

# 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, as this may give new insights into the local scale at which adaptation is possible. 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. 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 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 eco-morphological 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.,

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36 This selective pressure may drive the local adaptation of communication signals.

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38 One potential barrier to the maintenance of localized signal divergence is the homogenizing effect  
39 of gene flow. Population genetics theory suggests that gene flow may counteract local adaptation  
40 between localities and prevent divergence altogether, especially at small spatial scales, because  
41 of the inflow of maladapted alleles or because of the breaking of linkage between coevolving loci  
42 (Felsenstein, 1976; García-Ramos and Kirkpatrick, 1997; Dieckmann and Doebeli, 1999; Lenormand,  
43 2002; Hendry et al., 2007a). This genetic homogenization has been confirmed empirically  
44 in systems such as stick-insects (Nosil and Crespi, 2004) and stickleback (Hendry et al., 2007b).  
45 Yet, examples of microgeographic adaptation, i.e. adaptation at smaller scales than the range of  
46 dispersal, exist, highlighting the potential of some organisms to respond to selection in the face of  
47 gene flow (see Richardson et al. 2014 and references therein). Examples include small scale adap-  
48 tation in fragmented areas in Australian fruit flies (Willi and Hoffmann, 2012), or local adaptation  
49 to predation pressure in North American salamanders (Richardson and Urban, 2013). Therefore,  
50 despite evidence that local adaptation may be particularly difficult at small spatial scales where  
51 gene flow tends to cause adjoining populations to remain genetically homogeneous, the potential  
52 adaptive response of species traits, in particular communication signals, to localized differences in  
53 habitats remains relatively unknown (Richardson et al., 2014).

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55 Lizards of the neotropical genus *Anolis* are an excellent group for studying the eco-evolutionary  
56 dynamics of local adaptation and natural selection (Losos, 2009). A particularly conspicuous trait  
57 of anoles is their dewlap; an extensible flap of skin that is typically sexually dimorphic and used as  
58 a communication signal in courtship (Sigmund, 1983; Driessens et al., 2014, 2015) and territorial  
59 displays (Losos, 1985; Macedonia and Stamps, 1994; Macedonia et al., 2013) as well as in predator  
60 deterrence (Leal and Rodríguez-Robles, 1995, 1997; Leal and Rodriguez-Robles, 1997). Dewlap  
61 characteristics vary widely among the approximately 400 species of the genus (Nicholson et al.,  
62 2007). Interspecific variation in dewlap coloration is implicated in species recognition (Rand and  
63 Williams, 1970; Williams, 1969; Williams and Rand, 1977; Losos, 1985; Macedonia and Stamps,  
64 1994; Fleishman, 2000; Macedonia et al., 2013), and this function could have had a role in initiating  
65 and/or reinforcing reproductive isolation during speciation (Lambert et al., 2013; Geneva et al.,  
66 2015; Ng et al., 2017).

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68 Within species, studies have shown a link between variation in dewlap coloration and differ-  
69 ences in habitats or climatic conditions (Macedonia, 2001; Leal and Fleishman, 2002; Thorpe and  
70 Stenson, 2002; Thorpe, 2002; Leal and Fleishman, 2004; Vanhooydonck et al., 2009; Ng et al., 2012,  
71 2013, 2016; Vanhooydonck et al., 2009; Driessens et al., 2017). Some studies suggest that those  
72 differences may be adaptive, and that dewlaps may have evolved to maximize detectability given  
73 local light conditions (Fleishman and Persons, 2001; Leal and Fleishman, 2002, 2004). Although  
74 this claim is further supported by recent findings that dewlap colors are perceived differently un-  
75 der different levels of shading (Fleishman et al., 2020), other studies found conflicting patterns  
76 of between-habitat variation that did not support the sensory drive hypothesis (Fleishman et al.,  
77 2009; Ng et al., 2012; Macedonia et al., 2014).

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79 Previous studies investigating variation in anole dewlaps compared populations at relatively  
80 large geographical scales, e.g. between islands (Vanhooydonck et al., 2009; Driessens et al., 2017)  
81 or within large islands such as Puerto Rico (Leal and Fleishman, 2004) or Hispaniola (Ng et al.,  
82 2012, 2016). These large scales and marine barriers should reduce gene flow (Ng and Glor, 2011;  
83 Lambert et al., 2013; Richardson et al., 2014; Ng et al., 2017). That said, examples do exist of  
84 divergence in dewlap coloration at smaller scales or between populations with high degrees of gene  
85 flow (Thorpe and Stenson, 2002; Thorpe, 2002; Stapley et al., 2011; Ng et al., 2016).

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87 The species *Anolis sagrei* is widespread across islands of the West Indies (Reynolds et al., 2020).  
88 It has been the subject of numerous studies concerning local adaptation (Losos et al., 1994, 1997,  
89 2001; Kolbe et al., 2012), biological invasion (Kolbe et al., 2008), sexual selection (Tokarz, 2002;  
90 Tokarz et al., 2005; Tokarz, 2006; Driessens et al., 2014; Steffen and Guyer, 2014; Driessens et al.,  
91 2015) among many other topics. Between-island variation in the mainly orange-red color of its  
92 dewlap was shown to be better explained by climatic variables (Driessens et al., 2017) than by  
93 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 promiscuous 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 across multiple islands.

Our sampling design included sites in close proximity; the median distance between two sites within an island was 11.2km. Combining reflectance spectrometry and supervised machine learning, we tested for divergence in dewlap phenotype between habitats within islands and between islands across the range of *A. sagrei*. We predicted that if light conditions in the environment indeed drive color evolution, dewlaps should be most similar between beach scrub and mangrove forest, which both have high levels of light irradiance, compared to the darker, closed-canopy coppice forest. Similar, if detectability is maximized given the local conditions, we expected darker and more contrasting dewlaps in high irradiance habitats. Finally, if habitat characteristics are strong determinants of dewlap color variation, similar patterns should be observed across multiple islands (Losos, 2011).

120 **Methods**

121 **Data collection**

122 We sampled 466 male *Anolis sagrei* from seven islands in the Bahamas Archipelago – Abaco,  
123 North Andros, South Andros, South Bimini, Eleuthera, Long Island, Ragged Island – and two  
124 in the Cayman Islands – Cayman Brac and Little Cayman (Figure S1A). These islands were  
125 chosen to span the breadth of the West Indian range of *A. sagrei*, because they have highly similar  
126 habitat 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). Sample sizes are given in Table S1. Our sampling  
133 design enabled us to test for differences between habitats at a coarse and fine geographical scale.  
134 The median distance between two localities within an island was ~ 11km, with some islands being  
135 sampled at smaller or larger scales (Figure S1B), and 80.3% of all pairwise distances within islands  
136 were less than 50km. Additionally, there are no major barriers to dispersal (such as mountains or  
137 grassland) on any of the islands that we sampled.

138 **Reflectance measurements**

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

148 **Analysis**

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

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168 All analyses in this study were performed in R 3.6.1 (R Core Team, 2019) and the source code  
169 can be found at <https://github.com/rscherrer/dewlap>, presently private.

## Dimensionality reduction

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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.

## Among-island variance partitioning

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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).

## Within-island machine learning

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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.

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.

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.

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 5 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

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

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

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

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

## Univariate analyses

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

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

To know which habitat-populations were different from which in dewlap coloration, we performed different post-hoc multiple comparison tests (all implemented in the PMCMRplus package, Pohlert, 2020), depending on which assumptions were met. In cases where normality and homoskedasticity were met (i.e. OLS-ANOVA was the best fit), we used Tukey's honest significant difference test. When normality was met but not homoskedasticity (i.e. GLS-ANOVA was the 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.

## 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. Because often only a few sites were sampled per island, we could not get meaningful results from tests that use sites as units of observation, such as Moran's I test (Gittleman and Kot, 1990). Instead, we designed a permutation test where we randomly reshuffled individual lizards across sites within islands 1,000 times each, and systematically recalculated Pearson's correlation coefficient between geographic distances (computed as geodesic distances in the R package geosphere; Hijmans 2019) and phenotypic distances. We used the resulting null distributions of correlation coefficients to assess the significance of the observed spatial autocorrelation for each island.

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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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## 295 Results

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

305

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

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319 The accuracy of Random forest classification exceeded random expectation more often than  
320 expected by chance for all islands. Accuracy was as high as 73

321 On Abaco, dewlaps from the mangrove habitat were the best discriminated, while dewlaps  
322 from the beach scrub habitat were often mistaken for dewlaps from the coppice habitat (Fig. 2A).  
323 Importance analysis revealed that beach scrub and mangrove lizards mostly differed in reflectance  
324 in the UV-end of the spectrum (below 400nm, Fig. 2B), where mangrove dewlaps had higher UV  
325 reflectance relative to beach scrub lizards, and coppice lizards had an intermediate UV reflectance  
326 between the two other habitats (Fig. 2C). Consistent with this, our analyses of variance detected  
327 significantly lower PC2 scores in mangrove lizards than in the two other habitats, representing a  
328 higher UV-reflectance relative to red (Fig. 2D, E, Table 3). Beach scrub lizards also scored lower  
329 on PC3, indicating less curvature of the reflectance profile and relatively higher reflectance at in-  
330 termediate wavelengths (blue-to-yellow) than at the ends of the range (Fig. 2D, E). Differences  
331 were detected between sites both at large ( $\sim 100\text{km}$ ) and short ( $< 1\text{km}$ ) distances (Fig. 2F, G).

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333 On Bimini, the random forests mostly correctly classified lizards from the coppice and mangrove  
334 habitats while often misclassifying lizards from the beach scrub habitat (Fig. S3A). Relatively flat  
335 importance profiles for beach scrub lizards suggested that brightness was used instead of a partic-  
336 ular wavelength to identify some of the beach scrub dewlaps (Fig. S3B). Indeed, some beach scrub  
337 dewlaps were substantially brighter than the rest (Fig. S3C), a pattern that was captured by our  
338 analysis of variance along PC1 (i.e. brightness, Fig. S3D, E, Table 3). The random forests also  
339 used UV reflectance to discriminate between coppice and mangrove dewlaps (Fig. S3B), which  
340 could reflect the significant difference we detected along PC3 between these two habitats (Fig.  
341 S3D, Table 3). Beach scrub lizards were characterized by elevated red reflectance relative to UV  
342 (as represented by PC2, (Fig. S3D, E)), and beach scrub and mangrove lizards were characterized  
343 by a more even distribution of the reflectance along the spectrum (as represented by PC3, (Fig.  
344 S3D, E)), in contrast to coppice lizards which harbored a stronger curvature at intermediate wave-  
345 lengths (Fig. S3D, E). On this island, the beach scrub and coppice habitats were separated by a  
346 few hundred meters, making this contrast the smallest geographical scale at which differences in  
347 coloration were found in our study (Fig. S3F, G).

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349 On Cayman Brac, all three habitats could be well discriminated against each other (Fig. S4A),  
350 with UV reflectance appearing to be an important variable differentiating beach scrub and man-  
351 grove dewlaps (Fig. S4B). In contrast, coppice dewlaps had a relatively flat importance profile,

suggesting that brightness made them more distinct rather than any particular wavelength (Fig. S4B). Consistent with this, coppice dewlaps were significantly different from all other dewlaps along PC1 (Fig. S4D, Table 3). At a distance between 2 and 3km (Fig. S4F, G), dewlaps in the beach scrub habitat reflected more red light (as represented by PC2, Fig. S4D, E) and more UV (as represented by PC3) than in the mangrove habitat. Coppice lizards were also characterized by a higher UV reflectance than mangrove dewlaps (PC3, Fig. S4D, E, Table 3).

Eleuthera 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. 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. S5D, 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. S6A). Mangrove dewlaps were most distinct with respect to their reflectance in short wavelengths (Fig. S6B), with significantly lower UV reflectance (as represented by PC2, Fig. S6D, 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. S6D, E, Table 3).

On Long Island the three habitats were relatively well discriminated (Fig. S7A). Importance profiles indicated that short wavelengths were used to discriminate between beach scrub and mangrove lizards (Fig. S7B). Beach scrub lizards had more curved reflectance profiles than in either of the two other habitats, with higher levels of UV and red reflectance relative to intermediate wavelengths (PC3, Fig. S7D, E, Table 3). Beach scrub lizards also differed from mangrove lizards along PC4 (Fig. S7D), which represented a rather small portion of the variance not already explained by the first three principal components, and is therefore difficult to interpret (Fig. S7E). Coppice lizards were significantly darker than mangrove and beach scrub lizards (PC1, Fig. S7D, E, Table 3).

On North Andros, although the random forest classification was significant ( $P = 0.0216$ , Table 1) and the average confusion matrix indicated that lizards from beach scrub were particularly well predicted (Fig. S8A), no significant univariate differences were detected along any of the four PCs (Fig. S8D, Table 3). Importance analysis of full-spectrum random forests showed higher importance scores near the UV-end of the spectrum in discriminating beach scrub dewlaps from the rest (Fig. S8B). Besides, reflectance curves of beach scrub dewlaps appeared more similar to each other in the UV range than dewlaps from other habitats (Fig. S8C), suggesting that the machines may have used this low within-habitat variance, as opposed to between-habitat differences in means, to correctly classify beach scrub lizards. A small sample size on this island may also have contributed to a lack of power in detecting univariate differences using analyses of variance (Table S1).

On South Andros beach scrub and coppice dewlaps could be discriminated better against each other than with mangrove dewlaps (Fig. S10A), with importance profiles supporting UV-reflectance as a predictor of coppice lizards (Fig. S10B). Coppice lizards had more curved reflectance profiles than beach scrub lizards (PC3), and lizards from both habitats differed along PC4, which is again more difficult to interpret (Fig. S10D, E, Table 3). Beach scrub lizards also differed from mangrove lizards in PC4 (Fig. S10D, E, Table 3).

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

## 405 Discussion

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

412

413 Detectable differences in dewlap color between habitat-populations are surprising, as habitats  
414 were often in close geographical proximity to each other (as close as a few hundred meters on  
415 Bimini and most of the time within ten kilometers). Indeed, given that (1) the populations were  
416 continuously distributed between the habitats, (2) different habitat-populations were not mono-  
417 phyletic with respect to mitochondrial haplotypes ([van de Schoot 2016 unpubl.](#)), and (3) *A. sagrei*  
418 have been shown to be a highly mobile species ([Kamath and Losos, 2018](#)), we would have expected  
419 more homogeneous distributions of color phenotypes within islands due to extensive gene flow,  
420 with fewer differences between populations, especially those in close proximity.

421

422 Several scenarios could account for these findings. One explanation is an adaptive one. Indeed,  
423 populations living in different habitats could be phenotypically adapted to their local environmen-  
424 tal conditions. Given that the brightly colored dewlap of *A. sagrei* is used as a communication  
425 signal, its color may be a target for selection if the transmission or perception of the signal differs  
426 from one habitat to another, for example because of differences in ambient light, according to the  
427 sensory-drive hypothesis ([Endler and McLellan, 1988; Endler, 1992, 1998](#)). The sensory-drive hy-  
428 pothesis has been tested multiple times for dewlap coloration in *Anolis* lizards, with mixed results.  
429 Some authors found support for it ([Leal and Fleishman, 2002, 2004](#)), while others did find differ-  
430 ences in dewlap coloration between habitats, but those were inconsistent with the sensory-drive  
431 hypothesis ([Fleishman et al., 2009; Ng et al., 2012](#)).

432

433 If our results were an example of sensory drive, we would have expected to see consistent dif-  
434 ferences between habitat-populations across islands (a pattern that would have been a compelling  
435 indicator of adaptation at all, [Losos 2011](#)). This is because environmental conditions that may be  
436 relevant to color signal detectability such as light, temperature, moisture and vegetation, are con-  
437 sistent within the three main and clearly distinct habitat-types found across the sampled islands,  
438 i.e. beach scrub, primary coppice and mangroves ([Howard, 1950; Schoener, 1968](#)). Moreover, the  
439 patterns of divergence expected under a sensory drive scenario should be consistent with increased  
440 detectability given the local light conditions, such as the high contrasts with background vegetation  
441 found in the UV-range by [Leal and Fleishman \(2002\)](#) and [Leal and Fleishman \(2004\)](#).

442

443 Instead, we found differences in the way dewlap color differs between habitats across islands.  
444 While short-wavelengths (UV reflectance) were often involved in color differences, they were not  
445 involved on all islands where significant differences were detected. On some islands, other or ad-  
446 ditional variables differed, such as brightness, red reflectance or the reflectance at the ends of the  
447 spectrum visible to *Anolis* lizards (UV and red, [Lazareva et al. 2012](#)) relative to intermediate  
448 wavelengths (blue-to-yellow). Similar portions of the spectrum were sometimes involved in oppo-  
449 site directions on different islands, such as on Abaco and Cayman Brac, where mangrove lizards  
450 had a higher UV-reflectance than beach scrub lizards on the former, but a lower UV-reflectance  
451 on the latter. Under a sensory-drive scenario, we would have expected the same variables to be  
452 consistently divergent between habitats, or at least in a consistent direction.

453

454 Consistent patterns across islands would have been a good clue for a sensory-drive explana-  
455 tion, but in particular consistent differences between habitats that are most different in their local  
456 conditions regarding the ecological function of the dewlap, such as ambient light. For example, if  
457 ambient light is an important factor shaping dewlap coloration, we would expect mangrove and  
458 beach scrub lizards, both inhabiting areas with high light penetration, to harbor more similar  
459 dewlaps, and to differ significantly from lizards from the coppice habitat, where irradiance is low.  
460 Overall, the observed heterogeneity of divergence patterns across islands provides no support to a  
461 sensory-drive explanation.

Phenotypic plasticity could be another cause for dewlap color variation between habitats, where different conditions would favor different phenotypes in different habitats, without genetic changes. Indeed, the yellow, orange and red colors in anoline dewlaps are produced by pterins and carotenoids (Ortiz, 1962; Ortiz et al., 1962; Ortiz and Williams-Ashman, 1963; Ortiz and Mal-  
donado, 1966; Macedonia et al., 2000; Steffen and McGraw, 2007, 2009). Animals can synthesize pterins from nucleotides, but lack the ability to synthesize carotenoids (Goodwin, 1984; Hill et al., 2002; Hill and McGraw, 2006). Different food qualities across sites within islands could therefore potentially cause detectable differences in coloration. Alternatively, more subtle effects on dewlap color could arise from developmental plasticity and depend, e.g. on differences in egg-rearing conditions. However, more data are needed to test these hypotheses, and although some work has shown plastic responses of dewlap color in response to parasites in *A. sagrei* (Cook et al., 2013), we find it unlikely to account for the widespread habitat differences we found. Besides, 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 another species with a carotenoid-based dewlap, *A. distichus*, found little support for phenotypic and developmental plasticity in dewlap coloration.

Genetic drift is another process that can account for differences in phenotype between localities, especially in small populations. One way this could proceed is through isolation-by-distance, where more distant populations accumulate more differences through time because of the reduced effect of gene flow at larger geographical scales relative to the dispersal range of the species (Rousset, 2004). Here, we only found a significant correlation between phenotypic and geographical distances on Eleuthera to support this scenario. On all the other islands, in contrast, populations from closer sites were not phenotypically more similar, which argues against isolation-by-distance. That said, there were often few sampling sites per island in our study, whose locations were not uniformly chosen within the islands, and so the true extent of isolation-by-distance may be difficult to test. Other, less trivial forms of drift may be at play than isolation-by-distance, but nevertheless, we did find significant differences in color phenotype at relatively small spatial scales, sometimes in neighboring habitats, on islands where gene flow is probably highly pervasive, as suggested by high rates of encounter between males and females (Kamath and Losos, 2018), making the divergence of habitat-populations by drift in relative genetic isolation an unlikely scenario.

A number of alternative explanations remain. First, there could be sexual selection for different dewlap colors in different locations. Indeed, although the sensory-drive hypothesis may include a sexual selection aspect, e.g. if the optimal male phenotype in a given habitat is a function of female perception, sexual preferences may also be arbitrary and independent of the habitat (Andersson, 1994), and so could differ across habitats and islands. Although one previous study has found no link between dewlap coloration and body size dimorphism in *A. sagrei* (a proxy for the intensity of sexual selection) in an among-island comparison (Baekens et al., 2018), within-island data are lacking to test the plausibility of this scenario.

Alternatively, selective pressures may be different in similar habitats from one island to another, because of other environmental variables not accounted for by the habitat-type classification we used (Howard, 1950; Schoener, 1968). The islands we sampled indeed exhibit variation in some climatic variables but also in densities of predators and anole congeners, which have all been shown to correlate with variation in *A. sagrei* mean dewlap coloration among islands (Vanhooijdonck et al., 2009; Baekens et al., 2018).

Finally, different island-populations could also respond differently to similar selective pressures, resulting in various between-habitat divergence patterns across islands. Several factors could account for this. For example, the founder populations of each island, which we know colonized the islands independently (van de Schoot, unpublished thesis; Driessens et al. 2017; Reynolds et al. 2020), could have exhibited different dewlap colors at the time of colonization, as may be suggested by the larger differences we observed between than within islands. In turn, different initial phenotypes could have led to different ways in which populations would have diverged between habitats. Moreover, the different founding populations may have also consisted in different subsets of the standing genetic variation of their Cuban ancestor due to potential bottlenecks (Reynolds et al., 2020), which may have constrained the way they would later respond to the local selective

520 pressures of their respective islands.

521

522 Altogether, our results show that dewlap color of *A. sagrei* commonly varies between habitat-  
523 types, even in close geographical proximity, within islands of the West Indies, and that coloration  
524 differs in different ways from one island to another. We discussed several non-mutually exclusive  
525 mechanisms that could explain these observations, but more data are needed to thoroughly test  
526 each of these. Nevertheless, heterogeneous patterns of divergence across islands do not support an  
527 adaptive sensory-drive scenario, and we propose that within-island dewlap color variation may  
528 be underlain by a more subtle mosaic of factors.

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529

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## 539 Figures

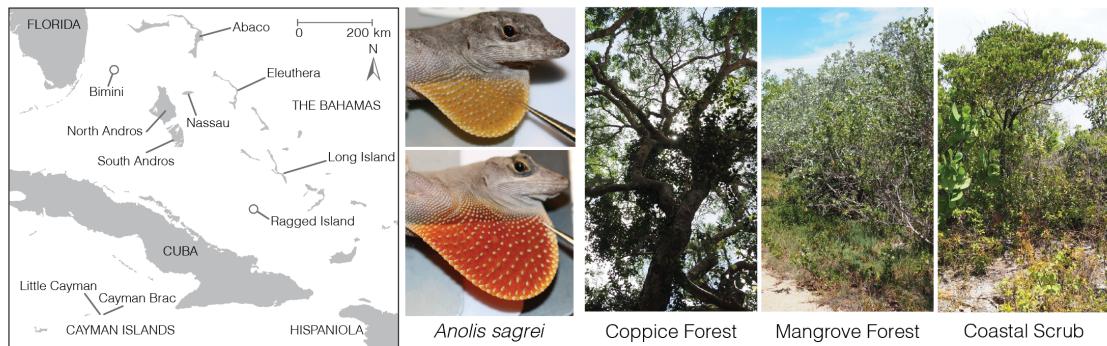


Figure 1: Overview of our study design, including a map of the Bahamas and the Cayman Islands, on which are indicated the nine islands we sampled, two representatives of our study species *Anolis sagrei* with their dewlaps deployed, and the three types of habitats we considered on each island.

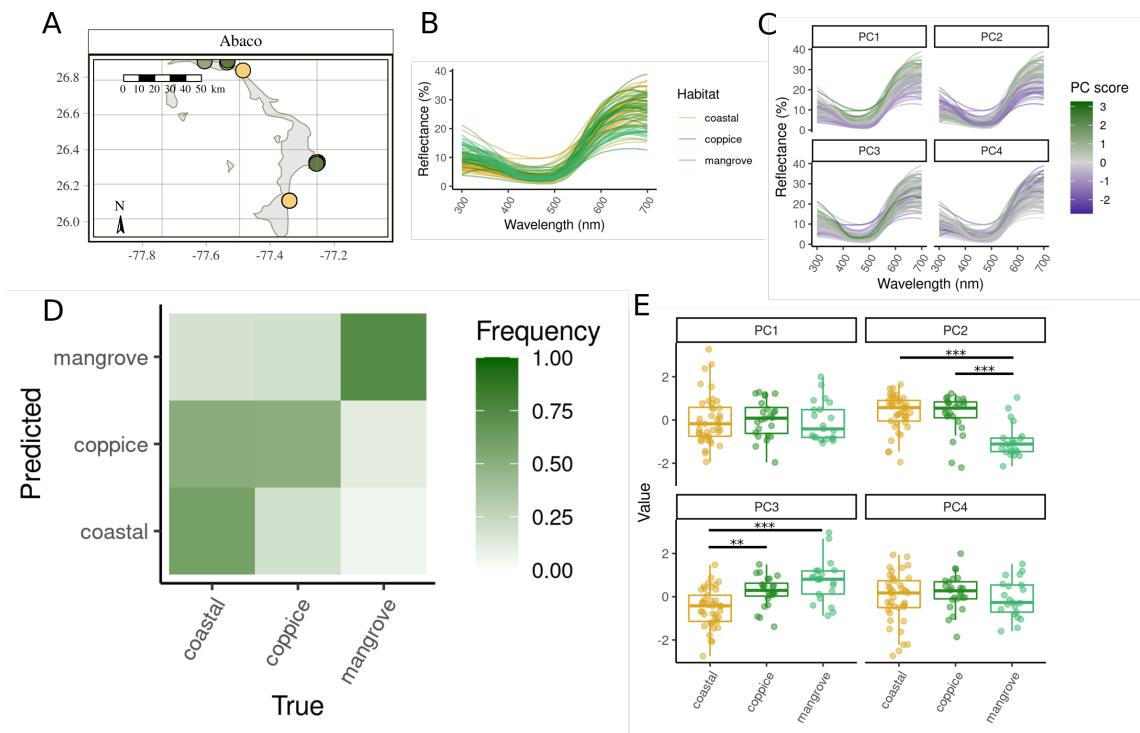


Figure 2: Comparison of dewlap coloration across habitats on Abaco. (A) Map of the island with the sampling sites colored by habitat. (B) Reflectance profiles of all the dewlaps on the island. (C) How reflectance profiles map onto the within-island principal components. (D) Confusion matrix showing the 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

540

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: 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 lizards were randomly permuted among sites (1,000 permutations). \*,  $P < 0.05$ ; \*\*,  $P < 0.01$ , \*\*\*,  $P < 0.001$ .

Island	$\rho$	$P$
Abaco	0.448	0.065
Bimini	0.810	0.137
Cayman Brac	-0.737	0.754
Eleuthera	0.844	0.006 **
Little Cayman	-0.042	0.625
Long Island	0.367	0.183
North Andros	0.051	0.505
Ragged Island	-0.363	0.620
South Andros	-0.979	0.904

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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## Supplementary Figures

792

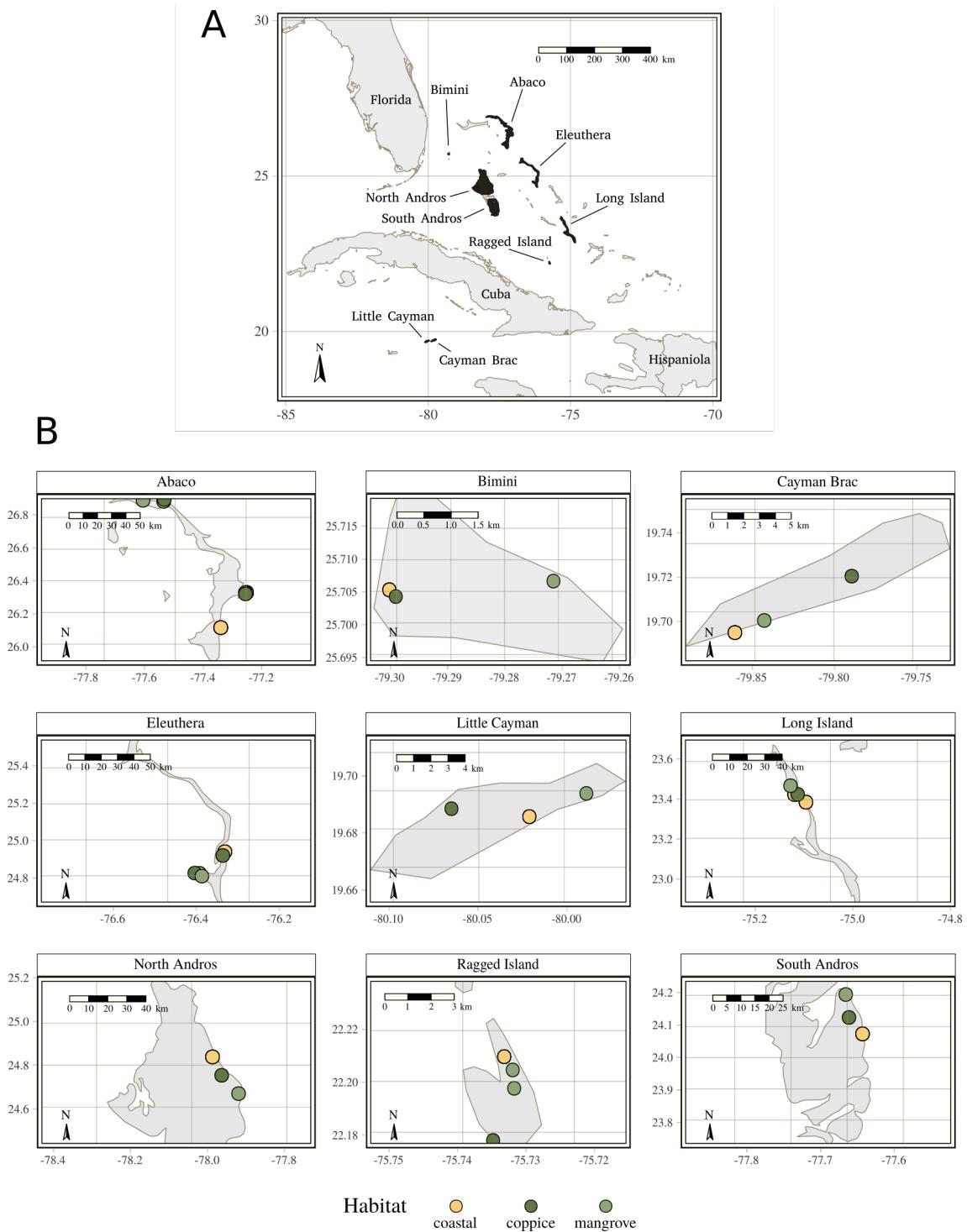


Figure S1: Maps of the islands. (A) Map of the West Indies with sampled islands highlighted in black. (B) Sampling sites within islands colored after their respective habitat-types.

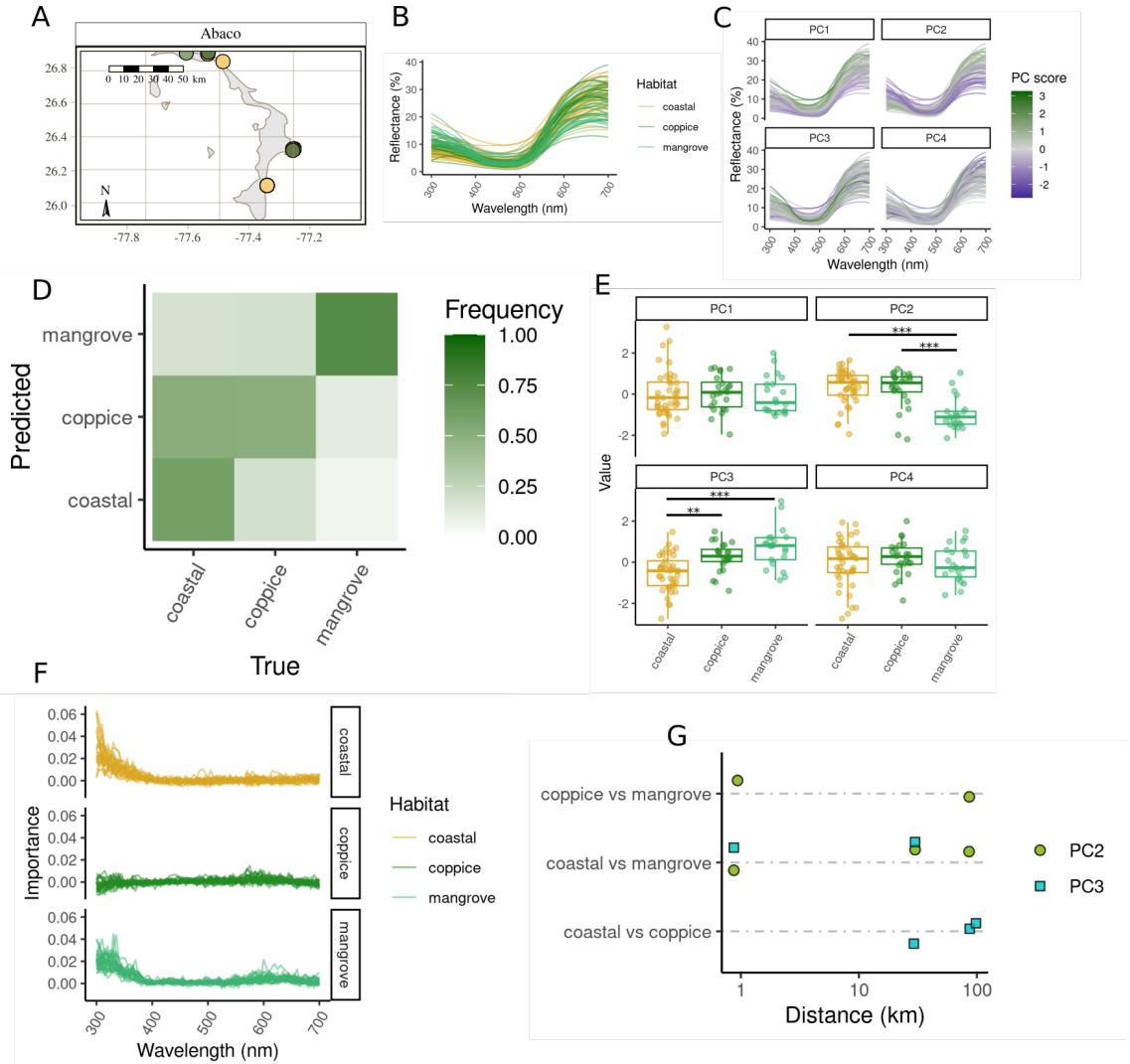


Figure S2: Comparison of dewlap coloration across habitats on Abaco, with extended results. (A–E) Legend as per Figure 2. (F) One-dimensional sensitivity analysis showing the relative importance (mean decrease in accuracy) of the various wavelengths in random forest classification of the whole spectrum. (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.

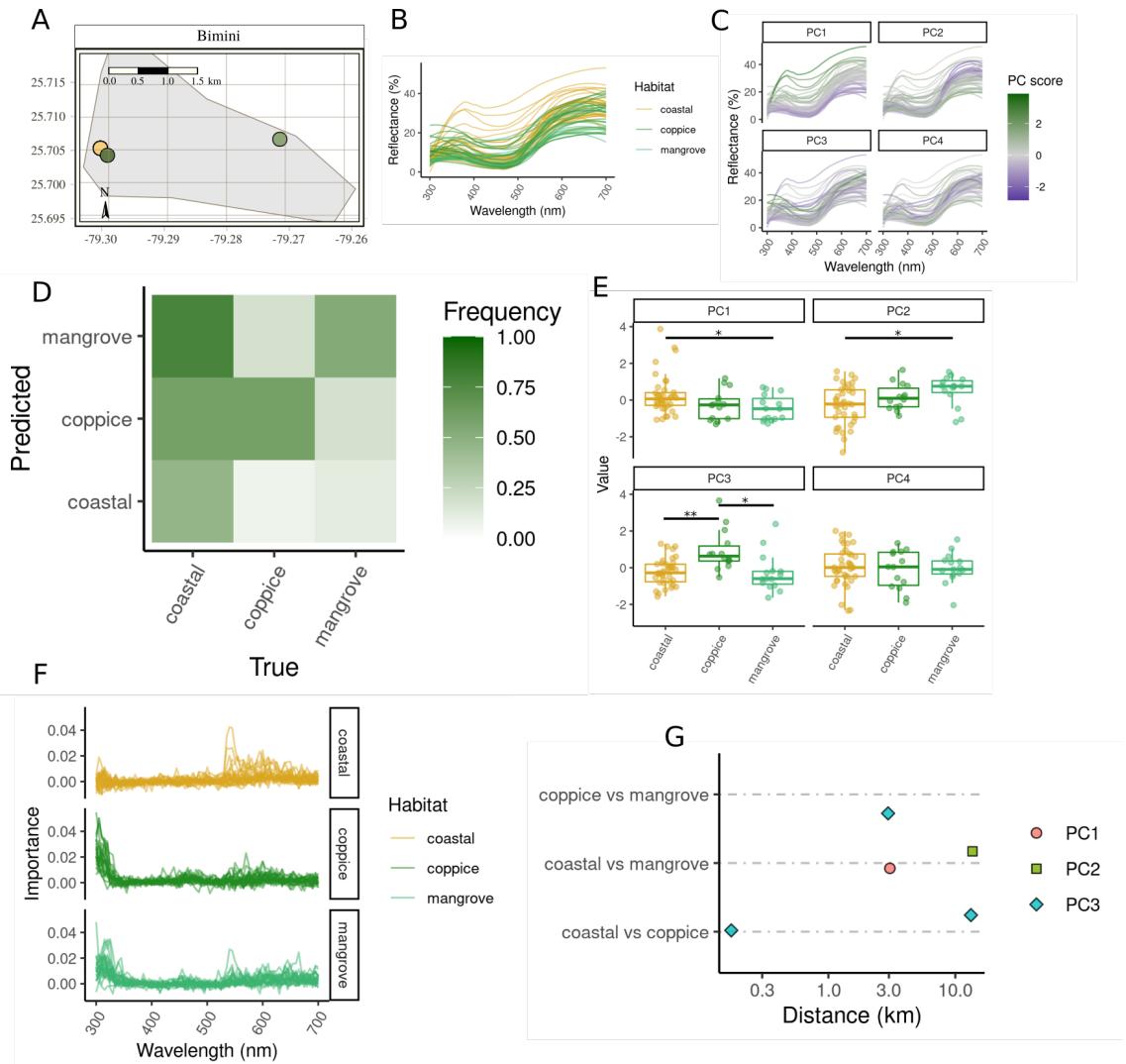


Figure S3: Comparison of dewlap coloration across habitats on Bimini. Legend is as per Figure S2.

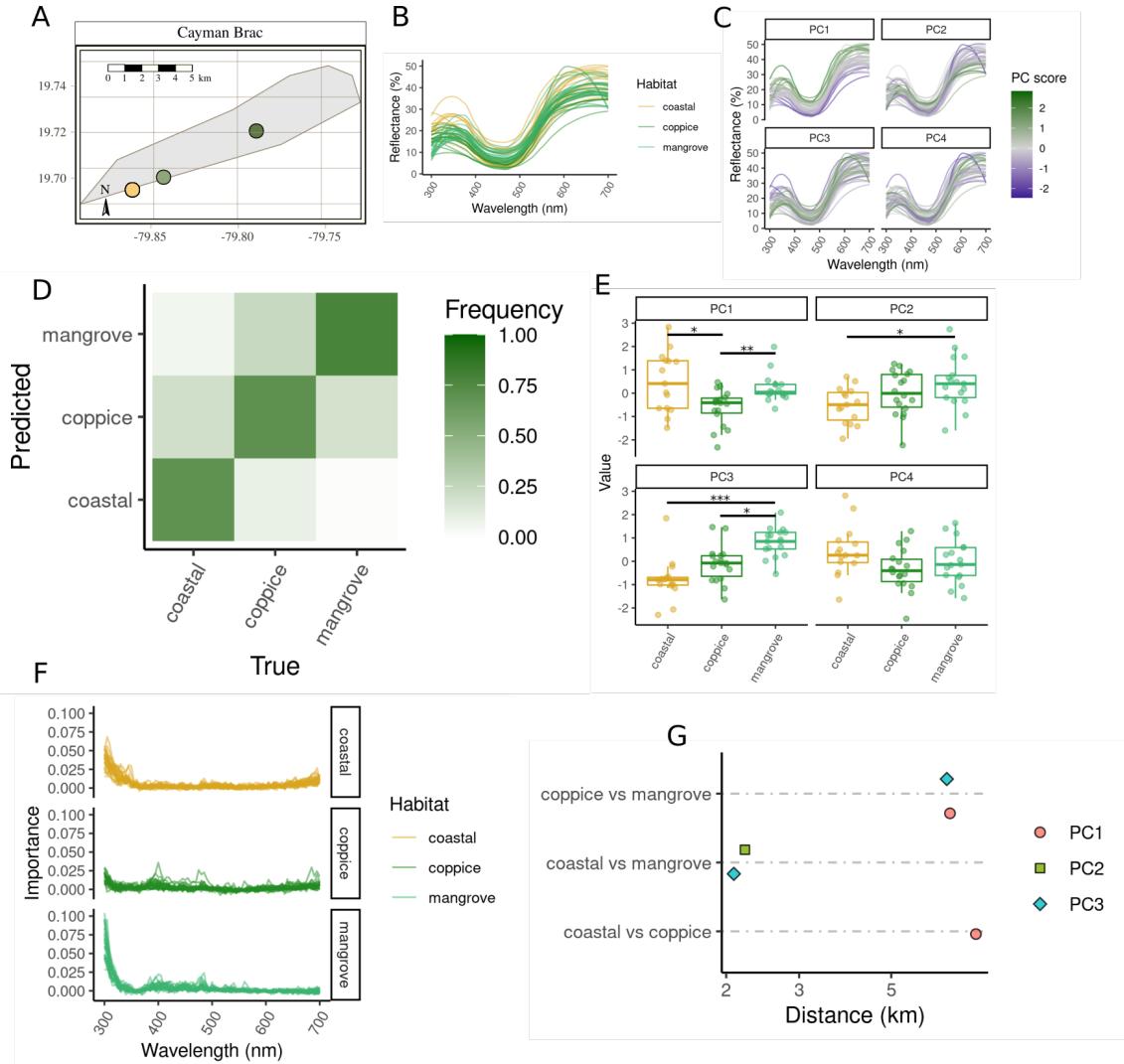


Figure S4: Comparison of dewlap coloration across habitats on Cayman Brac. Legend is as per Figure S2.

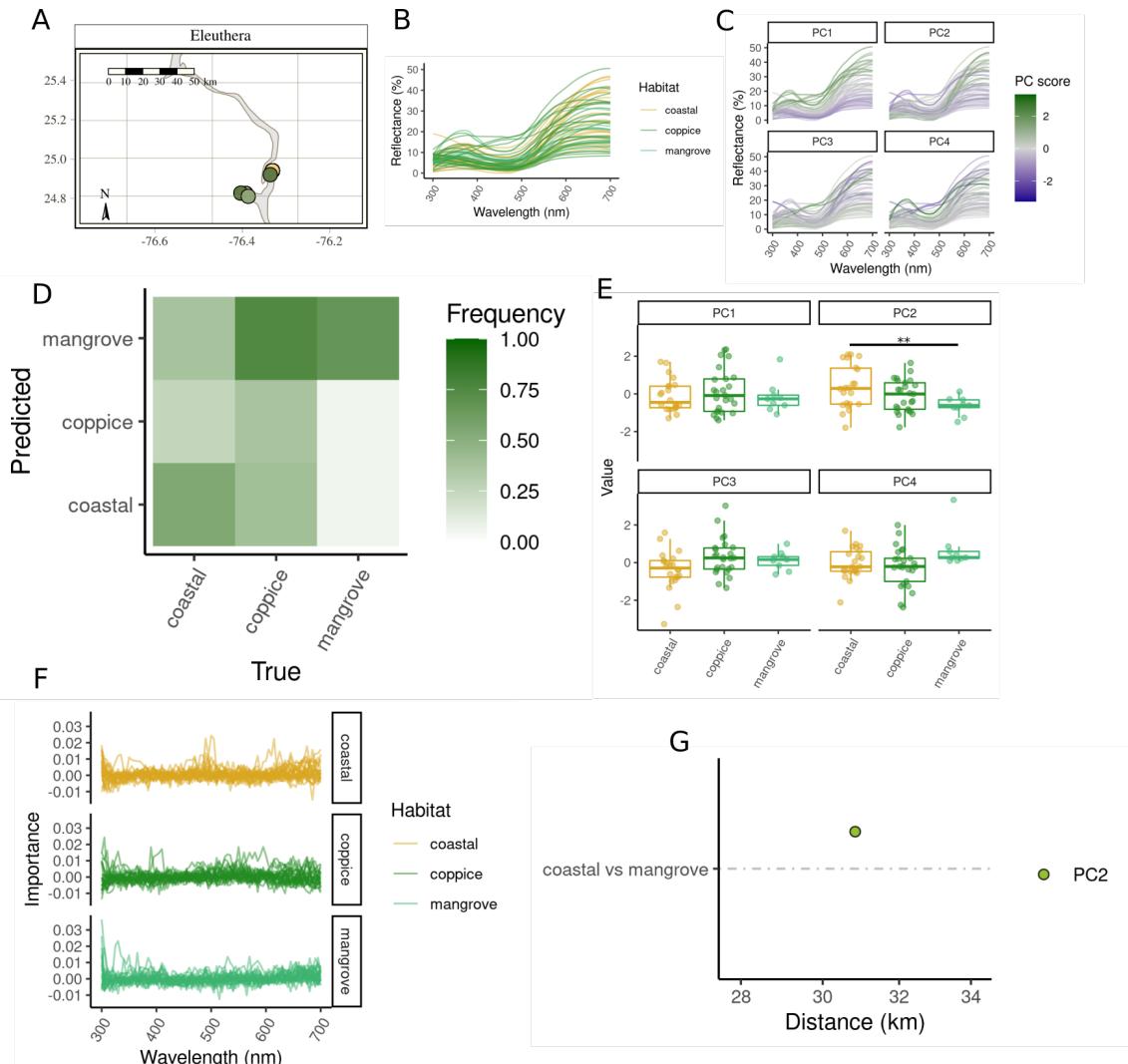


Figure S5: Comparison of dewlap coloration across habitats on Eleuthera. Legend is as per Figure S2.

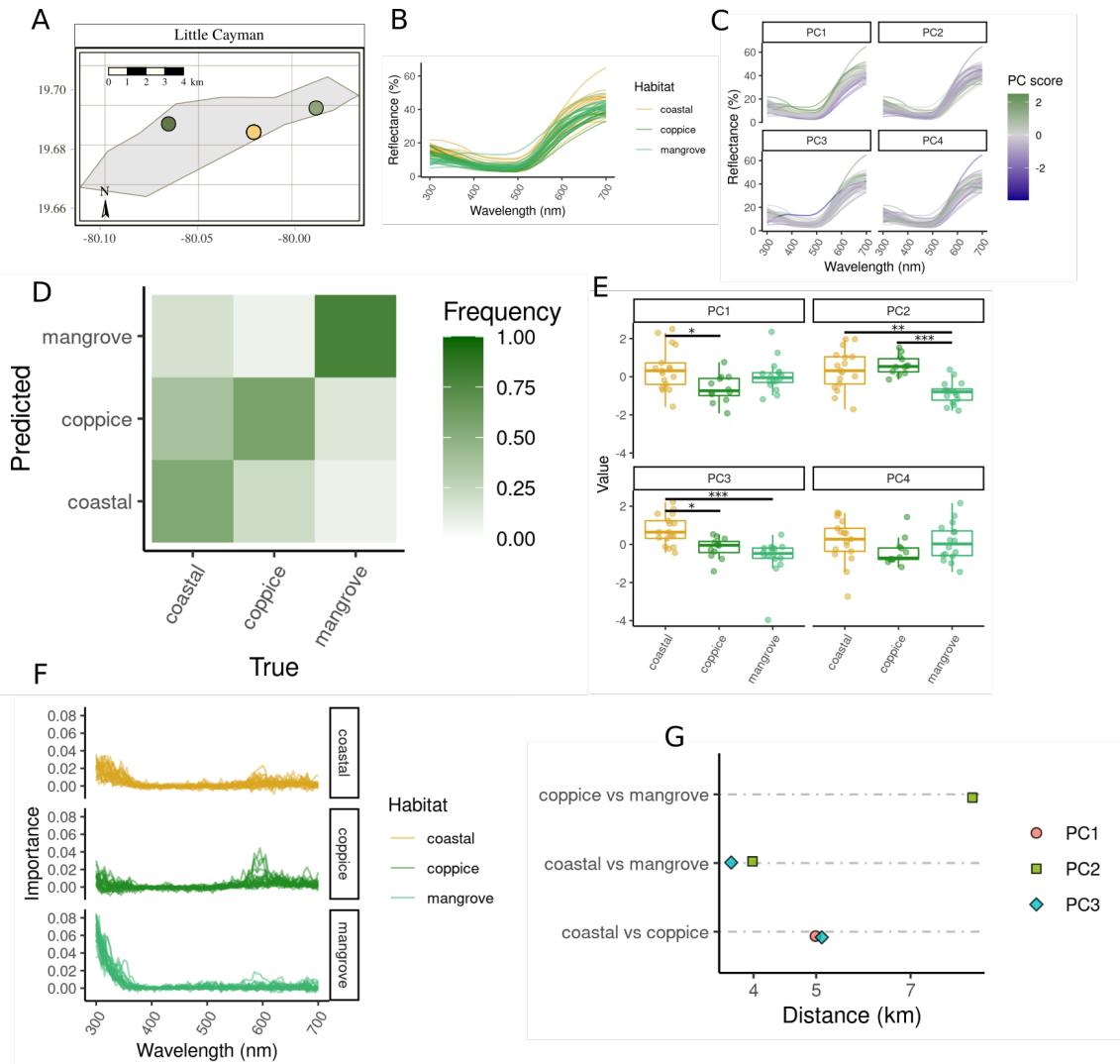


Figure S6: Comparison of dewlap coloration across habitats on Little Cayman. Legend is as per Figure S2.

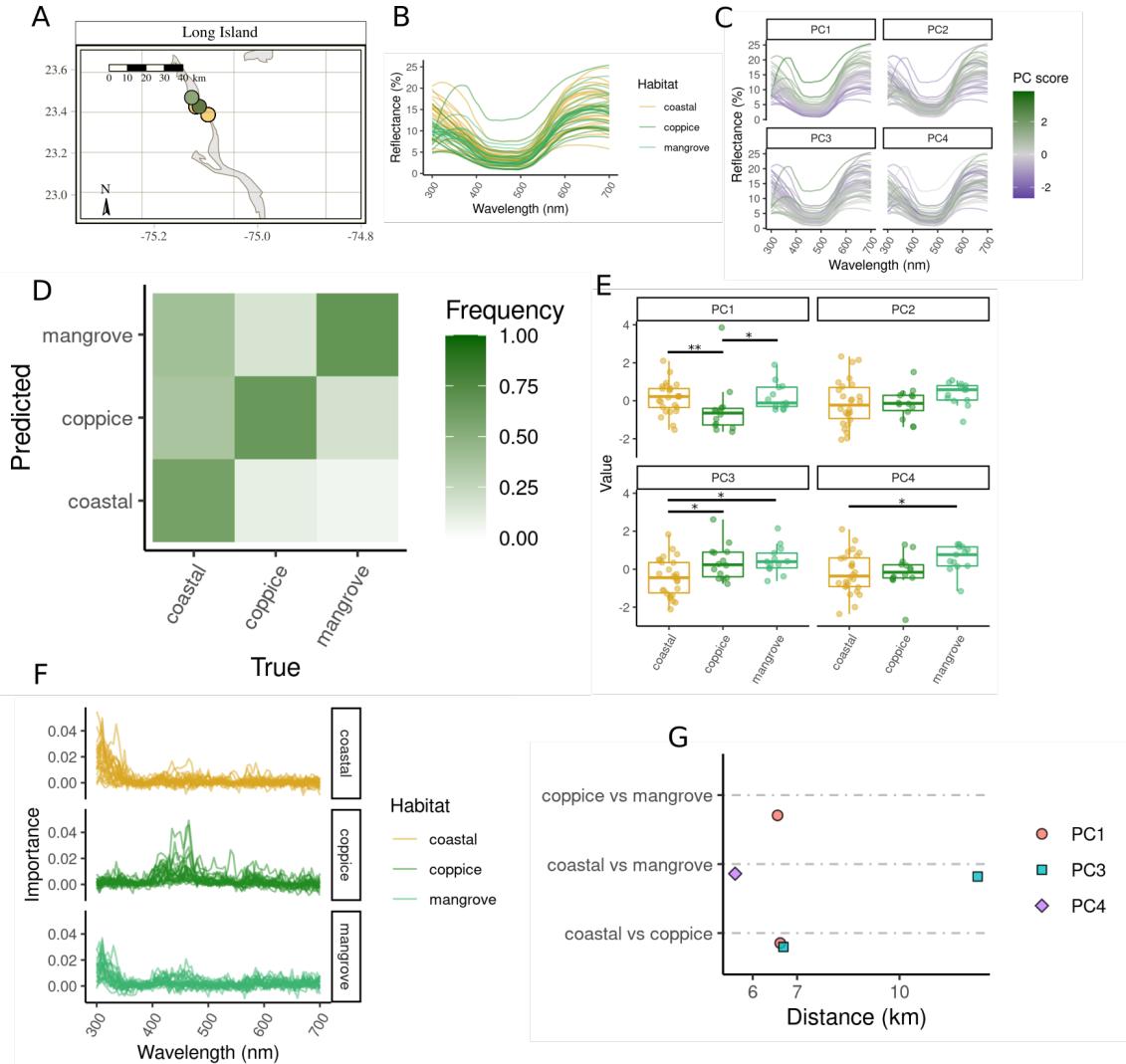


Figure S7: Comparison of dewlap coloration across habitats on Long Island. Legend is as per Figure S2.

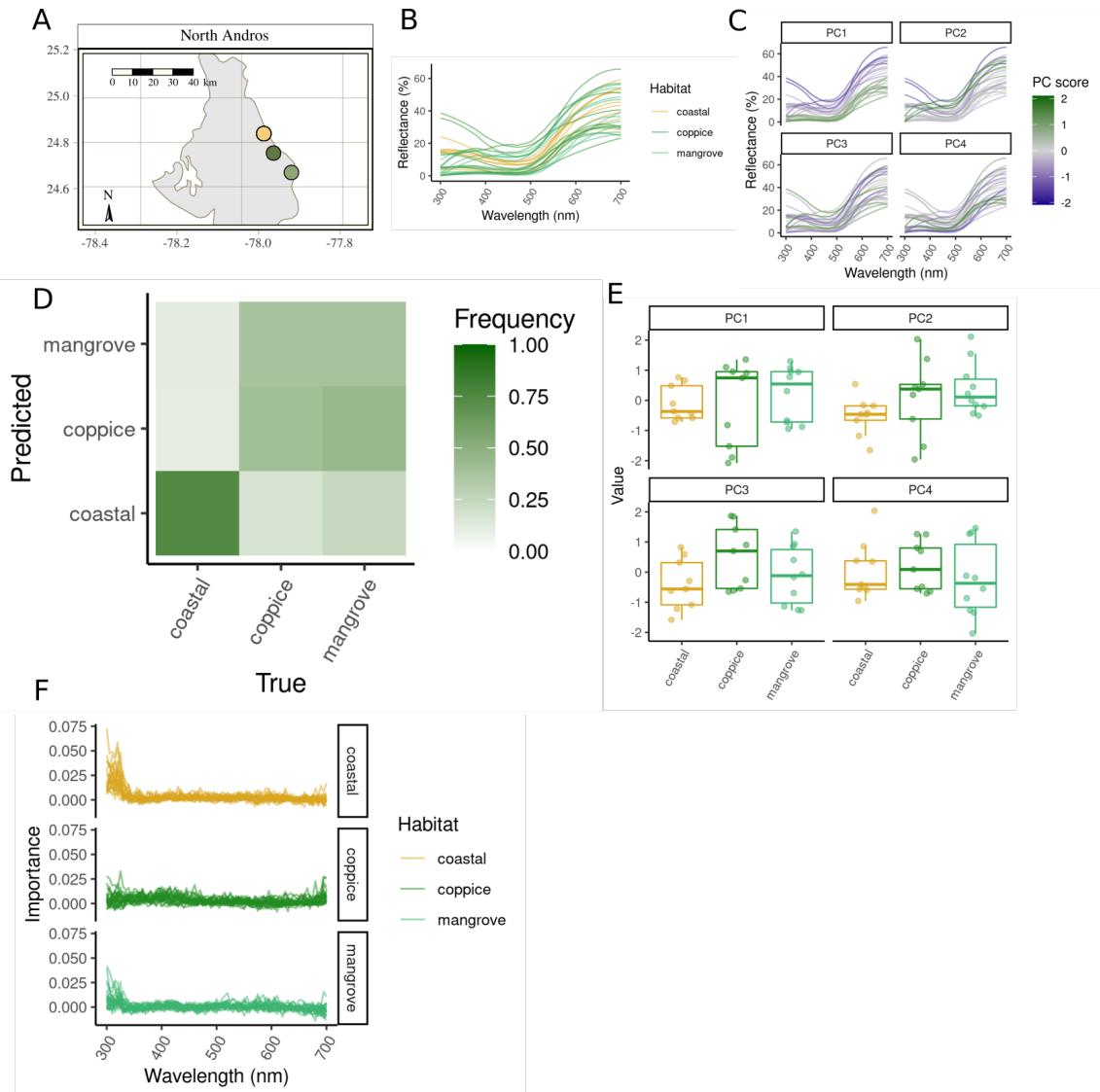


Figure S8: Comparison of dewlap coloration across habitats on North Andros. Legend is as per Figure S2, but without panel G.

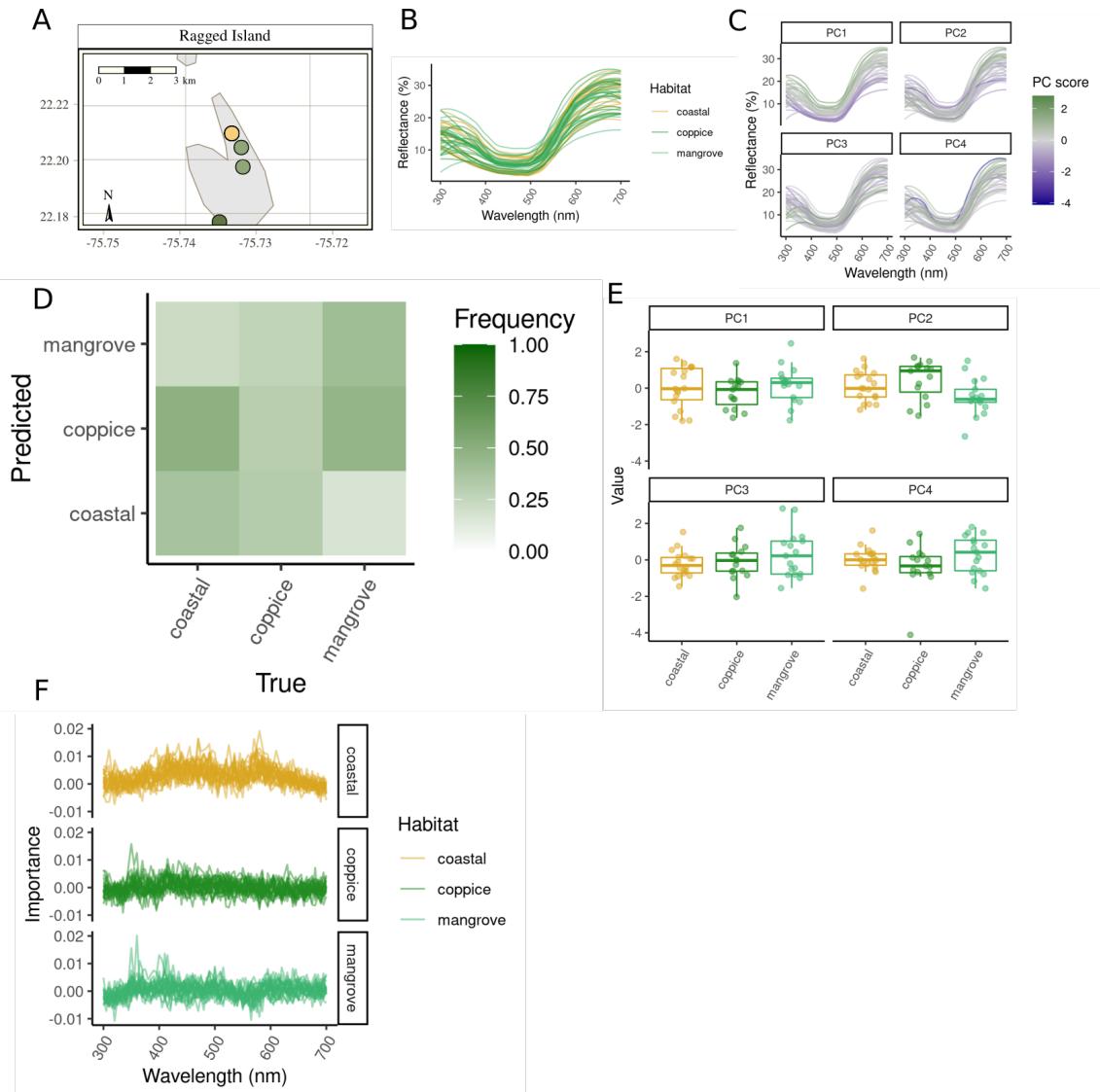


Figure S9: Comparison of dewlap coloration across habitats on Ragged Island. Legend is as per Figure S2, but without panel G.

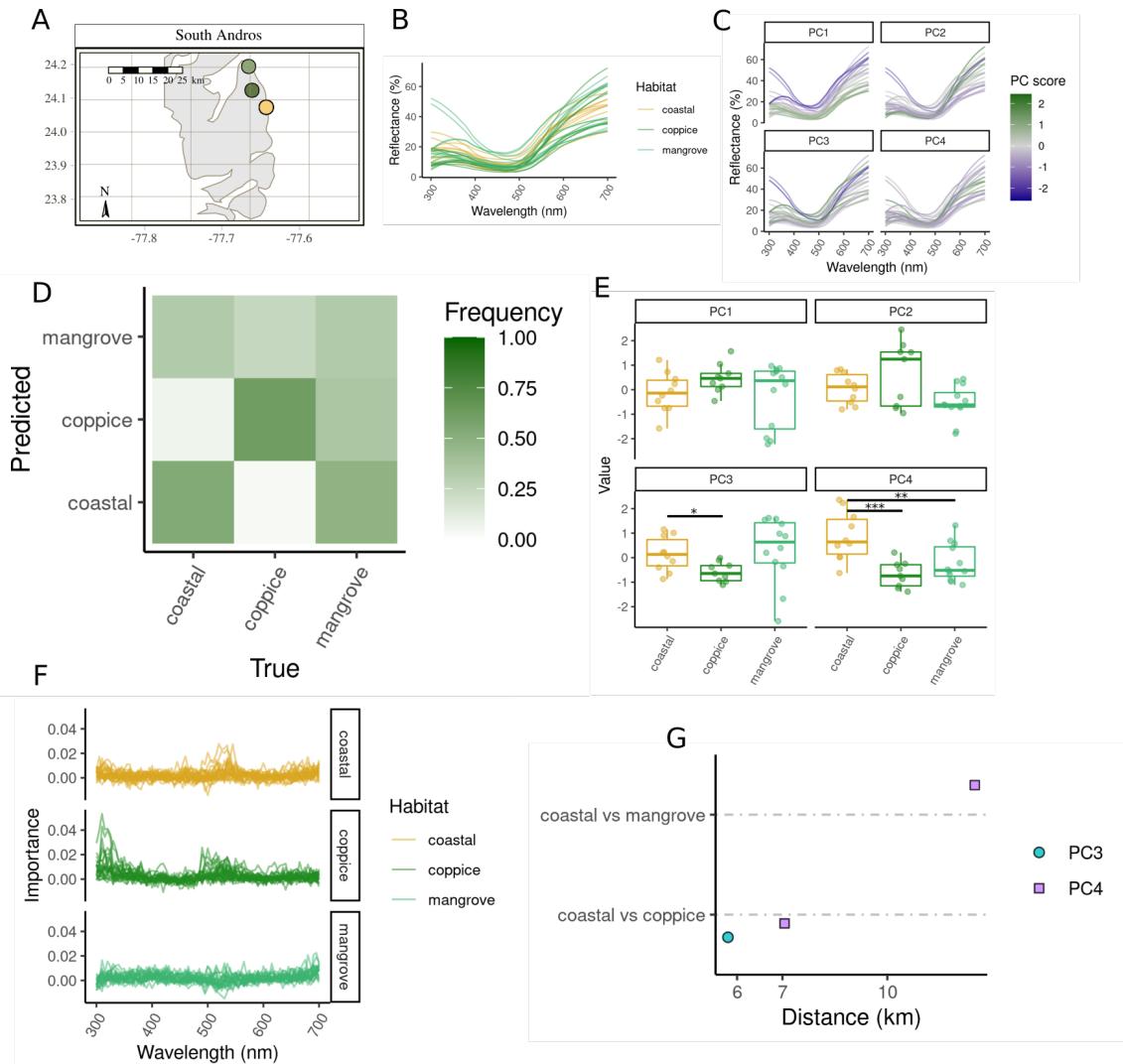


Figure S10: Comparison of dewlap coloration across habitats on South Andros. Legend is as per Figure S2.

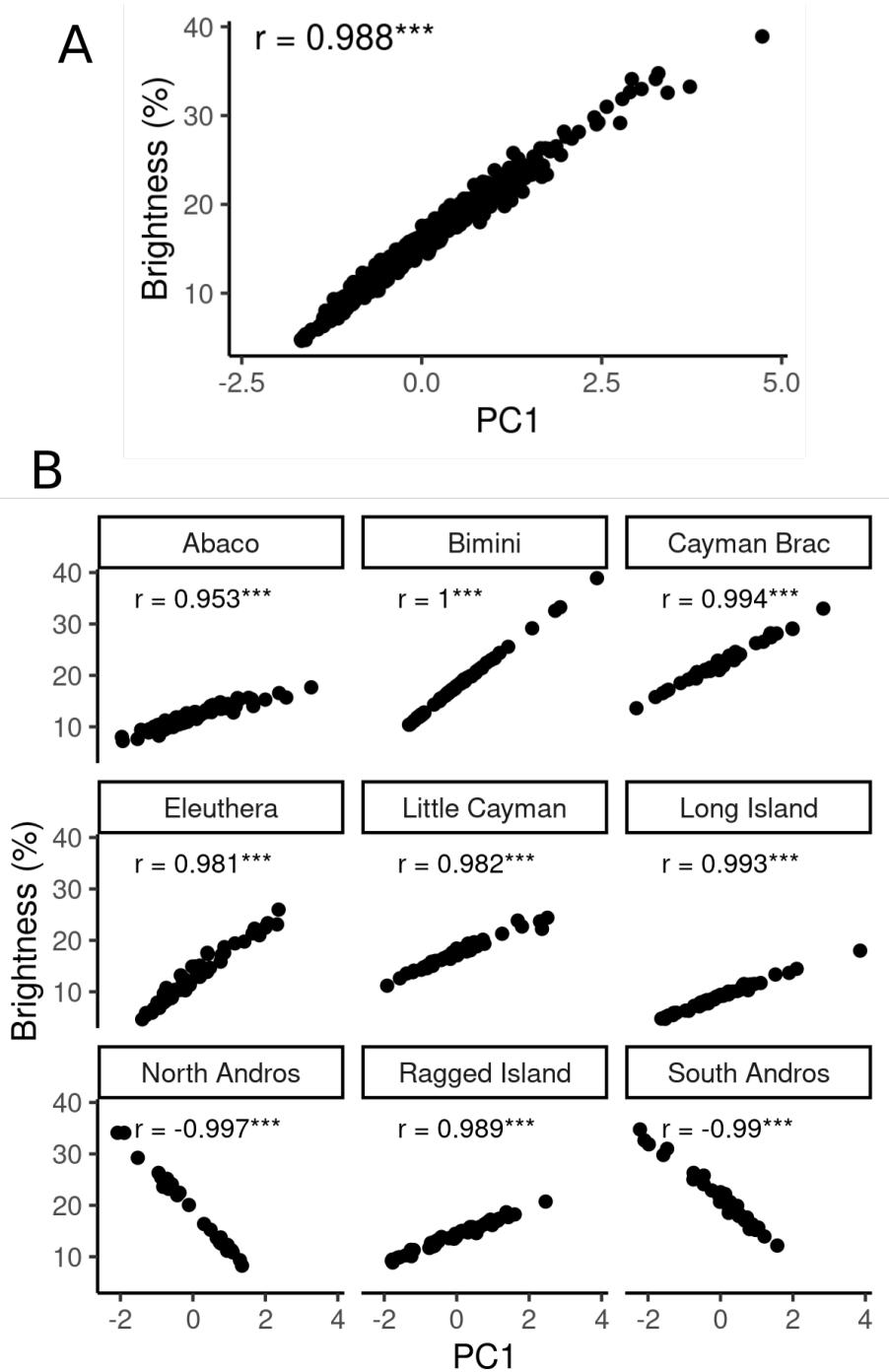


Figure S11: PC1 captures brightness across all islands. (A) Correlation between dewlap brightness (as measured by the mean reflectance from 300 to 700nm in wavelength) and PC1 score across all islands. (B) Correlation between brightness and within-island PC1, for each island. Pearson's correlation coefficients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

<sup>793</sup> **Supplementary Tables**

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	**