

An integrative approach to assess non-native iguana presence on Saba and Montserrat: are we losing all native *Iguana* populations in the Lesser Antilles?

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Research Article

Keywords: Caribbean, endangered, hybridization, Iguana iguana, incursion, invasive alien species identification

Posted Date: September 7th, 2022

DOI: <https://doi.org/10.21203/rs.3.rs-1867242/v1>

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Abstract

Invasive alien species are among the main drivers of the ongoing sixth mass extinction wave, especially affecting island populations. Although the Caribbean is well-known for its high species richness and endemism, also for reptiles, equally important is the regional contribution of non-native species to island biodiversity. The Lesser Antilles encompass high genetic diversity in *Iguana*, though most native populations either have gone extinct or are declining following competitive hybridization with invasive non-native iguanas. Here we assessed non-native presence in two poorly-studied native melanistic *Iguana iguana* populations using available genetic tools, and explored utilizing size-dependent body measurements to discriminate between native and non-native iguanas. Genetic samples from Saba and Montserrat were genotyped across 17 microsatellite loci with STRUCTURE and multivariate analyses indicating non-native iguanas presence only on Saba. This was corroborated by mtDNA and nDNA sequences, highlighting a non-native origin in Central America and the ABC islands. We identified preliminary evidence suggestive of hybridization. Morphological variation among size-dependent characteristics showed that non-native iguanas have significantly larger subtympanic plates than native iguanas. Non-native individuals also differed in scalation and coloration patterns. Overall, our findings demonstrate the need for continuous monitoring for non-native iguanas within remaining native *Iguana* populations in the Lesser Antilles, with those not directly threatened by non-native iguanas restricted to only 8.7% of the historic range. Although genetic data allows for identification of non-native or hybrid iguana presence, this field-to-lab workflow is time consuming. Rapid *in-situ* identification of non-native individuals is crucial for conservation management, and besides scale and coloration patterns, we have highlighted the utility of size-dependent variables for rapid diagnosis. We urge regional partners to build morphometric databases for native *Iguana* populations that will help to quickly detect future incursions of non-native iguanas and allow the rapid implementation of effective countermeasures during the early phase of invasion.

Introduction

Invasive alien species (IAS) are among the most prominent threats to global biodiversity (Bax et al. 2003; Buthart et al. 2010), contributing to what has been named the sixth mass extinction (McGeogh et al. 2010; Bellard et al. 2016). Although natural introductions (e.g. following hurricanes), of non-native species have shaped current patterns of species diversity and biogeography (Heinicke et al. 2007; Fonte et al. 2019; Kennedy et al. 2020), anthropogenically-mediated introductions continue to increase in frequency and are redefining biogeographic patterns (Capinha et al. 2015). IAS can impact native species through a range of mechanism, e.g., direct predation, (out)competition, replacement and hybridization, including their various combinations (Reaser et al. 2007).

Isolated insular populations and species are especially vulnerable to IAS (Tershy et al. 2015), including those of the Greater Caribbean region (Gleditsch et al. 2022). Within this region, human impact, especially in terms of high inter-island transport and poor biosecurity, drives high occurrence of non-native species, especially for reptiles (Cox et al. 2022; Jesse et al. 2022). In addition, hurricane events can also

translocate invasive alien reptile species to other islands (Censky et al. 1998), as can recovery aid campaigns triggered by natural disasters (van den Burg et al. 2021a).

Within the Greater Caribbean region, insular iguana species are being impacted by recently-arrived non-native iguanas (Knapp et al. 2021). While on Little Cayman the intergeneric hybridization of the native *Cyclura nubila caymanensis* with non-native *Iguana iguana* is alarming (Moss et al. 2018), the main invasive iguana hotspot is in the Lesser Antilles (Fig. 1). There, especially *Iguana delicatissima* has been impacted by non-native iguanas, which are considered the major factor behind its range-wide decline and its recent assignment as Critically Endangered (van den Burg et al. 2018a). Non-native iguanas are competitively hybridizing and outcompeting native *I. delicatissima* populations throughout the French West Indies (Vuillaume et al. 2015; Angin 2017), on Anguilla (Pounder et al. 2020), on St. Eustatius (van den Burg et al. 2018b), and most recently also on the Commonwealth of Dominica (van den Burg et al. 2020). Besides *I. delicatissima*, several native populations of the *I. iguana* complex also occur on Lesser Antillean islands (Stephen et al. 2013; Iguana Taxonomy Working Group 2016, 2022). Although these populations have received less attention, research and conservation interest in these native gene pools has increased recently.

Iguanas on St. Lucia, St. Vincent and the Grenadines were recently described as subspecies and later as the species *Iguana insularis*, with two subspecies (Breuil et al. 2019, 2022). The IUCN-SSC Iguana Specialist Group's Taxonomy Working Group (ITWG) currently does not recognize the proposed full species status, retaining the sub-species status, *Iguana iguana insularis* and *I. i. sanctaluciae* as originally described by Breuil et al. (2019) until further evidence is published (Iguana Taxonomy Working Group 2022). Among these southern Lesser Antillean islands, non-native iguanas are also established on or have hybridized with native iguanas, namely on St. Lucia, St. Vincent, and several Grenadine islands (Fig. 1; Morton 2008; Breuil et al. 2019, 2022). For Grenada, some evidence suggests non-native presence, but no samples have been analysed so far to confirm their presence and of potential hybridization (Breuil et al. 2019).

Iguana melanoderma was described by Breuil et al. (2020) for the Lesser Antillean iguana populations of Saba and Montserrat, while the ITWG still considers these as distinct subpopulations of *Iguana iguana iguana*, until further evidence is published (Iguana Taxonomy Working Group 2022). Here, we follow the proposed ITWG taxonomy. Breuil et al. (2020) found no evidence for the presence of non-native iguanas on Saba and Montserrat based on morphological characters and limited microsatellite and mtDNA sequence data. However, more extensive yet preliminary data collected during 2021 suggested that a few non-native iguanas might have since arrived on Saba (van den Burg et al. 2022), while reports of an apparent sudden increase of iguana numbers on Montserrat (pers. comm. Ernestine Corbett; pers. comm. Stephen Mendes) also gives reason for concern about the possible presence and spread of non-native iguanas with higher reproductive potential.

Non-native presence and potential hybridization in native *Iguana* populations are mostly assessed using microsatellite and sequence data in conjunction with genetic databases for native populations (e.g.,

Vuillaume et al. 2015; van den Burg et al. 2018b, 2021b; Pounder et al. 2020), especially for hatchling and juvenile iguanas given their high morphological similarity between island populations. Although some comparisons on scale and coloration patterns can also be used (Breuil 2013), the data underlying this reference dataset only covers a small part of the *Iguana iguana* complex and numerous characteristics are not informative. These morphological characteristics are mostly used to identify non-native *I. iguana* and *I. iguana* x *I. delicatissima* hybrids within native *I. delicatissima* populations (Vuillaume et al. 2015; van den Burg et al. 2018b, 2020; Pounder et al. 2020). However, correct diagnostic characterization of hybrid status using these patterns is not guaranteed (Vuillaume et al. 2015). Hence, non-native and hybrid identification within native *I. iguana* populations is more difficult and additional characteristics should be explored to strengthen rapid *in-situ* field identification.

Here, based on preliminary data from Saba and Montserrat, we aimed to assess the presence of non-native iguanas on two of the last Lesser Antillean islands that hold native *Iguana* populations. For this, we implemented genetic reference tools representing most of the native range of *Iguana iguana*. We also explored the use of additional variables to identify non-native and hybrid iguanas by assessing size-corrected body measurements for native and non-native iguanas.

Material And Methods

Fieldwork was conducted during August-September 2021 and July-December 2021 on Saba and Montserrat, respectively. We captured iguanas by means of lasso and pole, hand, or cage traps. We then collected photographic images and a genetic sample, either blood (ventrally from the caudal vein) or tissue (5 mm clip from largest dorsal spine), from the caught animals. On Saba 55 iguanas were caught and on Montserrat 80 were caught, from which, respectively, 29 and 40 were selected for genetic analyses. These were selected considering presence of potential non-native iguanas and a comparable geographic coverage for both islands (Fig. 2). Morphology was used to pre-identify potential non-native iguanas using the following characteristics: body coloration, the presence/absence of a melanistic patch between the eye and subtympanic plate and enlarged nasal scales (Breuil et al. 2020).

On Saba, sampling was done opportunistically during a population assessment (van den Burg et al. 2022), where we also collected the following morphometric measurements: snout-vent length, tail length, upper arm length, lower arm length, upper leg length, lower leg length, toe length, head width, head length, snout length, eye length, mouth length, head depth, tympanum height, tympanum width, sub-tympanic plate height, sub-tympanic plate width, mid-body spine length and the presence or absence of an enlarged nasal scale ("horn") (see Supplementary material). Although melanism was defined as one of the main characters of these populations (Breuil et al. 2020), a subsequent study on the Saba population found that very few animals were completely melanistic (van den Burg et al. 2022). Therefore, knowledge about native body coloration is still in need of further assessment, and we recorded only the presence of a melanistic patch between the eye and tympanum in adult iguanas as the main distinguishing color characteristic (Gerber 1999; Breuil et al. 2020).

Microsatellite laboratory procedures at Labofarm-GenIndexe (France) using identical methodologies as presented in Valette et al. (2013) were used to amplify 17 microsatellite loci (see van den Burg et al. 2021b). Then, PCR product analyses and allele scoring were performed following van den Burg et al. (2021b) and using sample IGD277 to standardize allele sizes.

A subset of five samples per island was then processed at the University of Amsterdam where DNA isolation and PCR methodology were performed to amplify the NADH dehydrogenase subunit 4 (ND4) and the MutL homolog 3 (MLH3), using PCR, respectively, following van den Burg et al. (2018) and Malone et al. (2017). These samples were selected based on microsatellite results and morphology (see above). Successful amplification was confirmed using gel electrophoresis, whereafter PCR products were sequenced both reverse and forward. Chromatograms were assessed, curated and aligned using Geneious Prime (2021.1.1). Sequences will be uploaded to GenBank prior to publication, and the microsatellite data will be added to the IguanaBase database (van den Burg et al. 2021).

Data Analyses

Genetic analyses using microsatellites were only performed on samples with < 20% missing data. These samples were combinedly analysed with those from IguanaBase (van den Burg et al. 2021c) to assess clustering with samples previously collected on Saba and Montserrat, as well as to identify potential presence of non-native iguanas and their geographic origin. Here, we followed van den Burg et al. (2021) by running STRUCTURE (Prithard et al. 2000) within GENODIVE (Meirmans 2020) using identical settings for K, ranging 1–8, and prepared subsequent visualizations using bar plots through Structure Harvester (Evanno et al. 2005; Earl and vonHoldt 2012) and distruct (Rosenberg 2004). Additionally, we assessed non-native iguana presence through implementation of the `predict.dapc()` function from the *adegenet* package (Jombart 2008) within the R environment (R Core Team 2022). This function assigns individuals to populations in a provided reference dataset, in this case IguanaBase. We performed multiple analyses on the reference data that differed in PCA-axes inclusion in order to test assignment robustness. The allele ranges for all loci were then visually compared to those published in IguanaBase (van den Burg et al. 2021c), especially for those loci so far regarded as fixed in the Saba and Montserrat populations; L3, L8, L13, L16, L17, L24. Lastly, relative allelic richness was calculated using the *GenPopReport* package for Saba and Montserrat separately (Adamack and Gruber 2014).

Mitochondrial (ND4) and nuclear (MLH3) sequence data were compared to data from the native *Iguana iguana* range available on GenBank as well as from a large unpublished dataset (van den Burg et al. unpublished data). Pairwise differences were used to identify available sequences with the highest nucleotide similarity and their geographic origin.

Morphological analyses were done on native iguanas by regressing individual length variables against SVL to assess size-dependence, whereafter we compared residuals of adults (> 20 cm SVL) between sexes to assess sex-linked differences using *t*-tests. Variables were initially checked for normality and equality of variance. We repeated SVL regressions for a dataset including non-native iguanas and used

resulting residuals to assess differences between sex and species status. These were tested using a two-way ANOVA or Kruskal-Wallis test depending on the results from homogeneity of variance and normality tests. Data handling and analyses were performed in RStudio Version 1.2.5033 (RStudio Team 2019).

Results

Genetic results

Microsatellite data

For microsatellite analyses we retained samples with < 20% missing data, including 29 individuals from Saba and 39 from Montserrat. Results from a STRUCTURE analysis for $K = 6$ (Fig. 3A and B), based on taxonomic and geographic clusters, indicated that all 2021-captured individuals had assignment scores of > 0.90 for the cluster with melanistic *I. iguana* from Saba and Montserrat, except for four samples from Saba and three from Montserrat. For Saba, specimen SAB58 was assigned to *I. i rhinolopha* (0.92), while the other three specimens indicated mixed origins for *I. i rhinolopha* (SAB08, 0.42; SAB57, 0.29), and *I. i rhinolopha* and *I. aff. iguana* Clade I (SAB04, 0.13, 0.43). For Montserrat, three specimens (Mont33, 0.35; Mont39, 0.22; Mont40, 0.10) had partial assignments to *I. i. iguana* and to several pre-2021 reference Montserrat samples.

DAPC posterior scores clustered all except four samples within the genetic morphospace from previously collected Saba and Montserrat samples, and minimally with *I. i iguana* (Fig. 4). Specimen SAB58 was assigned to *I. i rhinolopha*, while population assignment of the other three Saba specimens different depending on the number of included PCA axes; SAB04, *I. aff. iguana* Clade I; SAB57, *I. i iguana*; SAB08, mixed partial assignments to *I. i rhinolopha*, *I. aff. iguana* Clade I, *I. i iguana*, as well as Saba and Montserrat. All except three specimens from Montserrat were consistently assigned to the IguanaBase group of Saba and Montserrat; Mont33, Mont39 and Mont40 were also assigned to *I. i. iguana*.

Novel haplotypes for these populations were found across 15 loci (Fig. 3C). Compared to previously genotyped samples, 23 new haplotypes were identified from Saba, and five from Montserrat of which two overlapped. For 13 loci these new haplotypes fell outside the known size ranges for the Saba and Montserrat population.

On Saba, mean allelic richness was 2.82 for all the 29 genotyped samples, 1.59 when excluding the four identified non-native iguanas, and 1.75 when combining the 25 native samples with six Saba samples from IguanaBase. For the 39 2021-sampled iguanas from Montserrat, mean allelic richness was 2.13, and 2.30 including 14 Montserrat IguanaBase samples.

Sequence data

Generated ND4 (789 bp) and MLH3 (778 bp) sequences all matched 100% to published and unpublished available sequences from the native *I. iguana* range. For ND4, all five Montserrat specimens and SAB02

had the Caribbean CAR2 haplotype (GenBank accession HM352505, Stephen et al. 2013). From Saba, SAB08, SAB57 and SAB58 had the mtDNA Central-American haplotype CA4 (GenBank accession HM352508, Stephen et al. 2013), while SAB04 had a haplotype only known from the ABC-islands (van den Burg et al. unpublished data).

For MLH3, both islands had one sample with unreadable chromatogram data. The remaining four Montserrat specimens, as well as SAB02, were homozygous for a haplotype only found within *I. i. iguana* (H18; van den Burg et al. unpublished data). While SAB08 and SAB57 were heterozygous with H18, and, respectively, a haplotype known from El Salvador (H16; van den Burg et al. unpublished data) and one known only from Honduras (H15; van den Burg et al. unpublished data). SAB58 was heterozygous for H15 and H16.

Morphology results

Considering all life stages of native iguanas, each measured length variable was found to be size dependent, see Table 1: tail length ($R^2 = 0.98$), upper arm length (0.96), lower arm length (0.98), upper leg length (0.98), lower leg length (0.98), toe length (0.91), head width (0.96), head length (0.95), snout length (0.97), eye length (0.94), mouth length (0.97), head depth (0.94), tympanum height (0.88), tympanum width (0.72), subtympenic-plate height (0.92), subtympenic-plate width (0.78), mid-body spine length (0.82).

On Saba, differences in size-corrected residuals between native female and male adult iguanas were significant for 11 of 16 tested variables (Table 1); tail length was not tested given the high percentage of animals with a broken tail. Considering differences in sex and native/non-native status, nine length variables significantly differed between sex but not status, while subtympenic plate height significantly differed between status but not sex (Table 1, Figs. 5 and 6).

Nasal scalation assessment indicated that three iguanas (SAB08, SAB57, and SAB58) from Saba had an enlarged nasal scale (Fig. 6), while this was absent in all iguanas from Montserrat. The melanistic patch between the eye and tympanum was completely absent in two iguanas (SAB57 + 58), but faintly present in the two other iguana (SAB04 + 08) from Saba (Fig. 6). On Montserrat, all iguanas had melanistic scales within this patch, however, for Mont36 and Mont42 this feature was very limited.

Discussion

The Lesser Antilles are plagued by the continuing presence and spread of non-native *I. iguana*, which is a major threat to the native insular *Iguana* populations. Here we report on the presence of non-native iguanas on two of the last remaining islands previously believed to lack such invasive populations, Saba and Montserrat. Although no evidence of incursions was found for Montserrat, we did identify a recent non-native incursion on Saba as flagged by the integrated analysis of multiple genetic and morphological data. In addition to scale and coloration patterns, we highlight how morphometrics can aid the *in-situ*

identification of non-native and hybrid iguanas within native populations, especially in absence of rapid genetic analytic techniques.

Passenger- and cargo transport to Saba is mainly channelled through St. Maarten, though private vessels can have other origins. A recently reinstalled ferry service also first passes St. Eustatius, which is home to a native *I. delicatissima* population. Additionally, a biweekly service delivers cargo from Martin County (Florida, USA), where non-native *I. iguana* is present (Meshaka et al. 2004; iNaturalist, 2022). On St. Maarten *I. delicatissima* has already gone extinct and currently a large non-native *I. iguana* population is present. Based on microsatellite, ND4 and MLH3 data, our results identify that non-native iguanas on Saba have their ultimate genetic origin in Central America, as well as the ABC islands. However, given the high inter-island connectivity with St. Maarten and since iguanas from the same genetic backgrounds have been identified there (van den Burg et al. 2018b), their more immediate origin is presumed to be the large non-native iguana population on St. Maarten. Likewise did two other non-native reptiles that arrived in recent years from St. Maarten (van den Burg et al. 2021d).

Among the Lesser Antillean islands, individual non-native *Iguana iguana* have been traced back to several native range origins, e.g., mainland South America (Brazil), Central America (e.g., Honduras and El Salvador), the ABC islands, as well as Saba and St. Lucia (Vuillaume et al. 2015; van den Burg et al. 2018b; Breuil et al. 2019; Pounder et al. 2020). In addition, our work as well as that by others show that Lesser Antillean native *Iguana* populations have at some point or other, also made to other (neighboring) islands (Vuillaume et al. 2015; van den Burg et al. 2018b), be it either by natural or human-mediated dispersal. The demonstrated occurrence of Saba/Montserrat haplotypes on St. Maarten (van den Burg et al. 2018b) particularly shows the need to utilize multiple genetic markers when trying to identify non-native iguanas on Saba, as introgressed Saban iguanas could theoretically re-invade the original native population. Considering mtDNA data across non-native populations present in the Greater (De Jesús Villanueva et al. 2021) and Lesser Antilles (the current study and references above), non-native populations on Anguilla and St. Maarten stand out given their high genetic diversity given the presence of three out of the four major mtDNA clades of the *I. iguana* complex.

Although 26 novel alleles were identified across 15 loci for Saba and Montserrat, these can present either undiscovered native variation or the presence of a non-native gene pool. As relatively few samples were previously analysed from Saba and Montserrat it is not surprising that we identified novel alleles, also for Montserrat from where our data highlights an absence of non-native iguanas. Considering all loci, L20 stands out as novel alleles were identified on both islands, though we argue that their significance strongly differs. Namely, the 182 allele identified on Montserrat coincide closely with the previously known diversity suggesting this constitutes a rare haplotype. In contrast, the novel alleles from Saba suggest non-native presence given the large gap with known native diversity (but see Estoup et al. 2002). Given the large, combined sample size from Saba (35) and Montserrat (53), especially fixed loci can aid identification of non-native presence. As a total of four variable loci (L3, L8, L16, L17; Fig. 3) are fixed across both island populations (except for several aberrant samples from Saba) this provides additional

evidence that non-native iguanas are present on Saba but absent on Montserrat. This was corroborated by our nDNA and mtDNA sequence data.

Even though a complete overview is still lacking, scalation and coloration patterns have already been found to be highly variable throughout the *I. iguana* complex. We assessed presence of enlarged nasal scales and a melanistic facial patch in both the Saba and Montserrat population, given previous morphological assessments of these populations (Breuil et al. 2020; van den Burg et al. 2022). Among both populations only three Saba animals had nasal horns and were additionally assigned as non-native by microsatellite and sequence data. Similarly, these three iguanas, including one other individual from Saba, genetically identified as non-native, lacked a clear melanistic black patch (though two had some faint partial black scales). On Montserrat several adult iguanas genetically assigned as native had a patch with roughly 50% melanistic scales, while two individuals only had 10–20% melanistic scales. In other words, the melanistic patch of native Montserrat animals was less pronounced than in native animals of Saba. As we found no genetic evidence of non-native presence on Montserrat, we believe that the less evident melanistic patch is a native characteristic of the Montserrat population. This is corroborated by Breuil et al. (2020), who indicated that the Montserrat population is less melanistic.

In order to help distinguish non-native from native iguanas in invaded populations, we here explored the use of novel tools, in addition to molecular data (Stephen et al. 2013; Martin et al. 2015; Vuillaume et al. 2015; van den Burg et al. 2018, 2021; Miller et al. 2019; Pounder et al. 2020; Breuil et al. 2022; Mitchell et al. in prep) and scale and color pattern characters (Breuil 2013). Size-dependent comparisons across 16 variables indicated that on Saba non-native iguanas have larger subtympenic plate scales than native iguanas (Figs. 5 and 6). The diagnostic power and usage of these characteristics in distinguishing between non-native and native iguanas will depend not only on sample size but on the geographic and taxonomic origin and admixture of non-native individuals. In short, we hypothesize that if four iguanas with different genetic origins and admixture were to arrive on Saba, other SVL-dependent variable(s) could be used to identify non-native animals. Therefore, additional analyses on intraspecific variation among the measured variables in *I. iguana* should be useful (and are ongoing; van den Burg et al. unpublished data). This approach is likely most useful to apply in small insular populations as opposed to widely-connected mainland populations given the former tend to show less variation and given their vulnerability to non-native incursions. However, *ex-situ*, within the (illegal) pet trade (Noseworthy 2017; van den Burg and Weissgold 2020), these variables will remain of low value until a range-wide and high-sampled dataset is present. Therefore, we suggest that size-dependent variables will be of greatest value in distinguishing between more distantly related species and that they should especially be evaluated for use in conservation management for *I. delicatissima*.

While the initial presence of non-native species can be expected to be highest around the incursion point, an initial presence at locations more distant from the incursion point can occur when biosecurity regulations are bypassed (Brisbane et al. 2020). Saba knows two principal incursion points to the island. These are the Fort Bay harbor and the Juancho E. Yrausquin airport, situated on the southwest and northeast sides of the island, respectively. Non-native iguanas were mainly located at a site 0.4 km east

from the harbor, although one animal was found north of the settlement of Windwardside (Fig. 2). How this latter individual reached the center of the island is impossible to determine precisely. We speculate that it might concern an intentionally and illegally released pet (Jesse et al. 2016) arriving either via the airport or the harbour, or that it might have arrived at the harbour as an intra-island stowaway that originally arrived at the harbor. Occurrence of three non-native iguanas near the harbor strongly suggest their arrival via transport by boat or ship, which in the case of the neighboring island of St. Eustatius, also appears to be the principal way of incursion of non-native iguanas (Debrot et al. 2022).

Introgressive displacement has been identified as the main threat to *I. delicatissima* (Vuillaume et al. 2015). Hybrid and non-native iguanas are believed to be more territorially aggressive, and have higher fitness thanks to larger clutch sizes compared to *I. delicatissima* (van Wagensveld and van den Burg 2018). Although data on clutch size from the Montserrat and Saba populations are practically unavailable (Blankenship 1990; van den Burg et al. 2022), insular *Iguana* populations have generally been found to have smaller clutch sizes than continental populations (e.g., Fitch and Henderson 1977; van Marken Lichtenbelt and Albers 1993; Bock et al. 2018), even though this is assumed to be partially climate dependent (e.g., Novosolov et al. 2013). The available data suggest that the Montserrat and Saba population produce clutch size within the lower range recorded for *I. iguana*, potentially allowing reproductive and genetic swamping when non-native or hybrid iguanas would reproduce within these island populations.

Whether hybridization has occurred on Saba remains unresolved given our limited data, though preliminary data could suggest it is taking place. The presence of non-native iguanas on St. Maarten with a (partial) *I. i. iguana* origin limits our ability to assess whether the *I. i. iguana* nDNA from the three admixed iguanas on Saba represents native or non-native DNA. Although SAB58 could be the maternal parent of SAB08 and 57 given mtDNA, nDNA (MLH3) and body size, our microsatellite allele data rejects this hypothesis. All data combined, suggests that none of the non-native iguanas had an identical pair of parents. The relatively low number of non-native iguanas observed on Saba implies that on-island hybridization might still be absent or rare. However, the presence of two iguanas with faint melanism with the facial patch could further suggests that these animals are hybrids. Additional fieldwork and genetic assessment is needed to determine whether hybridization is still truly absent.

Our results in any case show that there is a pressing need for rapid action to completely remove non-native iguanas on Saba, and that prospects for this are relatively good in light of apparently lacking or only limited hybridization. However, only immediate action can result in the removal of non-native iguanas before they start interbreeding (Debrot et al. 2022). Failure to act at an early stage of the invasion process elsewhere has led to strong growth of invasive iguana populations, and serious existential threats to or even extinction of the original native iguana populations e.g., on Grand Cayman (Rivera-Milán and Haakonsson 2020) and in the French West Indies (Angin 2017). More extensive size-dependent morphometric data collection from the native population will additionally be essential to further help discern differences with non-native iguanas, which can help to rapidly identify and cull non-native and or hybrid iguanas.

We conclude by pointing out that our findings illustrate the need for continuous monitoring for non-native iguanas within the remaining native *Iguana* populations in the Lesser Antilles. Although genetic data from across the native *Iguana* range currently allows identification of non-native or hybrid presence, this field-to-lab workflow is often excessively time consuming. At present genetic assessment requires the shipment of samples and involves delays due the required permitting processes (e.g. CITES permits). Improved *in-situ* identification screening to rapidly distinguish non-native from native individuals is urgently needed. Here, in addition to scale and coloration patterns, we show size-dependent morphological variables can also be utilized for rapid screening. We urge our regional partners to build morphometric databases for native *Iguana* populations as soon as possible that can help to rapidly distinguish non-native iguanas for culling because even if biosecurity is eventually improved, incursions will from time to time continue to take place.

Declarations

Acknowledgements

We thank Luke Jones from the Durrell Wildlife Conservation Trust, as well as Ernestine Corbett and Annesta Fergus from the Government of Montserrat for permits and staffing. For data collection on Montserrat we further thank Lloyde Martin, James Daley, Stephen Mendes, Stephon Hixon and Tiffanie Williams from the Government of Montserrat, as well as Antwone Sinclair and Alecia Allison from the Montserrat National Trust, and Calvin Fenton. Sampling methodology on Montserrat was approved through a Durrell ethics review (ETH21/0202). We thank the Saba Conservation Foundation for on-island logistic support throughout our project on Saba, and the Executive Council of the island for permitting this research and sampling procedures (663/2021). This work flows forth from the Wageningen University BO research program (BO-43-117-006) and was financed by the Ministry of Economic Affairs, Agriculture and Innovation (EL&I) under project number 4318100346-1, as well as by the Durrell Wildlife Conservation Trust. For additional financial support we are grateful to the Dutch Iguana Foundation, Jill Jollay, and Julie Morningstar. We are grateful to David Schikorski and Betsie Voetdijk whom genotyped and sequenced the genetic samples, to Chloé Warret Rodrigues for translating the abstract to French, and to Lara Jansen for preparing Longmire field buffer.

Author Contributions Statement

MPB, MG and *AOD* conceived the ideas, designed methodology and acquired funding; *MPB, LB, TSW* and *KVR* collected the data; *MPB* analysed the data; *MPB* led the writing of the manuscript. All authors contributed critically to the drafts and gave final approval for publication.

Conflicts of Interest: The authors have no relevant financial or non-financial interests to disclose.

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Table 1

Table 1 is not available with this version.

Figures

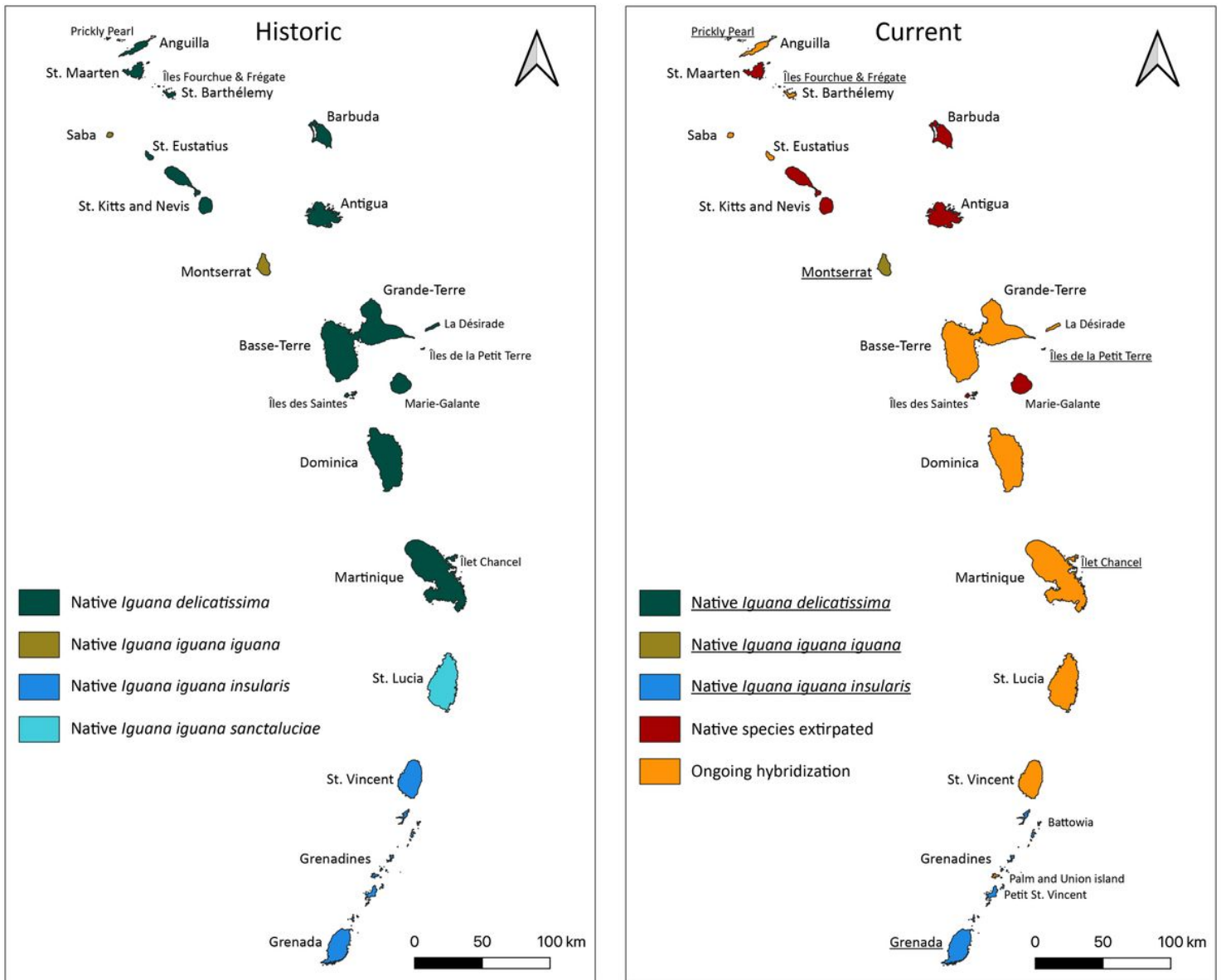


Figure 1

Historic and current status of *Iguana* sp. populations within the Caribbean Lesser Antilles, including ongoing hybridization and extirpated populations. Taxonomy follows the Iguana Taxonomy Working Group Supplement 2022. Only Grenadines islands with current hybrid populations are individually mentioned. Some areas on islands identified as having “Ongoing hybridization” still have isolated native populations.

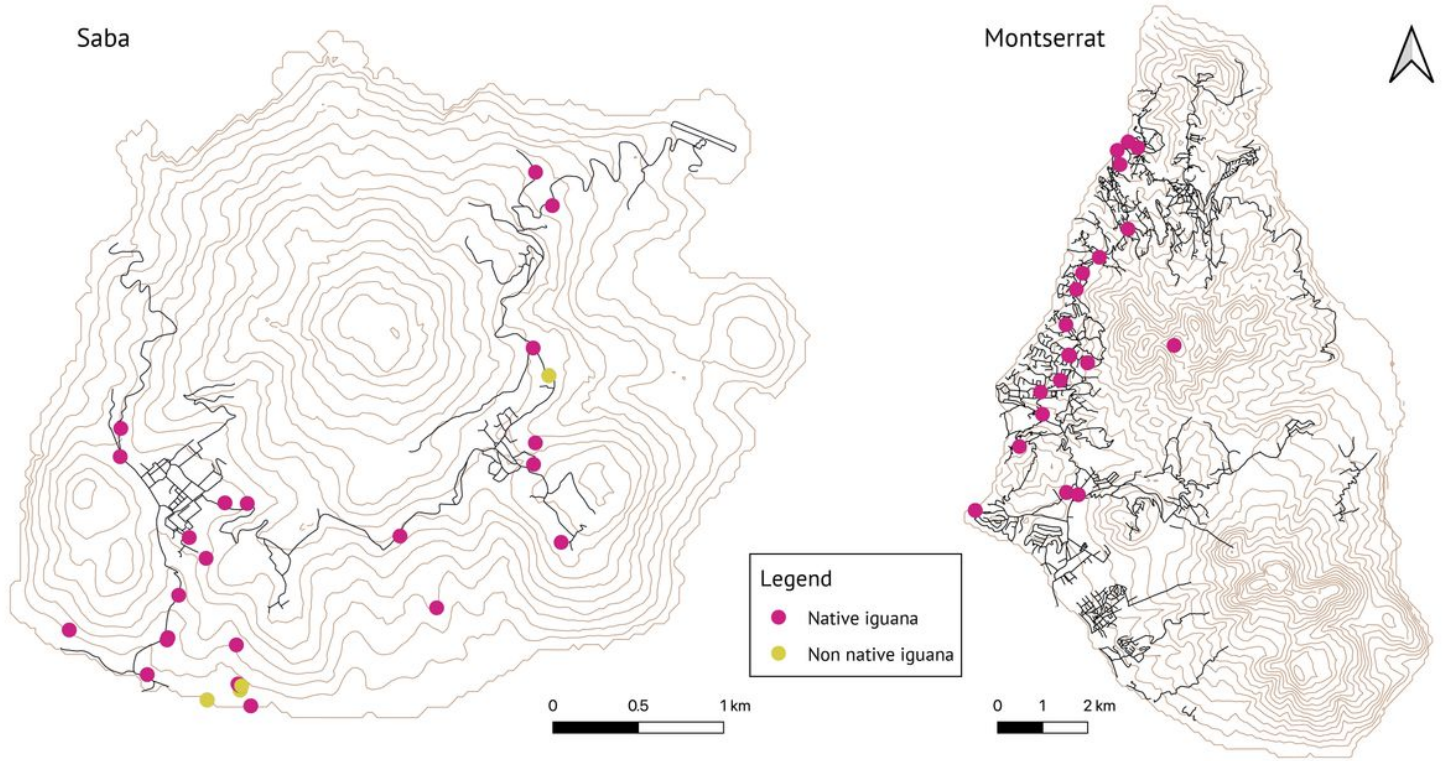


Figure 2

Maps of Saba and Montserrat showing locations of collected genetic samples, and distribution of non-native iguanas on Saba. Contour lines at 50-meter intervals.

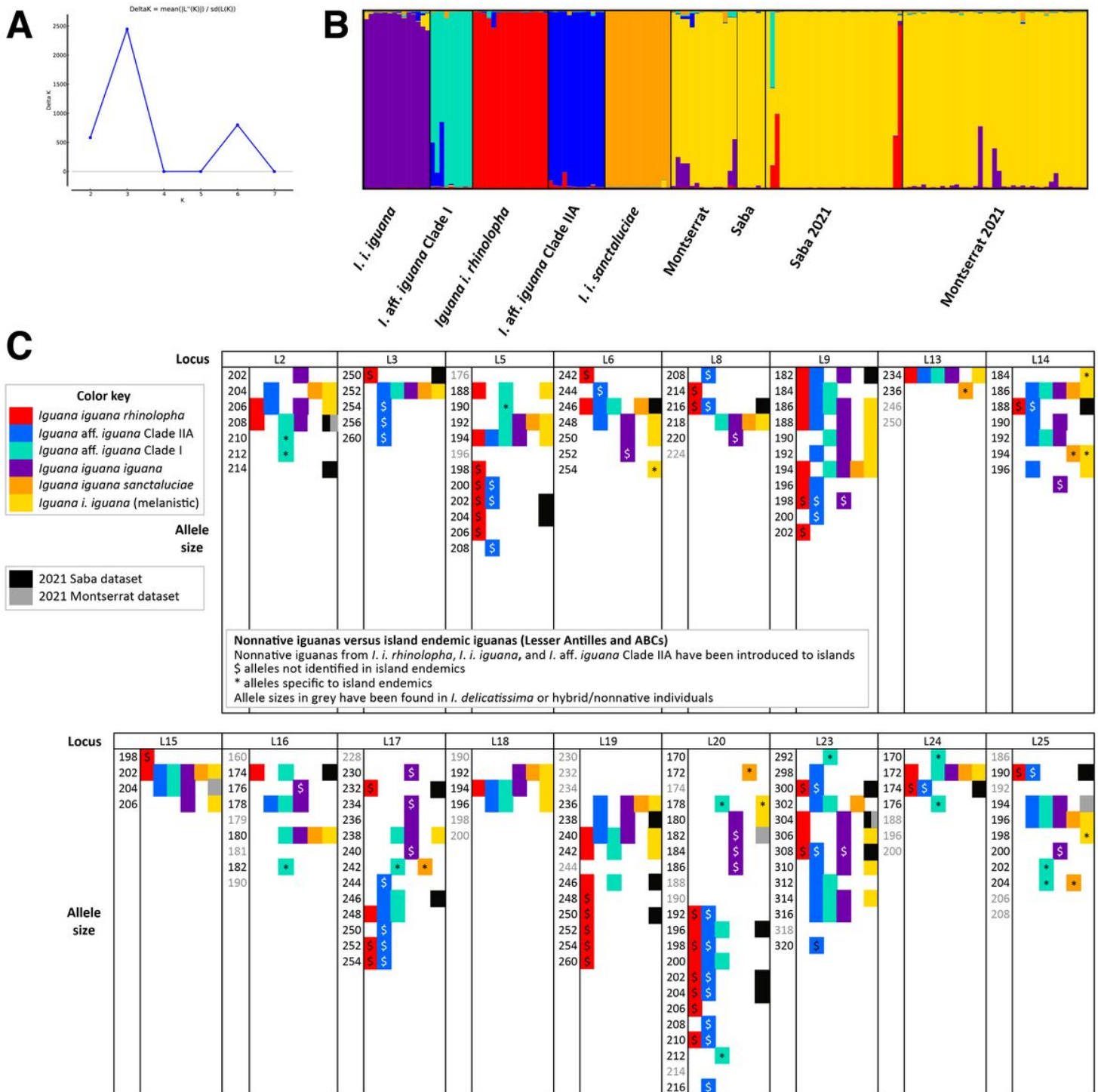


Figure 3

Genetic variation among 17 microsatellites for samples collected on Saba and Montserrat, and referenced to the *Iguana iguana* complex following IguanaBase (van den Burg et al. 2021c). A) Delta K and B) STRUCTURE plots for differentiation among five taxonomic units, and pre- and post-2021-sampled individuals on Saba and Montserrat. C) Allele variation among six units and 2021-sampled individuals.

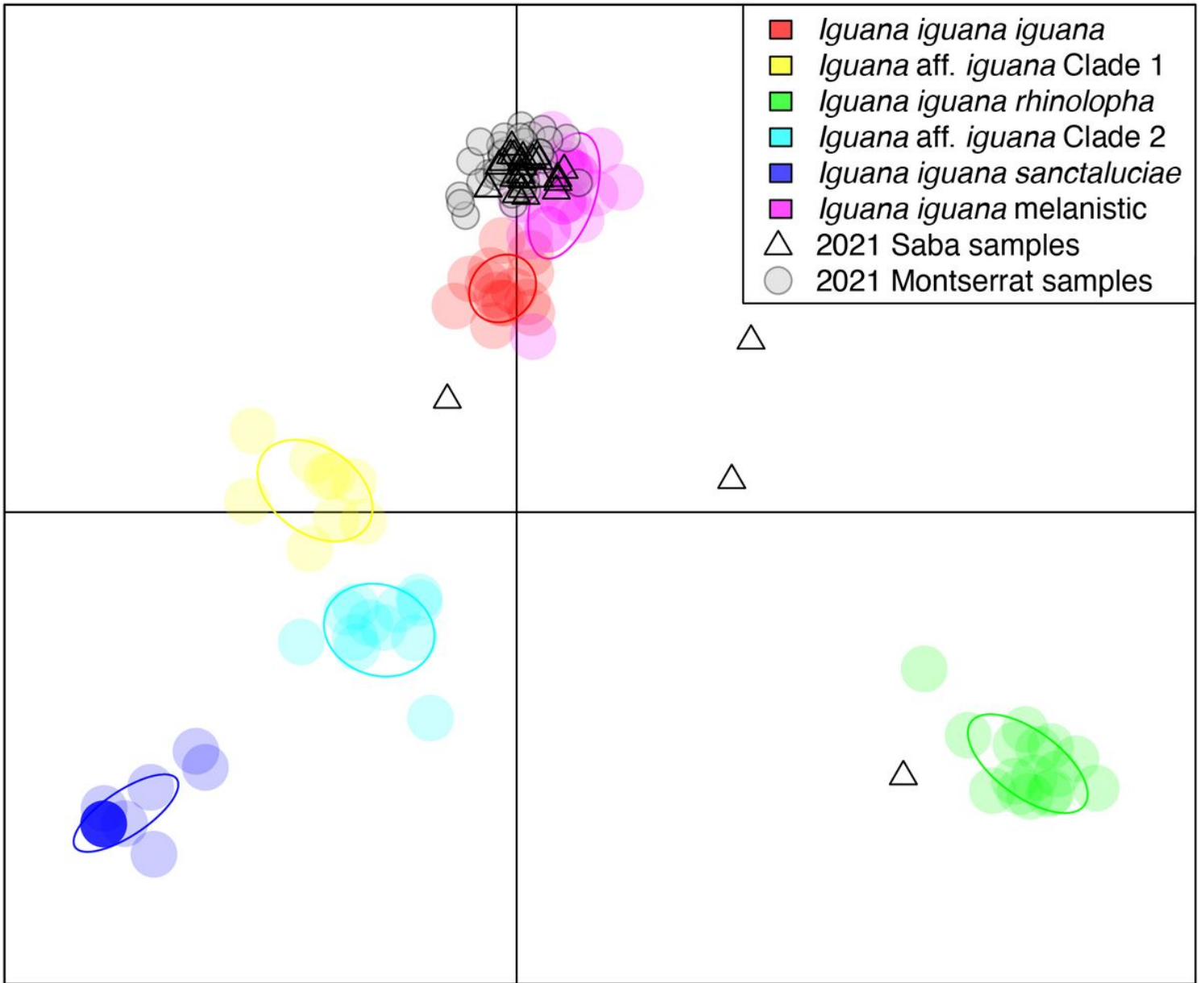


Figure 4

Discriminant Analysis of Principal Components (PC) scatterplot representing 17 microsatellite loci from reference IguanaBase individuals (colored circles) and assigned 2021-sampled individuals (black triangles) in the PC morphospace.

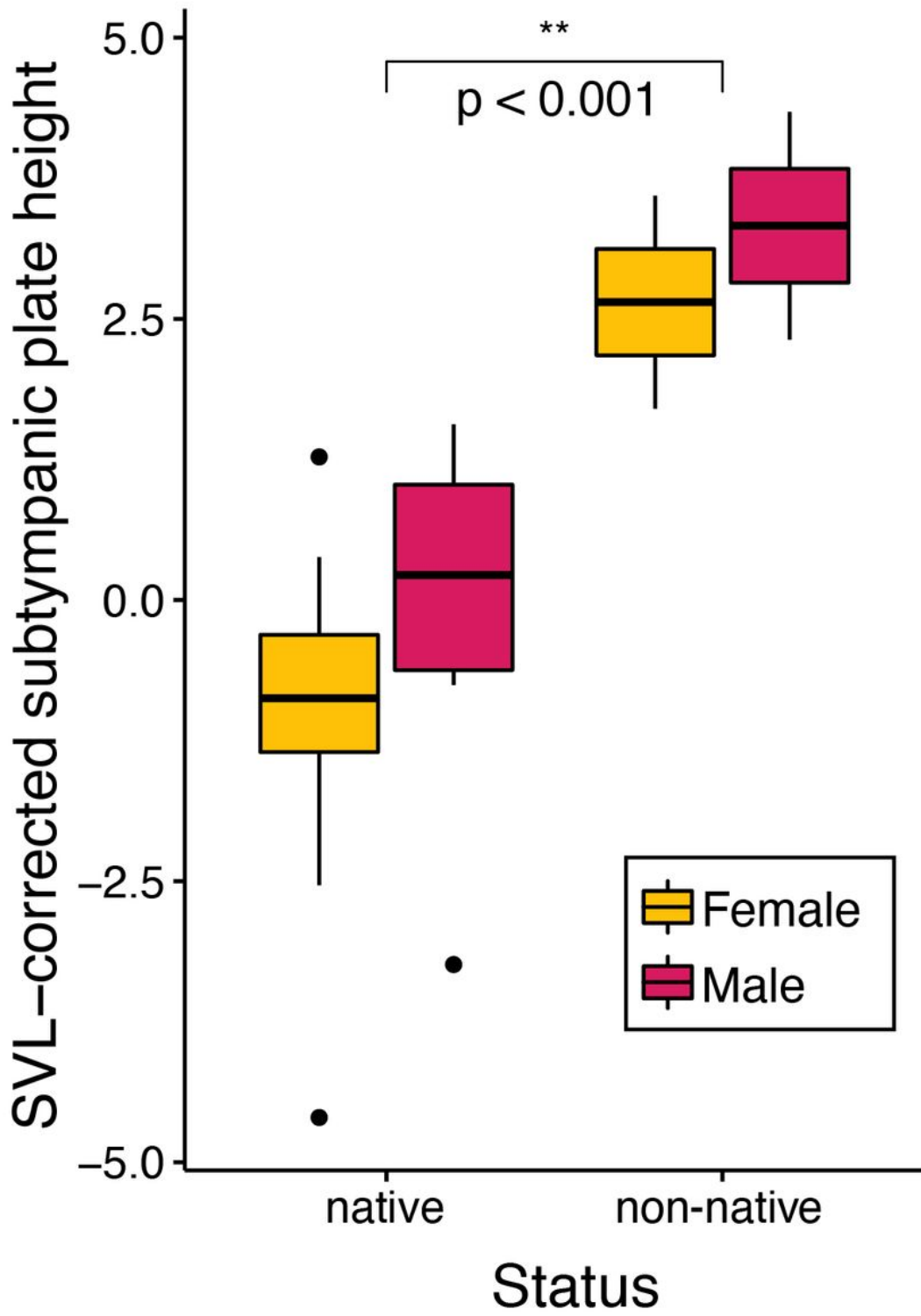


Figure 5

Difference in height of the subtympenic plate between non-native and native iguanas on Saba. Data are SVL-corrected residuals for genetically assessed native adult iguanas, >20cm SVL.



Figure 6

Lateral view of three iguanas captured on Saba in 2021. Left, native female SAB09, 390 mm SVL; absence of enlarged nasal scale, presence of melanistic patch between the eye and tympanum. Middle, non-native female SAB58, 375 mm SVL; presence of enlarged nasal scale, absence of melanistic patch between the eye and tympanum. Right, non-native male SAB08, 345 mm SVL; presence of enlarged nasal scales, faint presence of melanistic patch between the eye and tympanum.