Ecomorphology Within an Ecomorph: Variation in Morphology, Ecology, and Behavior Within Cybotoid Anoles

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Caribbean *Anolis* lizards exhibit a complex suite of ecological, morphological, and behavioral traits that allow their specialization to particular microhabitats. These microhabitat specialists, called ecomorphs, have independently evolved on the islands of the Greater Antilles, and diversification among anole ecomorphs has been the focus of many studies. Yet, further habitat specialization also occurs among species within the same ecomorph. In this study, we examined ecological, morphological, and behavioral divergence in three Hispaniolan trunk-ground anole species: *Anolis cybotes, A. marcanoi*, and *A. longitibialis*. We examined relationships among limb morphology, locomotor behavior, and perch use, and between sexual size dimorphism (a measure for the strength of sexual selection) and sexually-selected morphological traits and behaviors. We found significant differences among the three cybotoid species that mirror differences among the ecomorphs. Across anoles and within cybotoids, species that have longer limbs tend to move less frequently, occupy broader perches, and have smaller fourth toes with fewer lamellae. In addition, species that have high male-biased size dimorphism have larger heads, smaller dewlaps, and smaller testes. These results are consistent with the predictions of sexual selection theory, in that species with large male body size may have larger heads due to increased male-male combat, and smaller testes due to a trade-off between pre- and postcopulatory selection. Overall, this study suggests that a combination of local adaptation and sexual selection produce ecomorphological diversification within cybotoid anoles of the same ecomorph group.

Ecomorphology, the study of the relationships between an organism's ecology and its morphology, has revealed that evolution often shapes complex suites of traits to allow specialization to a particular environment. The evolution of such phenotypic suites has facilitated divergence into diverse habitats, driving adaptive radiations in multiple taxa (reviewed in Schluter, 2000). Further, in some groups, ecomorphological evolution has produced strikingly repeatable results, producing discrete types of species adapted to a set of niches (e.g., *Eleutherodactylus* frogs: Hedges, 1989; *Anolis* lizards: Losos et al., 1998; cichlid fish: Danley and Kocher, 2001; boas and pythons: Esquerré and Keogh, 2016). When habitat specialization in multiple, independent lineages results in the convergent evolution of ecology, morphology, and behavior, the resulting types are termed "ecomorphs" (following Williams, 1972), defined as specialists to a particular microhabitat. While the evolution of ecomorphs has been a focus of macroevolutionary studies, divergence *within* an ecomorph, which can result in further habitat specialization (Losos, 1996), has received far less attention.

Of the approximately 150 species of anole lizards (genus *Anolis*) in the Caribbean, most are categorized as one of six ecomorphs: trunk-ground, trunk-crown, trunk, grass-bush, twig, and crown giant (Williams, 1972, 1983; Losos, 2009). Specific ecomorphs have independently evolved on each of the islands of the Greater Antilles (Losos et al., 1998), and species within an ecomorph exhibit coordinated combinations of adaptations to their structural microhabitat. For example, trunk-ground species are generally medium-sized brown lizards with long limbs and a stocky build, and as sit-and-wait predators they perch low on tree trunks and forage on the ground and in low vegetation. Twig species, on the other hand, are mottled gray or brown, have short limbs and narrow bodies, and move slowly and cryptically on small branches in the canopy. There is a rich body of literature that compares a wide range of traits both among and

within the anole ecomorphs (reviewed in Losos, 2009), but the majority of studies focused on within-ecomorph variation has focused on variation in traits not directly associated with anole ecomorphology (e.g., thermal ecology: Ruibal, 1961, Rand, 1964, Hertz et al., 2013; body size: Muñoz et al., 2014; but see Wollenberg et al., 2013). In this study, we examined variation in the morphological, ecological, and behavioral traits that distinguish different ecomorphs within a group of species of a single ecomorph: the Hispaniolan trunk-ground anoles.

The closely-related cybotoid anoles in Hispaniola have spread into different macrohabitats across the Dominican Republic and Haiti; thus, this clade offers an ideal opportunity to test ecomorphological hypotheses within an ecomorph (Glor et al., 2003; Wollenberg et al., 2013). The group of cybotoid lizards includes *A. cybotes* (including *A. armouri* and *A. shrevei*, which are nested within *A. cybotes*; Wollenberg et al., 2013), *A. marcanoi*, *A. whitemani*, *A. longitibialis*, and *A. strahmi*. Here, we focus on three of these species (Fig. 1): *A. cybotes*, a macrohabitat generalist that occurs throughout the island of Hispaniola; *A. longitibialis*, a species restricted to rocky outcrops in the xeric southwestern Dominican Republic (Gifford et al., 2003); and *A. marcanoi*, a species that occurs in south-central Dominican Republic in semi-xeric to semi-mesic forest habitats (Glor et al., 2003). Using these three species, we tested the hypothesis that ecological traits associated with morphological differentiation *across* ecomorphs are also associated with morphological traits *within* an ecomorph.

First, one of the defining traits of anole ecomorphs is based on the relationship between limb morphology and perch diameter. Longer-limbed species (i.e., trunk-ground and trunk-crown species) predominantly use larger perches and exhibit higher sprint speeds on broad perches than shorter-limbed species (i.e., grass-bush and twig species; Losos, 1990; Irschick and Losos,

1999), while shorter limbs are generally associated with increased maneuverability on smaller perches (Losos and Sinervo, 1989; Irschick and Losos, 1998). In addition, toepad morphology is associated with clinging ability in anoles (Irschick et al., 1996), and the more arboreal anole ecomorphs generally have larger subdigital toepads, with more adhesive lamellae, than the more terrestrial ecomorphs (Glossip and Losos, 1997). Locomotor performance in anoles is also dependent on substrate type (smooth or rough) and angle of incline, with lizards running faster on rough substrates and larger males pausing or slipping more frequently on smooth, vertical substrates (Kolbe et al., 2015). Further, anoles preferentially use perches that allow maximum locomotor performance (Irschick and Losos, 1999, but see Kolbe et al., 2015). Based on these previous findings, we tested three predictions regarding limb morphology, perch use, and locomotor behavior. 1a) Species with longer limbs, larger toepads, and more lamellae on their toepads will use broader perches. 1b) Longer limbed species will perform more fast locomotor movements (i.e., runs and jumps), and shorter-limbed species will perform more slow movements (i.e., crawls). 1c) The macrohabitat generalist species (*A. cybotes*) will exhibit more variation in perch use, and thus locomotor behavior, than the two macrohabitat specialist species (*A. longitibialis* and *A. marcanoi*).

Second, anole ecomorphs differ in the extent of sexual size dimorphism (SSD), with trunk-ground and trunk-crown species exhibiting high male-biased SSD, and the other four ecomorphs with low SSD (Butler et al., 2000). This variation in SSD has been attributed to both ecological variation between the sexes and to intrasexual selection (Butler et al., 2000). Here, we use SSD as a proxy for the strength of pre-copulatory sexual selection, following the extensive empirical evidence that males in general, and particularly in lizards, evolve larger body sizes as the result of competition for mates or other resources (Andersson, 1994; Cox et al., 2003, 2007).

We tested the hypothesis that SSD in cybotoid anoles was associated with sexually-selected morphologies and behaviors, with the following predictions. 2a) Because intense male conflict in anoles may escalate to locking jaws (e.g., Greenberg and Noble, 1944; Jenssen et al., 2000), species with high SSD will exhibit relatively larger head dimensions, which offer stronger bite forces (e.g., Herrel et al., 1999, 2001). 2b) Male anoles perform displays that include extensions of the dewlap (a colorful throat fan), head-bobs, and push-ups in courtship and aggression (Jenssen, 1977). Thus, we predicted that species with high SSD will have larger dewlaps and more frequent dewlap and push-up display behaviors. 2c) Finally, because pre-copulatory male competition may be associated with an energetic trade-off with post-copulatory sperm competition (Preston et al., 2003; Fitzpatrick et al., 2012; Parker et al., 2013; Lüpold et al., 2014; Kahrl et al., *in press*), species with high SSD will have smaller testes.

MATERIALS AND METHODS

Measures of Behavior and Habitat Use.—We examined adult male anoles of the three focal species in the Dominican Republic during the summer breeding season in 2005, 2009, 2010, and 2015. We studied *Anolis cybotes* at Coralsol Beach Resort in La Ciénaga, Barahona (N 18° 03’42.2”, W -71° 06’39.9”); *Anolis longitibialis* in Manuel Goya (N 17° 50’8.5”, W -71° 27’0.5” and N 17° 48’ 9.216”, W -71° 26’ 51.792”); and *Anolis marcanoi* near El Matadero (N 18° 23’10.0”, W -70° 26’31.4”; N 18° 24’ 9.936”, W -70° 25’ 12.792”; and N 18° 19’ 30.18”, W -70° 17’ 20.508”). We performed behavioral observations and morphological measurements in 2015, and recorded habitat use in each of the four years of study.

We conducted 12-120 min focal observations on adult males of each species (*Anolis cybotes*: n = 43, 20.6 h observation; *Anolis longitibialis*: n = 31, 33.5 h observation; *Anolis*

marcanoi: n = 4, 6.5 h observation), as follows. We located undisturbed lizards by walking slowly through the habitat, and then observed the behavior of the focal lizard using binoculars, from a minimum distance of 5 m. During behavioral data collection, we recorded all display behaviors (focusing on dewlap extension and head-bob and push-up displays), locomotor movements (with each movement categorized as a run, crawl, or jump), and copulations. To prevent repeated observations of the same male, we only observed lizards on perches separated by a minimum of 10 m, and we systematically conducted observations in different areas of the locality each day. All observations were performed between 0930 and 1730, and never in inclement weather (i.e., rain). For each individual, we calculated rates of each display behavior (dewlap extensions, combined head-bobs and push-ups) per min, the total rate of locomotor movements per min, and the average duration of each dewlap extension in sec. We also determined the proportion of movements of each locomotor type (runs, crawls, and jumps) and the proportion of "fast" (runs or jumps) and "slow" (crawl) movements for each individual.

To obtain measures of habitat use, we noted the type of substrate on which each individual perched during behavioral observations. Perch diameter was collected for lizards that were seen before they were disturbed, and never for lizards that were first seen moving. Additionally, we recorded the orientation of the lizard on the perch as quasi-horizontal (0°-30°) or quasi-vertical (31°-90°).

Morphological Measurements.—To collect morphological measurements, we captured 7-17 adult males of each species by hand or noose. (These males were always not the same males on which behavioral observations were performed and perch data were collected.) We measured the snout-vent length (SVL) of each lizard to the nearest mm using a plastic ruler or digital calipers

to the nearest 0.01 mm. We also captured 11-26 adult females of each species from the same localities, and measured their SVL to obtain measures of SSD. We calculated the average male and female SVL for each species, and calculated the SSD of each species as:

$$SSD = \frac{Male\ SVL}{Female\ SVL} - 1$$

following Lovich and Gibbins (1992). If males are larger than females, then by convention this measure of SSD is positive.

For adult males, we measured a series of additional morphological traits. Mass was measured to the nearest 0.1 g using a Pesola spring scale, and external head and limb dimensions were each measured to the nearest 0.1 mm using digital calipers. Head length was measured as the distance from the parietal eye to the tip of the snout, head width was measured at the widest part of the skull (the anterior base of the cranium), and head depth was measured at the deepest part of the skull (immediately behind the eyes).We measured the femur length as the distance between the body wall and the most distal point of the knee, tibia length as the distance between the knee and the most distal point of the foot, and fourth toe length as the distance between the tip of the longest toe (metatarsal IV) not including the claw, and the point of insertion of the toe at the footpad. Three characteristics of the subdigital toepad on the fourth toe were also measured: number of lamellae on the toepad, pad length, and pad width. These traits were measured using digital flatbed scans with a resolution of 2400-3200 dpi, and were analyzed with the software Image J (NIH, Bethesda). All foot measurements were taken twice, and we used the mean of these measures in subsequent analyses (modified after Zani, 2000). We measured the hindlimb, fourth toe, and toepad on the right side of the lizard unless it was injured, in which case we measured the left side.

To measure dewlap size, we held the lizard's head parallel to a background of white graph paper and photographed the dewlap, fully extended using forceps, twice for each animal. We measured the area of both photographs of each dewlap using ImageJ (NIH), and then used the larger of the two measures in subsequent analyses.

Because these animals were also used in a series of other physiological studies, a subset of lizards on which morphological measurements were made were transported to Trinity University and euthanized by rapid decapitation. Immediately following euthanasia, we opened the body cavity and measured the length and width of the right testis, before the testis was removed from the animal. These measures were used to calculate testis volume using the formula for the volume of an ellipsoid ($4/3\pi a^2 b$), where a is the radius of the width of the testis and b is the radius of its length. We converted this measure of testis volume to a measure of mass using the density of testis tissue ($1 mm^3/mg$) reported by Licht and Pearson (1969). We then calculated gonadosomatic index (GSI, the ratio of testis mass to body mass) for each individual.

Statistical Analysis.—We conducted all statistical analyses in JMP (version 9.0, 2010; SAS Institute Inc., Cary, NC). All morphological traits were $\log_{10}$ transformed for our analyses. As head measurements are highly correlated, we used Principal Component Analysis (PCA) to summarize $\log_{10}$ head length, $\log_{10}$ head width and $\log_{10}$ head depth. This returned one significant PC ($\chi_2 = 135.34$, df = 5, $P < 0.001$) that explained 92.6% of the variance and had positive loadings of head length (0.95), depth (0.96), and width (0.97). We interpreted this PC as an overall measure of head size, and included this as Head Size PC in subsequent analyses.

To test for differences among species in body shape, we used body-sized corrected residuals from the regression of the $\log_{10}$ transformed trait on $\log_{10}$ SVL, which included all

individuals of all species. We then used a series of analyses of variance (ANOVA) to test for differences among the three species in $\log_{10}$ SVL, Head Size PC, GSI, number of lamellae on the fourth toepad, and the residuals of mass, head length, head width, head depth, femur length, tibia length, fourth toe length, fourth toepad length and width, and dewlap size. We used Tukey's HSD post-hoc tests for all pair-wise comparisons following significant ANOVA results.

To test for differences among species in rates of behavioral traits (dewlap extension, head-bobs and pushups, total movements), proportion of type of locomotor movements (runs, crawls, or jumps), and fast (runs and jumps) and slow (crawls) movements, and the average duration of dewlap extension among the species, we again used a series of ANOVA. Copulation was rarely observed during these observations, and thus could not be statistically analyzed. We used a series of Brown-Forsythe tests to assess the equality of variance in behavioral traits among the species. We also tested for differences in substrate use and substrate orientation (horizontal or vertical) between species using chi-square tests. Because our data on perch diameter for *A. longitibialis* was very limited, *A. longitibialis* was removed from the analysis of diameter. We then compared differences in perch diameter between *A. cybotes* and *A. marcanoi* using ANOVA.

RESULTS

Morphological analysis showed that the three cybotoid species differed significantly in body size and limb dimensions (Table 1, Fig. 2, Supplemental Table 1). *Anolis cybotes* and *A. longitibialis* did not differ in SVL, but *A. cybotes* had the largest relative mass, while *A. marcanoi* was the smallest species in both measures of overall size. We also found that *A. longitibialis* had significantly longer hindlimbs and shorter fourth toes with fewer lamellae than *A. cybotes* and *A.*

marcanoi, and *A. cybotes* had shorter hindlimbs, longer toes, and more lamellae than the other species (Table 1, Fig. 2).

We also found that the three species differed in locomotor behavior and perch type (Fig. 3, Supplemental Table 2). *Anolis cybotes* moved substantially more frequently than *A. longitbialis* ($F_{2,49} = 6.23$, $P = 0.003$, Fig. 3), and had a higher variation in the frequency of their movements ($F_{2,75} = 6.83$, $P = 0.001$). However, the proportion of each type of movement (runs, crawls, and jumps) did not differ among the three species (all $P > 0.17$; Fig. 3) nor did the proportion of fast movements (runs, and jumps) ($F_{2,69} = 0.10$, $P = 0.25$), or slow movements ($F_{2,69} = 2.38$, $P = 0.10$). We also did not find a significant difference in the variance of each of these types of movements (all $P > 0.18$).

Further, perch use differed substantially among the species ($\chi_2 = 122.9$, df = 10, $P < 0.001$), such that *A. longitibialis,* the species with the longest limbs and shortest toes and the fewest lamellae on their fourth toepads, was most often found on rocks, while *A. marcanoi* was most often found on fence posts and tree trunks. *Anolis cybotes* occupied the widest range of habitats, but was predominantly found on tree trunks and branches (Fig. 3), and was found on vertical branches more often than *A. marcanoi* and *A. longitibalis* ($\chi_2 = 13.05$, df = 2, $P = 0.001$). We found marginally significant differences in perch diameter between *A. cybotes* and *A. marcanoi* ($F_{1,26} = 3.06$, $P = 0.092$), where *A. cybotes* inhabited perches that were slightly narrower than *A. marcanoi*.

The three species also differed in the extent of SSD, with *A. longitibialis* (SSD = 0.352) exhibiting the most dimorphism, followed by *A. cybotes* (SSD = 0.169), and *A. marcanoi* (SSD = 0.085). Consistent with our predictions, the species with lowest SSD (*A. marcanoi*) had the smallest head (Head Size PC), but contrary to expectation, it exhibited the largest relative dewlap

(Table 1, Fig. 4D). Despite significant differences in the Head Size PC, the three species did not differ in head shape, as measured by the relative head length, width, and depth (Table 1). Further, our prediction that other morphological and behavioral traits associated with precopulatory sexual selection would be positively associated with the extent of SSD was not supported. *Anolis cybotes*, the species with intermediate SSD, performed a higher rate of dewlap extensions (Fig. 4E, $F_{2,77} = 12.20$, $P < 0.001$) and head-bobs/pushups (Fig. 4C, $F_{2,77} = 13.31$, $P < 0.001$) than *A. longitibialis,* although the species did not differ in the average duration of dewlap extension (Fig. 4F, $F_{2,59} = 2.18$, $P = 0.122$). The species also differed in testis morphology in the direction we predicted: the least dimorphic species (*A. marcanoi*) had a higher GSI that the two more dimorphic species (Fig. 4B, $F_{2,49} = 6.23$, $P = 0.003$).

DISCUSSION

Caribbean anoles are a classic example of adaptive radiation via niche partitioning, as ecomorphs have evolved to occupy a wide range of microhabitats (Williams, 1983; Losos, 2009). Yet even within the ecomorph groups, several clades have undergone further diversification within their particular microhabitat on a given island. Among the best-studied examples of intra-ecomorph diversification are the *Anolis sagrei* species group (Knouft et al., 2006), the cybotoid anoles (Glor et al., 2003; Wollenberg et al., 2013), and the *Anolis carolinensis* species group (Burnell and Hedges, 1990; Glor et al., 2004). These groups provide an ideal opportunity to study local adaptation and speciation within a clade. Here, we demonstrate significant differences in the morphology, ecology, and behavior of three species of closely-related cybotoid anoles. Consistent with our predictions, we show an association between substrate use, morphology and behavior, suggesting that each species of anole has experienced local adaptation to the variable

environments in the Dominican Republic. This pattern of adaptation mimics some patterns among anole ecomorphs, where species with larger perches tend to have longer limbs (Losos and Sinervo, 1989; Losos, 1990), but not others, as cybotoid species with longer limbs did not jump and run more often than crawl, as shown across ecomorphs (Losos, 1990). Our data also suggest that monomorphic species tend to have smaller heads and larger testes, consistent with theory on sexually selected traits in this group (Kahrl et al., *in press*). However, we found no relationship with dimorphism and social display behaviors. We explore these findings in more detail below.

Cybotoid anoles are a morphologically diverse group that inhabits a range of perch substrates and macrohabitats in the Dominican Republic (Glor et al., 2003; Wollenberg et al., 2013). We found that variation in microhabitat or substrate use has likely led to predicable changes in their morphology and behavior, similar to the patterns of ecomorphological divergence across Caribbean anoles (Losos and Sinervo, 1989; Losos, 1990). For these three species, we found an association between the use of narrower perches, shorter hindlimbs, and longer fourth toes with more lamellae on the toepad (Fig. 2, 3). This result mirrors patterns of adaption among species across ecomorphs, where species that live on smaller perches tend to have shorter limbs and with more lamellae, traits thought to aid in clinging ability while running on small perches (Cartmill, 1985; Pounds; 1988; Losos, 1990). It also corroborates weak associations between morphology and habitat previously described in this group (Glor et al., 2003; Wollenberg et al., 2013). In particular, *A. cybotes* was observed on small vertical perches most frequently, and had the shortest limbs, longest fourth toes, and most toepad lamellae of the three species. Together, these traits suggest that this species has likely adapted to climbing and running on narrow perches (Irschick et al., 1996; Zani 2000). On the other hand, *A. longitibialis*

likely has adapted to its saxicolous habitat with longer limbs that may enable this species to run faster and jump farther on broad rocky surfaces (Losos, 1990; Irschick and Losos, 1999).

Yet contrary to our hypothesis, there was no difference in the proportion of fast or slow movements between species of anoles. We did find, however, that the long-limbed saxicolous species (*A. longitbialis*) tended to move less frequently than short-limbed arboreal species (*A. cybotes*). Again, this relationship between microhabitat use and movement rate mimics a pattern observed among broader groups of anoles, where species that live on tree trunks or branches tend to have higher movement rates than those that live near or on the ground (Johnson et al., 2008). This variation in movement rate may be due to differences in foraging behavior among species, associated with variation in visibility from the lizard's perch (Johnson et al., 2008). In particular, microhabitats with a greater density of twigs and branches tend to obscure visibility, and lizards may move more frequently to survey the areas around them for prey or conspecifics (Moermond, 1979).

We also tested the hypothesis that generalist species, who use more diverse substrates, exhibit higher variation in their locomotor behavior. *Anolis cybotes* used the widest variety of substrates, while both *A. marcanoi* and *A. longitibialis* were observed on fewer substrate types, and on a single substrate type > 60% of the time (Fig. 3A). This difference in perch use may be a function of species-specific preferences for particular perches, perch availability, or an interaction of the two (Johnson et al., 2006). Consistent with our hypothesis, we also found that individual *A. cybotes* had higher variation in their overall movement rate than the other two species. Since *A. cybotes* occupies more diverse perch types, there may be higher variation in visibility and therefore, high variation in movement rates in this species (Moermond, 1979).

We measured several traits associated with pre- and postcopulatory selection in each of these three species to test for associations between the strength of sexual selection, morphology, and social behavior. These species vary in the extent of male-biased sexual size dimorphism (Fig. 4), which can result from precopulatory selection on male body size (Cox et al., 2003, 2007). We predicted that species that experience strong precopulatory selection (i.e., high SSD), would also have larger heads, and especially deeper and wider heads due to the use of the head for biting during male-male combat (Lailvaux et al., 2004; Lailvaux and Irschick, 2007). We found that the species with medium and high SSD (*A. longitibialis* and *A. cybotes*) had significantly larger heads (Head Size PC), than the species with low SSD, *A. marcanoi* (Fig. 4A). However, it is also possible that these species differ in head dimensions because of ecological factors such as the size of the prey available in each habitat (Roughgarden and Fuentes, 1977).

We also predicted that species with strong precopulatory selection would exhibit larger dewlaps and higher display rates, but we found that as SSD increased, dewlap area decreased, and there was no association between SSD and dewlap rate in these species (Fig. 4). There have been a variety of hypotheses proposed to explain variation in dewlap size and patterning among species of anoles, many of which have found weak or non-significant support for differences among ecomorph groups or for variation due to species recognition (Nicholson et al., 2007). Yet, Williams and Rand (1977) found that among islands, populations that contained few species of anoles did not differ in their dewlaps when other cues for species recognition were present (i.e., strong differences in other phenotypes). However, in populations where other cues were lacking, anoles tended to have more variable dewlap colors (William and Rand, 1977). Among the three species in this study, *A. marcanoi* has a reddish pink dewlap, while both *A. cybotes* and *A. longitibialis* have pale yellow or white dewlaps. As *A. cybotes* and *A. marcanoi* have slightly

overlapping geographical ranges, the observed differences in color and size of the dewlap may aid in species recognition, especially given the physical similarities between these two species. Additionally, dewlap size is weakly associated with habitat illumination, which may also be important in driving the evolution of the dewlap (Losos and Chu, 1998; Leal and Fleishman, 2004), although that was not a focus of the present study.

Finally, we observed that species with stronger precopulatory selection (SSD) had a smaller GSI (Fig. 4B). This pattern was consistent with our predictions of a trade-off between pre- and postcopulatory selection, and mirrors a trade-off that exists among other species of anoles (Lailvaux et al., 2004; Lailvaux and Irschick, 2007; Kahrl et al., *in press*). This suggests that the patterns of diversification in morphology due to sexual selection that occurs among species across ecomorphs are also occurring within ecomorph groups.

Our current results may inform patterns of divergence among other groups of sympatric and allopatric anole species within the same ecomorph in Cuba and Hispaniola that exhibit niche partitioning (Losos et al., 2003; Glor et al., 2004; Knouft et al., 2006) similar to the patterns observed among ecomorphs on each island (Williams, 1983; Losos, 1990; Losos et al., 1998). In particular, the cybotoid group of anoles show significant differences in their morphology that is independent of their phylogenetic relationships (Glor et al., 2003), and can be attributed to a combination of microhabitat and genetic differentiation between these species (Wollenberg et al., 2013). Here, we found that the closely related species *A. cybotes*, *A. longitibialis*, and *A. marcanoi* exhibited striking differences in their morphology, ecology, and behavior that are likely due to local adaptation to varying macro- and microhabitats and differences in the strength of sexual selection among species. In sum, ecomorphological variation can indeed produce divergence both among and within anole ecomorphs.

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506 **FIGURE LEGENDS**

507 **Figure 1**. Photographs of adult male *A. cybotes* (A), *A. marcanoi* (B), and *A. longitibialis* (C),
508 and their respective representative habitats: La Ciénaga, Barahona (D), the road to El Matedero
509 (E). and Manuel Goya (F).

510

511 **Figure 2**. Means ± standard error of $\log_{10}$ snout-vent length (SVL) (A), residual femur length
512 (B), residual tibia length (C), residual fourth toe length (D), number of lamellae on the fourth
513 hindlimb toe (E), and residual fourth toepad length (F) for *A. cybotes* (N = 17), *A. marcanoi* (N =
514 7), and *A. longitibialis* (N = 17). Species averages with different superscripts are significantly
515 different ($P < 0.05$).

516

517 **Figure 3.** Comparisons of ecology (substrate use, A), and behavior (mean ± S.E. of the
518 movement rate, B, and movement type, C) for *A. cybotes* (CYB), *A. marcanoi* (MAR), and *A.*
519 *longitibialis* (LON). Here, substrate use (A) is graphed as a proportion of total observation.
520 Movement rate (B) was calculated as the total number of movements (run, crawl, and jump) per
521 minute. Movement type (C) is expressed as a proportion of the total movements observed for
522 each species.

523

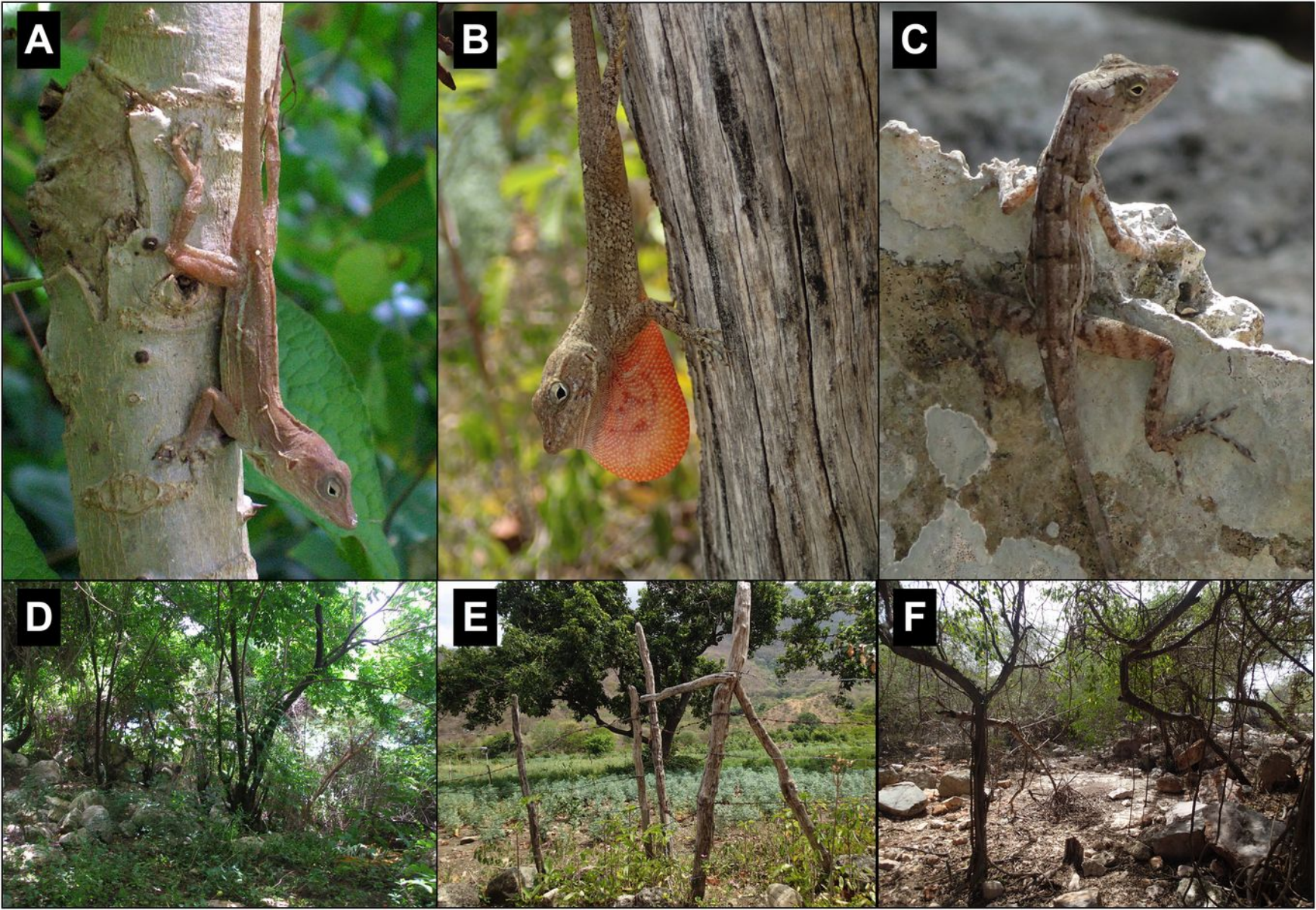
524 **Figure 4.** Comparisons of sexual size dimorphism (SSD) and other sexually selected traits in
525 cybotoid anoles. Means ± S.E. of Head Size PC (A), GSI (B), pushups per minute (C), residual
526 dewlap area (D), dewlap extensions per minute (E), and dewlap extension time (F) for *A. cybotes*
527 (N = 17), *A. marcanoi* (N = 7), and *A. longitibialis* (N = 17). These data are presented, from left

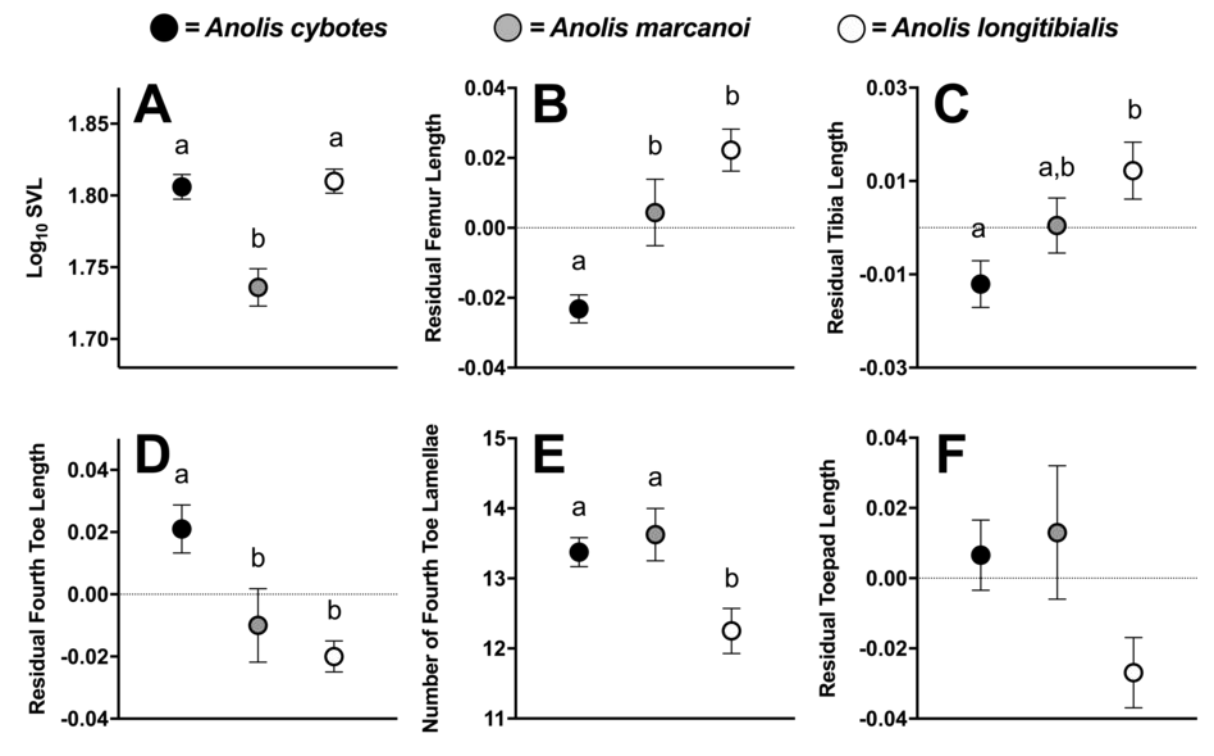
528 to right, by the extent of sexual size dimorphism in each species: *A. marcanoi* (SSD = 0.085), *A.*

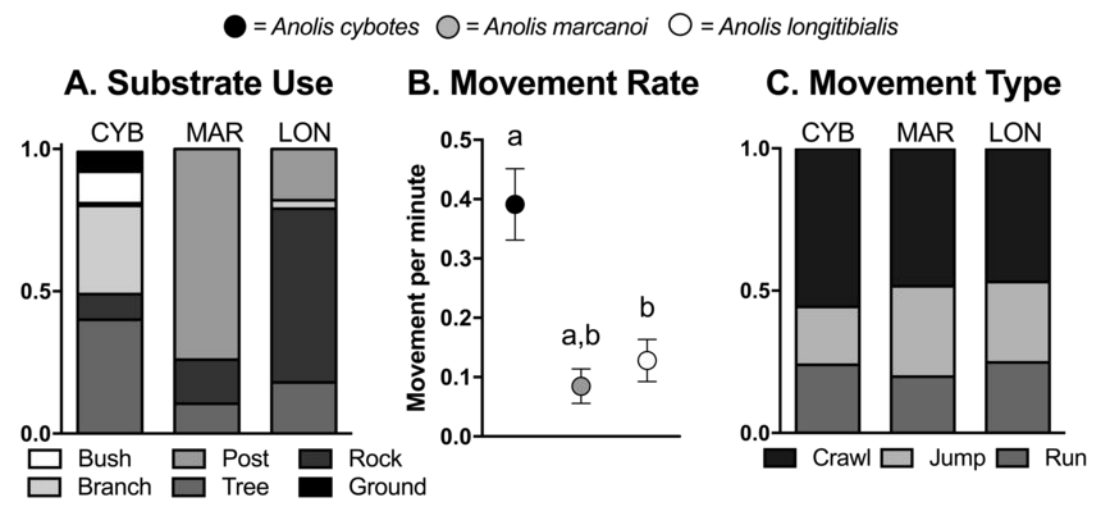
529 *cybotes* (SSD = 0.169), and *A. longitibialis* (SSD = 0.352).

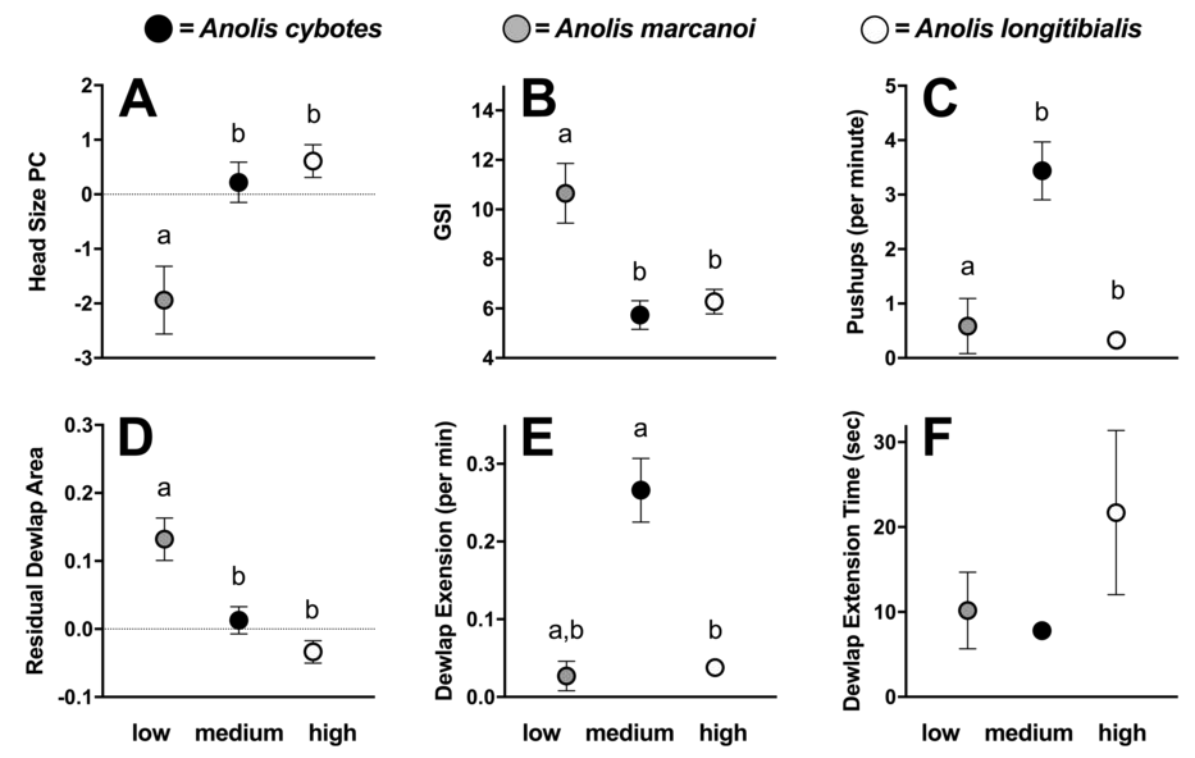
Table 1. Results from separate ANOVAs testing differences in log_{10}-transformed body size (SVL), Head Size PC, and body size corrected morphology (residuals of morphological traits regressed against SVL) in *Anolis cybotes*, *Anolis longitibialis*, and *Anolis marcanoi*. Significant comparisons are indicated with *.

	$F_{2, 39}$	P
Log_{10} SVL	12.24	<0.001*
Residual Mass	5.33	0.009*
Residual Head Length	2.86	0.069
Residual Head Width	0.35	0.740
Residual Head Depth	0.33	0.718
Head Size PC	8.15	0.001*
Residual Femur Length	16.16	<0.001*
Residual Tibia Length	4.66	0.015*
Residual Fourth Toe	9.47	<0.001*
Residual Toepad Length	2.12	0.159
Residual Toepad Width	3.30	0.069
Number of Lamellae	5.56	0.018*
Residual Dewlap Size	11.89	0.001*


A
B
C
D
E
F


= Anolis cybotes
= Anolis marcanoi
= Anolis longitibialis
A
Log10 SVL
1.85
1.80
1.75
1.70
a
b
a
B
Residual Femur Length
0.04
0.02
0.00
-0.02
-0.04
a
b
b
C
Residual Tibia Length
0.03
0.01
-0.01
-0.03
a
a,b
b
D
Residual Fourth Toe Length
0.04
0.02
0.00
-0.02
-0.04
a
b
b
E
Number of Fourth Toe Lamellae
15
14
13
12
11
a
a
b
F
Residual Toepad Length
0.04
0.02
0.00
-0.02
-0.04


● = Anolis cybotes ● = Anolis marcanoi ○ = Anolis longitibialis
A. Substrate Use
CYB
MAR
LON
1.0
0.5
0.0
Bush
Branch
Post
Tree
Rock
Ground
B. Movement Rate
Movement per minute
0.5
0.4
0.3
0.2
0.1
0.0
a
a,b
b
C. Movement Type
CYB
MAR
LON
1.0
0.5
0.0
Crawl
Jump
Run


= Anolis cybotes
= Anolis marcanoi
= Anolis longitibialis
A
Head Size PC
B
GSI
C
Pushups (per minute)
D
Residual Dewlap Area
E
Dewlap Exension (per min)
F
Dewlap Extension Time (sec)
low
medium
high
Sexual size dimorphism