

1 **Ecomorphological variation in three species of cybotoid anoles**

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3 ARIEL F. KAHRL¹, BRITTNEY M. IVANOV², KATHARINA C. WOLLENBERG VALERO³, AND MICHELE

4 A. JOHNSON^{2,4}

5 ¹ Zoologiska institutionen: Etologi, Stockholm University, Stockholm, Sweden SE-10691

6 ² Department of Biology, Trinity University, San Antonio, TX, USA 78212

7 ³ School of Environmental Sciences, University of Hull, Hull, HU6 7RX, United Kingdom

8
9 ⁴ CORRESPONDENCE: e-mail, mjohnso9@trinity.edu

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12 ABSTRACT: Caribbean *Anolis* lizards exhibit a complex suite of ecological,
13 morphological, and behavioral traits that allow their specialization to particular microhabitats.
14 These microhabitat specialists, called ecomorphs, have independently evolved on the four islands
15 of the Greater Antilles, and diversification among anole ecomorphs has been the focus of many
16 studies. Yet, habitat specialization has also occurred among species within the same ecomorph
17 group. Here we examined ecological, morphological, and behavioral divergence in three
18 Hispaniolan trunk-ground species, the cybotoid anoles: *Anolis cybotes*, *A. marcanoii*, and *A.*
19 *longitibialis*. We found differences in limb morphology, locomotor behavior, and perch use
20 among the three cybotoid species that mirror differences across the ecomorphs. Within these
21 species of cybotoids, species that have longer limbs tend to move less frequently, occupy broader
22 perches, and have smaller fourth toes with fewer lamellae. We also observed that the species
23 with higher male-biased size dimorphism had larger heads, smaller dewlaps, and smaller testes.
24 These results are consistent with the predictions of sexual selection theory, in that species with
25 large male body size may have larger heads due to increased male-male combat, and smaller
26 testes potentially due to a trade-off between pre- and postcopulatory selection. Overall, this study
27 suggests that a combination of local adaptation to different structural habitats and sexual
28 selection may produce ecomorphological diversification within cybotoid anoles of the same
29 ecomorph group.

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31 **Key words:** *Anolis*; Dewlap; Hindlimb; Locomotor behavior; Perch use; Sexual size
32 dimorphism

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

47 Of the approximately 150 species of anole lizards (genus *Anolis*) in the Caribbean, most
48 are categorized as one of six ecomorphs: trunk-ground, trunk-crown, trunk, grass-bush, twig, and
49 crown giant (Williams 1972, 1983; Losos 2009). Specific ecomorphs have independently
50 evolved on each of the islands of the Greater Antilles (Losos et al. 1998), and species within an
51 ecomorph exhibit coordinated combinations of adaptations to their structural microhabitat. For
52 example, trunk-ground species are generally medium-sized (male SVL between 50-75 mm)
53 brown lizards with long limbs and a stocky build, and as sit-and-wait predators they perch low
54 on tree trunks and forage on the ground and in low vegetation. Twig species, on the other hand,
55 are mottled gray or brown, have short limbs and narrow bodies, and move slowly and cryptically
56 on small branches in the canopy. There is a rich body of literature that compares a wide range of

57 traits both among and within the anole ecomorphs (reviewed in Losos 2009; Wollenberg et al.
58 2013; Kamath and Losos 2017), but the majority of studies about within-ecomorph variation has
59 focused on variation in traits not directly associated with anole ecomorphology (e.g., thermal
60 ecology: Ruibal 1961; Rand 1964; Hertz et al. 2013; body size: Muñoz et al. 2014). In this study,
61 we examined variation in the morphological, ecological, and behavioral traits that distinguish
62 different ecomorphs within several species of a single ecomorph: the Hispaniolan trunk-ground
63 anoles.

64 These closely related anoles, termed the 'cybotoids', have spread into different
65 macrohabitats across the Dominican Republic and Haiti. Thus, this clade offers an ideal
66 opportunity to examine patterns of diversification within an ecomorph class (Glor et al. 2003;
67 Wollenberg et al. 2013). The group of cybotoid lizards includes *A. cybotes* (including *A. armouri*
68 and *A. shrevei*, which are phylogenetically nested within *A. cybotes*; Wollenberg et al. 2013), *A.*
69 *marcanoii*, *A. whitemani*, *A. longitibialis*, and *A. strahmi*. Here, we focus on three of these species
70 (Fig. 1): *A. cybotes*, a macrohabitat generalist that occurs throughout the island of Hispaniola,
71 generally at elevations below 1800 m; *A. longitibialis*, a species restricted to rocky outcrops in
72 the xeric southwestern Dominican Republic (Gifford et al. 2003); and *A. marcanoii*, a species that
73 occurs in south-central Dominican Republic in semi-xeric to semi-mesic forest habitats (Glor et
74 al. 2003). Using these three species, we tested for differences in ecological, behavioral, and
75 morphological traits among species of cybotoid anoles, and compared these patterns with the
76 known patterns of among-ecomorph diversification.

77 One of the defining traits of anole ecomorphs is based on the relationship between limb
78 morphology and perch diameter. Longer-limbed species (i.e., trunk-ground and trunk-crown
79 species) predominantly use larger or broader perches and exhibit higher sprint speeds on broad

80 perches than shorter-limbed species (i.e., grass-bush and twig species; Losos 1990; Irschick and
81 Losos 1999), whereas shorter limbs are generally associated with increased maneuverability on
82 smaller perches (Losos and Sinervo 1989; Irschick and Losos 1998). In addition, toepad
83 morphology is associated with clinging ability in anoles (Irschick et al. 1996), and the more
84 arboreal anole ecomorphs generally have larger subdigital toepads, with more adhesive lamellae,
85 than the more terrestrial ecomorphs (Glossip and Losos 1997). Locomotor performance in anoles
86 is also dependent on substrate type (smooth or rough) and angle of incline, with lizards running
87 faster on rough substrates and larger males pausing or slipping more frequently on smooth,
88 vertical substrates (Kolbe et al. 2015). Further, anoles preferentially use perches that allow
89 maximum locomotor performance (Irschick and Losos 1999; but see Kolbe et al. 2015). Based
90 on these previous findings, we tested three predictions regarding limb morphology, perch use,
91 and locomotor behavior: (1a) Species with longer limbs, larger toepads, and more lamellae on
92 their toepads will use broader perches. (1b) Longer-limbed species will perform more fast
93 locomotor movements (i.e., runs and jumps), and shorter-limbed species will perform more slow
94 movements (i.e., crawls). (1c) We predict that the macrohabitat generalist species (*A. cybotes*)
95 will exhibit more variation in perch use and locomotor behavior, than the two macrohabitat
96 specialist species (*A. longitibialis* and *A. marcanoii*). Though a range of perch diameters may be
97 available in each habitat, the variation of perches available in the specialist habitats may be
98 reduced, especially in desert habitats.

99 Anole ecomorphs also differ in the extent of sexual size dimorphism (SSD), with trunk-
100 ground and trunk-crown species exhibiting relatively high male-biased SSD, and the other four
101 ecomorphs exhibiting relatively low SSD (Butler et al. 2000; Cox et al. 2007). This variation in
102 SSD has been attributed to both ecological variation between the sexes and to intrasexual

103 selection (Butler et al. 2000; Cox et al. 2007). Here, we use SSD as a proxy for the strength of
104 precopulatory sexual selection, following the extensive empirical evidence that males in general,
105 and particularly in lizards, evolve larger body sizes as the result of competition for mates or other
106 resources (Andersson 1994; Cox et al. 2003, 2007). We tested for differences in sexually-
107 selected morphologies and behaviors, with the following predictions. (2a) Because intense male
108 conflict in anoles may escalate to locking jaws (e.g., Greenberg and Noble 1944; Jenssen et al.
109 2000), species with high SSD will exhibit relatively larger head dimensions, which offer stronger
110 bite forces (e.g., Herrel et al. 1999, 2001). (2b) Male anoles perform displays that include
111 extensions of the dewlap (a colorful throat fan), head-bobs, and push-ups in courtship and
112 aggression (Jenssen 1977). Thus, we predicted that species with high SSD will have larger
113 dewlaps and more frequent dewlap and push-up display behaviors. (2c) Finally, because
114 precopulatory male competition may be associated with an energetic trade-off with
115 postcopulatory sperm competition (Fitzpatrick et al. 2012; Parker et al. 2013; Lüpold et al. 2014;
116 Kahrl et al. 2016), we predicted that species with high SSD will have smaller testes.

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MATERIALS AND METHODS

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Measures of Behavior and Habitat Use

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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 (18°03'42.2"N, -71°06'39.9"W, datum = WGS84); *Anolis longitibialis* in Manuel Goya (17°50'8.5"N, -71°27'0.5"W and 17°48'9.216"N, -71°26'51.792"W); and *Anolis marcanoii* near El Matadero (18°23'10.0"N, -70°26'31.4"W; 18°24'9.936"N, -70°25'12.792"W; and 18°19'30.18"N, -70°17'20.508"W).

126 We performed behavioral observations and morphological measurements in 2015, and recorded
127 habitat use in each of the four years of study.

128 We conducted 12–120 min focal observations on adult males of each species (*Anolis*
129 *cybotes*: $n = 43$, 20.6 h observation; *Anolis longitibialis*: $n = 31$, 33.5 h observation; *Anolis*
130 *marcanoi*: $n = 4$, 6.5 h observation), as follows. We located undisturbed lizards by walking
131 slowly through the habitat, and then observed the behavior of the focal lizard using binoculars,
132 from a minimum distance of 5 m. During behavioral data collection, we recorded all display
133 behaviors (focusing on dewlap extension and head-bob and push-up displays), locomotor
134 movements (with each movement categorized as a run, crawl, or jump), and copulations. To
135 prevent repeated observations of the same male, we only observed lizards on perches separated
136 by a minimum of 10 m, and we systematically conducted observations in different areas of the
137 locality each day. All observations were performed between 0930 h and 1730 h, and never in
138 inclement weather (i.e., rain). For each individual, we calculated rates of each display behavior
139 (dewlap extensions, combined head-bobs and push-ups) per min, the total rate of locomotor
140 movements per min, and the average duration of each dewlap extension in sec. We also
141 determined the proportion of movements of each locomotor type (runs, crawls, and jumps) and
142 the proportion of “fast” (runs or jumps) and “slow” (crawl) movements for each individual.

143 To obtain measures of habitat use, we noted the type of substrate on which each
144 individual perched during behavioral observations. Perch diameter was collected for lizards that
145 were seen before they were disturbed, and never for lizards that were first seen moving.

146 Additionally, we recorded the orientation of the lizard on the perch as quasi-horizontal (0–30°)
147 or quasi-vertical (31–90°).

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149 Morphological Measurements

150 To collect morphological measurements, we captured 20 male and 20 female *A. cybotes*,
151 17 male and 20 female *A. longitibialis*, and 12 male and 11 female *A. marcanoi*, by hand or
152 noose (these males were not always the same males on which behavioral observations were
153 performed and perch data were collected). We measured the snout–vent length (SVL) of each
154 lizard to the nearest mm using a plastic ruler (nearest 0.1 mm). We calculated the average male
155 and female SVL for each species, which was used to calculate the SSD of each species as:

$$156 \quad SSD = \frac{Male\ SVL}{Female\ SVL} - 1$$

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

159 We measured a series of additional morphological traits in the adult males. Mass was
160 measured to the nearest 0.1 g using a Pesola spring scale, and external head and limb dimensions
161 were each measured to the nearest 0.1 mm using digital calipers (Mitutoyo, Japan). Head length
162 was measured as the distance from the parietal eye to the tip of the snout, head width was
163 measured at the widest part of the skull (the anterior base of the cranium), and head depth was
164 measured at the deepest part of the skull (immediately behind the eyes). We measured the femur
165 length as the distance between the body wall and the most distal point of the knee, tibia length as
166 the distance between the knee and the most distal point of the foot, and fourth toe length as the
167 distance between the tip of the longest toe (metatarsal IV) not including the claw, and the point
168 of insertion of the toe at the footpad. Three characteristics of the subdigital toepad on the fourth
169 toe were also measured: number of lamellae on the toepad, pad length, and pad width. These
170 traits were measured using digital flatbed scans with a resolution of 2400–3200 dpi, and were
171 analyzed with the software ImageJ (NIH, USA). All foot measurements were taken twice, and

172 we used the mean of these measures in subsequent analyses (modified after Zani 2000). We
173 measured the hindlimb, fourth toe, and toepad on the right side of the lizard unless it was injured,
174 in which case we measured the left side.

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

179 Because these animals were also used in a series of other physiological studies, a subset
180 of lizards on which morphological measurements were made were transported to Trinity
181 University and euthanized by rapid decapitation. Immediately following euthanasia, we opened
182 the body cavity and measured the length and width of the right testis, before the testis was
183 removed from the animal. These measures were used to calculate testis volume using the formula
184 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
185 the radius of its length. We converted this measure of testis volume to a measure of mass using
186 the density of testis tissue ($1 \text{ mm}^3/\text{mg}$) reported by Licht and Pearson (1969). We then calculated
187 gonadosomatic index (GSI, the ratio of testis mass to body mass) for each individual.

188

189 Statistical Analysis

190 We conducted all statistical analyses in JMP (version 9.0, 2010; SAS Institute Inc.,
191 USA). All morphological traits were \log_{10} transformed for our analyses. As head measurements
192 are highly correlated, we used Principal Component Analysis (PCA) to reduce dimensionality of
193 \log_{10} head length, \log_{10} head width and \log_{10} head depth. This returned one significant PC ($\chi^2 =$
194 135.34, $df = 5$, $P < 0.001$) that explained 92.6% of the variance and had positive loadings of head

195 length (0.95), depth (0.96), and width (0.97). We interpreted this PC as an overall measure of
196 head size, and included this as Head Size PC in subsequent analyses.

197 To test for differences among species in body shape, we used body size-corrected
198 residuals from the regression of the \log_{10} -transformed trait on \log_{10} SVL, which included all
199 males of all species. We then used a series of analyses of variance (ANOVA) to test for
200 differences among the three species in \log_{10} SVL, Head Size PC, GSI, number of lamellae on the
201 fourth toepad, and the residuals of mass, head length, head width, head depth, femur length, tibia
202 length, fourth toe length, fourth toepad length and width, and dewlap size. We used Tukey's
203 HSD post-hoc tests for all pair-wise comparisons following significant ANOVA results.

204 To test for differences among species in rates of behavioral traits (dewlap extension,
205 head-bobs and push-ups, total movements); proportion of type of locomotor movements (runs,
206 crawls, or jumps), and fast (runs and jumps) and slow (crawls) movements; and the average
207 duration of dewlap extension among the species, we again used a series of ANOVA. Copulation
208 was rarely observed during these observations (in total, we saw only one pair of *A. cybotes*
209 copulate during this study), and thus this behavior could not be statistically analyzed. We used a
210 series of Brown-Forsythe tests to assess the equality of variance in behavioral traits among the
211 species. We also tested for differences in substrate use and substrate orientation (horizontal or
212 vertical, as defined above) between species using chi-square tests. We compared differences in
213 perch diameter between *A. cybotes* and *A. marcanoii* using ANOVA. Because our data on perch
214 diameter for *A. longitibialis* was very limited, *A. longitibialis* was removed from the analysis of
215 diameter.

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RESULTS

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

225 In addition, we found that the species differed in locomotor behavior and perch type (Fig.
226 3, Supplemental Table 2). *Anolis cybotes* moved substantially more frequently than *A.*
227 *longitibialis* ($F_{2,49} = 6.23$, $P = 0.003$, Fig. 3), and had a higher variation in the frequency of their
228 movements ($F_{2,75} = 6.83$, $P = 0.001$). However, the proportion of each type of movement (runs,
229 crawls, and jumps) did not differ among the three species (all $P > 0.17$; Fig. 3) nor did the
230 proportion of fast movements (runs, and jumps) ($F_{2,69} = 0.10$, $P = 0.25$), or slow movements
231 (crawls) ($F_{2,69} = 2.38$, $P = 0.10$). We also did not find a significant difference in the variance of
232 each of these types of movements (all $P > 0.18$).

233 Further, perch use differed substantially among the species ($\chi^2 = 122.9$, $df = 10$, $P <$
234 0.001), such that *A. longitibialis* (the species with the longest limbs, shortest toes, and the fewest
235 lamellae on their fourth toepads) was most often found on rocks, while *A. marcanoii* was most
236 often found on fence posts and tree trunks. *Anolis cybotes* occupied the widest range of habitats,
237 but was predominantly found on tree trunks and branches (Fig. 3), and was found on vertical
238 branches more often than *A. marcanoii* and *A. longitibialis* ($\chi^2 = 13.05$, $df = 2$, $P = 0.001$). We
239 found no significant difference in perch diameter between *A. cybotes* and *A. marcanoii* ($F_{1,26} =$
240 3.06 , $P = 0.092$).

241 The three species also differed in the extent of SSD, with *A. longitibialis* (SSD = 0.352)
242 exhibiting the most male-biased dimorphism, followed by *A. cybotes* (SSD = 0.169), and *A.*
243 *marcanoii* (SSD = 0.085). Consistent with our predictions, the species with lowest SSD (*A.*
244 *marcanoii*) had the smallest heads (Head Size PC), but contrary to expectation, it exhibited the
245 largest relative dewlaps (Table 1, Fig. 4D). Despite significant differences in the Head Size PC,
246 the three species did not differ in head length, width, or depth (Table 1). Further, our prediction
247 that other morphological and behavioral traits associated with precopulatory sexual selection
248 would show a directional pattern with the extent of SSD was not supported. *Anolis cybotes*, the
249 species with intermediate SSD, performed a higher rate of dewlap extensions (Fig. 4E, $F_{2,77} =$
250 12.20 , $P < 0.001$) and head-bobs/push-ups (Fig. 4C, $F_{2,77} = 13.31$, $P < 0.001$) than *A.*
251 *longitibialis*, although the species did not differ in the average duration of dewlap extension (Fig.
252 4F, $F_{2,59} = 2.18$, $P = 0.122$). The species also differed in testis morphology in the direction we
253 predicted: the least dimorphic species (*A. marcanoii*) had a higher GSI than the two more
254 dimorphic species (Fig. 4B, $F_{2,49} = 6.23$, $P = 0.003$).

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DISCUSSION

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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 (Burnell and Hedges 1990; Glor et al. 2003,
2004; Knouft et al. 2006; Wollenberg et al. 2013). The cybotoid anoles provide a model for
studying local adaptation and speciation within a clade because this group occupies a wide range
of habitats in Hispaniola, and exhibits morphological differences that may be attributed to this

264 ecological variation. Here, we demonstrate differences in the morphology, ecology, and behavior
265 of three species of closely-related cybotoid anoles. Consistent with our predictions, the
266 differences in substrate use, morphology and behavior, within this ecomorph mirror some
267 patterns of diversification among anole ecomorphs and among other species of lizards,
268 suggesting that each cybotoid species has experienced local adaptation to the variable habitats in
269 the Dominican Republic. For example, among anole ecomorphs, species that use broader perches
270 tend to have longer limbs (Losos and Sinervo 1989; Losos 1990). Our data also suggest that
271 species with low male-biased dimorphism tend to have smaller heads and larger testes, consistent
272 with theory on sexually selected traits in this group (Kahrl et al. 2016), though we found no
273 relationship between SSD and social display behaviors. Although we found differences in these
274 traits among species, we could not directly test for associations between morphology, ecology,
275 and behavior as we only examined these traits in three species of anoles. However, these data
276 provide one of the first comparisons of behavior and sexually-selected traits in this group.

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278 Morphology and Substrate Use

279 Cybotoid anoles are a morphologically diverse group that inhabit a range of perch
280 substrates and macrohabitats in the Dominican Republic (Glor et al. 2003). We hypothesized
281 accordingly, that variation in microhabitat or substrate use may have led to predictable changes in
282 morphology and behavior, similar to the patterns of ecomorphological divergence across
283 Caribbean anoles (Losos and Sinervo 1989; Losos 1990). For the three species in this study, we
284 found that the species that uses the narrowest perches also has shorter hindlimbs, and longer
285 fourth toes with more lamellae on the toepad (*Anolis cybotes*, Fig. 2, 3). This result is consistent
286 with patterns of adaption among species across ecomorphs, where species that live on smaller

287 perches tend to have shorter limbs with more lamellae, traits thought to aid in clinging ability
288 while running on small perches (Cartmill 1985; Pounds 1988; Losos 1990). Our results also
289 correspond to previous research that has shown deterministic evolution in morphology of *A.*
290 *cybotes*, which partially mirrored diversification in previous stages of this adaptive radiation
291 (Wollenberg et al. 2013). Together, these traits suggest that this species has likely adapted to
292 climbing and running on relatively narrow perches (Irschick et al. 1996; Zani 2000).

293 In addition, we found that the morphology associated with living on rocks in *A.*
294 *longitibialis* mimics the patterns of morphology seen in other species of lizards. In particular,
295 the longer limbs of *A. longitibialis* may be an adaptation to its saxicolous habitat that enable this
296 species to run faster and jump farther on broad, rocky surfaces (Losos 1990; Irschick and Losos
297 1999). Similar morphological features occur in other boulder-dwelling anoles, including *A.*
298 *eugenegrahami* and *A. aquaticus*, who have elongated limbs and flattened bodies (Leal et al.
299 2002, Muñoz et al. 2015), a morphological adaptation that is not limited to anoles, but is
300 common among other groups of lizards. In fact, the evolution of long limbs, toes, and flattened
301 bodies has been repeated in five other lineages of rock-dwelling lizards (Revell et al. 2007,
302 Goodman et al. 2008) and is linked with improved performance in jumping, sprinting, and
303 climbing on rocky habitats (Goodman et al. 2008). This suggests that the ecomorphological
304 adaptations seen in anoles can serve as a general model for how other species of lizards may
305 adapt to specialized habitats.

306 Because species may exhibit population-level variation, especially those that, like the
307 cybotoid anoles, exist across heterogeneous landscapes, it is important to recognize that there are
308 limitations in using data from one or a few populations to represent a species. Thus, the data
309 presented here are a “snap-shot” of the true diversity in behavior, morphology, and substrate use

310 in each species. In particular, we examined a lowland population of *A. cybotes* in this study, yet
311 this species occurs in montane areas as well. Parallel to the ecomorphological associations
312 among the three species reported in this study, *A. cybotes* exhibits similar ecomorphological
313 associations in limb dimensions and microhabitat along the altitudinal clines of Hispaniola
314 (Wollenberg et al. 2013, Muñoz et al. 2014). This intraspecific variation further supports the idea
315 that local ecomorphological adaptation in cybotoids is widespread, both within and among each
316 species.

317

318 Behavior and Substrate Use

319 Contrary to our hypothesis, we found no difference in the proportion of fast or slow
320 movements between species of anoles. We did find, however, that the long-limbed saxicolous
321 species (*A. longitibialis*) tended to move less frequently than the short-limbed tree-dwelling
322 species (*A. cybotes*). Again, this relationship between microhabitat use and movement rate
323 mimics relationships observed among older stages of the anole radiation, where species that live
324 on tree trunks or branches tend to have higher movement rates than those that live near or on the
325 ground (Johnson et al. 2008). This variation in movement rate may be due to differences in
326 foraging behavior among species, associated with variation in visibility from the lizard's perch
327 (Johnson et al. 2008). In particular, microhabitats with a greater density of twigs and branches
328 tend to obscure visibility, and lizards may move more frequently to survey the areas around them
329 for prey or conspecifics (Moermond 1979).

330 We also tested the hypothesis that generalist species, which use more diverse substrates,
331 exhibit higher variation in their locomotor behavior. *Anolis cybotes* used the widest variety of
332 substrates, while both *A. marcanoii* and *A. longitibialis* were observed on fewer substrate types,

333 and on a single substrate type > 60% of the time (Fig. 3A). This difference in perch use may be a
334 function of species-specific preferences for particular perches, perch availability, or an
335 interaction of the two (Johnson et al. 2006, Hermann et al. 2017). Consistent with our hypothesis,
336 we also found that individual *A. cybotes* had higher variation in their overall movement rate than
337 the other two species. Since *A. cybotes* occupies more diverse perch types, there may be higher
338 variation in visibility and therefore, high variation in movement rates in this species (Moermond
339 1979). However, we note that our behavioral observations of *A. marcanoi* were limited, and our
340 species-level description of their behavior may be estimated with some error.

341

342 Sexually Selected Traits

343 We measured several traits associated with pre- and postcopulatory selection in each of
344 these three species to examine patterns between SSD, morphology, and social behavior.
345 Although we could not statistically test this relationship among species, we observed patterns
346 consistent with hypotheses about how precopulatory selection might influence trait evolution
347 among species. These species vary in the extent of male-biased sexual size dimorphism (Fig. 4),
348 which can result from precopulatory selection on male body size (Butler et al. 2000; Cox et al.
349 2003, 2007). We predicted that species with high male-biased SSD would also have larger heads,
350 and especially deeper and wider heads due to the use of the head for biting during male-male
351 combat (Lailvaux et al. 2004; Lailvaux and Irschick 2007). We found that the species with
352 medium and high SSD (*A. longitibialis* and *A. cybotes*) had larger heads (Head Size PC) than the
353 species with low SSD, *A. marcanoi* (Fig. 4A). Though there is evidence in several species of
354 lizards that bite force or head size can predict the outcomes of male-male combat (Hews 1990,
355 Pratt 1992, López et al. 2002, Gvozdík and Damme 2003, Husak et al. 2006), little comparative

356 work in squamates has tested the hypothesis that species with high male-biased SSD also have
357 positive allometry for head size (Carothers 1984). Our study suggests a positive association
358 between SSD and head size, but many more species are required to statistically test this
359 hypothesis.

360 We also predicted that species with high male-biased SSD would exhibit larger dewlaps
361 and higher display rates, but we found that the species with the highest male-biased SSD had the
362 smallest dewlap area (Fig. 4). We also found no similarity between SSD and display rate in these
363 species (Fig. 4). A variety of hypotheses have been proposed to explain variation in dewlap size
364 and patterning among species of anoles, many of which have found weak or non-significant
365 support for differences among ecomorph groups or for variation due to species recognition
366 (Nicholson et al. 2007). Among islands, anole species that co-occurred with other species of
367 anoles did not differ in their dewlaps when other cues for species recognition were present (i.e.,
368 when there were strong differences in other phenotypic traits). However, in populations where
369 other cues were lacking, anoles tended to have more variable dewlap colors (William and Rand
370 1977). Among the three populations that we sampled, *A. marcanoii* had reddish-pink dewlaps,
371 whereas both *A. cybotes* and *A. longitibialis* had pale yellow or white dewlaps. These observed
372 differences in dewlap size and color may aid in species-recognition, especially given the physical
373 similarities between these species, though our data cannot test this hypothesis. Additionally,
374 dewlap size is weakly associated with habitat illumination, which may also be important in
375 driving the evolution of the dewlap (Losos and Chu 1998; Leal and Fleishman 2004), although
376 that was not a focus of the present study.

377 Finally, we observed that species with higher male-biased SSD had smaller testes (Fig.
378 4B). This pattern was consistent with our predictions of a trade-off between pre- and

379 postcopulatory selection, and mirrors a trade-off that exists both among other species of anoles
380 and across all squamates (Lailvaux et al. 2004; Lailvaux and Irschick 2007; Kahrl et al. 2016).
381 This suggests that the patterns of diversification in morphology due to sexual selection that
382 occurs among species are also occurring within ecomorph groups.

383

384 Our current results may inform patterns of divergence among other groups of sympatric
385 and allopatric anole species within the same ecomorph in Cuba and Hispaniola that exhibit niche
386 partitioning (Losos et al. 2003; Glor et al. 2004; Knouft et al. 2006) similar to the patterns
387 observed among ecomorphs on each island (Williams 1983; Losos 1990; Losos et al. 1998). In
388 particular, the cybotoid group of anoles show differences in morphology that are independent of
389 their phylogenetic relationships (Glor et al. 2003), and can be attributed to a combination of
390 microhabitat and genetic differentiation between these species (Wollenberg et al. 2013). Here,
391 we found that the closely related species *A. cybotes*, *A. longitibialis*, and *A. marcanoii* exhibited
392 striking differences in their morphology, ecology, and behavior that may be due to local
393 adaptation to varying microhabitats and differences in the strength of sexual selection among
394 species, which may represent a yet underexplored dimension of local adaptation.

395

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561 TABLE 1.—Results from separate ANOVAs testing for differences in log₁₀-transformed body
 562 size (SVL), Head Size PC, and body size corrected morphology (residuals of morphological
 563 traits regressed against SVL) among *Anolis cybotes*, *Anolis longitibialis*, and *Anolis marcanoii*.
 564 Significant comparisons are indicated with (*).

565

Trait	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*

566

567

FIGURE CAPTIONS

568

FIG 1.— Photographs of adult male *Anolis cybotes* (A), *A. marcanoii* (B), and *A.*

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longitibialis (C), and their respective representative habitats: La Ciénaga, Barahona (D), the road

570

to El Matedero north of Bani (E), and Manuel Goya (F) in the Dominican Republic.

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572

573

FIG 2.— Means \pm standard error of \log_{10} snout–vent length (SVL) (A), residual femur

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length (B), residual tibia length (C), residual fourth toe length (D), number of lamellae on the

575

fourth hindlimb toe (E), and residual fourth toepad length (F) for *Anolis cybotes* (N = 17), *A.*

576

marcanoii (N = 7), and *A. longitibialis* (N = 17). Superscripts denote significant differences

577

between species ($P < 0.05$).

578

579

580

FIG 3.— Comparisons of ecology (substrate use, A), and behavior (mean \pm S.E. of the

581

movement rate, B, and movement type, C) for *Anolis cybotes* (CYB), *A. marcanoii* (MAR), and

582

A. longitibialis (LON). Here, substrate use (A) is graphed as a proportion of total observation.

583

Movement rate (B) was calculated as the total number of movements (run, crawl, and jump) per

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minute. Movement type (C) is expressed as a proportion of the total movements observed for

585

each species.

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587

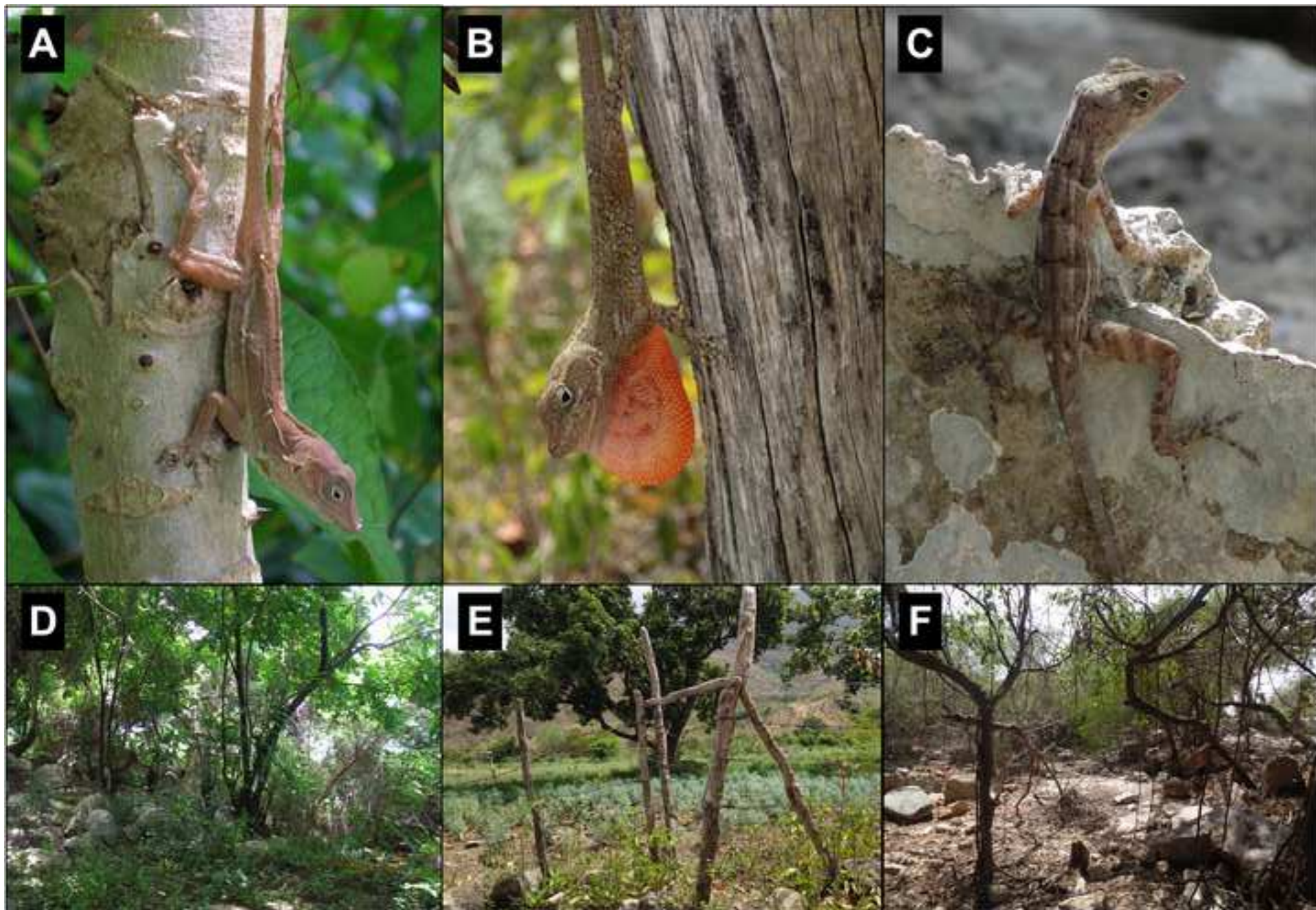
588

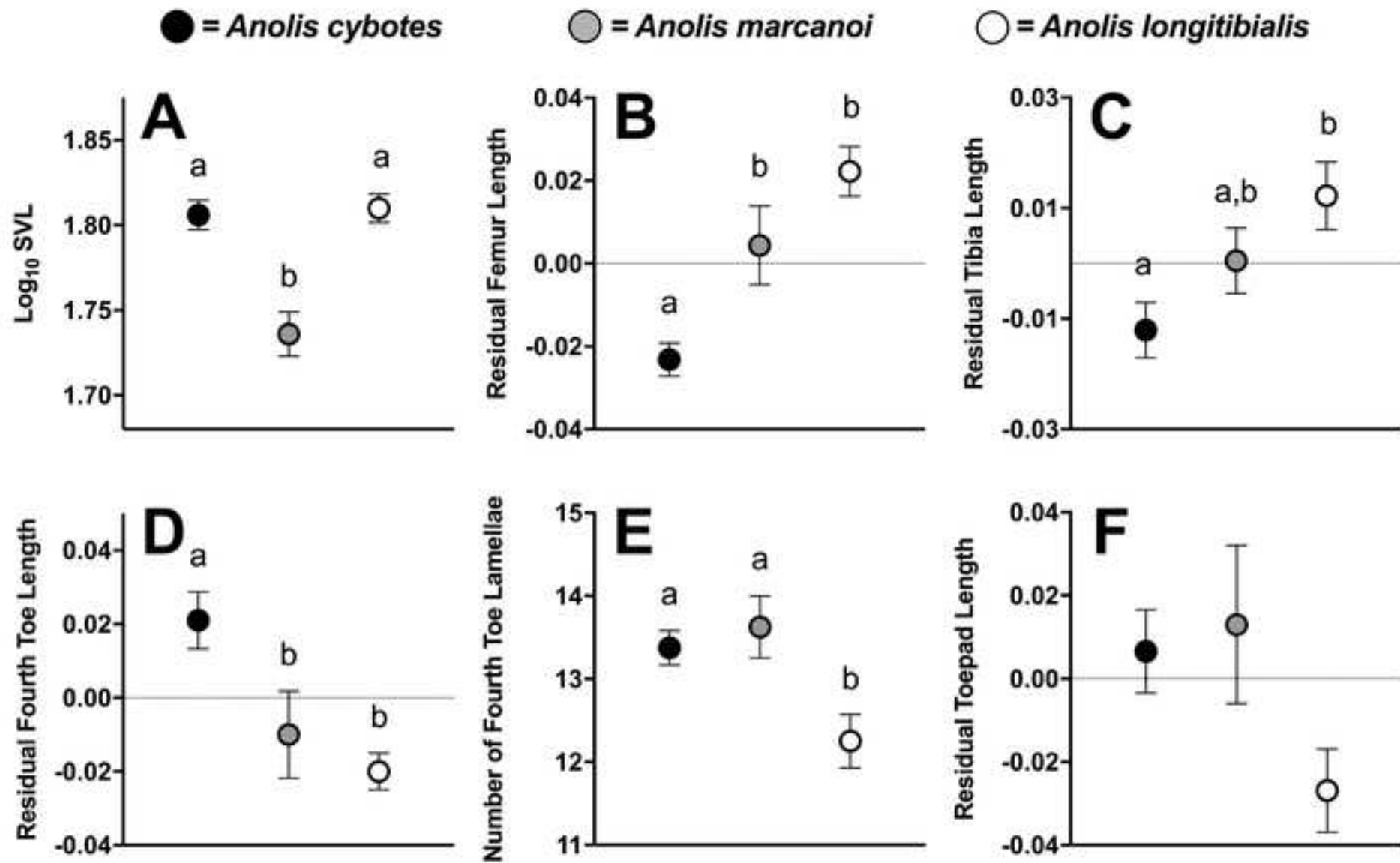
FIG 4.— Comparisons of sexual size dimorphism (SSD) and other sexually selected traits

589

in cybotoid anoles. Means \pm S.E. of Head Size PC (A), GSI (B), push-ups per minute (C),

590 residual dewlap area (D), dewlap extensions per minute (E), and dewlap extension time (F) for
591 *Anolis cybotes*, *A. marcanoi*, and *A. longitibialis*. These data are presented, from left to right, by
592 the extent of sexual size dimorphism in each species: *A. marcanoi* (SSD = 0.085), *A. cybotes*
593 (SSD = 0.169), and *A. longitibialis* (SSD = 0.352).





● = *Anolis cybotes* ● = *Anolis marcanoi* ○ = *Anolis longitibialis*

