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The microstructure of scales varies in four lizard species

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Abstract

The microstructure of lizard scales carries a phylogenetic signal in traits, reflecting key adaptations with high functional value. Using scanning electron microscopy, we examined the skin surface of four European Lacertidae species which occur in different habitats, as well as climatic conditions. We sampled *Podarcis carbonelli* and *P. virescens* from an oceanic-influenced climate, *Zootoca vivipara* from a temperate climate, and *Gallotia galloti* from three climatically distinct sites. In all species, dorsal midbody scales were proximally laminated. However, *G. galloti* differed from the other species, displaying spiny margins on lamellae, whereas *Podarcis* species and *Z. vivipara* had smooth margins. Within *G. galloti*, spiny microstructures were denser and larger in the northern, humid population compared to the high-elevation and southern, drier populations. These differences highlight the potential functional role of microstructures in skin adaptation to environmental conditions and emphasize their ecological significance in lizard species across climatic variation.

Keywords: Lacertidae, skin, morphology, microornamentation, functional morphology

The skin of reptiles represents an important boundary layer between the environment and the individual (Gatten, 1984). It consists of several layers, with the outermost layer being the highly keratinized epidermis (Alibardi, 2003). Mechanical protection, water permeability and UV protection are the most important functions of the skin for all amniotic vertebrates (Kardong, 2012). Different skin layers are involved in these main functions, with the outermost layer playing a wide role in all functions. Therefore, the main aim of the present study was to investigate the differences in the microstructure of the skin surface in four lizard species living in different climates and habitats.

The surface of reptile scales consist of two components, which are distingushed as follows: 1) macroornamentation, formed by the arrangement of the Oberhäutchen cell boundaries (MaO), and 2) microornamentation as a pattern of the Oberhäutchen cell surface (MiO) (Peterson, 1984a, b; Peterson and Bezy, 1985). Since the terminology in the studies on the microstructure of the scale surface is very inconsistent, we here use the term “microstructure” of the scale surface to refer to the two components of the general pattern (MaO and MiO). Using the scanning electron microscropy (SEM), microsturctures, early research on lizard skin microstructure has revealed significant interspecific and body-part differences (e.g. Stewart and Daniel, 1973), as well as adaptations bonded to ecological factors (e.g. Sammartano, 1976). Similar patterns of microstructure are found in lizard species with similar ecology but from different squamate families (e.g., the lamellar pattern for the same (dorsal) scales is described in species of Lacertidae, Scincidae, Xantusiidae) (e.g., Sammartano, 1976; Peterson, 1984a; Peterson and Bezy, 1985; Arnold, 2002; Gower, 2003). On the other hand, similar patterns can be observed in species with different ecology (landscape and/or climate preference) with the same families or even the same genus (e.g., *Chalcides ocellatus* and *C. chalcides*: Sammartano, 1976). These findings suggest that different microstructures have undergone important adaptations and have a functional value in lizards.

Flat, smooth lamellae are thought to reduce friction and improve locomotion, particularly in ground-dwelling species (Stewart and Daniel, 1973; Arnold, 2002). A smooth surface also facilitates dirt shedding, which is crucial for species inhabiting moist environments (Arnold, 2002). In contrast, species with spines or ridges tend to be more cryptic, as these structures suppress skin shininess (Arnold, 2002; Abdel-Hady et al., 2022). Spiny or ridged lamellae can also enhance friction, benefiting species that climb vegetation or originate from arid environments (Arnold, 2002; Allam, Abo-Eleneen and Othman, 2019; Abdel-Hady et al., 2022). These studies collectively indicate that lizard skin microstructure is shaped by both evolutionary history and functional needs, with different surface patterns enhancing locomotion, camouflage, or environmental resistance. Understanding these adaptations provides insight into the ecological significance of microstructural traits in lizards.

In our study, we used the shed skin (e.g. Spinner, Westhoff and Gorb, 2013) to analyze the microstructure of the surface of the skin. During shedding, when the superficial epidermis was removed, the *stratum basale* forms two new layers, the *stratum granulosum* and the *stratum corneum* (Maderson et al., 1998). The new layers have the same microornamentation as the old ones, which allows us to use shedded skin as a non-invasive sampling method to study the microstructure of the skin surface.

We investigated the surface microstructure of the dorsal scales from mid-body of four species: The Carbonell‘s wall lizard (*Podarcis carbonelli*), the Geniez‘s wall lizard (*P. virescens*), the viviparous lizard (*Zootoca vivipara*), and the Gallot‘s lizard (*Gallotia galloti*). Specimens of *P. carbonelli* (male, SVL = 51 mm) and *P. virescens* (male, SVL = 59 mm) were caught in the same location in Portugal in an urbanized area around 10 km inland from the Atlantic coast (Lat. 40.92, Lon. -85.42) with coastal-influenced climate (Average high annual temperature = 19˚C, Average low annual temperature = 9˚C). The *Z. vivipara* specimen (male, SVL = 50) originated from Slovenia (Lat. 46.06, Lon. 14.48) and a mixed forest habitat and temperate climate (Average high annual temperature = 14˚C, Average low annual temperature = 6˚C). For the species *G. galloti*, we examined samples obtained from three specimens that were collected from three different locations on the Tenerife island: NORTH (male, SVL = 132 mm, Lat. 28.57, Lon. -16.19) in a humid forested habitat with subtropical humid climate (Average high annual temperature = 24˚C, Average low annual temperature = 18˚C), SOUTH (male, SVL = 116, Lat. 28.06, Lon. -16.54) in a dry rocky and sandy habitat with arid climate (Average high annual temperature = 24˚C, Average low annual temperature = 17˚C), and at the VULCANO (male, SVL = 102 mm, Lat. 28.26, Lon. -16.62) in a volcanic rocky habitat with relatively dry and colder mountainous climate (Average high annual temperature = 6˚C, Average low annual temperature = -1˚C). All individuals were adult males and were freshly shedding skin when we collected it, thus, the stage of the shedding cycle was the same.

In a preliminary experiment, we compared SEM micrographs of chemically fixed or only air-dried samples of shed skin pieces. Since no obvious differences could be detected, we continued the images on air-dried skin pieces. A small piece (approx. 1 x 1 cm) was first washed in 75% alcohol to remove all particles and air-dried. The dried samples were mounted on a carbon tape disc on the aluminium holders, sputtered with 7 nm gold-palladium using a Precision Etching Coating System (682 PECS, Gatan), and examined with a field emission SEM (Jeol JSM-6500F). We obtained secondary electron images (SEI) with a working distance (WD) of 10 mm, an accelerating voltage of 15 kV, a spot size of 9, and approximately 0.7 nA probe current. Aperture 4 was used for high-resolution images. The scale bar and other imaging parameters are provided at the bottom of the SEM image. Imaging was done at different magnification, focusing at the middle position of one dorsal scale. High magnification images (5000x) were used to characterize the microstructural features of the skin surface.

The presence of lamellae was observed in all images (Figure 1), thus, we described the surface of the lamellae as (i) smooth or (ii) rough, the pattern of lamellae as (i) parallel lines or (ii) parallel lines with breaks, position of the lamellae as (i) overlapping or (ii) at an angle with visible spaces between them, and the margin of the lamellae as (i) smooth, (ii) serrated or (iii) with spines. Spines observed throughout the image area were described as (i) sharp only, (ii) rounded only, or (ii) sharp and rounded.

The skin surface of lamellae of both *Podarcis* species, such as *Z. vivipara* appeared smooth (Figure 1A-C), while of *G. galloti* appeared rough in all three samples (Figure 1D-F), but the roughness was most evident in the *G. gallotia* from the south (Figure 1F), indication adaptation for dry and hot conditions. The pattern of lamellae was parallel in *G. galloti* (Figure 1D-F), while breaks in parallel lines can be seen in all other species (Figure 1A-C). The angle of the lamellae varied between species; *Z. vivipara* had lamellae that were angled away from each other so that an opening was visible between them (Figure 1A), in *P. carbonelli* and *P. virescens*, the lamellae appeared to be arranged flat on top of each other with a narrow gap between them (Figure 1B,C), which was also partly visible in *G. galloti* from the volcano area (Figure 1D), while continous lamellae with no gaps visible between them were visible in northern and southern *G. galloti* (Figure 1E,F). The margin of lamellae was smooth for *Z. vivipara* and *P. carbonelli* (Figure 1A,B), while it was slightly serrated in *P. viresecens* (Figure 1C). Opossitely, all *G. galloti* had spines on the lamellar margin, which were either very dense and sharp in shape (northern site) (Figure 1E), dense and mostly rounded (southern site) (Figure 1F) or rounded and appeared together with serrated pattern (volcano site, Figure 1D).

Our study provides novel insights into the microstructure of the lizard skin of four lacertid species inhabiting distinct sites, with different habitat and climatic conditions, and we identified differences in the surface properties of the lamellae between and within species (*G. galloti*). The different morphology of the skin surface within one species, *G. galloti*, sampled under three different climatic conditions, suggests possible intraspecific functional adaptations to local environmental conditions (e.g. Sammartano, 1976). However, future systematic studies should be conducted with an appropriate sample size and include additional populations to cover the full heterogeneity of habitats and climatic zones of the islands to fully understand the local adaptation effects.

The lamellar arrangement observed across all species supports previous findings that this structural pattern is widespread among lizards (Arnold, 2002; Harvey and

Gutberlet, 1995). Variability in the lamellar pattern, surface, angle and margin highlights potential functional role. For instance, *Z. vivipara*, from temperate forested habitats, displayed lamellae with a smooth surface and angled margins forming visible openings. This structure could facilitate moisture retention on the skin surface or aid in the shedding of the dirt, corroborating with other studies of ground-dwelling species from similar climates (Arnold, 2002; Kardong, 2012). *Zootoca vivipara* is known to inhabit very humid areas even at high elevations or latitudes and has a relatively high rate of water loss (Žagar et al., 2017). On the other hand, more overlapping lamellae in *Podarcis carbonelli* and *P. virescens* that live in drier habitats may better retain water. Openings between the lamellae may facilitate movement of water across the skin (e.g. Kattan and Lillywhite, 1989), even though the most important waterproof barrier in the skin is located in the mesos-layer of the epidermis (Landmann, 1986; Lillywhite, 2006; Alibardi and de Nardo, 2013).

Regarding *G. galloti*, the spiny lamellar margins and rough surfaces were recorded in three different sites but varied in expression across the climatic zones and elevation. Rougher surfaces may suppress shininess for camouflage or improve traction in dry environments (Arnold, 2002; Abdel-Hady et al., 2022), which aligns with our finding that most spiny lamelle margin and rough surface was observed in *G. gallotia* from the most dry site – south. The roughness of lamellae in dry conditions suggests a condition to minimize water loss with microsturcures that trap moisture (Arnold, 2002; Abo-Eleneen and Allam, 2011; Alibardi, 2003; Kardong, 2012). A previous study comparing ecophysiological traits in populations of *G. galloti* across an elevational gradient showed that lizards from the southernmost population exhibited the lowest rates of cumulative water loss (Séren et al., 2023). However, spiny lamellae margin was also observed in a population of *G. galloti* sampled in the most humid area of the north of

Tenerife. This implies that these kinds of microstructures may have additional functions other than retaining water loss, which requires further research.

Our use of shed skin as a non-invasive sampling method offers a valuable tool for studying skin microstructure while adhering to the 3R principles (Russell and Burch, 1959). Additionally, the findings confirm that shed skin reliably reflects the microornamentation of the intact epidermis (Kardong, 2012). Future research should investigate the functional implications of these microstructural differences under controlled experimental conditions, such as water retention efficiency, dirt shedding, and traction enhancement. Additional comparative studies across additional lizard families and habitats (following Arnold, 2002) would also further illuminate the ecological and evolutionary significance of these adaptations.

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References

Abdel-Hady, A.E., El-Amir, S.M., Al-Muttri, N.S., El-Hariri, H.M. (2021): Scale microornamentation in some lizard species. Pak. J. Zool. **2021**: 1-7.

Abo-Eleneen, R.E., Allam, A.A. (2011): Comparative Morphology of the Skin of *Natrix tessellata* (Family: Colubridae) and *Cerastes vipera* (Family: Viperidae). Zoological Science, **28**: 743-748.

Allam, A.A., Abo-Eleneen, R.E., Othman, S. I. (2019): Microstructure of scales in selected lizard species. Saudi J.Biol. Sci. **26**: 129-136.

Alibardi, L. (2003): Adaptation to the land: The skin of reptiles in comparison to that of amphibians and endotherm amniotes. J. Exp. Zool. B **298B**: 12-41.

Alibardi, L., DeNardo, D.F. (2013): Ultrastructural and immunocytochemical features of the epidermis of the lizard *Heloderma suspectum* indicate richness in lipids and lack of a specialized shedding complex. Acta Zool. **94**: 35-43.

Arnold, E.N. (2002): History and function of scale microornamentation in lacertid lizards. J. Morphol. **252**: 145-169.

Gatten Jr, R.E. (1984): Biology of the Reptilia, Volume 13, Physiology D, Physiological Ecology.

Gower, D.J. (2003): Scale microornamentation of uropeltid snakes. J. Morphol. **258**: 249-268.

Harvey, M.B., Gutberlet Jr, R.L. (1995): Microstructure, evolution, and ontogeny of scale surfaces in cordylid and gerrhosaurid lizards. J. Morphol. **226**: 121-139.

Kattan, G.H., Lillywhite, H.B. (1989): Humidity acclimation and skin permeability in the lizard *Anolis carolinensis*. Physiol. Zool. **62**: 593-606.

Kardong, K.V. (2012): Vertebrates: Comparative Anatomy, Function, Evolution (6th ed.). McGraw-Hill.

Landmann, L. (1986): Epidermal permeability barrier: Transformation of lamellar granule-disks into intercellular sheets by a membrane fusion process. J. Investigat. Dermatol. **87**: 202-209.

Lillywhite, H.B. (2006): Water relations of tetrapod integument. J. Exp. Biol. **209**: 202-26.

Maderson, P.F.A., Rabinowitz, T., Tandler, B., Alibardi, L. (1998): Ultrastructural contributions to an understanding of the cellular mechanisms involved in lizard skin shedding with comments on the function and evolution of a unique lepidosaurian phenomenon. J. Morphol. **236**: 1-24.

Peterson, J.A. (1984a): The scale microarchitecture of *Sphenodon punctatus*. J. Herpetol. **18**: 40-47.

Peterson, J.A. (1984b): The microstructure of the scale surface in iguanid lizards. J. Herpetol. **18**: 437-467.

Peterson, J.A., Bezy, R.L. (1985): The microstructure and evolution of scale surface in xantusiid lizards. Herpetologica **41**: 298-324.

Russell, W.M. S., Burch, R.L. (1959): The Principles of Humane Experimental Technique. Methuen.

Sammartano, F. (1976): Microornamentazioni delle scaglie di alcuni Sauri. Animalia **3**: 195-205.

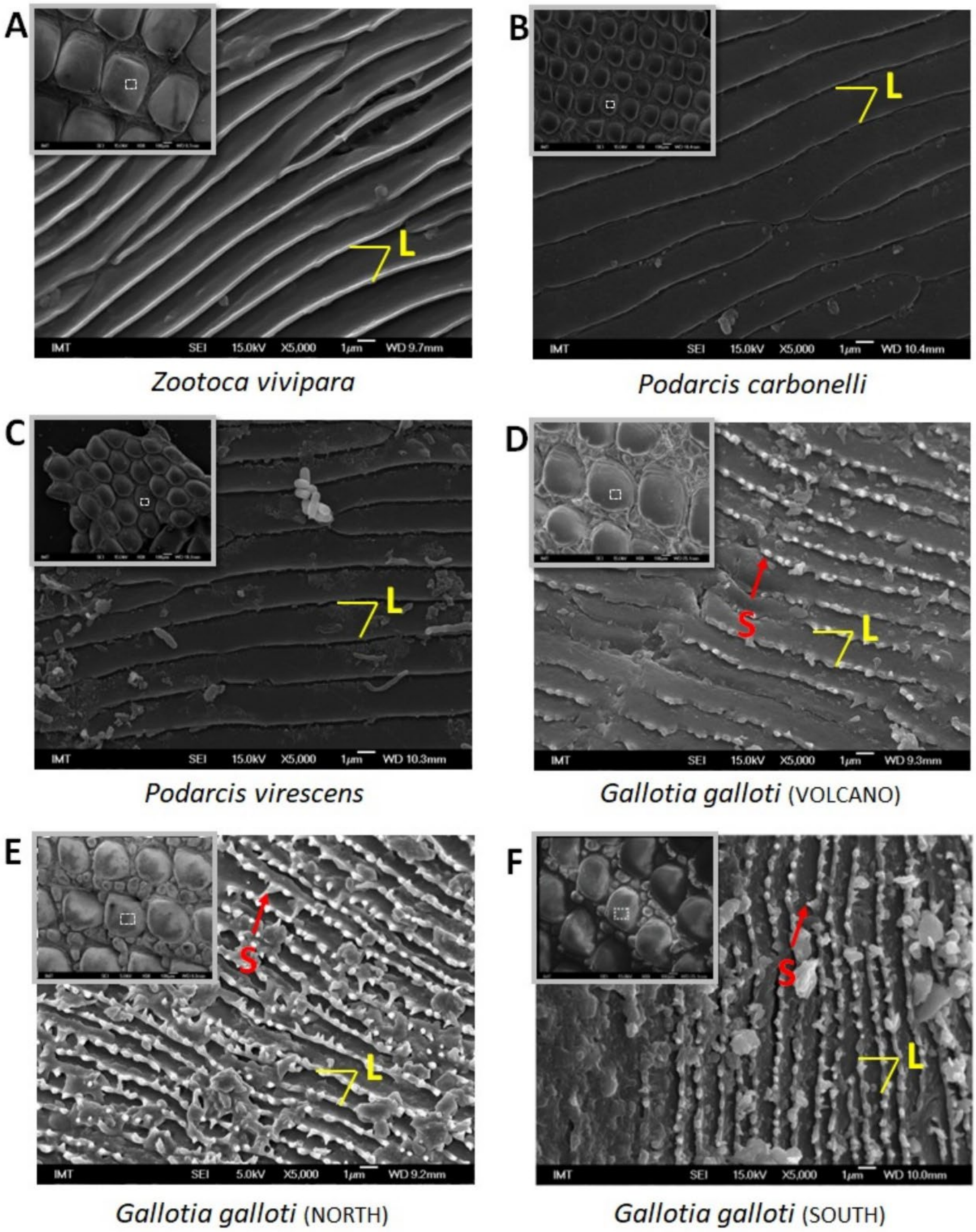
Serén, N., Megía-Palma, R., Simčič, T., Krofel, M., Guarino, F.M., Pinho, C., Žagar, A., Carretero, M.A. (2023): Functional responses in a lizard along a 3.5-km altitudinal gradient. J. Biogeogr. **50**: 2042-2056.

Spinner, M., Westhoff, G., Gorb, S.N. (2013): Subdigital and subcaudal microornamentation in chamaeleonidae—a comparative study. J. Morphol. **274**: 713-723.

Stewart, G.R., Daniel, R.S. (1973): Scanning electron microscopy of scales from different body regions of three lizard species. J. Morphol. **139**: 377-388.

Žagar, A., Vrezec A., Carretero, M.A. (2017): Does thermal and hydric physiology of *Zootoca* (*vivipara*) *carniolica* reflect their selected microhabitat conditions? Salamandra **53**: 153-159.

Figure 1. SEM micrographs of microstructure of the dorsal scales in four lacertid species. A: *Zootoca vivipara*, B: *Podarcis carbonelli*, C: *P. virescens*, D: *Gallotia galloti* (Volcano), E: *G. galloti* (North), F: *G. galloti* (South). The framed image in the upper left corner shows the scales at higher magnification (60x), and the white dashed square indicates the centre position in the scale where the close-up (at 5000x) was taken. One lamellae (L) and one spike (S) were labelled on each image for visual interpretation.


A
L
IMT SEI 15.0kV X5,000 1μm WD 9.7mm
Zootoca vivipara
B
L
IMT SEI 15.0kV X5,000 1μm WD 10.4mm
Podarcis carbonelli
C
L
IMT SEI 15.0kV X5,000 1μm WD 10.3mm
Podarcis virescens
D
S
L
IMT SEI 15.0kV X5,000 1μm WD 9.3mm
Gallotia galloti (VOLCANO)
E
S
L
IMT SEI 5.0kV X5,000 1μm WD 9.2mm
Gallotia galloti (NORTH)
F
S
L
IMT SEI 15.0kV X5,000 1μm WD 10.0mm
Gallotia galloti (SOUTH)