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Exploring the Potential of Morphometrics to Inform the Conservation of *Iguana delicatissima*

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Abstract: Invasive alien species severely impact native and endemic species, disproportionately affecting insular species like Caribbean reptiles. The Lesser Antillean iguana, *Iguana delicatissima*, is rapidly declining due to hybridization with non-native green iguanas (NNGIs). With numerous NNGI populations present in the region, the last *I. delicatisisma*-inhabited islands remain highly susceptible to invasion, whereupon the rapid in situ differentiation of NNGIs is essential. However, currently only few scale and coloration characters allow the in situ identification of NNGIs. Here, we assessed the differentiating power of an additional 20 meristic and length-dependent characteristics between the *I. delicatissima* population on St. Eustatius and the NNGI population on St. Maarten, the main source of NNGIs arriving on St. Eustatius. Of these, 13 significantly differed between *I. delicatissima* and NNGIs, with a ≥94% assignment accuracy in multi-variate predictive models. We recommend that these characteristics be assessed and validated, especially for hybrid individuals, allowing the identification of NNGIs even in populations where hybridization is ongoing. Our work demonstrates the value of investing in baseline morphometric datasets for *I. delicatissima* and other native iguanas to aid in the rapid in situ detection of NNGIs for removal.

Keywords: Caribbean; extinction; green iguana; hybridization; *Iguana iguana*; in situ detection; Lesser Antilles; reptiles

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1. Introduction

Invasive alien species are among the most severe actors currently adversely impacting global biodiversity (e.g., [1]). Insular species are often disproportionately negatively affected by the impacts of invasive species [2], and with the higher levels of mutualism found in insular ecosystems, the impacts can be wide-ranging [3]. Besides processes of direct impact, such as predation, processes like invasive hybridization (between native and non-native species) can also cause major declines and extinctions in local endemic populations [4–6]. Protecting native species from invasive hybridization is essential, especially for the rare and endangered species that often occur on islands. However, this can be problematic, since parental species and their hybrids can be morphologically very difficult to distinguish (e.g., [7]). Morphological identification can be further obscured when hybrids are fertile and introgression between (>)F1 hybrids and parental species occurs (e.g., [8]), and the presence of this displacing process can go undetected [9]. As cryptic invasions are common [10], local stakeholders are tasked with the quick detection of non-native presence and subsequent invasive hybridization to optimize species protection. Although genetic assessments often provide clarity, rapid in situ decision making based on morphology is

critical to effective removal campaigns, which should be based on previously identified diagnostic features during morphology-based assessments (e.g., [11]).

Within the Caribbean, native iguana species are threatened by both invasive hybridization and subsequent competitive replacement with non-native green iguanas (NNGIs) from the *Iguana iguana* complex [12–15]. This is especially true for the Lesser Antillean iguana (*Iguana delicatissima*), a sister species to *I. iguana*, that has seen its historical range from Anguilla to Martinique decrease by >80% through a multitude of anthropogenic factors [16]. In the absence of mitigation, its range is projected to decrease to only 1% of its Pre-Colombian range, and it is therefore considered as Critically Endangered by IUCN criteria [17]. Currently, *I. delicatissima* is confined to only a few populations, which either remain pure and inhabit small islets of <2 km^2 or remain present on larger islands were hybridization has occurred or is occurring [18–21]. Stakeholders throughout the region are trying to conserve these last remaining populations, a process that is extremely challenging. This is especially true given the number of regionally established non-native green iguana populations that have differing but only marginally studied (see [18,20,21]) geographic origins from throughout the highly variable *I. iguana* complex [22,23]. The same threat of hybridization is endangering other native regional iguana populations [13,15], and the need to rapidly identify non-native green iguanas and introgressed individuals in situ is highly urgent. Our purpose was to explore the potential utility of a multi-variate approach based on morphological characteristics for the rapid in situ distinction of NNGIs using the St. Eustatius *Iguana delicatissima* and the St. Maarten NNGI population as a test case.

Invasive hybridization with *I. delicatissima* commenced when NNGIs arrived in the Lesser Antilles during the 19th century [24]. However, it was not until the 1990s–2000s that this process' alarming impact on the conservation status of *I. delicatissima* became clear [25–29]. In fact, Day and Thorpe [27] had already suggested that multi-variate analyses combining morphometrics and color and scale patterns can quickly differentiate between *I. delicatissima* and *I. iguana*, but their data and results were never published and are believed lost [R.S. Thorpe, pers. comm.]. Later, Breuil [24] provided the only published morphological assessment between *I. delicatissima* and green iguanas focusing on scale morphology and coloration patterns. However, subsequent genetic work has indicated that introgression can compromise the in situ utility of morphological characters that are currently being used to identify non-native iguanas [18]. It is likely that a larger amount of differentiating morphological characters could further improve non-native identification; however, since Day and Thorpe [27], no study has reassessed SVL (snout–vent length)-dependent characters to aid in the differentiation of *I. delicatissima* from non-native green iguanas, even though a recent study did demonstrate that SVL-dependent characters can aid in non-native iguana identification within other native *Iguana* populations in the Lesser Antilles [15]. Here, we have built on the proposal of van den Burg et al. [15] to create morphometric baseline datasets of remaining native *Iguana* populations in the Lesser Antilles, with the intended goal of developing this methodology as part of the conservation toolbox for more effective protection of *I. delicatissima*. We did so by means of an exploratory assessment of variation in SVL-dependent characters and scalation characters between an *I. delicatissima* population and a regional established non-native green iguana population.

2. Materials and Methods

2.1. Study System

Sint Eustatius remains one of the few islands with native *Iguana delicatissima*; however, non-native green iguanas occasionally arrive with incoming cargo shipments, presumably from neighboring St. Maarten/Martin [20,30]. There is a single known case of hybridization between a pure green iguana and *Iguana delicatissima* on St. Eustatius from 2015. The parental non-native female was caught the following year within an ongoing monitoring program and just prior to laying another clutch [30]. The program has since resulted in the capture of 10 hybrid iguanas (the last of which in 2020) and additional green iguanas that arrived at the harbor as recently as March 2024 ([29], STENAPA unpublished data).

Currently, only a single non-native green iguana is known to occur on St. Eustatius, which appears to have arrived in early 2023 and remains to be captured despite hundreds of search hours. In short, the largely arrested process of hybridization on St. Eustatius stands in sharp contrast to other islands [16,18,21], with any currently present hybrids being the result of a 2023–2024 clutch of the recently arrived non-native green iguana in question.

2.2. Fieldwork

We collected data during fieldwork periods on St. Eustatius (2021–2024) and St. Maarten (2024), where we caught iguanas either by hand or using a noose and rope. Photographs were taken of all sides of the head, the anterior dorsal side of the body, the fourth toe of the hind claws, femoral pores, and mid-body ventral scales. We took size measures following van den Burg et al. [15], as well as data for additional characteristics: snout–vent length (SVL), tail length (TL), lengths of the upper and lower front (UF + LF) and hindleg (UH + LH), length of the fourth toe (TL4), head width (HW), head length (HL), head depth (HD), snout length (SL), eye length (EL), mouth length (ML), tympanum height (TH) and width (TW), and mid-body spine length (MSL). We also collected data on jaw-to-ear length (JE) and skull height (SH) from 2023 onwards. After data collection, we released all iguanas in healthy condition at their capture locations. We additionally scored several meristic characteristics using photographs: the highest number of femorals on either hindleg (FP), the number of supra digital scales on the fourth toe (SD), and the number of ventral mid-body scales within 1 square centimeter (VS). All data and measurements were collected by the first author, and unless specified otherwise, data for bilateral characteristics were collected from the right side [15]. See Supplementary Information for descriptions of how all measurements were taken.

2.3. Data Analyses

Apart from JE and SH, all other length variables are already known to be SVL-dependent and are free of allometry [15]. We first affirmed this for these two new variables. Prior to data analysis, we checked all variables for normality and equality of variance. SVL-dependent variables were then regressed against SVL for all iguanas of >20 cm SVL, representing late subadults to young and older adults. For each variable, using SVL as a covariate, we assessed differences between sexes (separately per island) and between *I. delicatissima* and non-native iguanas, using either an ANCOVA or Kruskal–Wallis test dependent on the normality of data distribution and homogeneity of variances. To test the differentiating power of multiple variables, we implemented multi-variate and cluster methodology using principal component (PCA) and discriminant analyses (LDA), which we performed for a dataset using all measured length-dependent variables (except for TL, given that numerous iguanas had regenerated tails, and the later 2023 initiation of the inclusion of VS, JE, and SH). We used nine different model-training percentages within our LDA analyses, between 0.50 and 0.90. We performed a subsequent significance test for this dataset using a multi-variate analysis of covariance (MANCOVA) for native vs. non-native status, using SVL as covariate. Lastly, we assessed differences between *I. delicatissima* and non-native iguanas for both meristic variables (FP and SD) using pairwise *t*-tests or Wilcox rank-sum tests depending on the normality of data distribution and homogeneity of variances. Given that we performed both ANCOVA and MANCOVA analyses (n = 54) using the same dataset, we corrected p-values using the HY-FDR methodology [31,32]. We used RStudio version 2023.12.1+402 [33] and R version 4.3.2 [34] to perform our analyses.

3. Results

We captured a total of 54 (37 female, 17 male) and 51 (23 female, 28 male) iguanas with an SVL over 20 cm on St. Eustatius and St. Maarten, respectively. An additional 13 smaller iguanas were captured on St. Eustatius, for which we only compared the meristic characters to those of larger animals (see below). All length variables, including VS, were SVL-dependent for both the St. Eustatius *I. delicatissima* and St. Maarten green

iguana population (Table 1). SVL-dependent variables significantly differed between male and female *I. delicatissima*, using SVL as a covariate, except for HD, EL, and VS (Table 1). Equally for green iguanas on St. Maarten, all SVL-dependent variables significantly differed between sexes, except TL4, HW, EL, HD, VS, and TW (Table 1). Individual comparisons between *I. delicatissima* from St. Eustatius and green iguanas from St. Maarten indicated that 11 SVL-dependent characters significantly differed between these groups. *Iguana delicatissima* had significantly larger upper and lower front legs, a wider head, a larger skull height, and a higher ventral scale density (Table 1). *Iguana iguana* from St. Maarten had a significantly larger head and snout length, a larger mid-body spine and tail (Figure 1), a wider tympanum, and a larger head depth (Table 1).

Table 1. Morphometric analysis results for *Iguana delicatissima* from St. Eustatius and non-native green iguanas from St. Maarten. R^2-values are given for snout–vent length regressions, as well as the results of ANCOVA tests between the sexes using SVL as covariate. Significant ANCOVA results are indicated by an asterisk for adjusted $p < 0.05$ using the HY-FDR method. Detail on the *Iguana delicatissima* and green iguana comparison is indicated in the footnote.

	***Iguana delicatissima* (St. Eustatius)**				**Green Iguanas (St. Maarten)**			
Variable	R^2	*F*	*df*	*Adj. p*	R^2	*F*	*df*	*Adj. p*
Upper frontleg length [a]	0.90	28.704	52	5.85×10^{-6} *	0.92	14.901	48	5.89×10^{-4} *
Lower frontleg length [a]	0.94	27.570	52	8.10×10^{-6} *	0.94	14.398	48	7.00×10^{-4} *
Upper hindleg length	0.89	15.928	52	3.66×10^{-5} *	0.92	22.826	48	4.05×10^{-4} *
Lower hindleg length	0.82	34.351	52	1.23×10^{-6} *	0.91	29.354	48	5.85×10^{-6} *
Length of 4th toe	0.62	18.779	52	1.46×10^{-4} *	0.82	4.515	47	5.27×10^{-2}
Head width [a]	0.90	0.183	52	7.25×10^{-1}	0.89	0.003	48	9.53×10^{-1}
Head length [a]	0.84	105.130	52	5.87×10^{-13} *	0.92	35.323	48	1.23×10^{-6} *
Snout length [a]	0.87	15.722	52	4.05×10^{-4} *	0.92	5.107	48	3.88×10^{-2} *
Eye length	0.76	2.607	52	1.37×10^{-1}	0.76	0.059	48	8.40×10^{-1}
Mouth length	0.89	22.443	52	4.01×10^{-5} *	0.94	13.256	48	1.09×10^{-3} *
Head depth [a]	0.75	18.019	51	1.92×10^{-4} *	0.91	0.952	48	3.84×10^{-1}
Tympanum height	0.75	55.68	52	6.13×10^{-9} *	0.78	7.696	48	1.17×10^{-2} *
Tympanum width [a]	0.81	11.527	52	1.54×10^{-3} *	0.74	3.503	48	8.41×10^{-2}
Mid-body spine length [a]	0.57	31.672 [b]	1 [b]	8.96×10^{-8} [b]*	0.71	31.362	48	3.44×10^{-6} *
Jaw height	0.65	69.165	37	4.87×10^{-9} *	0.82	56.679	48	6.84×10^{-9} *
Skull height [a]	0.72	91.006	37	1.76×10^{-10} *	0.83	54.969	48	9.23×10^{-9} *
Ventral scale density [a]	0.82	0.440	47	5.62×10^{-1}	0.76	4.274	42	5.93×10^{-2}
Tail length [a]	0.79	21.174	38	1.75×10^{-5} *	0.84	14.600	19	1.54×10^{-3} *

[a] Adj. $p < 0.05$ for comparison between *I. delicatissima* and green iguanas. [b] Results of Kruskal-Wallis chi squared test due to not normal and unequal variance.

The number of SD on the fourth toe differed significantly between sexes for *I. delicatissima* ($W = 152.5$, $p < 0.001$) but not for green iguanas from St. Maarten ($W = 265.5$, $p = 0.2812$). The number of SD was significantly higher for green iguanas than for *I. delicatissima* ($t = -12.915$, $df = 106$, $p < 2.2 \times 10^{-16}$; Figure 2B). The number of FP was not significantly different between sexes, but *I. delicatissima* had significantly more FP compared to green iguanas from St. Maarten ($W = 3051$, $p < 2.2 \times 10^{-16}$; Figure 2A). Femoral and supra digital counts for small-sized *I. delicatissima* fell within the range of those of larger *I. delicatissima*; $n = 13$; SD, 30–35; FP, 20–22.

The PCA analysis for all SVL-dependent variables (except TL, VS, JE, and SH) indicates only a minor overlap in morphospace between *I. delicatissima* from St. Eustatius ($n = 54$) and green iguanas from St. Maarten ($n = 50$) (Figure 3). PC1-axis contributes most to separation between sexes and PC2 between I. delicatissima and green iguanas. The highest loadings for PC1 were UH, LH, HL, SL, ML, and MSL, and, for PC2, were UF, LF, HW, SL, HD, and MSL. The nine LDA models using 50–90% training input had high accuracy (94–100%) for correctly assigning *I. delicatissima* and green iguanas. The one-way MANCOVA for island status was significant ($F_{14,88} = 45.203$, $p < 0.001$, Wilk's $\Lambda = 0.122$).

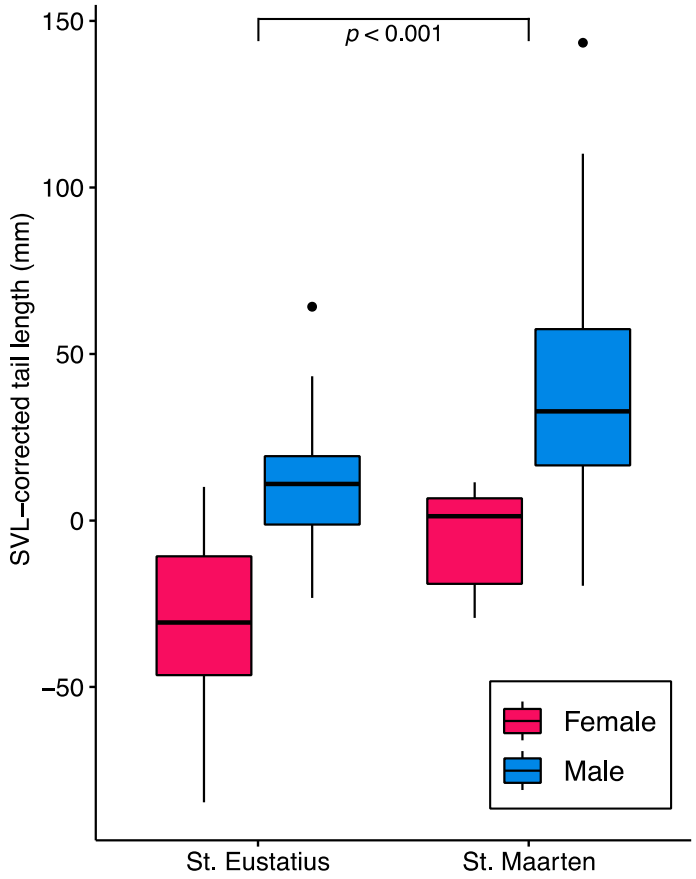


Figure 1. Significant differences in tail length between *Iguana* populations on St. Eustatius and St. Maarten. Data are SVL-corrected residuals from iguanas of >20 cm SVL.

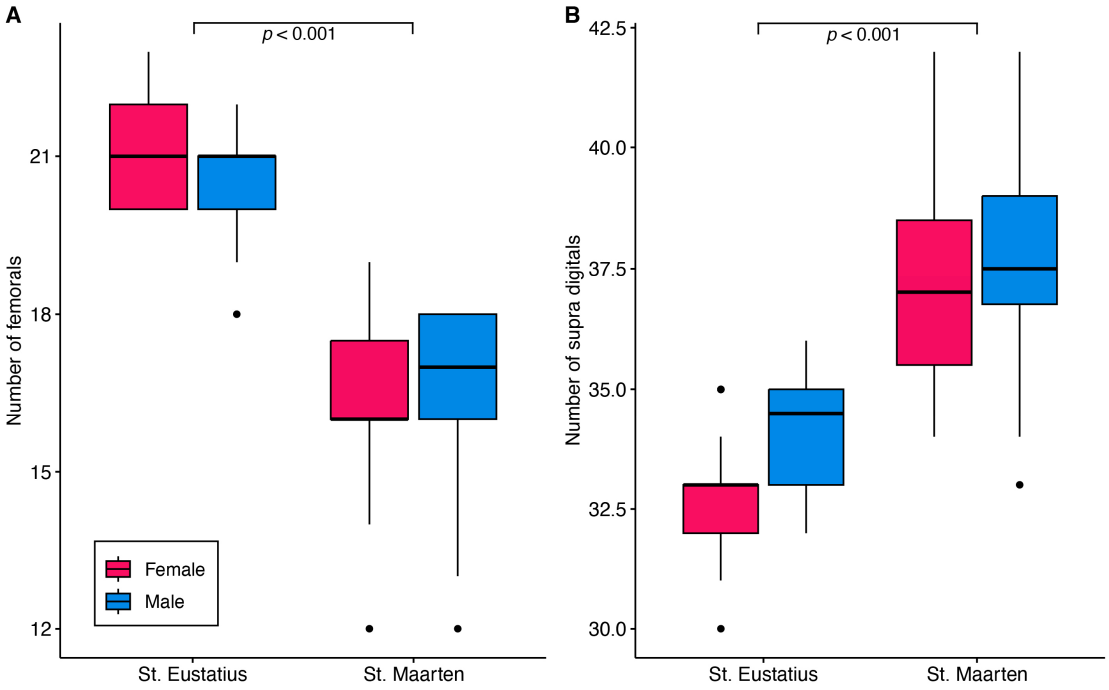


Figure 2. Variation in (**A**) femorals and (**B**) supra digitals on the fourth toe between sexes and *Iguana* populations on St. Eustatius and St. Maarten, indicating significant differences between both populations.

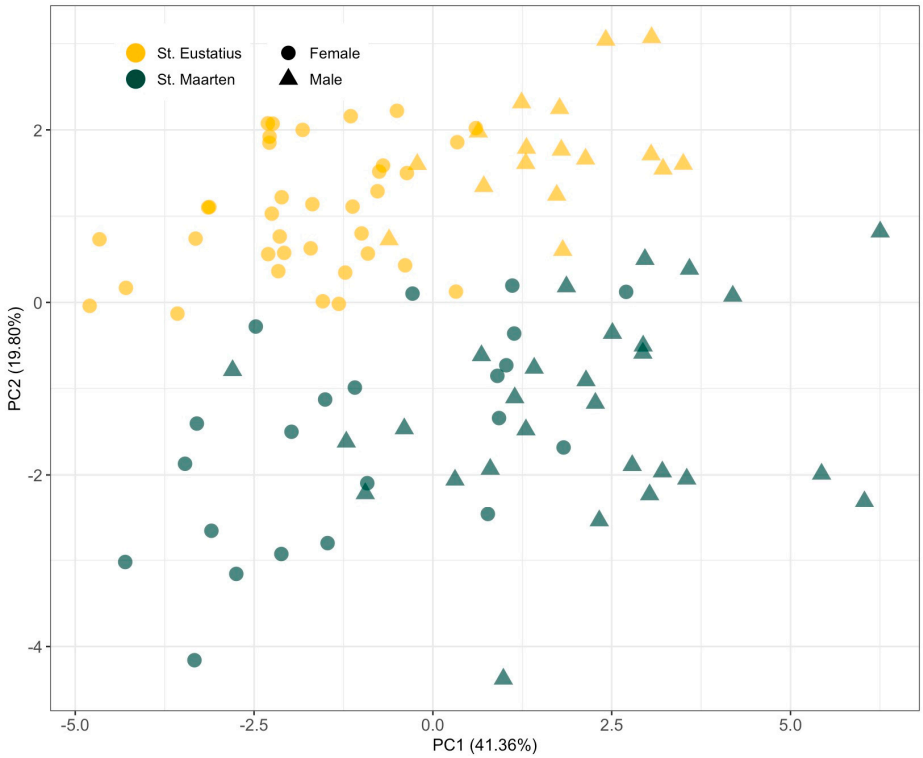


Figure 3. Principal component analysis plot summarizing variation across residuals of all SVL-dependent variables in morphospace, except tail length, dorsal scale density, jaw-to-ear length, and skull height given their percentage of missing data.

4. Discussion

Our assessment highlights numerous morphometric and meristic characters that allow the rapid in situ distinction of the key St. Maarten non-native green iguana (NNGI) source population from the St. Eustatius' *I. delicatissima* population. These results demonstrate that this approach can boost the on-ground conservation management of the remaining *Iguana delicatissima* populations and possibly other iguanid populations, as well. Although this study is restricted to a comparison between only two populations, our results demonstrate an important new potential tool to help protect the St. Eustatius *I. delicatissima* population from the threat of further hybridization. While Day and Thorpe [27] indicate the presence of intraspecific variation in *I. delicatissima*, which appears to be associated with differences in climate and island banks, our effort should be viewed as a time-saving exploratory example for our regional partners, to help them develop their own island-specific assessments. Islands where hybridization is ongoing allow us to assess to what extent and degree of introgression these characters are compromised (using complementary genetic analyses) and which combination of characters maximizes the correct distinction of *I. delicatissima* within a continuous introgression scenario.

Since Breuil [24], no additional differentiating morphological assessments between *I. delicatissima* and green iguanas have been published, despite the knowledge that morphometric differences exist [27]. Here, we identified an additional set of 13 characters that significantly differed between *I. delicatissima* from St. Eustatius and non-native green iguanas from St. Maarten (Table 1; Figures 2 and 3). Since we focused our exploratory effort on larger iguanas, follow-up assessments should investigate the utility of the identified 11 SVL-dependent characters for smaller-sized (<20 cm SVL) native and non-native individuals. Scale counts, other than SVL-dependent characters, are less likely to have ontogenetic variation, and we identified two useful meristic characters that were also used by Lazell [25]: the maximum number of femorals (Figure 2A) and the number of supra digitals on the fourth toe (Figure 2B). Data from some (n = 13) smaller iguanas indeed showed concordance with the variation found for larger individuals. Therefore, we urge

researchers to pay more attention to the identification of additional meristic characters with differentiating power between native and non-native iguanas. Although Lazell [25] contrarily did not find a difference between *I. delicatissima* and *I. iguana* for these two meristic characters, his results were likely undermined by the inclusion of introgressed populations from the Guadeloupe archipelago [24]. Lastly, here, we provide a preliminary comparison of total length between *I. delicatissima* and regionally established non-native green iguanas, which is an important dominance feature in iguanas [35]. The displacement of *I. delicatissima* by non-native green iguanas is well known, although the mechanisms underlying this process are yet to receive research attention. So far, aggressive outcompetition, including the greater strength of green iguanas, has been proposed [16,18], and a singular record of hybrid fecundity suggests these might have higher fitness compared to pure *I. delicatissima* [36]. Here, we provide a first data-driven insight on one aspect of male-to-male dominance between green iguanas and *I. delicatissima*, since our results show that tail size is larger for green iguanas of equal SVL (Figure 1). Green iguanas thus have a larger total length and lateral profile compared to *I. delicatissima*. We note that this assessment only included half of our dataset due to the large number of individuals with a partially regenerated tail (see *df* statistic in Table 1). We thus recommend that future studies identify a proxy for total tail length, to allow the inclusion of all animals in data analyses and usage within multi-variate analyses. We suggest that the circumference of the tail base at the cloaca or at an SVL-dependent distance from the cloaca would be first option to explore.

Compared to *I. delicatissima*, intraspecific variation is more evident within green iguanas [22–24,27], although a full range-wide assessment has not been performed. Established non-native *Iguana* populations within the Lesser Antilles have different, and often multiple, geographic origins ([18,20,21,37], M. Breuil pers. comm.). Although larger non-native sample sizes (>20) have only been analyzed from Anguilla, Antigua, Guadeloupe, and St. Maarten/Martin ([18,21,38], M. Breuil pers. comm.), some populations still await assessment, e.g., in Barbuda and Dominica. The St. Maarten/Martin population currently has the most complex introgressive history, with NNGIs from multiple major mtDNA clades hybridizing, as well as evidence of some remaining *I. delicatissima* introgression (M. Breuil pers. comm.). This high variation of mixed origins could explain some of the observed contrast in dimorphism concerning three variables compared to *I. delicatissima* (Table 1). Our results indicate that head depth, tympanum width, and the length of the fourth toe show dimorphic differences in *I. delicatissima* but not in NNGIs. Dimorphism for these characters was also present in the Saba *I. iguana* population [15]. The absence of dimorphism for these characters in the NNGI St. Maarten/Martin population might result from the diverse genetic composition of populations from multiple major mtDNA clades [20]. Hybridization with or among non-native species occurs in numerous systems and provides opportunities to study selection and adaptive introgression [39–42]. Since the native range of green iguanas includes multiple geographic and climatic regions, the non-native populations within the Caribbean, where different genetic lineages intermix, could provide insights into these processes.

Population-specific baseline datasets can aid in the rapid in situ identification of non-native iguanas and can replace the long process of genetic identification when adequate differentiating characters and datasets are available [15]. However, fieldwork duration and data collection are restrained by limited time and financial resources, and ideally each data-collected character has some degree of differentiating power between native and non-native individuals. Our preliminary assessment can help regional conservation partners in prioritizing characters to measure, especially if limited resources do not allow the recommended complete local assessments. Whilst SVL-dependent characters can only be measured in situ, photographing meristic characters can provide an opportunity for subsequent ex situ data collection. We used this combined in situ/ex situ workflow to assess the number of femorals and supra digitals and ventral scale density.

In conclusion, and adding to Breuil [24], we here have identified several morphological characters that can be used to aid the rapid distinction of *Iguana delicatissima* from non-native green iguanas, allowing the last remaining *I. delicatissima* populations to be better, and more rapidly, protected from the threat of invasive hybridization. With the native morphospace of the St. Eustatius *I. delicatissima* population defined, the rapid identification of any divergent iguana should be more straightforward using a number of measurements and meristics. We call on conservation and governmental bodies to provide local conservation organizations with rapid funding to construct local population-specific baseline datasets. For islands with ongoing introgression (St. Barthelemy, Martinique, La Desirade, and Dominica), financial aid should also include funding for an initial genetic assessment.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/conservation4040038/s1.

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Data Availability Statement: The raw data supporting the conclusions of this article will be made available by the authors on request.

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Conflicts of Interest: The authors declare no conflicts of interest.

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