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Ontogenetic scaling patterns of lizard skin surface structure as revealed by gel-based stereo-profilometry

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Abstract

The skin surface structure of squamate reptiles varies greatly among species, likely because it plays a key role in a range of tasks, such as camouflage, locomotion, self-cleaning, mitigation of water loss and protection from physical damage. Although we have foundational knowledge about squamate skin morphology, we still know remarkably little about how intraspecific variation in skin surface structure translates to functional variation. This gap in our understanding can be in part traced back to: (i) our lack of knowledge on how body size determines skin surface structure; and (ii) the lack of means to perform high-throughput and detailed analysis of the three-dimensional (3D) anatomy of reptilian skin surfaces in a non-destructive manner. To fill this gap, we explored the possibilities of a new imaging technique, termed gel-based stereo-profilometry, to visualize and quantify the 3D topography of reptilian skin surface structure. Using this novel approach, we investigated intra-specific and intra-individual variation in the skin surface morphology of a focal lizard species, *Anolis cristatellus*. We assessed how various characteristics of surface topography (roughness, skew and kurtosis) and scale morphology (area, height, width and shape) scale with body size across different body regions. Based on an ontogenetic series of *A. cristatellus* males, we show that skin roughness increases with body size. Skin patches on the ventral body region of lizards were rougher than on the dorsum, but this was a consequence of ventral scales being larger than dorsal scales. Dorsal surface skew and kurtosis varied with body size, but surfaces on the ventral skin showed no such relationship. Scale size scaled isometrically with body size, and while ventral scales differed in shape from dorsal scales, scale shape did not change with ontogeny. Overall, this study demonstrates that gel-based stereo-profilometry is a promising method to rapidly assess the 3D surface structure of reptilian skin at the microscopic level. Additionally, our findings of the explanatory power of body size on skin surface diversity provide a foundation for future studies to disentangle the relationships among morphological, functional and ecological diversity in squamate reptile skin surfaces.

Key words: *Anolis cristatellus*; lizard integument; scale morphology; skin surface topography.

Introduction

Reptiles evolved a specialized keratinous integument that played a crucial role in their successful colonization of terrestrial habitats during the Mesozoic (Landmann, 1986; Alibardi, 2003). The skin surface structure of squamate reptiles, in particular, has since evolved to be exceptionally diverse, with extensive morphological variability, even among closely related species (Arnold, 2002; Broeckhoven et al. 2018). In addition to its structural complexity across multiple length scales (Arnold, 2002; Gower, 2003; Schmidt & Gorb, 2012; Spinner et al. 2013a,b; Bucklitsch et al. 2016; Allam et al. 2019), squamate skin is also functionally diverse (Gorb, 2005, 2009), playing important roles in camouflage (Spinner et al. 2013a), communication (Cuervo et al. 2016), locomotion (Irschick et al. 1996; Autumn et al. 2000; Russell & Johnson, 2007; Baum et al. 2014a,b), self-cleaning (Watson et al. 2015), mitigation of water loss (Dmi'el, 2001) and protection from physical damage (Broeckhoven et al. 2015).

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For any of these specific functions, however, the evolution of an 'optimal' skin surface would depend on the particular environmental and ecological constraints imposed on the animal (Losos, 2011; Irschick & Higham, 2016; Riedel et al. 2019) — for example, in arid environments, natural selection might favor skin surfaces that limit water loss (Broeckhoven et al. 2018), and an arboreal lifestyle may drive the evolution of skin surfaces that exhibit increased adhesive forces (Losos, 1990). Moreover, environmental factors may not drive skin surface specialization of all body regions in a similar way. For example, an arboreal lifestyle will primarily affect the skin surfaces of body regions involved in locomotion (e.g. toepads in geckos; Russell & Johnson, 2007; Johnson et al. 2009). While valuable biomechanical (Spinner et al. 2013a,b; Baio et al. 2015) and morphological studies (Russell, 2006; Schmidt & Gorb, 2012; Riedel et al. 2015) have contributed to our knowledge of functional morphology and diversity in squamate integuments, we still lack an understanding of how skin surfaces vary in three-dimensions (3D) across an individual and how these surfaces change with body size. More importantly, we also lack the basic means of assessing the metrics of reptile skin in a high-throughput fashion for understanding how their form and function vary across a wide range of different squamate species.

When studying trait variation, both within and among species, it is critical to identify how much of this observed variability is dictated by body size (Schmidt-Nielsen, 1984; Spence, 2009). Information on the degree of size-dependent variation in skin surface structure can be used to identify how much of the observed skin diversity is the result of local adaptation or is simply the byproduct of growth. As such, knowledge of the explanatory power of body size on squamate surface diversity provides the foundation for future studies aimed at disentangling the relationships between morphological, functional and ecological diversity in skin architecture. While several previous studies have assessed how lizard scale and scale ornamentation relate to body size (Calsbeek et al. 2006; Oufiero et al. 2011; Wegener et al. 2014; Lourdais et al. 2017; Broeckhoven et al. 2018; Tulli & Cruz, 2018), the (ontogenetic) scaling relationships of their 3D surface structure, particularly measurements of scale height, have remained largely unexplored. With modern advances in high-resolution imaging techniques (Eggeling, 2018), however, the tools to rapidly collect detailed data on reptilian skin surface structures are now routinely available.

While imaging and quantifying the complexity of biological surface structures can be accomplished by various methods, such as atomic force microscopy (Huber et al. 2005), micro-computed tomography (Broeckhoven et al. 2017) and scanning electron microscopy (Russell & Johnson, 2014; Riedel et al. 2015), these techniques often require extensive specimen preparation and/or long image acquisition times, and are thus not well suited for the high-throughput analysis of large numbers of specimens in a non-destructive manner. In contrast, gel-based stereo-profilometry (Johnson & Adelson, 2009; Li & Adelson, 2013) has recently proved to be an exceptionally useful technique for the rapid 3D structural characterization of biological surfaces without any specimen preparation, permitting 3D visualization *in situ* and even *in vivo* (Lauder et al. 2016; Wainwright & Lauder, 2016; Wainwright et al. 2017; Ankhelyi et al. 2018; Wainwright & Lauder, 2018). Inspired by these previous studies, which have primarily focused on the structural characterization of fish scales, we here demonstrated that gel-based stereo-profilometry is also an effective tool for visualizing and quantifying the scaled surfaces of squamates.

The aim of the present study was twofold: (i) to collect detailed 3D reconstructions of squamate skin surface structure; and (ii) to examine how different surface features (i.e. surface topography and scale morphology) scale with body size across different body regions. To assess scaling patterns, we took a static allometric approach by studying different individuals of the same species during various ontogenetic stages (Shingleton, 2010). Studying ontogenetic scaling patterns may yield valuable broad insights into trait adaptations, and may aid in identifying size-related constraints within a species (Schmidt-Nielsen, 1984). Here, we focused on the anole lizard species *Anolis cristatellus*, a focal lizard for diverse studies including thermal biology (Battles & Kolbe, 2018), behavioral ecology (Dufour et al. 2018; Gunderson et al. 2018a), invasion biology (Kolbe et al. 2016; Kahrl & Cox, 2017), functional morphology (Kolbe, 2015; Winchell et al. 2018) and evolutionary biology (Williams, 1972; Losos, 1990; Gunderson et al. 2018b).

Materials and methods

Specimens

We examined 25 preserved specimens of *A. cristatellus*, ranging from young hatchlings to large-bodied adults [across a snout–vent length (SVL) range of 16–67 mm]. Specimens were preserved in 70% ethanol and obtained from the herpetological collections of the Museum of Comparative Zoology at Harvard University (MA, USA). All individuals originated from the same population (Mayaguez, Puerto Rico, Greater Antilles), and were collected during the same field expedition (Table S1). In order to eliminate any potential effect of intersexual variation in skin surface anatomy and to increase statistical power, only male lizards were included in this study. Sex determination for hatchlings, however, was not feasible as these smaller specimens were morphologically indistinguishable from one another. Prior to imaging of the lizards' skin surface, SVL of each individual was measured using digital calipers (Mitutuyo; precision = 0.01 mm).

While in this study only museum-preserved specimens were examined, exploratory follow-up tests demonstrated no significant effect of preservation on anole surface structure (see Supplementary materials for details; Table S2).

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Skin surface imaging

Three-dimensional surface reconstructions of (hydrated) squamate skin were performed using a gel-based stereo-profilometry system manufactured by GelSight™ (Waltham, MA, USA), as described by Wainwright et al. (2017). In this approach, a deformable transparent gel pad (diameter = 43 mm) with one opaque surface was pressed onto the skin area of interest on the lizard specimen, creating a surface impression of the lizard's skin. While the gel pad was still in contact with the skin, a series of photographs from six different illumination angles was acquired, and a topographical 3D map of the surface was subsequently created by merging the acquired images using the GelSight™ surface analysis software.

For each lizard specimen, we investigated surface structures at two body regions: one on the dorsum, posterior to the midpoint between the pectoral and pelvic girdle; and one on the ventrum, also posterior to the midpoint between the pectoral and pelvic girdle (Fig. 1a). Skin surfaces were lightly brushed prior to imaging to remove any surface debris. The resulting reconstructed areas from each specimen measured 4.46 × 2.98 mm with a pixel density of 5202 × 3465, giving pixel resolution of 0.86 μm in the plane of the scan. After the 3D reconstructions were performed using the GelSight™ software, MountainsMap (Digital Surf, Besançon, France) was used to quantitatively evaluate the 3D data set.

Quantifying skin surface structure

Using the 3D reconstructions of the skin, the following metrological variables were extracted to quantify the skin surface topography: root-mean-square roughness, skew and kurtosis. Roughness (Sq) is given by the square-root of the sum across the surface of the squared distance of each point from the mean height. Skew (Ssk) and kurtosis (Sku) are parameters concerning the shape of the distribution of heights across a surface. A normal distribution of heights results in a skew of zero and a kurtosis of three. High positive skew corresponds to surfaces with many peaks; while low, negative skew describes surfaces with many valleys. A kurtosis above three indicates extremely high peaks or valleys, while a kurtosis below three indicates relatively gradual (and non-extreme) surface heights. For details on these variables used in surface metrology, see Whitehouse (1994). All measurements were taken from 2 × 2 mm cropped regions from the original scans.

Aside from surface topography, we also examined scale morphology in order to fully assess overall variation in skin surface structure. Based on the raw 2D photographs of the skin surface acquired from the GelSight™ system, we measured several morphological features of the lizard scales (Fig. 2). First, the area, length and width of 10 dorsal and 10 ventral scales per individual were measured using ImageJ (Abràmoff et al. 2004). Because we obtained a repeatability of over 98% for each of the three variables (Lessells & Boag, 1987),

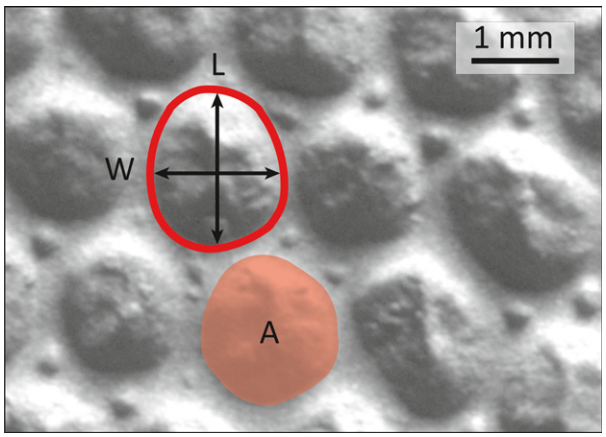

Fig. 2 Grayscale plan-view image of the dorsal body region of *Anolis cristatellus*. Annotations show scale length (L), width (W) and area (A).

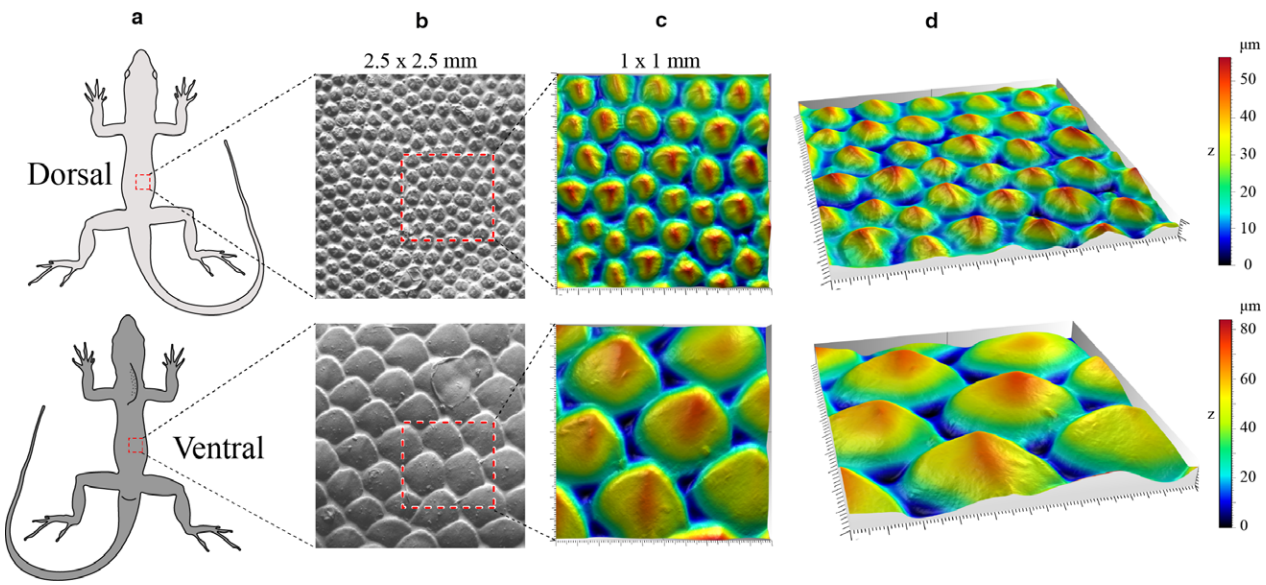

Fig. 1 Imaging the surface topography of lizard skin. (a) Illustration of the ventrum and dorsum of *Anolis cristatellus* with the red squares indicating the anatomical regions of study. (b) Grayscale plan-view images, (c) 3D reconstructions of the surface topography of the lizard skin displayed as colored elevation maps, and (d) oblique views of the same areas with *z*-height scale bars.

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we used (for each variable) the mean of the 10 measurements obtained for each individual for future statistical analyses. Second, we counted the number of scales along a 1-mm (anterior to posterior) transect. These scale counts were conducted 10 times on the ventrum and 10 times on the dorsum, and the average scale number for the dorsum and ventrum was calculated. Third, to characterize scale shape, we used an elliptic Fourier analysis (EFA). This type of analysis is commonly used to describe the shape of 2D outlines that do not possess clearly defined homologous landmarks by mapping the distance from the geometric center of the outline to each point on the contour with a polar coordinate function (Smith & Kriebel, 2017; Potier et al. 2018). This function can then be described in terms of a Fourier series with a series of harmonics; the lower harmonics approximate the coarse-scale features of outlines, whereas the higher harmonics capture more subtle variation. For more details on EFA, see Shen et al. (2009). From the raw 2D photographs, we first converted the scales on the images into scale silhouettes in Adobe Photoshop (San Jose, CA, USA) and, thereafter, we transformed the silhouettes into outlines using R package Momocs (Bonhomme et al. 2014). For the ventral and dorsal scales separately, we constructed outlines of five scales per individual and calculated the average scale shape for each individual using the function 'mshapes'. Inter-individual variation in average scale shape was thus quantified using EFA, which decomposed the outlines into nine harmonics (which gathered 99% of the total harmonic power; Fig. 3a,b). As there were four coefficients associated with each of the harmonics, EFA described the shape of each scale with a total set of 36 coefficients, which we summarized using a principal component analysis (PCA). All the analyses were conducted for the ventral and dorsal scales separately.

Data analysis

Prior to analysis, scale morphometrics (area, width and length) and SVL were $\log_{10}$-transformed, and scale counts were square root-transformed to meet the assumptions of normality. Traditional linear regressions were used to assess relationships between body size and skin topography (roughness, skew and kurtosis), body size and scale shape (scores of PC1, see above), and between scale size and scale counts. We used reduced major axis regressions (RMA) to explore allometric relationships between body size and scale size (width and length; which are expected to scale isometrically), and body size and scale area (expected to scale proportional to SVL to the second power). To test whether relationships differed between body regions, we included 'body region' (ventral and dorsal) as a factor in the statistical analyses.

Results

From a set of 25 *A. cristatellus* lizards ranging across a wide range of body size (SVL; min. = 16.46 mm, max = 67.30 mm), and using gel-based stereo-profilometry, we obtained data on a variety of skin features to explore ontogenetic scaling patterns in the skin surface structure of lizards. In total, we acquired 50 topographic images, with an average acquisition time (of imaging and digital reconstruction combined) of approximately 90 s per scan.

Results from these studies demonstrated that the surface topography of *A. cristatellus* skin varies considerably between the dorsal and ventral region of an individual's body (Fig. 4), and among individuals of different sizes (Fig. 5a). Specifically, large individuals have rougher skin than small individuals ($F_{1,46} = 385.55$, $P < 0.001$), and the skin on the ventral side of the body is consistently rougher than on the dorsum ($F_{1,46} = 71.47$, $P < 0.001$; Fig. 5a). The relationship (slope) between body size and skin roughness did not differ between body regions (interaction SVL*body

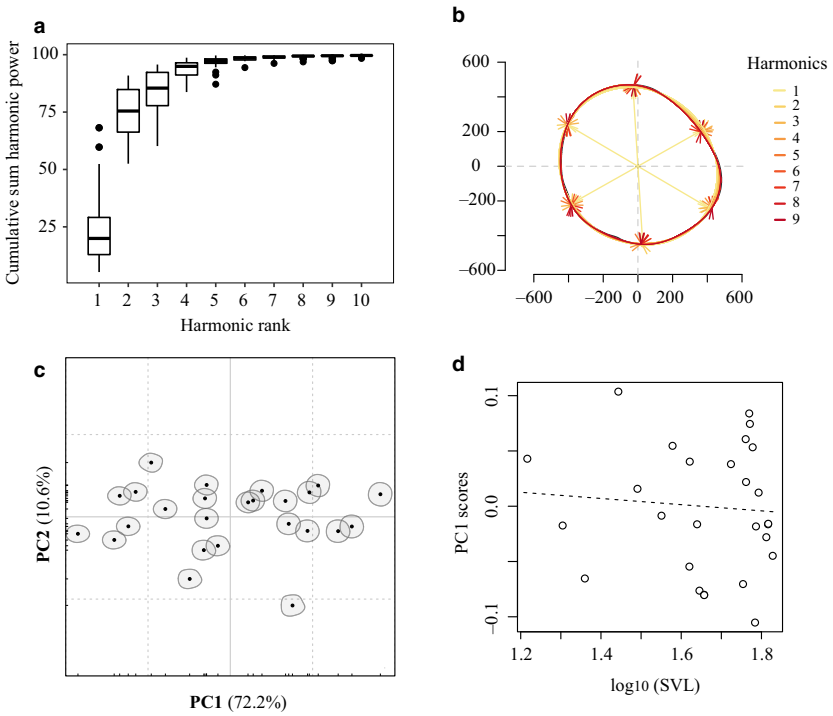

Fig. 3 Quantifying scale shape using elliptic Fourier analysis (EFA) in *Anolis cristatellus*. (a) Cumulated harmonic power of the first 10 ranks. (b) Visualization of the Ptolemaic ellipses on the outline of a dorsal scale, which illustrates intuitively the principle behind EFA. (c) Shape variation of dorsal scales plotted into morphospace along two principal component axes. (d) A scatterplot of body size [snout–vent length (SVL)] over dorsal scale shape (PC1). The relationship is statistically not significant ($F_{1,23} = 0.164$, $P = 0.689$).

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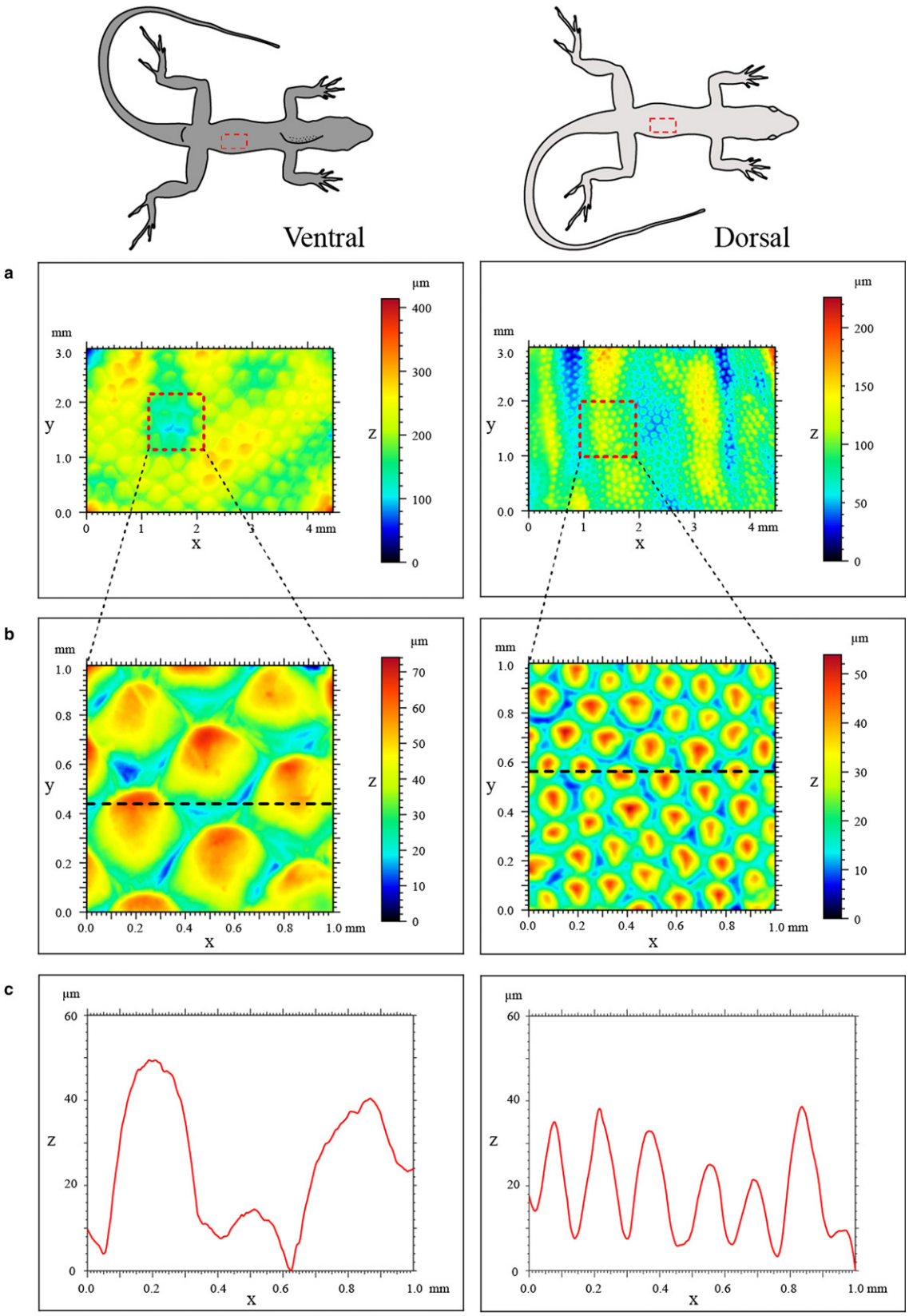

Fig. 4 Visualization of the variation in skin surface topography between dorsal and ventral body regions in *Anolis cristatellus*. A 2D colored elevation map of the (a) original (13.5 mm^2) and (b) cropped (1 mm^2) skin patch. (c) A profile line graph along the black dotted reference line from (b) showing surface topography.

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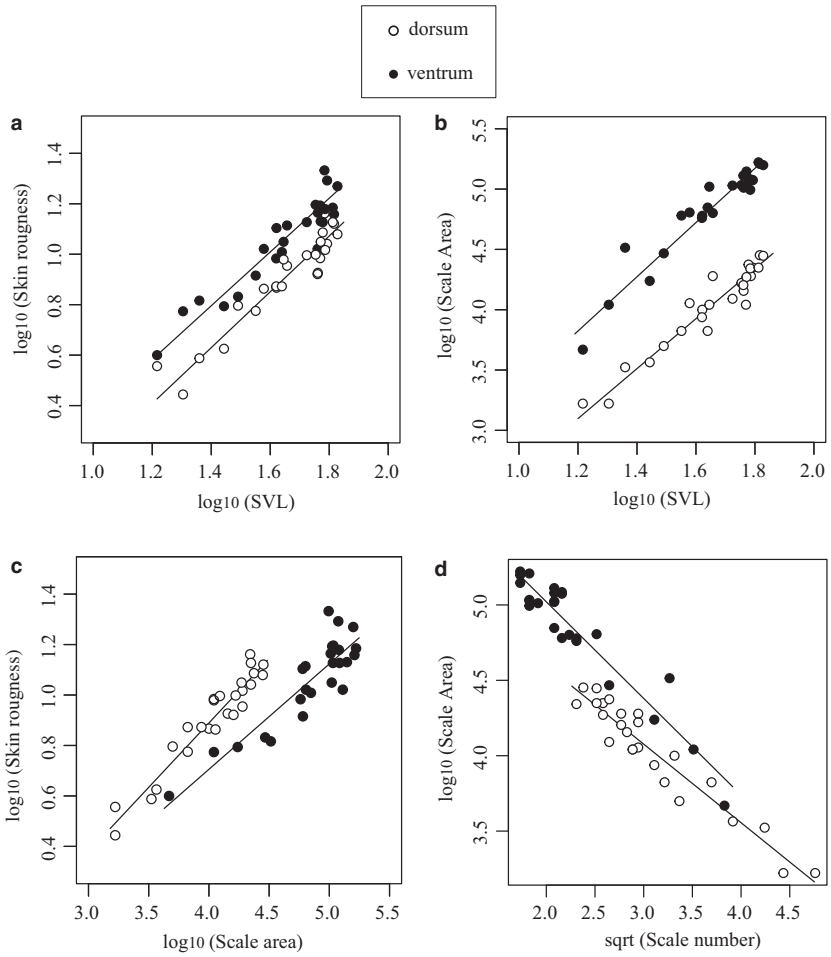

Fig. 5 Scatterplots showing the relationships between body size and (a) skin roughness and (b) scale area, and between scale area and (c) skin roughness and (d) scale number in *Anolis cristatellus*.

Table 1 Results of the linear regressions testing for the relationship between skin surface topography (roughness, skew, kurtosis) and body size (SVL) for the dorsal and ventral body region in *Anolis cristatellus*.

Body region	Topographic variable	*F*	*P*	R^2
Dorsal	Roughness	217.3	<0.001	0.90
	Skew	11.88	0.002	0.31
	Kurtosis	12.46	0.002	0.32
Ventral	Roughness	171.4	<0.001	0.88
	Skew	2.90	0.102	0.07
	Kurtosis	0.33	0.571	0.03

region; $F_{1,46} = 0.30$, $P = 0.590$; Fig. 5a) and, while dorsal surface skew and kurtosis scaled significantly with body size, ventral surfaces exhibited no such trend (Table 1; Fig. S1).

Detailed morphometric analyses of the dorsal and ventral skin scales showed that all dimensions for scale size (height, width and area) were strongly intercorrelated (all $P < 0.001$; Fig. S1), and strongly linked with body size (Table 2). Both dorsal and ventral scale size scaled isometrically with body size (Table 2), with the ventral scales being larger than the dorsal scales ($F_{1,46} = 701.50$, $P < 0.001$; Fig. 5b). The relationship between scale area and body size did not differ between body regions (interaction SVL*body region; $F_{1,46} = 0.86$, $P = 0.359$; Fig. 5b). The same was true for the length and width of the scales (Fig. S1).

Skin surfaces bearing large scales were more rough than surfaces with small scales ($F_{1,46} = 279.23$, $P < 0.001$, Fig. 5c). Subsequently, the finding that adult lizards had a rougher skin on their ventrum than on their dorsum (Fig. 5b) is a consequence of ventral scales being larger than dorsal scales: a patch of skin containing dorsal scales of a particular size is rougher than a patch of skin bearing ventral scales of the same size ($F_{1,46} = 56.48$, $P < 0.001$; Fig. 5c). The slope between scale area and skin roughness did not differ significantly between body regions (interaction scale area*body region; $F_{1,46} = 2.83$, $P = 0.100$; Fig. 5c). In addition, we found a strong correlation between scale area and scale number: individuals with large scales have a lower number of scales on a 1-mm transect ($F_{1,46} = 762.640$, $P < 0.001$; Fig. 5d). However, the relationship between scale area and scale counts differed between body regions (interaction scale number*body region; $F_{1,46} = 4.85$, $P = 0.033$; Fig. 5d).

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Table 2 Allometries of scale morphometrics versus SVL in *Anolis cristatellus*, obtained through RMA, with SVL as independent variable.

Body region	Scale variables	Intercept	2.5% CI	97.5% CI	Slope	2.5% CI	97.5 CI	R^2
Dorsal	Area (mm^2)	0.61	0.17	1.00	2.07	1.83	2.34	0.92
	Length (mm)	0.25	−0.07	0.52	1.10	0.93	1.29	0.86
	Width (mm)	0.27	0.01	0.49	1.08	0.94	1.23	0.90
Ventral	Area (mm^2)	1.12	0.63	1.55	2.25	1.98	2.55	0.92
	Length (mm)	0.50	0.18	0.77	1.17	0.99	1.36	0.88
	Width (mm)	0.68	0.43	0.91	1.10	0.97	1.25	0.91

Scale number explained 82.0% (dorsal) and 82.3% (ventral) of the intraspecific variation in scale area.

The PC analysis of harmonic coefficients from the EFAs captured 72.2% of the variation in dorsal scale shape in the first component (PC1; Fig. 3c). A second PC analysis on the ventral scale shapes explained 59% of the variation in PC1. Subsequently, we used the respective scores of PC1 as an index for scale shape. A linear regression was unable to find a significant relationship between body size and dorsal scale shape ($F_{1,23} = 0.164$, $P = 0.689$; Fig. 3d), and ventral scale shape ($F_{1,23} = 0.668$, $P = 0.422$).

Discussion

Understanding the 3D microstructural surface details of biological systems can provide critical insight into how species interact with their environments. In the example provided here, we describe a new tool, gel-based stereo-profilometry, for investigating squamate skin structure to help address these needs. Using this approach, we were able to successfully assess ontogenetic scaling patterns of the 3D skin surface structure of *A. cristatellus* lizards. The findings of this study clearly demonstrate the utility of gel-based stereo-profilometry for the detailed and rapid micro-scale visualization and quantification of the 3D surface structure of lizard skin.

Body size and skin topography

A number of recent studies have documented substantial variation in a range of morphological characteristics of the skin surface structures of squamate reptiles (Arnold, 2002; Gower, 2003; Schmidt & Gorb, 2012; Spinner et al. 2013a,b; Allam et al. 2019). Remarkably, however, only a few studies have quantified the 3D structure of squamate skin surfaces, with data limited to microstructures on the ventral scales of snakes (Baum et al. 2014a,b), and no study to date has evaluated the relationship between 3D scale structure and body size variation. In our present study, we found that the skin surface topography of *A. cristatellus* lizards is tightly linked with body size. For example, body size explained as much as 88% (ventrum) and 90% (dorsum) of the observed intraspecific variation in skin roughness. This result likely has strong functional implications, as roughness is known to affect the optical and mechanical properties of biological surfaces (Gorb, 2009). Compared with smooth surfaces, for example, rough surfaces typically reflect light more diffusely (e.g. facilitating camouflage in vipers; Spinner et al. 2013a), are more hydrophobic (e.g. enabling self-cleaning in geckos; Watson et al. 2015) and create more friction (e.g. allowing undulating locomotion in snakes; Hazel et al. 1999). For example, many snakes and legless lizards locomote via an undulatory behavior (Jayne, 1986; Gasc & Gans, 1990), which requires ventral skin to provide high, directional friction in order to support forward motion, and slide along the substrate (Hu et al. 2009). In these species, size-related variation in skin roughness might influence locomotory abilities, and future experimental work using the techniques described here could be used to assess whether size-related variation in skin surface topography also reflects size-related variation in functionality or performance.

Gel-based stereo-profilometry is unique in that it allows rapid 3D microstructural characterization of biological surfaces *in situ* and *in vivo* (Wainwright et al. 2017) at sizes between tens of millimeters to a few hundred microns. As such, this method is well suited for high-throughput analysis of large numbers of specimens in a non-destructive manner. However, nanoscale structural analyses still require labor-intensive and destructive techniques, such as scanning electron microscopy (Russell & Johnson, 2014) or atomic force microscopy (Huber et al. 2005). Different research questions thus demand different technical approaches, and ultimately it is the combination of compatible techniques that will offer the most complete assessment of an animal's skin surface structure.

Body size and scale morphology

Aside from skin surface topography, our findings also show considerable intraspecific variation in scale size among lizards at different ontogenetic stages. Information on scale size and its body size-dependent variation is functionally relevant because keratinized scales have been proposed to help reduce evaporative water loss (Bentley & Schmidt-

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Nielsen, 1966; MacLean, 1985; Eynan & Dmi'el, 1993; Dmi'el et al. 1997). Surprisingly, almost no physiological research has been conducted that experimentally tests the direct effect of scale size on water loss and, as such, future work to explore these potential correlations is warranted.

In our study, we found a strong positive relationship between scale size and body size in *A. cristatellus* (scale size scales isometrically with body size). This result is not unexpected, as the scale-size to body-size link has been documented (predominantly on the interspecific scale) in many lizards groups, such as *Liolaemus* (Tulli & Cruz, 2018), *Sceloporus* (Oufiero et al. 2011) and *Anolis* (Wegener et al. 2014). Interestingly, most studies do not measure the actual size of the scale, but instead count the number of scales along a defined transect on the skin and use the inverse of these scale counts as a proxy of scale size (Kerfoot, 1970; Thorpe & Baez, 1987; Malhotra & Thorpe, 1997; Oufiero et al. 2011; Wegener et al. 2014). Indeed, scale number per unit length is often a strong predictor of scale size, as it explains approximately 95% of the interspecific variation in scale size among *Liolaemus* species, for instance (Tulli & Cruz, 2018). Based on microscopically detailed images, however, our study shows that scale number could only explain approximately 82% of the intraspecific variation in *A. cristatellus*, leaving 18% still unaccounted for. In addition, we found that the relationship between scale size and scale number differed between the body regions. Together, these findings underline that researchers should keep thinking critically when using scale number as the inverse measure of scale size, especially in correlative tests aiming to disentangle the environmental drivers and constraints of scale size divergence.

Using modern geometric morphometric techniques (EFA; Bonhomme et al. 2014), we also successfully quantified scale shape in *A. cristatellus* and found no indication of shape changes during ontogeny. Although the shape of scales can vary considerably among squamate species, scale shape is known to be particularly species-specific and fixed at birth (Harvey & Gutberlet, 1995). The conservative character of scale shape has not gone unnoticed by naturalists for whom it serves an important trait for both early and modern taxonomic and systematic investigations (Leydig, 1873; Fitzsimons, 1901; Boulenger, 1921; Bucklitsch et al. 2016). While earlier work has typically scored scale shape qualitatively (e.g. circular, oval, elongated, guttiform), it would be opportune for future comparative studies to make use of modern methods to quantify shape, such as the EFA described in the present study to precisely quantify interspecific shape variation and increase statistical power.

Intra-individual variation in skin surface structure

Our examination of *A. cristatellus* skin not only showed that various features of the skin surface structure of lizards are size-dependent, but also that they can vary substantially among body regions within the same individual. Importantly, the relationship between body size and all measured skin surface variables did not differ substantially between the ventral and dorsal body region in our study. Whereas qualitative intra-individual diversity in scale size and shape has been documented for most squamate species (Fitzsimons, 1901; Boulenger, 1921; Peterson & Williams, 1981; Peterson, 1983; Arnold & Ovenden, 2004), the use of gel-based stereo-profilometry permits the ability to rapidly and quantitatively compare skin 3D topography between body regions. We demonstrate that the roughness of a 1-mm^2 skin patch is higher on the ventral body region of an individual lizard than on its dorsum. This finding appears to be a consequence of ventral scales being larger than dorsal scales (Fig. 5a–c) because skin patches carrying similar-sized dorsal and ventral scales (e.g. a hypothetical ventral skin patch of a small lizard and dorsal patch of a large lizard, so that in these two examples the scales are size-matched) are rougher on the dorsum than on the ventrum. The observed intra-individual variation in skin topography (which is interrelated with scale size and shape) might thus mirror the diversity of tasks the specific parts of the skin must perform, and may provide critical insights into the functional significance of these differences.

Novel imaging techniques open new avenues of investigation

In addition to the ability to quantify the micro- and macro-structural details of squamate skin, the 3D data sets acquired using gel-based stereo-profilometry can be directly converted into surface meshes, which can in turn be 3D printed. These tangible models can then be directly employed for studies to investigate the role of scale geometry on animal–substrate interactions, or enlarged for educational purposes to illustrate key differences between different squamate taxa. The techniques described here thus open exciting new avenues for investigating structure–function relationships in squamate skin and, as such, we encourage future squamate researchers to examine intra-individual variation in surface topography in an attempt to help link structural with functional variation in these ecologically and morphologically diverse reptiles.

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Author contributions

SB, DJI, JBL conceived and designed the study; SB, DKW, JCW collected the data; SB conducted statistical analyses, prepared figures, and drafted and revised the manuscript; all authors aided in interpreting the results and contributed to editing the final paper.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1 List of the museum specimens used in the current study, with information on the Museum of Comparative Zoology (MSZ) identification number, SVL and sampling locality.

Table S2 Results of the repeated-measure ANOVAs testing for differences in a range of measures that characterize the anole skin surface structure.

Fig. S1 Scatterplots showing the relationships between body size and two characteristics of skin topography (a, skew; b, kurtosis) and scale size (c, width; d, length).

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