

Variation in tail morphology across urban and forest populations of the crested anole (*Anolis cristatellus*)

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Anolis lizards are well known for their specialist ecomorphs characterized by the convergent evolution of suites of traits linked to the use of particular microhabitats. Many of these same traits evolve rapidly in response to novel selection pressures and have been very well studied. In contrast, the tail crest, a feature present in a subset of lineages, has been almost entirely overlooked. Variation in tail crest morphology within and among species remains largely unstudied, as does the function of the trait. Here, we use the natural experiment provided by urbanization to ask whether tail crest size differs between urban and forest populations of the crested anole (*Anolis cristatellus*) across the Caribbean island of Puerto Rico. We find that tail crest size differs primarily between regions; however, within regions, crests are invariably larger in urban than in forest environments. This difference in size is correlated with the hotter, drier conditions and sparser distribution of perches that typify urban sites, leading to the intriguing possibility that the tail crest might be under differential natural selection for signalling and/or because of the thermoregulatory challenge of urban habitats. Further study is required to shed light on the functional significance and evolution of this under-studied trait.

ADDITIONAL KEYWORDS: anole – environmental variation – morphology – Puerto Rico – tail crest – urbanization.

INTRODUCTION

Recent years have witnessed a substantial increase in attention to the ecological and evolutionary effects of urbanization (Rivkin *et al.*, 2019). Urbanization results in the loss of suitable habitat for many species, ultimately leading to diminished biodiversity in urban areas compared with more pristine habitats close by (McKinney, 2008). Nonetheless, considerable evidence is beginning to accumulate that many species persist in drastically modified urban environments, where they often use novel anthropogenic niche spaces (e.g. Winchell *et al.*, 2018). These environments create new challenges related to the numerous structural and climatic habitat changes that typify urbanization (reviewed by Forman, 2014). Urban habitats tend to be more open and structurally simplified and

are dominated by anthropogenic substrates and structures. They also tend to be hotter and drier than nearby unmodified areas, a well-known phenomenon called the urban heat island effect (Oke, 1973). These various differences between urban habitats and more natural environments nearby can create novel selection pressures, leading to adaptive divergence in behaviour, physiology and morphology in many urban plants and animals (reviewed by Johnson & Munshi-South, 2017).

Lizards in the genus *Anolis* (called anoles) are perhaps best known for the repeated convergent evolution of similar microhabitat specialization across different Caribbean islands (Losos, 1998, 2009). Habitat specialists (termed ecomorphs) are characterized by suites of convergent morphological features that are highly correlated with habitat use. Studies of anole ecomorphology have shown that traits including body size, tail size, limb and head dimensions

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and toepad area determine the abilities of a given species to locomote in different microhabitats, and can evolve rapidly in response to new environmental circumstances (Losos *et al.*, 1997, 2004; Stuart *et al.*, 2014; Winchell *et al.*, 2016). This evolutionary history of rapid adaptation and morphological convergence make anoles an ideal taxon for investigating urban adaptive responses. To date, researchers have documented behavioural (Chejanovski *et al.*, 2017; Lapiedra *et al.*, 2017; Aviles-Rodriguez & Kolbe, 2019), morphological (Winchell *et al.*, 2016; Thawley *et al.*, 2019) and physiological (Battles & Kolbe, 2019; S.C. Campbell-Staton and K.M. Winchell, unpublished observations) divergence in urban anoles compared with their forest counterparts.

One anole trait that has received relatively little attention to date is the tail crest, which is a thin, dorsally positioned sail of skin-covered tissue that remains erect (i.e. the lizard cannot extend or retract the crest at will, as with the dorsal and nuchal crests of many species in this group). Prominent tail crests are not particularly common among anoles. Although, to our knowledge, the total number of *Anolis* species with tail crests has not been assessed comprehensively, we are aware of a total of 25 different species exhibiting the trait (*Anolis acutus*, *Anolis allogus*, *Anolis baleatus*, *Anolis barahonae*, *Anolis cristatellus*, *Anolis cooki*, *Anolis cuvieri*, *Anolis desecheensis*, *Anolis ernestwilliamsi*, *Anolis ferreus*, *Anolis garmani*, *Anolis gundlachi*, *Anolis homolechis*, *Anolis jubar*, *Anolis lineatus*, *Anolis luteosignifer*, *Anolis mestrei*, *Anolis oculatus*, *Anolis monensis*, *Anolis quadriocellifer*, *Anolis ricordi*, *Anolis roosevelti*, *Anolis rubribarbus*, *Anolis sagrei* and *Anolis scriptus*; Poe, 2004), among a total of 427 described *Anolis* (Uetz & Stylianou, 2018). Among these species, not all species possess equally prominent crests, and in all species in which tail crests are prominent, only males possess a large crest (Schwartz & Henderson, 1991; Malhotra & Thorpe, 1997; Brandley & de Quieroz, 2004; Charles & Ord, 2012). In iguanians, sexually dimorphic ornaments, such as crests and spines, are often assumed to be products of sexual selection (Andersson, 1994; Stuart-Fox & Ord, 2004). Nevertheless, although a number of anoles and other iguanian lizards exhibit tail crests, relatively little attention to date has been given to this particular trait (especially compared with other sexually dimorphic characters, such as the dewlap), and its function remains essentially unknown. In anoles, two main hypotheses regarding tail crest function have been proposed: signalling and thermoregulation.

The signal detection hypothesis posits that the tail crest serves primarily as a long-range signal between males communicating with one another across large distances in open habitats (Charles & Ord, 2012). Male anoles principally signal to one another using

the dewlap, a flap of retractable skin under the chin that is extended in agonistic interactions and, in many species, also with extendable (soft-tissue) dorsal and nuchal crests (Nicholson *et al.*, 2007; Losos, 2009). In addition, some species (both with and without tail crests) have also been known to move their tails as part of agonistic and anti-predator displays (Echelle *et al.*, 1971; Leal & Rodriguez-Robles, 1995; Elmasri *et al.*, 2012). Whether male anoles in crested species also use the tail crest itself for intraspecific signalling is not yet known, but evidence suggests that it is a possibility. For instance, within a clade of primarily crested species on Puerto Rico and surrounding islands, Charles & Ord (2012) found that species in which males perch relatively far from one another have prominent tail crests, in contrast to species lacking tail crests that perch more densely. They also reported that males whose territories overlapped with many females had larger crests than males whose territories overlapped with fewer females. This work suggests that the tail crest might be used as a signal for male–male displays in species in which males are physically separated by large distances. Likewise, females may be able to see males with larger crests more easily and may therefore be more likely to approach and mate with them rather than with males that have smaller, less-visible crests.

Tail crests could also serve a thermoregulatory function. As ectotherms, many anoles must thermoregulate actively to maintain their body temperature (Huey & Slatkin, 1976). The ability of an individual to achieve and maintain an optimal body temperature is influenced by both behaviour and physiology. A prominent tail crest could play a significant role in thermoregulation by providing a greater surface area across which heat can be exchanged. Similar anatomical structures that increase surface area (e.g. dewlaps) have been shown to function in heat exchange in ungulate mammals (Bro-Jørgensen, 2016) and iguanas (Morgareidge & White, 1969). In addition, palaeontological models suggest that crest-like structures found in Permian synapsids (dorsal sails) might have helped to regulate body temperature through both heat absorption (Haack, 1986; Bennett, 1996; Florides, 1999) and heat dissipation (Bennett, 1996), although these claims are not uncontroversial (Tomkins *et al.*, 2010).

Evidence of a thermoregulatory role for the tail crest in anoles is limited. For example, in Puerto Rico, three closely related species that have tail crests differ widely in their degree of thermoregulatory specificity: *A. gundlachi*, the yellow-chinned anole, is a thermoconformer that hardly regulates its body temperature at all; *A. cristatellus*, the crested anole, is a thermoconformer in some habitats and a thermoregulator in others; and, finally, *A. cooki*, Cook's pallid anole, is a strict thermoregulator that

maintains a relatively precise body temperature throughout its active period (Hertz, 1992; Hertz *et al.*, 1993). Although all three of these species possess tail crests, crest size has not been compared quantitatively between them, and it is certainly possible that they could differ substantially one from the other. The limited analyses of intraspecific tail crest variation relative to habitat has likewise provided little evidence to support a relationship between habitat and tail crest size. For example, on the island of Dominica, tail crest size in *A. oculatus* varied among populations across the small island range of the species, but it was not correlated with temperature or with any other habitat variable (Malhotra & Thorpe, 1997). In addition, a series of unpublished experiments by Perry and LeVering (referenced by Perry, 2005) found no benefits or costs of tail crests in terms of desiccation or thermoregulation in *A. cristatellus*.

We supposed that the large environmental and ecological differences between urban and forest environments could lead to shifts in tail crest morphology among lizard populations that might shed light on this understudied trait. In particular, focusing on the crested anole (*A. cristatellus*) of Puerto Rico, we asked:

How do climatic and structural environments vary regionally across the island, and between urban and forest habitats?

Do the tail crests of *A. cristatellus* differ regionally across the island, and between urban and forest habitats within each region?

MATERIAL AND METHODS

FIELD DATA COLLECTION

In 2012, we captured 319 adult male *A. cristatellus* as encountered at six sites consisting of an urban and a forest location from each of three municipalities (San Juan, Mayagüez and Ponce). Urban sites were dominated by impervious surfaces and minimal canopy cover, whereas forest sites were mature secondary forests, with minor human impacts at two of the sites. The forest site in Ponce has encroaching urban development along one edge, and the forest site in San Juan is transected by several paved footpaths and is surrounded by relatively dense urbanization (Fig. 1). We transported lizards to a field laboratory, where we measured snout–vent length (all measurements were taken by K.M.W.) and took high-resolution lateral

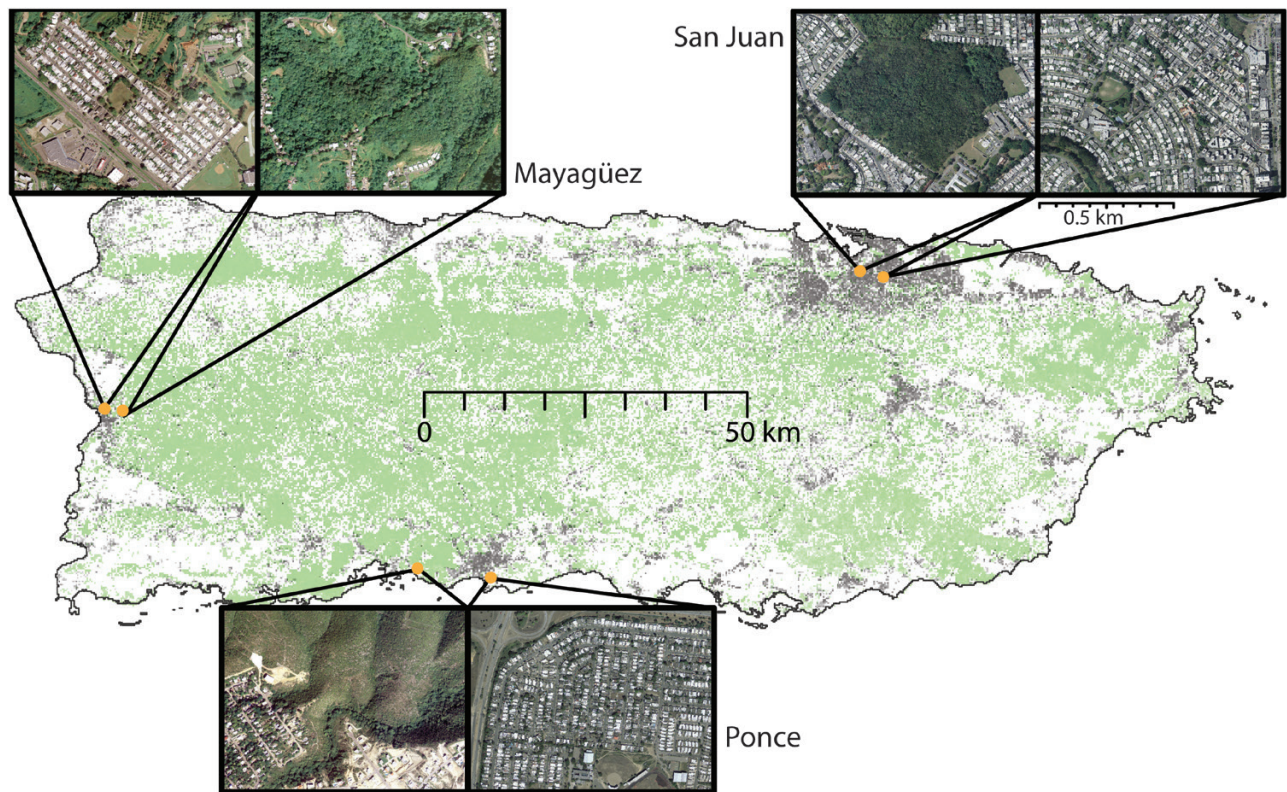


Figure 1. Satellite imagery of each site, with 0.5 km scale bar for all inset images. Grey and green shading on the map represent patterns of high levels of impervious surface and tree canopy coverage, respectively. Land cover is from the National Land Cover Database (Xian *et al.* 2011; Homer *et al.* 2015). Imagery by U.S. Geological Survey (2012).

images of the tail on a flatbed scanner (Epson V300, 2100 dpi). All lizards were returned to their site of capture after measurement.

GIS ANALYSIS

We obtained BIOCLIM raster layers from the WorldClim 2 dataset at 30' resolution (Fick *et al.*, 2017). We increased the spatial resolution of rasters by a factor of ten, with nearest neighbour (i.e. raster cells are assigned the value of their parent cell) resampling using the 'Resample' tool in ArcMAP. We determined the centroid of the sampled area at each site and created 500 m circular buffers, for which we extracted climate data for mean annual temperature and total annual precipitation BIOCLIM variables (BIO1 and BIO12) per raster cell. In addition, we quantified habitat openness within each sampled area using two measures of perch distribution estimated from aerial imagery: perch isolation and perch abundance. For both measures, we first randomly distributed 50 points (with a minimal distance of 5 m between points) within each site using the create random points function in ArcMAP. Using orthoimagery (U.S. Geological Survey, 2012), we then estimated perch isolation by calculating the distance from each point to the edge of the nearest tree canopy edge or anthropogenic structure (other than the structure on which the point landed). We estimated perch abundance by site by counting the number of points that fell on large structures that could be used as perches (tree canopy or anthropogenic structures). We performed all spatial analyses in ArcGIS (ArcMAP v.10.4.1; ESRI, 2016).

MEASUREMENT OF TRAITS

We measured only tails that were intact and not regenerated up to the end of the crest ($N = 225$). Exclusion from the dataset based on tail damage was random with respect to crest presence (Supporting Information, Appendix S1). Some individuals had very minimal crests, which comprised only a row of keeled scales on the dorsum of the tail. We therefore defined the end of the crest as the point at which the dorsal scale row transitioned from keeled to uniform. To capture variation in multiple aspects of crest size, we measured the following tail traits using the ObjectJ plugin (Visher & Nastase) in the FIJI distribution of ImageJ software (Schindelin *et al.*, 2012; Rueden *et al.*, 2017): crest length, crest area, and tail height at the five highest points along the tail crest (Fig. 2). We retained the maximal tail height value from these five measurements. We identified the base of the tail as the point at which the scales of the lizard

changed from uniform (as on the body and legs) to a distinct ring pattern around the tail. We measured all traits three times each and used the average of these measurements for all subsequent analyses.

STATISTICAL ANALYSES

We used three separate two-way ANOVAs, with municipality interacting with habitat type (urban or forest), to test whether urban and forest sites within each municipality differed in climate [summarized by BIOCLIM variables BIO1 (mean annual temperature) and BIO12 (total annual precipitation)] and/or in perch isolation. For perch abundance, we analysed perch presence/absence at random points using a binomial linear model by habitat type, with municipality as a covariate. The interaction between municipality and habitat type was significant for both climate analyses (temperature, $P < 0.001$; precipitation, $P < 0.001$) and for the perch abundance analysis ($P = 0.003$), but was not significant for perch isolation ($P = 0.742$; and was therefore dropped from this model). For all significant interaction effects, we analysed differences between municipalities and between urban–forest pairs within each municipality using the 'contrast' function, with multivariate adjustment, in the R package *emmeans* (v.1.3.4; Lenth, 2019).

We quantified repeatability of our three tail crest measurements (length, area and maximal height) using the intraclass correlation coefficient (ICC) implemented in the R package *ICC* (Wolak *et al.*, 2012). We investigated the relationships between our three measurements of tail crest morphology using a single linear model of ln-transformed tail crest area by the other two ln-transformed variables, with ln-transformed body size (snout–vent length) as a covariate. We investigated the possibility of an interaction of body size with each of these traits (Supporting Information, Appendix S2) and found that all three traits were positively correlated with body size consistently across sites and municipalities. Body size was not a predictor of crest presence (i.e. some large individuals had minimal to no crests; Supporting Information, Appendix S2).

To test whether tail crest morphology differed by habitat (urban or forest), we compared the three ln-transformed morphological traits (crest area, maximal crest height and length of crest) between urban and forest lizards using a two-way MANCOVA ('manova' in R base package *stats*), with municipality interacting with habitat, and body size as a covariate. We verified multivariate normality of the model residuals (Supporting Information, Appendix S3). We included body size as a covariate in order to assess variation in tail crest morphology relative to body size across sites. [Body size might also be a target of selection in these populations, but given that we have previously documented significant but inconsistent

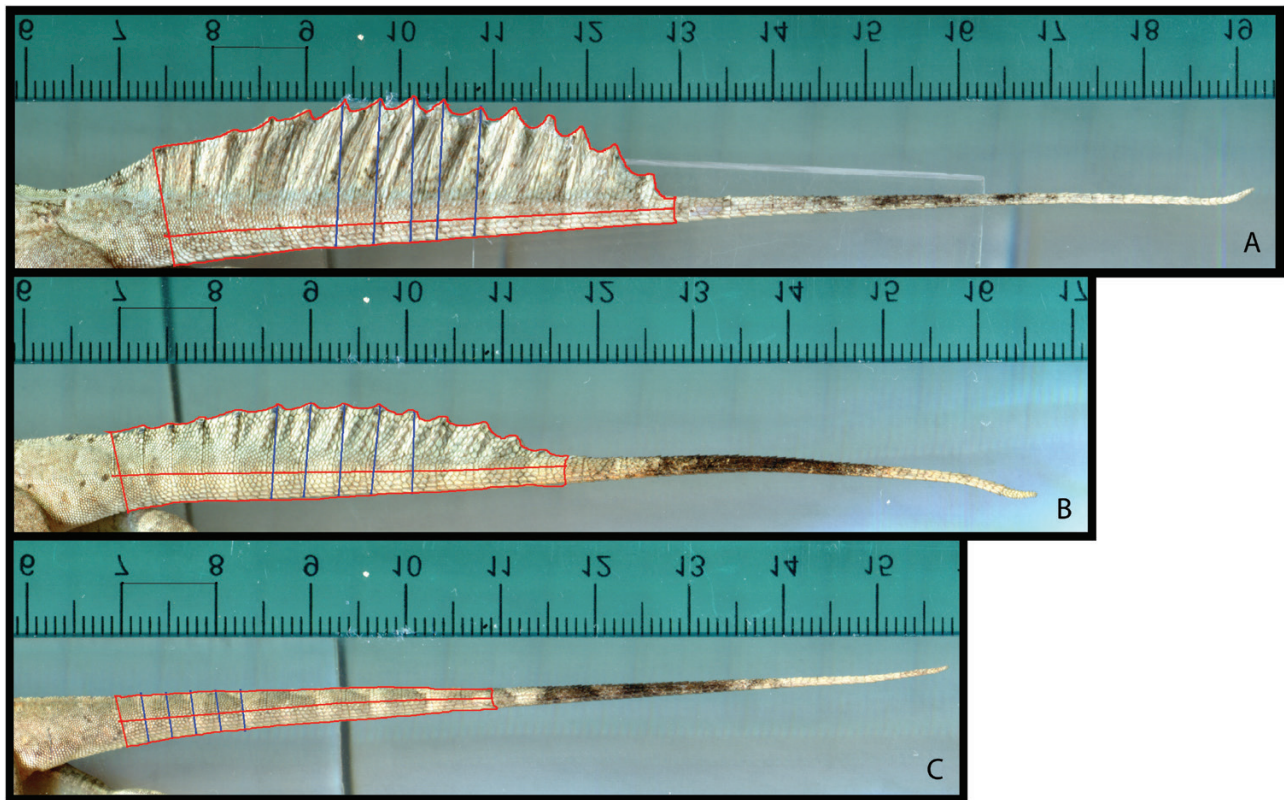


Figure 2. Representative images of tail crests used for data analysis. Coloured lines show measurements taken in ImageJ for tail crest heights (blue), length (horizontal red line) and area (red trace around crest). Individuals are from urban (A) and natural (B, C) sites in Ponce.

differences in body size between these same urban and forest populations (Winchell *et al.*, 2016), we conduct no further analyses here on this highly variable trait.] A significant habitat \times municipality interaction in our MANCOVA would indicate that the differences between urban and forest populations varied by municipality. To determine which specific traits differed between municipalities and habitats, we performed an ANOVA on the MANCOVA model ('summary.aov' in R base package *stats*). We also investigated differences between municipalities and between urban and forest habitats within each municipality for each significant effect using the 'contrast' function with multivariate adjustment in the R package *emmeans* v.1.3.4 (Lenth, 2019).

We did not exclude individuals with minimal crests from the analyses. We reasoned that any function that the crest serves (thermoregulation, signalling or other) is likely to augment a role already played by the tail, rather than creating an entirely new function, and thus concluded that excluding animals with minimal or no crests was not appropriate. For instance, the tail-wagging displays of *A. cristatellus* are visible with or without a crest; a large crest simply amplifies that signal. Likewise, heat exchange occurs across all body parts and extremities, and a large tail crest would thus serve to

facilitate heat exchange by increasing surface area-to-volume ratio, compared with a lizard that possesses a crestless caudal appendage. We therefore measured all crest variables (crest length, crest area and tail height) for all individuals, including those with little to no crest. Nonetheless, to ensure that this approach did not impact our findings, we performed several additional analyses (Supporting Information, Appendix S4). First, we tested whether the number of animals with minimal crests differed between urban and forest habitats and between regions. Second, we repeated our MANCOVA by first conservatively excluding any individuals without prominent crests but keeping individuals with ambiguous (small) crests ($N = 166$ individuals included), then by more liberally excluding individuals with any visible crest at all ($N = 139$ individuals included).

We performed all statistical analyses using R v.3.5.3 (R Core Team, 2019).

RESULTS

HABITAT

We found significant differences in both mean annual temperature and total annual precipitation across

municipalities (temperature, $F_{2,670} = 1298.3$, $P < 0.001$; precipitation, $F_{2,670} = 17694.4$, $P < 0.001$). Ponce sites were hottest and driest, whereas Mayagüez sites were the coolest and wettest ($P < 0.001$ for all contrasts between municipalities; Fig. 3). Within municipalities, urban sites were consistently hotter (Mayagüez urban, 0.4 ± 0.03 °C, $P < 0.001$; Ponce urban, 0.6 ± 0.02 °C, $P < 0.001$; San Juan urban, 0.2 ± 0.03 °C, $P < 0.001$; Fig. 3) and drier (Mayagüez urban, -16.71 ± 0.84 cm, $P < 0.001$; Ponce urban, -19.86 ± 0.72 cm, $P < 0.001$; San Juan urban, -4.54 ± 0.83 cm, $P < 0.001$; Fig. 3).

Perch isolation, our first metric of habitat openness, differed significantly between urban and forest habitats across all municipalities (Fig. 4A; $F_{1,296} = 108.022$, $P < 0.001$). The differences in perch isolation were consistent across municipalities (habitat \times municipality, $F_{2,294} = 0.293$, $P = 0.746$), and there was no significant variation between municipalities (municipality, $F_{2,294} = 0.046$, $P = 0.955$). Urban areas were significantly more open than forest habitats; in urban environments, the nearest potential perch (e.g. tree, building, fence) was on average 2.1 ± 0.2 m farther from our randomly placed points than in forested sites ($t = 10.393$, $P < 0.001$).

Perch abundance, our second metric of habitat openness, also differed between urban and forest habitats. Urban habitats had fewer potential perches than forest habitats (d.f. = 1, 296, $P < 0.001$; Fig. 4B),

suggesting an overall more open environment. Across urban habitats, only 60–72% of random sample points fell on potential perches compared with 90–100% in forests. The magnitude of the differences between urban and forest sites for perch abundance differed between municipalities (habitat \times municipality, d.f. = 2, 294, $P = 0.003$; Fig. 4B), but consistently differed in the same direction, such that the urban habitat in each municipality had fewer available perches than the forest habitat (municipality: d.f. = 2, 297, $P = 0.481$; Mayagüez, $\chi^2 = 14.037$, $P < 0.001$; Ponce $\chi^2 = 4.159$, $P = 0.041$; San Juan, $\chi^2 = 22.562$, $P < 0.001$). In urban habitats, in each municipality at least half of the random sample points that fell on environmental features were found on anthropogenic structures (73.5% in Mayagüez, 58.8% in Ponce and 83.3% in San Juan), whereas no anthropogenic structures were sampled in forest habitats.

MORPHOLOGY

We had extremely high repeatability of our tail crest measurements (ICC, 0.951–0.994), with the within-group variance being far lower than the among-group variance for all variables. Tail crest area was strongly and significantly related to maximal tail height (estimate, 1.085 ± 0.018 , $t = 59.562$, $P < 0.001$) and tail crest length (estimate, 0.733 ± 0.051 , $t = 14.391$, $P < 0.001$).

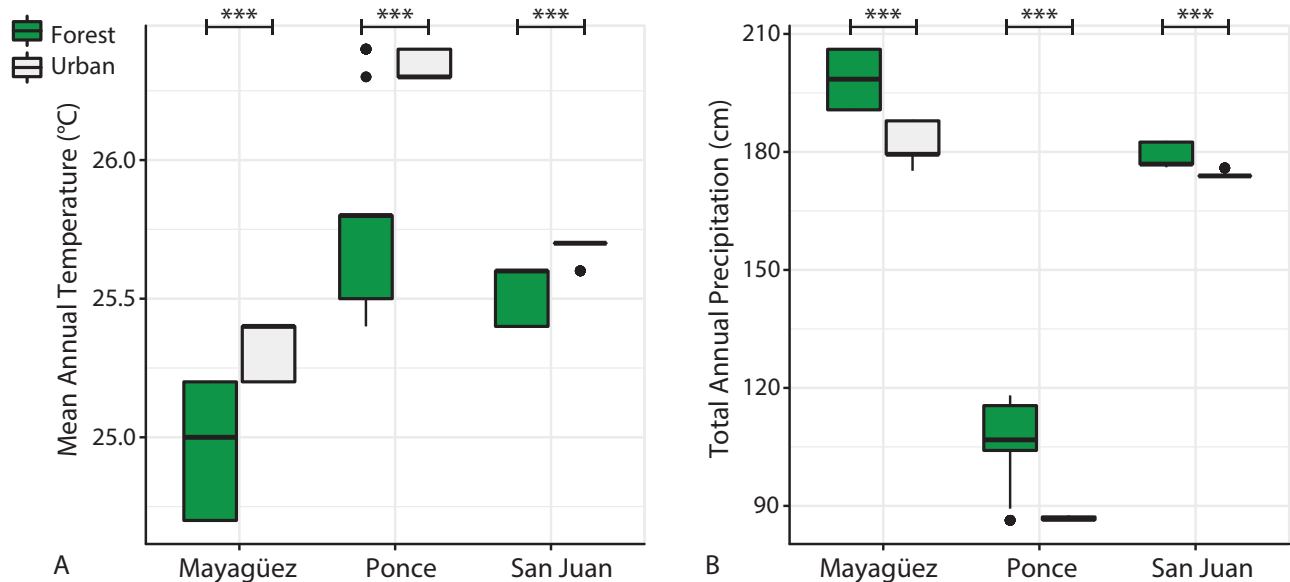


Figure 3. Summary of climatic variation by site described by two BIOCLIM variables: BIO1, mean annual temperature (in degrees Celsius), and BIO12, total annual precipitation (in centimetres). Within each municipality, urban sites are hotter and drier than the nearby forest site ($P < 0.001$ for all pairwise contrasts, indicated by *** above each urban-forest pair). Between municipalities, irrespective of habitat type, temperature and precipitation also differed: coolest and wettest conditions were in Mayagüez, and hottest and driest conditions in Ponce (all pairwise contrasts between municipalities $P < 0.001$).

Our results showed substantial regional variation in tail crest morphology (MANCOVA, $F_{6,356} = 19.272$, $P < 0.001$; Table 1). Tail crests differed in area and maximal height (but not length) between municipalities (ANCOVA; Table 2; Fig. 5). In particular, lizards in Ponce had significantly larger tail crests than lizards in San Juan (Ponce–San Juan contrasts: area $13.9 \pm 4.0\%$ larger, $P < 0.001$; maximal height $13.6 \pm 3.3\%$ taller, $P < 0.001$), which in turn had larger tail crests than lizards in Mayagüez (San Juan–Mayagüez contrasts: area $21.0 \pm 3.7\%$ larger, $P < 0.001$; maximal height $14.8 \pm 3.1\%$ taller, $P < 0.001$).

Within municipalities, all three tail crest measures differed by habitat (urban vs. forest), with significant habitat \times municipality interactions for crest length only (Tables 1 and 2). Across all three municipalities, urban lizards had relatively larger crests ($10.1 \pm 3.0\%$ larger, $P < 0.001$) and taller crests at the maximal height ($6.4 \pm 2.4\%$ taller, $P = 0.009$; Fig. 5). Relative

tail crest length was also significantly longer in urban compared with forest populations in Ponce ($8.0 \pm 1.9\%$ longer, $P < 0.001$) and in Mayagüez ($4.9 \pm 1.8\%$ longer, $P = 0.007$), but not in San Juan ($P = 0.627$; Fig. 5).

In some individuals, the tail crest is minimal or absent (Fig. 2C). The frequency of lizards with prominent crests did not differ between urban and forest habitats ($P = 0.545$) and thus is unlikely to impact the difference in average tail crest size that we measured between habitat types in this study. We did, however, encounter significant regional differences in tail crest frequency when conservatively treating this trait as a discrete binary character rather than a continuous trait. In particular, across both urban and forest habitats fewer lizards in Mayagüez had prominent tail crests compared with animals from San Juan and Ponce ($P < 0.001$; Supporting Information, Appendix S4). Out of an abundance of caution, we repeated our analyses excluding any individuals

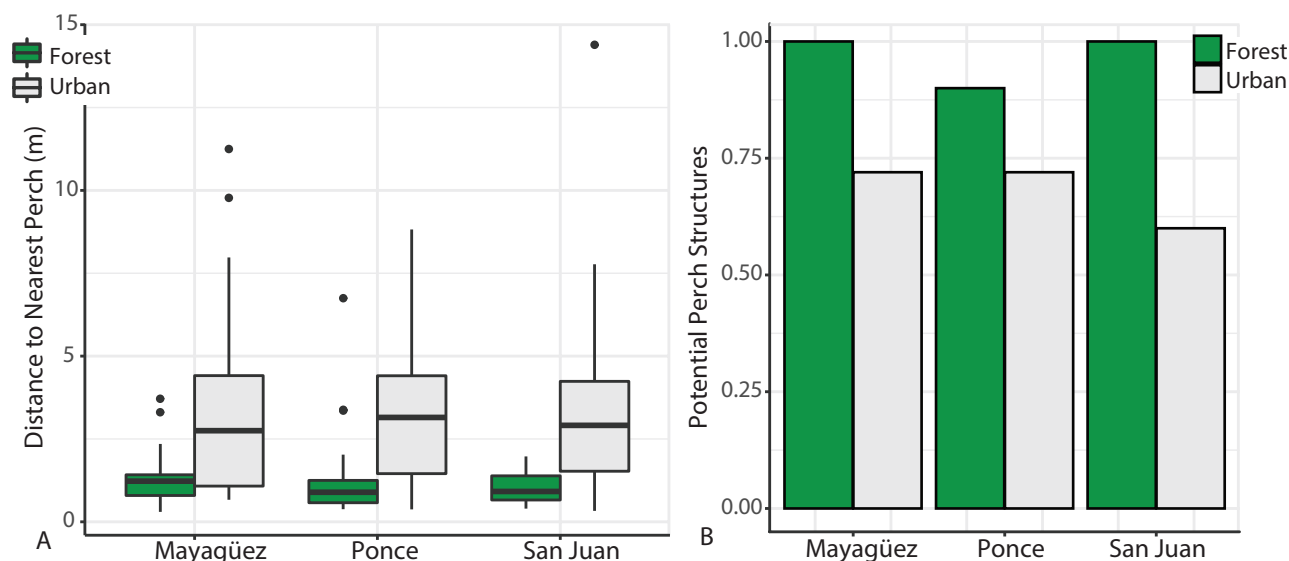


Figure 4. Urban habitats have perches that are more isolated compared with forest habitats and fewer potential perch sites overall. A, perch isolation, i.e. distance from randomly sampled locations to the nearest (alternative) structure. Differences between urban and forest pairs were significant across all municipalities, with no significant regional differences ($***P < 0.001$ for the main effect of habitat type across all municipalities). B, perch abundance, i.e. proportion of randomly sampled locations within each site that fell on a large permanent structure (e.g. tree, building, fence). There were no overall differences between municipalities, but there was a significant interaction of habitat type \times municipality (statistical significance for urban–forest contrasts within each municipality shown above each pair of bars: $*P < 0.05$, $***P < 0.001$).

Table 1. Results from MANCOVAs for the three morphological variables across habitats within municipalities

	Wilks' λ	d.f.	F	P -value
Habitat	0.922	3, 178	5.055	0.002
Municipality	0.57	6, 356	19.272	< 0.001
Habitat \times municipality	0.899	6, 356	3.244	0.004

Significant effects are indicated in bold. A significant interaction effect indicates that the effect of habitat on tail crest morphology differs by municipality.

Table 2. Results from ANCOVAs, subsequent to the MANCOVA in Table 1

	d.f.	F	P-value
Habitat			
Area of crest	1, 180	12.771	< 0.001
Maximal crest height	1, 180	8.779	0.003
Crest length	1, 180	11.305	< 0.001
Municipality			
Area of crest	2, 180	45.787	< 0.001
Maximal crest height	2, 180	43.440	< 0.001
Crest length	2, 180	1.818	0.165
Habitat × municipality			
Area of crest	2, 180	0.179	0.836
Max. crest height	2, 180	0.152	0.859
Crest length	2, 180	5.927	0.003

Significant effects are indicated in bold. A significant interaction effect under ‘‘Habitat × municipality’’ indicates that the effect of habitat on tail crest morphology differs by municipality.

without tail crests or with only very small crests. Our results were qualitatively unaffected and still showed a significant effect of urbanization on tail crest morphology (Supporting Information, Appendix S4).

DISCUSSION

Although morphological evolution in *Anolis* has been studied extensively (reviewed by Losos, 2009), we are aware of relatively few studies that have explored the evolution, behavioural ecology or functional significance of the anole tail crest. Here, we measured the tail crest in individuals of the crested anole (*A. cristatellus*) in different geographical locations and in urban and forest habitats throughout their native range on the island of Puerto Rico. We found that *A. cristatellus* tail crests differed regionally across the island and locally between urban and forest habitats.

At the regional scale, we found that lizards in environments that are hot and dry tended to have larger tail crests. The three municipalities that we sampled, Mayagüez, Ponce and San Juan, differed significantly from one another in a range of climatic variables. Intriguingly, tail crest size covaried with climate among our sites, consistent with the hypothesis that the tail crest plays a thermoregulatory role in this species. Specifically, in Ponce (which is the hottest and driest of our three municipalities), lizards had the largest tail crests, and in Mayagüez (where the climate is coolest and wettest), lizard tail crests were smallest. However, the three municipalities did not differ in habitat openness or perch abundance as measured by aerial imagery. Thus, at the regional scale, we found support for the thermoregulation hypothesis, but not for signal detection.

Nevertheless, we need to be careful not to overstate the significance of this pattern. In particular, if tail crest size and climate differed in a completely random way among sites, with only three localities the probability of finding the largest tail crests in the hottest site and the smallest tail crests in the coolest is one in six. Furthermore, it is hard to say whether our spatial analysis has captured habitat structure at the scale most relevant to signal detection. In particular, although habitat openness clearly differs between habitats, it is not equally obvious whether or not greater openness invariably translates to longer signalling distances, a key component to the signalling hypothesis for the tail crest (Charles & Ord, 2012). For instance, in the most open habitats, lizards might tend towards a clumped distribution in the few regions where suitable perches are found.

Within municipalities, we found that tail crests differed consistently between urban and forest environments. Compared with forest lizards, urban lizards have tail crests that are larger in area and taller in height, relative to body size, across all three municipalities. Our finding of phenotypic differences between urban and forest lizards is consistent with previous research on the same individuals sampled in this study showing that urban lizards have relatively longer limbs and toes with more subdigital lamellae (Winchell *et al.*, 2016). Genetic analysis also demonstrated that urban and forest populations within each region are not differentiated for a mitochondrial marker and, conversely, that paired populations of each of the three regions form genetically distinct clades (Winchell *et al.*, 2016). This suggests that the pattern of morphological divergence between urban and forest lizards that we have measured, if genetically based, probably arose independently in each of the three geographical regions. Moreover, the convergent pattern of tail crest variation suggests that this morphological shift might have occurred in response to similar selective pressures across urban sites.

Urban environments worldwide tend to be significantly warmer than nearby forest habitats, and our sites follow this common pattern. Differences in thermal environments in urban areas can create strong selection pressures on anoles, leading to elevated body temperatures and higher thermal tolerances in urban lizards (Winchell *et al.*, 2016; S.C. Campbell-Staton and K.M. Winchell, unpublished observations). Consistent with the regional pattern of tail crest variation, lizards within each municipality have significantly larger tail crests in the hot and dry urban habitats. These results are thus also consistent with some type of relationship between tail crest morphology and climate. Our findings contrast with a study of intraspecific variation in tail crest size of *A. oculatus*, which found that tail crest variation across the island

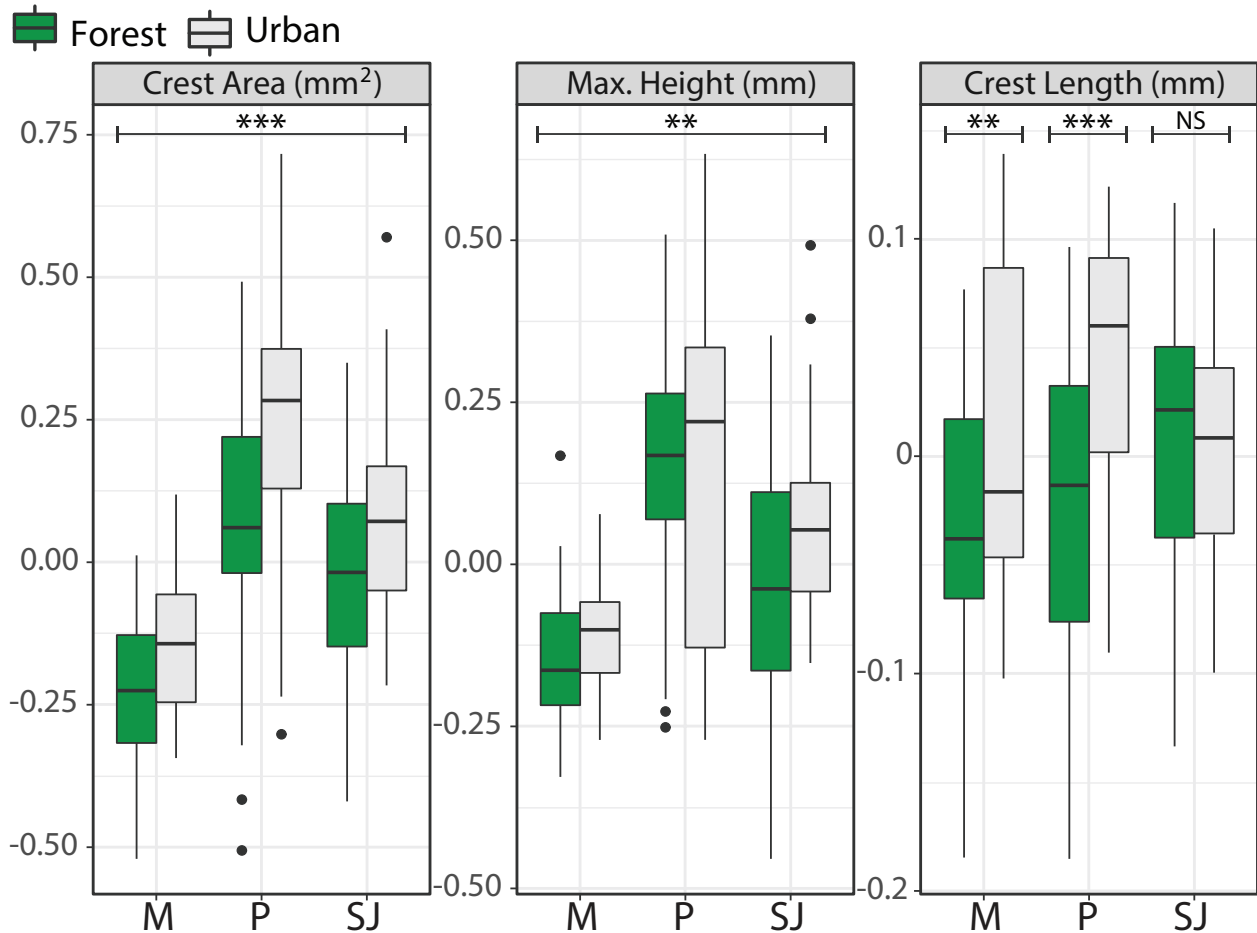


Figure 5. All three tail crest measurements differed consistently between urban and forest populations except for tail crest length, which was not significantly different between urban and forests habitats in San Juan and did not differ between municipalities (significance levels: NS, $P > 0.05$, $**P < 0.01$, $***P < 0.001$). Traits are shown here as relative trait values (residuals of body size) on a logarithmic scale. Municipalities: M, Mayagüez; P, Ponce; SJ, San Juan. Pairwise contrasts for urban vs. forest habitats within each municipality are shown for tail crest length only because the interaction of municipality and habitat type (urban/forest) was not significant for the other two measures.

of Dominica was not correlated with any climatic variables, including temperature (Malhotra & Thorpe, 1997). However, *A. oculatus* is a forest shade species that occupies variable microhabitats throughout Dominica (Knox *et al.*, 2001), whereas *A. cristatellus* is a mixed/open forest species and a trunk-ground ecomorph, generally occupying low trunk habitats throughout its range (Losos, 2009). As such, the two species might not experience the same range of thermal environments and might therefore exhibit different patterns in tail crest morphology. Moreover, urban environments may contain areas with elevated temperatures (e.g. car parks) or decreased hydric stress (e.g. in heavily landscaped areas with supplemental watering). This microclimatic heterogeneity may not be captured adequately by remote-sensing data but can impact lizard activity and thermoregulatory

strategies (Ackley *et al.*, 2015). Although our results are consistent with the thermoregulatory hypothesis for *A. cristatellus*, future research should investigate the functional relevance of tail crests in hot and dry environments and how this varies with microclimate within urban environments.

In addition to climatic differences, urban and forest habitats also differed in their perch abundance and perch isolation. Urban habitats tended to have fewer large structures on which lizards can perch (e.g. trees, buildings, fences) and were significantly more open compared with forests. We found that lizards had significantly larger tail crests in the open, more sparse urban habitats than in the closed forest habitats within each municipality. Although these patterns between habitat types do not mirror regional patterns across municipalities (i.e. structural habitat did not

differ across municipalities), it is still possible that differences in tail crest morphology between urban and forest populations are related to differences in habitat openness and perch distribution at this local scale. Larger tail crests in anoles might be important as long-range signals in open environments where males must perch farther from one another and from potential mates (Charles & Ord, 2012). That being said, as we noted previously, a key component of this hypothesis is that the mean signalling distance is larger in open than in closed habitats; a prediction that may or may not hold for our study sites and that is dependent on behaviour in addition to the structural environment.

Our results clearly show that tail crest morphology varies at both regional (municipality) and local (habitat) scales. Overall, municipality explains more variance than habitat (Fig. 5; Table 1), corresponding to greater differences in tail crest morphology between different areas on the island of Puerto Rico than between urban and forest habitats, within a region. This result is not entirely surprising given the evolutionary history of these populations. Previous work on these same lizards showed that the populations from three municipalities were genetically distinct from one another, as evidenced by high levels of Φ_{ST} for a mitochondrial marker. Within each municipality, urban and forest populations showed relatively little genetic divergence, probably reflecting much more recent separation or ongoing gene flow between the two habitat types (Winchell *et al.*, 2016). It is therefore not surprising to find greater morphological variation between regions than between habitats within a region. The fact that we also found consistent morphological differences between habitats within each region, and that these differences were correlated with similarly consistent differences in habitat characteristics between sites, nonetheless suggests that similar underlying factors might underlie morphological divergence between urban and natural environments in each region.

In addition to the abiotic differences we documented, it is possible that biotic factors relevant to tail crest variation also differ between urban and forest environments. Predation might be one such factor affecting tail crest size in *A. cristatellus*, although the differences in the magnitude and effect of predation pressure between urban and forested sites are generally not well documented. Nevertheless, prior research showing that urban lizards can have higher rates of tail autotomy and injury suggests that urban lizards might face higher predation pressure, altered predator communities and/or increased intraspecific aggressive interactions (Tyler *et al.*, 2016; Winchell *et al.*, 2019). *Anolis cristatellus* anti-predator displays are thought to include tail movement (Leal & Rodriguez-Robles, 1995). If so, and if predation is indeed higher in urban

areas, then urban lizards with larger tail crests might be favoured by natural selection if a larger tail crest results in a more effective predator display. In our dataset, rates of tail autotomy did not differ between individuals with crests and individuals without crests (Supporting Information, Appendix S1). However, tail autotomy is but one proxy for predation, and might not fully reflect either predation pressure or predator efficacy (e.g. Lovely *et al.*, 2010).

It is important to note that we cannot rule out the possibility that the differences in crest size that we observed between urban and forest habitats might be the result of phenotypic plasticity, rather than being genetically based. In anoles, a number of physiological, behavioural and morphological traits have been shown to be plastic in certain conditions, including limb length, thermal tolerance and display behaviour (Kolbe & Losos, 2005; Ord *et al.*, 2010; Kolbe *et al.*, 2014). Whether tail crest morphology is influenced likewise by environmental conditions is unknown. Although the effects of temperature on the growth and development of specific traits have not been well studied, the altered thermal profile of urban environments has been shown to influence the developmental rate in anoles. Tiatragul *et al.* (2017) showed that increased temperatures in urban areas accelerated embryonic development in *A. cristatellus* and *A. sagrei*, although hatchling phenotypes were unaffected. Other studies have shown little evidence for temperature-induced plasticity in morphological traits that lasts beyond the egg or hatchling stages (Elphick & Shine, 1998; Goodman & Heah, 2010; Pearson & Warner, 2016). In addition, it is possible that the crest continues to grow after an individual reaches sexual maturity and might therefore be affected by environmental conditions throughout its entire lifetime, not only during the embryonic and hatchling/juvenile stages. Further work focusing on the development and growth of the tail crest in different environments would be extremely informative.

In conclusion, the patterns of repeated morphological variation between lizards from urban and forest habitats across Puerto Rico suggest that the tail crest is an important trait that may be shaped by similar natural or sexual selection pressures across cities, where larger tail crests seem to be favoured. Although there has been limited study on the function of tail crests, we find some support for a thermoregulatory hypothesis at both regional and local habitat scales, and some support for a signal detection hypothesis at the local habitat scale. Of course, it is also possible that an unknown selective factor that differs between urban and forest habitats underlies the tail crest patterns that we have observed. Thus, perhaps the most important finding of this study is merely that tail crests differ between urban and forest habitats in

a consistent way, regardless of the underlying cause. Consequently, it seems probable that differential natural selection between habitats might be shaping this characteristic. Further studies of both intra- and interspecific variation in the tail crest and its association with climate, structural habitat and social context will be essential to our understanding of the roles of natural and sexual selection in the evolution of this interesting trait.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article at the publisher's web-site:

Appendix S1. Exclusion of autotomized tails.

Table S1. Number of lizards per site that were excluded because of autotomy before the end of the tail crest and those with intact tails analysed.

Appendix S2. Relationship between body size, tail damage and tail morphology across sites.

Table S2a. Model summary of binomial generalized linear model of crest presence interacting with habitat type.

Table S2b. Type III ANOVA for the three tail crest morphology models analysed to investigate potential interactions between body size, habitat and municipality.

Figure S2. The relationship between crest area and body size differed by site in the slope (but not the direction) of the relationship between municipalities. Overall, crest area scaled positively with body size across all sites, and the relationship did not differ significantly between forest and urban habitats.

Appendix S3. Analysis of normality for MANCOVA.

Figure S3. Residuals of the MANCOVA for all three morphological variables were normally distributed.

Table S3. Mardia's multivariate normality test.

Appendix S4. Analysis of tail crest presence and effect of 'absent' crests on analyses.

Figure S4a. Dotplots for the raw values of the three tail crest measurements by municipality and colored by habitat type (green for natural forest habitats and gray for urban habitats).

Figure S4b. Presence of tail crests did not differ between urban and forest habitat types but did differ regionally.

Table S4a. Comparison of results from MANCOVAs for the three morphological variables excluding individuals without tail crests but including ambiguous tails.

Table S4b. Comparison of results from ANCOVAs, subsequent to the MANCOVAs above, for the three morphological variables. Model A includes all individuals, model B excludes individuals without tail crests but includes ambiguous tails, and model C excludes all individuals that did not have an obvious tail crest.

SHARED DATA

Data used in this paper may be downloaded from the Harvard Dataverse repository (doi:10.7910/DVN/UU5Y1D).