# On the origin of frictional adhesion in geckos: small morphological changes lead to a major biomechanical transition in the genus *Gonatodes*

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The evolutionary history of vertebrate locomotion is punctuated by innovations that have permitted expansion into novel ecological niches. Frictional adhesion of geckos is an innovation renowned for enabling locomotion on vertical and inverted smooth surfaces. Much is known about the microstructure and function of the fully-expressed gekkotan adhesive apparatus, although how it originated is poorly understood. Therefore, identifying species that exhibit the earliest stages of expression of frictional adhesion will provide significant insights into the evolution of this trait. Our previous investigation of digital proportions, shape, scalation, skeletal form, and subdigital epidermal micro-ornamentation in the genus *Gonatodes* led us to hypothesize that *Gonatodes humeralis* expresses incipient frictional adhesion. To test this, we first conducted a phylogenetic analysis of *Gonatodes* and related sphaerodactyl genera to clarify the historical context of the evolution of frictional adhesive capability in the genus. We then measured the ability of *G. humeralis* and its close relatives to generate frictional adhesive force, examined their locomotor capabilities on low-friction surfaces, and observed animals in their natural habitat. After accounting for body mass and phylogenetic relationships, we found that *G. humeralis* generates frictional adhesive force essentially equivalent to that of *Anolis*, and can scale vertical smooth surfaces. *Gonatodes vittatus*, a species that lacks elaborated epidermal setae, generates negligible frictional adhesive force and can only ascend smooth inclined surfaces with a pitch of $\leq 40°$. We conclude that the ostensibly padless *G. humeralis*, with feet lacking the musculoskeletal, tendinous, and vascular modifications typical of pad-bearing geckos, nevertheless can employ frictional adhesive contact to assist locomotion. As in *Anolis*, the release of frictional adhesive contact occurs when the foot is plantar flexed after the heel has lifted from the surface. Our findings indicate that the origin of frictional adhesion was likely gradual but that, ultimately, this led to major shifts in ecology and function. © 2016 The Linnean Society of London, *Biological Journal of the Linnean Society*, 2017, **120**, 503–517.

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## INTRODUCTION

Animal locomotion is replete with examples of elaborate behavioural and morphological novelties that enhance performance (Irschick & Higham, 2016). The adhesive apparatus of geckos is one such innovation, permitting locomotion in challenging microenvironments, such as on vertical smooth surfaces. This remarkable system generally involves a complex hierarchy of components: setae (microscopic beta-keratin hair-like structures), scansors (expanded digital scales), and modified skeletal elements, muscles, and tendons of the foot and other parts of the limb (Russell, 1975). The effective use of these morphological modifications is associated with altered locomotor kinematics facilitating the deployment of the system (Birn-Jeffery & Higham, 2014; Higham *et al.*, 2015), including changes in how the gecko pushes off at the end of stance in association with active hyperextension of the tips of the digits (Russell, 2002), as well as how the limbs are moved in relation to foot contact (Birn-Jeffery & Higham, 2014, 2016; Zhuang

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& Higham, 2016). Such an adaptive syndrome has originated, and become secondarily reduced or lost, independently on multiple occasions within the Gekkota (Gamble *et al.*, 2012; Higham *et al.*, 2015).

Although active hyperextension of the distal portions of the digits, for disengagement of the system prior to the heel being lifted from the surface, is regarded as being synonymous with gekkotan frictional adhesion (Russell & Higham, 2009), it is not necessary for frictional adhesion to be effective among lizards (Irschick *et al.*, 1996; Russell & Bels, 2001; Irschick, Herrel & Vanhooydonck, 2006). Specifically, instead, lizards of the genus *Anolis* roll the distal tips off the substrate at the end of the stance phase. This action, termed passive hyperextension (Russell & Bels, 2001), is shared with the locomotor kinematics of primitively padless lizards (Snyder, 1952; Renous & Gasc, 1977; Brinkman, 1980; Arnold, 1998). During the evolution of the complex adhesive apparatus of geckos, it is likely that the transformation of subdigital spinules into adhesive setae was the initial key step in altering the dynamics of traction (Russell, 1976; Peterson, 1983; Peattie, 2008; Russell *et al.*, 2015), after which the morphology, control, and kinematics of the system were modified further (Russell, 1976).

Multiple instances of reduction and loss of the adhesive apparatus have been identified. For example, *Rhoptropus afer* in Namibia occupies relatively horizontal surfaces, is markedly cursorial, and has a reduced adhesive apparatus compared to that of its close relatives, which are predominantly climbers (Higham & Russell, 2010; Collins, Russell & Higham, 2015). Despite the plethora of examples that illustrate transitions towards secondary loss of frictional adhesion, a demonstration of the transition from frictional to frictional adhesive attachment has remained elusive. Although it has been noted that some groups of geckos possess enlarged subdigital scales that bear small setae (Russell, 1976), no functional data associated with such presumed transitions are available.

Recent comparative studies and ecological observations led us to the ancestrally 'padless' diurnal sphaerodacyline genus *Gonatodes*. We previously reported on macroscopic and microscopic aspects of the digital anatomy of this genus, and one small species, *Gonatodes humeralis*, exhibits elaborated epidermal structures that are potentially capable of inducing adhesive attachment (Russell *et al.*, 2015). Compared to its congeners, its epidermal spinules in the vicinity of the digital inflections are longer and are elaborated into branched, spatulate-tipped setae located on the free distal margin of the sub-inflection scales. *Gonatodes humeralis*, in common with its 29 congeners, lacks the modifications of the digital musculotendinous, circulatory, and skeletal systems that are generally considered to be essential for the operation of a gekkotan adhesive apparatus (Russell *et al.*, 2015).

We first conducted a phylogenetic analysis of *Gonatodes* and related sphaerodactyl genera to clarify the historical context of the origin of the hypothesized adhesive capability within the genus, and to provide context for subsequent observations. Then, using multiple species of *Gonatodes* from both Trinidad & Tobago (TT) and French Guiana (FG), we examined their adhesive clinging ability and locomotor capabilities on smooth, low-friction inclined substrata. Based on previous research with padless and pad-bearing lizards, we tested three predictions: (1) *G. humeralis* exhibits passive digital hyperextension via pedal plantar flexion (Brinkman, 1980), at the end of stance, in a similar fashion to *Anolis* (Russell & Bels, 2001); (2) *G. humeralis*, but no other *Gonatodes* species, is capable of generating frictional adhesive force; and (3) *G. humeralis*, but no other species in the genus, can scale steep, low-friction smooth surfaces.

## MATERIAL AND METHODS

### PHYLOGENETIC METHODS

We estimated phylogenetic relationships among the species of *Gonatodes* and related sphaerodactyl geckos aiming to better understand the historical context of adhesive digit evolution in the genus. DNA sequences were assembled from previously published molecular phylogenies (Gamble *et al.*, 2008, 2015; Geurgas, Rodrigues & Moritz, 2008; Schargel *et al.*, 2010; Russell *et al.*, 2015). The concatenated dataset included 20 *Gonatodes* species and 42 outgroups, including representatives from all sphaerodactyl genera. Sequence data were composed of 5182 bp of aligned DNA from fragments of six nuclear protein-coding genes (ACM4, CMOS, PDC, NT3, RAG1, and RAG2) and three mitochondrial genes (ND2 and associated tRNAs, 12S, and 16S) (see Supporting Information, Table S1).

A time-calibrated Bayesian phylogeny was constructed using BEAST, version 1.8.1 (Drummond *et al.*, 2012), with a lognormal relaxed clock and Yule tree prior, implemented on the CIPRES (Cyberinfrastructure for Phylogenetic Research) Science Gateway (Miller, Pfeiffer & Schwartz, 2010). Optimal data partitioning and model selection were conducted employing PARTITIONFINDER, version 1.1.0 (Lanfear *et al.*, 2012), using the greedy search algorithm and Bayesian information criterion model selection. The tree root was calibrated using a lognormal prior (mean = 1.5; SD = 1.0) and a minimum age of 112 Myr based on the fossil gekkotan *Hoburogekko suchanovi* (Daza, Alifanov & Bauer, 2012).

Divergence between the clade containing *Teratoscincus scincus* and the clade containing *Teratoscincus roborowskii*, based on the Tien Shan-Pamir uplift in western China (Macey *et al.*, 1999), used a lognormal prior (mean = 0.0; SD = 1.0) with a minimum age of 9 Myr. Finally, the minimum age for the genus *Sphaerodactylus* was set at 15 Myr using a lognormal prior (mean = 1.5; SD = 1.0) based on the amber fossils *Sphaerodactylus dommeli* and *Sphaerodactylus ciguapa* (Kluge, 1995; Iturralde-Vinent & Mac-Phee, 1996; Daza & Bauer, 2012). Bayesian analyses were run in duplicate for 5 million generations, sampling every 10 000 generations. Output files were checked for convergence using TRACER (Rambaut & Drummond, 2007), and both runs, minus burn-in, were combined to estimate tree topology and divergence times.

We reconstructed the potential for digital frictional adhesive capability among sampled gecko species using maximum-likelihood with the ace function in the R package APE 3.1-4 (Paradis, Claude & Strimmer, 2004). Frictional adhesion was categorized as being 'present' or 'absent' in sampled species (Russell, 1972; Gamble *et al.*, 2012), based upon digit structure. We compared transition models with equal and different rates and used the 'all rates different' model as it best fit the data, as determined by the Akaike information criterion.

### FIELD COLLECTIONS AND MEASUREMENTS

We collected four species of *Gonatodes* in FG and TT during multiple trips from 2014 to 2015, all occurring in November/December: *G. humeralis* (both FG and TT), *Gonatodes vittatus* (TT), *Gonatodes ocellatus* (TT), and *Gonatodes ceciliae* (TT). In Trinidad, *G. humeralis* was collected on the north-west coast, *G. vittatus* in the Arima Valley and near Manzanilla Beach, and *G. ceciliae* near the village of Biche. In Tobago, *G. ocellatus* was collected just west of Charlotteville. In FG, *G. humeralis* was collected at the Pararé site of the Nouragues field station (Gasc, 1981). In addition, we collected several *Thecadactylus rapicauda* in FG. This species is sympatric with *G. humeralis* and exhibits a structurally-complex adhesive apparatus (Russell, 1975, 2002), providing for a useful comparison.

Ecological observations were recorded only for individuals and species collected in TT. Observations were made during normal activity periods, between 10.00 h 15.00 h. Upon sighting an animal, we recorded the type of substrate on which the animal was found. Animals were then collected by hand and transported to our field laboratory for morphological and biomechanical measurements, as well as videography. All fieldwork was approved by permits from the *Centre National de la Recherche Scientifique* in France (for FG), the Wild Life Section of the Forestry Division in Trinidad, and the Department of Natural Resources & the Environment in Tobago.

### MORPHOLOGY

Following capture, we obtained body mass using a 10 g spring scale (Pesola), and measured limb segment lengths and snout–vent length with digital calipers. Micro-morphological data were taken from Russell *et al.* (2015) and were used in the present study to correlate frictional adhesive force with the microscopic anatomy of the adhesive apparatus.

### FRICTIONAL ADHESIVE FORCE MEASUREMENTS

We measured the adhesive force of each lizard using a Mark-10 Force Gauge (Model M5-10, accuracy = ±0.1% full-scale), with a capacity for measuring forces up to 50 N. We hung a clear section of acrylic (~5 × 3 cm; thickness 0.6 cm) from the output hook. Maximum tensile frictional adhesive force of the forelimbs was quantified by allowing the animal to attach and then pulling it away from, and in line with, the output hook. Animals were slowly pulled by hand and moved no more than 3 cm. *Sensu* Gilman *et al.* (2015) and Irschick *et al.* (1996), we measured the adhesive force produced by the forelimbs only, although differences in forelimb force were assumed to be representative of overall differences in force. Measurements were repeated at least five times, and the maximum value was recorded. All trials were performed during daylight hours to mirror activity patterns in nature. Prior to each trial, the acrylic was wiped with 100% ethanol and allowed to dry. Forces of many other species have been measured in this manner and are reported by Irschick *et al.* (1996). These values enabled us to assess how much force *Gonatodes* is able to generate in comparison to geckos with a well-developed adhesive apparatus, as well as in comparison with anoles and adhesively-capable skinks (the latter generating the weakest clinging forces recorded for any lizards tested to date). We also calculated the safety factor by dividing the adhesive force by body weight (body mass × acceleration as a result of gravity).

To account for body mass, we used an ordinary least squares (OLS) regression of body mass vs. maximum clinging force. The residuals were obtained and scaled to the average frictional adhesive force reported for skinks by Irschick *et al.* (1996), given that they exhibit a very low level of frictional adhesive force. To account for phylogenetic relationships, we also used phylogenetic generalized least squares (PGLS) (Grafen, 1989) analysis in R, version 3.3.1

(R Foundation for Statistical Computing) using the packages APE (Paradis *et al.*, 2004), nlme (Pinheiro *et al.*, 2014), and Phytools (Revell, 2012) to obtain the scaling exponent and residuals for mass vs. clinging force. We used a well-sampled molecular phylogeny of squamates (Zheng & Wiens, 2016) to perform the phylogenetic regression. The original maximum-likelihood tree consisted of 4162 lizard and snake species and was time-calibrated using penalized likelihood. We pruned the tree to include just taxa matching our adhesion data (Irschick *et al.*, 1996) and data collected in the present study. None of the skink species with adhesion data were included in the original phylogeny; in those cases, we used closely-related species instead. The three *Prasinohaema* species with adhesion data were replaced by three *Insulasaurus*, and *Lipinia leptosoma* was replaced with *Lipinia pulchella*. Two different phylogenies have both recovered *Prasinohaema* and *Insulasaurus* as the sister clade to *Lipinia*, yet neither study included the other genus (Linkem, Diesmos & Brown, 2011; Skinner, Hugall & Hutchinson, 2011). It is therefore presumed that *Prasinohaema* and *Insulasaurus* are an equal phylogenetic distance from *Lipinia* and that these taxon substitutions should have limited influence on the subsequent comparative analyses.

## LOCOMOTOR CAPABILITIES AND MECHANISM OF DIGITAL HYPEREXTENSION

Geckos were encouraged to move up an acrylic trackway, which was cleaned with 100% ethanol prior to each trial. We performed such trials for *G. humeralis* (*N* = 5) and *G. vittatus* (*N* = 5), which represent the two morphological extremes documented in our previous study (Russell *et al.*, 2015). To assess the ability of geckos to move up an inclined acrylic trackway, we incrementally (by 10°) altered the pitch of the substrate. We started with the acrylic sheet oriented vertically and subsequently reduced the angle of incline until the gecko was able to ascend. The shallowest angle used was 40°, being the steepest angle that could be ascended by both species.

Using a high-speed camera (Edgertronic) operating at 500 fps, we obtained high-speed videos of *G. humeralis* to determine the pattern of digital hyperextension at the end of stance. To quantify the sequence of events, we determined the end of stance (which was the frame in which the contact of the foot with the surface was completely broken), the time of initial elevation of the heel (when the heel was no longer in contact with the substrate), and the first frame in which no toe tip was in contact with the surface. The timing between these events (i.e. relative to stance) was quantified and compared with similar measurements from an ancestrally padless gecko (*Eublepharis macularius*), a pad-bearing gecko (*Chondrodactylus bibronii*), and a species of gecko with a secondarily simplified adhesive apparatus (*R. afer*). This research complied with an Animal Use Protocol (A20140028) issued by the University of California at Riverside Institutional Animal Care and Use Committee.

## STATISTICAL ANALYSIS

We used SYSTAT, version 13 (Systat Software Inc.) for all of the statistical analyses. To compare the frictional adhesive force of *G. humeralis* with that of other pad-bearing geckos, anoles, and skinks, we used one-way analysis of variance (ANOVA) with group (*G. humeralis*, other pad-bearing geckos, anoles, and skinks) as the independent variable and frictional adhesive force (residuals) as the dependent variable. We did this using the residuals from the OLS and the PGLS regressions. Tukey's post-hoc test was used to determine the pairwise differences between groups. One-way ANOVA and Tukey's post-hoc test were used to determine the differences in the timing of digital hyperextension relative to the elevation of the heel during stance. All values in the results are reported as the mean ± SEM.

# RESULTS

## PHYLOGENETICS

Phylogenetic relationships among sphaerodactyl genera and *Gonatodes* species were largely congruent with previously published phylogenies at well-supported nodes (Gamble *et al.*, 2008; Schargel *et al.*, 2010; Pyron, Burbrink & Wiens, 2013; Russell *et al.*, 2015) (Fig. 1; see also Supporting Information, Fig. S1) and exhibited concordant divergence times (Gamble *et al.*, 2015). Within *Gonatodes*, *G. humeralis* formed a strongly supported clade with *G. antillensis* and *G. concinnatus*.

Mapping the capability for frictional adhesion (based upon digit structure) onto the phylogeny supports multiple, independent origins of frictional adhesion among the Sphaerodactylidae from a common ancestor that lacked this property. This is similar to the results reported by Gamble *et al.* (2012). In particular, frictional adhesion in *G. humeralis* was independently derived, this species being deeply nested within *Gonatodes* (Fig. 1).

## ECOLOGY

*Gonatodes vittatus* was found among fallen palm fronds and on rough tree trunks and branches along
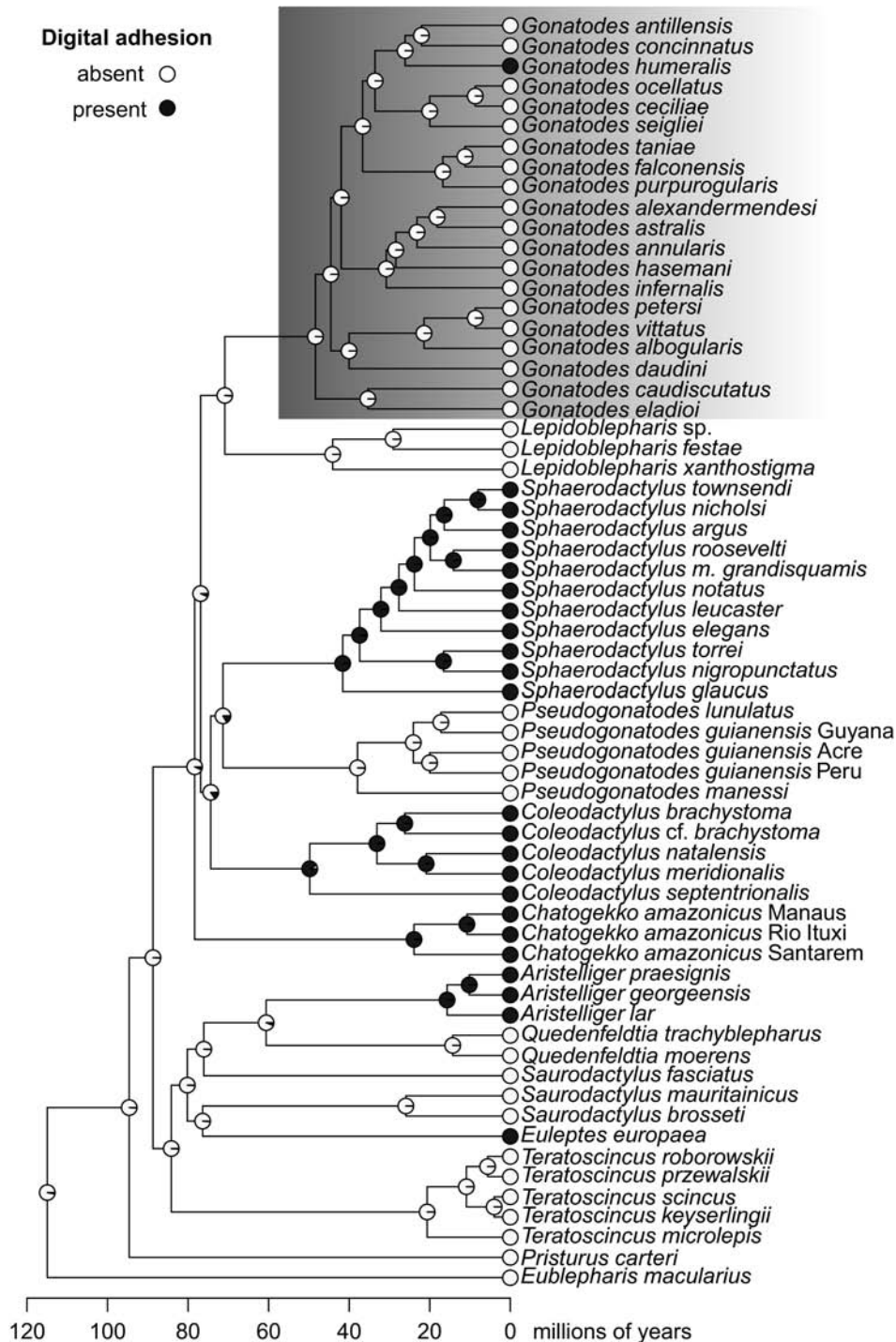
**Figure 1.** Evolution of the capability for frictional adhesion reconstructed on a time-calibrated, Bayesian phylogeny of sphaerodactyl geckos. Genus *Gonatodes* is highlighted by a grey box. White circles indicate species considered to lack frictional adhesive capabilities and black circles indicate species that exhibit this capability. Proportion of black to white in the area of the pie-charts at internal nodes within the phylogeny indicates relative support for the presence of the capability to generate frictional adhesion as deduced from the maximum likelihood reconstruction using a two-rate model.

the beach, as well as on horizontal surfaces around human-made structures. It is active in direct sunlight. *Gonatodes ocellatus* was found on rocks in Tobago, often in shaded, very humid areas. *Gonatodes ceciliae* was found on rough tree trunks and was also active in shaded areas. *Gonatodes humeralis* was active in varying light levels but generally in the shade, and was found on rough tree trunks and smooth vertical bamboo culms, with the latter constituting a low-friction surface (Fig. 2). It has also been reported to occupy smooth leaf surfaces when resting and sleeping (for details, see Discussion). Sample sizes for these observations varied among species but were based on a minimum of five
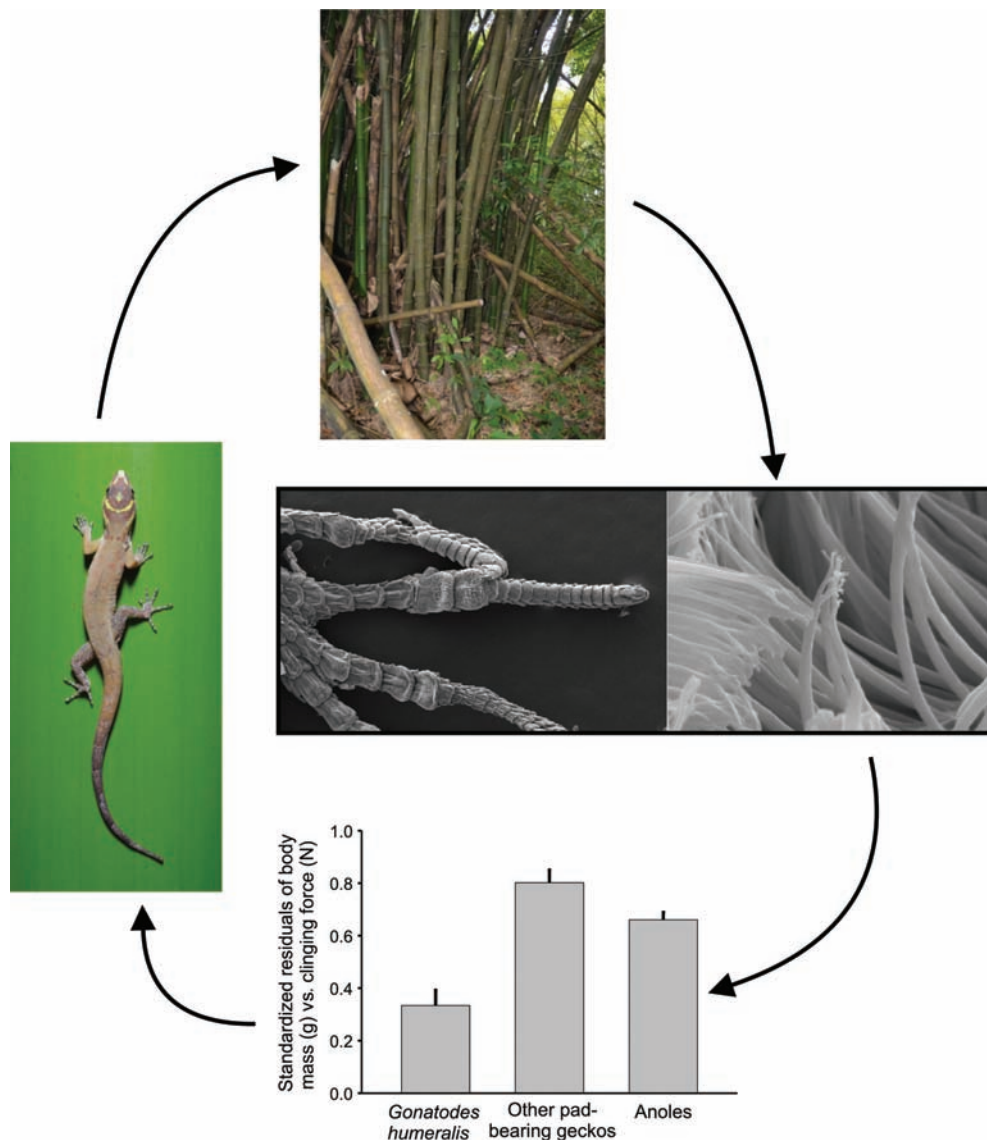


**Figure 2.** Composite illustration of the structural basis and ecological role of incipient frictional adhesion in *Gonatodes humeralis*. Upper photo shows bamboo shoots in the natural habitat of *G. humeralis* in Trinidad. Individuals were found perched on these vertical or steeply-inclined surfaces. The middle right image presents scanning electron micrographs of the ventral surface of the pedal digits and of individual setae of *G. humeralis*. Lower graph shows the residuals of a regression of body mass (g) vs. clinging force (N) of *G. humeralis* ($N = 19$), other pad-bearing geckos, and anoles. All residuals are relative to the values for skinks as reported by (Irschick *et al.*, 1996). Multiple sources were used for values for anoles (Irschick *et al.*, 1996) and other geckos (Irschick *et al.*, 1996; Bergmann & Irschick, 2005; Stewart & Higham, 2014), including data we collected for *Thecadactylus rapicauda* ($N = 9$). Values are the mean $\pm$ SEM. Middle left image depicts an individual *G. humeralis* station-holding on a vertical bamboo culm in the laboratory.

individuals (*G. ceciliae*), although often exceeded 30 individuals. Our observations are corroborated by those reported in the literature.

## MORPHOLOGY

The mean ± SD body mass of *G. humeralis* from TT ($N = 10$) and FG ($N = 9$) was 0.62 ± 0.08 and 0.79 ± 0.12 g, respectively. The mean ± SD body mass of the five *G. vittatus* individuals used in our frictional adhesive force and locomotion trials was 0.93 ± 0.08 g. The mean ± SD body mass of the *T. rapicauda* ($N = 9$) was 2.48 ± 0.15 g. Detailed analyses of digit morphology are provided in Russell *et al.* (2015).

## FRICTIONAL ADHESIVE FORCE MEASUREMENTS

*Gonatodes humeralis* ($N = 19$) generated substantial frictional adhesive forces (Fig. 2), and this did not differ between the TT and FG populations. The results are presented for the combined forelimbs. The *G. humeralis* individuals from TT ($N = 10$) and FG ($N = 9$) had a mean ± SD frictional adhesive forces of 0.11 ± 0.01 and 0.10 ± 0.01 N, respectively. By contrast, the *T. rapicauda* ($N = 9$) had a mean ± SD frictional adhesive force of 4.67 ± 0.39 N (Fig. 2). None of the *G. vittatus*, *G. ocellatus*, and *G. ceciliae* specimens from TT generated any detectable frictional adhesive force (sample sizes = 6 each). The OLS regression of log body mass vs. log maximum frictional adhesive force for geckos, anoles, and skinks combined resulted in a scaling exponent of 0.99 ($r^2 = 0.70$, $P < 0.01$) (Fig. 3A). With skinks removed, the scaling exponent became 1.08 ($r^2 = 0.92$, $P < 0.01$) (Fig. 3A). The PGLS regression resulted in a comparable scaling exponent of 0.95 with all species included. The one-way ANOVA comparing the residual frictional adhesive force (from the OLS regression) between *G. humeralis*, other pad-bearing geckos, anoles, and skinks was significant ($F = 14.2$, d.f. = 3, $P < 0.001$), and the results were comparable using the residuals from the PGLS regression. Tukey's post-hoc test revealed that size-corrected frictional adhesive force of *G. humeralis* was significantly smaller than that of other pad-bearing geckos ($P < 0.01$), and significantly greater than that of skinks ($P < 0.01$), although not significantly different from that of anoles ($P = 0.68$). Both pad-bearing geckos and anoles generated a greater frictional adhesive force than did skinks (both $P < 0.01$).

Safety factor ranged from just over 80 in pad-bearing geckos to approximately 30 for *G. humeralis* and less than 20 for skinks (Fig. 3B). Both anoles and pad-bearing geckos exhibited significantly higher safety factors than *G. humeralis*, although the latter
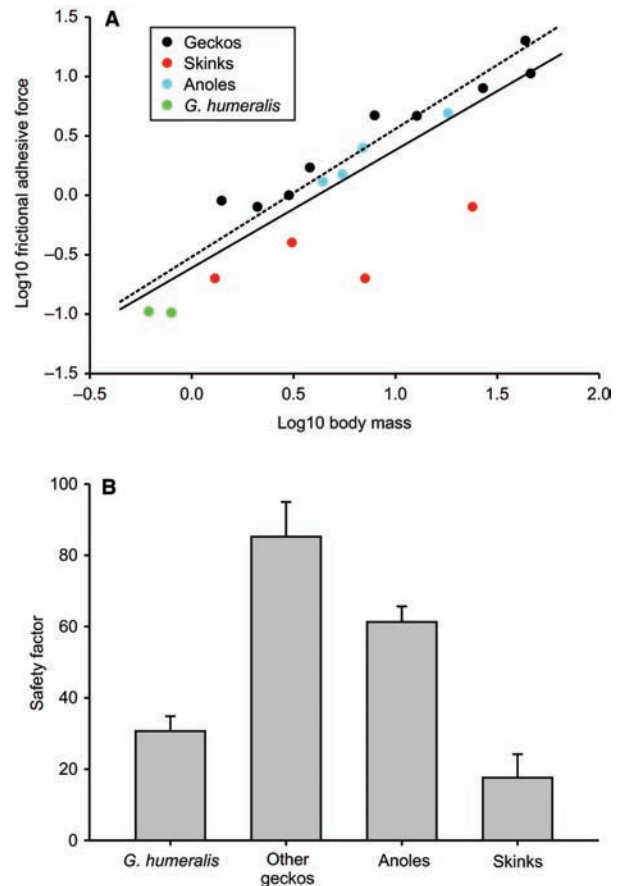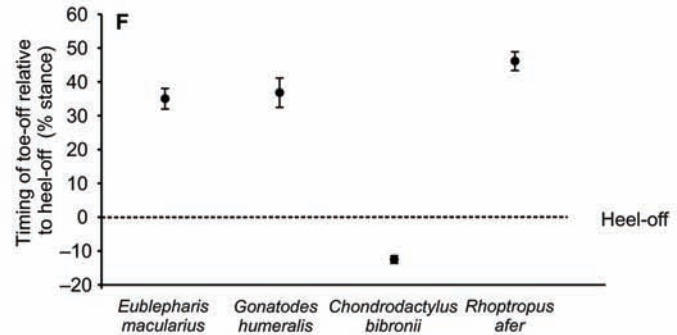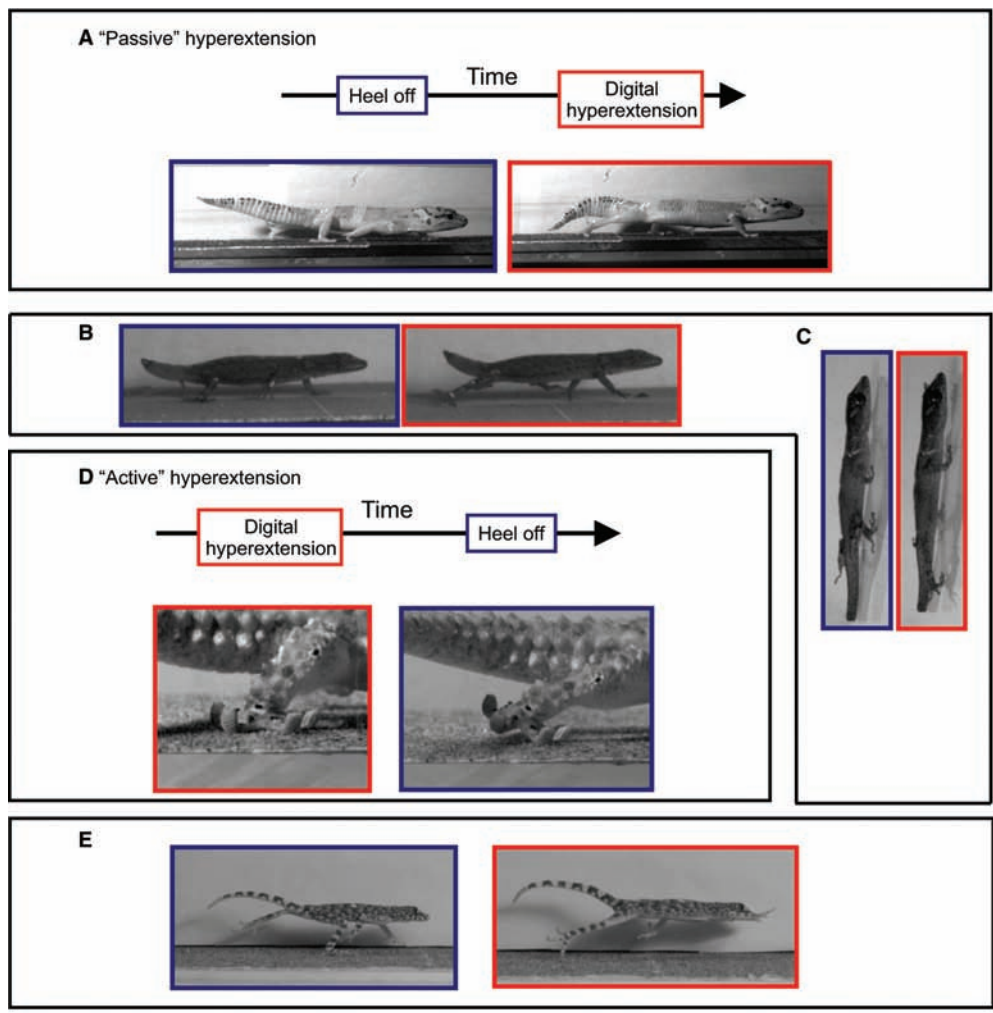


**Figure 3.** Linear regressions of log body mass vs. log maximum frictional adhesive force for geckos, skinks, and anoles combined (A, solid regression line) and for only geckos and anoles combined (A, dashed regression line). The scaling exponent for all species was 0.99 ($r^2 = 0.70$, $P < 0.01$). With skinks removed, the scaling exponent became 1.08 ($r^2 = 0.92$, $P < 0.01$). The phylogenetic generalized least squares regression yielded a scaling exponent of 0.95 (all species included, $P < 0.01$). B, the average safety factor (frictional adhesive force/body weight) for *G. humeralis*, pad-bearing geckos, anoles, and skinks. Data for geckos, anoles, and skinks not included in the present study were taken from the studies listed in the caption of Figure 2. Values are the mean ± SEM.

was significantly greater than the values for skinks (Fig. 3B). When comparing safety factor with body mass (OLS regression), there was no significant relationship ($r^2 = 0.002$, $P > 0.05$).

## LOCOMOTOR CAPABILITIES AND DIGITAL HYPEREXTENSION

*Gonatodes humeralis* was consistently capable of moving up the vertical acrylic sheet (see Supporting Information, Video Clip S1). By contrast, *G. vittatus*

was incapable of moving on any incline > 40°, and slipped and skidded when attempting to do so. On all surfaces, *G. humeralis* passively hyperextended its digits at the end of stance (Fig. 4), and the timing of this relative to heel elevation was not significantly different from that of *E. macularius* or *R. afer* (Fig. 4). However, all three of these species differed significantly from *C. bibronii* (ANOVA, *P* < 0.01), for which digital hyperextension preceded heel elevation (Fig. 4).

## DISCUSSION

The results of the present study are consistent with *G. humeralis* exhibiting the initial stages of a key

**Figure 4.** Patterns of digital hyperextension among geckos. A, showing what is typically referred to as 'passive hyper-extension' (PDH of the present study; see Discussion), which involves rolling off the tips of the toes following heel elevation at the end of stance. Shown in the video is a leopard gecko (*Eublepharis macularius*) walking on a level surface. B, C, *Gonatodes humeralis* executing the same pattern as in (A) on horizontal and vertical surfaces, respectively, indicating that the acquisition of adhesive capabilities need not be coupled with anything more than the ancestral pattern of digital hyperextension. D, showing what is typically referred to as 'active hyperextension' (DPH of the present study; see Discussion) in which the distal ends of the toes are elevated prior to heel elevation. Images show this pattern in *Chondrodactylus bibronii*, a pad-bearing gecko. Secondary simplification of the adhesive apparatus in geckos also results in the re-acquisition of 'passive hyperextension' (PDH) during high speed horizontal running, as shown here for *Rhoptropus afer* (E). F, average timing of toe-off relative to heel-off, displayed as a percentage of stance. Values > 0 indicate that the heel was lifted from the substrate prior to the tips of the toes, and values < 0 indicate that the elevation of the distal ends of the toes (carrying the adhesive pads) preceded heel elevation. Dashed line indicates the boundary between PDH (above the line) and DPH (below the line). Timing did not differ among geckos without toepads or with a simplified adhesive system [analysis of variance (ANOVA), $P > 0.05$], although all three of these species differed significantly from the species with fully-expressed pads (ANOVA, $P < 0.01$). For each species, $N = 4$ and the values are the mean ± SEM.

transition from friction-enhanced to frictional adhesion-enhanced traction, thus providing a window into the evolution of the adhesive system of geckos. Our observations show that the relatively simple expression of setae on the ventral surface of the digits of *G. humeralis*, in the absence of any specialized anatomical systems to control them (Russell *et al.*, 2015), is associated with a dramatic shift in function. Indeed, *G. humeralis* can generate sufficient frictional adhesive attachment forces (Fig. 2) to permit it to scale a vertical clean acrylic sheet (see Supporting Information, Video Clip S1), which constitutes a smooth, low-friction surface (Russell & Higham, 2009). By contrast, a closely-related species (*G. vittatus*) was incapable of ascending an incline > 40°, generates no detectable frictional adhesive attachment force, and slipped badly on this surface, even at this relatively shallow pitch. Informatively, we found *G. humeralis* to sometimes occupy vertical bamboo shoots in its natural habitat (Fig. 2), whereas other species in the genus generally employ rough tree trunks, rocks, fallen palm trees, and fronds, as well as even the ground. Thus, the present study reveals not only the existence of a morphologically and functionally intermediate stage between frictional and frictional adhesive attachment, but also the ecological and biomechanical implications of such a transition. The adhesive system of *G. humeralis* not only is structurally simple relative to that of other geckos, but also is indicative that slight modifications in form can dramatically influence functional outcomes and the ecological niches that can be exploited.

### IMPLICATIONS FOR THE ORIGIN OF FRICTIONAL ADHESION IN GECKOS

Many of the major transitions in vertebrate evolution are intricate and complex, and are reflective of a number of steps being necessary prior to their functioning in a new context. For example, the origin of terrestrial locomotion in vertebrates involved correlated changes in a suite of anatomical and physiological features (Shubin, Daeschler & Jenkins, 2015). One of the key attributes of the gekkotan frictional adhesive apparatus is its ability to promote adhesive attachment to smooth surfaces. Work over the past few decades has identified the mechanisms of such attachment, which involves van der Waals forces and may involve electrostatic interactions (Autumn *et al.*, 2000, 2002; Autumn & Peattie, 2002; Loskill *et al.*, 2012; Izadi, Stewart & Penlidis, 2014), facilitated by microscopic setae arranged on enlarged subdigital (and even subcaudal) scales. Although an elaborate hierarchy of muscles, tendons, and vascular modifications typifies the gekkotan adhesive apparatus, our findings show that adhesive attachment and detachment can occur in the absence of such adnexa. The transformation of subdigital spinules into setae, as found in *G. humeralis* (Russell *et al.*, 2015), is also encountered in other lizard groups, such as anoles (Ruibal & Ernst, 1965). Friction-enhancing filaments occur as the ancestral condition on subdigital scales and friction pads of *Gonatodes* (Russell *et al.*, 2015) and they are widespread among geckos (Peattie, 2008). Similar friction-enhancing filaments are also present in chameleons (Spinner, Westhoff & Gorb, 2013; Khannoon *et al.*, 2014). Much like anoles, the setae of *G. humeralis* are relatively simple (compared to those of other pad-bearing geckos) and short (Russell *et al.*, 2015), and are located adjacent to friction-enhancing spinules. The setae provide a dramatic advantage in sectors of the habitat typified by smooth inclined surfaces, such as leaves, as well as hard and slippery stems (as exemplified here by bamboo culms).

The frictional adhesive forces generated by the forelimbs of *G. humeralis* are striking, especially given that no other species in the genus examined

thus far possesses setae or is capable of generating any frictional adhesive force. The amount of frictional adhesive force (relative to body mass) generated exceeds that of skinks (Irschick *et al.*, 1996) and statistically equates with that of anoles, although it does not attain the levels recorded for other geckos (Irschick *et al.*, 1996; Bergmann & Irschick, 2005; Stewart & Higham, 2014) that are categorized as 'pad-bearing' (Gamble *et al.*, 2012). Thus, our findings demonstrate that environmentally significant frictional adhesive forces employable in the locomotor cycle can be generated by gekkotans possessing a relatively simple, incipient adhesive system.

### SCALING AND SAFETY FACTOR

The biomechanical consequences of scaling can result in considerable constraints on function and body size (Biewener, 2005). For animals that rely on adhesion for holding station or moving in their natural habitat, scaling has become a central issue (Labonte & Federle, 2015; Labonte *et al.*, 2016). Much of the attention devoted to this has focused on the scaling of pad area to body mass. This approach, however, is not applicable to *G. humeralis* because, although it exhibits the ability to generate frictional adhesive forces, it lacks toe pads. We focused, therefore, on the scaling of frictional adhesive force to body mass, and the scaling exponent is not significantly different from 1 across geckos (Fig. 3A), which mirrors that found in leaf-cutting ants (Labonte & Federle, 2015). This 'functional similarity' is also comparable to that reported by Irschick *et al.* (1996) for pad-bearing lizards. These results collectively indicate a potential disconnect between pad area and frictional adhesive capabilities. If they were directly related to each other, then the scaling exponents relative to body mass should be similar. This suggests that something other than pad area is responsible for the magnitude of frictional adhesion that is achievable, such as details of the actual structure and configuration of the setae that make up the attachment surface, rather than the area of the pad itself. For example, setal density and length, among other factors, can differ between species (Russell *et al.*, 2015), and even from location to location within the pads of a single species (Johnson & Russell, 2009). A scaling exponent of 1 (for frictional adhesive force vs. body mass) also deviates from the expected isometric exponent of 0.66 for the scaling of area to body mass. Therefore, we must conclude that the relationship of pad area to frictional adhesive capability is unclear and that we do not currently understand what is driving the scaling exponent of the relationship between body mass and frictional adhesive force.

Safety factor was high in all species examined (Fig. 3B). In addition, there was no relationship between safety factor and body mass, which is surprising (Labonte & Federle, 2015). This is likely a result of functional similarity between frictional adhesive force and body mass, with scaling exponents not significantly different from 1.0 regardless of whether phylogeny is taken into account (Fig. 3A). Future work should be directed toward clarifying the mechanisms underlying the constant safety factors observed across geckos, as this might be a result of selection and could permit the wide range of body sizes observed.

### ECOLOGY

The ecological circumstances occupied by *G. humeralis* are likely related to its possession of an incipient adhesive system. We found that, although it occurs on rough tree trunks, it also occupies smooth vertical bamboo shoots (Fig. 2), which present a very challenging, low-friction surface that is likely impossible to traverse without the aid of an adhesive system. Smooth surfaces are beneficial for a gecko with an adhesive system given that more of the setae will be capable of making contact with them (Russell & Johnson, 2007). However, hard smooth surfaces pose a major challenge to lizards without an adhesive system, given that claws are rendered nonfunctional when they cannot penetrate the surface or interdigitate with surface irregularities (Zani, 2000).

Laboratory observations revealed that *G. humeralis* can cling to fresh bamboo shoots (Fig. 2), whereas other species cannot. Although bamboo is not native to Trinidad, it is widespread there and is hospitable to *G. humeralis*. Additionally, *G. humeralis* has been observed resting on smooth leaf surfaces (Murphy, 1997) and sleeping at night on leaves (Avila-Pires, 1995; Henle & Knogge, 2009). In our laboratory trials, *G. humeralis* was able to climb vertical smooth, low-friction surfaces (see Supporting Information, Video Clip S1), whereas other species (*G. vittatus*) could not. Thus, the incipient adhesive system of *G. humeralis* is consistent with it being able to exploit naturally-occurring smooth, low-friction surfaces. Occupancy of these allows it to avoid competition with congeners that lack frictional adhesive capability, as well as with other species of arboreal lizards (Harmon, Harmon & Jones, 2007). It also enables it to occupy locations less accessible to predators. The ability of *G. humeralis* to employ such surfaces is likely associated with its broad range relative to that of other species of *Gonatodes* (it has by far the largest geographical range in its genus; Avila-Pires, 1995). We are currently exploring the factors involved in its range expansion.

## PATTERNS OF DIGITAL HYPEREXTENSION

Although all lizards exhibit digital hyperextension, the actuation of this motion is expressed in two dramatically different ways. The term 'active hyperextension' has been commonly associated with the gekkotan adhesive apparatus, and involves a rearrangement of the sequence of toe and heel lifting, such that the distal tips of the toes are lifted prior to heel elevation at the end of stance (Fig. 4). This unique sequence of events during the latter part of the stance phase shifts the transmission of force away from the tips of the toes, leading to a rearrangement of kinematics (Russell, 2002; Russell & Higham, 2009) and load transfer (Autumn *et al.*, 2006). By contrast, so called 'passive hyperextension' typifies the ancestral condition (for *Iguana*: Renous & Gasc, 1977; for *Tupinambis*: Brinkman, 1980) in which the metatarsus is first rotated around its long axis, becoming vertical with its extensor surface facing anteriorly. This is the initial phase of pedal plantarflexion, which then proceeds to the extension of the ankle joint, and ultimately concludes when the first three toes roll off the substrate at the end of the propulsive phase (Brinkman, 1980). Thus, digital hyperextension in this case is a necessary byproduct of the ankle being extended and force being transferred distally to the toes during the final moments of propulsion (Snyder, 1952; Renous & Gasc, 1977; Brinkman, 1980) (Fig. 4). The terminology for the two patterns of digital hyperextension is therefore misleading given that both are actively driven by the neuromuscular system. Therefore, we propose that the pattern of hyperextension be named in accordance with the actual sequence of events: proximodistal hyperextension (PDH; formerly 'passive hyperextension') and distoproximal hyperextension (DPH; formerly 'active hyperextension').

DPH is not employed by *G. humeralis* (Fig. 4), despite the presence of frictional adhesion-enhancing setae, thus establishing that gekkotan frictional adhesion need not be accompanied by the suite of morphological and physiological changes associated with DPH. Why then would selective pressures favour DPH as frictional adhesive capabilities increase? Although several hypotheses exist (Autumn *et al.*, 2006; Stewart & Higham, 2014; Xu *et al.*, 2015), the most probable explanation is the association of DPH with a reconfiguration of foot structure in geckos, whereby the digits become symmetrically arrayed around a broad arc (Russell, Bauer & Laroiya, 1997), with the digit tips no longer being aligned with the direction of progression (Russell & Oetelaar, 2016), thus rendering limb-driven PDH untenable.

## THE ORIGIN OF INNOVATIONS AND THE TEMPO OF EVOLUTION

Evidence for intermediate forms during the loss of a structure (or structures) is prevalent. For example, the reduction or loss of limbs in squamates (lizards and snakes), has occurred approximately 25 times (Wiens, Brandley & Reeder, 2006; Brandley, Huelsenbeck & Wiens, 2008). Fewer examples, however, are known that highlight intermediate forms during the evolutionary gain of novel structures, especially those that can be regarded as key innovations (Frazzetta, 1975). One such example, however, is that relating to the cichlid pharyngeal jaw apparatus (Liem, 1973). The neotropical species, *Cichla ocellaris*, possesses elements that are transitional between the ancestral form and the more derived forms of cichids (Liem, 1973). Our discovery of a functionally intermediate form in the transition to frictional adhesion in a lineage of geckos highlights a statement by Mayr (1960): 'Perhaps most astonishing is the relative slightness of reconstruction that seems to be necessary for successful adaptation to rather drastic shifts of adaptive zones'. The slight modifications resulting in adhesive capability in *G. humeralis* have likely triggered a major ecological shift, and a potential range expansion that eclipses other members of its genus. We are not advocating that this intermediate form will necessarily lead to a fully developed adhesive apparatus, although it can be taken to represent middle ground between the absence of frictional adhesive capability and its full expression as exhibited by pad-bearing geckos. Overall, *Gonatodes* offers a number of attractive possibilities for assessing the origin of evolutionary innovations.

Although the evolution of adhesive morphology appears to have occurred in small incremental steps in *Gonatodes*, the biomechanical ramifications of such changes are profound. This highlights the discordance in the tempo of evolution between subordinate traits (relatively small shifts) and higher-level emergent functional traits (relatively large shifts). Generally speaking, such shifts in function can result from small changes in body size or small changes in a physiological or morphological trait (Webb, 1982; Koehl, 1996). Unlike shifts in the tempo of evolution related to many-to-one mapping of form to function (Collar & Wainwright, 2006), in which multiple combinations of morphological traits can result in the same level of performance, we focus our attention on small changes in morphology that 'tip the scale' from one category of function to another. Such punctuational shifts are likely common in evolution given that, upon reaching a certain morphological threshold, a major transition in

function can occur and have widespread and significant impacts on ecology (Bock, 1959). Other examples of this include the impact of morphology on parachuting in gekkonid lizards (Russell, 1979), the impact of small changes in morphology on locomotor and feeding performance/function in fishes (Liem, 1973; Webb, 1982), and the quantum shifts in mechanical function that result from small morphological changes in the closure of the mammalian secondary palate (Thomason & Russell, 1986). Perhaps one of the most striking examples is the simple thinning of the cuticle covering the leg of terrestrial crabs of the genus *Scopimera* that expands the function of the leg from a locomotor structure to an organ of respiration (Burggren, 1992). Extending our study to encompass other independent origins of frictional adhesion in geckos will allow us to determine whether the prevalence of such dramatic shifts in function that result from relatively simple changes in morphology are characteristic of geckos in general.

## CONCLUSIONS

Innovations often involve complex and hierarchical systems of integration of physiology and anatomy. They likely begin, however, with a trigger that provides the focus for further integrated change. Such triggers are generally difficult to identify but can be regarded as key innovations. We have shown that a rather modest morphological transformation, the elaboration of epidermal spinules into frictional adhesion-promoting setae, can serve as the key innovation that acts as the foundation for more complex and elaborate subsequent changes. The morphological shifts in digit form in *Gonatodes* documented by Russell *et al.* (2015) are quite subtle but presage the pattern seen in the complexly-organized, basally-derived adhesive pads of numerous lineages of geckos. The snapshot of evolutionary transformation displayed by *G. humeralis* is consistent with the attainment of morphological change that has enabled release from ecological constraints. Such changes may lead to rapid diversification or an expansion of habitat or resource use, which is something that requires further investigation for *G. humeralis*. Overall, the pattern of expression of the adhesive system is somewhat analogous to that evident in the genus *Anolis*, which possesses an adhesive system but lacks distal to proximal digital hyperextension (Russell & Bels, 2001). Anoles exhibit adhesive forces weaker than those of most geckos (Fig. 2), as well as morphological features of the digits that are consistent with this lower capacity for force generation, providing an appropriate comparative model for the early evolution of the gekkotan adhesive system, as exemplified by *G. humeralis*.

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# SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article:

**Figure S1.** Bayesian, time-calibrated phylogeny of sphaerodactyl geckos from six nuclear protein coding genes and three mitochondrial genes. Bayesian posterior probabilities are shown at the nodes.

**Table S1.** GenBank IDs for samples of six nuclear protein coding genes (ACM4, CMOS, PDC, NT3, RAG1, and RAG2) and three mitochondrial genes (ND2 and associated tRNAs, 12S, and 16S) used to estimate phylogenetic relationships among *Gonatodes* species.

**Video Clip S1.** A high-speed video (500 frames $s^{-1}$) slowed down 10 times. This shows an individual *Gonatodes humeralis* climbing a clean vertical acrylic sheet using its incipient adhesive system.