

# Dewlap color variation in *Anolis sagrei* is maintained among habitats within islands of the West Indies

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## Abstract

Animal signals evolve in an ecological context. Locally adapting animal sexual signals can be especially important for initiating or reinforcing reproductive isolation during the early stages of speciation. Previous studies have demonstrated that dewlap color in *Anolis* lizards can be highly variable between populations in relation to both biotic and abiotic adaptive drivers at relatively large geographical scales. Here, we investigated differentiation of dewlap coloration among habitat types at a small spatial scale, within multiple islands of the West Indies, to test the hypothesis that similar local adaptive processes occur over smaller spatial scales. We explored variation in dewlap coloration in the most widespread species of anole, *Anolis sagrei*, across three characteristic habitats spanning the Bahamas and the Cayman Islands, namely beach scrub, primary coppice forest and mangrove forest. Using reflectance spectrometry paired with supervised machine learning, we found significant differences in spectral properties of the dewlap between habitats within small islands, sometimes over very short distances. Passive divergence in dewlap phenotype associated with isolation-by-distance did not explain our results. On the other hand, these habitat-specific dewlap differences varied in magnitude and direction across islands, and thus our primary test for adaptation – parallel responses across islands – was not supported. We suggest that neutral processes or selection could be involved in several ways, including sexual selection. Our results shed new light on the scale at which signal color polymorphism can be maintained in the presence of gene flow, and the relative role of local adaptation and other processes in driving these patterns.

**Keywords** — reflectance, adaptation, sexual signal, machine learning, polymorphism

## Introduction

The staggering diversity of animal communication signals has long been of interest to evolutionary biologists. Animals use chemical, mechanical, electromagnetic, and visual signals to communicate in a wide variety of contexts, including, competition for mates, species recognition, aposematism, and cooperation (Bradbury and Vehrenamp, 2011). A primary evolutionary factor shaping communication signals is the sensory system and behavior of recipients (the sensory drive hypothesis; Endler and McLellan 1988; Endler 1992, 1998). Over the past decades, scientists have established that signals evolve in an ecological context and are dependent on environmental conditions (Endler, 1992, 1993a,b). Just as different habitats may favor different combinations of ecomorphological traits to maximize performance and fitness (Arnold, 1983), they may also shape different forms of a signal, so as to maximize its transmission and detection (e.g. Seehausen 1997), or reduce its detection by unintended recipients such as predators (Endler, 1984, 1990, 1991; Halfwerk et al., 2014). This selective pressure may drive the local adaptation of communication signals.

One potential barrier to the maintenance of localized signal divergence is the homogenizing effect of gene flow. Population genetics theory suggests that gene flow may counteract local adaptation between localities and prevent divergence altogether, especially at small spatial scales, because of the inflow of maladapted alleles or because of the breaking of linkage between coevolving loci (Felsenstein, 1976; García-Ramos and Kirkpatrick, 1997; Dieckmann and Doebeli, 1999; Lenormand, 2002; Hendry et al., 2007a). This genetic homogenization has been confirmed empirically in systems such as stick insects (Nosil and Crespi, 2004) and stickleback (Hendry et al., 2007b). Yet, examples of microgeographic adaptation, i.e. adaptation at smaller scales than the range of dispersal, exist, highlighting the potential of some organisms to respond to selection in the face of

gene flow (see Richardson et al. 2014 and references therein). Examples include small scale adaptation in fragmented areas in Australian fruit flies (Willi and Hoffmann, 2012), and local adaptation to predation pressure in North American salamanders (Richardson and Urban, 2013). Therefore, despite evidence that local adaptation may be particularly difficult at small spatial scales where gene flow tends to cause adjoining populations to remain genetically homogeneous, the potential adaptive response of species traits, in particular communication signals, to localized differences in habitats remains relatively unknown (Richardson et al., 2014). Lizards of the neotropical genus *Anolis* are an excellent group for studying the eco-evolutionary dynamics of local adaptation and natural selection (Losos, 2009). A particularly conspicuous trait of anoles is their dewlap, an extensible flap of skin that is typically sexually dimorphic and used as a communication signal in courtship (Sigmund, 1983; Driessens et al., 2014, 2015) and territorial displays (Losos, 1985; Macedonia and Stamps, 1994; Macedonia et al., 2013) as well as in predator deterrence (Leal and Rodríguez-Robles, 1995, 1997; Leal and Rodriguez-Robles, 1997). Dewlap characteristics vary widely among the approximately 400 species of the genus (Nicholson et al., 2007). Interspecific variation in dewlap coloration is implicated in species recognition (Rand and Williams, 1970; Williams, 1969; Williams and Rand, 1977; Losos, 1985; Macedonia and Stamps, 1994; Fleishman, 2000; Macedonia et al., 2013), and this function could have had a role in initiating or reinforcing reproductive isolation during speciation (Lambert et al., 2013; Geneva et al., 2015; Ng et al., 2017).

Within species, studies have shown a link between variation in dewlap coloration and differences in habitats or climatic conditions (Macedonia, 2001; Leal and Fleishman, 2002; Thorpe and Stenson, 2002; Thorpe, 2002; Leal and Fleishman, 2004; Vanhooydonck et al., 2009; Ng et al., 2012, 2013, 2016; Vanhooydonck et al., 2009; Driessens et al., 2017). Some studies suggest that those differences may be adaptive and that dewlaps may have evolved to maximize detectability given local light conditions (Fleishman and Persons, 2001; Leal and Fleishman, 2002, 2004). Although this claim is further supported by recent findings that dewlap colors are perceived differently under different levels of shading (Fleishman et al., 2020), other studies found conflicting patterns of between-habitat variation that did not support the sensory drive hypothesis (Fleishman et al., 2009; Ng et al., 2012; Macedonia et al., 2014).

Previous studies investigating variation in anole dewlaps compared populations at relatively large geographical scales, e.g. between islands (Vanhooydonck et al., 2009; Driessens et al., 2017) or within large islands such as Puerto Rico (Leal and Fleishman, 2004) or Hispaniola (Ng et al., 2012, 2016). These large scales and marine barriers should reduce gene flow (Ng and Glor, 2011; Lambert et al., 2013; Richardson et al., 2014; Ng et al., 2017). That said, examples do exist of divergence in dewlap coloration at smaller scales or between populations with high degrees of gene flow (Thorpe and Stenson, 2002; Thorpe, 2002; Stapley et al., 2011; Ng et al., 2016).

*Anolis sagrei* is widespread across islands of the West Indies (Reynolds et al., 2020). It has been the subject of numerous studies concerning local adaptation (Losos et al., 1994, 1997, 2001; Kolbe et al., 2012), biological invasion (Kolbe et al., 2008), and sexual selection (Tokarz, 2002; Tokarz et al., 2005; Tokarz, 2006; Driessens et al., 2014; Steffen and Guyer, 2014; Driessens et al., 2015) among many other topics. Between-island variation in the mainly orange-red color of its dewlap was shown to be better explained by climatic variables (Driessens et al., 2017) than by proxies for biotic factors such as sexual selection or predation pressure (Vanhooydonck et al., 2009; Baeckens et al., 2018). How intra-island differences in habitat may contribute to the diversity of dewlap coloration, however, remains unexplored, and may reveal new insights into the scale of local differentiation despite gene flow.

Here, we analyzed the color characteristics of *A. sagrei* dewlaps within nine islands in the Bahamas and Cayman Islands. These island systems presently, if not historically, comprise relatively small islands, with no major geographic barriers within islands limiting dispersal for this species (Kamath and Losos, 2018). These islands all share three characteristic native West Indian small-island habitat-types – beach scrub bush, closed-canopy primary coppice forest, and mangrove forest – that are often spatially intermingled. These habitats contrast in environmental parameters including vegetation community, light irradiance, humidity, and temperature (Howard, 1950; Schoener, 1968). The Cayman Islands and the Bahamas have been colonized independently by *A. sagrei* from Cuba (van de Schoot 2016 unpublished thesis; Reynolds et al. 2020), such that these

archipelagos constitute an ideal suite of natural replicates to explore within-island dewlap diversity 107  
across multiple islands. 108  
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Our sampling design included sites in close proximity; the median distance between two sites 110  
within an island was 11.2km. Combining reflectance spectrometry and supervised machine learning, 111  
we tested for divergence in dewlap phenotype between habitats within islands and between islands 112  
across part of the range of *A. sagrei*. We predicted that if light conditions in the environment 113  
indeed drive color evolution, dewlaps should be most similar between beach scrub and mangrove 114  
forest, which both have high levels of light irradiance, compared to the darker, closed-canopy 115  
coppice forest. If detectability is maximized given the local conditions, we expected darker and 116  
more contrasting dewlaps in high irradiance habitats. Finally, if habitat characteristics are strong 117  
determinants of dewlap color variation, similar patterns should be observed across multiple islands 118  
119 (Harvey and Pagel, 1991; Losos, 2011).

120 **Methods**

121 **Data collection**

122 We sampled 466 male *A. sagrei* from seven islands in the Bahamas Archipelago – Abaco, North  
123 Andros, South Andros, South Bimini, Eleuthera, Long Island, and Ragged Island – and two in the  
124 Cayman Islands – Cayman Brac and Little Cayman (Figure S1A). These islands were chosen to  
125 span the breadth of the West Indian range of *A. sagrei*, because they have highly similar habitat  
126 types, and because the *A. sagrei* on each island group are derived from ancient and distinct  
127 colonization events from Cuba (i.e. relatively evolutionarily independent, Reynolds et al. 2020).  
128 Three habitats were sampled on each island based on characterizations by Howard (1950) and  
129 Schoener (1968). Each habitat is clearly distinguishable by its dominant vegetation type — xeric  
130 beach scrub (open, relatively dry habitat consisting of low vegetation or isolated trees), primary  
131 coppice forest (closed-canopy forest) and mangrove forest (wet coastal habitat with trees growing  
132 in brackish water and high light penetration, although lizards were sampled in dry soil areas).  
133 Sample sizes are given in Table S1. Our sampling design enabled us to test for differences between  
134 habitats at a coarse and fine geographical scale. The median distance between two localities within  
135 an island was ~ 11km (Figure S1B), and 80.3% of all pairwise distances within islands were less  
136 than 50km. Additionally, there are no major barriers to dispersal (such as mountains or grassland)  
137 on any of the islands that we sampled.

138 **Reflectance measurements**

139 We measured reflectance between 300nm and 700nm wavelength, a range from ultraviolet to red  
140 that encompasses the colors visible to most lizards and vertebrates in general (Lazareva et al.,  
141 2012). Measurements were taken with an Ocean Optics USB4000 spectrometer, a pulsed Xenon  
142 light source (PX-2, Ocean Optics, Largo, FL, USA) and a reflectance probe protected by a black  
143 anodized aluminum sheath. Measurements were taken with a 45-degree inclination to prevent  
144 specular reflection (Endler, 1990). The device was regularly standardized with a Spectralon white  
145 standard (Labsphere, North Sutton, NH, USA). Reflectance was measured at the center of the  
146 dewlap. Reflectance curves were smoothed using the R package pavo (Maia et al., 2013) as well  
147 as with custom R functions, down to one reflectance value at each nanometer in wavelength from  
148 300 to 700nm.

149 **Analysis**

150 We tested for detectable differences in dewlap coloration between populations from different habi-  
151 tats across islands by following an analytic pipeline in several steps. First, we used multivariate  
152 analyses of variance to assess the relative contributions of islands, habitats and habitat-by-island  
153 interactions on the partitioning of variation in color space. Second, and provided that habitat-by-  
154 island interactions were found, we investigated habitat-differences in dewlap color for each island  
155 separately using machine learning classification. Third, for each island where multivariate differ-  
156 ences were detected using our machine learning pipeline, we used univariate analyses of variance  
157 to decompose the signal among the different dimensions of color space. Fourth, for each signifi-  
158 cant between-habitat variation found in univariate analyses, we used post-hoc tests to determine  
159 which habitats were responsible for the differences. Last, to get insights into the spatial scale of  
160 phenotypic variation, for each significant contrast between two habitats detected along a given  
161 dimension on a given island, we performed multiple pairwise Wilcoxon tests to assess differences  
162 in dewlap coloration among the sites involved in that significant contrast, and recorded the geo-  
163 graphical distance between sites that were found significant. In parallel, we tested a possible effect  
164 of isolation-by-distance, an alternative cause of phenotypic divergence between localities, based on  
165 diffusion approximation and dispersal distance, irrespective of habitat types. We did so using a  
166 permutation test to assess the significance of the correlation between geographical distances and  
167 phenotypic distances among sites within each island.

168 All analyses in this study were performed in R 3.6.1 (R Core Team, 2019).

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<b>Dimensionality reduction</b>	170
Because neighboring wavelengths are highly collinear and redundant in reflectance, we reduced the dimensionality of the data using principal component analysis (PCA), as per Cuthill et al. (1999) and Leal and Fleishman (2002). We performed PCA on data from all islands combined, as well as on each island separately and systematically retained the first four principal components (PC), which together always explained more than 88.8% of the variance across islands (Table S2). PCs need not represent the same wavelengths across islands because they are fitted on different datasets. Nevertheless, PC1 was highly collinear with brightness for all islands (Figure S11), while the other PCs captured the chromatic variation (i.e. irrespective of brightness) in dewlap color.	171 172 173 174 175 176 177 178
<b>Among-island variance partitioning</b>	179
We performed a two-way nonparametric multivariate analysis of variance (PERMANOVA, Anderson 2001, R package vegan, Oksanen et al. 2019) to identify differences in coloration between islands, habitats and habitats within islands, using principal components fitted on data from all islands together. We used a nonparametric test because although no multivariate outliers were detected based on the Mahalanobis distance, the assumption of multivariate normality was violated in several habitats on several islands (Henze-Zirkler's test, Henze and Zirkler 1990, R package MVN, Korkmaz et al. 2014, $P < 0.05$ , Table S3).	180 181 182 183 184 185 186
<b>Within-island machine learning</b>	187
We performed a machine learning classification analysis on the first four principal components within each island separately, using random forests (Breiman, 2001). Random forests are a versatile, intuitive, and powerful algorithm commonly used in machine learning, using decision trees to predict the labels of particular observations based on their multivariate coordinates. These coordinates, or variables, are passed through a series of successive decision nodes, each examining a given variable of any given observation (James et al., 2013). The prediction for each observation is an aggregate over a large number of decision trees, each tree being trained on a subset of observations sampled with replacement from the dataset, and each tree being allowed to examine only a subset of the variables. This allows the random forest to overcome the individual errors of all trees in the predictions it makes.	188 189 190 191 192 193 194 195 196 197 198
To detect differences in dewlap coloration between habitats, we measured the success of random forests in reassigning individual lizards to their correct habitat of origin, based solely on their principal component scores. In machine learning, this so-called cross-validation procedure is typically done in two steps (James et al., 2013). First, a random forest is trained in recognizing features of dewlap coloration most associated with the different habitats, by being presented with multiple observations, making predictions about them, and updating its own decision rules based on whether the prediction deviates from the truth. Then, once trained, the patterns that the random forest has learned to recognize are tested by presenting new, previously unseen observations to the random forest, and measuring the proportion of correct predictions. This proportion, or success score, can then be statistically assessed against random guessing using a binomial test.	199 200 201 202 203 204 205 206 207 208 209
The cross-validation procedure requires that the data be split into a training set and a testing set. To remove any bias due to the set that is being sampled for training, it is common practice to use k-fold cross-validation (James et al., 2013), where the data are split into $k$ random bins and $k$ independent machines are trained, each taking one of the bins as a testing set and the rest for training, and where classification success is measured by summing all correct classifications from the $k$ machines.	210 211 212 213 214 215 216
Here, we used a k-fold cross-validation procedure with $k = 5$ , where each training set consisted of 80% of the data and the machine was tested on the remaining 20%. Each training set was conditioned on containing at least five lizards from each of the three habitats. We also down-sampled the training set to the sample size of the least represented habitat, to ensure that the different habitats were equally represented. To further remove any bias due to the specific random split into the different bins, we replicated each k-fold cross-validation five times. We then averaged the five resulting confusion matrices across replicates, where each confusion matrix shows the number	217 218 219 220 221 222

224 of lizards from each habitat reassigned into each habitat. For each island, we then used the average  
225 proportion of correctly reassigned lizards (i.e. the proportion of observations on the diagonal  
226 of the average confusion matrix) as an estimate of classification success. This score was tested  
227 against random guessing by comparing it to a binomial distribution with number of trials being  
228 the number of lizards on that island and success probability 1/3, representing the rate of successful  
229 classification by chance when three habitats are involved.

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231 We used the machine learning fitting functions in the R package rminer (Cortez, 2020), which  
232 calls random forest routines from the randomForest package (Liaw and Wiener 2002, implemen-  
233 tation from the original random forest algorithm Breiman, 2001). For each random forest, we  
234 optimized the number of trees in the forest and the number of variables examined by each tree  
235 using the grid hyperparameter search procedure implemented in rminer, to choose between two  
236 numbers of trees (500 or 1,000) and four numbers of principal components examined per tree (1  
237 to 4), using rminer's ordered holdout validation method with 2/3 of the data used for training.

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239 We validated the results of our analysis by using two other widely used machine learning  
240 classification methods: linear discriminant analysis and support vector machines (Cristianini and  
241 Shawe-Taylor, 2000; James et al., 2013), both accessible in rminer (Cortez, 2020).

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243 To know which wavelengths were most used to assign data points to each habitat, we trained  
244 another set of random forests, this time directly on reflectance data (taken every 5nm from 300 to  
245 700nm) instead of principal components. We recorded the relative importance of each wavelength  
246 for each habitat, as measured by the mean decrease in accuracy during wavelength permutation,  
247 implemented in the randomForest package (Liaw and Wiener, 2002).

## 248 Univariate analyses

249 For each island where significant differences in dewlap coloration were detected between habitats,  
250 we used multiple univariate analyses of variance (ANOVA) to identify possible principal compo-  
251 nents underlying the observed differences. We constructed our ANOVA models in two steps, as  
252 per Zuur (2009). In a first step, we accounted for heterogeneity of variances across groups by  
253 systematically comparing the goodness-of-fit of an ANOVA model estimated with ordinary least  
254 squares (OLS) with that of a model estimated with generalized least squares (GLS), which al-  
255 lowed one estimate of residual variance per habitat (using the R package nlme, Pinheiro and Bates  
256 2000; Pinheiro et al. 2020). Both models were fitted with restricted maximum likelihood (REML).  
257 Goodness-of-fit was estimated using Akaike's Information Criterion corrected for small sample sizes  
258 (AICc, R package MuMIn, Bartoń 2019), and the estimation method yielding the lowest AICc was  
259 retained. In a second step, we re-fitted the retained model with maximum likelihood (ML) to  
260 test for the effect of habitat type using likelihood ratio tests (LRT) between a model including a  
261 habitat-term and a null model lacking the habitat-term.

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263 We evaluated the normality of the standardized residuals (residuals divided by their standard  
264 error, which can differ among habitats in a GLS model) of each fitted ANOVA model using Shapiro-  
265 Wilk's test, with P-values adjusted for multiple testing using the Benjamini-Hochberg correction  
266 (Benjamini and Hochberg, 1995). In cases where significant deviations from normality were de-  
267 tected ( $P_{adj} < 0.05$ , Table S4) we performed Kruskal-Wallis's nonparametric test to back up the  
268 ANOVA results.

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270 To know which habitat-populations were different from which in dewlap coloration, we per-  
271 formed different post-hoc multiple comparison tests (all implemented in the PMCMRplus package,  
272 Pohlert, 2020), depending on which assumptions were met. In cases where normality and ho-  
273 moscedasticity were met (i.e. OLS-ANOVA was the best fit), we used Tukey's honest significant  
274 difference test. When normality was met but not homoscedasticity (i.e. GLS-ANOVA was the  
275 best fit), we used Dunnett's T3 test. Finally, whenever we used Kruskal-Wallis's test because the  
ANOVA residuals were not normally distributed, we used Nemenyi's test for post-hoc comparisons.

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## Spatial autocorrelation

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We tested for within-island spatial autocorrelation between the geographical distances among sampling sites and their Euclidean distances in multivariate color space (mean PC1 to PC4 per site, Table S5), regardless of habitat type. For this, we performed Mantel's test (Legendre and Legendre 2012, R package vegan; Oksanen et al. 2019) on each island, using 999 permutations and geographical distances computed as geodesic distances from latitude and longitude data (R package geosphere, Hijmans 2019).

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## Site differences

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In this study, we were interested in the minimum spatial scale at which significant differences between habitats could be detected within islands. We performed multiple pairwise nonparametric Wilcoxon-Mann-Whitney tests (Hollander et al., 2013) to compare dewlap coloration between sites with different habitat types, for each pair of habitats and each variable where significant differences were detected with our analyses of variance. The P-values were adjusted using a Benjamini-Hochberg correction for multiple testing (Benjamini and Hochberg, 1995).

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292 **Results**

293 We tested for variation in *A. sagrei* dewlap coloration between populations living in three charac-  
294 teristic habitat types across nine islands that span the West Indian range of the brown anole (beach  
295 scrub, primary coppice and mangroves). We found that most of the variation in coloration was  
296 partitioned between islands (two-way PERMANOVA,  $F(df = 8) = 43.7$ ,  $P = 0.0001$ , explained  
297 variance  $R^2 = 40.9\%$ ). Nonetheless, we did find evidence for differences in dewlap coloration  
298 between habitat types, and those were mostly island-specific (habitat-by-island interaction term,  
299  $F(16) = 3.53$ ,  $P = 0.0001$ ,  $R^2 = 6.6\%$ ), with a significant portion of the variation explained by  
300 an habitat effect across all islands, but this effect was relatively small ( $F(2) = 4.7$ ,  $P = 0.0001$ ,  
301  $R^2 = 1.1\%$ ).

302 We subsequently tested for differences in dewlap coloration between habitat-populations within  
303 each island, using within-island principal component scores (to maximize the variation captured  
304 for each island, see Methods). Our within-island random forest classification analyses revealed  
305 detectable differences in dewlap coloration on eight out of the nine islands in our sample: Abaco,  
306 Bimini, Cayman Brac, Eleuthera, Little Cayman, Long Island, North Andros and South Andros.  
307 The accuracy of random forest classification exceeded random expectation more often than ex-  
308 pected by chance for all these islands (Table 1). Accuracy was as high as 73% for Cayman Brac.  
309 We obtained similar results using other machine learning approaches such as support vector ma-  
310 chines (Table S7) and linear discriminant analysis (Table S8), except that these methods did not  
311 detect significant differences on Eleuthera and North Andros. We describe in details the specific  
312 differences detected on each island in the Appendix, and focus here on the general patterns emerg-  
313 ing from our data.

314 Overall, we found significant differences in dewlap coloration between populations that were  
315 often in close geographical proximity. On Bimini, notably, we found a significant difference be-  
316 tween dewlaps from beach scrub and primary coppice forest, at a distance of a few hundred meters,  
317 making this contrast the smallest geographical scale at which differences in coloration were found  
318 in our study (Fig. S3). We also detected significant differences in dewlap coloration at distances  
319 below one kilometer on Abaco (Fig. S2G), and at distances between one and ten kilometers on  
320 Bimini (Fig. S3G), Cayman Brac (Fig. S4G), Little Cayman (Fig. S6G), Long Island (Fig. S7G)  
321 and South Andros (Fig. S10G).

322 We found evidence of spatial autocorrelation in dewlap coloration between the sites within is-  
323 lands for Abaco (Table 2), suggesting that populations from closer sites tend to have more similar  
324 dewlaps on this island than expected by chance. Abaco was the island we sampled at the largest  
325 scale, with some sites nearly a hundred kilometers away from each other (Fig. 2A). That said, some  
326 sites were also in close proximity, and significant differences in coloration were detected between  
327 habitats sometimes less than a kilometer away (Fig. S2G), suggesting that differences in dewlap  
328 coloration between distant sites may be partly attributable to isolation-by-distance, but this may  
329 not necessarily be the case for sites in close proximity. We did not find evidence for spatial auto-  
330 correlation on other islands than Abaco (Table 2).

331 A striking feature of our data was inconsistencies in between-habitat differences among islands,  
332 in terms of which habitats differ from which, which dimensions of coloration were involved, and  
333 in which direction. For example, while on Cayman Brac the random forests could well distinguish  
334 between all three habitats (Fig. S4D), on Abaco dewlaps from beach scrub and primary coppice  
335 were often mistaken, and on Bimini beach scrub dewlaps were more often classified into primary  
336 coppice or mangrove than into beach scrub (Fig. S3D). In terms of variable importance, for multi-  
337 ple islands the random forests used information in the UV range to discriminate between at least  
338 some habitats, particularly on Abaco (Fig. S2F), Bimini (Fig. S3F), Cayman Brac (Fig. S4F),  
339 Little Cayman (Fig. S6F) and Long Island (Fig. S7F), but differences in UV reflectance involved  
340 different habitats and were in different directions among these islands.

## Discussion

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Two main insights follow from our results. First, we detected significant differences in dewlap coloration between habitats within seven out of the nine islands investigated (excluding North Andros where the follow-up univariate analyses were not significant), suggesting a putatively high potential for local differentiation of dewlap coloration in *Anolis sagrei*. Second, we found differences in coloration along different dimensions of color space, and in different directions, on different islands.

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Detectable differences in dewlap color between populations are surprising, as habitats were often in close geographical proximity to each other (as close as a few hundred meters on Bimini and most of the time within ten kilometers). Indeed, given that (1) the populations were continuously distributed between the habitats, (2) populations from different habitats were not monophyletic with respect to mitochondrial haplotypes (van de Schoot 2016 unpublished thesis), and (3) *A. sagrei* is highly mobile (Kamath and Losos, 2018), we would have expected more homogeneous distributions of color phenotypes within islands due to gene flow, with fewer differences between populations, especially those in close proximity.

Several scenarios could account for these findings. One explanation is an adaptive one: populations living in different habitats could be phenotypically adapted to their local environmental conditions. Given that the brightly colored dewlap of *A. sagrei* is used as a communication signal, its color may be a target for selection if the transmission or perception of the signal differs from one habitat to another, for example because of differences in ambient light, according to the sensory-drive hypothesis (Endler and McLellan, 1988; Endler, 1992, 1998). The sensory-drive hypothesis has been tested multiple times for dewlap coloration in *Anolis* lizards, with mixed results. Some authors found support for it (Leal and Fleishman, 2002, 2004), while others found differences in dewlap coloration between habitats inconsistent with the sensory-drive hypothesis (Fleishman et al., 2009; Ng et al., 2012).

If our results were an example of sensory drive, we would have expected to see consistent differences between populations from different habitats across islands, given the apparent environmental consistency each of the three habitat types across the islands we sampled. In particular, we would have expected divergence in line with increased detectability given local light conditions, such as the high contrasts with background vegetation found in the UV range in Leal and Fleishman (2002) and Leal and Fleishman (2004). We might also have expected mangrove and beach scrub lizards, both inhabiting areas with high light penetration, to have more similar dewlaps, and to differ significantly from lizards from the coppice habitat, where irradiance is low. Instead, we found inconsistencies in the way dewlap color differed between habitats across islands. While short-wavelengths (UV reflectance) were often involved in color differences, they were not involved on all islands where significant differences were detected. On some islands, other or additional variables differed, such as brightness, red reflectance or the reflectance at the ends of the spectrum visible to *Anolis* lizards (UV and red, Lazareva et al. 2012) relative to intermediate wavelengths (blue-to-yellow). Similar portions of the spectrum were sometimes involved in opposite directions on different islands, such as on Abaco and Cayman Brac, where mangrove lizards had a higher UV-reflectance than beach scrub lizards on the former, but a lower UV-reflectance on the latter. Overall, the observed heterogeneity of divergence patterns across islands provides no support to a sensory-drive explanation.

It is presently not known if the reported differences in coloration have a genetic basis. Yet, we find it unlikely that these differences arose through phenotypic plasticity, as although the carotenoids that partly make up the red and orange colors of anole dewlaps must be found in the diet (Goodwin, 1984; Hill et al., 2002; Hill and McGraw, 2006), studies testing the effect of carotenoid deprivation (Steffen et al., 2010; Ng et al., 2013) and heritability (Cox et al., 2017) of dewlap coloration in *A. sagrei* and *A. distichus* (another species with a carotenoid-based dewlap), found little support for phenotypic and developmental plasticity in dewlap coloration. One exception is a study demonstrating that lizards heavily parasitized by skin mites had duller dewlaps (Cook et al., 2013), but we found no sign of that in our study.

402 We found no evidence for a role of genetic drift in explaining the observed patterns either.

403 First, *A. sagrei* was distributed across the islands continuously, usually at relatively high popula-  
404 tion densities, rather than in small and isolated populations where drift might be expected to have  
405 a strong effect. Second, we found no evidence of isolation-by-distance except on Abaco, which was  
406 sampled at the largest geographical scale, with sites nearly a hundred kilometers apart from each  
407 other. Hence, while isolation-by-distance may explain long-range differences on this island, most of  
408 the differences among habitats across the rest of the sampling region are unlikely to be explained  
409 by genetic drift, as habitats were often in close proximity (less than 10km).

410  
411 In this study, we found larger differences among than within islands, a pattern already reported  
412 and linked to climatic conditions ([Vanhooydonck et al., 2009](#)) and to densities of predators and of  
413 anole congeners ([Baeckens et al., 2018](#)). Differences among habitats within islands, however, are  
414 still difficult to account for. Remaining hypotheses may include, for example, runaway sexual selec-  
415 tion arbitrarily operating in different directions across islands ([Andersson, 1994](#)), but no evidence  
416 so far suggests that dewlap is a target of mate choice in anoles ([Tokarz, 2002; Tokarz et al., 2005;](#)  
417 [Nicholson et al., 2007](#)). Another hypothesis is that the different genetic constitutions of different  
418 islands, perhaps resulting from founder effects (the islands have been colonized independently, [van](#)  
419 [de Shoot 2016 unpubl.; Driessens et al. 2017; Reynolds et al. 2020](#)), may have predisposed pop-  
420ulations to adapt differently to similarly selective circumstances. Either way, new data would be  
421 needed to test these hypotheses.

422  
423 Altogether, our results show that dewlap color of *A. sagrei* commonly varies between habitat  
424 types, even in close geographical proximity, within islands of the West Indies. However, coloration  
425 differs in different ways across similar habitats from one island to another. We discussed several  
426 non-mutually exclusive mechanisms that could explain these observations. Nevertheless, heteroge-  
427 neous patterns of divergence across islands do not support an adaptive sensory-drive scenario, and  
428 we propose that within-island dewlap color variation may be underlain by a more subtle mosaic of  
factors.

429

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430

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## Figures

44 3

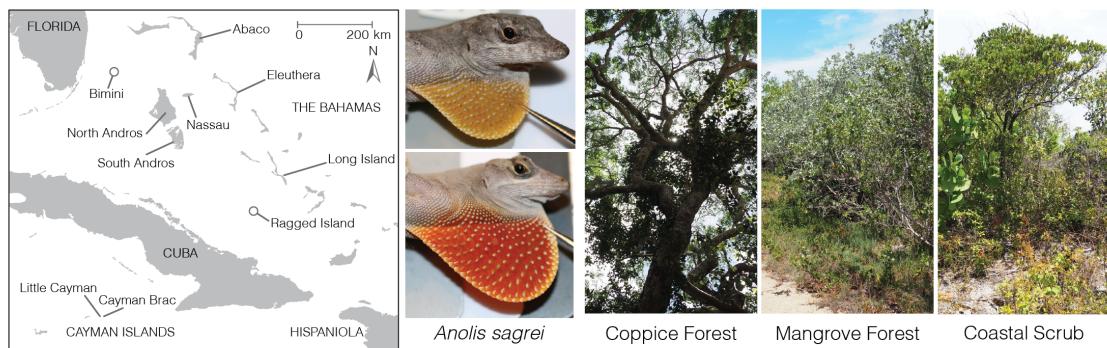


Figure 1: Overview of our study design, including a map of the Bahamas and the Cayman Islands, on which are indicated the nine islands we sampled, two representatives of our study species *Anolis sagrei* with their dewlaps deployed, and the three types of habitats we considered on each island.

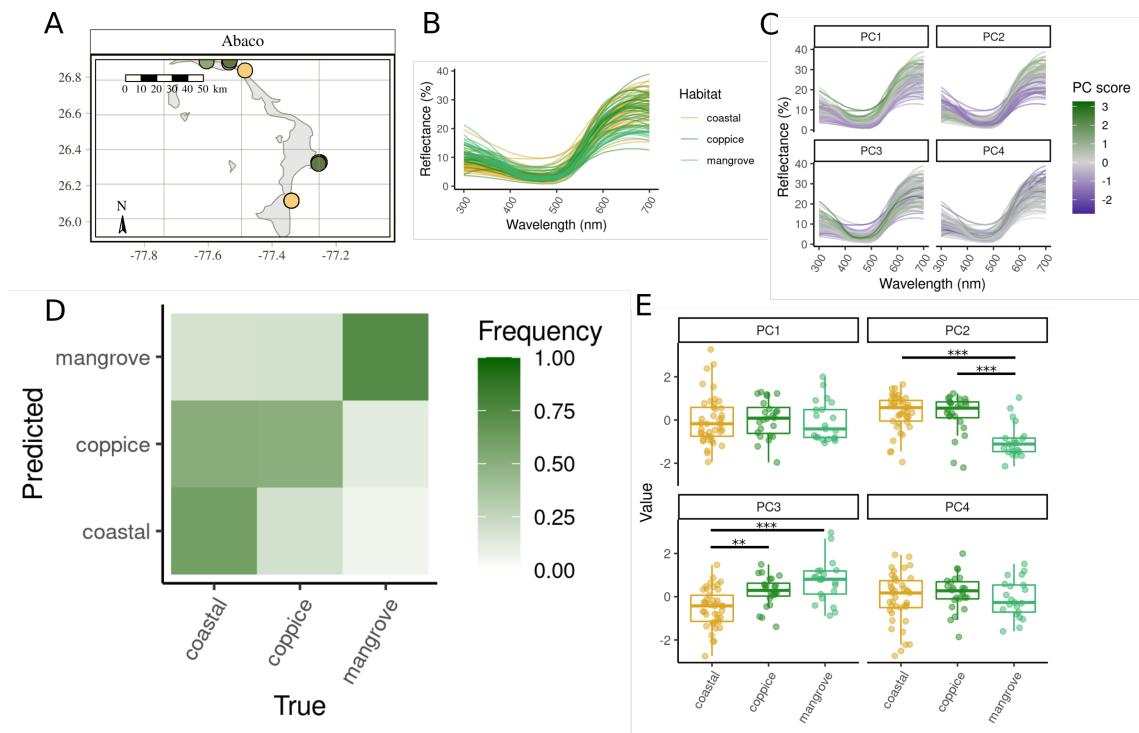


Figure 2: Comparison of dewlap coloration across habitats on Abaco. (A) Map of the island with the sampling sites colored by habitat. (B) Reflectance profiles of all the dewlaps on the island. (C) How reflectance profiles map onto the within-island principal components. (D) Confusion matrix showing the proportion of lizards from each (true) habitat reassigned to each (predicted) habitat by the random forests, based on the first four within-island principal components and averaged across replicates. Each column sums to one. (E) Within-island principal component scores across habitats. Bars indicate significant contrasts. \*,  $P < 0.05$ ; \*\*,  $P < 0.01$ ; \*\*\*,  $P < 0.001$ .

## Tables

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Table 1: Random forest classification results. For each island are shown the sample size ( $N$ ) and the proportion of correctly reassigned observations (or success score). P-values were computed using a binomial test and assess the significance of the observed success score relative to the score expected under random guessing. \*,  $P < 0.05$ ; \*\*,  $P < 0.01$ , \*\*\*,  $P < 0.001$ .

Island	$N$	Score	$P$
Abaco	86	0.612	< 0.0001 ***
Bimini	67	0.510	0.0011 **
Cayman Brac	50	0.728	< 0.0001 ***
Eleuthera	56	0.493	0.0072 **
Little Cayman	45	0.649	< 0.0001 ***
Long Island	53	0.634	< 0.0001 ***
North Andros	28	0.507	0.0216 *
Ragged Island	50	0.368	0.2874
South Andros	31	0.484	0.0270 *

Table 2: Mantel's test of spatial autocorrelation. For each island are shown the correlation (Pearson's  $\rho$ ) between the matrix of phenotypic distances between populations from each site and the matrix of geographic distances between sites, where phenotypic distances are Euclidean distances between the mean phenotypes of each site in the multivariate space consisting of the first four within-island principal components. P-values assess the significance of the observed correlation against the correlation expected if population means were randomly permuted among sites (999 permutations). \*,  $P < 0.05$ .

Island	$\rho$	$P$
Abaco	0.448	0.033 *
Bimini	0.810	0.250
Cayman Brac	-0.737	0.833
Eleuthera	0.844	0.058
Little Cayman	-0.042	0.667
Long Island	0.367	0.333
North Andros	0.051	0.667
Ragged Island	-0.363	0.708
South Andros	-0.979	1.000

Table 3: Significance of habitat differences in dewlap coloration, using ANOVA for all islands where significant multivariate differences in dewlap coloration were detected by random forests. Model, best-fitting model (either OLS or GLS). AICc, corrected AIC score of the best-fitting model.  $\Delta\text{AICc}$ , difference in AICc between the best-fitting model and the OLS-model. AICcw, AICc weight. Log-lik., log-likelihood.  $\chi^2$ , likelihood ratio. df, degrees of freedom. \*,  $P < 0.05$ ; \*\*,  $P < 0.01$ ; \*\*\*,  $P < 0.001$ .

Island	Variable	AICc	$\Delta\text{AICc}$	AICcw	Model	Log-lik.	$\chi^2$	df	P
Abaco	PC1	255.81	2.16	0.746	OLS	-121.45	0.14	2	0.9308
Abaco	PC2	225.32	4.02	0.882	OLS	-105.66	31.74	2	< 0.0001 ***
Abaco	PC3	229.53	2.01	0.732	OLS	-107.84	27.37	2	< 0.0001 ***
Abaco	PC4	254.64	0.78	0.596	OLS	-120.85	1.36	2	0.5070
Bimini	PC1	194.16	0.77	0.595	OLS	-90.87	7.40	2	0.0248 *
Bimini	PC2	193.49	1.29	0.656	OLS	-90.52	8.09	2	0.0175 *
Bimini	PC3	184.22	-0.23	0.529	GLS	-83.46	10.39	2	0.0056 **
Bimini	PC4	200.91	3.54	0.854	OLS	-94.40	0.33	2	0.8499
Cayman Brac	PC1	136.64	-4.05	0.884	GLS	-59.29	13.81	2	0.0010 **
Cayman Brac	PC2	144.75	3.51	0.853	OLS	-66.24	8.41	2	0.0149 *
Cayman Brac	PC3	127.13	2.77	0.800	OLS	-56.86	27.16	2	< 0.0001 ***
Cayman Brac	PC4	147.37	4.33	0.897	OLS	-67.63	5.63	2	0.0600
Eleuthera	PC1	168.72	2.42	0.770	OLS	-78.46	1.00	2	0.6074 **
Eleuthera	PC2	160.03	-2.20	0.750	GLS	-70.89	11.34	2	0.0034 **
Eleuthera	PC3	163.49	-0.20	0.525	GLS	-72.69	5.57	2	0.0617
Eleuthera	PC4	164.08	3.49	0.852	OLS	-76.01	5.89	2	0.0525
Little Cayman	PC1	130.60	2.50	0.777	OLS	-59.26	8.18	2	0.0167 *
Little Cayman	PC2	112.66	-3.61	0.859	GLS	-46.74	29.76	2	< 0.0001 ***
Little Cayman	PC3	118.32	1.41	0.669	OLS	-52.68	21.34	2	< 0.0001 ***
Little Cayman	PC4	135.58	2.53	0.780	OLS	-61.92	2.85	2	0.2410
Long Island	PC1	154.54	-2.09	0.740	GLS	-68.62	2.91	2	0.2331
Long Island	PC2	155.80	-3.08	0.823	GLS	-68.92	4.52	2	0.1043
Long Island	PC3	150.54	3.67	0.862	OLS	-69.08	11.24	2	0.0036 **
Long Island	PC4	155.05	2.38	0.767	OLS	-71.47	6.46	2	0.0395 *
North Andros	PC1	88.64	0.27	0.534	OLS	-38.84	0.75	2	0.6864
North Andros	PC2	85.36	2.17	0.748	OLS	-37.01	4.42	2	0.1100
North Andros	PC3	85.31	5.82	0.948	OLS	-36.98	4.48	2	0.1065
North Andros	PC4	88.45	4.83	0.918	OLS	-38.74	0.96	2	0.6194
South Andros	PC1	95.12	0.44	0.554	OLS	-41.93	3.10	2	0.2125 *
South Andros	PC2	89.93	-0.05	0.506	GLS	-35.84	7.76	2	0.0206 *
South Andros	PC3	87.21	-6.14	0.956	GLS	-34.05	10.35	2	0.0056 **
South Andros	PC4	83.01	2.94	0.813	OLS	-35.23	16.51	2	0.0003 ***

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## Appendix

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Here we describe more precisely the patterns identified on each island.

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On Abaco, dewlaps from the mangrove habitat were the best discriminated, while dewlaps from the beach scrub habitat were often mistaken for dewlaps from the coppice habitat (Fig. 2D). Importance analysis revealed that beach scrub and mangrove lizards mostly differed in reflectance in the ultraviolet (UV) end of the spectrum (below 400nm, Fig. S2F), where mangrove dewlaps had higher UV reflectance relative to beach scrub lizards, and coppice lizards had an intermediate UV reflectance between the two other habitats (Fig. 2B). Consistent with this, our analyses of variance detected significantly lower PC2 scores in mangrove lizards than in the two other habitats (Fig. 2E, Table 3), representing a higher UV-reflectance relative to red (Fig. 2C). Beach scrub lizards also scored lower on PC3 (Fig. 2E, Table 3), indicating less curvature of the reflectance profile and relatively higher reflectance at intermediate wavelengths (blue-to-yellow) than at the ends of the range (Fig. 2C). Differences were detected between sites both at large ( $\sim 100\text{km}$ ) and short ( $< 1\text{km}$ ) distances (Fig. S2G). Abaco was the only island where we detected significant spatial autocorrelation (Table 2), that is, sites that were closer geographically tended to have populations of lizards with more similar dewlap colors.

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On Bimini, the random forests mostly correctly classified lizards from the coppice and mangrove habitats while often misclassifying lizards from the beach scrub habitat (Fig. S3D). Relatively flat importance profiles for beach scrub lizards suggested that brightness was used instead of a particular wavelength to identify some of the beach scrub dewlaps (Fig. S3F). Indeed, some beach scrub dewlaps were substantially brighter than the rest (Fig. S3B, C), a pattern that was captured by our analysis of variance along PC1 (i.e. brightness, Fig. S3C, E, Table 3). The random forests also used UV reflectance to discriminate between coppice and mangrove dewlaps (Fig. S3F), which could reflect the significant difference we detected along PC3 between these two habitats (Fig. S3C, E, Table 3). Beach scrub lizards were characterized by elevated red reflectance relative to UV (as represented by PC2, (Fig. S3C, E)), and beach scrub and mangrove lizards were characterized by a more even distribution of the reflectance along the spectrum (as represented by PC3, (Fig. S3C, E)), in contrast to coppice lizards which harbored a stronger curvature at intermediate wavelengths (Fig. S3C, E). On this island, the beach scrub and coppice habitats were separated by a few hundred meters, making this contrast the smallest geographical scale at which differences in coloration were found in our study (Fig. S3G).

On Cayman Brac, all three habitats could be well discriminated against each other (Fig. S4D), with UV reflectance appearing to be an important variable differentiating beach scrub and mangrove dewlaps (Fig. S4F). In contrast, coppice dewlaps had a relatively flat importance profile, suggesting that brightness made them more distinct rather than any particular wavelength (Fig. S4F). Consistent with this, coppice dewlaps were significantly different from all other dewlaps along PC1 (Fig. S4E, Table 3). At a distance between 2 and 3km (Fig. S4G), dewlaps in the beach scrub habitat reflected more red light (as represented by PC2, Fig. S4C, E) and more UV (as represented by PC3, Fig. S4C, E) than in the mangrove habitat. Coppice lizards were also characterized by a higher UV reflectance than mangrove dewlaps (PC3, Fig. S4C, E, Table 3).

On Eleuthera, although random forests detected between-habitat differences in dewlap color, other approaches did not (Tables S8 and S7), suggesting that the differences may be small. Consistent with this, the only significant univariate difference detected was for PC2 between beach scrub and mangrove lizards, where beach scrub lizards had higher levels of red reflectance and mangrove lizards higher levels of UV reflectance (Fig. S5C, E, Table 3).

Little Cayman was characterized by a better discrimination of mangrove lizards from the rest than between beach scrub and coppice lizards (Fig. S6D). Mangrove dewlaps were most distinct with respect to their reflectance in short wavelengths (Fig. S6F), with significantly lower UV reflectance (as represented by PC2, Fig. S6C, E, Table 3). Beach scrub lizards were characterized by brighter dewlaps than coppice lizards (PC1), and also more convex curves, i.e. slightly higher UV and red reflectance (as represented by higher PC3 scores), than lizards from the other two habitats (Fig. S6C, E, Table 3).

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739 On Long Island the three habitats were relatively well discriminated (Fig. S7D). Importance  
740 profiles indicated that short wavelengths were used to discriminate between beach scrub and man-  
741 grove lizards (Fig. S7F). Beach scrub lizards had more curved reflectance profiles than in either of  
742 the two other habitats, with higher levels of UV and red reflectance relative to intermediate wave-  
743 lengths (PC3, Fig. S7C, E, Table 3). Beach scrub lizards also differed from mangrove lizards along  
744 PC4 (Fig. S7E), which represented a rather small portion of the variance not already explained  
745 by the first three principal components, and is therefore difficult to interpret (Fig. S7C). Coppice  
746 lizards were significantly darker than mangrove and beach scrub lizards (PC1, Fig. S7C, E, Table  
747 3).

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749 On North Andros, although the random forest classification was significant ( $P = 0.0216$ , Table  
750 1) and the average confusion matrix indicated that lizards from beach scrub were particularly well  
751 predicted (Fig. S8D), no significant univariate differences were detected along any of the four PCs  
752 (Fig. S8E, Table 3). Importance analysis of full-spectrum random forests showed higher impor-  
753 tance scores near the UV-end of the spectrum in discriminating beach scrub dewlaps from the rest  
754 (Fig. S8F). Besides, reflectance curves of beach scrub dewlaps appeared more similar to each other  
755 in the UV range than dewlaps from other habitats (Fig. S8B), suggesting that the machines may  
756 have used this low within-habitat variance, as opposed to between-habitat differences in means,  
757 to correctly classify beach scrub lizards. A small sample size on this island may also have con-  
758 tributed to a lack of power in detecting univariate differences using analyses of variance (Table S1).

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760 On South Andros beach scrub and coppice dewlaps could be discriminated better against  
761 each other than with mangrove dewlaps (Fig. S10D), with importance profiles supporting UV-  
762 reflectance as a predictor of coppice lizards (Fig. S10F). Coppice lizards had more curved re-  
763 flectance profiles than beach scrub lizards (PC3), and lizards from both habitats differed along  
764 PC4, which is again more difficult to interpret (Fig. S10C, E, Table 3). Beach scrub lizards also  
765 differed from mangrove lizards in PC4 (Fig. S10C, E, Table 3).

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767 Classification success was not significantly better than expected by chance on Ragged Island  
768 (Table 1) where nearly no habitat could be differentiated from any other based on reflectance.

<sup>769</sup> Supplementary Figures

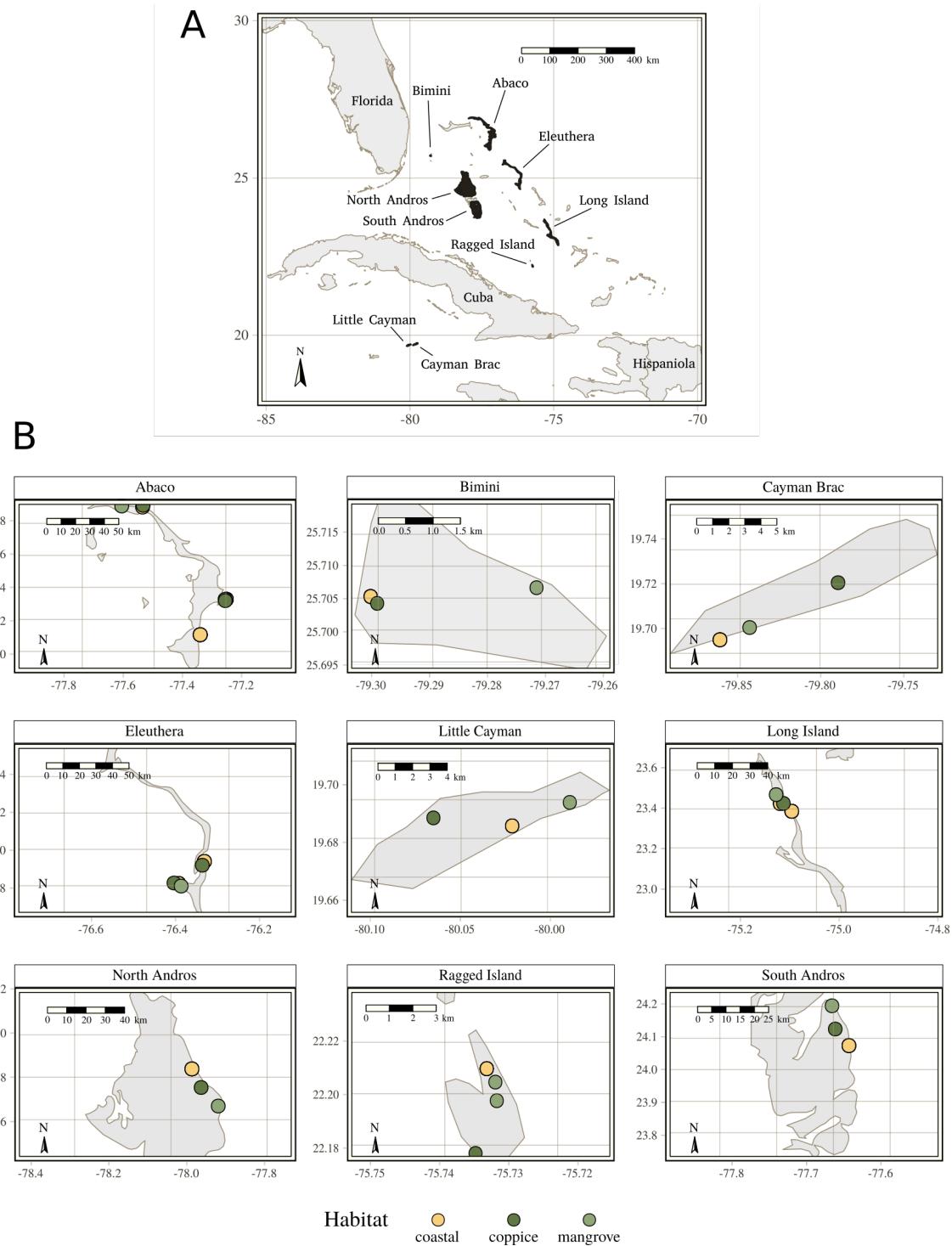


Figure S1: Maps of the islands. (A) Map of the West Indies with sampled islands highlighted in black. (B) Sampling sites within islands colored after their respective habitat types.

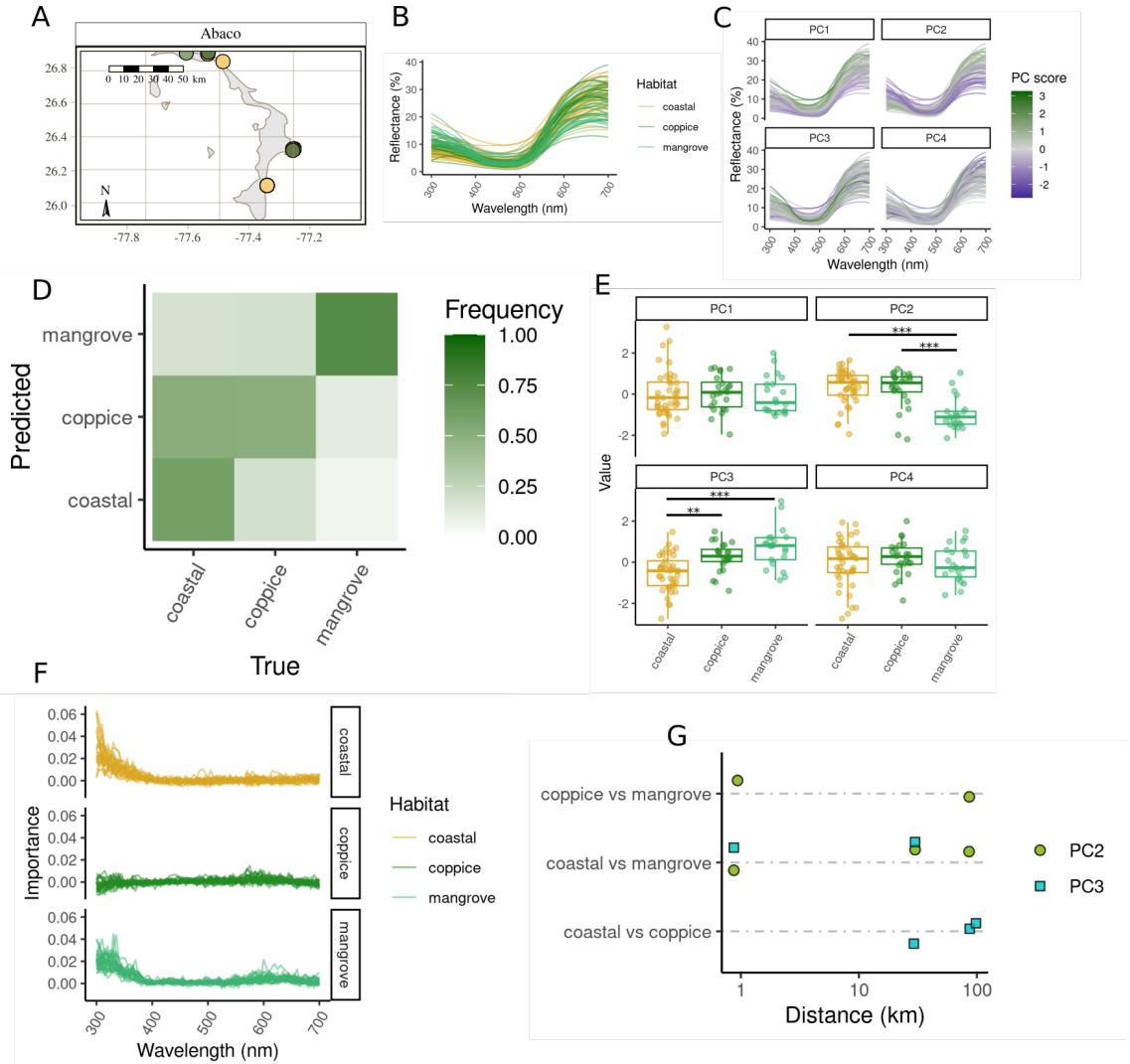


Figure S2: Comparison of dewlap coloration across habitats on Abaco, with extended results. (A–E) Legend as per Figure 2. (F) One-dimensional sensitivity analysis showing the relative importance (mean decrease in accuracy) of the various wavelengths in random forest classification of the whole spectrum. (G) Geographical distance between sites where significant differences were detected in within-island principal component scores (Wilcoxon test, Benjamini-Hochberg correction,  $P < 0.05$ ), including only pairs of sites whose habitats were involved in between-habitat dewlap differences.

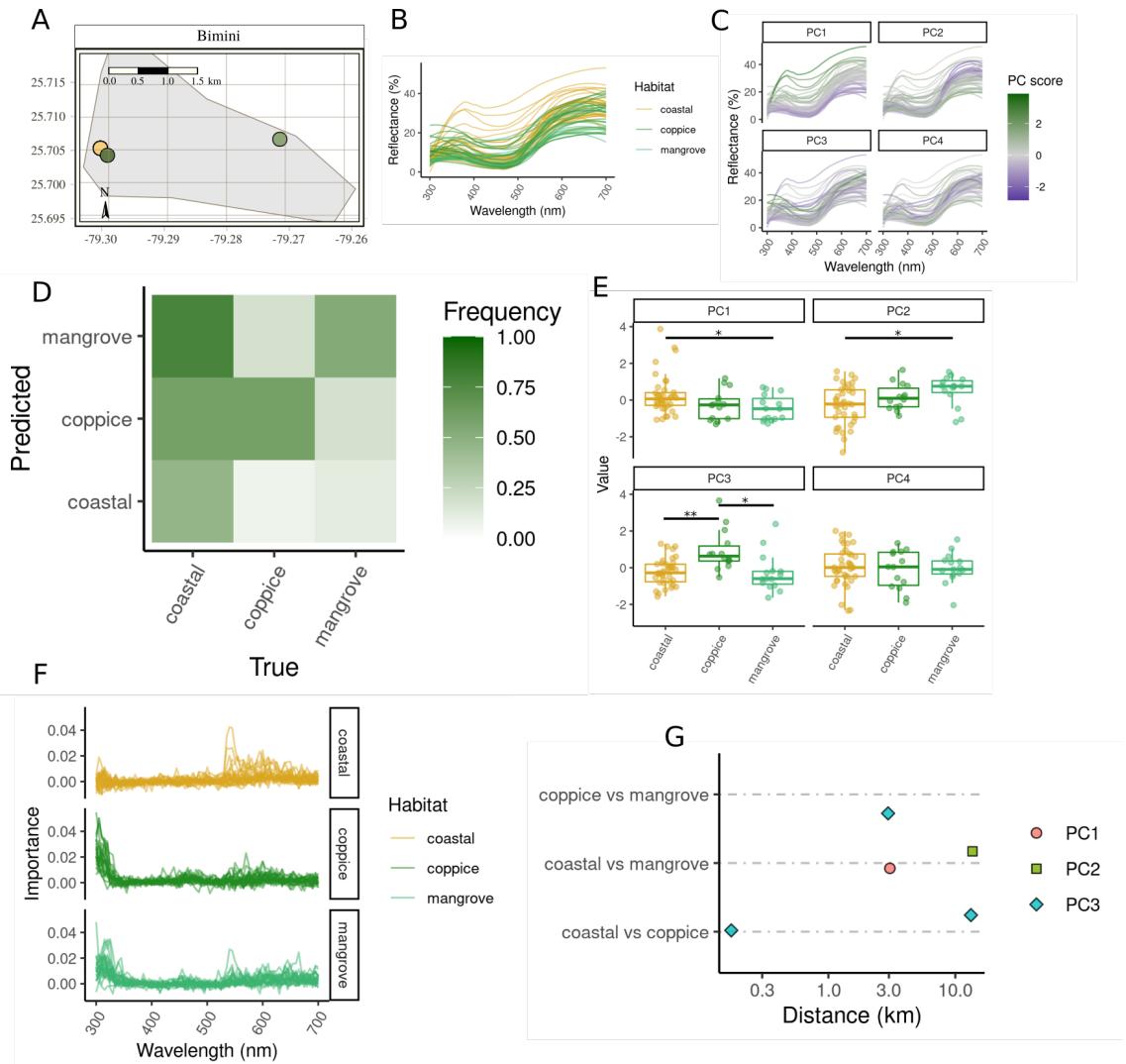


Figure S3: Comparison of dewlap coloration across habitats on Bimini. Legend is as per Figure S2.

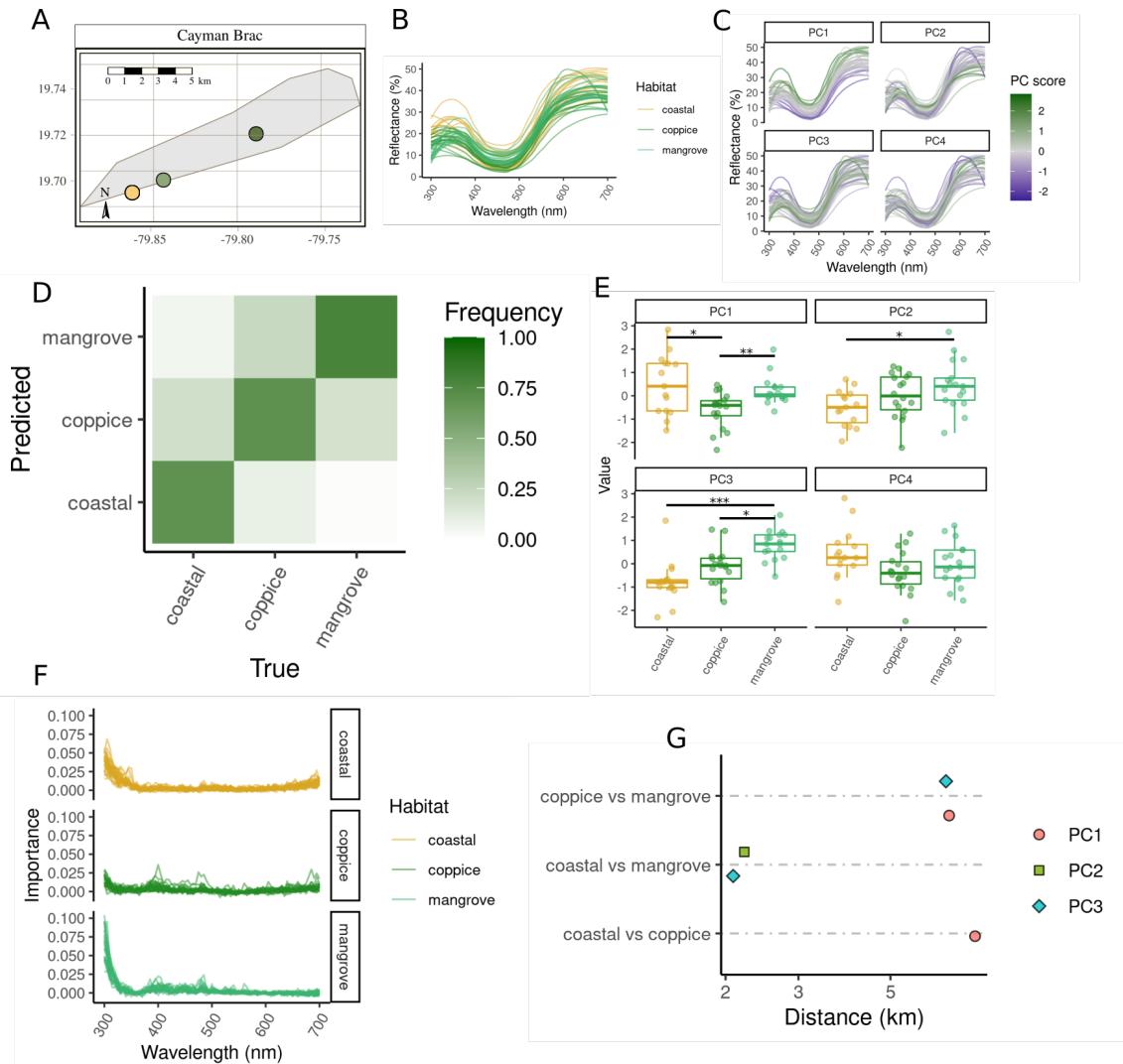


Figure S4: Comparison of dewlap coloration across habitats on Cayman Brac. Legend is as per Figure S2.

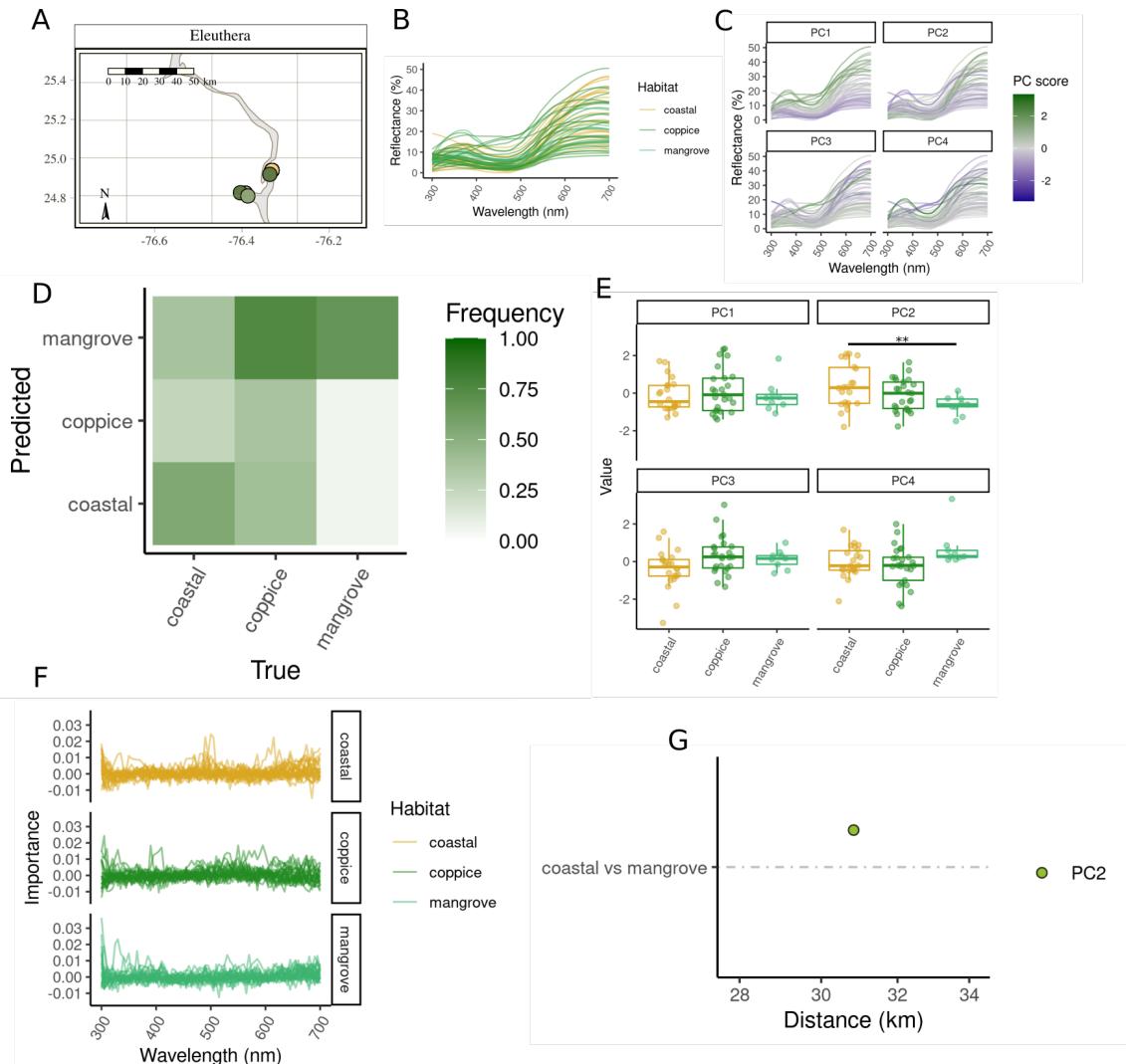


Figure S5: Comparison of dewlap coloration across habitats on Eleuthera. Legend is as per Figure S2.

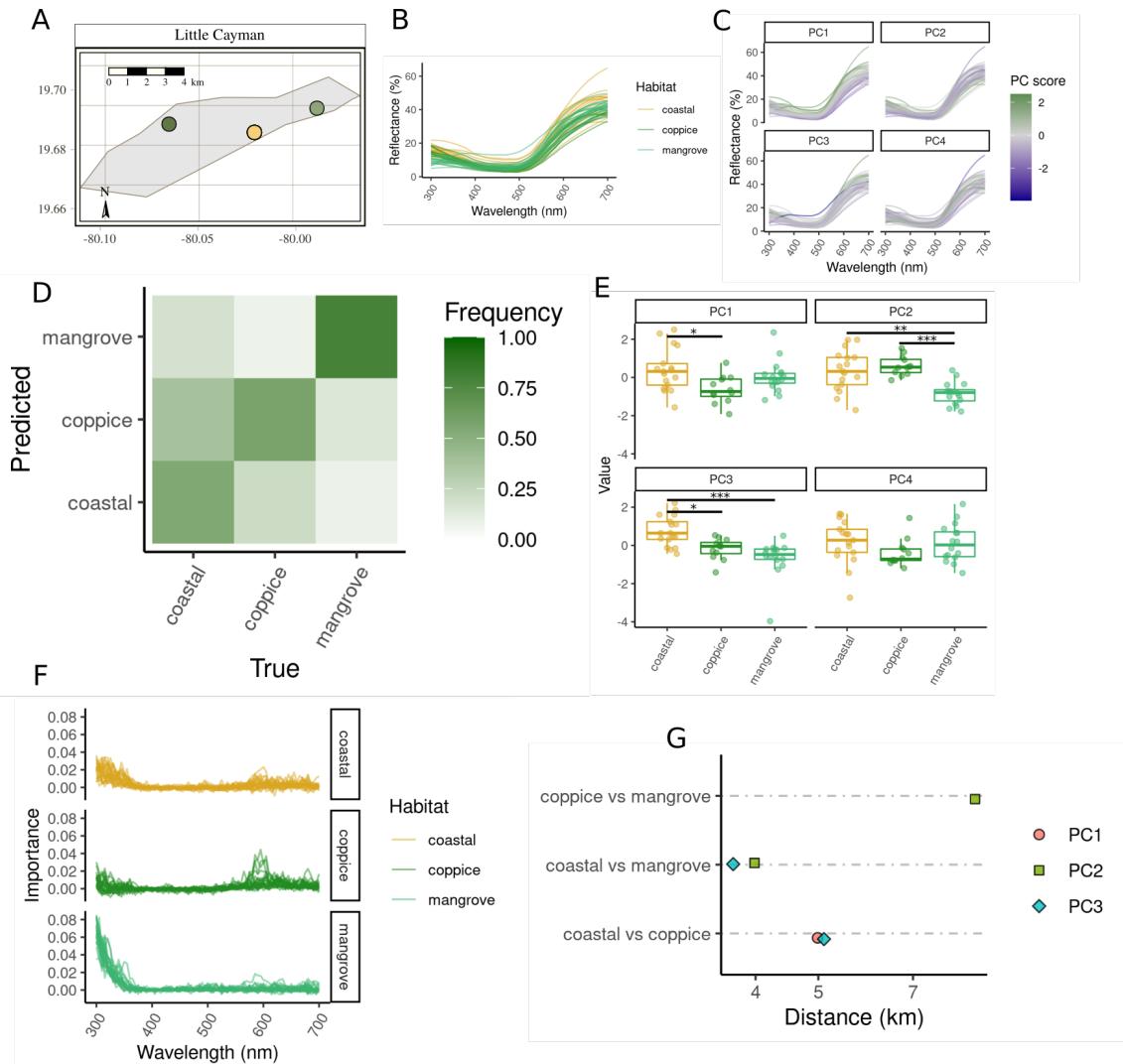


Figure S6: Comparison of dewlap coloration across habitats on Little Cayman. Legend is as per Figure S2.

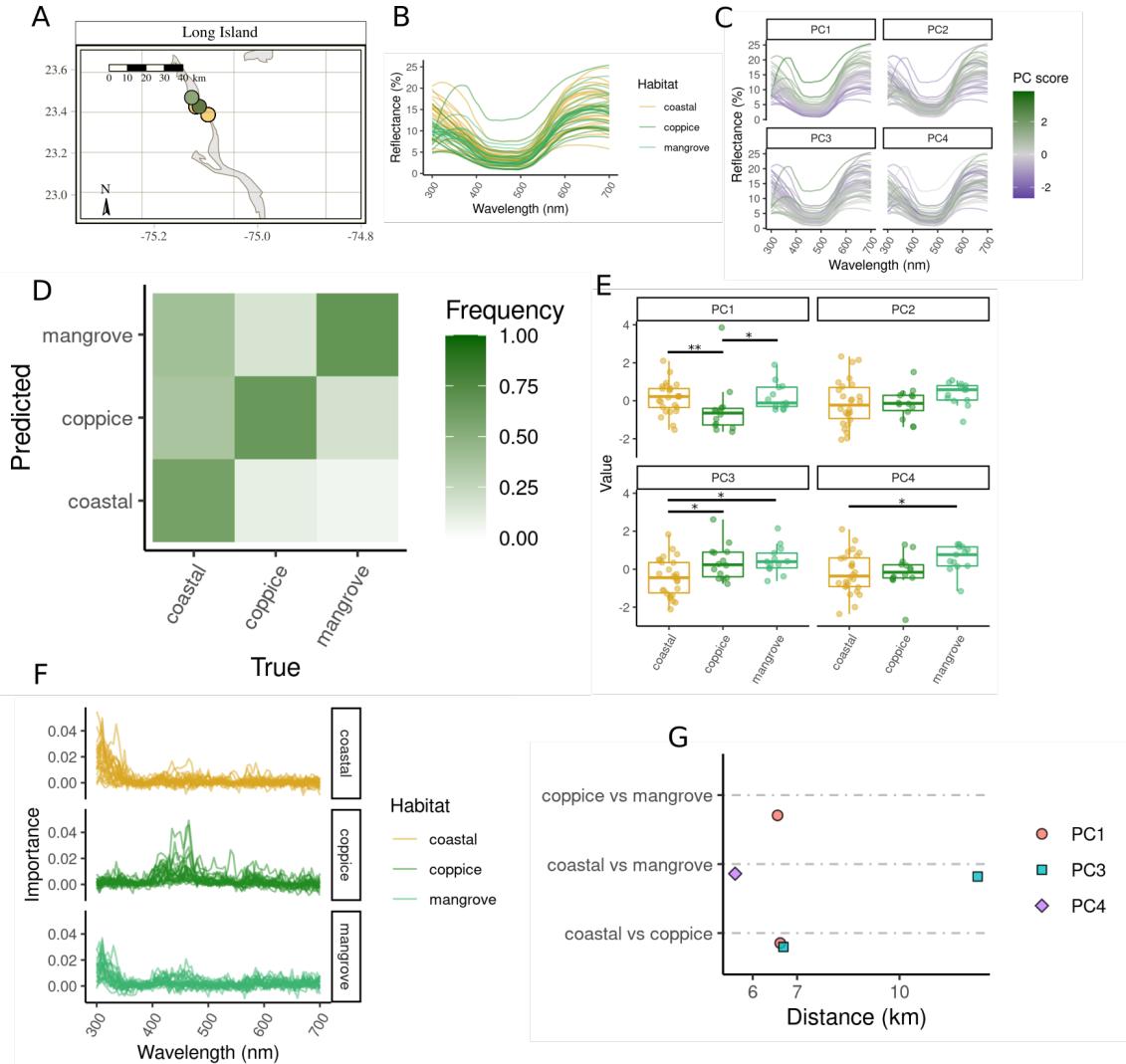


Figure S7: Comparison of dewlap coloration across habitats on Long Island. Legend is as per Figure S2.

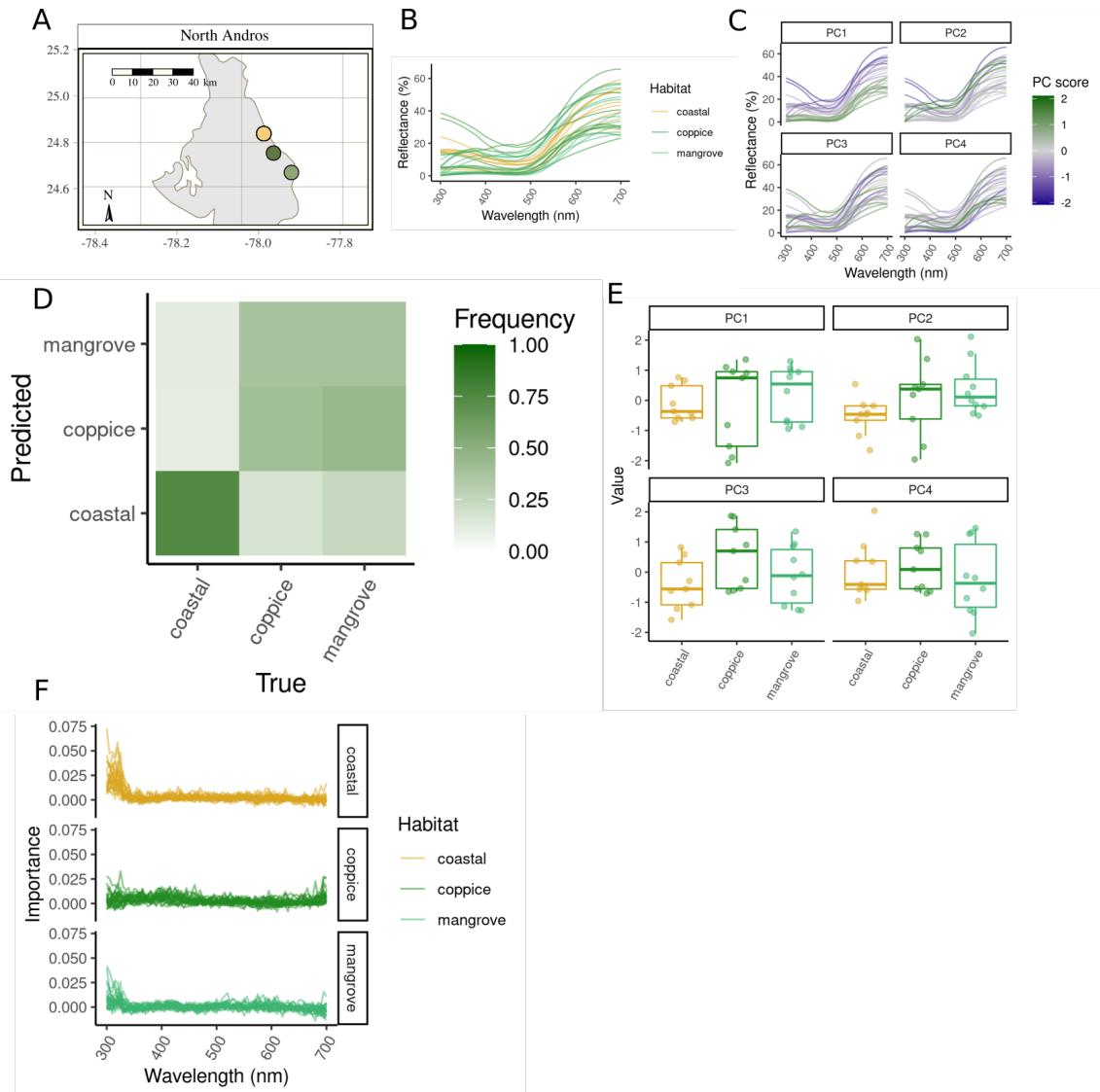


Figure S8: Comparison of dewlap coloration across habitats on North Andros. Legend is as per Figure S2, but without panel G.

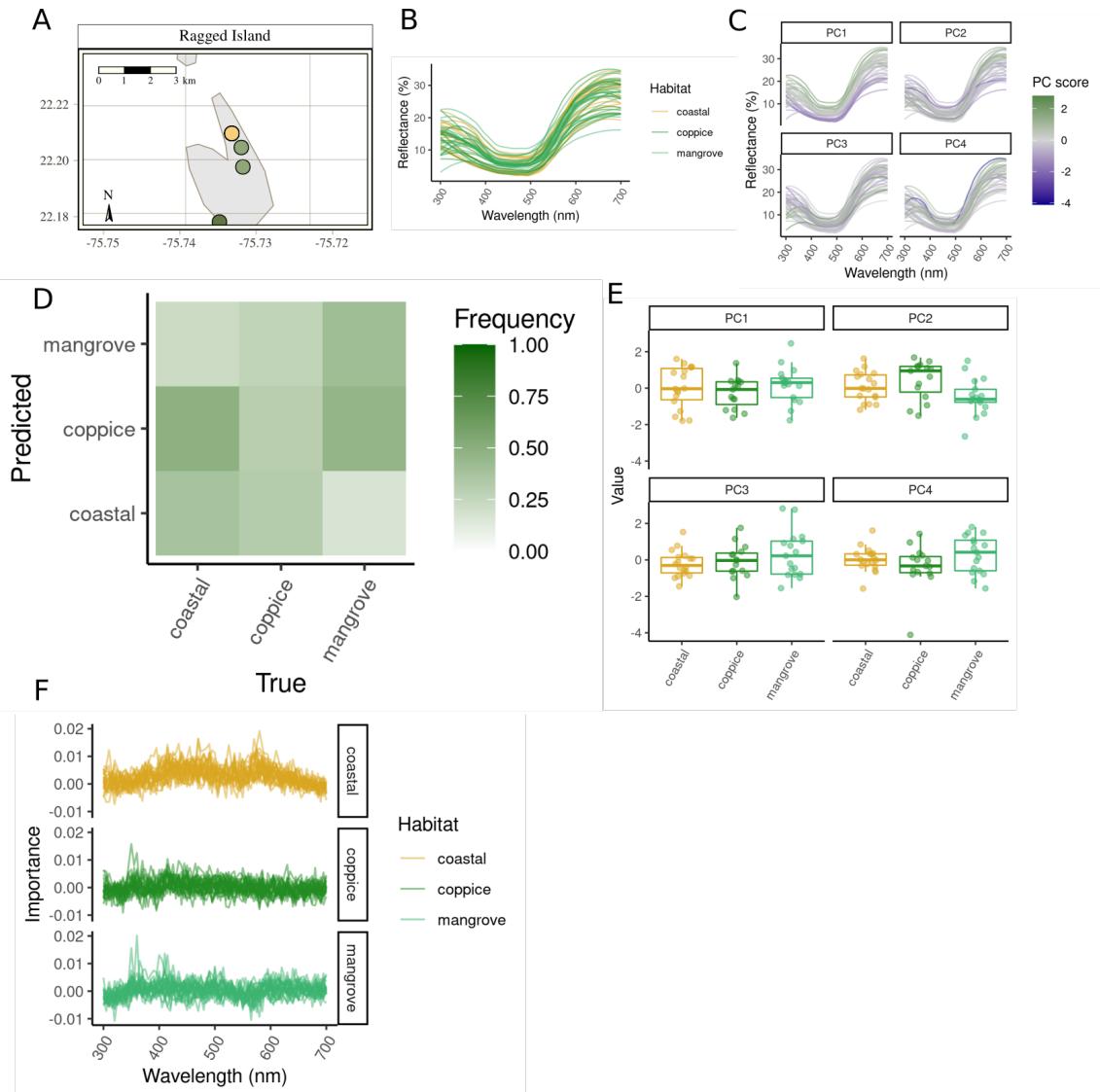


Figure S9: Comparison of dewlap coloration across habitats on Ragged Island. Legend is as per Figure S2, but without panel G.

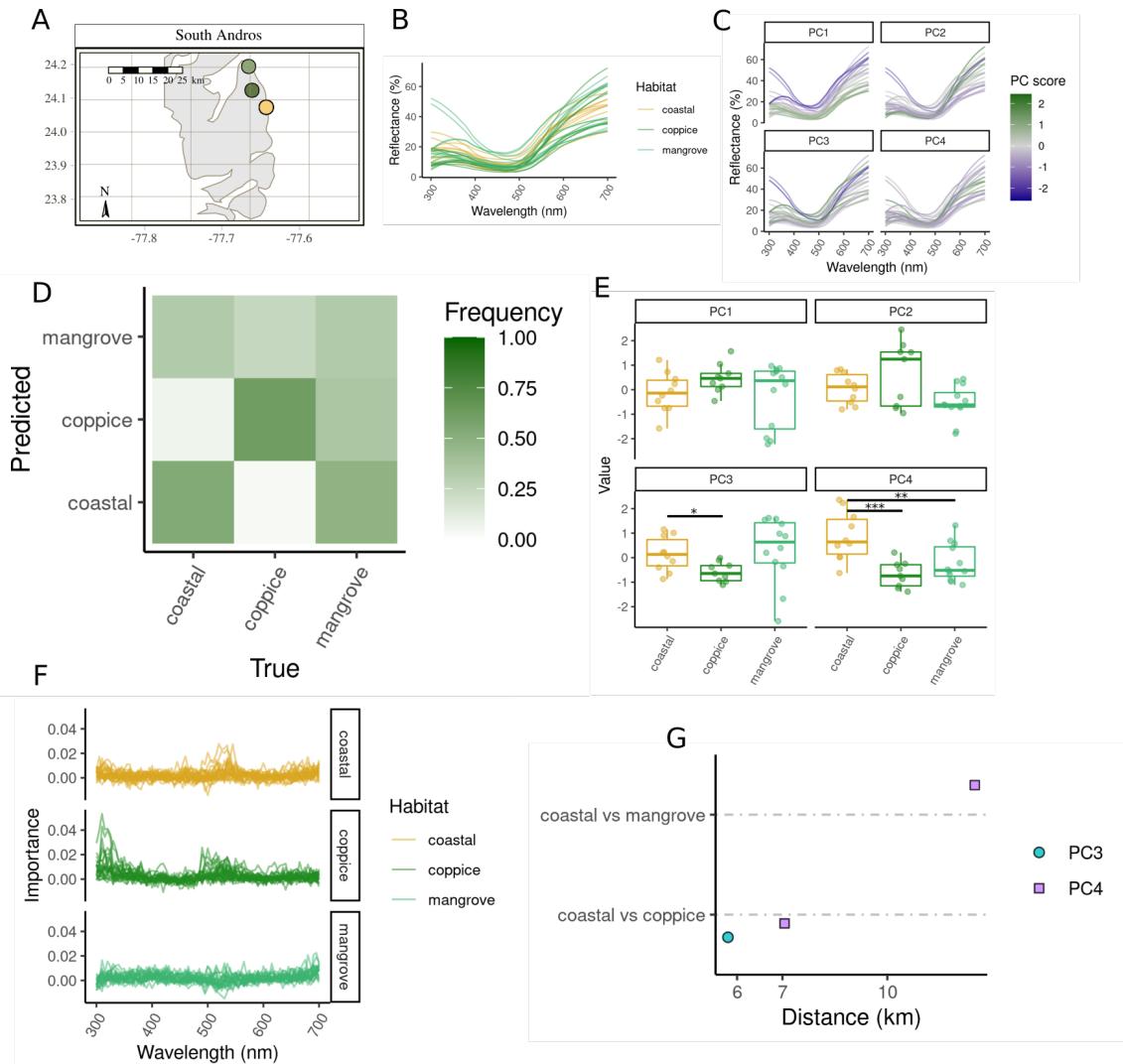


Figure S10: Comparison of dewlap coloration across habitats on South Andros. Legend is as per Figure S2.

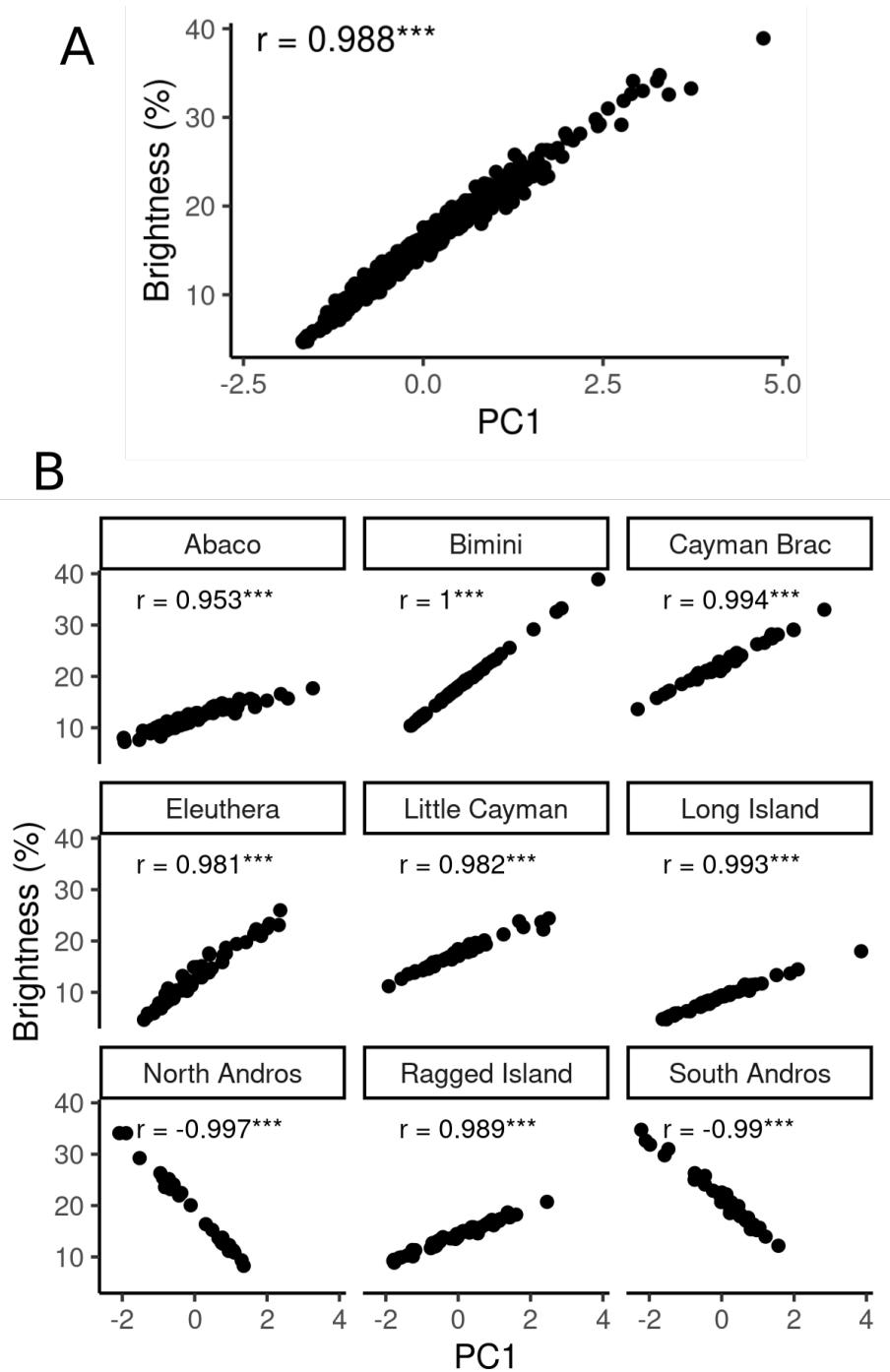


Figure S11: PC1 captures brightness across all islands. (A) Correlation between dewlap brightness (as measured by the mean reflectance from 300 to 700nm in wavelength) and PC1 score across all islands. (B) Correlation between brightness and within-island PC1, for each island. Pearson's correlation coefficients are reported. \*\*\*,  $P < 0.001$ .

## Supplementary Tables

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Table S1: Number of lizards sampled in each habitat on each island.

	coastal	coppice	mangrove
Abaco	41	24	21
Bimini	38	14	15
Cayman Brac	15	18	17
Eleuthera	22	25	9
Little Cayman	17	12	16
Long Island	26	14	13
North Andros	9	9	10
Ragged Island	18	15	17
South Andros	10	9	12

Table S2: Proportion of variance explained by the first four principal components on each island, as well as in the PCA performed on all islands together (last row).

Island	Total	PC1	PC2	PC3	PC4
Abaco	0.906	0.400	0.279	0.147	0.079
Bimini	0.921	0.502	0.208	0.160	0.051
Cayman Brac	0.888	0.438	0.190	0.155	0.105
Eleuthera	0.926	0.490	0.233	0.138	0.066
Little Cayman	0.907	0.441	0.212	0.176	0.078
Long Island	0.925	0.515	0.205	0.161	0.043
North Andros	0.937	0.560	0.170	0.152	0.054
Ragged Island	0.907	0.483	0.226	0.127	0.072
South Andros	0.948	0.488	0.247	0.146	0.067
All islands	0.913	0.473	0.197	0.164	0.079

Table S3: Henze-Zirkler's test of multivariate normality, performed on global principal components (i.e. fitted on data from all islands together) in each habitat and on each island. The number of outlier points detected based on the Mahalanobis distance is reported.  $HZ$ , test statistic. \*,  $P < 0.05$ ; \*\*,  $P < 0.01$ ; \*\*\*,  $P < 0.001$ .

Island	Habitat	Outliers	$HZ$	$P$
Abaco	coastal	0	1.099	0.0027 **
Abaco	coppice	0	1.074	0.0022 **
Abaco	mangrove	0	1.063	0.0023 **
Bimini	coastal	0	1.281	< 0.0001 ***
Bimini	coppice	0	0.850	0.0482 *
Bimini	mangrove	0	1.191	0.0001 ***
Cayman Brac	coastal	0	0.647	0.5311
Cayman Brac	coppice	0	0.701	0.3940
Cayman Brac	mangrove	0	0.657	0.5357
Eleuthera	coastal	0	1.614	< 0.0001 ***
Eleuthera	coppice	0	1.481	< 0.0001 ***
Eleuthera	mangrove	0	0.729	0.1423
Little Cayman	coastal	0	0.624	0.6599
Little Cayman	coppice	0	0.638	0.4867
Little Cayman	mangrove	0	0.873	0.0413 *
Long Island	coastal	0	0.824	0.1468
Long Island	coppice	0	0.923	0.0150 *
Long Island	mangrove	0	0.773	0.1289
North Andros	coastal	0	0.658	0.3174
North Andros	coppice	0	0.763	0.0900
North Andros	mangrove	0	0.668	0.3185
Ragged Island	coastal	0	0.756	0.2268
Ragged Island	coppice	0	0.797	0.1115
Ragged Island	mangrove	0	0.542	0.9022
South Andros	coastal	0	0.660	0.3451
South Andros	coppice	0	0.659	0.3154
South Andros	mangrove	0	0.911	0.0144 *

Table S4: Shapiro-Wilk's test of univariate normality of the standardized residuals from OLS and GLS-ANOVAs performed on each island where significant differences were detected by random forest classification.  $W$ , test statistic. \*,  $P_{adj} < 0.05$ ; \*\*,  $P_{adj} < 0.01$ ; \*\*\*,  $P_{adj} < 0.001$ .

Island	Variable	$W$	$P$	$P_{adj}$	
Abaco	PC1	0.961	0.0109	0.0497	*
Abaco	PC2	0.960	0.0089	0.0473	*
Abaco	PC3	0.988	0.5938	0.7308	
Abaco	PC4	0.982	0.2709	0.4816	
Bimini	PC1	0.890	0.0000	0.0008	***
Bimini	PC2	0.984	0.5179	0.6906	
Bimini	PC3	0.959	0.0281	0.0997	
Bimini	PC4	0.980	0.3386	0.5418	
Cayman Brac	PC1	0.986	0.8236	0.8785	
Cayman Brac	PC2	0.989	0.9299	0.9299	
Cayman Brac	PC3	0.934	0.0079	0.0473	*
Cayman Brac	PC4	0.981	0.5926	0.7308	
Eleuthera	PC1	0.930	0.0031	0.0330	*
Eleuthera	PC2	0.975	0.3090	0.5204	
Eleuthera	PC3	0.981	0.4989	0.6906	
Eleuthera	PC4	0.970	0.1788	0.4082	
Little Cayman	PC1	0.955	0.0821	0.2539	
Little Cayman	PC2	0.982	0.6854	0.7833	
Little Cayman	PC3	0.891	0.0005	0.0081	**
Little Cayman	PC4	0.977	0.4858	0.6906	
Long Island	PC1	0.937	0.0074	0.0473	*
Long Island	PC2	0.989	0.9039	0.9299	
Long Island	PC3	0.971	0.2170	0.4341	
Long Island	PC4	0.983	0.6261	0.7420	
North Andros	PC1	0.937	0.0952	0.2539	
North Andros	PC2	0.978	0.8075	0.8785	
North Andros	PC3	0.905	0.0147	0.0587	
North Andros	PC4	0.949	0.1913	0.4082	
South Andros	PC1	0.941	0.0879	0.2539	
South Andros	PC2	0.946	0.1199	0.2952	
South Andros	PC3	0.965	0.3950	0.6019	
South Andros	PC4	0.957	0.2480	0.4668	

Table S5: Locations of the sampling sites across islands, with mean within-island principal component scores per site.

Island	Longitude	Latitude	Habitat	PC1	PC2	PC3	PC4
Abaco	-77.7	26.9	mangrove	1.006	0.117	-0.057	-1.259
Abaco	-77.6	26.9	coastal	0.155	0.532	0.079	-2.046
Abaco	-77.6	26.9	coppice	-0.084	0.033	0.636	-0.652
Abaco	-77.2	26.1	coastal	0.304	-0.093	-0.987	-0.133
Abaco	-77.0	26.3	mangrove	-0.316	-1.224	0.899	0.185
Abaco	-77.0	26.3	coppice	0.092	0.314	0.124	0.483
Abaco	-77.0	26.3	coastal	-0.337	0.750	-0.207	0.540
Bimini	-79.3	25.6	coastal	-0.263	-1.270	-0.185	0.164
Bimini	-79.3	25.7	coastal	0.468	0.083	-0.242	0.011
Bimini	-79.3	25.7	coppice	-0.270	0.171	0.926	-0.124
Bimini	-79.3	25.7	mangrove	-0.447	0.533	-0.288	-0.014
Cayman Brac	-79.9	19.7	coastal	0.483	-0.523	-0.781	0.443
Cayman Brac	-79.8	19.7	mangrove	0.219	0.453	0.810	-0.015
Cayman Brac	-79.8	19.7	coppice	-0.610	0.008	-0.114	-0.355
Eleuthera	-76.3	24.8	coppice	-0.123	-0.857	0.045	0.117
Eleuthera	-76.3	24.8	coastal	-0.520	-0.642	-0.492	-0.538
Eleuthera	-76.3	24.8	mangrove	-0.163	-0.651	0.126	0.685
Eleuthera	-76.2	24.9	coppice	0.391	0.602	0.540	-0.539
Eleuthera	-76.1	24.9	coastal	0.254	1.228	-0.315	0.402
Little Cayman	-80.1	19.7	coppice	-0.621	0.614	-0.179	-0.404
Little Cayman	-80.0	19.7	coastal	0.395	0.355	0.743	0.175
Little Cayman	-80.0	19.7	mangrove	0.047	-0.838	-0.655	0.118
Long Island	-75.2	23.5	mangrove	0.207	0.366	0.484	0.587
Long Island	-75.2	23.4	coastal	-0.097	-0.798	-0.140	-0.347
Long Island	-75.2	23.4	coppice	-0.485	-0.133	0.366	-0.148
Long Island	-75.1	23.4	coastal	0.316	0.317	-0.626	-0.130
North Andros	-77.9	24.8	coastal	-0.098	-0.516	-0.403	0.066
North Andros	-77.8	24.8	coppice	-0.139	0.090	0.519	0.192
North Andros	-77.8	24.7	mangrove	0.213	0.384	-0.104	-0.232
Ragged Island	-75.7	22.2	coppice	-0.269	0.440	-0.088	-0.360
Ragged Island	-75.7	22.2	coastal	0.049	0.076	-0.221	0.029
Ragged Island	-75.7	22.2	mangrove	0.246	-0.273	0.118	0.933
Ragged Island	-75.7	22.2	mangrove	0.099	-0.749	0.588	-0.636
South Andros	-77.6	24.2	mangrove	-0.250	-0.539	0.316	-0.211
South Andros	-77.6	24.1	coppice	0.466	0.657	-0.586	-0.687
South Andros	-77.5	24.1	coastal	-0.120	0.055	0.149	0.872

Table S6: Nonparametric Kruskal-Wallis tests performed on each variable on each island where deviations from normality were detected. \*,  $P < 0.05$ ; \*\*,  $P < 0.01$ ; \*\*\*,  $P < 0.001$ .

Island	Variable	$\chi^2$	df	$P$
Abaco	PC1	0.74	2	0.6924
Abaco	PC2	23.13	2	< 0.0001 ***
Bimini	PC1	7.38	2	0.0250 *
Cayman Brac	PC3	22.46	2	< 0.0001 ***
Eleuthera	PC1	0.29	2	0.8666
Little Cayman	PC3	19.95	2	< 0.0001 ***
Long Island	PC1	10.98	2	0.0041 **

Table S7: Support vector machine classification results. Legend is as per Table 1.

Island	<i>N</i>	Score	<i>P</i>	
Abaco	86	0.581	< 0.0001	***
Bimini	67	0.555	< 0.0001	***
Cayman Brac	50	0.708	< 0.0001	***
Eleuthera	56	0.443	0.0513	
Little Cayman	45	0.711	< 0.0001	***
Long Island	53	0.664	< 0.0001	***
North Andros	28	0.429	0.1039	
Ragged Island	50	0.424	0.0756	
South Andros	31	0.574	0.0040	**

Table S8: Linear discriminant analysis classification results. Legend is as per Table 1.

Island	<i>N</i>	Score	<i>P</i>	
Abaco	86	0.644	< 0.0001	***
Bimini	67	0.585	< 0.0001	***
Cayman Brac	50	0.780	< 0.0001	***
Eleuthera	56	0.425	0.0871	
Little Cayman	45	0.738	< 0.0001	***
Long Island	53	0.574	0.0001	***
North Andros	28	0.414	0.1911	
Ragged Island	50	0.400	0.1259	
South Andros	31	0.587	0.0013	**