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Micro-ornamentation patterns in different areas of the epidermis in the gecko *Tarentola mauritanica* reflect variations in the accumulation of corneous material in Oberhautchen cells

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Abstract

Micro-ornamentations characterize the surface of scales in lepidosaurians and are summarized in four main patterns, i.e., spinulated, lamellated, lamellate-dentate, and honeycomb, although variations of these patterns are present in different species. Although geckos are known to possess a spinulated pattern derived from the Oberhautchen layer, also other pattern variations of the spinulated micro-ornamentation are present such as those indicated as dendritic ramification, corneous belts, and small bare patches. The present study mainly describes the variation of micro-ornamentations present in scales of different skin regions in the Mediterranean gecko *Tarentola mauritanica* using scanning and transmission electron microscopy. The study reports that the accumulation of corneous material in Oberhautchen cells is not homogenous in different areas of body scales and, when mature, this process gives rise to different sculpturing on the epidermal surface generating not only spinulae but also transitional zones leading to the other main patterns. It is hypothesized that spinulae formation derives from the vertical and lateral symmetric growth of tuberculate, non-overlapped scales of geckos. Sparse areas also result smooth or with serpentine-ridges likely revealing the beta-layer located underneath and merged with the Oberhautchen. The eco-functional role of this variable micro-ornamentation in the skin of lizards however remains largely speculative.

Keywords Gecko · Epidermis · SEM · Micro-ornamentation · Sensory organs

Introduction

The outer surface of the scales in lizards is sculptured in a variably complex series of micro-ornamentation that reflect the accumulation of corneous material in the outmost layer of the epidermis termed Oberhautchen (Stewart and Daniel 1973; Peterson 1984a, b; Irish et al. 1988; Harvey 1993; Harvey and Gutberlet 1995; Arnold 2002; Gower 2003; Dujsebajeva et al. 2021; Riedel and Schwarzkopf 2022). The above studies using the scanning electron microscope (SEM) have concluded that there are four main micro-ornamentation patterns in lepidosaurians, i.e., spinulated, lamellated,

lamellate-dentated, and honeycomb, with further sub-specific variations. The formation of micro-ornamentation in different species of lizards derives from a process of molding of one epidermal layer termed clear layer on a connected layer called Oberhautchen (Ernst and Ruibal 1966; Alexander and Parakkal 1969; Maderson et al. 1972; Hiller 1972; Alibardi 1999a, b). After the two layers are mature, they split one from another giving rise to skin shedding with the elimination of a molt (Alibardi 1998; Maderson et al. 1998). In contrast with the spinulated, lamellated, and lamellated-spinulated micro-ornamentation, the “honeycomb” pattern of lizard scales derives from the fusion of three or more Oberhautchen cells along their perimeter (imbrication, see Peterson 1984a, b; Irish et al. 1988; Alibardi 1999b, 2000), and the role of the clear layer in the process is even more complex (and unclarified) than for the other patterns.

While over most scales of geckos and anoline lizards the Oberhautchen forms short 0.5–3- μm -long protrusions assuming a spiny aspect (spinulated pattern), in the ventral part of digits (fingers and toes), the shedding complex gives rise to setae of 2–4- μm diameter by 10–100 μm length in

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the digital or caudal adhesive pads (Ernst and Ruibal 1966; Bauer 1998; Russel 2002; Alibardi and Meyer-Rochow 2017; Alibardi and Bonfitto 2019; Bonfitto et al. 2022; Griffing et al. 2021). As lizards undergo somatic growth and cleaning the epidermal surface from dust, particles, and micro-organisms, the epidermis undergoes a cyclical shedding cycle (Maderson et al. 1998; Alibardi 2014). During the cycle, a new epidermis is formed underneath the old one, determining epidermal renewal. In particular, beneath the Oberhautchen layer, newly formed cells accumulate the hardest corneous material indicated as “corneous beta material” since it is mainly composed of corneous beta pro-teins (CBPs), formerly indicated as beta-keratins (Maderson et al. 1998; Sawyer et al. 2000; Alibardi 2014). Beta cells eventually merge with Oberhautchen cells forming a mechanically resistant beta-corneous layer covered by micro-ornamentation. Other epidermal layers are formed

underneath, collectively forming at maturity an alpha-layer that is limiting water loss and is mechanically elastic and flexible allowing some scale motility.

Recent studies on numerous skin areas in the geckos *Lygodactylus conraui* and *L. capensis* have indicated that the spinulated micro-ornamentation of these lizards is in reality more complex than previously believed, revealing the distribution over the body of other complex patterns in addition to the classical spinulated one. Corneous material is accumulated on the surface of the epidermis in different areas of the body forming patterns indicated as “corneous belts” or as “corneous dendritic ramifications” (Bonfitto et al. 2022; Fig. 1A, B). Sparse small “naked or bare” areas, where the Oberhautchen and beta-layer meet forming smooth or slightly corrugated regions, indicated as “serpen-tine microridges” (Maderson et al. 1998), were also noted. These micro-ornamentation patterns are likely the result of

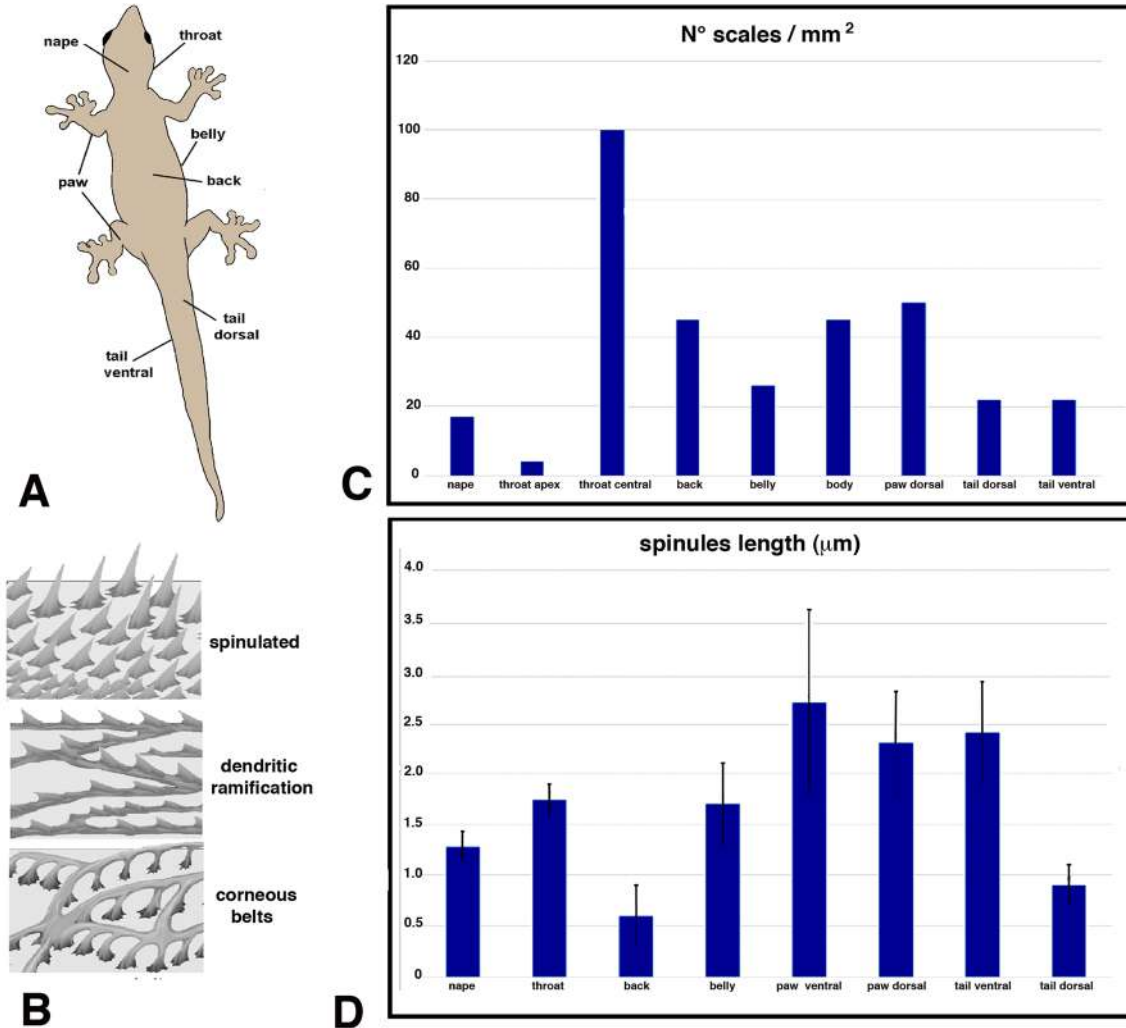


Fig. 1 Schematic drawings (A, B) and summarizing histograms (C, D). **A** Areas analyzed. **B** drawing summarizing the three main microstructure patterns. **C** Histogram reporting scale density in each area analyzed. **D** Histogram reporting spinulae length in each area analyzed

different modalities of accumulation of corneous material in Oberhautchen cells and their fusion with cells of the beta-layer, but further analysis on this process is needed. In order to confirm the presence of similar or alternative patterns of micro-ornamentation, we have examined another species of gecko, the Mediterranean gecko *Tarentola mauritanica* using SEM and transmission electron microscopy (TEM) analyses in different skin areas. Aside from the specific study on a gecko, the present survey aims to generalize the cytological origin of micro-ornamentations on the scales of all lepidosaurian reptiles.

Materials and methods

Biological material and SEM preparation

The present survey has utilized two specimens of the Mediterranean gecko *Tarentola mauritanica*, accidentally dead and preserved in formalin for some hours followed by a longer storage in 70% of ethanol. From one specimen, numerous body regions of the skin were collected from the nape, throat, back, abdomen, fingers and toes, and the tail. The second sample served for the collection of missing areas from the former specimen, namely throat and scalp.

After about 5 days of drying at room temperature under covering (to avoid dust pollution), the samples of 2–3 mm were immobilized on alluminium stubs of 5 mm in diameter that were previously coated with double-sticky tape for SEM analysis. The samples were double coated with gold using a metalizer device (BIO-RAD SEM Coating System, SC502), and observed at various magnifications under a SEM Hitachi S-2400 operating at 15 kV. Other observations at higher magnification have employed a “Thermofisher Quattro S” powered by a field emission gun for electron source.

The collected SEM images have been analyzed by a J-imaging software (NIH free software, USA) for image analysis that allows to measure and analyze statistically details of the length, wide, and density of the surface

micro-ornamentations of the epidermis such as scale, spinulae, and setae.

Transmission electron microscopy

For light (LM) and TEM analysis, small pieces of fingers and tail skin were collected from two specimens and they were immediately fixed for about 10 h in 2.5% glutaraldehyde in 0.1 M phosphate buffer at neutral pH, dehydrated in ethanol and propylene oxide and embedded in Durcupan resin for TEM. After sectioning using an ultramicrotome (LKB, Nova), semithin sections (1–3 μm thick) were collected on glass slides, dried on a hot plate, and stained with 0.5% Toluidine blue for general histology and tissue orientation. Thin sections (40–60 nm thick) were collected using the ultramicrotome on copper or nickel grids (200 mesh). The sections were stained using routine stain with uranyl acetate and lead citrate, and observed under a CM-100 Philips and Zeiss 10C/CR transmission electron microscope operating at 60–80 kV. Images were collected by a digital camera and fed into a computer for image storage, study, and figure composition using a Photoshop program (8.0).

Results

SEM observations on the tail and limbs

In Fig. 1 A are indicated the main areas surveyed in the present study, and in Fig. 1B are shown the general aspects of the three patterns previously observed in geckos. The histograms (Fig. 1C–D) show the scale density (n. scales/ mm^2) in different skin areas, and the size of spinulae or setae and of the sensory organs detected. Table 1 reports some characteristics of scales and their micro-ornamentation in different regions of the skin.

Tail scales often appear overlapped one with another and in numerous cases on the dorsal surface, their Oberhautchen layer, the superficial micro-ornamentation, features

Table 1 Reports some values for the different studied areas

	Scale shape	Scale Nb/ mm^2	Spinulae and/or setae	Spinulae length (mm)	Nb sensilli/scale	Interscale
Nape	Polygonal and rectangular	17	Spinulae	1.28 ± 0.14	Max 20	Present
Throat	Polygonal and rectangular	Variable	Spinulae	1.74 ± 0.15	Max 20	Present
Back	Roundish	45	Spinulae	0.6 ± 0.3	1 ÷ 6	Present
Abdomen	Roundish	26	Spinulae	1.7 ± 0.4	0	Present
Legs ventral Side	Absent	0	Spinulae and setae	2.7 ± 0.9	1 ÷ 2	Present
Legs dorsal side	Oval or rectangular	50	Spinulae	2.3 ± 0.5	0 ÷ 3	Absent
Tail ventral side	Rectangular	22	Spinulae	2.4 ± 0.5	0 ÷ 3	Present
Tail dorsal Side	Rectangular	22	Spinulae	0.9 ± 0.2	1 ÷ 2	Scarce

Legs ventral side setae length = max 50 mm

numerous central bumps or reliefs of 5–15 μm (Fig. 2A–C). In the ventral scales of the tail, superficial bumps are only seen in those located by the tail tip. Spinulae are very dense and 0.8–2.4 μm high in tail scales and 1–3 sensilli contain-ing 1–2 sensorial hairs are present by the tip of sparse tail scales (Fig. 2D; Fig. 1C, D; Table 1). At higher magnification, the spinulated Oberhautchen reveals a more or less uniform layer of curved spinulae (Fig. 2E).

Leg or arm scales, including those of the proximal area attached to the trunk, vary in their shape and size according to the examined area but tend to be oval-shaped and also slightly overlapped (Fig. 3A). Like in the tail scales, the micro-ornamentation of dorsal limb scales vary from a uniform layer of spinulae in most scales to regions of transitions with other patterns indicated as “dendritic

ramification” (Fig. 3B–D). The size of spinulae can reach the largest dimension in limbs, 1.8–3.4 μm (Fig. 1C). Sensilli on the scale tip show 1–5 sensorial hairs and are surrogando by a mix of isolated and short spinulae and sparse dendritic ramifications of the Oberhautchen surface (Fig. 3E–F). On the dorsal side, digits show a row of central and larger rectangular scales and 1–2 rows of smaller scales with a square shape (Fig. 4A). In the mid-level of the digits, the central scales show numerous small bumps while the hinge (inter-scale) regions, most occupied by the softer alpha-layer, appear wrinkled (Fig. 4B). The outer (dorsal) surface of scales where no bumps are present shows a uniform spinulated pattern of the Oberhautchen in the proximal (basal) area that tran-sits into the ramification dendritic pattern in the distal

Fig. 2 SEM images of the tail region. **A** General aspect of tail scales in dorso-lateral view. Bar 1 mm. **B** Detail of scales with smooth surface (arrows) and with bumps (arrowheads). Bar 100 μm . **C** Distal region of scale with two sensilli (arrow-heads). The sulci (arrows) like represent Oberhautchen cell perimeter. Bar 20 μm . The insert shows a small bare area (arrowhead) on the outer scale surface. Bar 50 μm . **D** Detail showing bumps (arrows) on some Oberhautchen cells surrounding a sensilla (arrowhead). Bar 30 μm . **E** Higher magnification view on the curved spinulae present on the outer scale surface. Bar 6 μm

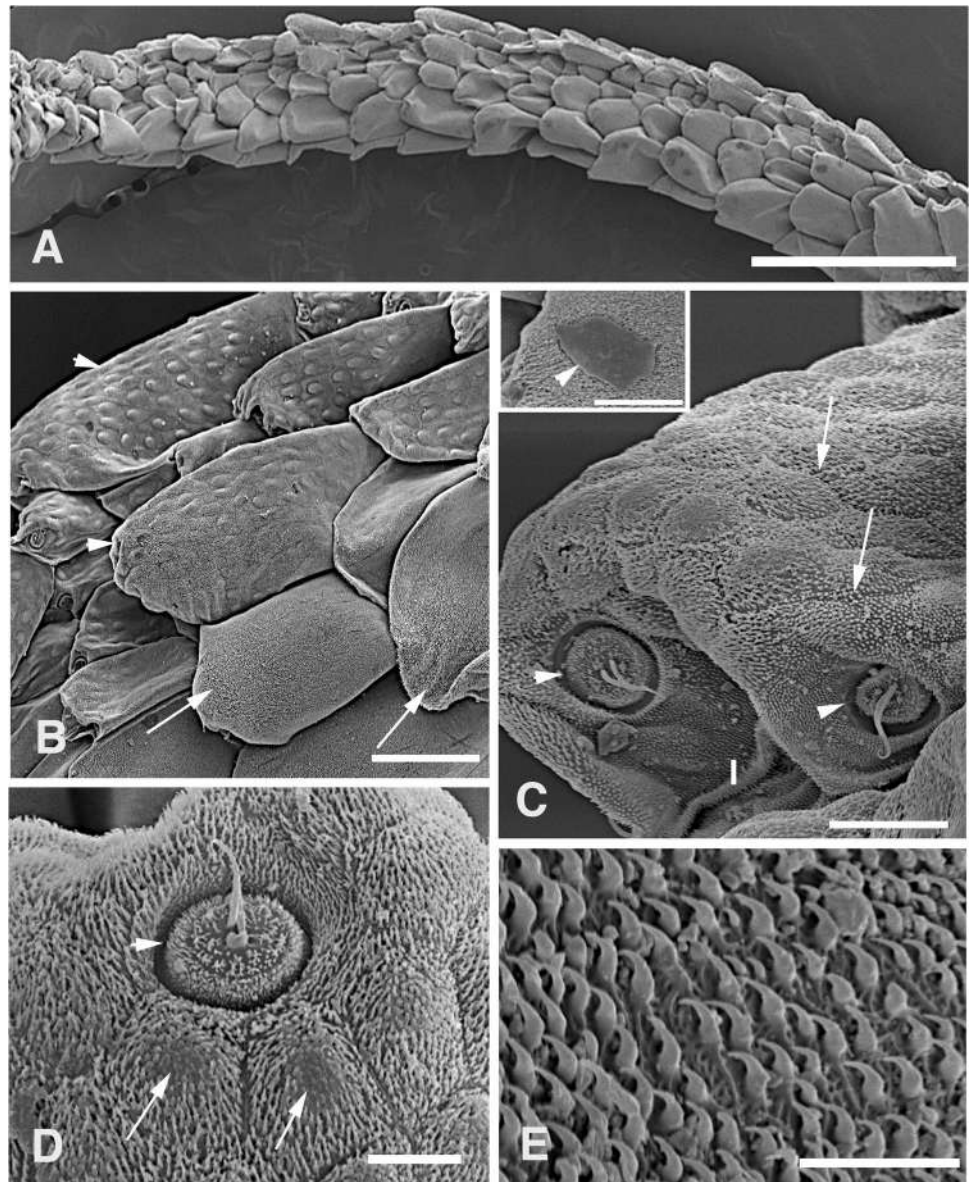
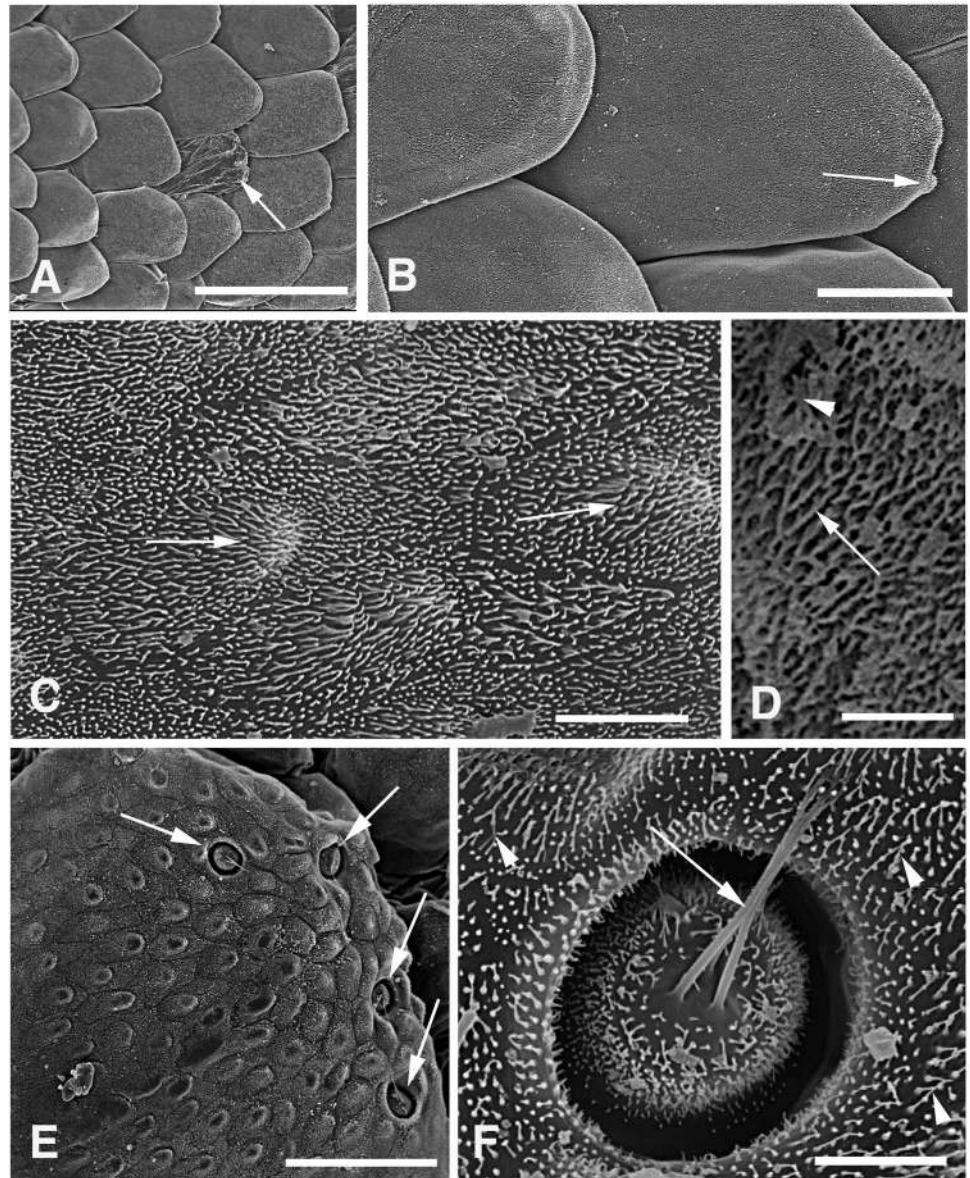


Fig. 3 SEM view of limb scales. **A** Slightly overlapped scales. The arrow indicates a scale that has lost the outer layers during preparation evidencing the alpha layers present underneath. Bar 300 μm . **B** Close-up to limb scales with a uniform Oberhautchen surface made of isolated spinulae. The arrows indicate the position of a sensilla on the anterior border. Bar 60 μm . **C** Detailed dorsal view of the spinulated microornamentation of the outer (dorsal) surface with central bumps (arrows). Bar 10 μm . **D** Higher mag showing the dendritic pattern of microornamentation (arrow). The arrowhead points to some remnants likely from the clear layer. Bar 10 μm . **E** Most of cells show bumps in this leg scale that also contains various sensilli (arrows). Bar 60 μm . **F** Detail on a sensilla with 2 sensory hairs (arrow) implanted on a bare Oberhautchen surface. Arrowheads indicate the dendritic microornamentation. Bar 6 μm

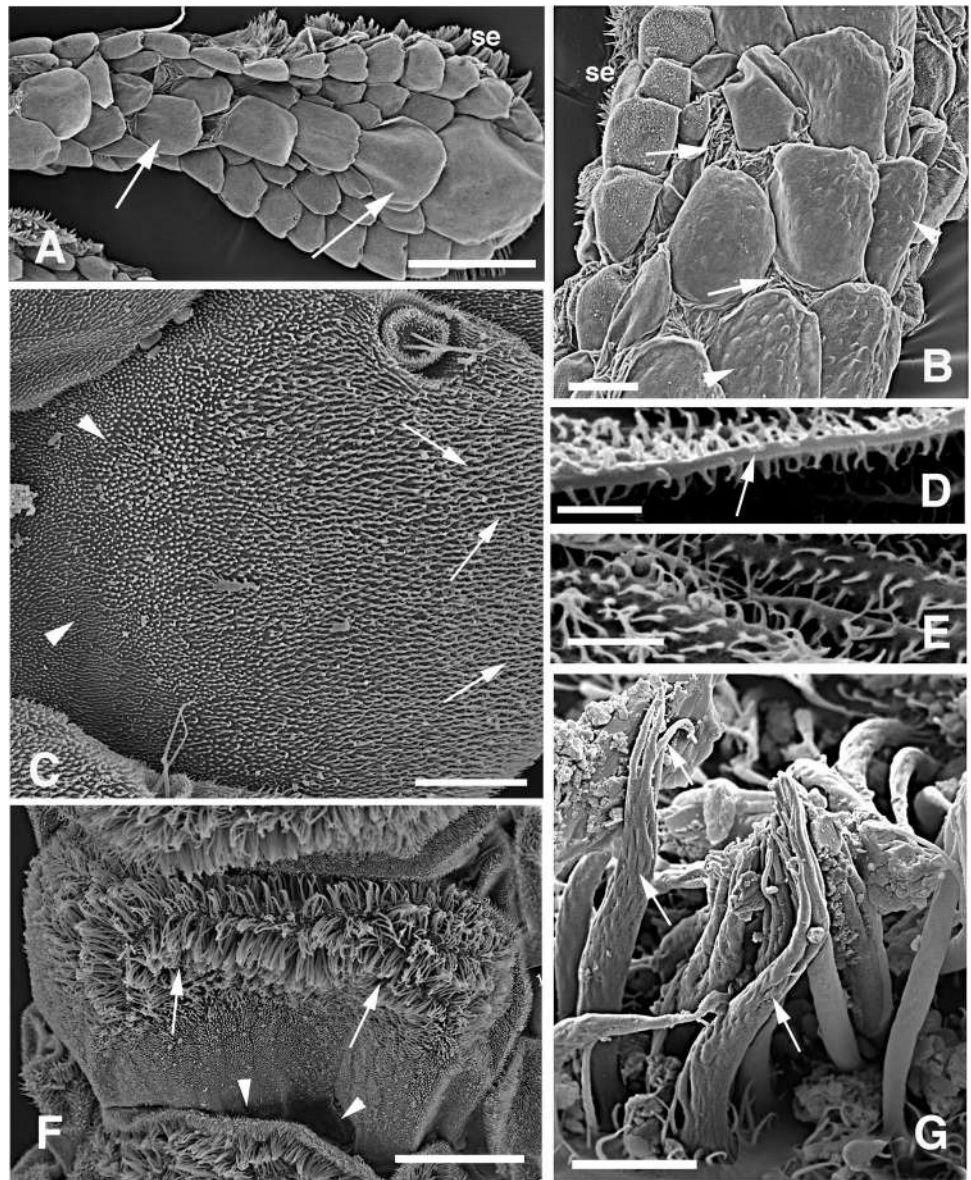


area and near the scale tip (Fig. 4C). Tiny and separated spinulae of 0.5–1.0 μm are present at the border between outer and inner scale surfaces (Fig. 4D), and in the hinge regions (Fig. 4E). On the ventral side of the digits, a series of overlapped pad lamellae are present and these modified scales include the long adhesive setae (Hiller 1972; Fig. 4F). The longer setae (30–50 μm in length) are observed at about 1/3 distal of the outer surface of a pad lamellae, the exposed area that contact the substrate for adhesion. Thinner setae of intermediate length, composed by merging ropes of corneous material, are seen along the perimeter of the lamella in continuation with the setae (Fig. 4G).

SEM observations on ventral and dorsal regions

Most ventral (belly) and lateral scales of the trunk are round-ish and nonoverlapped (tuberculate) and the interscale region (hinge) is visible among them (Fig. 5A). Sensilli are sparse or even absent in most scales and the outer surface shows an evenly bed of spinulated microornamentation with occasional naked areas of 5–10 μm (Fig. 5B, C). In the lateral scales of the trunk, small and roundish scales are also seen and they are mainly covered by a spinulated pattern (Fig. 5D). In ventral and lateral scales, some of the long spinules present a laminar shape with connecting thin bridges of corneous material (Fig. 5D, E). The size of spinulae in

Fig. 4 SEM view of digital scale. **A** Panoramic dorsal view featuring the different scale size and the larger central scales (arrows) of a digit; (se) setae (located on the ventral side). Bar 300 μm . **B** Dorsal view of the proximal part of a digit showing numerous scales featuring bumps (arrowheads). Arrows indicate the wrinkled interscale layers; (se) setae (located on the ventral side). Bar 100 μm . **C** Heterogenous patterns of a scale with the passage from a spinulated surface (arrowheads) in the proximal region to a dendritic or corneous belt pattern (arrows) in the distal (apical) part (arrows). Bar 20 μm . **D** Perimetral border of a scale (arrow) along which only spinulae are present. Bar 4 μm . **E** Isolated spinulae in the hinge (interscale) region. Bar, 2 μm . **F** Pad lamellae with apical setae (arrows). The proximal region of the lamella shows a uniform spinulated surface. Arrowheads indicate the direction of the proximal surface that is located underneath (hidden from) the previous lamella. Bar 80 μm . **G** Close-up to some short setae located along the perimeter of a pad lamella, and showing the agglutinated corneous ropes (arrows) that form these corneous elongations. Bar 10 μm

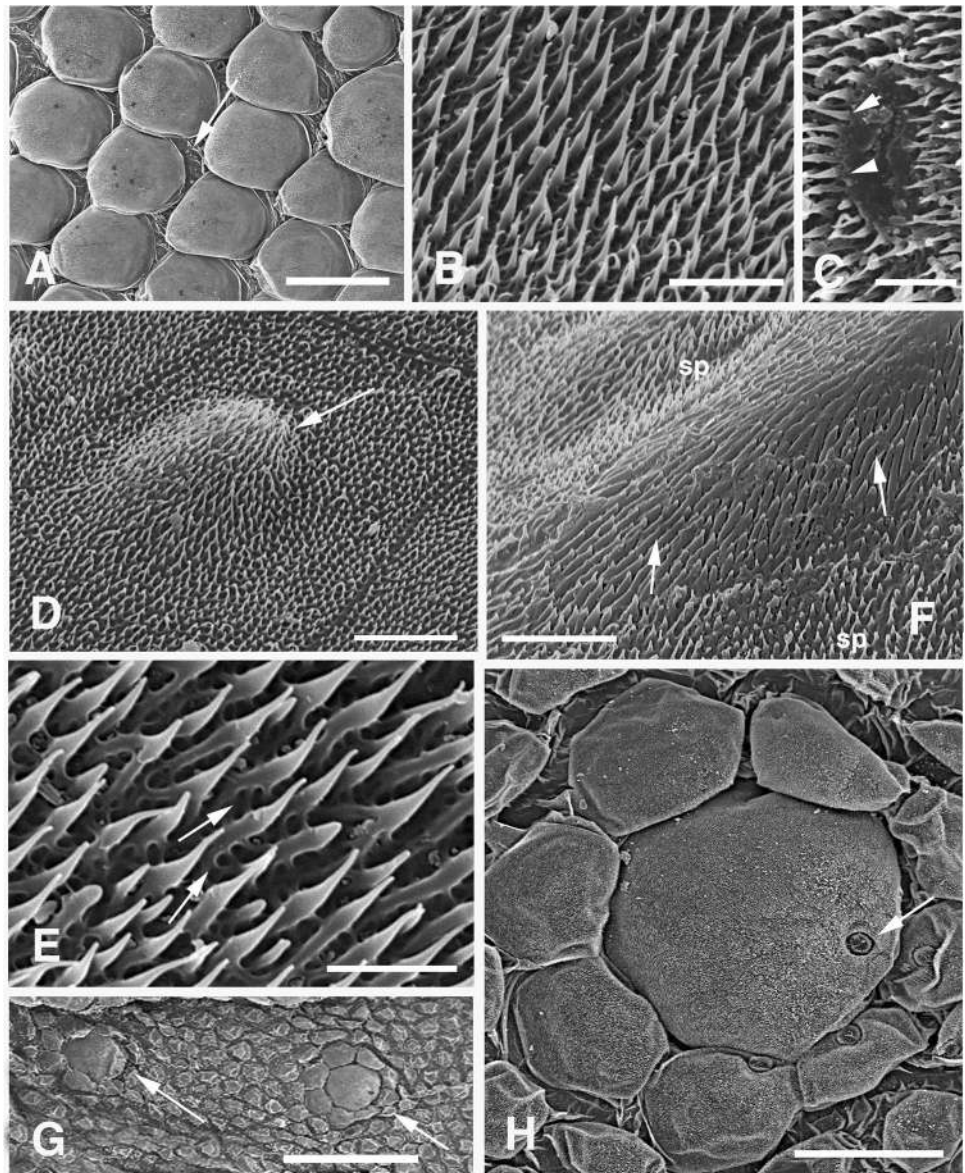


the belly and ventro-lateral scales varies, with a mean of 1.7 μm (Fig. 1C, Table 1).

A different and characteristic pattern is observed on the disposition, size, and shape of scales on the dorsal region (back). In this part of the skin, scales form an ordered disposition here indicated as “rosette-like aggregation” made by multiscale units with a longitudinal disposition along the skin (Fig. 5G). Each rosette is composed by a larger central and roundish scale surrounded by 7–8 smaller scales, here indicated as “companion scales” (Fig. 5H). Rosette are separated by rows of smaller scales here indicated as “inter-rosette scales.” Scales in the rosette-like aggregation may have a flat Oberhautchen surface or form superficial bumps (Fig. 6A, B). The prevalent micro-ornamentation on both types of surfaces is the spinulated patterns that is made from the shortest spinulae of the entire skin surface, 0.3–0.8 μm

(Table 1; Fig. 6C). The largest spinulae are present in the central scale of the rosette while in the surrounding companion scales the spinulae are the smallest in the skin, about half of the larger spinule (see Table 1). The broader variation in Oberhautchen microornamentation is observed in these dorsal scales, forming laminar oriented spinules and dendritic ramifications (Fig. 6D, E). In other areas, the Oberhautchen forms typical short spinulae, and they appear connecting the border of adjacent cells, probably merged one with another with the maturation of the Oberhautchen layer (Fig. 6F). The surface of companion scales shows very short spinulae, isolated from variable regions of bare Oberhautchen or spinulae connected by thin corneous bridges or roots, and merged in a corneous dendritic ramification (Figs. 6G, H; 7A, B). Fragments of cornified clear layer, compenetrated by the tip of spinulae, are sparsely observed above the Oberhautchen

Fig. 5 SEM view of ventral (A–C), lateral (D, E) and dorsal (G, H) scales. **A** Roundish shape with hinge regions (arrow). Bar 200 μm . **B** Higher magnification view showing the long separated spinulae. Bar 6 μm . **C** Small bare area connected to the base of spinulae (arrowheads). Bar 5 μm . **D** Bump (arrow) on the otherwise spinulated surface. Bar 5 μm . **E** Detail of spinulae with multiple corneous roots (arrows). Bar 4 μm . **F** Spinules (sp) turning into laminar pointed structures (arrows) in the central area of a scale. Bar 5 μm . **G** Low magnification view of dorsal (back) area occupied by small dorsal scales with two rosette-like aggregations (arrows). Bar 1 mm. **H** Detail on a rosette-like aggregation of scales featuring an evenly spinulated surface and an apical (caudal) sensilla (arrow) on the large and central scale. Bar 100 μm



layer (Fig. 7C). The hinge and inner scale surface of these dorsal scale, like for the scales of other regions, shows a more or less uniform bed of small and isolated spinulae (Fig. 7D).

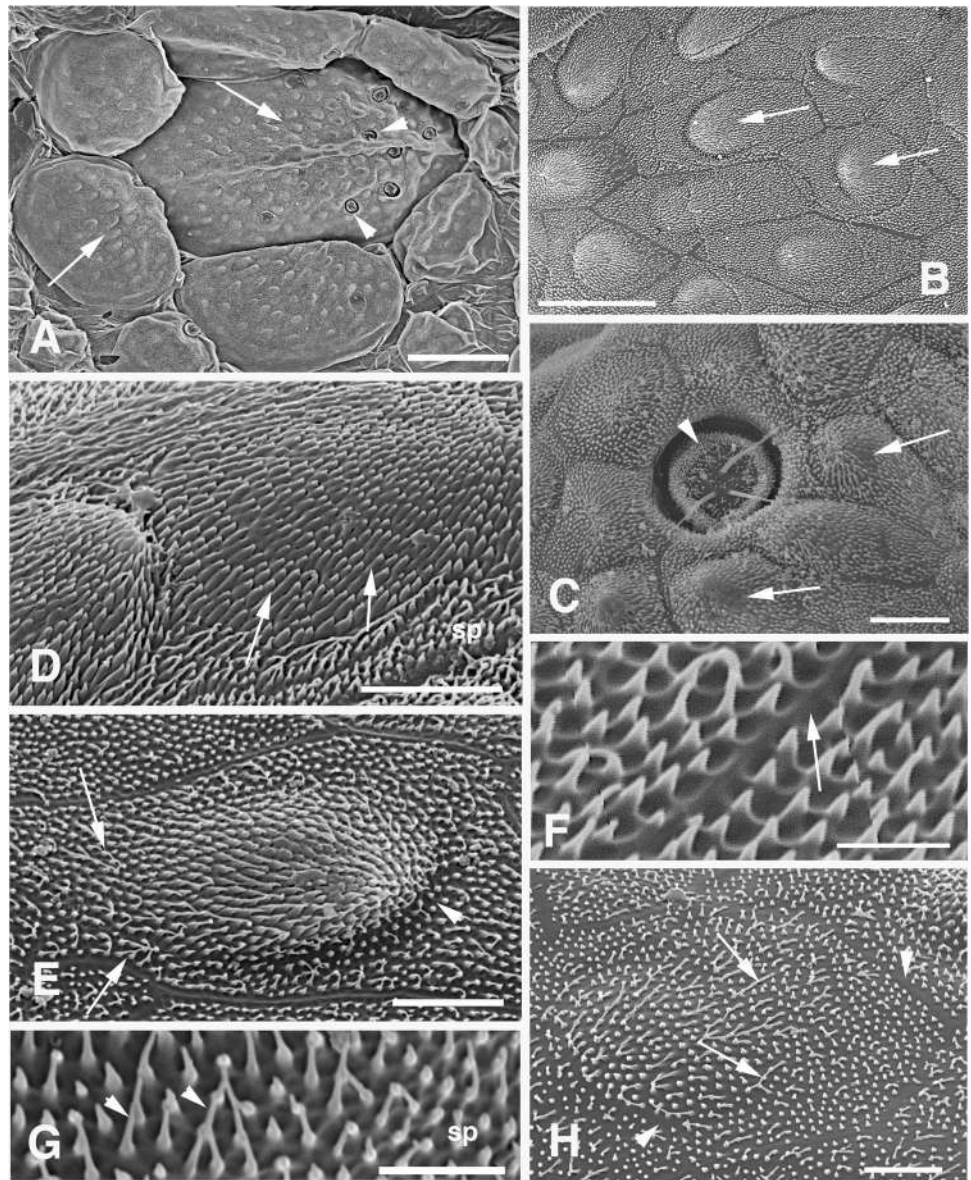
The larger scales in the center of a rosette are richer in sensilli, 3 to 6, and they are located on the distal side of the scale (Fig. 6A, C). Companion scales possess only 1–2 sensilli by their distal side and the inter-rosetta scales generally only 1 sensilla. These sensorial organs possess a variable number of sensorial hairs, 2 to 5 (Table 1).

SEM observations on head and neck scales

Most head scales, especially those of the mandibular sides, have the largest size compare to other skin regions and their density per square millimeter is consequently much lower

(Table 1). The shape of head scales varies according to the examined area: polygonal on the nape, rectangular and keeled around the orbits, rectangular and very large along the jaw perimeter, small and round in the throat, and organized in a rosette-like pattern in the neck (Figs. 7E–J; 8A, B). Almost all head scales possess sensilli with peripheral or central location on the different scales. Most scales also possess “bumps” with spinulae (mean length of 1.3 μm) and dendritic pattern as described in other areas (Fig. 8A–C). The pattern of micro-ornamentation is very variable in dorsal (forehead), nape, and labial scales, giving rise to areas on the scale surface with complex isolated or merged corneous dendritic ramifications (Fig. 8C–E) and also “corneous belts” (Fig. 8F). Sensilli are particularly numerous in peri-orbital, nape, and jaw scales, and they can reach as many as 20 per scale,

Fig. 6 SEM view of dorsal scales. **A** Large rosette-like scale aggregation in central dorsal area of the skin, made by bumped scales (arrows) with sensilli (arrowheads). Bar 100 μm . **B** Closer view of the bumps (arrows). Bar 20 μm . **C** Sensilla (arrowhead) of central scale surrounded by bumps (arrows) of Oberhautchen cells. Bar 10 μm . **D** Another central area of dorsal scale showing laminar spinulae (arrows) near isolated spinulae (sp). Bar 6 μm . **E** Variation of micro-ornamentation, from spinulated in front of a bump (arrow) to corneous-belt (arrows). Bar 5 μm . **F** Typical isolated small spinulae on the surface of “companion scales” (around the central scale) with the possible line of separation between oberhautchen cells (cornified at maturity). Bar 2 μm . **G** Variation of the micro-ornamentation pattern from corneous belt (arrowheads) to isolated spinulae (sp). Bar 2 μm . **H** Companion scale with separated spinulae although corneous belts are sparsely formed (arrows). Bar 5 μm



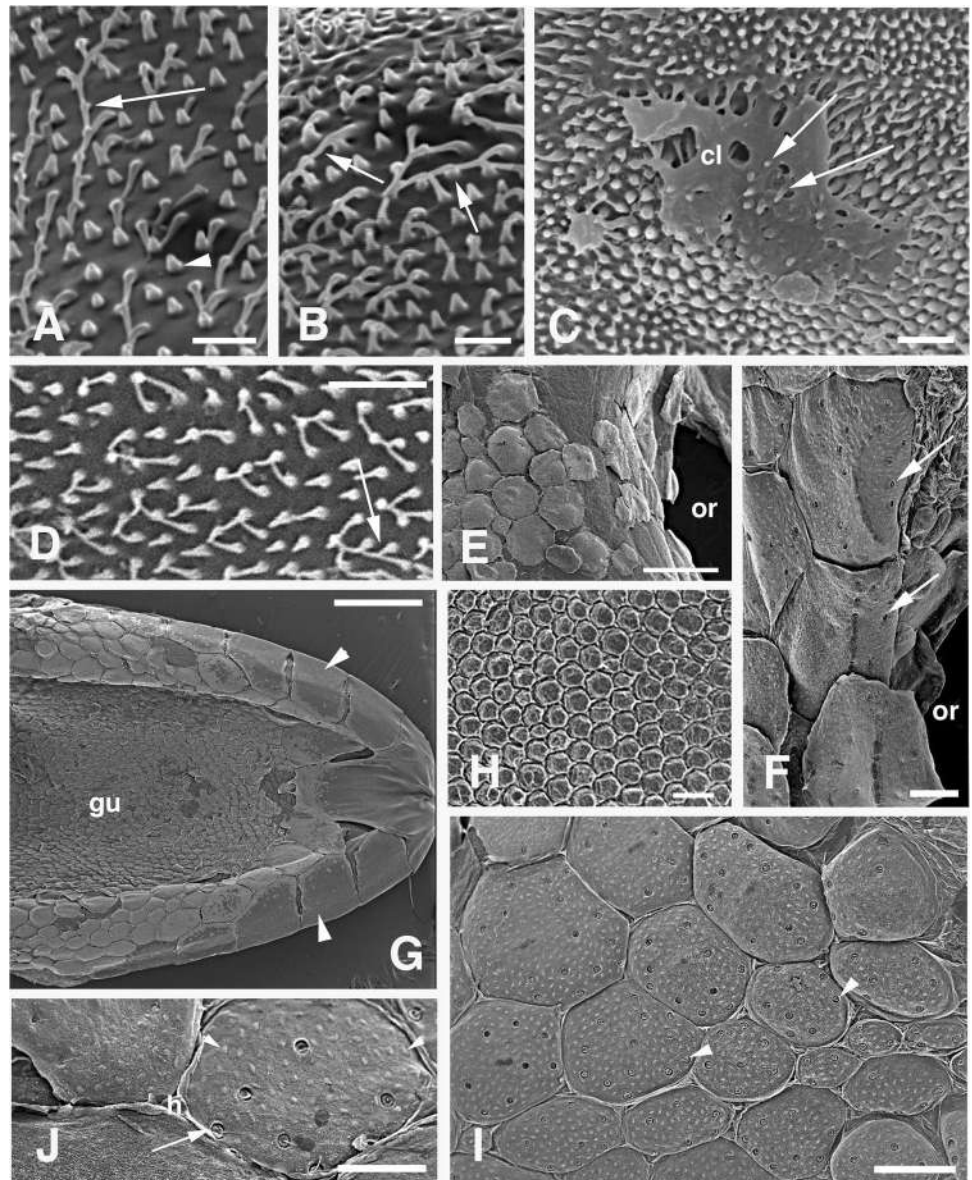
with a longitudinal distribution (periorbital scales) or with a random distribution in the other scales (Figs. 7F, I, J; 8A, B; Table 1). On the forehead, the number of sensilli is much lower than that in the other scales, about 2 per scale (Table 1), and all sensilli possess 1–3 sensory hairs. In some preparations, the circular dark tissues (naked Oberhautchen) of the sensilli is crossed by numerous corneous roots (Fig. 8G).

TEM observations on digital and caudal scales

The analysis of tail and digital scales during epidermal renewal (stages 3–4–5 of the shedding cycle) shows the formation of spinulae by the coalescence of numerous beta-packets and/or beta-bundles at their base (Fig. 9A). Oberhautchen and clear cells are initially (stage 3) cubic and electron-paler but they

rapidly shrink and become dark in successive stages (stages 4–5). An electron-dense, fibrous material is present in the interfaced, specular denticles of clear cells, and numerous but sparse keratin filaments are detected in the cytoplasm. In scales that show the duplication of epidermal generations, under-neath the mature outer beta-layer and immature alpha-layer, a new Oberhautchen with differentiating beta-cells is present (Fig. 9B, C). A row of spinulae sustained by a narrow and dark Oberhautchen appears in contact with a flat clear layer of the outer epidermal generation (Fig. 9D, stage 4). The clear layer turns corneous and dark later (corresponding to stage 5), and it surrounds the mature spinulae of the inner Oberhautchen (Fig. 9D, E). Once the clear layer is shed, only the spinules remain and they are particularly small and isolated in the hinge region where the beta-layer is absent (Fig. 9E). While a compact beta-layer is present on the outer scale surface

Fig. 7 SEM view of dorsal (back, **A–D**) and head (**E–I**) scales. **A** Detail of micro-ornamentation showing a dendritic pattern (arrow) and isolated spinulae (arrowhead). Bar 2 μm . **B** Another region featuring corneous belts (arrows) among isolated spinulae. Bar 2 μm . **C** Detail showing a likely fragments of the clear layer (cl) not yet shed but instead pricked by the tips (arrows) of numerous spinulae of the Oberhautchen. Bar 5 μm . **D** Detail on an areas occupied by isolated spinulae (arrow indicate a corneous belt). Bar 3 μm . **E** Shape of scales near the ocular orbit (or). Bar 1 mm. **F** Higher view of scales located along the border of the orbit (or). Arrows indicate some of the numerous sensilli. Bar 300 μm . **G** Gular region (gu) composed by small scales surrounded by the large scales of the jaw (arrowheads). Bar 1 mm. **H** Low magnification of the central gular region made by small circular scales. Bar 500 μm . **I** General roundish shape of non-overlapped nape scales where numerous sensilli are seen (arrowheads). Bar 300 μm . **J** Higher view of nape scale with sensilli (arrow) and numerous bumps (arrowheads). The interscale or hinge region (h) is very narrow. Bar 200 μm



(not shown), the spinulae of the inner scale surface and hinge region form a very thin Oberhautchen while the beta-layer is missing and only thin meso-cells, 0.1–0.2 μm , are present underneath (Fig. 9B, E, F). Although the Oberhautchen appears spinulated under TEM examination, this imaging only reflects the plane of sectioning while the real 3D structure only appears under SEM examination, as previously described.

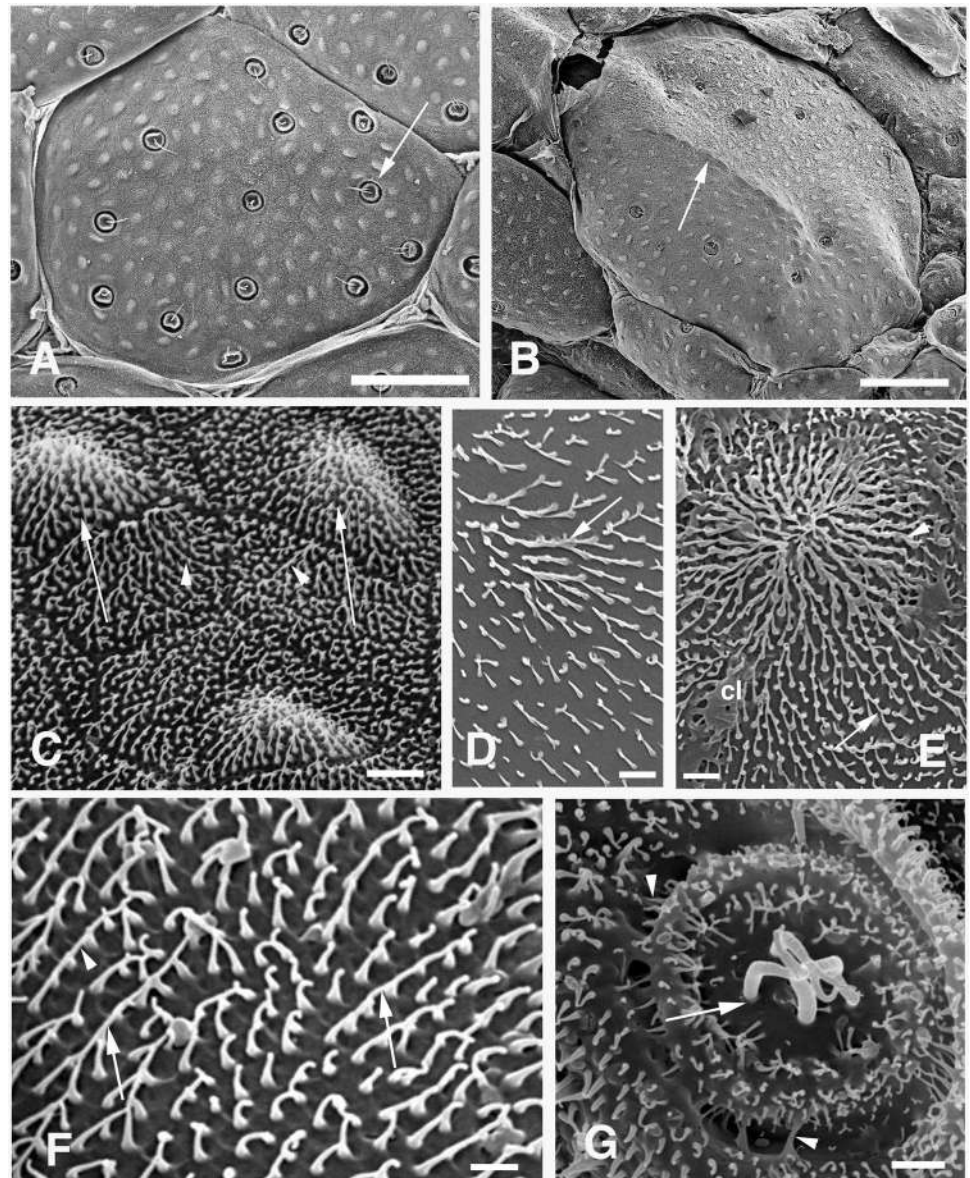
Discussion

Micro-ornamentation of body scales

The present observations, coupling SEM and TEM analyses, have documented numerous variations of the micro-ornamentation pattern present in scales of *T. mauritanica*,

showing that sculpturing of the epidermal surface is more complex than previously known (Fig. 10). Differently from previous studies on gecko micro-ornamentations (Hiller 1972, 1977; Spinner et al. 2013; Dujsebajeva et al. 2021; Riedel and Schwarzkopf 2022), the present study has detected a broad variability in the distribution of diverse patterns of micro-ornamentation in scales of various body regions, as previously documented for the geckos *Lygodactylus capensis* and *L. conraui* (Bonfitto et al. 2022). This is likely in connection with the variation in the process of deposition of corneous material on the surface of Oberhautchen cells interfaced with the cytoplasm of clear cells in different areas of the epidermis (Hiller 1972; Alibardi 1999a; Maderson et al. 1998). Heterogenous areas of micro-ornamentation with transitional regions or even with a basal-apical gradient of micro-ornamentation were also noted in the scales of

Fig. 8 SEM view of head scales with details of micro-ornamentation. **A** Nape scale near the neck featuring numerous sensilli (arrow). Bar 80 μm . **B** Rosette-like aggregation in the neck with a keel visible (arrow) in the central scale. Bar 100 μm . **C** Higher magnification of the bumped surface (arrows) where long corneous belts (arrowheads) are present. Bar 8 μm . **D** Detail on a nape scale showing isolated spinulae and a dendritic-pattern (arrow). Bar 6 μm . **E** Complex micro-ornamental surface made by apparently convergent corneous belts (arrows) mixed with more irregular dendritic corneous ramifications (arrowhead). Sparse corneous fragments of likely clear layer (cl) are observed. Bar 6 μm . **F** Close-up to a surface containing isolated small spinulae mixed with corneous belts micro-structure (arrows). Bar 2 μm . **G** Dorsal front view of a sensilla of a dorsal head scale (arrow indicate the sensory hairs) showing the corneous roots (arrowheads) connected with the surrounding Oberhautchen layer. Bar 4 μm



numerous species of snakes, where micro-ornamentation is indicated with the general term microdermatoglyphics (Price and Kelly 1989). Previous and the present observations indicate that the thinner the clear cells in hinge regions, the thinner is the resulting Oberhautchen and spinulae remain isolated (Fig. 10). In contrast, on the outer surface of scales, thicker clear and Oberhautchen cells give rise to longer spinulae but also to transitional regions where the deposition of corneous material gives rise to dendritic or corneous belt patterns and even to small bare areas (Fig. 10).

Also previous studies on other geckos have reported at least the three different patterns of micro-ornamentation here illustrated in addition to the classical “spinulated pattern” (Griffing et al. 2021; Bonfitto et al. 2022). A more irregular pattern was indicated as “dendritic ramification” and another

as apical “corneous belts,” two patterns that occupied variably extended areas of scales mixed with areas occupied by typical spinulae (Fig. 10). These different patterns can also be observed in the “superficial bumps” or reliefs here observed in the dorsal scales of the tail, limbs, digits and toes, back, and nape while they are almost absent in the ventral scales of the same regions where a even bed of spinulae is instead present. The nature of these bumps or central scale reliefs or “spinulated hillocks” (Irish et al. 1988) remains uncertain, namely whether they represent fidelity images of the surface or are instead generated from the artifactual cohartation resulting from the ethanol storage of the sample. Also, the induction of folds during sample preparation that deform the micro-ornamentation surface may distort the spinulated pattern giving rise to the apperent other dendritic

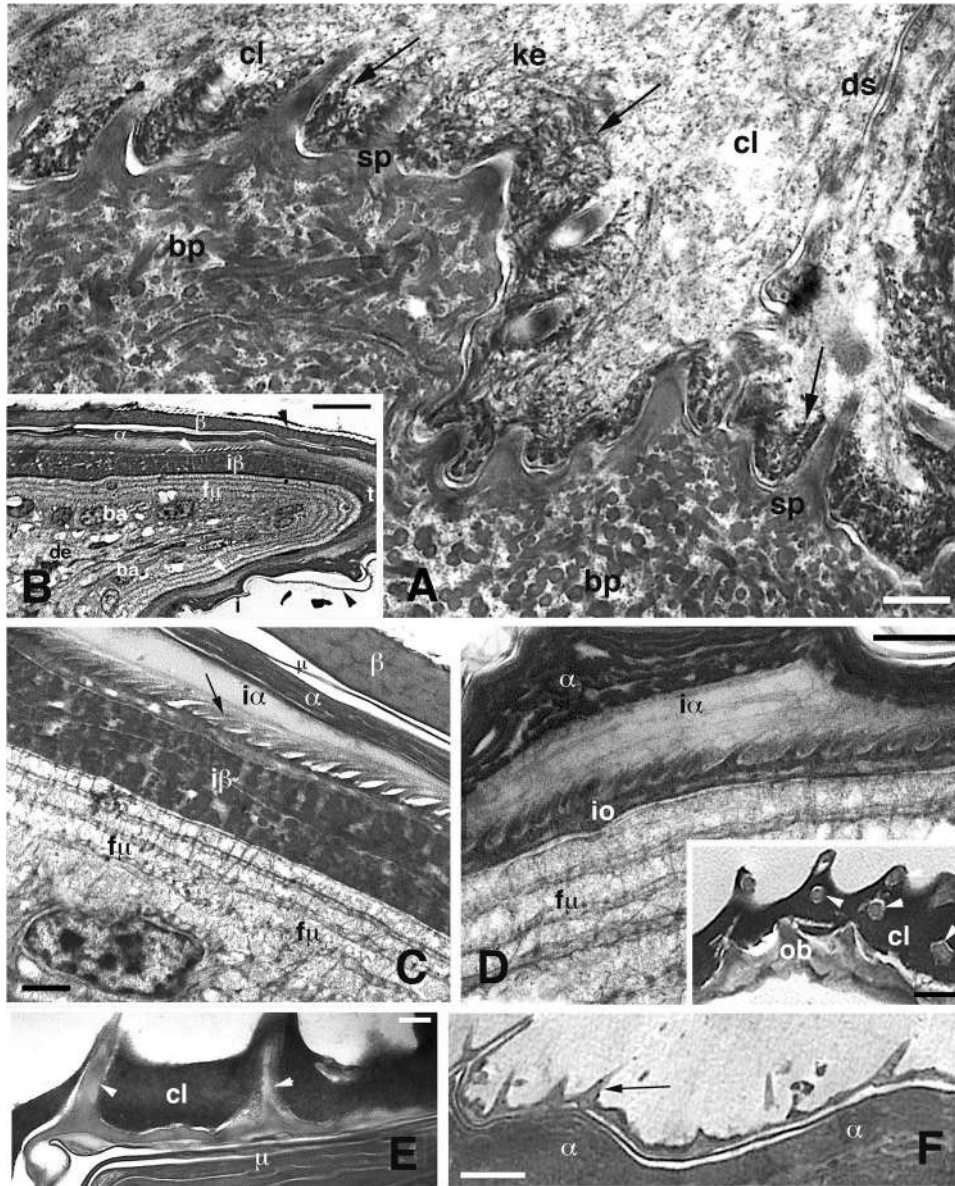


Fig. 9 TEM images of Oberhautchen cell differentiation in digital dorsal scales at stages 3–5 of the shedding cycle with duplication of epidermal generations. **A** Detail on the accumulation of beta-packets (bp) in the forming spinulae (sp) at stages 3–4. A dense fibrous material (arrows) in the clear layer (cl) surrounds the spinulae while sparse keratin filaments (ke) are present. Bar 200 nm. **B** Tip of a scale with epidermal duplication at stage 4. The black arrowheads indicate the outer Oberhautchen while the white arrowheads point to the inner Oberhautchen. Bar 10 μ m. **C** Area of the outer scale surface showing the entire layer sequence with the maturing inner Oberhautchen (arrow) and the underlying inner beta-layer (arrowheads). Bar 2 μ m. **D** Detail near the hinge region where no beta-cells are present but

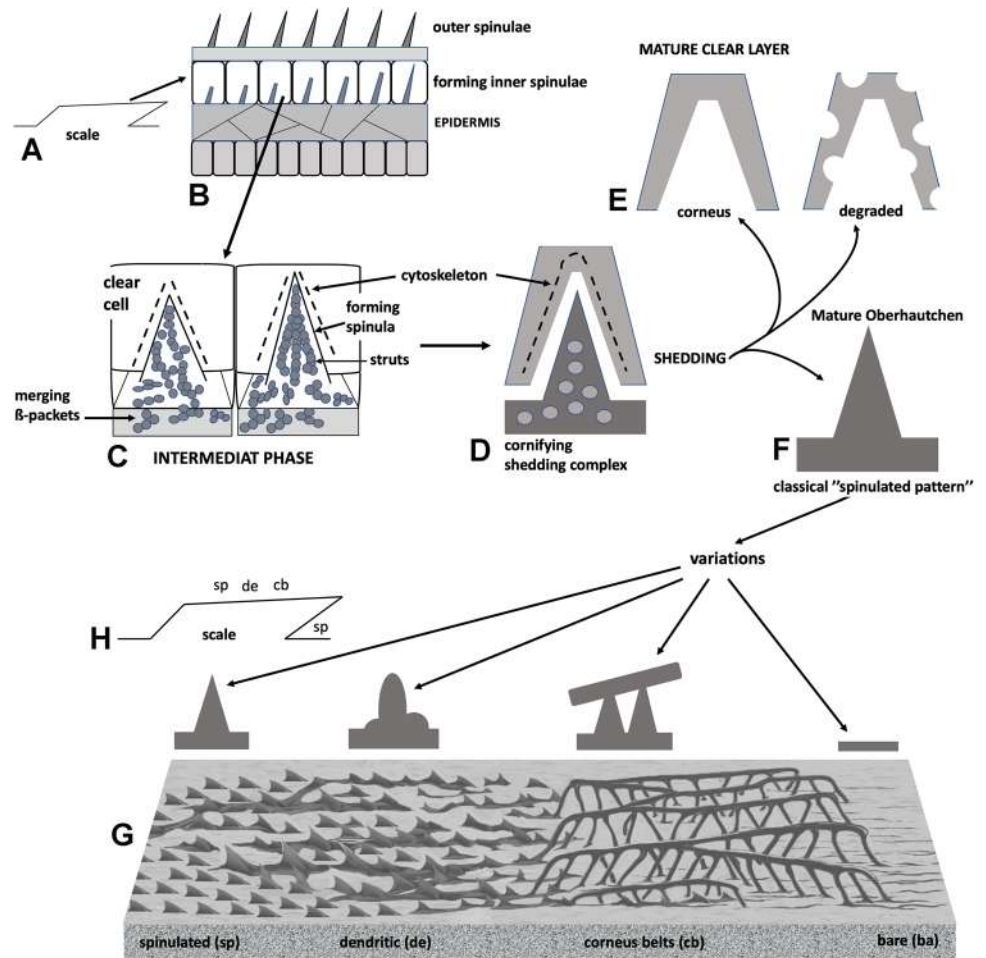
only the forming (inner) Oberhautchen. Bar 2 μ m. The inset (Bar 0.5 μ m) details on the spinulae of the Oberhautchen (arrowheads) inserted in the mature (dark) clear layer. **E** Another view of mature spinulae (arrowheads) within the dark clear layer before shedding (stage 5). Bar 200 nm. **F** Very thin mature Oberhautchen with spinulae in the hinge region. Bar 1 μ m. Legends: α , outer alpha-layer; β , outer beta-layer; ba, basal epidermal layer; bp, beta-packets/bundles; cl, clear cell layer; de, dermis by the scale tip; ds, desmosome; f μ , forming (inner) mesos layer; i, inner scale surface; i α , immature alpha-layer (lacunar); i β , forming inner beta-layer; io, inner Oberhautchen; ke, keratin filaments; μ , outer mesos-layer; ob, oberhautchen; sp, spinulae; t, by the scale tip

or corneous belt patterns, although this possibility remains remote and however indicate a certain plasticity of gecko micro-ornamentation.

However, the bumps are often localized in rows on central scales of the tail or limbs while they are absent in other

surrounding scales or are almost absent in the ventral skin region, so an artifact of preparation seems to be unlikely. Also, the bumps are centrally located within the cell borders, and these details indicate that they are not artifacts but true variations due to the central lifting of the Oberhautchen

Fig. 10 Drawing summary of the present observations. The epidermis of scales in renewal phase present two generations of epidermis and their spinulae (A, B). C illustrates the accumulation of corneous beta-material into the spinulae while they grow inside clear cells. As they mature, spines are fully corneous while clear cells also accumulate fibrous (cytoskeletal) material. At shedding from corneous or degenerating clear cells (E), fully corneous spinulae (F) remain on the surface of the epidermis. According to the variable superficial accumulation of corneous beta-material, at least four variations of micro-ornamentation are produced (G). While in the hinge region only small spinulae are formed, on the outer (dorsal) scale surface a mix of all four patterns can be found, including rare small bare areas (H)



surface. Similar central reliefs are also reported for other species of lepidosaurians (Irish et al. 1988). The higher length of spinulae in ventral areas of the trunk, head, and tail in comparison to the dorsal sides (Table 1, Fig. 1C), may be related to increasing frictional forces in the ventral zones of the skin in contact with the substrate, a tendency also observed in *Lygodactylus* sp. (Bonfitto et al. 2022). This phenomenon does not occur in hinge regions and in dorsal scales where a selection for friction is absent while sensorial input is increased in comparison to ventral areas. Short micro-ornamentation in these regions may have other roles such as increasing surface reflectivity, cleaning from dusts or fouling in dorsal scales and generally in hinge regions (Fahrenbach and Knutson 1975; Peterson 1984a; Arnold 2002; Gower 2003). Finally, the sparse or occasional tiny smooth or bare dark spots detected over various scales, likely represent naked Oberhautchen areas where likely the beta-layer is visible, smooth or in form of “serpentine ridges” (sensu Maderson et al. 1998; Bonfitto et al. 2022). While some of these regions may result from some artifacts introduced during the SEM preparation, others such as the “serpentine ridges” instead are very likely true superficial

discontinuities of the Oberhautchen layer revealing the underlying corneous material accumulated in beta-cells.

The chemical composition of the corneous material accumulated in Oberhautchen cells is mainly derived from CBPs, but also other unknown proteins are synthesized in this transitional layer formed underneath the old epidermal generation of alpha- and clear-cells (Maderson et al. 1998; Alibardi 2014; Alibardi and Toni 2006; Gamble 2019). All the different micro-ornamentation patterns and their transitional regions (Fig. 10) very likely derive from the almost perpendicular deposition of corneous material composed by CBPs and unknown dense material, as scales in geckos are mainly tuberculate, namely symmetric and not overlapped like in most other lizards and snakes. This means that these roundish scales grow peripherally, expanding their epidermal surface on a vertical direction and therefore generating roughly vertically pointed or slightly tilted spinulae. In contrast, in scales of other lizards and in most snakes, the growth is also lateral, generating overlapped scales (Irish et al. 1988; Price and Kelly 1989). It is therefore likely that the accumulation of the initially soft corneous beta material formed in Oberhautchen cells is subjected to a traction force

toward the direction of scale growth. This process might progressively shift the deposition of the corneous material within Oberhautchen cells in the direction of scale elongation giving eventually rise to a slanted dentate or dentate-lamellate aspect of the mature micro-ornamentation (Irish et al. 1988; Price and Kelly 1989; Gower 2003). This hypothesis however needs specifically designed experiments to be confirmed (or denied), especially altering the cytoskeleton present in clear cells of different size.

Types and distribution of sensory organs

The present study reports the presence of numerous sensilli in *T. mauritanica*, like for other geckos. In previous studies on geckos' micro-ornamentation, sensory organs included essentially the "spinulate type of sensilli" with 1–5 sensory hairs terminating with a single ending (Hiller 1977; Dujsebajeva 1995). In *T. mauritanica*, they are more frequent on the dorsal than in ventral scales (Table 1) but the specific reason for this localization, like for other reptilian species, is presently only speculative (Ananjeva et al. 1991; Harvey and Gutberlet 1995; Matveyeva and Ananjeva 1995; Ananjeva and Matveyeva-Dujsebajeva 1996; Povel and Van Der Kooij 1997). The thinning of beta- and alpha-layers of the epidermis observed for these microscopically localized areas of the epidermis of reptiles are likely favoring the innervation and sensory perception (Von Düring and Miller 1979). Spinulated sensilli are particularly numerous in head scales, and they are mainly localized in labial and orbicular/orbital scales (Table 1). Similar observations have been reported also in lizards and snakes, indicating that these scales on the head are particularly important for sensory reception (Matveyeva and Ananjeva 1995; Povel and Van Der Kooij 1997).

Spinulated sensilli with sensory hairs are considered mainly mechanical receptors (Hiller 1978) although the detection of other sensorial stimuli such as thermal or chemical (taste or smell) have been proposed (Von Düring and Miller 1979). The association of some melanocytes with nerves in sensory organs of some agamid lizards suggested that these cells may influence the thermosensitivity of these sensilli (Ananjeva et al. 1991). However, the specific function of sensilli localized in different body scales can only derive from experimental physiological studies. Like for the remaining epidermis, also the new spinulated sensilli derive from a duplication of the epidermis and by the regeneration of a new innervation (Hiller 1977; Whimster 1980; Dujsebajeva 1995). Specifically, regenerating nerves from dorsal root ganglia appears necessary for the regeneration of sensilli in the epidermis (Whimster 1980). Finally, the presence of localized sensilli bearing sensory setae further supports the idea that local cytological specialization of clear-cell cytology can give rise to tiny local variations on scale epidermis. This process also occurs in

the outer surface of the pad lamella where spinulae are abruptly turned into longer setae.

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Declarations

Conflict of interest The authors declare no competing interests.

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