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Frictional adhesion and toe pad micro-morphology of mainland *Anolis*

Adhesive toe pads are a defining characteristic of the *Anolis* clade, found on all but one described species (Peterson and Williams 1981). Adhesive toe pads may be a key innovation that facilitated occupation of niches unavailable to non-clinging vertebrates (Losos, 2009). The macrostructure of toe pads have been extensively studied, yet little is known about how the pad microstructure, which enables adhesion, contribute to adhesive performance at the organismal level. However, the micromechanics and adhesive performance of the complex, branched adhesive setae of geckos have received considerable attention recently (Autumn 2006).

Anolis toe pads (Fig. 1b) consist of modified subdigital scales termed lamellae (Fig. 1c) that contain setae (Fig. 1d) – microscopic (avg. 20 μm tall) hair-like structures made of beta-keratin (Ruibal and Ernst 1965) that facilitate adhesion.

Microscopic spatulae (Fig. 1e) at the tips of the setae interact with the surface. In geckos, adhesion is largely due to intermolecular van der Waals forces (Autumn et al. 2002). Initiation of attachment is reliant on mechanical application to the surface. Setae, lamellae, and whole toes are load dependent and directional; only with correct orientation, preload force, and drag can attachment occur

(Autumn 2000). While preliminary research suggests a similar mechanism in *Anolis* (unpublished), the adhesion micromechanics remain untested until now.

The specific toe pad microstructure is likely the result of the material requirements of the adhesive, phylogenetic constraint, and adaptation (for review see Autumn 2006; Irschick et

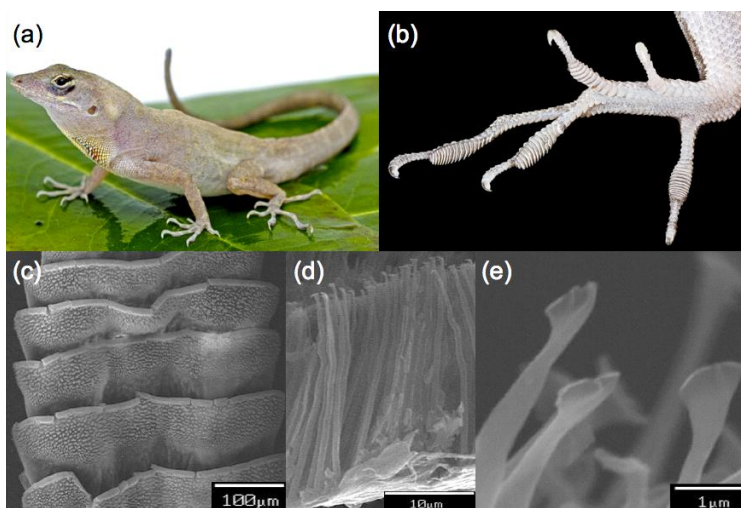


Fig. 1. Anatomy of the *Anolis* toe pad, depicted at increasing levels of detail (see text for explanation).

al. 2006). Despite considerable variation in morphology of adhesive microstructures (Ruibal and Ernst 1965; Peterson and Williams 1981), a comparison of adhesive structures between *Anolis* species has not been conducted. Models of van der Waals interaction suggest that geometry, including spatula shape, may determine the limits of seta function (Autumn 2006). Spatula shape, seta density and length are important in production of frictional adhesion in geckos (Peattie 2007). Models presented by Campolo et al. (2003) and Sitti and Fearing (2003) suggest that longer setae will be able to conform to rougher surfaces. If we model a single seta as a cantilever beam (as described in Autumn, 2006), the effective stiffness will be inversely proportional to the length squared: longer setae will have lower stiffness. The cantilever model can further be used to examine the angle of the seta to the substrate (Sitti and Fearing, 2003), and ultimately suggests that longer setae, with lower stiffness, may be able to adhere at a lower angle. If this is true, then seta shear force could be greater due to the lower contact angle, and shown by Tian et al. (2006).

In this paper we present preliminary comparative data to examine the consequences of toe pad microstructure on adhesive capabilities in mainland *Anolis* species. To do so, we measured microstructure morphology and organismal-level performance. Specifically, we address the hypothesis that longer setae will allow a higher shear stress (force/area) production.

Methods

In this study we explored adhesive capabilities of 5 *Anolis* species from Costa Rica. The species measured were: *A. capito*, *A. humilis*, *A. lemurinus*, *A. limifrons*, *A. polylepsis*, and *A. cupreus*. On the day of capture in the field, we took shear force measurements using an acetate covered glass microscope slide mounted to a dual-range force sensor (40 Hz; Vernier). I applied the longest hind toe to the acetate sheet, with slight manual preload if necessary. Shear force was recorded as the subject slid at a constant rate until detaching from slide. Each subject underwent three repeated trials for the left and right fourth toes. When necessary, individuals

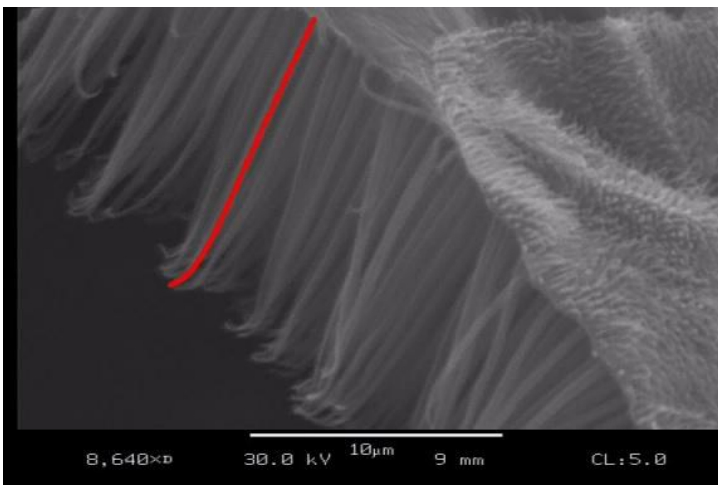


Fig. 2. I measured seta length from images of the proximal tip of the lamella. Setae were traced from the tip of the spatula to the base of the array. The maximum observed value was used in subsequent analysis

of rare species participated in up to 5 trials. Shear force data was standardized per toe pad area to determine stress (force/area).

I took a whole toe from individuals in the field post-performance trials. Prior to analyses, toes were stored in 70% ethanol. Using forceps, I isolated and air-dried single lamellae from the center of the preserved pad. I mounted the lamellae on an aluminum SEM stub with LocTite 410 instant adhesive. A Hummer VI sputtering system (Technics) coated all specimens with a thin layer of platinum. I took images of

the toe pad microstructure with an Amray 1810 Scanning Electron Microscope (SEM) the day of sputter-coating. Images were digitally recorded with ImageDV (v1.3, Evological), and analyzed using ImageJ (1.40g, Rasband).

I measured seta length from images of the proximal tip of the lamella (see figure). Setae were traced from the tip of the spatula to the base of the array. The maximum observed value was used in subsequent analysis.

We also performed our analysis on independent contrasts (Felsenstien 1985) using PDAP 6.0 (Garland 1999) based on the most complete phylogenetic tree of *Anolis* (Nicholson et al. 2005) to account for potential similarities due to shared ancestry.

Results and Discussion

Microstructure analysis included six mainland anole species from Costa Rica. A strong correlation between body mass and seta length exists (Pearson Correlation = 0.86, $P=0.03$ [*]). For all subsequent analysis, seta length was log transformed to account for potential non-linear changes of seta length with body size.

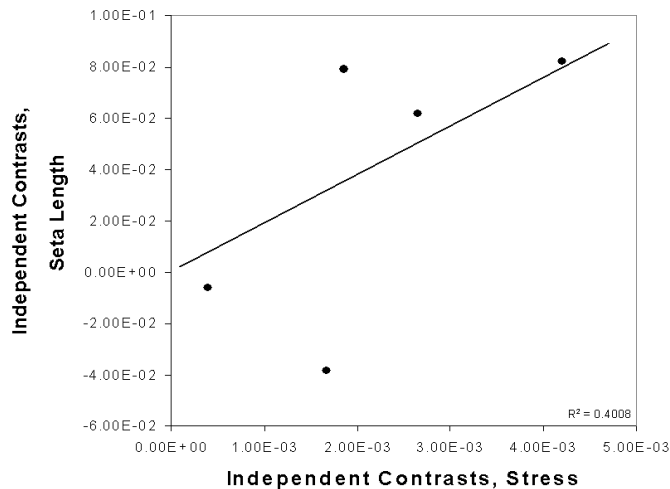


Fig. 3: Relationship between toe pad performance and seta length.

species, but did find a significant correlation between length and width, and width and shear force, likely due to an increase in spatula tips per seta. The microstructure of *Anolis* toe pads, however, allows an analysis of length independent of spatula tip density, because unlike in geckos, each seta has a single spatula. Setae ranged in length from 16 to 30 μm , inconsistent with the hypothesis (Peattie 2007) that *Anolis* are historically constrained from developing longer setae. While the data show a positive correlation between stress and seta length, further analysis of isolated setae and an increase in number of sampled species will be necessary to tease apart the relevance of setal length to attachment force.

Further Research

Data presented here covers one of the relationships between microstructure morphology and adhesive capacity of mainland anoles that we have explored. The varying toe pad

A positive correlation exists between phylogenetically corrected maximum seta length and stress (Fig) ($r^2=0.40$). However, this relationship is not statistically significant (ANOVA $f=1.94$, $df=1,3$, $P=0.23$ [NS]). Although this correlation supports our hypothesis that microstructure morphology is broadly correlated with performance, further data are needed. In a comparative analysis of single setae of eight gecko species, Peattie (2007) did not find a significant correlation between seta length and shear force in gecko

microstructure within the *Anolis* clade offers a unique opportunity to test a variety of mechanical models pertaining to fibrillar adhesives, and additional data will allow us to test these models more thoroughly. Further understanding of the *Anolis* adhesive will also allow us to test hypotheses concerning habitat use and mechanical abilities, and will ultimately allow us to test why mainland and Caribbean radiations differ in toe pad structure.

Although the data we report in this paper did not yield a statistically significant result, this is likely due to the small number of species sampled. Over the next year, we hope to increase our sample size by collecting data from additional mainland anole species in Panama.

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