Diversity of functional microornamentation in slithering geckos *Lialis* (Pygopodidae)

M. Spinner1,2, S. N. Gorb1 and G. Westhoff2,3

1Functional Morphology and Biomechanics, Zoological Institute, Kiel University, Am Botanischen Garten 9, 24118 Kiel, Germany
2Institute of Zoology, University of Bonn, Poppelsdorfer Schloss, 53115 Bonn, Germany
3Tierpark Hagenbeck gGmbH, Lokstedter Grenzstrasse 2, 22527 Hamburg, Germany

The skin of geckos is covered with countless microscopic protuberances (spines). This surface structure causes low wettability to water. During evolution, representatives of the recent gekkotan clade Pygopodidae started slithering on the ground. This manner of locomotion affected limb reduction resulting in a snake-like body. Regarding abrasion and frictional properties, a surface covered with gekkotan spines is a topography that hampers the snake-like locomotion mode. Using scanning electron microscopy, we investigated the shed skins of two pygopodid lizards, *Lialis jicari* (Papua snake lizard) and *Lialis burtonis* (Burton’s legless lizard), in order to show epidermal adaptations to limbless locomotion. Our data showed that Pygopodidae differ from their relatives not only anatomically, but also in their epidermal microstructure. Scales of *L. jicari* have five different structural patterns on various body regions. Ventral scales have nanoridges, similar to those found on the ventralia of snakes. Surfaces of scales covering the jaw bones, have flattened spine-like microstructures that might be an adaptation to reduce abrasion. Dorsal scales have oblong microscopic bulges covered with nanoridges. Spines cover the undersides and the interstices of scales over the entire body of both species and in *L. jicari* also the top of dorsal head scales. Our measurements of surface wettability (surface free energy) show superhydrophobic properties of the spiny surfaces in comparison with the other microstructural patterns of other body parts.

1. Introduction

Gecko skin is covered with numerous microscopic protuberances called spines, spinules or microspinules [1,2] connected with radial struts [2,3]. Such spines with a species-specific distance to each other in the range of 0.2–0.7 μm and a length of 0.3–2.0 μm are found in all examined genera of the Gekkota [2,4–7]. The widespread appearance of these structures in Gekkota gives rise to the assumption that spines are a basal epidermal feature of this group [2]. Measuring the contact angle of water droplets on spiny gecko skin, Hiller [7] substantiated the water-repellent character of this epidermal microstructure and suggested its self-cleaning function.

Pygopodidae (flap-footed lizards) are a clade of the Gekkota which is endemic to Australia and New Guinea [8–10]. Pygopodidae differ from their gekkotan relatives in that there is a total absence of forelimbs, and the hind limbs are reduced to rudimental flaps. Such a transition from a lizard-like to a snake-like body is a general trend in many squamate reptiles. A concerted evolution of limb reduction, digit loss and body elongation [11,12] took place in numerous other squamate clades, such as Gymnophthalmidae, Scincidae, Amphisbaenia, Dibamiidae, Anguidae and Serpentes [12]. Besides skeletal modifications, the disappearance of limbs also entails epidermal transitions [11]. In snakes, the most prominent and well-studied representatives of limbless reptiles, ventral scales (ventralia) have several macroscopic and microscopic features that can be interpreted as adaptations to limbless locomotion [13]. The ventralia are enlarged.
and show an imbricate arrangement. In comparison to a side-by-side arrangement of scales, free-scale edges in a posterior direction of overlapping scales provide advantageous frictional properties for forward locomotion [11,13]. Interlocking between scale edges and surface asperities prevents back slipping during uphill locomotion [11]. Keels or folds of ventralia were also interpreted as friction controlling or a wear resistance adaptation [11]. On the microscale, snakes also feature adaptations to limbless locomotion. Their scale surfaces feature microscopic indentations and elevations. Also, these structures are formed by the uppermost epidermal layer, the oberhautchen [14]. Such microstructure also occurs in other squamate reptiles [15] and is called microornamentation [16].

In many snake species, microornamentation of ventral scales consists of posteriorly oriented denticulations and nanoridges that are aligned along the longitudinal axis of the body [13,17]. Anisotropic frictional properties of these surface geometries, low friction during forward motion and higher friction in lateral and backward motion, suggest their contribution to limbless locomotion [13,17].

One can assume that limbless locomotion generally requires a particular epidermal surface structure with advantageous frictional properties. In pygopodid geckos, owing to their water-repellent and presumably self-cleaning properties, epidermal spines might retain their original function on the dorsal side of the limbless body. However, we assumed a convergence in the development of epidermal microstructure on the ventral scales similar to that of snakes. In this paper, we examined, for the first time to our knowledge, the microornamentation of different body regions in the exuviae of two pygopodid species, Lialis jicari (Papua snake lizard) and Lialis burtonis (Burton’s legless lizard) using scanning electron microscopy (SEM). Measurements of contact angles of water, diiodomethane and ethylene glycol on different body regions were used to estimate surface free energy (SFE). The combination of structural and experimental data served as a functional interpretation of various epidermal microstructures in limbless geckos.

2. Material and methods

For the examination of microornamentation, we used freshly shed exuviae of two individuals of L. jicari BOULENGER, 1903 and L. burtonis GRAY, 1835. Small strips of exuviae (from dorsal to ventral part) were dissected from the median region of the body. In addition, we investigated the eyes and the head of both species. Skin pieces were mounted on aluminium stubs using double-sided carbon conductive tape (Plano, Wetzlar, Germany) and sputter coated in a Balzers Union SCD040 unit (Balzers Union Ltd., Balzers, Liechtenstein) with a 10 nm thick layer of gold–palladium. All samples were studied in a SEM Hitachi S-4800 (Hitachi High-Technologies Corp., Tokyo, Japan) operating at an accelerating voltage of 3 kV. Dimensions of microornamentation were measured in SEM images on three scales of each body region of each individual using Imagej v. 1.45 software. Height profiles of microornamentation were taken from cross-sections of three individual scales from each individual. Additionally, microornamentation dimensions were determined in all scales of a transition from dorsal to ventral body side in one individual of L. jicari. For this purpose, 10 values for each variable, such as (i) distance of nanoridges, (ii) bulge width and (iii) bulge length, were taken from each scale.

Contact angles between 1 μl droplets of water, diiodomethane and ethylene glycol on both dorsal and ventral body scales, as well as dorsal scales of the head, were measured with a contact angle measuring device (OCA 20, Data Physics Instruments GmbH, Filderstadt, Germany) under room conditions (temperature = 20–24 °C, relative humidity = 40–60%) on eight individual scales of each body region. Droplets were placed on single scales using the application needle of the micro dispenser. Because of the superhydrophobicity of the dorsal head region, water droplets could not detach from the needle and be set down on the scales. For this reason, measurements of water droplets on this scale type were performed using the needle-in method. According to the established method for SFE calculations [18], the OWKR-model [19–21] was applied for SFE calculations of the contact angle data of the three fluids. The SFE of dorsal head scales of L. jicari was calculated according to the Wu-model [22]. All calculations were done with eight contact angle values per fluid measured on eight different scales of each body region from each individual exuvia. Surface tension data of the three applied liquids were taken from previous studies (water, diiodomethane: [23]; ethylene glycol: [24]).

3. Results

(a) Microornamentation

SEM images showed that the entire body of both species L. jicari and L. burtonis is covered with spines. Spines connected with radial struts were found in scale interstices in all examined body regions of both species (figure 1a). Also, overlapped upper surfaces of the dorsal, lateral and ventral body scales were covered with this spine pattern (figure 1b). Lower surfaces of these scales featured a similar pattern to that of small spines. Additionally, larger spines were evenly spread throughout the lower surfaces of the scales (figure 1c). Cutaneous sensilla were distributed over the upper sides of dorsal head scales and near the edges of dorsal body scales (figure 1d). Their depressions were also covered with spiny coverage and featured 1–10 large hairy structures in their centre (figure 1e).

Upper sides of scales that were not covered by the overlying scale featured a highly differentiated microornamentation in the different body regions. In L. jicari, the dorsal head scales were also covered with spines over their entire upper sides (figure 1f). The density of spines averaged 4.7 ± 0.34 spines μm⁻² (mean ± s.d.). Their length was about 1 μm and their averaged distance to each other was about 0.49 ± 0.04 μm. Spines were also found on the entire upper side of scales in the middle of the head region (figure 2a). Scales of the head region, covering the jaw bones (dentale), anterior part of the chin and throat, had spines at the overlapping edges, but at their medial surfaces, they were covered with flattened spine-like overlapping structures (figure 2a,b). The tips of these structures were oriented in a posterior direction (figure 2b). Flattened spines had a density of about 2.5 ± 0.05 spines μm⁻². Average distance between them, measured in lateral direction, was about 0.47 ± 0.02 μm. Dorsal scales, with the exception of the overlapping zone, showed a microstructure of oblong bulges with an average width of 5.3 ± 0.56 μm and a length of 120 ± 10 μm (figures 3a and 4a). Bulges were oriented along the longitudinal axis of the body. Towards the belly bulging decreased (figure 3b). Thus, lateral scales featured only slight bulges and ventral scales no bulges at all but rather nanoscale structures (figure 3b,c). All dorsal, lateral and ventral scales were additionally covered by nanoridges (nanostructures). On ventral scales, ridges were arranged parallel to the longitudinal
axis with an average distance of $0.65 \pm 0.09 \mu m$ and featured scattered free tips (figures 3c and 4h). On dorsal scales, nanoridges fused together along the centre of the bulges (figure 3a). As in ventral scales, the dorsal nanoridges were oriented along the longitudinal axis and had a similar distance to each other (figure 4b). Representatives of *L. burtonis* showed microornamentation patterns of the same dimensions on different body regions, similar to those of *L. jicari* with the exception of the absence of spines at the exposed surfaces of dorsal head scales. In *L. burtonis*, non-overlapped regions of dorsal head scales featured the same micro- and nanostructure as those of dorsal body scales. Outer surfaces of the eye scales in both species had polygonal oberhautchen cells (figure 5a) with pits (figure 5b). This pattern was disrupted only by imprints of the borders of clear layer cells, the bottom-most layer of the overlying former skin generation that was shed (figure 5i).

(b) Wettability
The epidermal patterns found on three different body regions, dorsal bulges and ventral nanoridges of *L. jicari* and *L. burtonis* and spines on dorsal head scales of *L. jicari*, showed different wettability and SFE (figure 6). Especially on spiny head scales of *L. jicari*, water droplets

---

**Figure 1.** Scanning electron micrographs of different body regions of *L. jicari*. (a) Scale interstice between dorsal scales is covered with spines. (b) Upper surface of a ventral scale at the edge of the overlapped zone which is also covered with spines. Exposed area shows a ridged microstructure. Free tips marked with arrows. (c) The underside of a ventral scale is covered with evenly spread smaller and larger spines. (d) Cutaneous sensilla (marked with white arrows) are distributed at the edge of dorsal body scales. (e) Cutaneous sensilla on a head scale. (f) Spiny microornamentation in the centre of a dorsal head scale.
featured high contact angles larger than 160° (figures 6b and 7). The calculated SFE was about 5.5 mN m\(^{-2}\) (Wu-model). Wettability (figure 6b) and SFE of ventral and dorsal scales were much higher in both species. Droplets of all fluids were circular on all surfaces, with the exception of dorsal scales. On these surfaces, diiodomethane droplets showed an oval shape. In this case, contact angles were measured from the rostral and lateral side of the scales and SFE was calculated for both directions (figure 6a). In ventral scales, SFE was about 24 mN m\(^{-2}\) (OWRK-model). Dorsal scales of all four individuals had an SFE of 27 mN m\(^{-2}\) measured from a lateral direction and 17 mN m\(^{-2}\) from a rostral direction.

4. Discussion

(a) From spines to different microornamentation patterns

Our study showed that representatives of the limbless gekkotan clade Pygopodidae have highly differentiated microornamentation, confined to certain body regions. Considering the omnipresence of epidermal spines in representatives of Gekkota and strong evidence that the setae of adhesive subdigital pads of arboreal geckos arose from the gekkotan spines [25], it is likely that pygopodid microornamentation patterns also evolved from these basal gekkotan structures. All microornamentation patterns found in *Lialis* can be traced back to spines or modifications of the cell shape. At exposed surfaces of the head region of *Lialis*, typical gekkotan spines are transformed to flattened protuberances overlapping each other and featuring endings that are oriented in a posterior direction. The dimensions and the scattered free tips found on the nanoridges on dorsal, lateral and ventral body scales suggest that these structures also originate from spines tilted on their side in a posterior direction and merged together. The dimensions of dorsal bulges range within the dimensions of cells. Hence, it can be assumed that bulges originate from convex-shaped oberhautchen cells. Pits on the eye scales can be interpreted as equivalent to the pits between the struts and spines which cover the rest of the body.

(b) Functional aspects of different microornamentation patterns

Distribution of different microornamentation patterns in different body regions that are involved in locomotion and sensory processes may hint at the functionality of epidermal microstructure [15,26,27]. It seems that pygopodid epidermal surfaces that are often in contact with the ground have structural adaptations. Flattened, posterior-oriented overlapping spines on exposed sites of the chin, especially on scales covering the jaw bone (dentale), may be interpreted as an adaptation to resisting mechanical loads and abrasion which are, in these areas, more intense when the head lies on the ground. Epidermal surfaces that are in contact with the ground during locomotion also show a specific spatial arrangement of microstructures which strongly points toward an adaptation. In contrast to spiny microornamentation or ophthalmic pits, which do not show any orientation relative to the body axis (isotropic geometry), ridges on ventral scales and the posterior-oriented endings of flattened spines on the chin have such a preferred orientation. In comparison to uniform spines and pits, which presumably have the same frictional properties in any direction, the oriented epidermal microstructure implies direction-dependent frictional properties (anisotropy). In chin microornamentation, flattened spines with backward oriented free tips, different friction can be expected during forward, backward and sideward sliding. Particularly in backward motion, interlocking of free tips with the substrate asperities could increase friction. Ridges on ventral scales are presumed to decrease friction in movement parallel to the ridges along the longitudinal (rostro-caudal) axis of the animal compared...
with the movement perpendicular to the ridges along the transversal (dextro-sinistral) axis.

(c) Anisotropic frictional properties in snake ventralia

Anisotropic frictional properties were previously documented in ventral scales (ventralia) of snakes. Ventralia of *Corallus hortulanus* feature ridges with an average distance of 0.33 μm [13] similar to that of *Lialis*. In their study, Berthé et al. [13] demonstrated friction minimising properties of nanoridges in a rostral direction compared with friction that occurs perpendicular to the ridges. On the contrary, the honey-comb-like microstructure of dorsal scales bears frictional isotropy. Convergent occurrence of ventral ridges with a distance of 0.3–0.7 μm in snakes and limbless geckos confirms the importance of this kind of microstructure for limbless locomotion in reptiles. The anisotropic frictional properties arising from the nanoridges have also been emphasized, in kinematic studies on limbless locomotion, to be a crucial feature for propulsion generation [28,29]. Microstructure responsible for frictional anisotropy fulfils the contradictory requirements of high friction for efficient propulsion but low friction promoting forward sliding. In contrast to the filigree gekkotan spines with a high aspect ratio (the quotient of structure height and diameter), ridges might further have a much higher abrasion resistance owing to their compactness.

**Figure 3.** Scales of *L. jicari* and scanning electron micrographs of their microornamentation at different magnifications at the (a) back, (b) the flank and (c) the belly. Back scales (a) feature bulges (B) covered with nanoridges (N). Ventral scales (c) feature only nanoridges (N). Free tips on nanoridges are marked in all scales of all regions with white arrow heads. In the images, the rostrad is on the upper part of the figure.
the spiny microstructure of geckos. However, the quadruped self-cleaning effect as the main advantage resulting from droplets. In his discussion, Hiller [7] has emphasized the not adhere well and can then be removed by rolling water both low SFE and microscale geometry of such surfaces do experimental and theoretical studies [38,39].

Figure 4. Dimensions of microornamentation in a transition from dorsal to ventral scales in L. jicari. (a) Length (dotted line) and width (solid line) of dorsal and lateral bulges. (b) Distance between nanoridges in dorsal, lateral and ventral scales. Standard deviation of 10 measurements per scale is shown by error bars.

(d) Functional significance of gekkotan spines
Despite ventral and dorsal epidermal adaptations to limbless locomotion in Lialis, typical gekkotan spines were found on some sites of the body. In both species, the spines were found in scale interstices, as well as on scale undersides and scale surfaces hidden by overlapping scales. Moreover, in L. jicari, exposed areas of dorsal head scales are also covered with spines. The important functional significance of such spiny surfaces may explain why spines remained in the gecko evolution in certain body regions. In quadruped geckos, Hiller [7] experimentally proved the water-repellent properties of spiny surfaces.

The contact angle of a water droplet on a surface is a measure of the surface wettability. Our comparative measurements showed that on spiny head scales of L. jicari, droplets of water had a contact angle larger than 150°, which corresponded to the hydrophobic state of the surface [30]. It was less wettable than dorsal and ventral scales and head scales of L. burtonis. Also, the calculated SFE of head spines was low. Assuming that all these surfaces have very similar keratin-based chemical composition, differences in wettability must be based on the differences in surface geometry. The hydrophobicity-enhancing function of the microstructure even leads to the superhydrophobicity of hydrophobic surfaces previously reported in numerous publications (e.g. [31–33]). All theoretical models show that pillar structures in the range of gekkotan spines correspond to the geometry that provides strong water repellence [34–37]. The oval shape of diiodomethane droplets on the dorsal epidermis of Lialis species is owing to the specific surface microstructure. Such an effect of anisotropic ridged surface geometry on droplet shape is substantiated by previous experimental and theoretical studies [38,39].

If a superhydrophobic surface has a low roll-off angle, it is additionally self-cleaning [40,41]. Contaminants owing to both low SFE and microscale geometry of such surfaces do not adhere well and can then be removed by rolling water droplets. In his discussion, Hiller [7] has emphasized the self-cleaning effect as the main advantage resulting from the spiny microstructure of geckos. However, the quadruped geckos are not fossorial [42], and therefore skin contamination with ground material, except on foot surfaces, and contamination with dust, is unlikely in these species. Such self-cleaning surfaces at scale interstices in Lialis might be a protection against fungi, which would have an optimal microclimate for growing there.

Furthermore, in the poikilothermic reptiles, water-repellent surfaces can contribute to thermoregulation because it prevents chilling which could arise from the evaporation of water droplets on the skin.

The self-cleaning function is even more important in depressions of cutaneous sensilla which were found in both Lialis species studied as well as in the pygopodid species Pygopus leptodapus [43] and many other gecko species [44]. Distribution and shape of large sensilla and their depressions in Lialis coincides with the data on other pygopodids [45].

It is difficult to interpret the species-specific differences in head microornamentation of Lialis species studied. Dorsal head scales of L. burtonis feature the same bulged and ridged microstructure as dorsal body scales, whereas L. jicari has spines in this region. This finding was all the more surprising, because, apart from this, both species show only little differences in their surface microstructure. Indeed, both species look so similar that only slight variation of ventral scales and the number of particular head scales and preanal pores are used as characters for determination [46]. Also the habitat and lifestyle of these two species are similar. Both species are sympatric, although L. burtonis have a larger distribution area. It is found in almost all of Australia and parts of New Guinea, with the exception of the very humid rainforests, whereas L. jicari inhabits only New Guinea. Both species are ground-living species that ambush their prey, catching it by raising their head and anterior body above the ground at an angle of about 30°. They can also follow their prey in a slithering manner through the grass and leaf litter [46,47]. Hence, the behaviour cannot explain the interspecific differences in microornamentation. However, the locomotion and ambushing behaviour indicate the different functional role of dorsal epidermis on the head and body. Because the dorsal part of the body is partially in contact with the substrate when an animal slithers through the grass and leaf litter, selective pressure towards abrasion resistant surface structures can be considered as high in both species. By contrast, dorsal areas of the anterior part of the body hardly come in contact with the substrate, as the animal is normally in an upright position. Consequently, these areas were not under selective pressure leading to the development of abrasion resistant microstructure in the form of compact ridges. Additionally, this microstructure may contribute to camouflage, hiding the body in such an environment.

(e) Microornamentation of the eye scales
Measurements on the wettability of eye scales (brille or spectacle) of Gekko gecko showed that the equilibrium contact angle of a water droplet on this surface is smaller than 100° indicating neither a superhydrophobic nor a self-cleaning surface [48]. Structure-based anti-adhesive self-cleaning eye surfaces are known in some insect species [49] and would also be an advantage for geckos. Spine absence on the spectacle surface is even more astonishing because geckos, also Pygopodidae, with the exception of the basal Eublepharidae, have neither movable eyelids nor lachrymal fluid that might aid in eye cleaning. It can be assumed that the self-cleaning
The effect of spines on spectacles is reduced owing to the stronger selective pressure towards enhancement of optical properties. It is very likely that the discovered microrough surface covered with pits has better anti-reflective, refractive and diffractive properties for vision than a spiny surface. In spite of lacking a self-cleaning surface structure, geckos may keep transparent spectacles owing to their periodical shedding when the skin of the entire body is shed. Additionally, geckos have another solution for dealing with eye contamination: they are able to clean their eyes with their tongues [50]. This behaviour was also observed in the examined species (figure 8 and see also the electronic supplementary material, video S1).

**Figure 5.** Scanning electron micrographs of eye scales of *L. jicari*. (a) Microornamentation of an eye scale. Borders of oberhautchen cells are marked with a black arrow. Imprints of borders of clear layer cells of the shed overlaying skin are marked with a white arrow. (b) The oberhautchen cells of eye scales have numerous nanoscopic pits on their exterior surface.

**Figure 6.** (a) Contact angles between droplets of water (H$_2$O, crosshatched bars), diiodomethane (CH$_2$I$_2$, white bars), and ethylene glycol (C$_2$H$_6$O$_2$, black bars) on ventral and dorsal scales of *L. jicari* and *L. burtonis* and dorsal head scales of *L. jicari*. Standard deviations of contact angles of each of eight dorsal and ventral scales of two individuals of each species, and eight dorsal head scales from the two individuals of *L. jicari* are shown with error bars. (b) Contact angles of (i) water droplets on ventralia, (ii) dorsal head scales and (iii) dorsal body scales. Contact angles of water on dorsal head scales were measured with the needle-in method.
Figure 7. Lialis jicari (Papua snake lizard) from the family Pygopodidae maintains a dry head, when it is sprinkled with water. As in its quadruped gekkotan relatives, the dorsal head scales of this species are covered with microscopic spines providing superhydrophobicity.

5. Outlook

This study has demonstrated the importance of functional surface micro- and nanostructure in the biology of reptiles. It shows that the same keratinous material fulfills different functions by modifying the surface topography on different body regions in the gekkotan genus Lialis, whereas, under similar selective pressures, similar structural solutions have simultaneously evolved in Serpentes and Gekkota.

Aside from Serpentes and Gekkota, limblessness also evolved independently in several other squamate lineages (Amphisbaenians, Anguidae, Anniellidae, Cordylidae, Dibamidae, Gerrhosauridae, Gymnophthalmidae and Scincidae; [11,12]). As microstructures were also found in all examined species of these clades [26,51–54], investigation of ventral scale surfaces of these clades could expand the knowledge of structural epidermal adaptations to limbless locomotion or even show a general surface geometry for this type of locomotion. However, different modes of locomotion and morphological specializations that may influence locomotion (such as osteoderms in Anguidae [55]) suggest different surface geometries whose frictional properties are adapted to the specific locomotion mode of the species. Finally, one must consider that not only surface geometry but also chemical composition and inner architecture can be modified to improve epidermal properties for locomotion and related aspects, such as abrasion resistance [56,57].

Acknowledgements. We thank Prof. Dr Horst Bleckmann (Rheinische Friedrich-Wilhelms-Universität Bonn, Institut für Zoologie, Bonn, Germany) for supporting this study with advice and material.

Funding statement. This work was partially supported by the German Science Foundation (DFG Graduiertenkolleg Bionik, GRK 1572) and by the Federal Ministry of Education, Science and Technology, Germany (BMBF project Biona 01RB0812) to S.N.G. and G.W.

References
