

Parameters of the adhesive setae and setal fields of the Jamaican radiation of anoles (Dactyloidae: *Anolis*): potential for ecomorphology at the microscopic scale

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Received 20 April 2022; revised 6 June 2022; accepted for publication 10 June 2022

The subdigital adhesive pads of Caribbean *Anolis* lizards are considered to be a key innovation that permits occupation of novel ecological niches. Although previous work has demonstrated that subdigital pad morphology and performance vary with habitat use, such investigations have only considered the macroscale aspects of these structures (e.g. pad area). The morphological agents of attachment, however, are arrays of hair-like fibres (setae) that terminate in an expanded tip (spatula) and have not been examined in a similar manner. Here we examine the setal morphology and setal field configuration of ecologically distinct species of the monophyletic Jamaican *Anolis* radiation from a functional and ecological perspective. We find that anoles occupying the highest perches possess greater setal densities and smaller spatulae than those exploiting lower perches. This finding is consistent with the concept of contact splitting, whereby subdivision of an adhesive area into smaller and more densely packed fibres results in an increase in adhesive performance. Micromorphological evidence also suggests that the biomechanics of adhesive locomotion may vary between *Anolis* ecomorphs. Our findings indicate that, in a similar fashion to macroscale features of the subdigital pad, its microstructure may vary in relation to performance and habitat use in Caribbean *Anolis*.

ADDITIONAL KEYWORDS: adaptive radiation – adhesion – bio-inspired adhesion – clinging ability – contact splitting – fibrillar adhesion – setae – spatulae.

INTRODUCTION

Caribbean lizards of the genus *Anolis* are recognized as a model system for the study of evolutionary ecology and adaptive radiation because of the well-supported ecomorphological paradigm with which they are associated (Losos, 1994, 2011; Irschick *et al.*, 2006). Phylogenetically and geographically distant species of Caribbean *Anolis* that inhabit similar microhabitats exhibit remarkable morphological convergence (Williams, 1972; Losos, 1990a, b; Mahler *et al.*, 2013). Six ecomorph classes have been identified based upon their shared microhabitat, morphology and behaviour

(Losos, 1990a, 2011). Crown-giant anoles, for example, generally use trunks and branches positioned within the crowns of trees, are large-bodied, and possess relatively short limbs and long tails (Beuttell & Losos, 1999; Losos, 2011). These morphological features are important adaptations for effective arboreal locomotion, allowing crown-giant anoles to resist the force of gravity in a three-dimensional, complex environment (Cartmill, 1985; Losos & Irschick, 1996; Beuttell & Losos, 1999; Irschick & Losos, 1999). In contrast, trunk-ground anoles move on broad substrates close to the ground (e.g. low tree trunks and the forest floor), are intermediately sized, and possess relatively long limbs and long tails (Beuttell & Losos, 1999; Losos, 2011), which permit effective terrestrial locomotion via enhanced stride length and sprint

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speed on broad substrates (Losos & Irschick, 1996; Beuttell & Losos, 1999; Irschick & Losos, 1999).

All species of anole, with the exception of the sand dune specialist *Anolis onca* (Peterson & Williams, 1981), possess subdigital adhesive pads that permit reversible attachment to and locomotion on vertical substrates (Ruibal & Ernst, 1965; Williams, 1972; Losos, 1990a, 2011; Garner *et al.*, 2019). Substrate use, perch height and locomotor modes of *Anolis* ecomorphs vary considerably, so it is not surprising that researchers have investigated the ecomorphological associations of their subdigital adhesive system (e.g. Williams, 1972; Pounds, 1988; Losos, 1990a, b; Glossip & Losos, 1997; Irschick *et al.*, 1997; Beuttell & Losos, 1999; Macrini *et al.*, 2003; Elstrott & Irschick, 2004; Schaad & Poe, 2010; Donihue *et al.*, 2018; Yuan *et al.*, 2019, 2020; Huie *et al.*, 2021; Miller & Stroud, 2022).

Previous studies have focused primarily on the ecomorphological associations of three macroscale features of anoline adhesive subdigital pads: width, area and the number of adhesive scales termed lamellae (e.g. Pounds, 1988; Glossip & Losos, 1997; Macrini *et al.*, 2003; Elstrott & Irschick, 2004). Each of these variables, even when adjusted for body size, scales positively with perch height amongst species, indicating that arboreal species possess relatively larger subdigital pads with a greater number of lamellae than terrestrial species (Pounds, 1988; Glossip & Losos, 1997; Macrini *et al.*, 2003; Elstrott & Irschick, 2004). The ecological associations of these macromorphological features suggest that such variation is critical in their effective exploitation of particular microhabitats (Elstrott & Irschick, 2004).

Elstrott & Irschick (2004) found that the subdigital pad area of 12 species of Caribbean *Anolis* scaled positively with increased clinging force, even when body size was taken into consideration, indicating that anoles with relatively larger subdigital pads display relatively greater clinging ability. Based on these findings and those of a previous study (Irschick *et al.*, 1996), Elstrott & Irschick (2004) asserted that subdigital pad size is the major determinant of clinging ability in *Anolis*. Clinging ability also scales positively with perch height, suggesting that arboreal anoles are capable of generating greater clinging forces than terrestrial anoles, purportedly attributable to their relatively larger subdigital pads (Elstrott & Irschick, 2004).

Embedded within such findings is the unstated assumption that the attributes of the individual setae (hair-like fibres) and the configuration of setae into fields on subdigital lamellae (Ruibal & Ernst, 1965; Peterson & Williams, 1981; Williams & Peterson, 1982; Peterson, 1983a, b) are invariable. It is evident, however, that clinging ability is a function of the direct interaction of the setae with

the locomotor substrate. The setae of anoles, unlike those of geckos, terminate in a single, expanded, triangular-shaped tip, known as a spatula (Ruibal & Ernst, 1965; Williams & Peterson, 1982; Peterson, 1983b; Garner *et al.*, 2021b). Spatulae are placed into intimate contact with the substrate's surface during locomotion, inducing intermolecular adhesion primarily via van der Waals forces (Autumn *et al.*, 2000, 2002; Autumn & Peattie, 2002). Despite this recognition, few studies have examined the morphology of adhesive setae of *Anolis* (Garner *et al.*, 2019), and even fewer have considered them from a functional or ecological perspective. As a result, whether interspecific variation in subdigital pad microstructure also varies with subdigital pad performance and habitat use remains unknown.

To explore this possibility, we examine the setal morphology and setal field configuration of five species of the monophyletic Jamaican radiation of *Anolis* to assess whether setal characteristics vary significantly between species that occupy distinct microhabitats. We selected this particular radiation because it is small but exhibits a high degree of ecological divergence. Specifically, the five species we examine represent four of the six anole ecomorphs. Our investigative design enables us to examine whether setal characteristics have differentiated in accordance with microhabitat use and whether such characteristics have any bearing on effective functioning in particular structural habitats. Furthermore, our results will allow us to evaluate the possibility of ecomorphological evolution of the adhesive microstructures of anoline subdigital pads at broader phylogenetic scales, with the potential for exciting avenues of future interdisciplinary research.

MATERIALS AND METHODS

SPECIMENS

The Jamaican radiation of *Anolis* comprises a monophyletic group of seven species: *Anolis conspersus*, *A. grahmi*, *A. garmani*, *A. opalinus*, *A. lineatopus*, *A. reconditus* and *A. valencienni*. *Anolis garmani* is a crown-giant anole, *A. grahmi* and *A. opalinus* are trunk-crown anoles, *A. valencienni* is a twig anole, and *A. lineatopus* is a trunk-ground anole (Fig. 1A). Perch height data for these five species included in our study were collected from published literature (Losos, 1990a; Losos & Irschick, 1996; Butler & Losos, 2002; Vanhooydonck *et al.*, 2007; Cooper, 2010) and averages were calculated for each species. We used these values to order species by perch height on plots (Table 1). *Anolis conspersus* and *A. reconditus* were not examined. *Anolis conspersus* is native to the Cayman Islands and thus does not share habitat with the Jamaican species, and *Anolis*

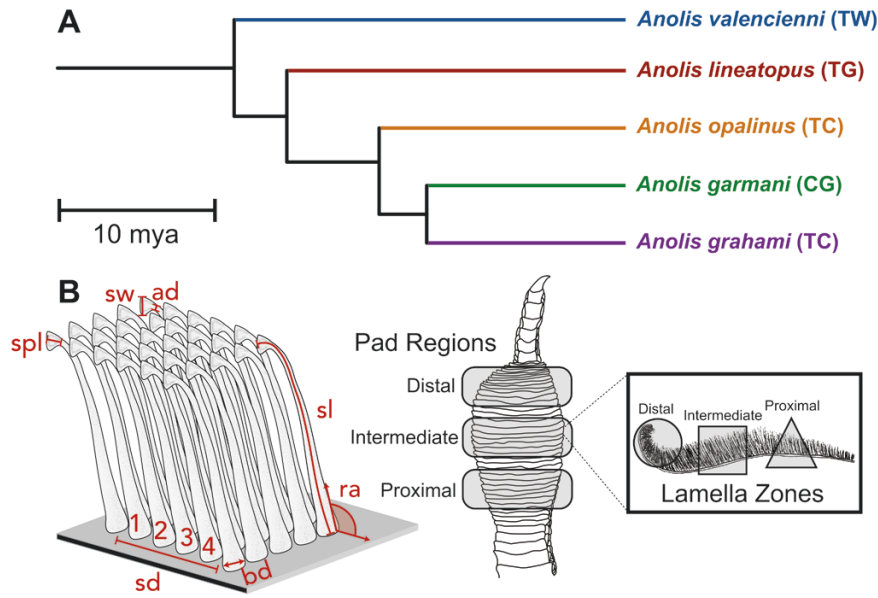


Figure 1. A, time-calibrated (Poe *et al.*, 2017) phylogeny of the five Jamaican *Anolis* species examined in this study and their ecomorph designation. CG = crown-giant; TC = trunk-crown; TW = twig; TG = trunk-ground. B, the left schematic displays a setal array and the various morphological parameters examined in this study. The right schematic (modified from Garner *et al.*, 2021b) displays a subdigital pad indicating that three lamellae were chosen from distal, intermediate and proximal regions of the pad to examine setal characters. Setal characters were then sampled from distal (Dist), intermediate (Int) and proximal (Prox) zones of individual lamellae. *ad*, apex diameter; *bd*, base diameter; *ra*, resting angle; *sd*, setal density; *sl*, setal length; *spl*, spatula length; *sw*, spatula width.

Table 1. Mean perch heights, sample sizes, sexes and the ecomorph designation of the five species of Jamaican *Anolis* examined in this study. Values were obtained from published literature (Losos, 1990a; Losos & Irschick, 1996; Butler & Losos, 2002; Vanhooydonck *et al.*, 2007; Cooper, 2010) and are presented as means ± SEM

Species	N	Females	Males	Ecomorph	Perch height (m)
<i>A. garmani</i>	3	–	3	Crown-giant	3.3 ± 0.3
<i>A. grahami</i>	4	2	2	Trunk-crown	2.0 ± 0.2
<i>A. opalinus</i>	4	1	3	Trunk-crown	1.6 ± 0.2
<i>A. valencienni</i>	3	1	2	Twig	1.9 ± 0.5
<i>A. lineatopus</i>	4	1	3	Trunk-ground	0.9 ± 0.1

reconditus, while endemic to Jamaica, has not been assigned to an ecomorph class.

Three or four ethanol-preserved specimens per species were obtained from the herpetology collections of the Museum of Comparative Zoology of Harvard University and The Academy of Natural Sciences of Drexel University. Accession numbers of individual specimens are provided in the [Supplementary Information \(Table S1\)](#).

SAMPLE PREPARATION AND SUBDIGITAL PAD MORPHOMETRICS

Methods for sample preparation, measurement of setal morphometric data and estimation of material properties of setae generally followed those of Garner *et al.* (2021b) and Johnson & Russell (2009), and are briefly summarized here.

Digit IV of the right pes was removed from each specimen and its ventral surface examined via light microscopy (Olympus SZX16 Stereomicroscope, Olympus Corp., Japan) for measurement of subdigital pad morphometrics using ImageJ (Schneider *et al.*, 2012). The digit was isolated from the image background via colour thresholding. True lamellae were defined as subdigital scales with frayed free margins and width to length ratios >1 (Peterson & Williams, 1981). All regions of the digit not carrying lamellae were subsequently removed from the image by hand and the number of lamellae counted. Images of isolated subdigital pads were converted to 8-bit greyscale and then to a binary image. Subdigital pad area was then calculated via the *Analyze particles* function in ImageJ.

Following this, digits were sectioned parasagittally to the right of the phalanges, yielding longitudinal sections of the digit and its setal arrays. Sections were

prepared for scanning electron microscopy (SEM) via critical point drying, affixed to SEM stubs via carbon tape and sputter coated with gold–palladium for 30 s. The parasagittal sections incorporating the phalanges were positioned on SEM stubs such that exposed setae were oriented perpendicular to the electron beam, permitting measurement of setal morphometrics along the proximodistal axis of the digit. Sections were viewed with a high-vacuum field-emission scanning electron microscope (JEOL 7401 FESEM; JEOL USA, Inc., Peabody, MA, USA).

SETAL/LAMELLAR MORPHOMETRICS

Setal/lamellar characters were measured following the definitions of [Garner *et al.* \(2021b\)](#): total setal length, length of the setal stalk, setal base diameter, setal apex diameter, setal resting angle, setal density and the exposed length of lamellae ([Fig. 1B](#)). Setal spacing was observed to be similar mediolaterally and proximodistally within species and individuals, and thus setal density could be calculated by counting the number of setae along a measured proximodistal sector of a lamella and then squaring this to obtain an estimate of the number of setae per μm^2 . This estimate was then converted to the number of setae per mm^2 .

For each longitudinal section, three lamellae, from different anatomical regions of the pad (proximal, intermediate and distal pad regions), were selected for measurement of setal morphometrics ([Fig. 1B](#)). Images of the seta-bearing regions of lamellae were taken at 500 \times magnification to permit measurement of lamella length. Each seta-bearing portion of the lamella was further visually subdivided into three zones (proximal, intermediate and distal) and a single image consisting of a subset of setae within each lamella zone was obtained at higher magnification (2000 \times) ([Fig. 1B](#)). Within each image, five setae were selected for measurement on a per-character basis (outlined in the previous paragraph). Setae were chosen based upon the ability to accurately measure the particular feature(s). Setal dimensions were measured three times and averaged to obtain an estimate of each feature. A total of 11 790 measurements were made.

SPATULAR MORPHOMETRICS

One parasagittal section per species that excluded the phalanges was positioned on an SEM stub such that the ventral surfaces of lamellae were oriented perpendicular to the electron beam. This permitted representative measurements of spatular morphometrics (spatula width and spatula length). Spatula width is defined as the dimension at its

widest point and spatula length as the span from the point of expansion of the spatular tip to the distal edge of the spatula ([Fig. 1B](#)). The thin, flexible nature of setae rendered it difficult to obtain many images of spatulae that were of sufficient quality for accurate morphometric data to be obtained. Spatulae from the distal zone of proximal, intermediate and distal lamellae were most conducive to quality imaging and five individual spatulae per image were selected for acquisition of spatular morphometrics.

CONTACT AREA RATIO

We used the product of setal density and spatula area at different regions along the pad of each species to calculate the ratio between the actual area available for contact (total spatula area) and projected area (total area of a region of the pad), hereafter referred to as the contact area ratio. A greater contact area ratio indicates proportionally greater area available for contact. Spatula area was obtained by approximating the spatulae as isosceles triangles and multiplying spatula width by spatula length and halving that value.

ESTIMATED EFFECTIVE BENDING STIFFNESS OF ANOLINE SETAE

Anoline setae are tapered structures and tapering can reduce the effective bending stiffness (k_{eff}) of the fibres. Based upon work by [Caliaro *et al.* \(2013\)](#), [Garner *et al.* \(2021b\)](#) calculated k_{eff} of anoline setae by multiplying the bending stiffness of a fixed radius cylinder (k) by a tapering ratio (t):

$$k_{\text{eff}} = k \cdot t = \frac{3\pi R_b^4 E}{4L^3} \cdot \frac{R_a}{R_b} \quad (1)$$

where R_b is the setal base radius, E is the elastic modulus of anoline setae (assumed to be 1 GPa based on gekkotan setae; [Autumn *et al.*, 2006](#)), L is length of the setal stalk, and R_a is the setal apex radius.

STATISTICAL ANALYSES

Mean values of setal morphometrics and k_{eff} were obtained for each specimen as a function of pad region and lamella zone. This generated nine values per individual corresponding to each combination of pad region and lamella zone.

The effect of size on setal morphometrics, lamella length, k_{eff} , subdigital pad area and lamella number was examined using linear regressions with snout–vent length (SVL) as the independent variable. Mean values per individual were used in these analyses. Subdigital pad area ([Supplementary Information, Fig. S1](#); $R^2 = 0.89$,

$P < 0.0001$), number of lamellae (Fig. S2; $R^2 = 0.31$, $P = 0.021$), setal apex diameter (Fig. S3; $R^2 = 0.5$, $P = 0.001$) and k_{eff} (Fig. 4A; $R^2 = 0.25$, $P = 0.04$) varied significantly with SVL, and thus residuals were used in subsequent analyses as size-corrected data. All other variables did not vary significantly with SVL, and for them, non-size-corrected data were used.

We examined differences in setal morphometrics as a function of species, pad region, lamella zone and all possible interactions using a series of analyses of variance (ANOVA). ANOVA was also used to investigate differences in lamella length, but only as a function of species, pad region and their interaction. Interspecific differences in size-corrected subdigital pad area, number of lamellae, setal apex diameter and k_{eff} were examined using ANOVA with species modelled as a fixed factor. Interactions that were not significant were removed from all subsequent final analyses. In the case of significant main effects, pairwise comparisons were performed using *post hoc* Tukey Honest Significant Differences (HSD) tests. The normality of residuals was examined using the Shapiro–Wilks test, and homogeneity of variance between species, pad region and lamella zone was tested using a series of Levene’s test. Setal length was natural log transformed prior to analysis to meet the assumption of homogeneity of variance. The variance of setal density was not homogeneous as a function of species, pad region or lamella zone (Levene’s test: $P < 0.05$) and no transformation alleviated this; thus, the non-parametric Welch’s ANOVA was used to examine differences in setal density as a function of species, pad region and lamella zone. In the case of significant main effects, pairwise comparisons were performed *post hoc* using non-parametric Games–Howell tests.

RESULTS

SUBDIGITAL PAD MORPHOMETRICS

Size-corrected subdigital pad area varied significantly as a function of species (Supplementary Information, Fig. S4; d.f. = 4, $F = 9.51$, $P = 0.001$). *Anolis valencienni* has significantly smaller subdigital pad area compared to all other species (all pairwise comparisons $P < 0.05$). No other species varies significantly in size-corrected subdigital pad area (all pairwise comparisons $P > 0.05$).

Number of lamellae varies significantly as a function of species when corrected for body size (Supplementary Information, Fig. S5; d.f. = 4, $F = 5.28$, $P = 0.01$). *Anolis garmani* and *A. grahmi* have significantly more lamellae on their subdigital pad compared to *A. lineatopus* when body size is taken into account ($P < 0.05$). No other species varies significantly in the

number of lamellae when corrected for body size (all pairwise comparisons $P > 0.05$).

SETAL/LAMELLAR MORPHOMETRICS

Setal length varies significantly as a function of pad region (Fig. 2A; d.f. = 2, $F = 19.94$, $P < 0.0001$) and lamella zone (d.f. = 2, $F = 16.99$, $P < 0.0001$). There was, however, a significant species interaction with each of these effects (pad region \times species: d.f. = 8, $F = 3.45$, $P = 0.001$; lamella zone \times species: d.f. = 8, $F = 2.68$, $P = 0.009$), suggesting that the variation in setal length along the proximodistal axis of the subdigital pad differs significantly amongst species (Fig. 2A). Setal length is significantly greater in the distal pad regions than the proximal pad regions of *A. garmani*, *A. valencienni* and *A. opalinus* (all $P < 0.05$), whereas it does not vary significantly along the pad regions of *A. grahmi* and *A. lineatopus* (all $P > 0.05$). Setae within the distal zone of each lamella are significantly shorter than those of the intermediate and proximal zone of the same lamella of *A. garmani* (all $P < 0.05$), whereas the setal length of all other species does not exhibit significant variation between the zones of each lamella (all $P > 0.05$).

Setal base diameter varies significantly between species (Fig. 2B; d.f. = 4, $F = 3.54$, $P = 0.009$), pad regions (Supplementary Information, Fig. S6; d.f. = 2, $F = 8.29$, $P = 0.004$) and lamella zones (Fig. S6; d.f. = 2, $F = 25.74$, $P < 0.0001$). *Anolis lineatopus* carries setae of significantly greater diameter than those of *A. valencienni* ($P = 0.005$), but there are no other significant differences in setal base diameter amongst species (all $P > 0.05$). Setal bases are significantly smaller in diameter in the distal pad region compared to the proximal pad region of all five species ($P = 0.0002$), although the setae of the intermediate pad region do not vary significantly in their base diameter compared to those of the distal or proximal pad regions (intermediate vs. distal: $P = 0.14$; intermediate vs. proximal: $P = 0.08$). Setae decrease significantly in base diameter proximodistally along lamella zones of all five species (all pairwise comparisons $P < 0.05$).

Size-corrected setal apex diameter does not vary significantly as a function of species (Supplementary Information, Fig. S7; d.f. = 4, $F = 1.86$, $P = 0.18$).

Setal resting angle varies significantly as a function of pad region (d.f. = 2, $F = 21.40$, $P < 0.0001$) and lamella zone (Supplementary Information, Fig. S8; d.f. = 2, $F = 31.90$, $P < 0.0001$). That of the distal and intermediate pad regions is significantly lower than that of the proximal pad regions of all five species ($P < 0.0001$), although setal resting angle does not vary significantly between distal and intermediate pad regions of all five species ($P \gg 0.05$). Setal resting angle decreases significantly proximodistally along

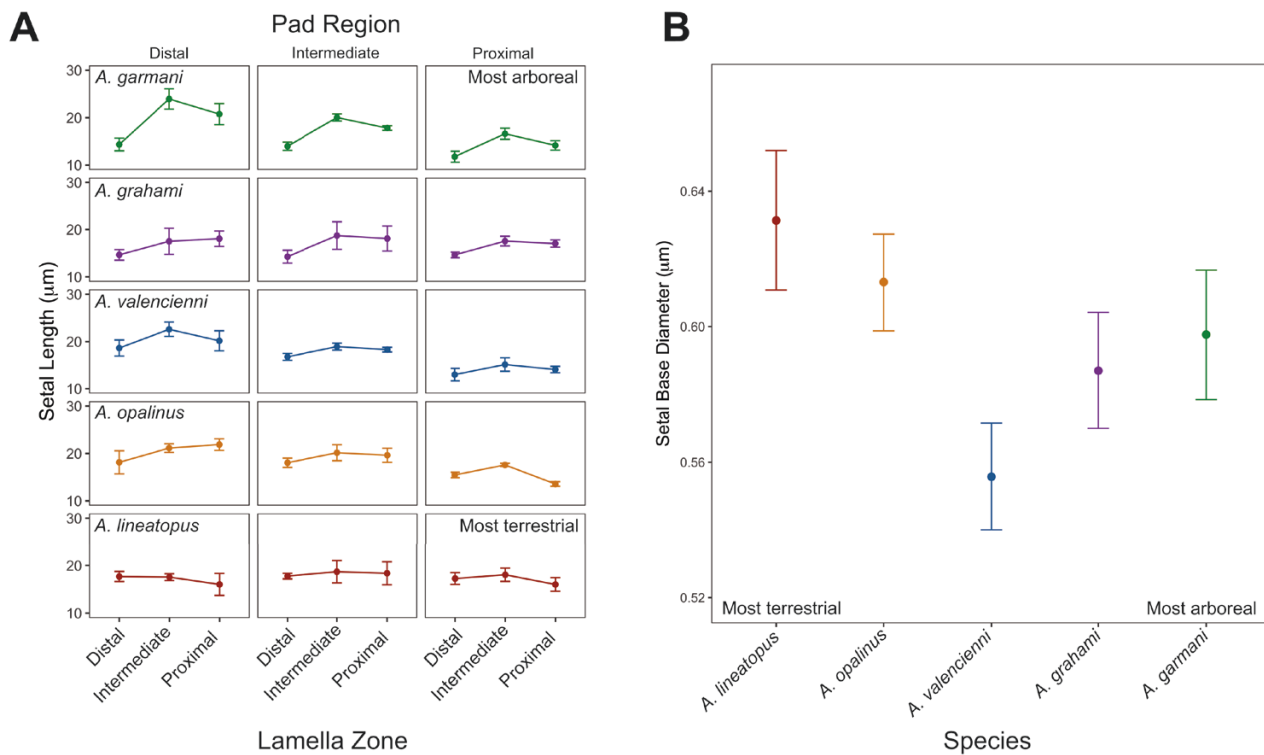


Figure 2. A, interspecific variation in setal length as a function of pad region and lamella zone. *Anolis garmani*, *A. valencienni* and *A. opalinus* exhibit increases in setal length proximodistally along pad regions (all $P < 0.05$). *Anolis grahami* and *A. lineatopus* exhibit no significant variation in setal length along the proximodistal axis of the digit (all $P > 0.05$). The setae of *A. garmani* are greatest in length in the intermediate zone of each of its lamellae (all $P < 0.05$). No other species exhibit significant variation in setal length across lamella zones (all $P > 0.05$). Green arrow indicates increase in perch height from the bottom to the top of the key. B, interspecific variation in setal base diameter. *Anolis valencienni* bears setae that are significantly thinner than those of *A. lineatopus* ($P = 0.005$). All other comparisons of setal base diameter were not significantly different from one another (all $P > 0.05$). Degree of arboreality increases from left to right.

the lamella zones of all five species (all pairwise comparisons $P < 0.05$).

Setal density varies significantly as a function of species (Fig. 3A; Welch's ANOVA: $F_{4,73} = 72.65$, $P < 0.0001$), but does not vary significantly between pad regions ($F_{2,96} = 1.76$, $P = 0.18$) or lamella zones ($F_{2,98} = 0.44$, $P = 0.64$). *Anolis garmani* possesses significantly greater setal density than all other species (all pairwise comparisons via Games–Howell $P < 0.05$). *Anolis valencienni* possesses significantly greater setal density than *A. opalinus* (Games–Howell $P = 0.006$) and exhibits a general trend of greater setal density than *A. lineatopus*, although this comparison is marginally not significant (Games–Howell $P = 0.07$). The remaining species do not differ significantly in setal density (Games–Howell $P > 0.05$).

Lamella length varies significantly as a function of pad region (Supplementary Information, Fig. S9; d.f. = 2, $F = 18.15$, $P < 0.0001$) but does not vary significantly as a function of species (d.f. = 4, $F = 1.50$, $P = 0.22$) or its interaction with pad region (d.f. = 8,

$F = 0.44$, $P = 0.89$). Distal lamellae are significantly longer than intermediate and proximal lamellae (distal vs. proximal: $P < 0.0001$; distal vs. intermediate: $P = 0.0004$). Proximal and intermediate lamellae do not vary significantly in length ($P = 0.27$).

SPATULA AREA

Spatula area varies considerably amongst species (Fig. 3B). *Anolis garmani* tends to have the smallest spatula area of all species, while *A. lineatopus* tends to have the largest. *Anolis grahami*, *A. valencienni* and *A. opalinus* have similar spatula area relative to the variance in this measure but tend to possess spatulae with greater areas than *A. garmani* and smaller areas than *A. lineatopus*.

Contact area ratio

Although spatula area and setal density vary considerably between species, interspecific differences in contact area ratio are minimal, particularly

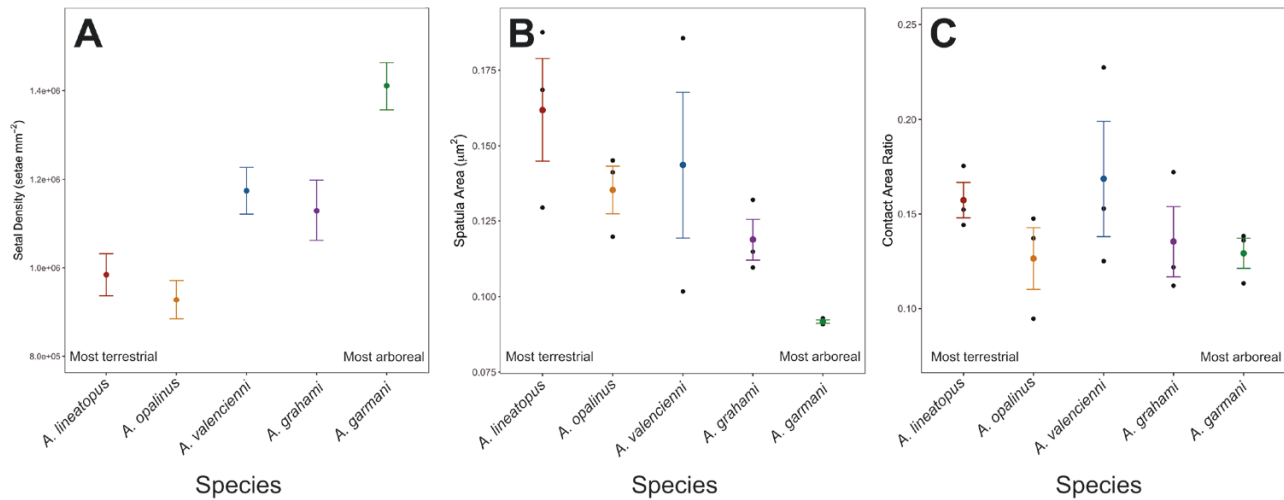


Figure 3. A, interspecific variation in setal density. *Anolis garmani* exhibits significantly greater setal density than all other species examined (all $P < 0.05$). *Anolis valencienni* has significantly greater setal density than *A. opalinus* ($P = 0.006$) and exhibits a general trend of greater setal density than *A. lineatopus*, although this is not statistically significant ($P = 0.07$). B, interspecific variation in the area of spatulae. *Anolis garmani* tends to possess spatulae with the smallest area, while *A. lineatopus* tends to possess spatulae with the greatest area. *Anolis grahami*, *A. valencienni* and *A. opalinus* have similar spatula area relative to the variance in this measure but tend to possess spatulae with greater area than *A. garmani* and smaller area than *A. lineatopus*. Small, black circles indicate average measurements of spatula area from different regions of the pad from a single individual of each species (see Methods). C, interspecific variation in contact area ratio. There do not appear to be substantial differences in contact area ratio between species relative to the variance, indicating that the area available for adhesive contact is similar between species. Small, black circles indicate average estimates of contact area ratio from different regions of the pad. Degree of arboreality increases from left to right in all plots.

considering the range of variation in setal density and spatula area within each species (Fig. 3C).

Effective bending stiffness (k_{eff})

Size-corrected effective bending stiffness (k_{eff}) does not vary significantly as a function of species (Fig. 4; d.f. = 4, $F = 0.44$, $P = 0.77$).

DISCUSSION

THE CONCEPT OF CONTACT SPLITTING AND ITS RELEVANCE TO FIBRILLAR ADHESION IN *ANOLIS*

Maximum attachment force of a fibrillar adhesive is partially dependent on the total amount of contact area that the adhesive fibres can accomplish (Autumn *et al.*, 2002; Arzt *et al.*, 2003; Spolenak *et al.*, 2005). Previously it has been suggested that the increase in size-corrected clinging ability of Caribbean *Anolis* associated with increasing perch height is a consequence of relatively larger subdigital pads (Macrini *et al.*, 2003; Elstrott & Irschick, 2004). Larger subdigital pads were presumed to bear a greater number of spatulae than smaller ones, and thus the total area available for contact and maximum attachment force should be greater in species with relatively larger subdigital pads

(Elstrott & Irschick, 2004). This assertion, however, implicitly assumes that setal density and spatula area remain consistent between species. In our sample of five Jamaican *Anolis*, we found that setal density and spatula area vary significantly between species. *Anolis garmani*, a crown-giant anole, possesses setal densities that are significantly greater than those of all other species that tend to perch lower in trees. Setal densities tend to be lowest in the species that are known to perch closer to the ground (e.g. *A. opalinus* and *A. lineatopus*). Spatula area, on the other hand, varies inversely with setal density (Fig. 3A, B), with *A. garmani* tending to have the smallest area per spatula, *A. lineatopus* having the greatest area per spatula and the remainder of the species possessing values relatively intermediate between these.

The trade-off between setal density and spatula area potentially represents the balancing of geometrical constraints associated with setal field configuration; a greater number of setae per unit area necessitates smaller spatulae to reduce interference with adjacent fibres. Fibrillar adhesion design theory, however, also predicts that a greater density of fibres with smaller adhesive tips, when modelled as hemispheres, will generate greater pull-off force than a fibrillar adhesive with less densely packed fibres with larger adhesive tips (Arzt *et al.*, 2003; Spolenak *et al.*, 2005;

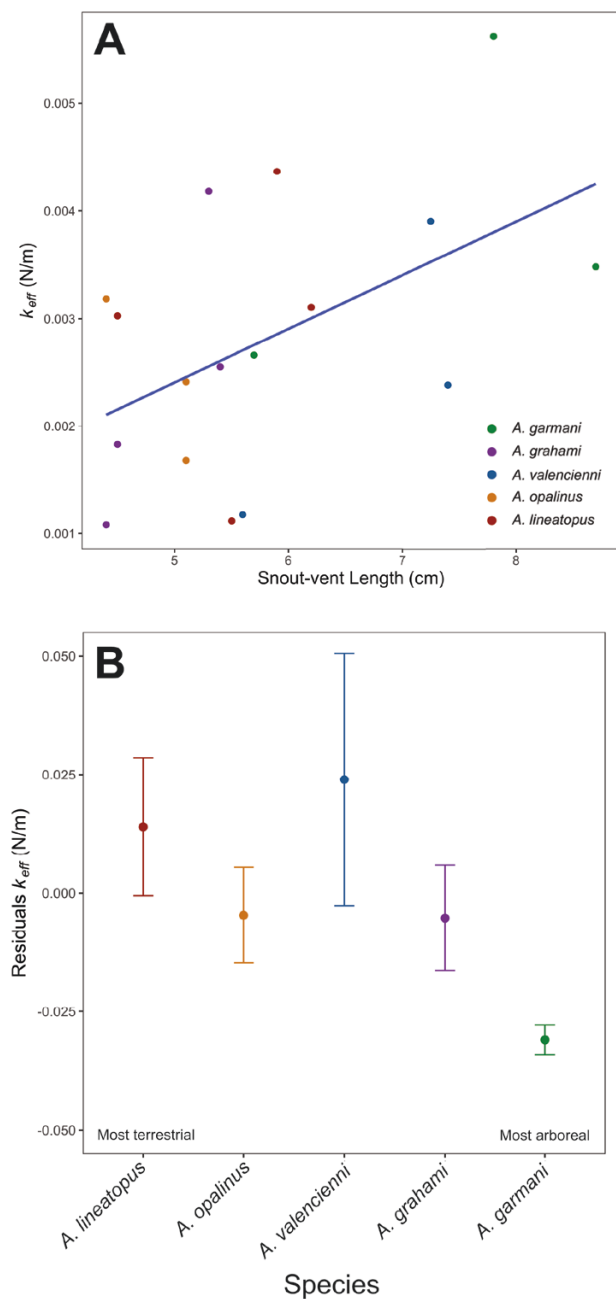


Figure 4. A, linear regression of k_{eff} as a function of snout-vent length (SVL) and species. k_{eff} varies positively and significantly with SVL ($R^2 = 0.25$, $P = 0.04$). B, there is no significant variation in size-corrected k_{eff} (via residuals of a linear regression of k_{eff} by SVL) between the species examined ($P = 0.77$). Degree of arboreality increases from left to right.

Kamperman *et al.*, 2010). This concept is known as contact splitting and its benefits for adhesive contact strength (σ_{adh} ; force per unit area) are demonstrated by eqn (2) (Spolenak *et al.*, 2005):

$$\sigma_{\text{adh}} = \frac{3A\gamma}{2R} \quad (2)$$

Here A is the contact area ratio, γ is the work of adhesion and R is the tip radius of the adhesive fibres. If two fibrillar arrays possess identical contact area ratios but different tip radii, eqn (2) predicts that the array with smaller tip radii (and as a consequence greater fibrillar density) will generate greater adhesive contact strength (Spolenak *et al.*, 2005). In their investigations of the functional ecomorphology of the subdigital pads of Caribbean *Anolis*, Elstrott & Irschick (2004) largely explained the positive correlation between clinging ability and perch height as a result of relatively greater subdigital pad area and, therefore, greater area available for contact. Interestingly, however, we found that the contact area ratio does not vary noticeably between the five species examined here. As such, our findings that Jamaican *Anolis* that perch higher in the tree canopy possess greater setal densities and smaller spatulae than those that perch closer to the ground suggest that anoles benefit from the functional outcome of contact splitting.

The concept of contact splitting makes a number of critical assumptions regarding contact geometry and force application (Johnson *et al.*, 1971; Arzt *et al.*, 2003). Although there is support for other contact geometries benefiting from subdivision into finer contacts that may more closely approximate anoline spatulae (e.g. Ghatak *et al.*, 2004; Chung & Chaudhury, 2005; Spolenak *et al.*, 2005), the ability of these models to predict the adhesion of anoline setae and setal arrays remains to be determined empirically (Garner *et al.*, 2019). Furthermore, the functional consequences of contact splitting can arise from a multitude of non-mutually exclusive mechanisms related to crack initiation and propagation, conformation to surface roughness, scaling, and stress distributions (Kamperman *et al.*, 2010). Thus, although our morphological findings are consistent with contact splitting theory, future theoretical and empirical work is needed to identify and evaluate the specific mechanisms driving the performance variation observed in Caribbean *Anolis*.

Contact splitting was first advocated to explain the mechanism by which larger animals possessing fibrillar attachment systems maintain strong attachment to surfaces despite increases in body mass relative to surface area (Arzt *et al.*, 2003). Such analyses were generally applied across broad phylogenetic scales, noting how setal density scaled positively and spatula size scaled negatively with body size of organisms ranging from small arachnids to large squamates (Arzt *et al.*, 2003). Nevertheless, recent work has reported evidence of contact splitting in comparisons of closely related families of arachnids. Frost *et al.* (2018) examined the morphology and

performance of the foot pads of three species of hunting spider (representing three different families) and found that attachment performance was greatest for the species that possesses the smallest and most densely packed adhesive microstructures, even though the area available for contact was similar to that of the other two species. Our morphological findings provide the first evidence of contact splitting within a single family and genus. Furthermore, the anoles selected for examination in this study are part of a larger monophyletic group of *Anolis* that diverged ~25 Mya and contains only two additional species (not examined in this work) (Poe *et al.*, 2017). Although ecomorphological divergence in a small subset of closely related anole species suggests that such trends may be apparent at broader phylogenetic scales, future work is needed to test whether this hypothesis is corroborated more extensively within the Caribbean *Anolis* radiation.

SETAL FIELD CONFIGURATION IN JAMAICAN ANOLIS

Beyond setal morphology, the configuration of setal fields and how setal form and dimensions vary along the proximodistal axis of the subdigital pad and its lamellae have been hypothesized to influence the function of the entire adhesive pad (Russell *et al.*, 2007; Johnson & Russell, 2009; Webster *et al.*, 2009; Garner *et al.*, 2021b; Russell & Garner, 2021). Most notably, setal length varies substantially and predictably along the proximodistal axis of the subdigital pads and scansors (analogous to the anoline lamellae) of geckos (Russell *et al.*, 2007; Johnson & Russell, 2009; Webster *et al.*, 2009) and *Anolis equestris* (Garner *et al.*, 2021b). This variation in setal length has been hypothesized to influence subdigital pad function in three main ways: (1) enhancement of adaptability and adhesion to rough substrates; (2) minimization of interference between adjacent setae during attachment and detachment; and (3) enabling simultaneous release of all setae carried on a single scansor/lamella (Johnson & Russell, 2009; Garner *et al.*, 2021b). Recent comparative morphological work has supported the third hypothesis (Garner *et al.*, 2021b; Russell & Garner, 2021). Gecko setae, and presumably those of anoles, detach from the surface when their inclination relative to the substrate reaches a critical angle (~30° for geckos; Autumn *et al.*, 2000; Autumn & Peattie, 2002). Johnson & Russell (2009) hypothesized that the proximodistal increase in setal length along a single scansor permits the critical release angle to be attained by all setae simultaneously, enabling detachment of the entire scansor from the substrate instantaneously rather than each row of setae detaching sequentially.

Examination of the setal field configuration of *A. equestris* revealed that the variation in setal length is

effectively opposite to that observed in numerous gecko species (Garner *et al.*, 2021b). Importantly, anoles and pad-bearing geckos differ in the manner in which their subdigital pads are detached from the surface; most pad-bearing geckos peel their adhesive pads from the substrate via distoproximal hyperextension (Russell, 1975), whereas anoles peel their adhesive pads in the opposite direction (proximodistally, as is the ancestral squamate condition) (Russell & Bels, 2001). Therefore, the differences in setal length variation between the two groups is consistent with the mechanism of subdigital pad peeling, being essentially equal but opposite in the two cases (Garner *et al.*, 2021b; Russell & Garner, 2021). Additional support for this hypothesis is derived from consideration of a species of gecko that possesses an incipient adhesive apparatus and rudimentary subdigital pads, *Gonatodes humeralis*, which detaches its adhesive pads from the substrate proximodistally. Its proto-lamellae exhibit a proximodistal decrease in setal length, identical to the condition observed in *A. equestris* (Russell *et al.*, 2015; Higham *et al.*, 2017a; Russell & Garner, 2021).

In our study comparing five species of Jamaican *Anolis*, we found that the proximodistal variation in setal length differs significantly between species. *Anolis garmani*, the crown-giant anole, exhibits variation in setal length that is identical to that observed in another crown-giant anole, *A. equestris*. The remaining species do not show significant variation in setal length along the proximodistal axis of lamellae. Although the influence of setal length variation on peeling performance has yet to be investigated empirically, our findings suggest that peeling performance and/or the biomechanics of peeling may differ between these species, and potentially ecomorphs in general, with the crown-giant anoles exhibiting enhanced peeling performance.

EFFECTIVE BENDING STIFFNESS OF ANOLINE SETAE

Fibrillar adhesion design theory also predicts that the aspect ratio of the fibres can influence adhesive contact strength. Fibres with greater aspect ratios, assuming equivalent material properties, will have lower bending stiffness, which may ultimately permit greater contact with the surface (and therefore adhesion), than fibres with lower aspect ratios (Spolenak *et al.*, 2005; Barreau *et al.*, 2016). We estimated effective bending stiffness (k_{eff}) of anoline setae by modelling them as tapered cylindrical beams and examined whether k_{eff} varied as a function of species. We found that k_{eff} did not vary significantly between species when adjusted for body size. Fibre aspect ratio (and therefore bending stiffness), however, is thought to be limited by the propensity for fibre condensation, in which fibres with particularly high aspect ratios are prone to aggregating

into a mat because interfibre adhesion is stronger than the fibre's ability to resist deformation during bending (Spolenak *et al.*, 2005). This condensed state is thought to reduce adhesive contact; thus, this trade-off between aspect ratio and fibre condensation may limit interspecific variability in fibre aspect ratio. Although we did not find statistically significant differences in k_{eff} theoretical models that connect quantitative differences in k_{eff} to quantitative differences in fibrillar adhesion are lacking. Therefore, it is possible that differences in k_{eff} (whether statistically significant or not) may be functionally relevant and we simply lack the theoretical models to understand how such differences relate to fibrillar adhesion. Clearly, future work is needed to allow us to understand how setal parameters such as setal base diameter, setal apex diameter and bending stiffness influence attachment of both individual setae and entire setal arrays.

SCALING OF THE ATTRIBUTES OF ANOLINE SUBDIGITAL PADS WITH BODY SIZE

Of all the parameters measured in this study, only four varied significantly with body size (as represented by SVL): subdigital pad area, number of lamellae, setal apex diameter and k_{eff} . Both subdigital pad area and the number of lamellae have been previously reported to vary with body size in Caribbean *Anolis* (Pounds, 1988; Glossip & Losos, 1997; Macrini *et al.*, 2003; Elstrott & Irschick, 2004), and thus our findings are in accord with those.

Although previous work found that Caribbean *Anolis* co-vary in size-corrected subdigital pad area and perch height (Macrini *et al.*, 2003; Elstrott & Irschick, 2004), we found that only *A. valencienni*, with significantly smaller size-corrected area, differed significantly from the remainder of the species examined in subdigital pad area once body size was accounted for. Therefore, it does not appear that size-corrected subdigital pad area co-varies with microhabitat use in our sample of anoles. It is not clear why our sample of Caribbean *Anolis* does not support the previous findings of covariance between size-corrected subdigital pad area and microhabitat use. One possibility may be methodological and relate to differences in measurement of subdigital pad area. Most studies recording this dimension for *Anolis* generally do not unambiguously report their criteria for delimiting what portion of the ventral surface of the digit constitutes the subdigital pad (e.g. Elstrott & Irschick, 2004; Winchell *et al.*, 2018). Others report that they consider the proximal extremity of the subdigital pad to be demarcated by the first lamella that is wider than the previous one (Crandell *et al.*, 2014; Donihue *et al.*, 2018; Dufour *et al.*, 2018; Yuan *et al.*, 2019; Avilés-Rodríguez *et al.*, 2021). Here we

used the criteria proposed by Peterson & Williams (1981), which details that the scales that compose the subdigital pad of anoles are those that have an aspect ratio (length/width) >1 and possess a frayed edge (representing the epidermal free margin; Russell & Eslinger, 2017). Clearly, there is no single unified definition of the subdigital surface in *Anolis*, and thus future work may consider identifying the internal and/or external anatomy that demarcate the subdigital pad in *Anolis* and other adhesive pad-bearing lizards.

We also found that k_{eff} varied significantly and positively with body size, and this appears to be driven by a similar relationship between setal apex diameter and body size. Elstrott & Irschick (2004) found that the clinging ability of Caribbean *Anolis* scales isometrically with body mass, indicating that larger anoles have relatively poorer clinging ability than smaller ones relative to their body size. Although this was largely explained by larger anoles having relatively smaller subdigital pad areas relative to their body size, it is possible that increased setal effective bending stiffness as a result of greater setal apex diameters could also result in this relatively reduced adhesive performance. Synthetic fibrillar arrays with lower fibre aspect ratios tend to generate lower pull-off force than those with greater aspect ratios (Barreau *et al.*, 2016). As mentioned above, the lack of theoretical models connecting k_{eff} to adhesion of isolated fibres or fibrillar arrays complicates this potential functional relationship.

TO STICK OR NOT TO STICK? BEHAVIOURAL ECOLOGICAL CONSIDERATIONS OF ADHESION IN *ANOLIS*

It is interesting to consider why clinging ability covaries with perch height in a behavioural ecological context. In other words, why would anoles that perch higher in trees (i.e. in the canopy) require greater attachment performance relative to those that perch lower on trees (i.e. below the canopy)? Elstrott & Irschick (2004) opined that enhanced clinging ability may be necessary for anoles that occupy greater perch heights because of the potential for sustaining injury and energetic risks associated with falling from the canopy. Given that the body size of anole species increases with perch height and that the terminal velocity (and therefore the force of impact) of a falling object increases with the object's mass, it is not unreasonable to predict that larger species of anole may be at a relatively greater risk of severe injury after a fall from high perches than are individuals of smaller species (Elstrott & Irschick, 2004; Higham *et al.*, 2017b). Additionally, anoles that fall from high in the tree canopy would probably have to expend considerable energy to regain their previous perch (Elstrott & Irschick, 2004). The attachment forces

generated by adhesive pad-bearing lizards on smooth, laboratory substrates (e.g. acrylic or glass) are much greater than those necessary to support their body mass during vertical station holding and locomotion (i.e. they possess a very large safety factor) (Irschick *et al.*, 1996; Autumn *et al.*, 2000; Wright *et al.*, 2021). Several studies have revealed that fibrillar adhesion can be significantly reduced on non-ideal substrates (e.g. dirty, wet, soft or rough) (Hansen & Autumn, 2005; Huber *et al.*, 2007; Russell & Johnson, 2007, 2014; Pugno & Lepore, 2008; Stark *et al.*, 2012, 2013; Hu *et al.*, 2012; Gillies & Fearing, 2014; Klittich *et al.*, 2017; Wright *et al.*, 2021; Garner *et al.*, 2021a). Thus, it is possible that the large safety factor relates to the maintenance of effective attachment capabilities when the substrate conditions are not ideal and adhesively effective contact is reduced to small patches. Little is known about the substrate properties used by free-ranging adhesive pad-bearing lizards (Niewiarowski *et al.*, 2016, 2019; Garner *et al.*, 2019; Higham *et al.*, 2019), and thus it is possible that substrates higher in the tree canopy present considerable challenges to the anoline adhesive system and that adhesive force-generating capacity must be greater for effective attachment and locomotion on them. Alternatively, substrates high in the tree canopy may be ideal for attachment via subdigital adhesive pads (Wright *et al.*, 2021). Many species of anole, for example, have been observed clinging to vertically oriented leaves while sleeping at night (Singhal & Johnson, 2007). Although leaf surface topography can vary considerably (Higham *et al.*, 2019; Kumar *et al.*, 2019; Naylor & Higham, 2019), some leaves are quite smooth and can induce considerable attachment force from subdigital adhesive pads (Naylor & Higham, 2019; Wright *et al.*, 2021). Anoles that perch high in the canopy may also require greater attachment performance than those that perch in lower habitats so that falls may be effectively arrested. Higham *et al.* (2017b) predicted the terminal velocities and impact forces of falling geckos landing on leaves in the tree canopy and found that such geckos may theoretically reach or exceed their maximum attachment capacity during such manoeuvres. Therefore, anoles may possess differential clinging abilities in relation to perch height to prevent falls from occurring, or potentially for arresting a fall once it has occurred.

It is also pertinent to consider the alternative: why would anoles occupying lower habitats exhibit lesser clinging ability relative to those occupying the tree canopy? One possibility may be related to the demands placed on lizards employing subdigital adhesive pads during locomotion. Russell & Higham (2009) found that engagement and subsequent disengagement of the adhesive pads of a pad-bearing gecko (*Tarentola mauritanica*) results in a significant speed decrement

when the adhesive pad is engaged with the substrate. Furthermore, they observed that geckos only employed their adhesive pads on inclines $> 10^\circ$, suggesting that they did not engage their adhesive pads until necessary to assist with traction. Many geckos (including *T. mauritanica*) are capable of actively hyperextending their subdigital pads from the substrate via specialized digital musculature (Russell, 1975) and can carry them in a continuously hyperextended state when not being employed in attachment. Anoles, on the other hand, lack the musculature that permits such a carriage of the digits, and subdigital pads are instead passively hyperextended by co-opting the ancestral squamate digital kinematic pattern (Russell & Bels, 2001). As such, ecological variation in adhesive performance in Caribbean *Anolis* may indicate a balancing of the speed–attachment trade-off when adhesive attachment is not deployed. The properties of substrates lower to the ground may also be challenging for effective attachment via adhesive fibres. Substrates may possess a particular roughness that makes fibrillar adhesion inadequate for attachment (Huber *et al.*, 2007; Pugno & Lepore, 2008; Gillies & Fearing, 2014) or may be covered in particulate matter that can clog and disable the adhesive arrays (Hansen & Autumn, 2005; Hu *et al.*, 2012; Russell & Delaugerre, 2017). The form and function of other attachment organs, such as claws, may also invoke a trade-off with those of the subdigital pads. Indeed, recent work found ecomorphological associations of the claws of Greater Antillean *Anolis*, suggesting that claw function may also vary with microhabitat use (Yuan *et al.*, 2019), although this has yet to be investigated empirically. Such notions suggest several exciting lines of inquiry that require future interdisciplinary work. Studies combining observations of the behavioural ecology, morphology and performance of adhesive pad-bearing lizards with examinations of the properties of the surfaces used by free-ranging lizards are particularly needed to better understand the environments and circumstances in which subdigital pads and claws are used.

CONCLUSION

Previous work identified correlations between clinging ability on smooth substrates, subdigital pad macromorphology and habitat use, and found that anoles that perch in the tree canopy exhibit greater clinging abilities than those that perch in lower habitats (Macrini *et al.*, 2003; Elstrott & Irschick, 2004). These findings were largely suggested to be the result of variation in subdigital pad area (Elstrott & Irschick, 2004), yet the morphology of the microstructures that primarily govern attachment to smooth substrates

(setae) was not investigated. Here we examined the setal morphology and setal field configuration of five species of *Anolis* from the island of Jamaica that generally use different microhabitats. We found that Jamaican anoles that perch in the tree canopy tend to have greater setal densities and smaller spatulae than those that perch in lower habitats, consistent with the theory of contact splitting and its impacts on adhesive performance. We also found interspecific differences in the proximodistal variation in setal length, engendering the hypothesis that peeling performance and/or biomechanics vary with habitat use. Although we expect the trends observed here to be consistent at larger phylogenetic scales, future work is needed to determine whether our findings are simply characteristic of this particular group of anoles or if they are indications of ecomorphological evolution of the adhesive setae of Caribbean *Anolis* and whether ecomorphological characteristics that are macroscopically evident extend to the microscopic level.

ACKNOWLEDGEMENTS

We thank Harvard University Museum of Comparative Zoology and the Academy of Natural Sciences at Drexel University for access to their collections and for the specimens used in this study. We also thank Dr Bojie Wang for assistance with SEM. Finally, we acknowledge the helpful support and insight of the Gecko Adhesion Research Group and the Dhinojwala Research Group. We have no conflicts of interest to declare. M.C.W. was funded by Lubrizol Advanced Materials under a biomimicry fellowship. A.P.R. acknowledges financial support from a Natural Science and Engineering Research Council of Canada Discovery Grant (9745-2008). A.D. acknowledges financial support from the National Science Foundation (NSF DMR-1610483).

DATA AVAILABILITY

The data underlying this article are available in Figshare at <https://doi.org/10.6084/m9.figshare.19488476>.

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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:

Table S1. Museum specimens used for this study and their accession numbers.

Figure S1. Linear regression of subdigital pad area as a function of snout-vent length (SVL) and species. Subdigital pad area varies positively and significantly with SVL ($R^2 = 0.31$, $P = 0.02$).

Figure S2. Linear regression of number of lamellae as a function of snout-vent length (SVL) and species. Number of lamellae varies positively and significantly with SVL ($R^2 = 0.89$, $P < 0.0001$).

Figure S3. Linear regression of setal apex diameter as a function of snout-vent length (SVL) and species. Setal apex diameter varies positively and significantly with SVL ($R^2 = 0.5$, $P = 0.001$).

Figure S4. Size-corrected (via residuals of Fig. S1) subdigital pad area varies significantly between the species examined ($DF = 4$, $F = 9.51$, $P = 0.001$). *Anolis valencienni* has significantly smaller size-corrected subdigital pad area than all of the other species (all $P < 0.05$). Species are ordered by perch height with perch height increasing from left to right (depicted by green arrow). Different letters indicate statistical differences.

Figure S5. Size-corrected (via residuals of Fig. S2) number of lamellae varies significantly between the species examined ($DF = 4$, $F = 5.28$, $P = 0.01$). *Anolis garmani* and *A. grahamsi* have significantly lower size-corrected number of lamellae than *A. lineatopus* (all $P < 0.05$). Species are ordered by perch height with perch height increasing from left to right (depicted by green arrow). Different letters indicate statistical differences.

Figure S6. Setal base diameter as a function of pad region and lamella zone. Setal bases are significantly smaller in the distal pad region compared to the proximal pad region of all five species ($P = 0.0002$). Setae decrease significantly in setal base diameter proximodistally along lamella zones of all five species (all pairwise comparisons $P < 0.05$).

Figure S7. Size-corrected (via residuals from Fig. S3) setal apex diameter does not vary significantly between any of the species examined ($DF = 4$, $F = 1.86$, $P = 0.18$). Species are ordered by perch height with perch height increasing from left to right (depicted by green arrow).

Figure S8. Setal resting angle as a function of pad region and lamella zone. Setal resting angles are significantly lower in the distal and intermediate pad regions compared to the proximal pad region of all five species ($P < 0.0001$). Setae decrease significantly in setal resting angle proximodistally along lamella zones of all five species (all pairwise comparisons $P < 0.05$).

Figure S9. Lamella length as a function of pad region. Lamella length varied significantly across pad regions ($DF = 2$, $F = 18.15$, $P < 0.0001$) with distal lamellae being significantly shorter than intermediate and proximal lamellae (distal vs. proximal: $P < 0.0001$; distal vs. intermediate: $P = 0.0004$). Different letters indicate statistical differences.