



Research

Cite this article: Griffing AH, Sanger TJ, Epperlein L, Bauer AM, Cobos A, Higham TE, Naylor E, Gamble T. 2021 And thereby hangs a tail: morphology, developmental patterns and biomechanics of the adhesive tails of crested geckos (*Correlophus ciliatus*). *Proc. R. Soc. B* **288**: 20210650.

<https://doi.org/10.1098/rspb.2021.0650>

Received: 16 March 2021

Accepted: 24 May 2021

Subject Category:

Morphology and biomechanics

Subject Areas:

developmental biology, evolution, biomechanics

Keywords:

Diplodactylidae, Gekkota, caudal adhesion, setae, embryology

Author for correspondence:

Aaron H. Griffing

e-mail: aaron.griffing@marquette.edu

Electronic supplementary material is available online at <https://doi.org/10.6084/m9.figshare.c.5448688>.

And thereby hangs a tail: morphology, developmental patterns and biomechanics of the adhesive tails of crested geckos (*Correlophus ciliatus*)

Aaron H. Griffing¹, Thomas J. Sanger², Lilian Epperlein², Aaron M. Bauer³, Anthony Cobos⁴, Timothy E. Higham⁴, Emily Naylor⁴ and Tony Gamble^{1,5,6}

¹Department of Biological Sciences, Marquette University, PO Box 1881, Milwaukee, WI 53201, USA

²Department of Biology, Loyola University Chicago, 1032 W. Sheridan Road, Chicago, IL 60660, USA

³Department of Biology and Center for Biodiversity and Ecosystem Stewardship, Villanova University, 800 Lancaster Avenue, Villanova, PA, USA

⁴Department of Evolution, Ecology, and Organismal Biology, University of California, 900 University Avenue, Riverside, CA 92521, USA

⁵Milwaukee Public Museum, 800 W. Wells Street, Milwaukee, WI 53233, USA

⁶Bell Museum of Natural History, University of Minnesota, Saint Paul, MN 55108, USA

id AHG, 0000-0001-8441-1330; TJS, 0000-0003-0047-1884; LE, 0000-0003-0741-3919; AMB, 0000-0001-6839-8025; TEH, 0000-0003-3538-6671; EN, 0000-0002-0703-7530; TG, 0000-0002-0204-8003

Among the most specialized integumentary outgrowths in amniotes are the adhesive, scale-like scansors and lamellae on the digits of anoles and geckos. Less well-known are adhesive tail pads exhibited by 21 gecko genera. While described over 120 years ago, no studies have quantified their possible adhesive function or described their embryonic development. Here, we characterize adult and embryonic morphology and adhesive performance of crested gecko (*Correlophus ciliatus*) tail pads. Additionally, we use embryonic data to test whether tail pads are serial homologues to toe pads. External morphology and histology of *C. ciliatus* tail pads are largely similar to tail pads of closely related geckos. Functionally, *C. ciliatus* tail pads exhibit impressive adhesive ability, hypothetically capable of holding up to five times their own mass. Tail pads develop at approximately the same time during embryogenesis as toe pads. Further, tail pads exhibit similar developmental patterns to toe pads, which are markedly different from non-adhesive gecko toes and tails. Our data provide support for the serial homology of adhesive tail pads with toe pads.

1. Introduction

The adhesive capabilities of lizards have captivated naturalists since Aristotle, over 2000 years ago (e.g. [1–8]). The lamellae and scansors (*sensu* [5]) of gecko and *Anolis* adhesive toe pads are highly specialized. Adhesive ability is facilitated through hair-like, hypertrophied elaborations of the epidermis known as setae [9,10]. At a gross morphological level scansors possess tendinous connections to the digits and either reticular vascular networks or adipose pads to facilitate control while lamellae lack these structures [6,11]. Geckos exhibit a spectrum of digital morphologies, including toe pads with scansors, toe pads with a combination of scansors and lamellae or no adhesive structures at all [5,11,12], whereas *Anolis* only have toe pads with adhesive lamellae [13]. Excluding an analogous, yet poorly understood, evolutionary origin of digital adhesion in scincid lizards [14], adhesive toe pads are hypothesized to have evolved independently approximately 15 times (approx. 14 gains in gekkotans and one gain in *Anolis*; [8,12,15]). However, toe pads are not the only adhesive, setae-bearing structures of lizards. Several gecko lineages exhibit setae-bearing, adhesive scansors at the venterodistal tip of the tail. These

lineages are geographically and phylogenetically disparate and comprise species in 21 genera in three of the seven gecko families (electronic supplementary material, S1 and S3). While no phylogenetic analyses of adhesive tail evolution have been done, it appears these structures evolved independently at least five times—once each in the families Sphaerodactylidae and Diplodactylidae and three times in the Gekkonidae [16]. The adhesive tail pads of diplodactylid geckos, henceforth called tail pads, are perhaps the most well-studied and are hypothesized to be serial homologues of adhesive toe pads [17]. Serial homologues are morphological structures that are present as multiple copies in the same organism and share a set of developmental constraints, such as fore- and hindlimbs of tetrapods [18–21]. Bauer's [17] hypothesis is based upon striking morphological similarities between adult adhesive toes and tails: reticular networks of blood vessels in addition to muscle fibres attaching directly to the dermal cores of scansors to provide control of the adhesive apparatus, adipose tissue to function as a cushion for the scansors, and of course, fields of setae covering the distal subcaudal tip [17]. Further evidence for the serial homology between tail and toe pads is their apparent evolutionary coupling. The absence of taxa exhibiting tail pads, but no toe pads, suggests that the evolution of toe pads is a prerequisite for evolving tail pads [17,22]. Corroborating the identity of a character as a serial homologue requires developmental data. The only developmental data available to Bauer [17] were a small post-natal series of *Rhacodactylus auriculatus*, preventing any further corroboration of serial homology.

The development of lizard toe pads, in general, is poorly known. In some geckos, the first scansorial ridges form at the distal half of the digit and then develop along the entire length of the digit while becoming more asymmetrical in the proximal–distal direction (*Tarentola*, *Ptyodactylus*; [23–26]). In *Anolis*, the beginning of lamella development follows similar patterns of lepidosaurian scale development [27]; however, the epidermis subsequently undulates, giving rise to asymmetrical lamellae [28,29]. Alternatively, previous studies suggest that all other body scales, with the exception of tail scales, arise from individual, dome-like epidermal papillae (i.e. placodes; [30,31]). This suggests that the developmental programme that gives rise to adhesive toe pads is derived. With the exception of a handful of works [17,22,32–35], the evolutionary morphology of adhesive tail pads has been largely ignored and remains enigmatic and the function and development of these pads have not yet been investigated. The combination of developmental and functional data will provide a robust test of the serial homology hypothesis posited by Bauer [17]. Here we (i) characterize the anatomy and micro-anatomy of *Correlophus ciliatus* (Diplodactylidae) tail pads, (ii) characterize the functional capability of an adhesive tail pad in relation to its toe pads and (iii) reinvestigate Bauer's [17] serial homology hypothesis by using developmental data to identify potential developmental constraint in the evolution of adhesive digits and tails.

2. Methods

Correlophus ciliatus are large-bodied (108 mm average snout–vent length, SVL), arboreal geckos native to New Caledonia [36–38]. Like all other New Caledonian diplodactylids, *C. ciliatus* exhibit

not only adhesive toe pads, but also an adhesive tail pad at the tip of a robust, prehensile tail (figure 1a). This species was not included in Bauer's [17] investigation because it was thought to be extinct and only 're-discovered' in the mid-1990s [41,42]. To compare tail development, we collected embryos of *C. ciliatus* and embryos of a digital pad-bearing, but non-adhesive-tailed gecko (*Lepidodactylus lugubris*) following protocols of Sanger *et al.* [43] and Griffing *et al.* [44], respectively. Embryos from both species were collected from captive colonies housed at Marquette University. Protocols for gecko husbandry are detailed elsewhere [37,44]. Using 286 collected embryos, we produced an embryonic staging series for *C. ciliatus*, the first staging series for any pygopodoid gecko, using published gecko staging series as a reference [45,46].

(a) Morphology

We examined tails and feet of pre- and post-natal specimens using scanning electron microscopy (SEM). Through a separate investigation of embryonic apoptosis, we also identified areas of substantial apoptotic activity within early tail pad development (stage 35 embryo) using Lysotracker Red DND-99 [47]. Setal densities of SEMs were estimated, following Bauer [17]. We investigated the relationship between setal density and maximum seta height in a phylogenetic context combining our *C. ciliatus* data with data from Bauer [17] and Schleich & Kästle [48] using the phylogeny from Skipwith *et al.* [49] and phylogenetic independent contrasts [50–53]. We also examined internal anatomy of post-natal tails using computed tomography [54], clearing and staining [55] and histology [56].

(b) Adhesive performance measurements and scaling

We measured frictional adhesive performance from the tails and forelimbs of 10 *C. ciliatus* over a range of body sizes (6.0–41.3 g) following the methods of Higham *et al.* [57], in which peak tensile force (Newtons) is obtained by placing the animal's adhesive pad(s) onto a pristine section of acrylic and slowly pulling the animal (or autotomized tail) in parallel opposition to an attached portable force gauge until pad slipping occurs; a single maximum force value was taken after multiple trials for each forelimb and tail. Obtaining reliable measurements often required the tail be autotomized. Once disconnected from the body, the adhesive strength of the tail was measured as above. Adhesive performance in geckos does not require active control [58], and removing the tail first avoided any variation due to behaviour or motivation (i.e. prehension). That said, we obtained tail adhesive performance before and after autotomy for most geckos.

We quantified the scaling relationships between adhesive force and body mass using linear regressions. Variables were first log-transformed to linearize the data. The slope of the regression represents the scaling exponent, with a slope of one representing the expected relationship from previous studies of interspecific scaling [7,57]. Scaling relationships were obtained for both the manus and the tail. Additionally, we calculated the potential for the adhesive tail tip to support the entire mass of the animal in a vertical orientation, as might occur when hanging from a branch. To do this we calculated tail adhesive safety factor, the ratio of maximum adhesive force of the tail to the force due to gravity (body mass $\times$ acceleration due to gravity). A value greater than one indicates the tail alone could support the body.

We investigated scaling of toe pad and tail pad area with relation to SVL using 24 formalin-fixed specimens, ranging from hatchlings to adult (36.8–108.3 mm). After log-transformation, we tested for differences between toe pad and tail pad area versus SVL scaling using linear regressions and analysis of variation [59].

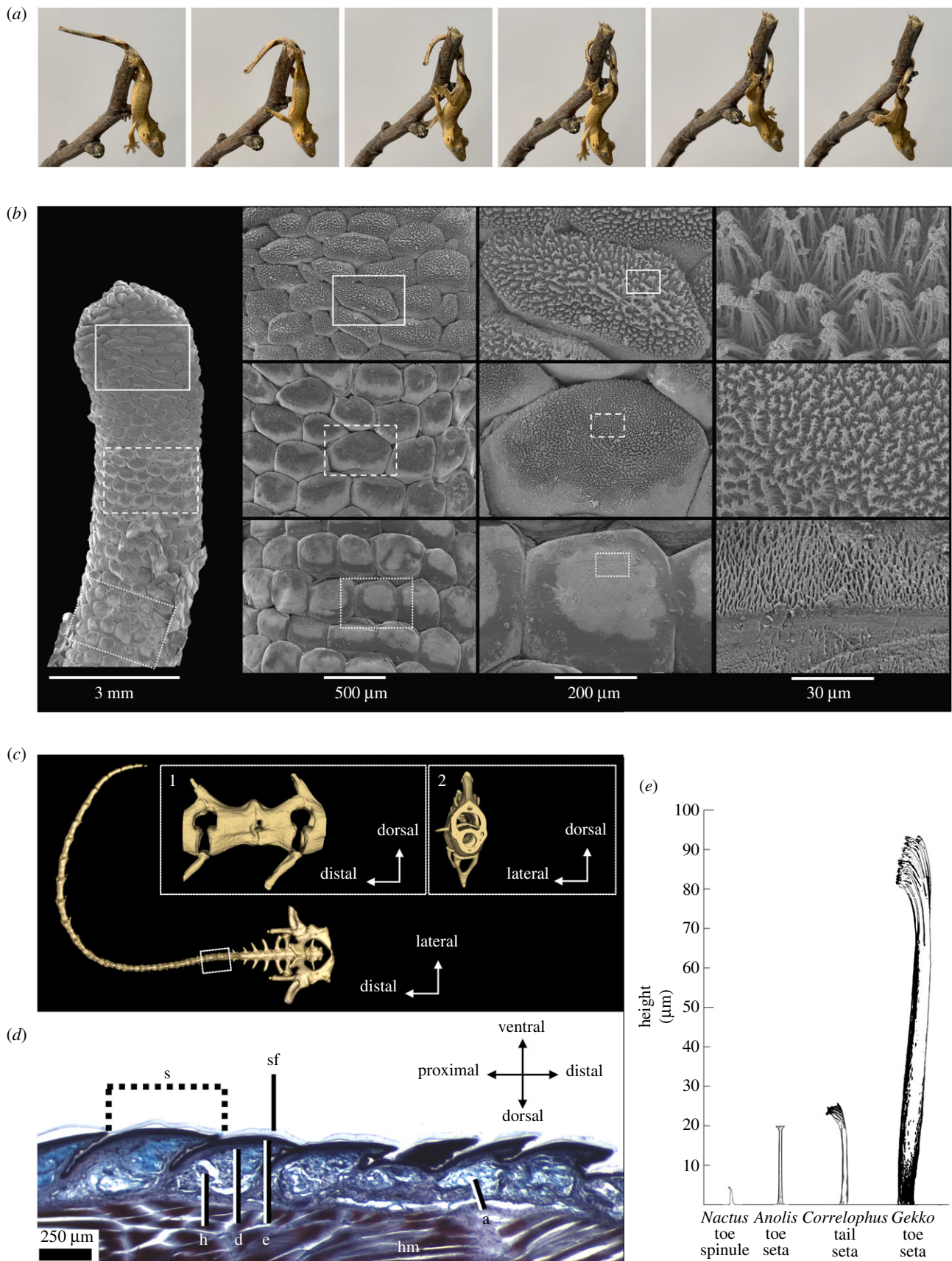


Figure 1. (a) A time-lapse of a subadult *Correlophus ciliatus* using its prehensile tail with adhesive tail pad to climb down a branch. (b) Scanning electron micrographs in ventral view of an adult *C. ciliatus* tail pad. Magnified images of different areas of the tail tip are framed by solid white boxes (distal), dashed white boxes (middle) and dotted white boxes (proximal). Each column is equally magnified relative to the three regions of the tail. Note that the distal tip of the pad exhibits dense fields of setae and more proximal regions have increasingly shorter spinules. (c) Caudal osteology of *C. ciliatus* depicted through μ CT. Ventral view of caudal and sacral vertebrae and portion of pelvic girdle. Dashed white box illustrates autotomic vertebrae magnified in lateral view (1) and transverse view (2). (d) Hall–Brunt Quadruple stained sagittal section of the *C. ciliatus* adhesive tail pad. Scansor rows 7–12. a, Adipose tissue; d, dermis; e, epidermis; h, hypodermis; hm, hypaxial muscle; s, scansorial unit; sf, setal field. (e) Average heights of a non-pad-bearing gecko (*Nactus*) spinules, *Anolis* toe pad setae, *Correlophus* tail pad setae and *Gekko* toe pad setae. Figure adapted from Russell [39] and Peattie [40]. (Online version in colour.)

3. Results

(a) Adult morphology of the adhesive tail pads

The tail of *C. ciliatus* comprises 27 amphicoelous caudal vertebrae with reduced transverse vertebral processes, similar to other functionally prehensile-tailed geckos (e.g. *Aleuroscalabotes felinus*; [60]; figure 1c; electronic supplementary material, S4). Unlike most geckos, no autotomy planes are visible distal to the eighth vertebra. Hypaxial muscle bundles are larger than epaxial muscle bundles (electronic supplementary material, S4). Fields of long, branching setae cover the ventrodiscal tip of the adult tail (figure 1b,d). This field occupies the distal most 13–16 scale/scansor rows, with proximal scale rows exhibiting shorter setae, and ultimately, non-branched spinules (figure 1b). Mean height of tail setae = 20.0 μm ($N = 30$ setae, measured from a ventrolateral tail scansor), the longest measuring 26.6 μm (figure 1b,e; electronic supplementary material, S2). Mean height of toe setae = 23.8 μm ($N = 30$), the longest measuring 32.7 μm (electronic supplementary material, S5). Setal densities between tail and toe pads are close to each other: tail = 32 950 setae mm^{-2} ; and toe = 30 000 setae mm^{-2} . There is an inverse relationship between maximum setal height and setal density in toe pads ($r^2 = 0.2889$, $F = 5.468$, $p = 0.0414$), but not tail pads ($r^2 = 0.2909$, $F = 3.282$, $p = 0.1076$; electronic supplementary material, S6). Distal scansors/scales are more imbricate and asymmetrical than proximal scales or the scales on the dorsal surface of the tail (figure 1d; electronic supplementary material, S4). Unlike the dorsal scales, the dermal core of scansors sits above a thick hypodermal layer of adipose tissue (figure 1d; electronic supplementary material, S4).

(b) Adhesive performance measurements and scaling

The adhesive performance of the manus ranged from 1.33 N to 8.12 N, with larger geckos clinging with greater force ($r^2 = 0.95$, $p < 0.001$; figure 2a). Adhesive performance of the manus scaled with $\text{mass}^{1.04}$. The adhesive performance of the tail pad ranged from 0.22 N to 1.19 N, and also increased with body size ($r^2 = 0.88$, $p < 0.001$; figure 2a). Adhesive performance of the tail scaled with $\text{mass}^{0.97}$. The safety factors for the tail pad ranged from 2.45 (largest gecko) to 5.57 (moderately sized gecko). Given an expected scaling exponent of 2 under isometry, the toepad area scaled with isometry (scaled with $\text{SVL}^{1.88 \pm 0.183}$), whereas the tail pad area scaled with negative allometry (scaled with $\text{SVL}^{1.54 \pm 0.298}$; figure 2b).

(c) Pre-natal development of adhesive structures

Correlophus ciliatus toe pad development begins shortly after interdigital webbing recession (stage 36; figure 3; electronic supplementary material, S7). Four subdigital scansorial ridges initially form in the widest, distal portion of the digit. Shortly after, a small number of new ridges form distally and many more ridges form proximally, all while simultaneously expanding laterally (figure 3). Individual scansors become more imbricate with one another until toe pad development is complete at stage 42 (figure 3).

Tail tip shape changes drastically during *C. ciliatus* embryonic development. The tail tip is initially pointed and subsequently sculpted away into a wide, blunt end through apoptosis (electronic supplementary material, S8; figure 3). Tail pad development occurs immediately after toe pad development begins. Prior to any signs of scansor

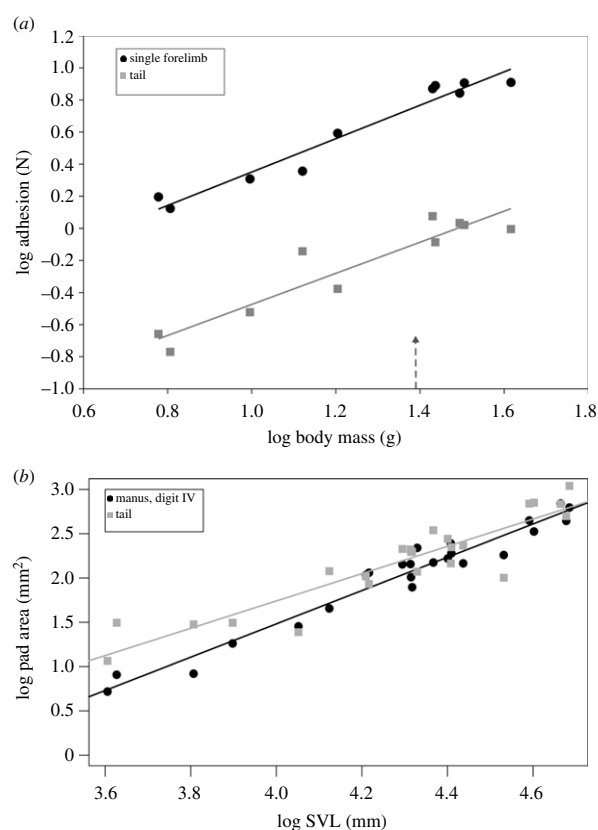


Figure 2. (a) Adhesive performance of *Correlophus ciliatus* tail pads and forelimbs. Points to the right of the dashed arrow denotes adults from juveniles/subadults. (b) Isometric scaling of toe pad area and negative allometric scaling of tail pad area with respect to SVL.

development, a subcaudal sulcus forms along nearly the entire length of the tail (stage 36). This sulcus is associated with enlarged hypaxial muscle bundles found in other prehensile-tailed geckos [17]. The distal-most portion of the tail exhibits lateral outgrowths, creating a distal pad which is somewhat wider than the rest of the tail (figure 3; early stage 36). Shortly after (mid stage 36), large lateral scansorial ridges form in the distal portion of the pad before forming new ridges in a distoproximal direction (stage early–mid stage 37). At stage 38, the distal scansorial ridges begin subdividing into numerous, raised units within a scansorial ridge (figure 3). We henceforth refer to this process as granularization. Subsequently (stage 39), the distal portion of the tail expands further laterally, forming a spatulate pad. The pad expands laterally and scales granularize in a distoproximal direction until tail pad development is complete by stage 42 (figure 3). Embryonic development of the non-padded tail of *L. lugubris* is notably different from *C. ciliatus*. *Lepidodactylus lugubris* does not exhibit a subcaudal sulcus and the first signs of tail scale development occur at stage 39 when approximately eight evenly spaced annular scale rows form simultaneously along the length of the tail (figure 3). Further annular scale rows form in between these initial rows by stage 40 and then granularize simultaneously by stage 41 (figure 3).

4. Discussion

(a) Adhesive tail tip structure and homoplasy

Correlophus ciliatus has been considered unique among adhesive-tailed geckos in having a paddle-shaped distal tail tip, thus

expanding the adhesive field compared to tail pads of other species [17,61]. However, despite this unique paddle-shape, the surface morphology and histomorphology of the *C. ciliatus* adhesive pad is largely similar to other gecko tail pads [17,62,63]. Setae are branched and of comparable height to toe setae, hypaxial muscle bundles are enlarged to presumably assist with prehension, and subdermal adipose tissue likely plays a role in cushioning scansors against surfaces (figure 1b*e*; electronic supplementary material, S2 and S4; [5,17,64–66]).

Setal heights and densities are largely similar between toe and tail pads (electronic supplementary material, S2). We demonstrate a significant inverse relationship between setal height and density in diplodactylid toe pads. The lack of this relationship in tail pads is likely due to a small sample size. In the gekkonid gecko genus *Gekko*, Bauer & Good [67] hypothesized that as body size increases between species, setal height and density increase and decrease, respectively. This relationship generally holds true for New Caledonian diplodactylids, but not New Zealand diplodactylids (electronic supplementary material, S2; [17]). Small-bodied *Bavayia* exhibit moderate setal heights (29–32 μm) with extremely dense fields of setae (35 600–42 900 setae mm^{-2}) while the large-bodied *Rhacodactylus* exhibit larger setal heights (34–38 μm) with less dense fields of setae (13 700–18 000 setae mm^{-2}). The microanatomy of *C. ciliatus* tail pads deviates from these trends. Although they exhibit setae of a comparable height to other large-bodied New Caledonian diplodactylids, setal densities of *C. ciliatus* tail and toe pads are much higher, on par with densities exhibited by *Bavayia sauvagii* toes and tails (electronic supplementary material, S4 and S2). Further, the setal tip width (i.e. the amount of branching) is larger than any other studied diplodactylids (electronic supplementary material, S2), with both toe and tail setae having a setal tip that is two to three times wider than the closely related *Correlophus sarasinorum*. The increased density of tail setae, coupled with large setal tip width, may provide *C. ciliatus* with adhesive ability which exceeds that of other diplodactylid geckos. However, it should be noted that setal densities can vary on gecko toe pads depending on location of measurement [68–70].

(b) Functional significance of tail adhesion

Digital adhesion in *C. ciliatus* is similar to some of the highest absolute adhesive forces recorded for geckos (see [7,57] for comparative values). *Gekko gecko* is the only species with higher recorded forces [7,58]. *Correlophus ciliatus* tail adhesion is substantial; forces frequently exceeded 1 N, which, in those cases, represented up to 80.2% of the force estimated to be produced by a single digit. *Correlophus ciliatus* tail adhesion values far exceed digital adhesion of *Anolis*, another pad-bearing group of lizards often studied in the context of digital adhesion. With both forelimbs engaged, *A. carolinensis* and *A. sagrei* generate 1.5 and 1.3 N of adhesive force, respectively [7]. Thus, crested gecko tail adhesive force often exceeds the forces that these anoles can generate with a single manus.

Safety factors for tail adhesion are, in all cases, sufficient to support the mass of the entire body in a vertical orientation, which means that *C. ciliatus* could potentially hang from a branch using only their tail. In fact, the maximum value of safety factor exceeds five, indicating that a single tail could theoretically hold up to five *C. ciliatus* without losing grip. Additionally, the tails are capable of prehension, adding yet

another component to their clinging ability. Although it is unclear how much grasping force the tail could exert on a perch, some lizards (e.g. chameleons) can exert up to 35 times their own body mass in grip force with their tail [71].

The scaling factors of digital and tail adhesion with respect to body mass in *C. ciliatus* were not significantly different from 1, which is comparable with previous research on geckos and other pad-bearing lizards [7,57], as well as leaf-cutting ants [72]. This ‘functional similarity’ is not found when examining the relationship between toepad area and adhesive force [7], where it largely follows the predicted scaling factor of 0.67 with respect to body mass. Therefore, other aspects of adhesive morphology, such as setal dimensions or density, are likely driving the functional similarity. Although the scaling exponents of adhesive pad area of the tail and digits (with respect to SVL) are not significantly different from each other, only toepad area scaled with isometry. By contrast, the tail pad scaled with negative allometry, possibly indicating that tails are more important for clinging in smaller geckos. This could be attributed to a shift in habitat use through ontogeny, although little is known about substrate use in nature.

(c) Revisiting serial homology of adhesive tail tips to adhesive digits

Correlophus ciliatus toe and tail pads exhibit strikingly similar patterns of pad subdivision and extension during development (figure 3). Additionally, the onset of pad formation and subdivision occurs near synchronously in both structures. The development of adhesive toe pads in *C. ciliatus* is similar to *Tarentola* geckos [24–26] suggesting comparable developmental mechanisms underly the formation of adhesive structures in both species. Consequently, our data provide further support to Bauer’s [17] hypothesis that the adhesive tail pads of some diplodactylid geckos are serially homologous to their toe pads. Our results only support this conclusion for the single origin of adhesive tail pads which includes *C. ciliatus* (clade comprising *Pseudothecadactylus* + New Caledonian geckos; [49]). A comparative developmental investigation of other groups (e.g. New Zealand geckos, *Lygodactylus*, *Euleptes*, etc.) is required to corroborate serial homology in other tail pad-bearing taxa. Further evidence of serial homology between tail and toe pads comes from similarities of setal development on the digits and regenerating tail of the New Zealand diplodactylid, *Woodworthia maculata* [62]. Such an experiment is impossible with *C. ciliatus* as they do not regenerate a full tail after autotomy, nor amputation [73]. Although there are underlying differences between toe and tail pads in skeletal, muscular and tendinous morphologies, these likely reflect the distinct ancestry upon which the adhesive pads evolved [17,41] and do not detract from the serial nature of the tail and toe adhesive apparatus.

The degree to which tail pads and toe pads are evolving independently (paramorphs; *sensu* [19]) or in tandem (homomorphs; *sensu* [19]) is unclear. Although the presence of tail pads appears linked to the presence of adhesive toe pads, anecdotal evidence suggests toe pad shape and size does not predict tail pad shape and size [17,74], suggesting some degree of independent evolution. Further investigations into other tail pad-bearing taxa may determine whether they exhibit different ranges of covariation between tail and toe pad shape and size [75].

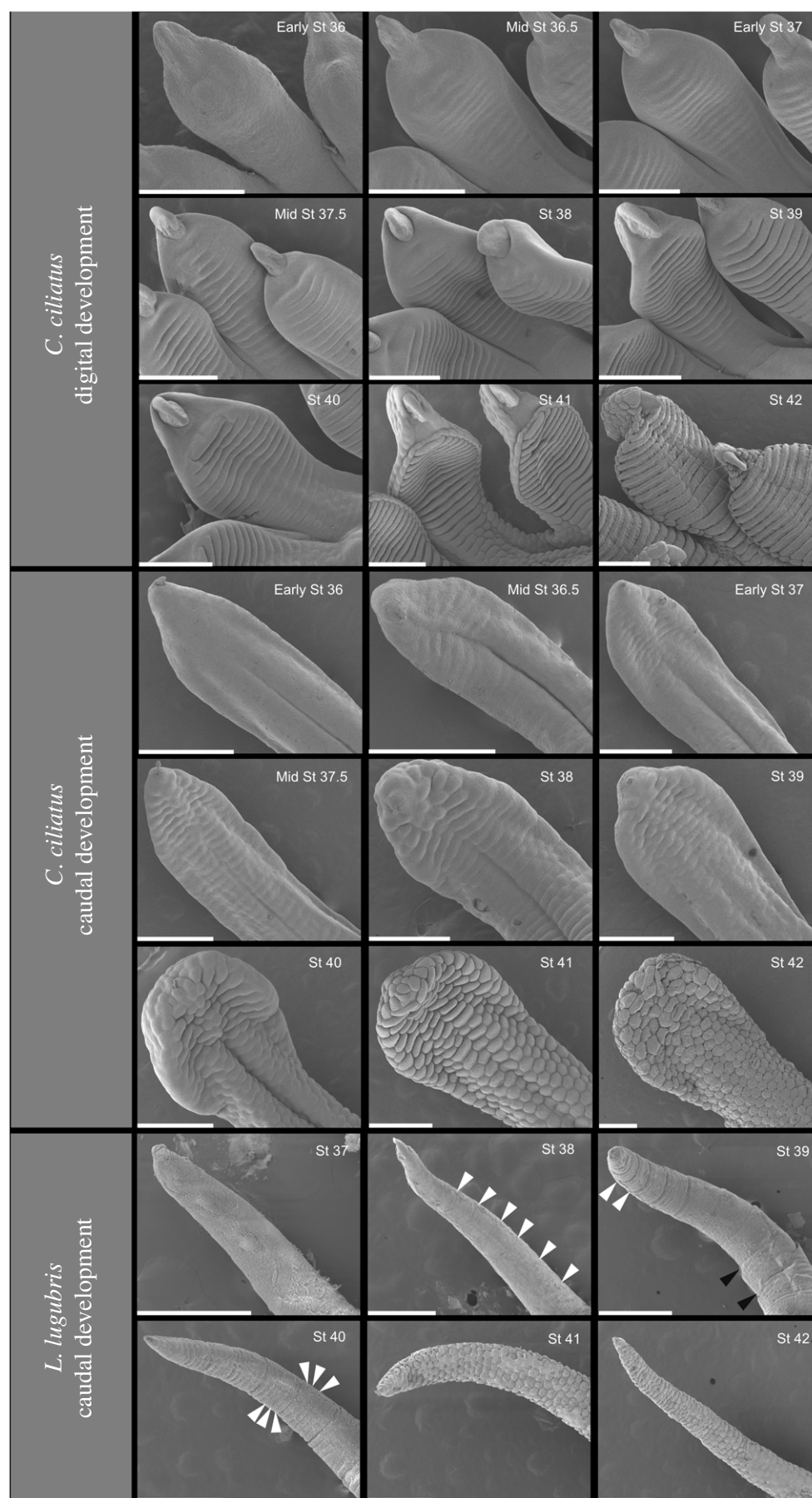


Figure 3. Scanning electron micrographs of digital and caudal development in *Correlophus ciliatus* and caudal development in *Lepidodactylus lugubris*. Embryonic stages 36–42. Plantar views of left manus, digit IV and ventral views of the distal tail tip. In *C. ciliatus*, both toe pads and tail pads exhibit a distal-toproximal development of scansor rows, which subsequently subdivide into what will become the adult scansors. In *L. lugubris*, scale annuli form synchronously along the entire length of the tail and wrap around the circumference of the structure (stage 38, white arrows). Initial annuli become more distinct (stage 39, black arrow) with annuli appearing near the distal tail tip (stage 39, white arrows). Eventually additional annuli form in between the initial annuli (stage 40, white arrows) and subsequently become granular (stages 41–42). Scale bars = 500 μ m.

Following pad subdivision and extension, tail pads deviate from the developmental pattern seen in toe pads and begin to exhibit granularization of the individual scansors (figure 3). We posit the adhesive scansor developmental programme, via homeosis, was supplanted onto the tail tip, resulting in markedly similar development of scansorial ridges. Soon after, the scansors granularize, creating numerous placode-like structures, which resemble typical reptile body scale development [76]. Unlike *C. ciliatus*, other adult gecko tail pads, like those of *Pseudothecadactylus* and *Lygodactylus*, exhibit mediolaterally broad scansors with few to no granular scansors [17,77]. To our knowledge, this derived, granularizing pattern has not been documented in other studies of amniote integumentary development [76,78]. Identifying the patterns of activator and inhibitor morphogens in tail pad development is required to determine whether this is a two-step process of lateral inhibition to form tail scansors [79]. These derivations further demonstrate that our current understanding of epidermal development is incredibly simplified and requires further descriptive embryology to fully characterize the diversity of epidermal developmental patterns [78]. Further investigation into the molecular patterns and processes that produce both digital and tail adhesive pads is necessary to definitively determine the degree of homology the two structures share.

Tail pads of *C. ciliatus*, but not the non-adhesive-tailed *L. lugubris*, appear to pass through the three main stages exhibited by developing toe pads (figure 3; electronic supplementary material, S8). Adhesive toe pads of *C. ciliatus* and *Tarentola* [24,25] exhibit these stages following digital webbing reduction: (i) pad formation, (ii) distal scansorial/lamellar ridge formation and (iii) distal-to-proximal and lateral ridge extension. By contrast, non-padded lizards of the genus *Pogona* develop all plantar scales synchronously across the length of the digit [78]. Following these stages, the scansorial rows of developing tail pads begin to granularize, presumably being released from previous constraint (figure 3). Further developmental research is required to determine: (i) if the pattern exhibited by *Pogona* is the ancestral state, and (ii) if there are biases in the production of morphological variation during pad morphogenesis (i.e. developmental constraint; [80]).

5. Conclusion

Our in-depth investigation into the structure, function and development of *C. ciliatus* reveals their tail pads are largely

similar to other diplodactylid lizards [17,62], with the exception of their extraordinarily dense fields of setae for their body size and large branching setal tips. The adhesive tail pad of *C. ciliatus* is highly functional, with adhesive capabilities on par with an entire *Anolis* manus. Paradoxically, the highly functional *C. ciliatus* tails do not regenerate, unlike nearly all other gecko species which autotomize their tails, including their close relatives, *C. sarasinorum*. Finally, we add evidence that toe pads and tail pads are serial homologues. Investigation into the molecular underpinnings of toe and tail pad development are required to definitively corroborate serial homology and identify the degree to which developmental constraint has affected the evolution of these enigmatic structures.

Ethics. All *Correlophus ciliatus* were housed at Marquette University (IACUC protocol AR-279) or University of California, Riverside (IACUC protocol 20170039) following standard husbandry protocols.

Data accessibility. Morphological and adhesion measurements: Figshare doi:10.6084/m9.figshare.14220098; raw μ CT data: Figshare doi:10.6084/m9.figshare.14220134, doi:10.6084/m9.figshare.14222834, doi:10.6084/m9.figshare.14222993; 3D μ CT models: Sketchfab <https://sketchfab.com/SangerLab>.

Authors' contributions. A.H.G.: conceptualization, data curation, formal analysis, investigation, methodology, resources, visualization, writing—original draft, writing—review and editing; T.J.S.: conceptualization, data curation, investigation, methodology, resources, supervision, visualization, writing—original draft, writing—review and editing; L.E.: data curation, formal analysis, visualization, writing—review and editing; A.M.B.: investigation, writing—original draft, writing—review and editing; A.C.: formal analysis, investigation, methodology, writing—original draft, writing—review and editing; T.E.H.: conceptualization, data curation, formal analysis, methodology, visualization, writing—original draft, writing—review and editing; E.N.: formal analysis, investigation, methodology, writing—original draft, writing—review and editing; T.G.: conceptualization, data curation, formal analysis, funding acquisition, investigation, methodology, resources, supervision, visualization, writing—original draft, writing—review and editing.

All authors gave final approval for publication and agreed to be held accountable for the work performed therein.

Competing interests. We declare we have no competing interests.

Funding. This study was funded by the National Science Foundation (DEB 1657662 to T.G.; MRI 1726994 to Jacob Ciszek (Scanning Electron Microscope facility, Loyola University in Chicago)). A.M.B. was supported by the Gerald M. Lemole Endowed Chair Funds through Villanova University.

Acknowledgements. We thank student employees of the Marquette University animal facility; Ryan Kerney for advice about Hall-Bunt Quadruple stain; Tony Russell for comments concerning scansorial identity and translation of [32].

References

1. Aristotle. 1918 (*Translated by Thompson DAW*) *Historia animalium*, book IX. Oxford, UK: Clarendon.
2. Schneider JG. 1812 Kritische Uebersicht der einzelnen Arten aus der Gattung von Eidechsen, welche ich Wandkletterer nenne, Linné aber und andere, Geckonen. *Denkschr. Bayer. Akad. Wiss. München* **1811/1812**, 31–71.
3. Hora SL. 1923 The adhesive apparatus on the toes of certain geckos and tree-frog. *J. Asiat. Soc. Bengal* **19**, 137–145.
4. Maderson PFA. 1964 Keratinized epidermal derivatives as an aid to climbing in gekkonid lizards. *Nature* **203**, 780–781. (doi:10.1038/203780a0)
5. Russell AP. 1972 The foot of gekkonid lizards: a study in comparative and functional anatomy. PhD thesis, University of London, London.
6. Russell AP. 1986 The morphological basis of weight-bearing in the scansors of the tokay gecko (Reptilia: Sauria). *Can. J. Zool.* **64**, 948–955. (doi:10.1139/z86-144)
7. Irschick DJ, Austin CC, Petren K, Fisher RN, Losos JB, Ellers O. 1996 A comparative analysis of clinging ability among pad-bearing lizards. *Biol. J. Linn. Soc.* **59**, 21–35. (doi:10.1111/j.1095-8312.1996.tb01451.x)
8. Gamble T, Greenbaum E, Jackman TR, Russell AP, Bauer AM. 2012 Repeated origin and loss of adhesive toepads in geckos. *PLoS ONE* **7**, e39429. (doi:10.1371/journal.pone.0039429)

9. Maderson PFA. 1970 Lizard hands and lizard glands: models for evolutionary study. *Forma et Functio* **3**, 179–204.
10. Ruibal R, Ernst V. 1965 The structure of the digital setae of lizards. *J. Morphol.* **117**, 271–294. (doi:10.1002/jmor.1051170302)
11. Russell AP, Stark AV, Higham TE. 2019 The integrative biology of gecko adhesion: historical review, current understanding, and grand challenges. *Integr. Comp. Biol.* **59**, 101–116. (doi:10.1093/icb/icz032)
12. Russell AP, Gamble T. 2019 Evolution of the gekkotan adhesive system: does digit anatomy point to one or more origins? *Integr. Comp. Biol.* **59**, 131–147. (doi:10.1093/icb/icz006)
13. Russell AP. 2017 The structure of anoline (Reptilia: Dactyloidae: *Anolis*) toe pads in relation to substratum conformity. *Acta Zool.* **98**, 300–309. (doi:10.1111/azo.12180)
14. Williams EE, Peterson JA. 1982 Convergent and alternative designs in the digital adhesive pads of scincid lizards. *Science* **215**, 1509–1511.
15. Losos JB. 2009 *Lizards in an evolutionary tree*. Berkeley, CA: University of California Press.
16. Gamble T, Greenbaum E, Jackman TR, Bauer AM. 2015 Into the light: diurnality evolved multiple times in geckos. *Biol. J. Linn. Soc.* **115**, 896–910. (doi:10.1111/bij.12536)
17. Bauer AM. 1998 Morphology of the adhesive tail tips of carphodactyline geckos (Reptilia: Diplodactylidae). *J. Morphol.* **235**, 41–58. (doi:10.1002/(SICI)1097-4687(199801)235:1<41::AID-JMOR4>3.0.CO;2-R)
18. Wagner GP. 1989 The biological homology concept. *Ann. Rev. Ecol. Syst.* **20**, 51–69. (doi:10.1146/annurev.es.20.110189.000411)
19. Wagner GP. 2014 *Homology, genes, and evolutionary innovation*. Princeton, NJ: Princeton University Press.
20. Hall BK. 1995 Homology and embryonic development. In *Evolutionary biology*, vol. 28 (eds MK Hecht, RJ Macintyre, MT Clegg), pp. 1–37. Boston, MA: Springer.
21. Ruvinsky I, Gibson-Brown JJ. 2000 Genetic and developmental bases of serial homology in vertebrate limb evolution. *Development* **127**, 5233–5244. (doi:10.1242/dev.127.24.5233)
22. Nussbaum RA, Raxworthy CJ, Pronk O. 1998 The ghost geckos of Madagascar: a further revision of the Malagasy leaf-toed geckos (Reptilia, Squamata, Gekkonidae). *Misc. Publ. Mus. Zool. Univ. Mich.* **186**, 1–26.
23. Rosenberg HI, Russell AP, Cavey MJ. 1992 Development of the subdigital adhesive pads of *Ptyodactylus guttatus* (Reptilia: Gekkonidae). *J. Morphol.* **211**, 243–258.
24. Khan Noon ER. 2015 Developmental stages of the climbing gecko *Tarentola annularis* with special reference to the claws, pad lamellae, and subdigital setae. *J. Exp. Zool. B Mol. Dev. Evol.* **324**, 450–464. (doi:10.1002/jez.b.22630)
25. Khan Noon ER, Russell AP, Tucker AS. 2015 Developmental mechanisms underlying differential claw expression in the autopodia of geckos. *EvoDevo* **6**, 8. (doi:10.1186/s13227-015-0003-9)
26. Alturk WIM, Khan Noon ER. 2020 Ontogeny of the Moorish gecko *Tarentola mauritanica* with emphasis on morphogenesis of the skin and its derivatives. *J. Exp. Zool. B. Mol. Dev. Evol.* **334**, 294–310. (doi:10.1002/jez.b.22951)
27. Maderson PFA. 1985 Some developmental problems of the reptilian integument. In *Biology of the reptilia*, vol. 14 (eds C Gans, F Billet, PFA Maderson), pp. 525–598. New York, NY: John Wiley & Sons.
28. Alibardi L. 1997a Morphogenesis of the digital pad lamellae in the embryo of the lizard *Anolis lineatopus*. *J. Zool. (Lond.)* **243**, 47–55. (doi:10.1111/j.1469-7998.1997.tb05755.x)
29. Alibardi L. 1997b Ultrastructural and autoradiographical analysis of setae development in the embryonic pad lamellae of the lizard *Anolis lineatopus*. *Ann. Sci. Natur. (Paris)* **18**, 51–61.
30. Dhoulailly D, Maderson PFA. 1984 Ultrastructural observations on the embryonic development of the integument of *Lacerta muralis* (Lacertilia, Reptilia). *J. Morphol.* **179**, 203–228. (doi:10.1002/jmor.1051790302)
31. Alibardi L. 1996 Scale morphogenesis during embryonic development in the lizard *Anolis lineatopus*. *J. Anat.* **188**, 713–725.
32. Tornier G. 1899 Ein Eidechschenschwanz mit Saugscheibe. *Biol. Zentralbl.* **19**, 549–552.
33. Underwood G. 1954 Categories of adaptation. *Evolution* **8**, 365–377. (doi:10.1111/j.1558-5646.1954.tb01503.x)
34. Maderson PFA. 1971 The regeneration of caudal epidermal specializations in *Lygodactylus picturatus keneinsis* (Gekkonidae, Lacertilia). *J. Morphol.* **134**, 467–477. (doi:10.1002/jmor.1051340407)
35. Vitt LJ, Ballinger RE. 1982 The adaptive significance of a complex caudal adaptation in the tropical gekkonid lizard *Lygodactylus klugei*. *Can. J. Zool.* **60**, 2582–2587. (doi:10.1139/z82-332)
36. Bauer AM, Sadlier RA. 2000 *The herpetofauna of New Caledonia*. Ithaca, NY: Society for the Study of Amphibians and Reptiles.
37. Seipp R, Henkel FW. 2000 *Rhacodactylus: biology, natural history, & husbandry*. Frankfurt, Germany: Edition Chimaira.
38. Bauer AM, Jackman TR, Sadlier RA, Whitaker AH. 2012 Revisions of the giant geckos of New Caledonia (Reptilia: Diplodactylidae: *Rhacodactylus*). *Zootaxa* **3404**, 1–52. (doi:10.11646/zootaxa.3404.1.1)
39. Russell AP. 1976 Some comments concerning interrelationships amongst gekkonine geckos. In *Morphology and biology of reptiles* (eds AA Bellairs, CB Cox), pp. 217–244. London: Academic Press.
40. Peattie AM. 2008 Subdigital setae of narrow-toed geckos, including a eublepharid (*Aeluroscalabotes felinus*). *Anat. Rec.* **291**, 869–875. (doi:10.1002/ar.20706)
41. Bauer AM. 1990 Phylogenetic systematics and biogeography of the Carphodactylini (Reptilia: Gekkonidae). *Bonn. Zool. Monogr.* **30**, 1–220.
42. Seipp R, Klemmer K. 1994 Wiederentdeckung von *Rhacodactylus ciliatus* Guichenot 1866 im Süden Neukaledoniens (Reptilia: Sauria: Gekkonidae). *Senck. Biol.* **24**, 199–204.
43. Sanger TJ, Hime PM, Johnson MA, Diani J, Losos JB. 2008 Laboratory protocols for husbandry and embryo collection of *Anolis* lizards. *Herpetol. Rev.* **39**, 58–63.
44. Griffing AH, Sanger TJ, Matamoros IC, Nielsen SV, Gamble T. 2018 Protocols for husbandry and embryo collection of a parthenogenetic gecko, *Lepidodactylus lugubris* (Squamata: Gekkonidae). *Herpetol. Rev.* **49**, 230–235.
45. Wise PAD, Vickaryous MK, Russell AP. 2009 An embryonic staging table for in ovo development of *Eublepharis macularius*, the leopard gecko. *Anat. Rec.* **292**, 1198–1212. (doi:10.1002/ar.20945)
46. Griffing AH, Sanger TJ, Daza JD, Nielsen SV, Pinto BJ, Stanley EL, Gamble T. 2019 Embryonic development of a parthenogenetic vertebrate, the mourning gecko (*Lepidodactylus lugubris*). *Dev. Dyn.* **248**, 1070–1090. (doi:10.1002/dvdy.72)
47. Fogel JL, Thein TZT, Mariani FV. 2012 Use of LysoTracker to detect programmed cell death in embryos and differentiating embryonic stem cells. *J. Vis. Exp.* **68**, e4254. (doi:10.3791/4254)
48. Schleich HH, Kästle W. 1986 Ultrastrukturen an Gecko-Zehen (Reptilia: Sauria: Gekkonidae). *Amphibia-Reptilia* **7**, 141–166. (doi:10.1163/156853886X00361)
49. Skipwith PL, Bi K, Oliver PM. 2019 Relicts and radiations: phylogenomics of an Australasian lizard clade with East Gondwanan origins (Gekkota: Diplodactyloidea). *Mol. Phylogenet. Evol.* **140**, 106589. (doi:10.1016/j.ympev.2019.106589)
50. Felsenstein J. 1985 Phylogenies and the comparative method. *Am. Nat.* **125**, 1–15. (doi:10.1086/284325)
51. Harmon LJ, Weir JT, Brock CD, Glor RE, Challenger W. 2008 GEIGER: investigating evolutionary radiations. *Bioinformatics* **24**, 129–131. (doi:10.1093/bioinformatics/btm538)
52. Revell LJ. 2012 phytools: an R package for phylogenetic comparative biology (and other things). *Methods Ecol. Evol.* **3**, 217–223. (doi:10.1111/j.2041-210X.2011.00169.x)
53. Pinheiro J, Bates D, DebRoy S, Sarkar D, R Core Team. 2020 nlme: Linear and nonlinear mixed effects models. See <https://CRAN.R-project.org/package=nlme>.
54. Rawson SD, Maksimcuka J, Withers PJ, Cartmell SH. 2020 X-ray computed tomography in life sciences. *BMC Biol.* **18**, 21. (doi:10.1186/s12915-020-0753-2)
55. Hanken J, Wassersug RJ. 1981 The visible skeleton. *Funct. Photog.* **16**, 22–26.
56. Kerney R, Wassersug R, Hall BK. 2009 Skeletal advance and arrest in giant non-metamorphosing African clawed frog tadpoles (*Xenopus laevis*: Daudin). *J. Anat.* **216**, 132–143. (doi:10.1111/j.1469-7580.2009.01176.x)
57. Higham TE, Gamble T, Russell AP. 2017 On the origin of frictional adhesion in geckos: small morphological changes lead to a major

- biomechanical transition in the genus *Gonatodes*. *Biol. J. Linn. Soc.* **120**, 503–517. (doi:10.1111/bij.12897)
58. Stewart WJ, Higham TE. 2014 Passively stuck: death does not affect gecko adhesion strength. *Biol. Lett.* **10**, 20140701. (doi:10.1098/rsbl.2014.0701)
 59. R Core Team. 2020 *R: language and environment for statistical computing*. Vienna, Austria: R Foundation for Statistical Computing
 60. Koppetsch T, Böhme W, Büsse S, Gorb SN. 2020 Comparative epidermal microstructure anatomy and limb and tail osteology of eyelid geckos (Squamata: Eublepharidae): implications of ecomorphological adaptations. *Zool. Anz.* **287**, 45–60. (doi:10.1016/j.jcz.2020.05.005)
 61. Guichenot A. 1866 Notice sur un nouveau genre de sauriens de la famille geckotiens, du Muséum de Paris. *Mém. Soc. Sci. Nat. Cherbourg* **12**, 248–252.
 62. Alibardi L, Meyer-Rochow VB. 2017 Regeneration of adhesive tail pad scales in the New Zealand gecko (*Hoplodactylus maculatus*) (Reptilia; Squamata; Lacertilia) can serve as an experimental model to analyze setal formation in lizards generally. *Zool. Res.* **38**, 191–197. (doi:10.24272/j.issn.2095-8137.2017.046)
 63. Alibardi L, Bonfitto A. 2019 Morphology of setae in regenerating caudal adhesive pads of the gecko *Lygodactylus capensis* (Smith, 1849). *Zoology* **133**, 1–9. (doi:10.1016/j.zool.2019.01.003)
 64. Russell AP, Bauer AM. 1988 Paraphalangeal elements of gekkonid lizards: a comparative survey. *J. Morphol.* **197**, 221–240. (doi:10.1002/jmor.1051970208)
 65. Hale ME. 1996 Functional morphology of ventral tail bending and prehensile abilities of the seahorse, *Hippocampus kuda*. *J. Morphol.* **227**, 51–65. (doi:10.1002/(SICI)1097-4687(199601)227:1<51::AID-JMOR4>3.0.CO;2-S)
 66. Zippel KC, Glor RE, Bertram JEA. 1999 On caudal prehensility and phylogenetic constraint in lizards: the influence of ancestral anatomy of function in *Corucia* and *Furcifer*. *J. Morphol.* **239**, 143–155. (doi:10.1002/(SICI)1097-4687(199902)239:2<143::AID-JMOR3>3.0.CO;2-O)
 67. Bauer AM, Good DA. 1986 Scaling of scansorial surface area in the genus *Gekko*. In *Studies in herpetology* (ed. Z Rocek), pp. 363–366. Prague, Czech Republic: Charles University and Societas Europaea Herpetologica.
 68. Autumn K. 2006 Properties, principles, and parameters of the gecko adhesive system. In *Biological adhesives* (eds A Smith, J Callow), pp. 225–256. Berlin, Germany: Springer-Verlag.
 69. Russell AP, Johnson MK. 2007 Real-world challenges to, and capabilities of, the gekkotan adhesive system: contrasting the rough and the smooth. *Can. J. Zool.* **85**, 1228–1238. (doi:10.1139/Z07-103)
 70. Russell AP, Garner AM. 2021 Setal field transects, evolutionary transitions and gecko–anole convergence provide insights into the fundamentals of form and function of the digital adhesive system of lizards. *Front. Mech. Eng.* **6**, 621741. (doi:10.3389/fmech.2020.621741)
 71. Herrel A, Measey GJ, Vanhooydonck B, Tolley KA. 2012 Got it clipped? The effect of tail clipping on tail gripping performance in chameleons. *J. Herpetol.* **46**, 91–93. (doi:10.1670/10-301)
 72. Labonte D, Federle W. 2015 Scaling and biomechanics of surface attachment in climbing animals. *Phil. Trans. R. Soc. B* **370**, 20140027. (doi:10.1098/rstb.2014.0027)
 73. Griffing AH, Gamble T, Cohn MJ, Sanger TJ. Submitted. Evolutionary and developmental constraints shape the repeated evolution of adhesive toe pads among lizards.
 74. Bauer AM. 1986 Systematics, biogeography and evolutionary morphology of the Carphodactylini (Reptilia: Gekkonidae). PhD thesis, University of California, Berkeley, CA.
 75. Young NM, Hallgrímsson B. 2005 Serial homology and the evolution of mammalian limb covariation structure. *Evolution* **59**, 2691–2704. (doi:10.1111/j.0014-3820.2005.tb00980.x)
 76. Di-Poi N, Milinkovitch MC. 2016 The anatomical placode in reptile scale morphogenesis indicates shared ancestry among skin appendages in amniotes. *Sci. Adv.* **2**, e1600708. (doi:10.1126/sciadv.1600708)
 77. Loveridge A. 1947 Revision of the African lizards of the family Gekkonidae. *Bull. Mus. Comp. Zool.* **98**, 1–469.
 78. Cooper RL, Lloyd VJ, Di-Poi N, Fletcher AG, Barrett PM, Fraser GJ. 2019 Conserved gene signaling and a derived patterning mechanism underlie the development of avian footpad scales. *EvoDevo* **10**, 19. (doi:10.1186/s13227-019-0130-9)
 79. Noramly S, Morgan BA. 1998 BMPs mediate lateral inhibition at successive stages in feather tract development. *Development* **125**, 3775–3787. (doi:10.1242/dev.125.19.3775)
 80. Maynard-Smith J, Burian R, Kauffman S, Alberch P, Campbell J, Goodwin B, Lande R, Raup D, Wolpert L. 1985 Developmental constraints and evolution: a perspective from the Mountain Lake Conference on development and evolution. *Quart. Rev. Biol.* **60**, 265–287. (doi:10.1086/414425)