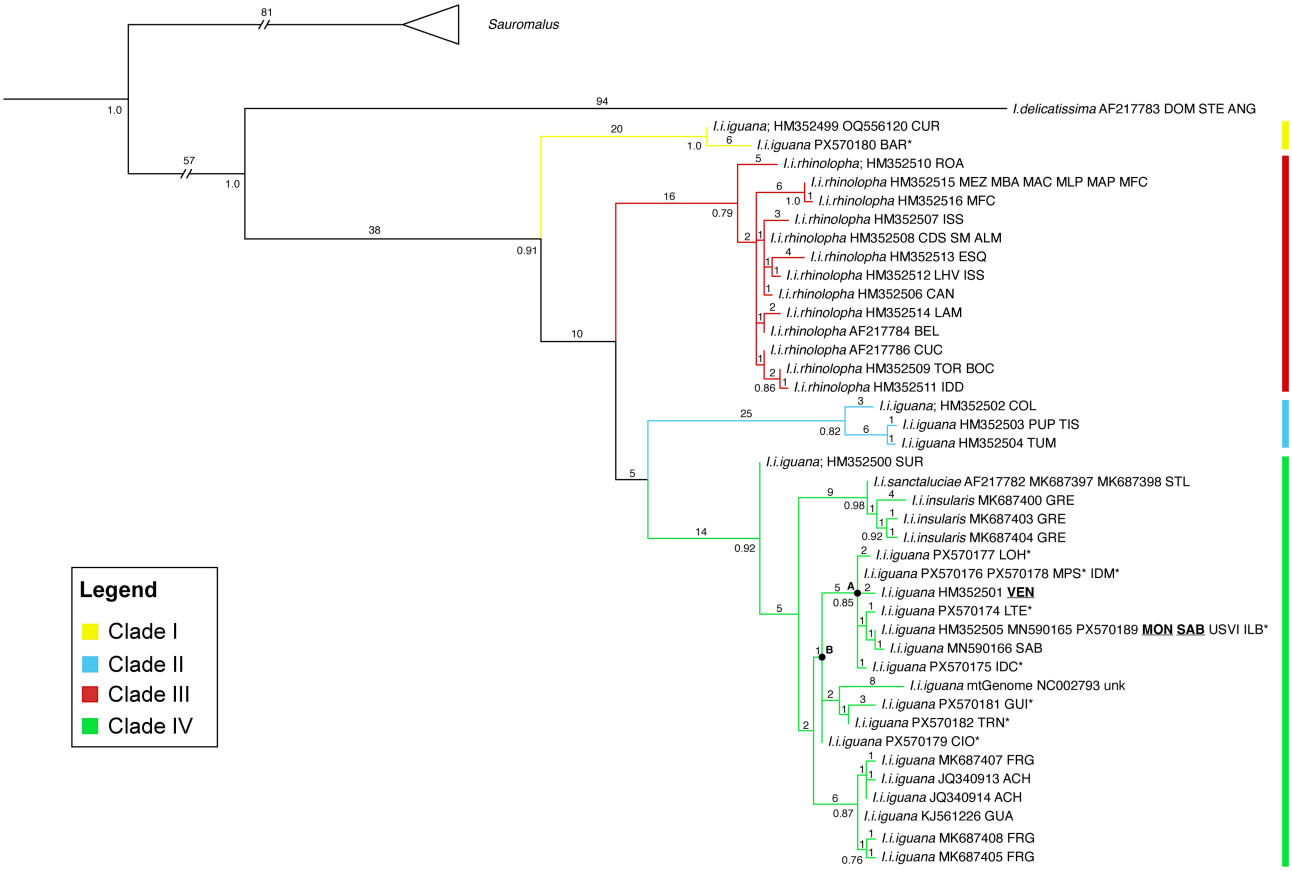


**FIGURE 2.** Estimated mtDNA ND4 phylogeny for the *Iguana iguana* species complex. Numbers above branches are the estimated nucleotide substitutions (converted from ML substitutions per site) and bootstrap support for nodes is indicated below, when > 0.70. The four color-coded clades are those previously identified within the *I. iguana* species complex (Stephen *et al.* 2013). Taxonomy for the *I. iguana* species complex follows ITWG (2016, 2022). Bold and underlined locality codes indicate samples assigned to *I. melanoderma* by Breuil *et al.* (2020); A and B indicate: Subclade A, the smallest subclade including all haplotypes previously referred to the proposed *I. melanoderma* and Subclade B, the largest subclade including those haplotypes and excluding all haplotypes previously referred to species other than the proposed *I. melanoderma*. Locality codes marked with asterisks indicate sequences generated in the current study.

## Discussion

Here we have extended the mtDNA datasets of Breuil *et al*. (2020) and Stephen *et al*. (2013) to include haplotypes in Janke *et al*. (2001; UNK), Martin *et al*. (2015; GUA), and from 10 new localities in the northern coastal region of South America, nine that are especially relevant to the taxonomic issue at hand. Our phylogenetic results indicate that the proposed *I. melanoderma* from Saba, Montserrat, and Cumana, Venezuela (Breuil *et al*. 2020) is less distinct than suggested, with the addition of many new haplotypes eroding the distinction between the already shallow divergences among the populations within Clade IV of the *I. iguana* species complex (Fig. 2). Additionally, if the proposed *I. melanoderma* were to be equated with either Subclade A or B in Figure 2, pairwise genetic distances between members within that subclade can be higher than distances between that subclade and non-subclade members (Table 1). Despite the localities added in this study, major sampling gaps remain in northern South America (e.g., CIO to SUR is 915 km), along with their likely haplotype variants.

Populations assigned to the proposed *Iguana melanoderma* included iguanas on the "Venezuelan coastal islands, Saba-Montserrat Puerto Rico Bank, Virgin Islands, St. Croix Bank", as well as those within a poorly defined mainland region: "north-eastern Venezuela" (Breuil *et al*. 2020: 123). We show that these populations are closely related to those in the lowland coastal region of eastern Venezuela (north of the Cordillera de la Costa), between Río Chico and the Paria Peninsula, and the continental and oceanic islands north of that area (Los Testigos LTE,

Isla Margarita IDM, Isla de Caribes IDC, Isla La Blanquilla ILB, and Islas Los Hermanos LOH) as well as to populations deeper in Venezuela (Ciudad Orinoco CIO, Anzoátegui) (Figs. 1 and 2).

The close ancestry of the proposed *I. melanoderma* to mainland and insular Venezuelan populations (within Clade IV) is further substantiated by the low sequence divergence (0.11% over 16,626 bases) between the published mtDNA genome from a Saban iguana (OQ076335.1) and that of the Isla la Blanquilla sample (RMNH. RENA.29501[PX570189]). Indeed, the p-distance between the common ND4 haplotype of the proposed *I. melanoderma* on Saba and Montserrat and its closest relatives in northern South America (ILB, LTE) is similar (0–0.28%) to that between the haplotype of the non-native *I. iguana* population from Guadeloupe (GUA) and its closest South American relatives (0.13–0.29% for FRG, ACH). As *I. iguana* on Guadeloupe (GUA) were introduced from French Guyana (FRG) (Breuil 2013; Vuillaume *et al*. 2015), the presence of iguanas on Saba and Montserrat also likely represents a recent colonization of those islands, whether natural or anthropogenic. Such translocations could have occurred prior to European presence within the Caribbean, as pre-colonial seafarers have spread both mainland plant and animal species to Caribbean islands (Giovas 2019).

Of the specimens from the additional nine localities we sequenced within the Clade IV range, only one (ILB) contained a haplotype previously known in *Iguana*; the most commonly sampled mtDNA ND4 allele in SAB and MON (HM352505, Stephen *et al.* 2013; Breuil *et al*. 2020; van den Burg *et al*. 2023b; van den Burg *et al*. 2025a). Further, new haplotypes from TRN (Trinidad), GUI (Sucre, Venezuela) and the inland locality of CIO, greatly diminish the distinction of the proposed *I. melanoderma*. The discovery of a new haplotype at almost each new sampled location, within just this limited geographic region, demonstrates the large gap that remains in our understanding of the distribution of genetic diversity within Clade IV. We can reasonably expect that continued expansion of geographic coverage will continue to reduce the differences between currently identified subclades within this mtDNA clade.

We also contend that the microsatellite dataset used to argue for genetic differentiation between the proposed *I. melanoderma* and other *Iguana* populations (Breuil *et al*. 2020) is too limited in its geographic sampling scheme to support that proposal. The population structure analysis compared iguanas from Montserrat and Saba to a geographically very distant *Iguana* population in mainland French Guiana and to those from the islands of St. Lucia and the Grenadines. A more appropriate dataset for such an analysis would have included multiple populations from the northern region of South America. Although microsatellites can be powerful markers for assessing connectivity between populations within natural dispersal limits, they are far less informative when sampling geographically distant or isolated populations.

Breuil *et al*. (2020) also used coloration to justify their recognition of *Iguana melanoderma*, comparing images of individuals within the proposed new species to very few populations in the broader geographic region of Clade IV. Coloration is known to be highly variable across the range of the *Iguana iguana* species complex, and melanism can be an adaptation to local environments (e.g., Lewis 1949; Rosenblum *et al*. 2007; Micheletti *et al*. 2012). The melanistic distinction of the proposed *I. melanoderma* decreases greatly after including images of partial and fully melanistic individuals from populations located outside of the range of the proposed species. This includes areas closer to the type locality of *I. iguana* in Suriname, where iguanas with at least partial melanism on the head are present (e.g., iNaturalist record 149447550), and the northeastern coast of Brazil, where melanistic *I. iguana* are also present (Fig. 3). Moreover, van den Burg *et al*. (2025b) recently showed that only 10% of iguanas on Saba are completely melanistic. As an example of how coloration can be unique at relatively small spatial scales, van Buchem *et al.* (2025) recently reported the presence of a regional piebaldism color morph along the mid- and south-Pacific coast of Costa Rica. In any case, the use of coloration to diagnose species in the *I. iguana* species complex requires an assessment, analysis, and resulting understanding of coloration across the extensive range of the species complex.

In addition to the problems with the evidence used to support the proposed *I. melanoderma* addressed above, the taxonomic proposal of Breuil *et al.* (2020), as summarized in their annotated maximum likelihood tree (their Fig. 3), is confusing because the geographically closest sample (ND4 haplotype HM352500, from Suriname) to the type locality of *I. iguana* (northern Suriname, ITWG 2016) was not assigned to that (or any) species. Instead, the populations from French Guiana and Alter do Chão (Brazil) were assigned to *I. iguana*, which is a questionable application of the name *I. iguana* (at least when populations from Suriname are considered to represent a different species from those from French Guiana and Alter do Chão). Therefore, the morphological datasets and comparisons (Breuil 2013; Breuil *et al.* 2020) did not include data from the population from which *I. melanoderma* should have been distinguished (*I. iguana* in Suriname).

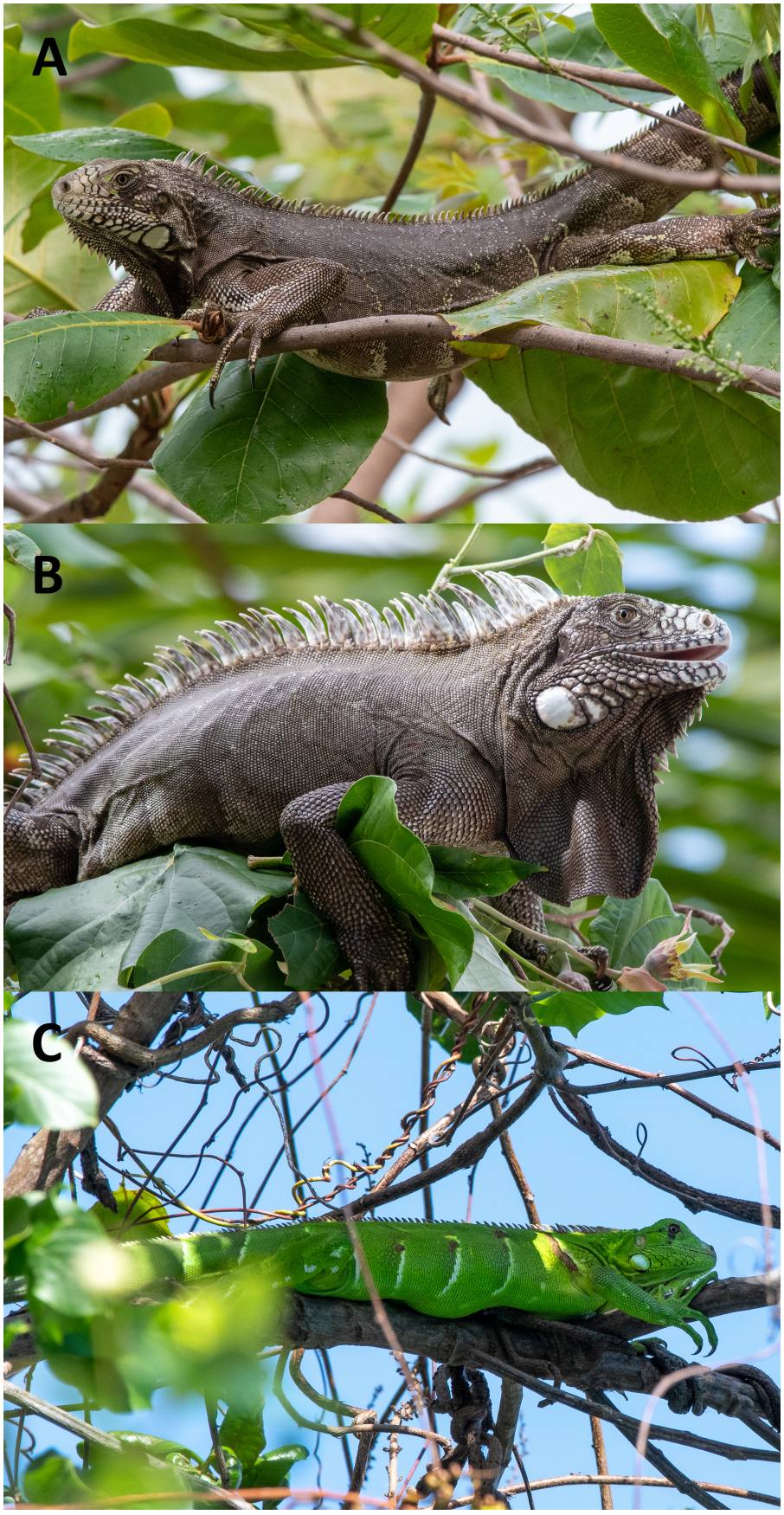


**FIGURE 3.** Photographs of melanistic iguanas from around the city of Pipa, Rio Grande do Norte, Brazil. A, young adult; B, adult; C, juvenile. Photos by M.P. van den Burg.

In the case of wide ranging and variable taxa, such as the *I. iguana* species complex, geographically limited datasets cannot substantiate conclusions about taxonomic distinctiveness, which instead require range-wide assessments. Such assessments should be based on a sampling scheme that includes representation across suspected barriers to gene flow (historical and present); for example, across the boundaries of distinct phytogeographic regions. These considerations are equally true for our understanding of both genetic and morphological variation. Our more extensive comparisons with insular and mainland samples demonstrate that the proposed *I. melanoderma* is substantially less distinct from other *Iguana* populations within Clade IV than originally presented. We thus recommend that, based on the current evidence, the proposed *I. melanoderma* not be recognized and instead be considered part of *I. iguana* (sensu ITWG 2016, 2022). We also do not support treating haplotype Subclades A or B within Clade IV as a subspecies of *I. iguana*, given the currently sparse sampling in northeastern South America and the known limitations of using only mtDNA to delimit species and subspecies (e.g., Wüster 2025).


## Acknowledgements

We want to extend our gratitude to Addison Wynn, Elza Duijm, and Ester Dondorp who helped us collate the necessary tissue samples and with shipment to the Netherlands. We thank Michel Breuil for his constructive comments and discussion on this work, as well as his support in our conclusion that subclades within Clade IV do not warrant species-level recognition. We appreciate the time and effort of our reviewers, whose comments resulted in significant improvement of the manuscript. This research was financed through the Wageningen University BO research program (BO-43-117-006) and the Ministry of Agriculture, Fisheries, Food Security and Nature (LVVN) under project number 4318100346-1. We thank Mike Rutherford for providing the Trinidad samples, and the Toledo Zoo and Arizona Desert Museum for *S. hispidus* and *S. varius* samples, respectively.


## References


Bianchini, G. & Sánchez-Baracaldo, P. (2024) TreeViewer: Flexible, modular software to visualise and manipulate phylogenetic trees. *Ecology and Evolution*, 14, e10873. https://doi.org/10.1002/ece3.10873

Bock, B., Malone, C.L., Knapp, C., Aparicio, J., Avila-Pires, T.C.S., Cacciali, P., Caicedo, J.R., Chaves, G., Cisneros-Heredia, D.F., Gutiérrez-Cárdenas, P., Lamar, W., Moravec, J., Perez, P., Porras, L.W., Rivas, G., Scott, N., Solórzano, A. & Sunyer, J. (2022) *Iguana iguana* (amended version of 2020 assessment). *The IUCN Red List of Threatened Species*, 2022, e.T174481A218317281. Available from: https://doi.org/10.2305/IUCN.UK.2022-2.RLTS.T174481A218317281.en (accessed 8 April 2024)

Breuil, M. (2013) Caractérisation morphologique de l'iguane commun *Iguana iguana* (Linnaeus, 1758), de l'iguane des Petites Antilles *Iguana delicatissima* Laurenti, 1768 et de leurs hybrides. *Bulletin Société Herpétologique de France*, 147, 309–346.

Breuil, M. (2016) *Morphological characterization of the common iguana Iguana iguana (Linnaeus, 1758), of the Lesser Antillean iguana Iguana delicatissima Laurenti, 1768 and of their hybrids*. International Reptile Conservation Foundation, Tucson, Arizona, 37 pp. [English translation of Breuil (2013)]

Breuil, M., Schikorski, D., Vuillaume, B., Krauss, U., Morton, M.N., Corry, E., Bech, N., Jelić, M. & Grandjean, F. (2020) Painted black: *Iguana melanoderma* (Reptilia, Squamata, Iguanidae) a new melanistic endemic species from Saba and Montserrat islands (Lesser Antilles). *ZooKeys*, 926, 95–131. https://doi.org/10.3897/zookeys.926.48679

Breuil, M., Vuillaume, B., Schikorski, D., Krauss, U., Morton, M.N., Haynes, P., Daltry, J.C., Corry, E., Gaymes, G., Gaymes, J. & Bech, N. (2019) A story of nasal horns: two new subspecies of *Iguana* Laurenti, 1768 (Squamata, Iguanidae) in Saint Lucia, St Vincent & the Grenadines, and Grenada (southern Lesser Antilles). *Zootaxa*, 4608 (2), 203–232. https://doi.org/10.11646/zootaxa.4608.2.1

Campos, P.F. & Gilbert, T.M. (2012) DNA extraction from formalin-fixed material. *In*: Shapiro, B. & Hofreiter, M. (Eds.), *Ancient DNA: methods and protocols*. Humana Press, Totowa, New Jersey, pp. 81–85. https://doi.org/10.1007/978-1-61779-516-9_11

Clusella-Trullas, S., Terblanche, J.S., Blackburn, T.M. & Chown, S.L. (2008) Testing the thermal melanism hypothesis: a macrophysiological approach. *Functional Ecology*, 22, 232–238. https://doi.org/10.1111/j.1365-2435.2007.01377.x

De Jesús Villanueva, C.N., Falcón, W., Velez-Zuazo, X., Papa, R. & Malone, C.L. (2021) Origin of the green iguana (*Iguana iguana*) invasion in the greater Caribbean Region and Fiji. *Biological Invasions*, 23, 2591–2610.

https://doi.org/10.1007/s10530-021-02524-5
Dunn, E.R. (1934) Notes on *Iguana*. *Copeia*, 1934 (1), 1–4.
https://doi.org/10.2307/1436422
Edler, D., Klein, J., Antonelli, A. & Silvestro, D. (2021) raxmlGUI 2.0: A graphical interface and toolkit for phylogenetic analyses using RAxML. *Methods in Ecology and Evolution*, 12 (2), 373–377.
https://doi.org/10.1111/2041-210X.13512
Giovas, C.M. (2019) The beasts at large–perennial questions and new paradigms for Caribbean translocation research. Part I: ethnozoogeography of mammals. *Environmental Archaeology*, 24 (2), 182–198.
https://doi.org/10.1080/14614103.2017.1315208
Hahn, E.E., Alexander, M.R., Grealy, A., Stiller, J., Gardiner, D.M. & Holleley, C.E. (2022) Unlocking inaccessible historical genomes preserved in formalin. *Molecular Ecology Resources*, 22 (6), 2130–2147.
https://doi.org/10.1111/1755-0998.13505
Hijmans, R.J., Cameron, S.E., Parra, J.L., Jones, P.G. & Jarvis, A. (2005) Very high resolution interpolated climate surfaces for global land areas. *International Journal of Climatology*, 25, 1965–1978.
https://doi.org/10.1002/joc.1276
Hummelinck, P.W. (1940) n.k. *In*: *Studies on the Fauna of Curaçao, Aruba, Bonaire and the Venezuelan Islands*. N.V. Drukkerij P. den Boer, Utrecht, pp. 59–108.
IWTG (2016) A checklist of the iguanas of the world (Iguanidae; Iguaninae). *In*: Iverson, J.B., Grant, T.D., Knapp, C.R. & Pasachnik, S.A. (Eds.), *Iguanas: Biology, Systematics, and 255 Conservation*. University of California Press, Berkeley, California, pp. 4–46.
Iguana Taxonomy Working Group, Buckley, L.J., de Queiroz, K., Grant, T.D., Hollingsworth, B.D., Malone, C.L., Pasachnik, S.A., Reynolds, R.G. & Zarza, E. (2022) A checklist of the iguanas of the world (Iguanidae; Iguaninae) 2022 supplement to: 2016. *Herpetological Conservation and Biology*, 11, 4–46 and 2019 Supplement. [https://www.iucn-isg.org/wp-content/uploads/2022/05/ITWG_Checklist_2022_Supplement.pdf]
Janke, A., Erpenbeck, D., Nilsson, M. & Arnason, U. (2001) The mitochondrial genomes of the iguana (*Iguana iguana*) and the caiman (*Caiman crocodylus*): implications for amniote phylogeny. *Proceedings of the Royal Society of London. Series B: Biological Sciences*, 268 (1467), 623–631.
https://doi.org/10.1098/rspb.2000.1402
Jaramillo, A.F., De La Riva, I., Guayasamin, J.M., Chaparro, J.C., Gagliardi-Urrutia, G., Gutiérrez, R.C., Brcko, I., Vilà, C. & Castroviejo-Fisher, S. (2020) Vastly underestimated species richness of Amazonian salamanders (Plethodontidae: *Bolitoglossa*) and implications about plethodontid diversification. *Molecular Phylogenetics and Evolution*, 149, 106841.
https://doi.org/10.1016/j.ympev.2020.106841
Kearse, M., Moir, R., Wilson, A., Stones-Havas, S., Cheung, M., Sturrock, S., Buxton, S., Cooper, A., Markowitz, S., Duran, C., *et al.* (2012) Geneious Basic: an integrated and extendable desktop software platform for the organization and analysis of sequence data. *Bioinformatics*, 28 (12), 1647–1649.
https://doi.org/10.1093/bioinformatics/bts199
Lazell, J.D. (1973) The Lizard Genus *Iguana* in the Lesser Antilles. *Bulletin of the Museum of Comparative Zoology*, 145, 1–28.
Lewis, T.H. (1949) Dark coloration in the reptiles of the Tularosa Malpais, New Mexico. *Copeia*, 1949 (3), 181–184.
https://doi.org/10.2307/1438984
Malone, C.L. & Davis, S.K. (2004) Genetic contributions to Caribbean iguana conservation. *In*: Alberts, A.C., Carter, R.L., Hayes W.K. & Martins E.P. (Eds.), *Iguanas: Biology and Conservation*. University of California Press, Berkeley, California, pp. 45–57.
https://doi.org/10.1525/california/9780520238541.003.0004
Martin, J.L., Knapp, C.R., Gerber, G.P., Thorpe, R.S. & Welch, M.E. (2015) Phylogeography of the endangered Lesser Antillean iguana, *Iguana delicatissima*: a recent diaspora in an archipelago known for ancient herpetological endemism. *Journal of Heredity*, 106 (3), 315–321.
https://doi.org/10.1093/jhered/esv004
Micheletti, S., Parra, E. & Routman, E.J. (2012) Adaptive color polymorphism and unusually high local genetic diversity in the side-blotched lizard, *Uta stansburiana*. *PLoS ONE*, 7 (10), e47694.
https://doi.org/10.1371/journal.pone.0047694
Nakahara, S., Zacca, T., Huertas, B., Neild, A.F., Hall, J.P., Lamas, G., Holian, L.A., Espeland, M. & Willmott, K.R. (2018) Remarkable sexual dimorphism, rarity and cryptic species: a revision of the '*aegrota* species group' of the Neotropical butterfly genus *Caeruleuptychia* Forster, 1964 with the description of three new species (Lepidoptera, Nymphalidae, Satyrinae). *Insect Systematics & Evolution*, 49 (2), 130–182.
https://doi.org/10.1163/1876312X-00002167
de Queiroz, K. (1995) Checklist and key to the extant species of Mexican iguanas (Reptilia: Iguaninae). *Publicaciones Especiales del Museu de Zoología*, 9, 1–48.
Rosenblum, E.B., Hickerson, M.J. & Moritz, C. (2007) A multilocus perspective on colonization accompanied by selection and gene flow. *Evolution*, 61, 2971–2985.
https://doi.org/10.1111/j.1558-5646.2007.00251.x

Stamatakis, A. (2014) Raxml version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. *Bioinformatics*, 30, 1312–1313.
https://doi.org/10.1093/bioinformatics/btu033

Stephen, C.L., Reynoso, V.H., Collett, W.S., Hasbun, C.R. & Breinholt, J.W. (2013) Geographical structure and cryptic lineages within common green iguanas, *Iguana iguana*. *Journal of Biogeography*, 40 (1), 50–62.
https://doi.org/10.1111/j.1365-2699.2012.02780.x

Sullivan, J., Abdo, Z., Joyce, P. & Swofford, D.L. (2005) Evaluating the performance of a successive-approximations approach to parameter optimization in maximum-likelihood phylogeny estimation. *Molecular Biology and Evolution*, 22 (6), 1386–1392.
https://doi.org/10.1093/molbev/msi129

Swofford, D.L. (2003) *PAUP*. Phylogenetic Analysis Using Parsimony (*and Other Methods)*. Sinauer Associates, Sunderland, Massachusetts. [program]

Tamura, K. & Nei, M. (1993) Estimation of the number of nucleotide substitutions in the control region of mitochondrial DNA in humans and chimpanzees. *Molecular Biology and Evolution*, 10 (3), 512–526.

van Buchem, R., van den Burg, M.P. & Meirmans, P.G. (2025) iNaturalist Observations Indicate Piebaldism in Iguana iguana Could be a Regional Color Morph: Response to López and Mora (2025). *Caribbean Journal of Science*, 55 (2), 592–597.
https://doi.org/10.18475/cjos.v55i2.a28

van den Burg, M.P., Grandjean, F., Schikorski, D., Breuil, M. & Malone, C.L. (2021) A genus-wide analysis of genetic variation to guide population management, hybrid identification, and monitoring of invasions and illegal trade in *Iguana* (Reptilia: Iguanidae). *Conservation Genetics Resources*, 13 (4), 435–445.
https://doi.org/10.1007/s12686-021-01216-5

van den Burg, M.P., Ramón-Laca, A., Carné Constans, A., Debrot, A.O. & Vieites, D.R. (2023a) The complete mitochondrial genome of the Critically Endangered Saba Green Iguana, *Iguana iguana* (Squamata: Iguanidae). *Mitochondrial DNA*, Part B, 8 (4), 475–478.
https://doi.org/10.1080/23802359.2023.2195510

van den Burg, M.P., Goetz, M., Brannon, L., Weekes, T.S., Ryan, K.V. & Debrot, A.O. (2023b) An integrative approach to assess non-native iguana presence on Saba and Montserrat: are we losing all native *Iguana* populations in the Lesser Antilles? *Animal Conservation*, 26 (2023), 813–825.
https://doi.org/10.1111/acv.12869

van den Burg, M.P., van der Horn, S.A., Ahlen, P.A., Jansen, L., Wulf, K. & Debrot, A.O. (2025a) Larger hybrid clutch size could drive regional displacement of native *Iguana* populations across the Lesser Antilles. *bioRxiv*, July 2025, 1–19.
https://doi.org/10.1101/2025.07.31.667849

van den Burg, M.P., Madden, H. & Debrot, A.O. (2025b) Population estimate and conservation of the melanistic Iguana iguana population on Saba, Caribbean Netherlands. *Herpetological Journal*, 35 (3), 176–186.
https://doi.org/10.33256/35.3.176186

Wüster, W. (2025) Shedding the mitochondrial blinkers: A long-overdue challenge for species delimitation in herpetology. *Vertebrate Zoology*, 75, 259–275.
https://doi.org/10.3897/vz.75.e161536

**APPENDIX 1.** New samples from members of the *Iguana iguana* species complex included in this study.

| Collection* or collector | Specimen | Locality# | Locality code (state, country) | GenBank accession |
|---|---|---|---|---|
| Naturalis | RMNH.RENA.29495 | Testigos, Morro de la Iguana | **LTE** (Federal Dependencies of Venezuela, Venezuela) | PX570174 |
| Naturalis | RMNH.RENA.29501 | Blanquilla, Valuchu | **ILB** (Federal Dependencies of Venezuela, Venezuela) | PX570189 |
| Naturalis | RMNH.RENA.29503 | Araya, Isla de Caribes | **IDC** (Nueva Esparta, Venezuela) | PX570175 |
| Naturalis | RMNH.RENA.48955 | Carupano, Puerto Santo | **MPS** (Sucre, Venezuela) | PX570176 |
| Naturalis | RMNH.RENA.48961 | Hermanos, Morro Pando | **LOH** (Federal Dependencies of Venezuela, Venezuela) | PX570177 |
| Naturalis | RMNH.RENA.48966 | Margarita, Guatamare, near El Valle | **IDM** (Nueva Esparta, Venezuela) | PX570178 |
| Smithsonian | USNM 27827 | 10.3167, -65.9833 | **RIO** (Miranda, Venezuela) | [no ND4, but anterior region] |
| Smithsonian | USNM 80625 | 8.16667, -63.5664 | **CIO** (Anzoátegui, Venezuela) | PX570179 |
| Smithsonian | USNM 216916 | 9.98, -69.53 | **BAR** (Lara, Venezuela) | PX570180 |
| Smithsonian | USNM 216918 | 10.63, -62.25 | **GUI** (Sucre, Venezuela) | PX570181 |
| Mike Rutherford | 2 wild specimens, 2013 | 10.50, -61.27 | **TRN** (Trinidad, Trinidad and Tobago) | PX570182 |

*Collections: Naturalis Biodiversity Center, Leiden, The Netherlands; National Museum of Natural History, Smithsonian Institution, Washington D.C., USA.

#Locality names are mentioned for specimens that lack GPS coordinates.

[A]No genetic data were obtained from four RMNH specimens; RMNH.RENA.48959, RMNH.RENA.48977, RMNH.RENA.48979, and RMNH.RENA.48981

**APPENDIX 2.** Locality codes used in Figure 1 not noted in Appendix 1. Order of localities follows Figure 2.

| Locality code | Clade |
|---|---|
| CUR (Curaçao) | I |
| ROA (Honduras, Roatan) | III |
| MEZ (Mexico, Oaxaca, Tututepec, Sta. Rosa) | III |
| MBA (Mexico, Guerrero, Acapulco, Las Brisas) | III |
| MAC (Mexico, Colima, Ahijadero) | III |
| MLP (Mexico, Guerrero, La Unión, La Paz) | III |
| MAP (Mexico, Michoacán, Apatzingán) | III |
| MFC (Mexico, Michoacán, Aquila, Puente el Chico) | III |
| ISS (El Salvador, Isla San Sabastion) | III |
| CDS (El Salvador, Costa Del Sol) | III |
| SM (El Salvador, San Miguel) | III |
| LHV (Honduras, Choluteca, Las Hormigas) | III |
| ESQ (Escuintla, Guatemala) | III |
| ALM (Aldea Las Mesas, Honduras) | III |
| CAN (El Salvador, Cangrejera) | III |

*.....continued on the next page*

**APPENDIX 2.** (Continued)

| Locality code | Clade |
|---|---|
| LAM (Los Amates Mariscos, Guatemala) | III |
| BEL (Belize) | III |
| CUC (El Salvador, El Cuco) | III |
| TOR (Costa Rica, Tortuguero) | III |
| BOC (Panama, Bocas del Toro) | III |
| IDD (Nicaragua, Rio San Juan, Isla del Diamente) | III |
| COL (Colombia, unknown) | II |
| PUP (Ecuador, Manabi, Punta Prieta) | II |
| TIS (Ecuador, Manabi, Reserva Titos Santos) | II |
| TUM (Tumbes, Peru) | II |
| ACH (Alter do Chão, Pará, Brazil) | IV |
| FRG (French Guyana) | IV |
| GRE (Saint Vincent and the Grenadines, Palm Island) | IV |
| GUA (French West Indies, Guadeloupe) | IV |
| MON (Montserrat) | IV |
| SAB (Caribbean Netherlands, Saba) | IV |
| STL (Saint Lucia) | IV |
| SUR (Suriname, unknown) | IV |
| USVI (United States Virgin Islands) | IV |
| VEN (Venezuela, Sucre, Cumana) | IV |